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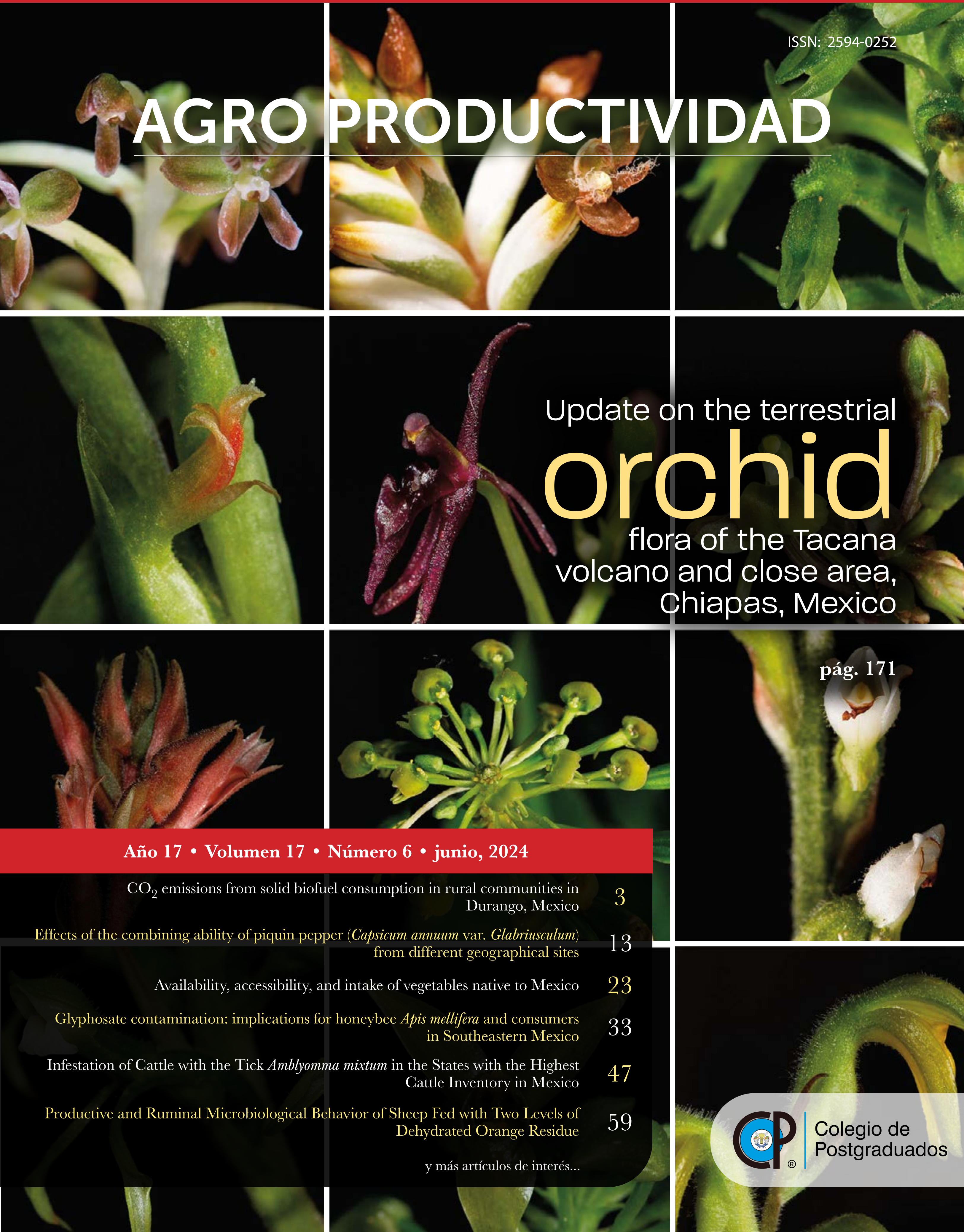

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Agradecimientos: Son opcionales y tendrán un máximo de tres renglones para expresar agradecimientos a personas e instituciones que hayan contribuido a la realización del trabajo.
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CO_2 emissions from solid biofuel consumption in rural communities in Durango, Mexico

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ABSTRACT

Objective: the objective of this study was to calculate the amount, in kilotons per year (kt a^{-1}), of CO_2 emissions from firewood consumption in rural communities of Durango in managed areas (UMAFOR - Forest Management Units) and at the municipality scale.

Design/Methodology/Approach: the firewood consumption was determined for each of the UMAFOR areas and the 39 municipalities into which the state of Durango is divided. Greenhouse Inventory Software® was used to determine CO_2 emissions.

Results: the annual CO_2 balance due to firewood consumption in Durango was 268.05 kt of CO_2. These emissions in relation to the national scale represent 1.52% per year. Those UMAFOR and the municipalities that are geographically located in the semi-arid zone of the state of Durango were those with the higher CO_2 emissions.

Findings/Conclusions: it is necessary to couple the consumption of firewood with eco-technologies that favor its efficient consumption, thus mitigating CO_2 emissions.

Keywords: firewood, greenhouse gases, rural localities, climate change.

INTRODUCTION

Air pollution affects all areas of the planet, and is defined by the effects on people's health as a result of their exposure to high concentrations of pollutants [1]; among other implications pertaining GHG emissions which generate changes in the global climate

Image by Andreas Lischka at Pixabay

[2]. Air pollutants are emitted by many sources that modify the quality of breathing air in a specific region [3]. The volume of CO_2 emissions from fuelwood consumption in developing countries is 825 million (Megagrams) Mg of CO_2 per year, and high CO_2 content has an environmental impact and consequences on human health [7].

In 2008, Mexico's National Emissions Inventory (INEM) included in its inventory estimates of pollutant emissions by source, state, and municipality [4]. However, INEM [5] mentioned that those published data are not comparable with previous inventories, since the methodologies implemented underwent some changes in order to improve the quality of accurate information. Therefore, it is necessary to recalculate previous inventories in order to make them comparable. Mexico has implemented three national GHG inventories, based on the methodology of the 1996 revised guidance of the Intergovernmental Panel on Climate Change (IPCC) [6].

Firewood is a primary source of energy for around 2.6 million people in rural communities in developing countries to meet the needs of cooking food, purifying drinking water, providing heat to households; as well as the production of clay products and the production of bread and other foods [8] The widespread use of this type of fuel is the leading cause of premature death for approximately 2.5 million people each year from inhaling large amounts of smoke [9].

CO_2 is a gas of natural origin, which is a by-product of combustion along with other gases, it is considered a greenhouse gas that affects climate change worldwide, affecting the natural balance of the planet [10]. In adequate quantities, CO_2 contributes to global habitable temperature, since without the presence of CO_2 global climatic conditions would be different. The importance of CO_2 is not due to be the most dangerous gas in terms of toxicity or permanence in the atmosphere, but in the concentration at which it is found. This is, 1000 times higher than any other product of industrial origin. Emissions of this gas are 50% of the greenhouse effect produced by human activity that forms part of global warming. CO_2 is a product of the combustion of fuels in automobiles and industrial heating, thermal power plants, residential chimneys, forest fires and the combustion of natural gas [11].

The origin of CO_2 emissions through anthropogenic activities varies by region. In the U.S., most of this gas originates from transportation. In China, by industries and thermal power plants [12]; in oil-producing countries by oil power plants; and in countries with lower human development indices, by the burning of firewood and other plant biomass fuels [13].

Mexico emits around 3.70 Mg of CO_2 *per capita*, a figure that is 4.02 Mg below the global average for emissions. Two-thirds of this volume corresponds to the various combustion processes in the energy, industrial, transports, and services sectors. The rest, about one third, originates from deforestation, land-use change, and wood burning [14, 16].

Nowadays, both developed and developing countries are adopting clean energy to mitigate global warming from GHG emissions. An example regarding the consumption of firewood in rural communities is the application of sustainable technologies such as solar stoves or ecological stoves [14]. Therefore, with the period 2021-2022 as a reference, the objective of this study was to calculate the amount, in kilotons per year (kt a^{-1}), of CO_2

emissions from firewood consumption in rural communities of Durango in managed areas (UMAFOR - Forest Management Units) and at the municipality scale.

MATERIALS AND METHODS

Study zone

This study was conducted in Durango, located in the Central-Northwest region of Mexico; at coordinates 26° 48' and 22° 19' N and 102° 28' and 107° 11' W. The bordering states are Chihuahua to the North, Coahuila and Zacatecas to the East, Sinaloa to the West, and Nayarit to the South (Figure 1).

Determination of total fuelwood consumption in Forest Management Units (UMAFOR) and at the municipal scale

The firewood consumption was determined in each of the UMAFOR and the 39 municipalities into which the state of Durango is divided. UMAFORs are areas defined according to the boundaries of watersheds, sub-watersheds or micro-watersheds, and are the basis for planning the efficient management of forest resources. Based on the above and within the framework of Mexico's General Law on Sustainable Forestry Development, CONAFOR-SEMARNAT, in coordination with the states, delimited 218 UMAFORs at the national scale, of which 13 are located in Durango (Figure 2). Also within the state administration framework, the consumption of firewood in the rural localities was determined, for the 39 municipalities composing the state of Durango (Figure 3).

The number of localities in each of the municipalities and the UMAFOR of Durango were identified and reclassified by parameters, leaving only the localities that met the established parameter, a human population between 100 and 2500 inhabitants [6].

Firewood consumption was estimated with the application of a survey in 100 randomly selected communities that included questions about the amounts used by the inhabitants

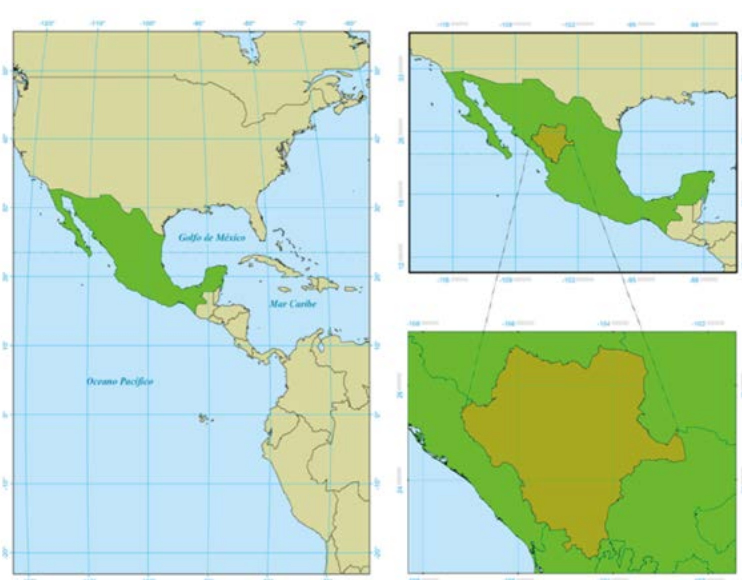

Figure 1. Geolocation of the state of Durango.

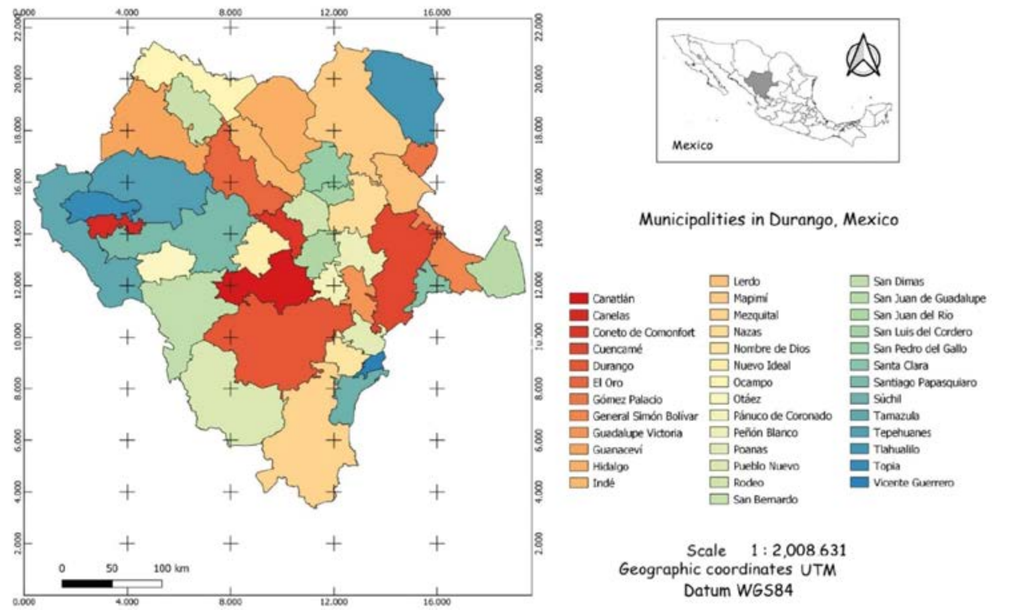

Figure 2. Geographical division by UMAFORs (Forest Management Units) in the state of Durango, Mexico. Graphics elaborated by the authors.

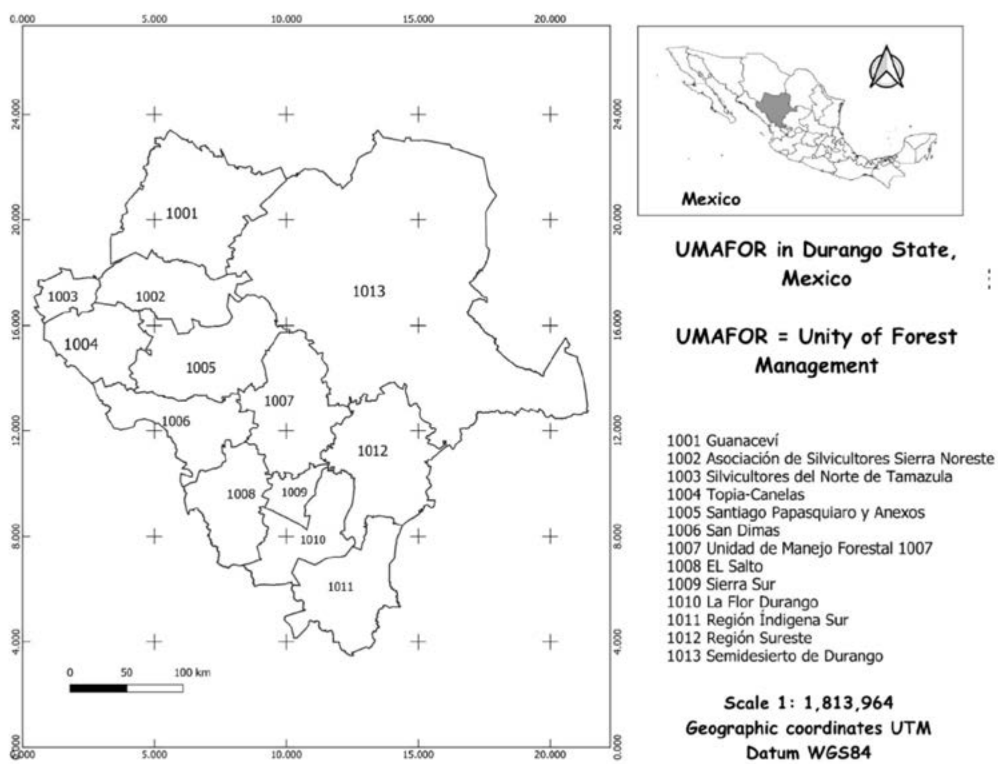

Figure 3. Geographical division by municipalities in the state of Durango, Mexico. Graphics elaborated by the authors.

(quantities estimated as transported by animals, vehicles, or manual handling) during 12 months. The questionnaire was applied in each community to 50 heads of households selected at random, of whom 75% were women and 25% were men with an average age of 41 years. Firewood was weighed in the quantities reported in the survey for a standardization of the units according to [15]. The firewood used was weighed for seven days (at the beginning and end of that period, T) according to the methodology proposed by [16] with the following equation to determine the consumption per day per person:

$$CL = \frac{Pi - Pf}{P * T} \quad (1)$$

where, CL: fuelwood consumption (kg); Pi: initial weight of firewood (kg); Pf: final weight of firewood (kg); P: number of people living in the household; T: number of days between Pi and Pf, in this case $T=7$.

Once the consumption of firewood per day was obtained, the result was multiplied by 365 to estimate the annual consumption.

Quantification of CO_2 emissions

The Greenhouse Inventory Software® was used to determine CO_2 emissions from firewood consumption. This software was designed in a series of steps based on the instructions of the 1996 Intergovernmental Panel on Climate Change Methodology [17], which includes the following formula:

$$EL = fC * fO * fCO_2 * CTL \quad (2)$$

where, EL: CO_2 emissions from the use of firewood (Mg CO_2 a^{-1}); fC=carbon content in wood (0.5); fO=percentage of oxidation of wood during combustion (87%); fCO_2=coal-to-CO_2 conversion factor (44/12); CTL=total fuelwood consumption in Megagrams per year (Mg a^{-1}).

The result obtained by equation 2 shows the total consumption of firewood in Megagrams per year (Mg a^{-1}). However it is required that the result is expressed in kt a^{-1}; therefore, the figure of firewood consumption to be entered into the IPCC-1996 software must be adjusted with equation 3 [18].

$$EL = \frac{fC * fO * fCO_2 * CTL}{1000} \quad (3)$$

With this, the estimation of CO_2 emissions from rural municipalities that burn wood in the state of Durango was obtained.

RESULTS AND DISCUSSION

Determination of firewood consumption at Forest Management Units (UMAFOR) and at the municipal scale

Firewood is used to cook most of the food consumed in homes, especially those that require long cooking times (preparation of tortillas, bean cooking, barbecue, etc.). The gas is mainly used to prepare food that does not require a lot of time on the fire. Firewood is the main source of fuel in rural communities because of the general culture of improving the taste of food, the ease of extraction and transport. The results of the estimation of the average amount of firewood by the direct and indirect methods was 3.5 kg a day per inhabitant for Durango, 76% of the interviewees mentioned using the three-stone stove to cook with firewood.

Quantification of CO_2 emissions in the UMAFOR and municipalities of Durango

According to the results, the annual balance of CO_2 released by firewood consumption in rural communities in Durango was 268.05 kt of CO_2. The contribution of each of the UMAFOR and the municipalities is presented in Tables 1 and 2. The UMAFOR and the municipalities that are located in the semi-arid part of the state (UMAFOR 1012, UMAFOR 1013, and the municipalities of Victoria de Durango, Gómez Palacio and Lerdo) are the ones that concentrate the greatest contributions to the CO_2 emitted by the consumption of firewood. This is due to the fact that those municipalities include the largest number of rural communities.

The sociodemographic profile of the semi-desert region in Durango presents environmental problems such as desertification, erosion, absence of official decrees to

Table 1. CO_2 emission from the use of firewood in Forest Management Units (UMAFOR) in kilotons per year (kt a^{-1}).

UMAFOR	No. of communities with more than 100 and less than 2,500 inhabitants	CO_2 emission in kt year^{-1}	%
1001 Guanaceví	44	7.55	2.81
1002 Tepehuanes	10	1.16	0.43
1003 Tamazula Norte	14	2.1	0.78
1004 Topia-Canelas	51	9.44	3.52
1005 Santiago Papasquiaro	78	11.73	4.37
1006 San Dimas	39	5.83	2.17
1007 Durango	92	20.9	7.79
1008 El Salto	52	8.98	3.35
1009 Sierra del Sur	13	4.07	1.51
1010 La Flor	34	7.5	2.79
1011 Mezquital	58	8.04	2.99
1012 Región Sureste	137	52.92	22.35
1013 Semidesierto de Durango	400	127.82	47.68
Total	1,022	268.04	100%

Table 2. CO_2 emission from the use of firewood at the municipal scale in kilotons per year (kt a^{-1}).

Municipality	No. of communities with more than 100 and less than 2,500 inhabitants	CO_2 emission in kt $year^{-1}$	%
Canatlán	35	11.9	4.43
Canelas	9	1.26	0.47
Coneto de Comonfort	10	2.72	1.01
Cuencamé	28	12.08	4.50
Durango	105	35.65	13.30
El Oro	20	2.57	0.95
General Simón Bolivar	22	6.67	2.48
Gómez Palacio	90	34.78	12.97
Guadalupe Victoria	10	7.07	2.63
Guanaceví	20	2.88	1.07
Hidalgo	10	2.44	0.91
Indé	17	2.65	0.98
Lerdo	43	19.98	7.45
Mapimí	18	3.41	1.27
Mezquital	68	9.52	3.55
Nazas	15	5.31	1.98
Nombre de Dios	27	8.17	3.04
Nuevo Ideal	14	3.25	1.21
Ocampo	41	9.08	3.38
Otáez	18	2.49	0.92
Pánuco de Coronado	15	4.66	1.73
Peñón Blanco	10	3.15	1.17
Poanas	14	8.97	3.34
Pueblo Nuevo	57	10.26	3.82
Rodeo	25	4.74	1.76
San Bernardo	10	1.42	0.52
San Dimas	41	6.07	2.26
San Juan de Guadalupe	15	2.94	1.09
San Juan del Río	26	5.17	1.92
San Luis del Cordero	3	1.29	0.48
San Pedro del Gallo	6	0.93	0.34
Santa Clara	5	1.78	0.66
Santiago Papasquiaro	53	9.08	3.38
Súchil	9	1.56	0.58
Tamazula	56	8.18	3.05
Tepehuanes	13	1.43	0.53
Tlahualilo	19	6.03	2.24
Topia	18	3.3	1.23
Vicente Guerrero	8	3.21	1.19
Total	1,022	268.04	100%

determine Protected Natural Areas and accentuated effects of climate change. These effects are accentuated by the excessive use of the scarce remaining forest resources, which intensifies the loss of biodiversity. Since there is a lot of pressure from the inhabitants on these areas, who generally have subsistence economies originated in the ways they obtain their food, household heating, and their productive micro-enterprises which involve the use of firewood [19].

Some studies (20) argue that as household incomes improve, solid biofuels are replaced by gas. In the semi-arid areas of Durango there is a deforestation crisis mainly due to families with incomes from subsistence activities who cannot easily afford the price of gas, or if they have the means, they usually combine firewood with the use of gas to compensate for the expenses.

Overall, in the homes of rural communities in Durango, the traditional three-stone stove is used for cooking, which coincides with what has been reported by [21]. This practice results in incomplete combustion that, in addition to generating other GHGs, is inefficient and causes environmental pollution problems and health problems in people [22]. It was observed that only 3.2% of the interviewees use proper wood stoves, which are designed with a closed combustion chamber, and have a chimney to keep away the gases produced by the household firewood combustion, this is, generated by the members of the household family [23].

CO_2 constitutes 49% to 57% of the total GHGs emitted, while the remainder is a contribution of other gases (CO, CH_4 and CN) according to [24]. If the goal is an effective reduction of the CO_2 emitted by firewood consumption, not only in Durango, but in all areas of the world where it is used, it is strictly necessary that developed eco-technologies do emit lower CO_2 rates than those emitted by traditional firewood burning, and those emission rates from the use of traditional fuels [24]. If a combination is made, of firewood with energy-saving stoves using of natural gas, it would be possible to obtain a decrease in the CO_2 released into the atmosphere according to [25].

In this regard, [6] recorded an inventory of greenhouse gases for Mexico using the IPCC-1996 methodology in the period between 1993 and 2002. Their results on firewood use, CO_2 emitted to atmosphere were 17 611 Gt per year, equivalent to 17 million 611 thousand Mg per year. If the emissions of the state of Durango in Mg of CO_2 are compared with those at the national scale, those represent 1.52%; and Mexico contributes with 2.13% of the global CO_2 emissions to the atmosphere.

CONCLUSIONS

Firewood consumption in the communities of Durango is important as a source of CO_2 emissions. If we add forest fires to this, and the agronomic and pasture burnings for livestock, the emission of vehicles and the CO_2 from industry, a total calculation would allow a better understanding of the environmental problems in Durango. Monitoring the air quality is necessary to implement solutions towards preventing air pollution in this state.

In Durango, as in all of Mexico and Latin American countries, there is still much to be quantified about CO_2 emissions and other GHGs, since it is yet a recent issue. Actions must be planned and implemented to address the damage, not only to natural resources but also to public health, with the highest emphasis on the contribution of this state to the inventories in process of the global warming.

Firewood is a fuel that has remained in force as a source of energy since the discovery of fire by humans. Projected scenarios show that this validity will continue in the near future. For this reason, it becomes necessary to couple that use with eco-technologies that help supporting firewood efficient consumption. Regarding the specific CO_2 emissions into the atmosphere, based on the data obtained in this research, Mexico is still far below within the figures of CO_2 released into the atmosphere, compared to other places in the world like the United States of America and China.

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Effects of the combining ability of piquin pepper (*Capsicum annuum* var. *Glabriusculum*) from different geographical sites

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ABSTRACT

Objective: To evaluate the effects of the general combining ability (GCA) and the specific combining ability (SCA) on the agronomic variables of piquin pepper (*Capsicum annuum* var. *Glabriusculum*) genotypes.

Methodology: A total of 36 F1 and nine parental crosses were used as plant material. The genotypes were distributed in a completely randomized block design with three replications. Ten agronomic variables were evaluated.

Results: Differences ($P \leq 0.01$) were found in all the evaluated variables, both in the genotypes and in GCA and SCA. Additive gene action influenced heritability, where following variables stood out: days to harvest (DTH), chlorophyll (CHL), plant height (PH), average fruit weight (AFW), fruit equatorial diameter (FED), and fruit polar diameter (FPD). One the one hand, genotypes G6 and G7 recorded the highest positive yield values for GCA, with 143.96 and 66.97 kg ha^{-1}, respectively. On the other hand, 58% of the SCA crosses obtained favorable yield results. Meanwhile, the highest positive values were obtained by the G6×G7, G8×G9, G5×G9, G3×G4, G4×G8, and G1×G8 crosses, which recorded 427.1, 190.5, 167.4, 146.8, 129.7, and 125.7 kg ha^{-1}, respectively.

Conclusions: According to the effects of GCA and SCA on the agronomic variables of piquin pepper, the genotypes G6 and G7 can be used to develop varieties, while the G6×G7, G8×G9, and G5×G9 crosses are recommended for hybrid formation within breeding programs.

Keywords: Diallelic, genetic variance, yield, GCA, SCA.

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INTRODUCTION

Genus *Capsicum* is grown all over the world. In the Americas, it can be found from the southern USA to Peru and northern Brazil (Perry *et al.*, 2007). It consists of approximately 38 species, of which, six species are the most widely cultivated: *C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, *C. pubescens*, and *C. assamicum* (Ramchiary and Kole, 2019). Mexico is the main center of domestication and diversification of the *Capsicum annuum*

Image by Ayounesao at Pixabay

species (Aguilar-Melendez *et al.*, 2009; Pickersgill, 2007). Its ancestor, *Capsicum annuum* var. *Glabriusculum* —commonly known as piquin pepper, chiltepín, quipín, timpinchile, mountain pepper, and wild pepper— is distributed throughout the country (Medina-Martínez *et al.*, 2010). The piquin pepper is a wild perennial shrub that develops under the shade of trees. It is a highly valuable phytogenetic resource of importance for the economy of rural households. As a result of its pungency and flavor, the price of piquin pepper is 40 times higher than serrano or jalapeño chillis. Piquin pepper is an important ingredient of Mexican cuisine (Alcalá-Rico *et al.*, 2019; González-Cortés *et al.*, 2015; Pedraza and Omez, 2008). Currently, the domestication and establishment of this species as a commercial crop has been the subject of some interest (Alcalá-Rico *et al.*, 2019). Genetic improvement is an alternative for its domestication and production increase. Consequently, determining the actions and the expression of its characteristics is fundamental. Genetic improvement of the desirable characteristics of a species depends on nature —the genetic variability of the interactions involved in the inheritance of characteristics, which can be estimated using diallel crosses (Zeinab and Helal, 2014). In this regard, diallelic analysis has been used as a tool to explore genetic effects (Huang *et al.*, 2015; Mendoza *et al.*, 2021). The general combining ability (GCA) is the average behavior of a genotype in hybrid combinations, while the specific combining ability (SCA) is the deviation of each cross on the average behavior of the parents involved in the crosses (Sprague and Tatum, 1942). As a result of the lack of information about the genetic effects of GCA and SCA on piquin pepper, the objective of this study was to determine the effects of the general combining ability (GCA) and the specific combining ability (SCA) on the agronomic variables of piquin pepper (*Capsicum annuum* var. *Glabriusculum*) genotypes.

MATERIALS AND METHODS

Location of the experimental site

The crosses and self-pollination of the genotypes of piquin pepper were carried out in the greenhouse of the plant breeding department of the Universidad Autónoma Agraria Antonio Narro. The evaluation of the F1 and the parental crosses was carried out under a 30% shade mesh, in the Forest Department of the said university, located at coordinates 25° 21’ 12” N and 101° 01’ 51” W, with an altitude of 1,779 m.a.s.l. According to the Köppen-Geiger climate classification, the climate of the area is temperate warm (Cfb), with a mean temperature of 16.4 °C and an average precipitation of 610 mm.

Plant material

Nine genotypes from different states: three from Nuevo León, two from Tamaulipas, two from Veracruz, one from Coahuila, and one from San Luis Potosí, Mexico, were used in the experiment. Genotypes were provided by the Las Huastecas experimental field of INIFAP (Table 1).

Pre-germination treatment and sowing

Before the sowing, the seeds were immersed in 5,000 mg kg^{-1} of gibberellic acid for 24 h, at room temperature. The aim was to break their physiological dormancy (Alcalá-

Table 1. Identification of the parental genotypes that were involved in the diallel crosses.

ID	Location	Municipality	State	Group
G1	Estación Álamo	Villaldama	N.L.	Piquin
G2	Ej. Potrero de Zamora	Aramberri	N.L.	Piquin
G3	Ej. Lázaro Cárdenas	Burgos	Tam.	Piquin
G4	Barranco Azul	San Carlos	Tam.	Piquin
G5	Castaños	Castaños	Coah.	Piquin
G6	Colatlán	IXMA	Ver.	PIHU
G7	Tiopancahuatl	IXMA	Ver.	PIHU
G8	Los Rincón	Linares	N.L.	Piquin
G9	La Labor	Rioverde	S.L.P.	Piquin

N.L.: Nuevo León, Tam.: Tamaulipas, Coah.: Coahuila, Ver.: Veracruz, S.L.P.: San Luis Potosí, IXMA: Ixhuatlán de Madero, PIHU: Piquin Huasteco.

Rico *et al*., 2019). Afterwards, two seeds were sown per cavity in the greenhouse, using 200-cavities polystyrene trays. Peat moss was used as substrate. Subsequently, the trays were piled-up and covered with black plastic to provide favorable conditions for the seeds and to speed up the germination process. When the apex of the seedlings emerged, the trays were separated and distributed all around the greenhouse. Two months after the sowing, the seedlings were transplanted to 30×30 polystyrene bags; vermicompost was used as substrate.

Parental crosses

Direct crosses and self-pollination of the parents were carried out during the flowering phenological stage, using Griffing's experimental method II (Griffing, 1956). In order to guarantee an effective cross or self-pollination, the pollinated flowers were covered and labelled. The seeds obtained from the F1, and the parentals were extracted from the ripe fruits. Subsequently, the seeds were sown using the same methodology mentioned in the section pre-germinative treatment and sowing. Subsequently, F1 and parental seedlings were placed on 1 m high metal structures to facilitate evaluation and agronomic management.

Experimental design

The F1 and parental crosses were distributed using a completely randomized block design, with three replicates. The useful plot had four plants per replication. There was a 0.5 m separation between plants, 0.9 m between rows, and 1.0 m between alleys.

Evaluated variables

Ten variables were evaluated: days to flowering (DTF), days to harvest (DTH), chlorophyll (CHL; SPAD units), plant height (PH; cm), production per plant (PPP; g), fruits harvested per plant (FHPP), average fruit weight (AFW; g), fruit equatorial diameter (FED; mm), fruit polar diameter (FPD; mm), and yield (YIE; kg ha^{-1}).

Analysis

The analysis of variance and the genetic effects of the general combining ability (GCA) and the specific combining ability (SCA) were performed according to the Griffing's experimental method II, model 1, using the DIALLEL-SAS05 computational routine, proposed by Zhang *et al.* (2005) with the SAS software version 9.4. The components of the genetic variance and the heritability were estimated using an expected mean square.

RESULTS AND DISCUSSION

Table 2 shows the effects of the combining ability obtained from the mean squares of the analysis of variance. The sources of variation of the genotypes, GCA and SCA recorded highly significant differences ($P \leq 0.01$) for the 10 variables. These results indicate that the expression of the characteristics of the piquin pepper genotypes is influenced by both the additive and non-additive effects, providing a different ability to pass on its characteristics to its descendants, in addition to presenting a diverse performance in specific hybrid combinations. These results match the findings of Sing *et al.* (2014), who determined that both the additive and non-additive variances were fundamental to control the expression of the characteristics of *Capsicum annuum* L. crosses.

In a genetic improvement program, the GCA and SCA variances enable the deduction of the type of the gene action and the importance of the expression of the characteristics (Rohini *et al.*, 2017). Table 3 shows that the CHL, PH, FED, and FPD variables recorded a higher GCA than SCA variance. Meanwhile, the rest of the variables recorded opposite results —SCA obtained the highest values. Rohini *et al.* (2017) pointed out that the characteristics with the highest SCA variations are controlled by the non-additive genes action. In addition, the GCA:SCA ratio recorded <1 values with a lower GCA variance and a higher SCA variance. When the values are >1, the results are the opposite. The variables

Table 2. Mean squares of the analysis of variance of diallel crosses, using Griffing's experimental method II.

SV	Rep	Genotype	GCA	SCA	Error	R^2
DF	2	44	8	36	88	
DTF	20.99	152.41 **	88.16 **	42.50 **	2.27	0.92
DTH	306.90 *	378.25 **	378.82 **	69.92 **	22.49	0.74
CHL	11.10 *	99.28 **	165.95 **	3.57 **	0.93	0.95
PH	94.61	1581.13 **	2600.12 **	66.36 **	19.18	0.93
PPP	25.83	161.71 **	107.80 **	41.93 **	9.64	0.74
FHPP	2278.10	4645.00 **	3098.74 **	1203.81 **	436.96	0.64
AFW	0.31 **	0.09 **	0.09 **	0.02 **	0.01	0.73
FED	0.48 **	0.63 **	0.95 **	0.04 **	0.02	0.88
FPD	0.31	10.95 **	19.21 **	0.19 **	0.05	0.97
YIE	12726.0	79693.0 **	53119.0 **	20663.0 **	4752.0	0.74

*, ** Significant at the ≤0.05 and ≤0.0.1 probability levels; SV: source of variation; Rep: repetition; GCA: general combining ability; SCA: specific combining ability; R^2: coefficient of determination; DF: degrees of freedom; DTF: days to flowering; DTH : days to harvest; CHL: chlorophyl; PH: plant height; PPP: production per plant; FHPP: fruits harvested per plant, AFW: average fruit weight; FED: fruit equatorial diameter; FPD: fruit polar diameter; YIE: yield.

that stood out in the additive variance included: DTH, CHL, PH, AFW, FED, and FPD. These results indicated that these characteristics can be inherited to the descendants. The dominance variance was related to the DTF, PPP, FHPP, and YIE variables. At the same time, this variance is associated with the dominance degree, registering the highest values in the same variables. Regarding the narrow-sense heritability estimations, the results fluctuated between 0.28 (DTF) and 0.96 (FPD). In addition, heritability was classified as high (>50%), medium (30-50%), and low (<30%) (Bhateria *et al.*, 2006). In this regard, 60% of the variables showed a high heritability, while 40% recorded a low to medium heritability. Additionally, the additive gene action influenced heritability. The information about these components of the variance is fundamental to determine the appropriate focus for the genetic improvement of the crop (Meena *et al.*, 2020).

Table 4 includes specific GCA data. Genotypes G6 and G7 recorded the highest positive and significant ($P \leq 0.01$) yield values, contributing 143.96 and 66.97 kg ha^{-1} above the mean for the different combinations. These genotypes likewise recorded the highest positive and significant values ($P \leq 0.01$) for the following variables: CHL (6.81 and 6.32 units SPAD), PH (32.09 and 12.24 cm), PPP (6.49 and 3.02 g), AFW (0.08 and 0.13 g), and FPD (2.04 and 2.41 mm). However, FED recorded significant negative values ($P \leq 0.01$), showing a 0.48 mm reduction in the different crosses.

Meanwhile, genotypes G1, G2, G5 and G9 recorded negative yield values with different levels of significance ($P \leq 0.05$ or 0.01) contributing a reduction of 39.76 - 81 kg ha^{-1} in combination with other genotypes. Likewise, these genotypes presented different negative values for CHL (1.56 - 3.19 SPAD), PPP (1.79 - 3.65 g) and FPD (0.28 - 1.21 mm).

On the other hand, genotypes G3, G4, and G8 showed no yield significance. Although genotype G3 recorded significantly negative ($P \leq 0.01$) for DTF (1.19 days), PH (14.47 cm), and FPD (0.48 mm) values. However, it also it obtained significantly positive ($P \leq 0.01$) FED (0.21 mm) values. With respect to the G4 genotype, showed significantly negative

Table 3. Variance and heritability components of the agronomic variables of piquin pepper.

Variables	σ^2_{GCA}	σ^2_{SCA}	GCA/SCA	σ^2_{A}	σ^2_{D}	ADD	h^2
DTF	7.81	40.23	0.19	15.61	40.23	2.27	0.28
DTH	32.39	47.43	0.68	64.79	47.43	1.21	0.54
CHL	15	2.64	5.69	30	2.64	0.42	0.91
PH	234.63	47.18	4.97	469.26	47.18	0.45	0.9
PPP	8.92	32.28	0.28	17.85	32.28	1.9	0.33
FHPP	241.98	766.85	0.32	483.96	766.85	1.78	0.35
AFW	0.01	0.01	0.76	0.02	0.01	1.15	0.56
FED	0.08	0.03	2.95	0.17	0.03	0.58	0.83
FPD	1.74	0.14	12.14	3.48	0.14	0.29	0.96
YIE	4397.01	15911.15	0.28	8794.02	15911.15	1.9	0.33

σ^2: variance; GCA: general combining ability; SCA: specific combining ability; A: additive; D: dominance; ADD: average degree of dominance; h^2: narrow-sense heritability; DTF: days to flowering; DTH: days to harvest; CHL: chlorophyll; PH: plant height; PPP: production per plant; FHPP: fruits harvested per plant; AFW: average fruit weight; FED: fruit equatorial diameter; FPD: fruit polar diameter; YIE: yield.

(P≤0.05) DTF (0.97 days) values and significantly negative (P≤0.01) CHL (1.29 SPAD) and PH (15.68 cm) values. In addition, it recorded significantly positive (P≤0.01) FED (0.22 mm) values. Finally, genotype G8 obtained significantly positive (P≤0.01) DTF (3.72 days), PH (5.64 cm), and FED (0.12 mm) values. Although it recorded significantly negative (P≤0.01) CHL (2.34 SPAD) and FPD (0.63 mm) values. Therefore, the genotypes have a different capacity to inherit their characteristics to their descendants. In this regard, determining the combination capacity of the genotypes that will be included in any genetic improvement program is fundamental for the effective transfer of desirable genes to the resulting progeny (Singh *et al*., 2014).

Regarding the effects of the specific combing ability (Table 5), 58% of the crosses recorded favorable yield results (*i.e.*, positive values). In addition, the highest significantly positive (P≤0.01) values of this variables were obtained by the G6×G7, G8×G9, and G5×G9 crosses, which recorded 427.1, 190.5, and 167.4 kg ha^{-1}, respectively. Meanwhile, the G3×G4, G4×G8, and G1×G8 crosses also recorded significantly positive (P≤0.05) values, obtaining an increase of 146.8, 129.7, and 125.7 kg ha^{-1}, respectively. Fifty percent of these crosses recorded negative GCA values for both parents, 33.3% obtained positive GCA values, and 16.7% showed one parent with negative GCA values and one

Table 4. Effects of the general combining ability (GCA) of nine genotypes of piquin pepper from different geographical sites.

Genotype	DTF	DTH	CHL	PH	PPP
G1	2.78 **	5.42 **	−3.16 **	1.68	−3.65 **
G2	0.39	3.42 *	−1.56 **	−0.46	−1.79 *
G3	−1.19 **	−1.40	0.49	−14.47 **	0.40
G4	−0.97 *	1.69	−1.29 **	−15.68 **	0.77
G5	−1.58 *	−5.79 **	−2.09 **	−10.69 **	−1.98 *
G6	1.51 **	−0.34	6.81 **	32.09 **	6.49 **
G7	−5.82 **	−12.12 **	6.32 **	12.24 **	3.02 **
G8	3.72 **	2.63	−2.34 **	5.64 **	−1.02
G9	1.15 *	6.48 **	−3.19 **	−10.35 **	−2.24 *
Genotype	**FHPP**	**AFW**	**FED**	**FPD**	**YIE**
G1	−15.43 *	−0.10 **	0.08 *	−1.01 **	−81.00 **
G2	8.25	−0.17 **	−0.13 **	−1.21 **	−39.76 *
G3	3.68	0.04	0.21 **	−0.48 **	8.87
G4	−1.26	0.04	0.22 **	0.06	17.13
G5	−18.51 **	0.04	0.22 **	−0.28 **	−43.86 *
G6	35.80 **	0.08 **	−0.43 **	2.04 **	143.96 **
G7	7.33	0.13 **	−0.52 **	2.41 **	66.97 **
G8	−4.56	−0.04	0.12 **	−0.63 **	−22.54
G9	−15.30 *	−0.02	0.23 **	−0.89 **	−49.78 **

*, ** Significant at the ≤0.05 and ≤0.0.1 probability levels; DTF: days to flowering, DTH: days to harvest; CHL: chlorophyll; PH: plant height; PPP: production per plant; FHPP: fruits harvested per plant; AFW: average fruit weight; FED: fruit equatorial diameter; FPD: fruit polar diameter; YIE: yield.

Table 5. Effects of the specific combining ability (SCA) of 36 F1 crosses of piquin pepper.

Crosses	DTF	DTH	CHL	PH	PPP
G1×G2	2.85 *	0.39	3.18 **	−4.15	−0.37
G1×G3	4.76 *	2.87	0.79	4.28	3.66
G1×G4	−8.78 **	−7.22	0.03	−2.85	5.19
G1×G5	−5.18 **	−12.73 **	0.66	5.92	0.28
G1×G6	3.07	2.15	−2.13 *	−3.12	−5.95 *
G1×G7	−0.60	2.93	1.40	3.82	−1.15
G1×G8	2.52	0.51	−0.07	−0.58	5.66 *
G1×G9	−6.90 **	−9.01 *	1.03	−1.25	3.31
G2×G3	−1.51	−2.13	−0.13	−2.51	1.30
G2×G4	−0.72	2.78	0.82	−1.47	5.43
G2×G5	0.88	8.27	0.51	3.34	4.91
G2×G6	−4.87 **	6.81	−1.14	1.35	−7.60 **
G2×G7	−4.87 **	−9.07 *	−0.09	14.95 **	−3.92
G2×G8	−1.75	−1.49	2.51 **	2.31	0.32
G2×G9	−6.51 **	0.99	2.10 *	−4.79	3.44
G3×G4	−2.81 *	−0.40	−0.08	−0.95	6.61 *
G3×G5	−8.21 **	−17.25 **	1.01	1.73	1.59
G3×G6	−6.96 **	−16.04 **	−1.15	−10.31 *	−4.12
G3×G7	−3.30 *	−4.25	0.65	6.88	0.85
G3×G8	0.16	−0.34	0.84	5.48	1.30
G3×G9	5.07 **	−0.52	1.20	−2.44	4.64
G4×G5	2.25	1.66	0.38	−2.44	2.69
G4×G6	−1.51	−2.13	−0.32	−2.01	−3.38
G4×G7	−4.18 **	−7.34	1.27	6.01	−7.11 *
G4×G8	−3.72 **	−3.10	1.56	−6.14	5.84 *
G4×G9	0.19	−2.61	−0.07	4.02	−1.01
G5×G6	−4.90 **	−9.31 *	2.65 **	−8.83 *	−3.11
G5×G7	−4.24 **	−2.19	2.10 *	−2.31	−3.20
G5×G8	−0.12	0.05	0.00	0.01	2.79
G5×G9	−0.87	−2.13	1.73	7.20	7.54 **
G6×G7	4.34 **	1.69	−0.82	−13.43 **	19.24 **
G6×G8	−5.21 **	3.60	−0.57	26.84 **	−5.30
G6×G9	−6.63 **	0.75	−1.03	7.25	−6.67 *
G7×G8	−6.21 *	−8.28	0.18	3.20	−6.55 *
G7×G9	−6.96 **	−0.46	−0.27	8.69 *	−7.19 *
G8×G9	−0.84	−2.22	0.63	−0.71	8.58 **
Crosses	**FHPP**	**AFW**	**FED**	**FPD**	**YIE**
G1×G2	−5.30	0.02	−0.03	0.01	−8.26
G1×G3	21.96	0.04	0.05	0.02	81.34

Table 1. continues...

Crosses	FHPP	AFW	FED	FPD	YIE
G1×G4	17.16	0.17 *	0.13	0.36	115.08
G1×G5	−1.18	0.07	0.14	0.70 **	6.24
G1×G6	−25.49	−0.03	0.02	−0.26	−132.00 *
G1×G7	11.65	−0.13	0.01	−0.59 **	−25.60
G1×G8	31.20	0.12	0.23	0.45 *	125.70 *
G1×G9	20.03	0.10	0.12	0.49 *	73.39
G2×G3	10.28	−0.05	−0.20	−0.05	28.82
G2×G4	50.40 *	0.01	−0.13	0.01	120.65
G2×G5	38.84 *	−0.03	0.03	0.06	108.90
G2×G6	−50.91 **	0.01	0.00	−0.03	−168.79 **
G2×G7	−30.11	0.01	0.06	0.03	−87.00
G2×G8	0.02	0.00	0.16	−0.09	7.13
G2×G9	29.85	−0.01	0.00	0.10	76.37
G3×G4	36.38	0.12	0.31 *	0.60 **	146.76 *
G3×G5	−3.96	0.20 *	0.20	0.36	35.33
G3×G6	−42.01 *	0.14	0.25 *	0.30	−91.39
G3×G7	5.29	0.06	0.02	0.20	18.84
G3×G8	10.17	−0.02	−0.03	0.05	28.80
G3×G9	30.84	0.03	0.10	0.30	103.03
G4×G5	2.82	0.17	0.23	0.48 *	59.64
G4×G6	2.85	−0.14	−0.24	−0.50 *	−74.91
G4×G7	−38.84 *	−0.01	−0.23	0.07	−157.95 *
G4×G8	23.29	0.18 *	0.30 *	0.21	129.70 *
G4×G9	−34.55	−0.25 **	0.11	−0.01	−22.51
G5×G6	−14.82	0.04	−0.14	−0.03	−69.04
G5×G7	−5.69	−0.10	−0.16	−0.35	−71.06
G5×G8	0.00	0.00	−0.04	−0.02	61.89
G5×G9	37.86	0.16	0.44 **	0.49 *	167.42 **
G6×G7	79.35 **	0.15	0.20	0.80 **	427.10 **
G6×G8	−16.61	−0.13	−0.13	−0.15	−117.57
G6×G9	−33.94	−0.06	−0.14	−0.48 *	−148.05 *
G7×G8	−35.22	0.05	−0.11	−0.06	−145.48 *
G7×G9	−32.39	−0.09	−0.07	−0.58 **	−159.68
G8×G9	52.66 **	0.09	0.06	0.32	190.50 **

*, ** Significant at the ≤0.05 and ≤0.0.1 probability levels; DTF: days to flowering, DTH: days to harvest; CHL: chlorophyll; PH: plant height; PPP: production per plant; FHPP: fruits harvested per plant; AFW: average fruit weight; FED: fruit equatorial diameter; FPD: fruit polar diameter; YIE: yield.

with positive GCA values. The second and the third cases match the results of Escorcia-Gutiérrez *et al.* (2010), who pointed out that the SCA effect of a single cross will be high and positive if at least one parent records a high GCA. Nevertheless, these results indicate that the crosses of parents with high GCA values will not necessarily obtain high SCA results. This information matches the findings of Picón-Rico *et al.* (2018), who concluded that the SCA effect cannot be totally predicted using the GCA of the parents. In addition, these crosses recorded an average increase of PPP (8.91 g), FHPP (43.46 fruits), AFW (0.14 g), FED (0.26 mm), and FPD (0.48 mm). Consequently, the SCA effects involved a non-additive gene action of the expression of one of the characteristics of the progeny resulting from a specific cross.

Meanwhile, there were significant negative values in the crosses G2×G6 ($P \leq 0.01$), G4×G7 ($P \leq 0.05$), G6×G9 ($P \leq 0.05$), G7×G8 ($P \leq 0.05$) and G1×G6 ($P \leq 0.05$). Who had a reduction in yield from 132 to 168.8 kg ha^{-1}. In this regard, 80% of the crosses had one GCA-negative and one GCA-positive parent. In average, these crosses recorded a reduction of PPP (6.85 g), FHPP (36.13 fruits), AFW (0.02 g), FED (0.09 mm), and FPD (0.22 mm).

CONCLUSIONS

The effects of the general combining ability in piquin pepper indicated the average potential of the hybrid combinations of the parents, while the effects of the specific combining ability provided data about the specific crosses with favorable or unfavorable expressions regarding the parents. This information is useful to select the genotypes of piquin pepper and to include them in genetic improvement programs with the aim of developing varieties or hybrids. The G6 and G7 parents are recommended in the development of piquin pepper varieties, because they recorded the highest significantly positive GCA values. Meanwhile, the best crosses were G6×G7, G8×G9, and G5×G9, whose remarkable yield components can be used for the creation of hybrids.

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Colegio de Postgraduados

Availability, accessibility, and intake of vegetables native to Mexico

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ABSTRACT

Objective: to analyze the intake of vegetables native to Mexico from 1980 to 2020, based on the food security approach.

Methodology: the availability and accessibility dimensions of food security were taken into consideration for this study; in addition, descriptive statistics and regression models were used.

Results: the apparent national intake of native vegetables increased during the study period, reaching 6.821 million tons in the year 2020, while the *per capita* intake was 148 grams in the same year. The actual income and the quarterly family expenses on vegetables, pulses, and seeds decreased from $1,890 Mexican pesos in 1980 to $1,082 Mexican pesos in 2020.

Study Limitations/Implications: the food utilization and stability dimensions that encompass food safety were not included in the study.

Conclusions: public food security policies must promote the production and intake of vegetables native to Mexico and increase the actual income of the most vulnerable Mexican families, facilitating accessibility to these products.

Key words: food security, food production, income.

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INTRODUCTION

Food security means that people have physical and economic access to nutritious food that does not damage their health. It implies the food availability, accessibility, utilization, and stability (FAO, 2013). Sometimes, food security is related to the regulation and control of food supply chains, nutritional insecurity, hunger, food trade, and food insecurity (Jones *et al.*, 2013; Leroy *et al.*, 2015; Burchi and Muro, 2015).

Food insecurity means poor food quality and lack of diversity in the physical availability, low accessibility, inappropriate utilization, and unstable accessibility to food (CONEVAL, 2019). Food security is important because an undernourished population is less economically productive (Jones *et al.*, 2013) and cannot participate in social and political life. Food security is a human right and must be sustainable (Burchi and Muro, 2015). Consequently, agricultural producers must use ecofriendly systems (Berry, 2015).

Several methodologies deal with food security, and they take into account food availability, accessibility, utilization, and stability (Jones *et al.*, 2013). The following

indicators were used: GDP, inflation, unemployment, wages, production, food demand, value chains, soil use, input costs, population, food expenses, education, indigenous households, marginalization, and subsidy indicators (Cruz *et al.*, 2022).

Food insecurity has been measured by such surveys as the Latin-American and Caribbean Food Security Scale (ELCSA) (Carmona *et al.*, 2017). However, alternative measurements of food security have been validated through alternative methodologies, including the use of correlation coefficients or regression models (Hoddinott, 2009; Jrad *et al.*, 2010).

These methodologies show that food security is related to food production (vegetables). In addition, this research used the dimensions established by the FAO, along with various indicators. Mexico is the center of origin of various agricultural products. The native vegetable production value amounts to 10.94% of the Gross Domestic Product (GDP) of the primary sector (SIAP-SIACON, 2020; World Bank, 2020). In addition, agricultural activities are a source of employment. Vegetables are a healthy source of food for Mexicans and some native produce stands out in international markets. Consequently, focusing on food security will help to determine the policies that should be implemented in the production, intake, and trade of native vegetables.

The hypothesis of this study was that, based on the food security focus, the intake of native vegetables can be explained by the production and the demand, as well as by the contrast between the income and the amount that a given household spends on vegetables, pulses, and seeds. The objective of this study was to analyze the intake of vegetables native to Mexico, using a food security focus and taking into account the availability and accessibility of the said vegetables.

MATERIALS AND METHODS

The following statistical variables were used in the analysis of the native vegetable availability and accessibility: maximum values, minimum values, averages, variances, standard deviation, coefficient of variation, growth rates, index numbers, and coefficient of correlation. In addition, regression models and trend analyses were developed (Infante and Zárate, 2012; Gujarati and Porter, 2010; Greene, 2018). The resulting statistical data provided the measurements used to characterize the two food security dimensions evaluated in this study.

In the first section (vegetable availability dimension), production (tons, t) was compared with domestic consumption (t). The national apparent consumption (NAC; production + importation − exportation) and individual consumption (NAC/population/360 days) were used to determine the demand. In addition, the value of the native vegetable production and the GDP of the primary sector were compared. The data about the production, the imports, and exports, the Mexican population, and the GDP of the agricultural sector were obtained from the Servicio de Información Agroalimentaria y Pesquera (SIAP-SIACON), the FAOSTAT, the INEGI, and the World Bank, respectively.

In the second section, the accessibility of vegetable intake was analyzed, including the following variables: quarterly actual income, transferences, and total actual expenses (beverages, tobacco, vegetables, pulses, and seeds). Data were obtained from the reports of the National Household Income and Expenditure Survey (ENIGH) and the Consumer Price Index (CPI) of the INEGI.

The structure of the analysis allowed an understanding of the availability of vegetables native to Mexico, because the production figures were compared with the domestic apparent consumption of these products. Studying the income and expenses of the families led to the identification of the economic resources of the families and how much they spend in vegetables, pulses, and seeds.

The list of the domesticated and collected vegetable species in Mexico were obtained from the inventory of the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO, 2008) and from the catalogue of native vascular plants of Mexico (Villaseñor, 2016). The analysis period was from 1980 to 2020. Accessibility (income and expenses) was only studied from 1984, due to the lack of data from previous years. The production, income, and expenses values were indexed based on the constant prices of 2018.

RESULTS AND DISCUSSION

Availability of Vegetables Native to Mexico

According to the SIAP-SIACON (2020), the production of vegetables native to Mexico plays a major role, contributing $90.601 billion Mexican pesos (2018 constant prices). Consequently, this sector is an important source of employment and produces 80.59% of the total vegetable production ($112,417 Mexican pesos) in 1,821,790 ha.

The volume and exports of the production of native vegetables recorded an increasing trend throughout time, while imports showed a slight decreasing trend (Figure 1). The increase of the first two variables can be explained by the adoption of free trade policies, introduced during the 1980s. These policies modified the food intake and production patterns of the country. Sánchez *et al.* (2019) reported that vegetable exports amounted to 10.29% of the total international exports in 2013. The crops that stood out were tomato, chili, squash, and bean.

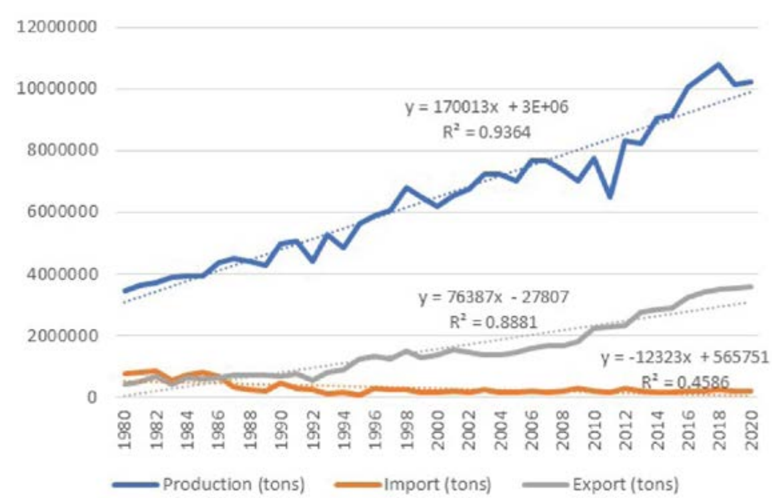

Figure 1. Production and trade of vegetables native to Mexico.

The minimum native vegetable production amounted to 3.462 million t in 1980, while the maximum native vegetable production reached 10.797 million t in 2018, and, in 2020, 10.236 million t were recorded. The vegetable produce that recorded the highest growth rates from 1980 to 2020 were: chayote (*Sechium edule*) (7,752.33%), chía (*Salvia hispanica*) (3,293.30%), and prickly pear (*Opuntia ficus-indica*) (969.86%). Meanwhile, the vegetables with the lowest growth rates included tomato (*Solanum lycopersicum*) (154.76%), sunflower (*Helianthus annuus*) (71.92%), and bean (*Phaseolus* spp.) (11.83%) (Table 1).

Some products have remarkable growth rates; however, they do not make up an outstanding proportion of the total production. For example, in 2020, chayote and chía recorded 2.08% and 0.05% growth rates, respectively. The vegetables that stood out included tomato (32.93%), chili (27.53%), bean (10.33%), prickly pear (8.43%), and squash (7.77%), which are part of the basic diet of Mexico. Chipilín (*Crotalaria longirostrata*) and jaltomate (*Jaltomata procumbens* C.), two native products consumed by Mexicans, are not included in governmental statistics.

Some products in Table 1 are not included in the NAC analysis. Consequently, the total production of these vegetables is used to meet the domestic market demand. Bean producers did not meet the demand during the study period: 95,371 t were imported in 2020 and the NAC decreased 16.94% from 1980 to 2020. A similar situation was faced by sunflower producers. Santos *et al.* (2017) pointed out that the lowest bean importation volume reached 2,909 t in 1992, while the highest bean importation volume amounted to 482 million t in 1982. These authors attributed the loss of production profitability and competitiveness to this phenomenon.

The NAC of the native vegetables increased from 3.781 million t in 1980 to 6.821 million t in 2020 (80.38% growth). The average daily individual consumption was 155 g in 1980, 160 g in 1990, 140 g in 2000, 139 g in 2010, and 148 g in 2020. From 1980 to 2020, the *per capita* intake of vegetables decreased by 4.31%. These results are the consequence of the immediate accessibility to low nutritional and high caloric food among the population, who disdain nutritional food, such as vegetables.

López and Alarcón (2018) pointed out that the *per capita* consumption of vegetables was 112 g in 1994 and 160 g in 2014. In addition, they mentioned that vegetable intake increases along with the age of the population.

Meanwhile, the production and intake of native vegetables are located in certain regions of the country, including: pipitza (Puebla, 148 t), huazontle (Puebla, 6,180; Tlaxcala, 298), quelite (Baja California, 2,245; Puebla, 22; Sonora, 544), pápalo (Guerrero, 4,597; Morelos, 1,209; Puebla, 1,008), and chilacayote (Mexico City, 40; State of Mexico, 996; Morelos, 718) (SIAP-SIACON, 2020). For example, chipilín is mainly produced and consumed in Chiapas. In order to increase the intake of these vegetables, their use should be promoted in the whole country.

Accessibility of the Population to Vegetables

In order to gain food accessibility and food intake in Mexico, the families need to earn a robust income to purchase this type of native vegetables. However, although the total actual income *per capita* has increased, the actual quarterly income of a Mexican family

Table 1. Production and National Apparent Consumption of native horticultural products in Mexico (tons).

Concept	Product / year	1980	1990	2000	2010	2020
Volume of production	Tomato (*Solanum* spp.)	1323148	1878415	2084443	2277791	3370827
	Chili (*Capsicum* spp.)	691264	850415	1428768	1942256	2818443
	Bean (*Phaseolus* spp.)	945358	1287610	887868	1157195	1057157
	Prickly pear (*Opuntia* spp.)	80640	174630	404460	723815	862733
	Pumpkin (*Cucurbita* spp.)	192547	332250	471350	533540	795299
	Green tomato (*Physalis philadelphica* Lam)	156915	271648	580247	719849	766515
	Jicama (*Pachyrhizus erosus* L.)	42374	87286	121665	184271	238980
	Chayote (*Sechium edule* J.)	2716	56316	128887	144413	213269
	Sweet potato (*Ipomoea batatas* L.)	19560	34116	52365	51064	75396
	Sunflower (*Helianthus annuus* L.)	4846	90	70	3797	8331
	Papalo (*Porophyllum macrocephalum*)	1138	392	2390	7459	6815
	Huazontle (*Chenopodium berlandieri* subsp. *Nuttalliae*)	1131	1442	1526	3568	6478
	Amaranth (*Amaranthus hypochondriacus* L.)	-	646	4240	3870	5625
	Chia (*Salvia hispánica* L.)	147	-	750	2914	4988
	Quelite (*Amaranthus cruentus* L.)	-	338	1841	1533	2812
	Epazote (*Dysphania ambrosioides*)	576	1010	1045	1606	2434
	Pipitza (*Porophyllum calcicola*)	-	140	105	208	148
	Chilacayote (*Cucurbita ficifolia* B.)	88	2382	2477	1423	-
Imports	Bean (*Phaseolus* spp.)	444306	330471	87661	117470	143635
	Sunflower (*Helianthus annuus* L.)	320111	114635	21802	13441	16299
	Chili (*Capsicum* spp.)	172	2445	13967	34030	29811
	Pumpkin (*Cucurbita* spp.)	-	-	1292	66	1945
	Tomato (*Solanum* spp.)	233	8034	44091	33049	255
Exports	Tomato (*Solanum* spp.)	373097	393237	689997	1509616	1826715
	Chili (*Capsicum* spp.)	22411	146154	339963	653863	1173331
	Pumpkin (*Cucurbita* spp.)	47674	162151	327419	47200	547450
	Bean (*Phaseolus* spp.)	2138	210	7091	30253	48264
	Sweet potato (*Ipomoea batatas* L.)	-	-	-	-	10732
	Sunflower (*Helianthus annuus* L.)	125	9	10	58	-
National Apparent Consumption	Chili (*Capsicum* spp.)	669025	706706	1102772	1322423	1674924
	Tomato (*Solanum* spp.)	950284	1493212	1438537	801224	1544366
	Bean (*Phaseolus* spp.)	1387526	1617871	968438	1244412	1152527
	Pumpkin (*Cucurbita* spp.)	144873	170099	145223	486406	249794
	Sweet potato (*Ipomoea batatas* L.)	19560	34116	52365	51064	64664
	Sunflower (*Helianthus annuus* L.)	324832	114716	21862	17180	24630

Source: own elaboration with data from SIAP-SIACON and FAO (consultation date January 20, 2023).

decreased during the study period, falling from $49,731 Mexican pesos in 1984 to $46,830 Mexican pesos in 2020 (Table 2). The total actual income per family decreased along with the size of the households.

When measuring the degree of association between the demand for native vegetables and the quarterly total actual income of Mexican families, a −0.2437878 negative correlation was obtained —*i.e.*, while the quantity variable increases, the income variable decreases.

The quarterly purchasing power of a Mexican family in 1984 ($49,731 Mexican pesos) would have allowed them to buy 45.96 times more vegetables than in 2020. Meanwhile, the income of a Mexican family in 2020 ($46,830 Mexican pesos) only covered 43.28 times this requirement —*i.e.*, the purchasing power of Mexican families has decreased because the actual family income has decreased.

The quarterly average income of the Mexican families during the study period was $50,009 Mexican pesos, $10,907 out of which came from government support and remesas

Table 2. Household income and expenditure by quarter at constant 2018 prices.

Year	Home size	Total current income	Total current income per person	Transfers	Gini coefficient	CME	SFBT	EFBCOH	SANB	TS	SVLS
1984	5.10	49731	9751	15498	0.425	34874	16116	7181	1935	1152	1890
1989	4.93	50800	10304	10097	0.469	34171	13884	8895	1227	851	1712
1992	4.72	56302	11928	13886	0.475	35558	12859	5817	1263	1086	1737
1994	4.60	58316	12677	11722	0.477	35900	12253	6844	1318	1101	1499
1996	4.52	42461	9394	10183	0.456	28461	10333	4255	1024	850	1328
1998	4.30	43588	10137	11005	0.476	29235	10071	4139	1169	797	1340
2000	4.16	50164	12059	12511	0.481	33915	10292	4725	1275	834	1116
2002	4.12	48747	11836	10596	0.454	33297	10236	5598	1163	1001	1110
2004	4.03	49456	12269	11260	0.460	36736	12531	5912	1192	949	1189
2006	3.96	55293	13977	11827	0.445	37401	11099	4683	1168	1019	1177
2008	4.00	54568	13628	11105	0.467	32556	11055	4472	1059	1124	1191
2010	3.88	47638	12291	10387	0.445	32553	10732	4896	1022	1103	1176
2012	3.72	48084	12939	11269	0.453	32397	11078	4741	1054	1311	1445
2014	3.79	46573	12301	10140	0.450	31063	10650	4470	998	1205	1065
2016	3.67	51887	14138	8057	0.449	31358	11021	2420	764	1120	980
2018	3.60	49724	13812	7625	0.426	31954	11254	2568	759	1123	1005
2020	3.55	46830	13191	8257	0.415	27841	10593	1415	795	1169	1082

Source: own elaboration with the ENIGH and with the INPC of the INEGI. CME = Current monetary expenditure; SFBT = Spending on food, beverages and tobacco; SFBT = Spending on food, beverages and tobacco; EFBCOH = Expenditure on food and beverages consumed outside the home; SANB = Spending on alcoholic and non-alcoholic beverages; TS = Tobacco spending. SVLS = Spending on vegetables, legumes and seeds.

(money transfers sent to the families). The maximum vegetable quarterly expense was \$1,890 Mexican pesos and the minimum was \$980 Mexican pesos. The highest dispersion regarding the mean was recorded by expenses in food and beverages consumed outside the households (37.08%), alcoholic and non-alcoholic beverages (24%), vegetables, pulses, and seeds (21.02%) (Table 3).

The expenses of a family in vegetables, pulses, and seeds and alcoholic and non-alcoholic beverages recorded a decreasing trend through time, while the expenses in tobacco registered a slightly increasing trend (Figure 2).

Although the NCA of native vegetables has doubtlessly increased over the years, the expenses in vegetables, pulses, and seeds decreased by 42.77% from 1980 to 2020. This decrease can be explained by the changes in the consumption patterns and the decrease in the actual prices of vegetables: consumers pay lower prices and producers receive lower prices for their products.

Table 3. Statistical analysis of household income and expenses by quarter.

Concept	Maximum	Minimum	Mean	Median	Variance	Standard deviation	Coefficient of variation
Total current income	58316	42461	50009	49724	18312268.42	4279.28	8.56
Transfers	15498	7625	10907	11005	3899263.42	1974.66	18.10
Current monetary expenditure	37401	27841	32898	32556	7725086.83	2779.40	8.45
Spending on food, beverages and tobacco	16116	10071	11533	11055	2491956.97	1578.59	13.69
Expenditure on food and beverages consumed outside the home	8895	1415	4884	4725	3280774.83	1811.29	37.08
Spending on alcoholic and non-alcoholic beverages	1935	759	1129	1163	73398.92	270.92	24.00
Tobacco spending	1311	797	1047	1101	21409.12	146.32	13.98
Spending on vegetables, legumes and seeds	1890	980	1297	1189	74245.65	272.48	21.02

Source: own elaboration with data from the ENIGH (1984-2020).

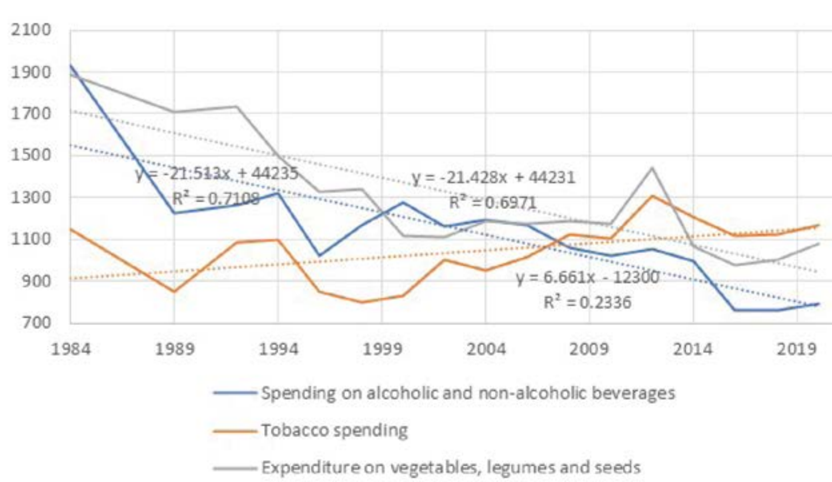

Figure 2. Quarterly expenses of Mexican families in beverages, tobacco, and vegetables.

The expenses in vegetables, pulses, and seeds had a slightly decreasing trend, in terms of the proportion of the income spent in food. The maximum and minimum proportions recorded were 13.50% and 8.89% in 1992 and 2016, respectively. The quarterly vegetable expenses per person decreased (17.78%) from $371 Mexican pesos in 1984 to $305 Mexican pesos in 2020 (Table 4).

The volume of the native vegetable production has certainly increased. The vegetable production value, as a proportion of the agricultural GDP in Mexico, has recorded the following results: 9.53% in 1980, 10.85% in 2000, and 10.94% in 2020 (SIAP-SIACON, 1980-2020; World Bank, 1980-2020). Consequently, the value of the native vegetable production has stagnated in comparison with the added value generated by the primary sector. Ayala *et al.* (2012) pointed out that the vegetable subsector accounted for 16% of the 2007-2010 domestic production. They concluded that the subsector is profitable and that it has a great dynamism.

The native products that stand out in the exportation sector are tomato, chili, squash, bean, sweet potato, and sunflower. Meanwhile, bean is one of the main imported foods (143,635 t beans in 2020), while chili impots increased from 172 t in 1980 to 29,811 t in 2020.

Table 4. Household spending on food by quarter (constant 2018 prices).

Year	Spending on food, beverages and tobacco (% of total current income)	Spending on alcoholic and non-alcoholic beverages (% of spending on food and tobacco)	Tobacco spending (% of spending on food and tobacco)	Spending on vegetables, legumes and seeds (% of spending on food and tobacco)	Growth rate of expenditure on vegetables, legumes and seeds (%)	Spending on vegetables, legumes and seeds per person (pesos)
1984	32.41	12.01	7.15	11.73	-	371
1989	27.33	8.84	6.13	12.33	−9.39	347
1992	22.84	9.83	8.45	13.50	1.41	368
1994	21.01	10.76	8.98	12.23	−13.70	326
1996	24.34	9.91	8.22	12.85	−11.40	294
1998	23.10	11.61	7.91	13.30	0.89	312
2000	20.52	12.38	8.11	10.84	−16.68	268
2002	21.00	11.37	9.78	10.85	−0.53	270
2004	25.34	9.52	7.57	9.49	7.11	295
2006	20.07	10.52	9.18	10.61	−0.98	298
2008	20.26	9.58	10.16	10.77	1.14	297
2010	22.53	9.52	10.28	10.95	−1.27	303
2012	23.04	9.51	11.83	13.04	22.92	389
2014	22.87	9.38	11.31	10.00	−26.28	281
2016	21.24	6.94	10.16	8.89	−8.03	267
2018	22.63	6.74	9.98	8.93	2.62	279
2020	22.62	7.50	11.04	10.21	7.58	305

Source: own elaboration with data from the ENIGH and with the INPC of the INEGI.

The NAC of native vegetables increased during the study period; however, the per capita consumption slightly decreased, from 155 g in 1980 to 148 g in 2020. The household quarterly expense in vegetables, pulses, and seeds (as proportion of the total actual income) decreased from 3.80% in 1980 to 2.31% in 2020. The monthly per capita expense in vegetables was $123.52 in 1984, $108.59 in 1994, $89.43 in 2000, $101.11 in 2010, and $101.56 Mexican pesos in 2020. Mundo *et al.* (2019) mentioned that Mexican families spent an average of $215.75 Mexican pesos/month in fruits and vegetables in 2018 (*versus* $93 Mexican pesos/month spent in native vegetables). The authors related the expenses with education, money transfers, and marginalization.

CONCLUSIONS

Food security in Mexico can be strengthened increasing the value of the vegetable products that have their origin center in the country. This situation would preserve the phenotype and genetic value of the plants, strengthen the agricultural production, and produce highly nutritional food for the society.

The value of the vegetable production could be increased improving productivity and providing appropriate fixed prices for producers. The native vegetable production will continue to meet the short-term demand, except in the case of the bean and sunflower production. Agricultural policies must be applied to increase the production of beans, chili, and other native vegetable products, consequently favoring exportations.

The decreasing trend in the per capita vegetable consumption will continue in the medium term. Policies aimed to promote the intake of healthy food, such as vegetables, should be implemented. In order to improve the intake of native vegetables, the actual income of the families should increase; consequently, new employments with higher income are required, as well as direct transfers, particularly for the most vulnerable population of the country.

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Glyphosate contamination: implications for honeybee *Apis mellifera* and consumers in Southeastern Mexico

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ABSTRACT

Objective: in a study conducted from June 2021 to May 2022 in two apiaries in southeastern Mexico, levels of glyphosate residues in pollen collected by bee *Apis mellifera* were analyzed to assess potential risks to both bees and humans.

Design/methodology/approach: the analysis used an immunoassay method after residue extraction using the QUECHERS method.

Results: the results revealed the presence of glyphosate in all samples, with concentrations ranging between 3.71 and 7.29 $\mu g\ kg^{-1}$. However, risk analysis, as indicated by the pollen hazard quotient, suggested that these quantities did not pose a serious threat to bees or humans. The levels were within the limits of the acceptable daily intake (ADI), the acute reference dose (ARfD) and the acceptable operator exposure level (AOEL).

Limitations/implications: although this study did not find any significant association between glyphosate and potential risks for both humans and bees, its persistence in the environment was demonstrated.

Findings/conclusions: Glyphosate levels at the study site were low, suggesting minimal risk to both humans and bees. However, the wide distribution of glyphosate in the region makes it necessary to emphasize long-term studies to understand the possible chronic effects of the pesticide on all species in the area.

Keywords: *Apis mellifera*, transgenic crops, herbicide, pollen hazard quotient.

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INTRODUCTION

For ease in weed control management, specific crops have been genetically altered to resist the herbicide glyphosate (GLY), resulting in a heightened global use of this wide-ranging systemic herbicide (Green, 2018; Székács & Darvas, 2012). GLY operates by blocking the activity of 5-enolpyruvylshikimate-3-phosphate synthase, an enzyme crucial for the biosynthesis of aromatic amino acids in plants (Duke & Powles, 2008; Shilo *et al.*,

2016). Because animals lack this enzyme, GLY is generally considered one of the least toxic pesticides for them (Duke & Powles, 2008). However, there is evidence indicating that GLY affects organisms beyond plants. For example, it has been demonstrated to decrease the reproduction of earthworms residing in the soil (Gaupp-Berghausen *et al.*, 2015). Moreover, GLY influences the growth of microalgae and aquatic bacteria and has detrimental effects on fish, amphibians, mammals, and birds (Benachour & Séralini, 2009; Relyea, 2005; Richard *et al.*, 2005). GLY is additionally associated with adverse effects on soil rhizosphere-associated bacterial communities (Newman *et al.*, 2016) and a decrease in mycorrhizal colonization (Helander *et al.*, 2018). Bees, as insects with a heightened risk of exposure, are especially susceptible due to various pathways of contact. They may encounter GLY while gathering nectar and pollen from flowering plants located several kilometers away, and subsequently transport any contaminants from these sources back to the hive (Agrebi *et al.*, 2019; Coupe *et al.*, 2012; Krupke *et al.*, 2012). The main routes of exposure involve interactions with agricultural crops, drift, and the widespread application of glyphosate formulations in urban environments for household and minor non-agricultural activities, such as weed control along railways, in parks, and within home gardens (Pasquale *et al.*, 2013; Silva *et al.*, 2018; Simon-delso *et al.*, 2017). Pesticide residues have been observed in a variety of bee-derived products, such as honey, pollen, propolis, wax, royal jelly, and honeycomb (Calatayud-Vernich *et al.*, 2017; de Oliveira *et al.*, 2016; Matin *et al.*, 2016; Pohorecka *et al.*, 2012; Ruiz-Toledo *et al.*, 2018; Tosi *et al.*, 2018; Valdovinos-Flores *et al.*, 2017; Zawislak *et al.*, 2019). Nevertheless, since 1990, the introduction and rapid spread of herbicide-resistant crops globally, including in Mexico (James, 2016), have led to increased GLY application and, consequently, to higher health risks for both honeybees and consumers (Agrebi *et al.*, 2019; Bohan *et al.*, 2005; Foulk, 2009; Rubio *et al.*, 2014).

Although GLY may not show significant toxicity to adult bees (Lewis *et al.*, 2016), concerns have been raised regarding potential chronic effects due to the accumulation of pesticide residues within beehives (Boily *et al.*, 2013; Crenna *et al.*, 2020; Herbert *et al.*, 2014; Weisbrod, 2020; J. Wu *et al.*, 2012; Zawislak *et al.*, 2019). For example, young adult bees exposed chronically to glyphosate formulations have displayed impaired associative learning and reduced sensitivity to sucrose (Gonalons & Farina, 2018; Herbert *et al.*, 2014; Luo *et al.*, 2021). Additionally, forager bees exposed to sublethal doses of such formulations have shown difficulties in navigating back to their hives (Balbuena *et al.*, 2015).

Concerning human health, numerous regulatory agencies and scientific organizations worldwide have reached a consensus that there is no conclusive evidence suggesting that glyphosate causes health problems (EFSA, 2015b; European Commission, 2002; USEPA, 1993). However, there is evidence indicating that residues of glyphosate found in the environment could potentially pose health risks to humans (Agrebi *et al.*, 2019). These risks include teratogenic, tumorigenic, and hepatorenal effects, which have been associated with endocrine disruption and oxidative stress, leading to metabolic alterations. The risk of exposure is further heightened by the fact that bee products are a part of the human supplementary diet. For instance, pollen is often considered an excellent dietary supplement for nutrition and is available in various forms on the market, such as granules, capsules, tablets, and powders (Komosinska-Vassev *et al.*, 2015; Kostić *et al.*, 2020). This potentially amplifies the risk to human health.

In our study area, beekeepers typically place their beehives in uncultivated areas that allow for natural plant succession. However, these locations are often surrounded by a landscape featuring various crops such as soybean, mango, beans, pumpkins, maize, and sesame (as observed by the authors). While soybean is the only genetically modified crop in this region, non-transgenic varieties are also cultivated. Consequently, foraging honeybees potentially encounter a range of pesticides, including GLY, in water, pollen, and nectar (Hladik *et al.*, 2016; Krupke *et al.*, 2012). Moreover, since many farmers have transitioned from non-transgenic to transgenic soybean varieties, it is assumed that the use of this herbicide has increased. As a result, transgenic soybean likely plays a significant role in the contamination by GLY. Therefore, we hypothesized that colonies located in areas where transgenic soybean pollen is present would exhibit higher levels of GLY. The objective of our study was to quantify glyphosate residues in pollen samples collected from honeybees (*Apis mellifera* L.) and assess the potential risk it may pose to honeybees and the health of consumers.

MATERIALS AND METHODS

Study period, site and sample collection

The study was carried out in the municipalities of Suchiate and Tapachula, in the Soconusco region, Chiapas, in southern Mexico. We selected two sites based on different land uses: Site 1 (14° 45"5.08"'N, 92° 15"46.87"'W) in Suchiate, characterized by 2% urban settlements, 36% preserved remnants of the original forest, and 62% cropland; and Site 2 (14° 45"19.20"'N, 92° 17"30.60"'W) in Tapachula, with 1% of land occupied by urban settlements, 17% covered by the original forest, and 82% designated as cropland. To minimize the influence of geographical factors, the sampling sites were located within the same ecoregion and separated by 3 km (Figure 1). We operated under the assumption that the foraging areas of the bees were relatively independent and restricted to their respective sites, given the perceived adequacy of food resources near the hives, as indicated by honey production levels. Throughout one year, we gathered monthly pollen samples from ten colonies of *A. mellifera* at each site, totaling 120 pollen samples. Each sample was preserved in a 15 mL Falcon tube and kept frozen at −20 °C until analysis. Simultaneous sampling was conducted at both sites.

Glyphosate extraction and quantification

GLY residues were extracted using the methodology developed by Wiest *et al.* (2011). Two grams of pollen were accurately weighed and placed in a 50 mL centrifuge tube. To this, 10 mL of water were added, and the mixture was vigorously shaken. Subsequently, 10 mL of acetonitrile, 3 mL of hexane, 4 g of anhydrous $MgSO_4$, 1.0 g of sodium chloride, 1 g of sodium citrate dihydrate, and 500 mg of disodium citrate sesquihydrate were added. The tube was promptly shaken by hand, vortexed for one minute, and then centrifuged for 2 minutes at 5000 g. Six milliliters of the supernatant were carefully transferred into a 15 mL PSA (primary secondary amine) tube, which contained 900 mg

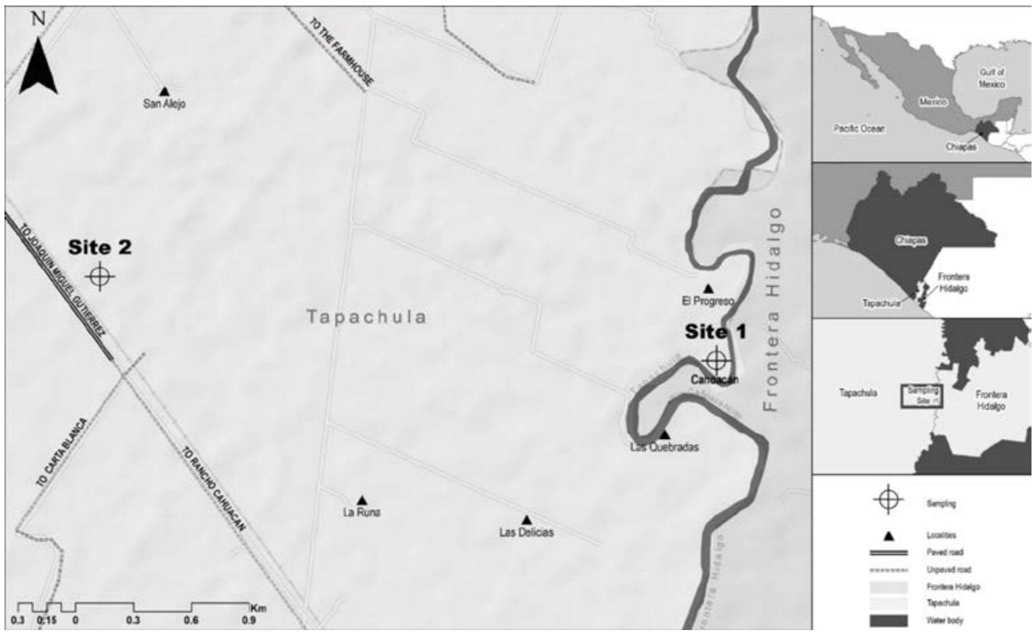

Figure 1. Sampling sites in the study. Sites were separated by approximately 3 km to keep foragers from visiting same resources.

of anhydrous $MgSO_4$, 150 mg of PSA bonded silica, and 150 mg of C18 bonded silica. This tube was immediately shaken by hand, vortexed for 10 seconds, and centrifuged for 2 minutes at 5000 g. Finally, 4 mL of the extract were transferred into a 10 mL glass, cone-ended centrifuge tube, evaporated until a final volume of 50 μL was achieved, and the sample was stored at −18 °C until analysis. All salts used for extraction were supplied by Agilent Technologies, Santa Clara, CA, USA.

GLY quantification was performed using an immunoassay kit from Abraxis LLC (Part number PN500084: Warminster, USA). The method demonstrated a limit of detection for GLY of 0.05 μg/L, a limit of quantification of 0.13 μg/L, a maximum detectable concentration of 4 μg/L, and an average recovery rate of 102%. For quantification, a four-point calibration curve was established (0.075, 0.2, 0.75, and 4 μg/L) with two replicates for each point. An analytical quality control solution at 0.5 μg/L was also employed. All samples underwent triplicate analysis, and for samples with GLY concentrations exceeding the calibration curve range, dilutions were performed until a reliable concentration estimate could be obtained. Possible cross-reactivity with other agrochemicals used in the study area, such as paraquat, spinosad, malathion, mancozeb, endosulfan, chlorothalonil, chlorpyrifos, and cypermethrin, was investigated. No interference was observed in the analysis, as confirmed by control tests in which these pesticides, at a concentration of 1 μg/L, did not react with our immunoassay test (Ruiz-Toledo *et al.*, 2014).

Exposure assessment and risk characterization to honeybee health

We calculated the average concentration of GLY found in the replicates and determined the Pollen Hazard Quotient (PHQ), which is a measure of the number of bees that, by consuming one kilogram of pollen, would reach the LD50 (dose required to kill 50% of the exposed population). To calculate the PHQ for honeybees, we followed the methods described by Stoner and Eitzer (2013a) and Traynor *et al.* (2016). This was done by dividing the concentration (μg/kg) of GLY in the sample by the oral LD50 of GLY (100 μg/bee), which is generally regarded as moderately toxic for adult bees (EFSA, 2015a; Lewis *et al.*, 2016) and then multiplying by 100. For reference, an adult bee that consumed 100 mg of pollen with a PHQ of 1000 would have ingested approximately 10% of the LD50 of the pesticide during its development stage as a nurse bee, which lasts about 10 days (Calatayud-Vernich *et al.*, 2018). If this 10% of the LD50 should not be exceeded (Atkins *et al.*, 1981), a PHQ value of 1000 would correspond to a critical threshold for bee health (Stoner & Eitzer, 2013b; Traynor *et al.*, 2016). A nurse bee typically consumes between 13 and 120 mg of pollen during its first 10 days of life (OECD/OCDE, 1998; Rortais *et al.*, 2005), with an average consumption of 65 mg (M.-P. Chauzat & Faucon, 2007). As a worst-case scenario, we considered the maximum consumption level of 12 mg of pollen per day (Rortais *et al.*, 2005). We then multiplied this highest consumption level by the highest observed glyphosate residues and compared the resulting exposure levels with the oral acute LD50 of GLY.

Risk to consumer's health

The toxicological reference values for GLY in this study were: 1) the acceptable daily intake (ADI) at 0.3 mg/kg bodyweight (Renwick, 2002a); 2) the daily acute reference dose (ARfD) at 0.5 mg/kg bodyweight (EFSA, 2015b); 3) the legally permitted maximum concentration of pesticide residues in or on food products or animal feed (MRL) at 0.05 mg/kg (EFSA, 2017); and 4) the Acceptable Operator Exposure Level (AOEL) at 0.1mg/kg (EFSA, 2015a; Luo *et al.*, 2021; Renwick, 2002b).

To assess the health risk associated with GLY residues in pollen for consumers, we relied on pollen consumption estimates obtained from data published in the EFSA Comprehensive European Food Consumption Database (EFSA, 2018). The highest 95th percentile value recorded corresponds to 69.55 g/person, which equates to 1.35 g/kg bodyweight for a person weighing 52 kilograms in France. In the most conservative scenario, we multiplied such high intake levels by the highest observed concentration of GLY residues. Finally, we compared the resulting exposure levels with the established toxicological reference values for GLY to determine the extent of the risk.

Statistical analysis

A descriptive analysis of GLY concentration was performed, which included calculating the geometric means, median, standard deviations, as well as minimum and maximum values. To identify any statistical differences among different sampling dates and between the two sites, a general linear mixed model ANOVA was conducted. In this analysis, the colony was treated as a random effect, while the site and the date of sampling were considered fixed effects. All statistical analyses were carried out using the R software package, and the significance level was set at 0.05 (R Development Core Team, 2020).

RESULTS AND DISCUSSION

Glyphosate residues in pollen

We detected GLY residues in all samples in the range of 3.71 to 7.29 μg/kg (Table 1). In site 1, a mean (±SD) GLY concentration of 5.07 μg/kg (±0.93) was found, while in site 2 it was 5.45 μg/kg (±0.84). No statistically significant differences were observed between sites ($p \geq 0.05$). The highest GLY concentration was found in site 1 in March (7.29 μg/kg). However, no significant difference was found between months ($p \geq 0.05$). In our study area, GLY residues were identified in all samples collected at both sites (site 1, 3.9 - 7.29 μg/kg and site 2, 3.71 - 6.68 μg/kg). Residues of pesticides, including GLY, have been identified in live honeybees, stored fresh pollen, and beeswax. Notably, the beeswax contains elevated levels of commonly employed acaricides in beekeeping (Kasiotis *et al.*, 2023). Thompson *et al.* (2014) identified this herbicide in brood samples at concentrations ranging from 1.23 to 19.5 mg/kg; Rubio *et al.* (2014) reported a mean concentration of 64 mg/kg and a maximum of 163 mg/kg in honey; Agrebi *et al.* (2019) found glyphosate residues in 91.4% of the bee bread samples, whose main component is pollen; the average concentration reported in this study is 55.52 mg/kg, seven times higher than in our study.

Exposure assessment and risk characterization of GLY residues in pollen for honeybees

In the estimation of HQ for GLY residues in pollen, we found an average of 5.79 for site 1 and 5.45 for site 2. This indicates that at site 1 an adult bee consumes 0.06% of the LD50 during its development stage, while in site 2 it is 0.05% (Table 1). In the worst case and with the maximum concentration of residues detected in pollen, we found that these concentrations could correspond to doses of 0.87 μg of GLY residues ingested per nurse

Table 1. Concentration of GLY in pollen (μg/Kg) in the samples from both study sites (S1 and S2); N, number of samples; [a] % of samples with detectable levels (%≥DL); [b] values reported as geometric mean (GM); (SD) standard deviation; (HQ) Hazard quotient; % of LD50 refers to the proportion of the LD50 ingested daily by a bee.

Year	Month	N	%≥DL [a]		GM [b]		Median		SD		HQ		% of LD50	
			S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
2021	June	10	100	100	5.03	6.02	6.14	5.88	1.15	0.36	5.03	6.02	0.05	0.06
	July	10	100	100	6.54	6.68	6.54	6.09	0.45	1.14	6.54	6.68	0.07	0.07
	August	10	100	100	7.29	6.09	6.47	5.06	0.68	1.21	7.29	6.09	0.07	0.06
	September	10	100	100	6.47	4.48	6.51	4.48	0.34	0.68	6.47	4.48	0.06	0.04
	October	10	100	100	5.93	3.71	6.14	5.29	0.32	1.14	5.93	3.71	0.06	0.04
	November	10	100	100	6.57	5.06	5.81	5.06	1.45	0.60	6.57	5.06	0.07	0.05
	December	10	100	100	6.34	5.98	6.46	5.98	1.20	0.74	6.34	5.98	0.06	0.06
2022	January	10	100	100	3.95	4.86	4.94	4.86	0.98	0.71	3.95	4.86	0.04	0.05
	February	10	100	100	4.94	6.26	5.91	6.26	0.57	0.60	4.94	6.26	0.05	0.06
	March	10	100	100	5.91	5.78	6.57	6.02	0.69	0.16	5.91	5.78	0.06	0.06
	April	10	100	100	6.35	5.66	6.35	5.72	0.70	0.49	6.35	5.66	0.06	0.06
	May	10	100	100	5.07	5.69	5.91	5.94	0.72	0.16	5.07	5.69	0.05	0.06

bee over 10 days (0.012 g × 7.29 μg/kg × 10 days). This exposure level corresponds to approximately 0.008% of the oral LD50 of GLY.

Based on our assessments, the GLY concentrations found here do not appear to be toxic to bees. The maximum concentration of GLY residues found (7.29 μg/kg) led to sub-lethal exposure (not acutely toxic to bees), equivalent to a dose of 0.87 μg/bee (0.008% of its LD50), ingested over the first 10 days of life of a nurse bee. Nevertheless, some studies have suggested that even minute amounts of GLY may compromise the immune system of bees (Samsel & Seneff, 2013). Studies have shown that it increases the susceptibility of bees to the effects of other pesticides (J. Y. Wu *et al.*, 2011), perhaps working as a synergist (Botías *et al.*, 2017; A. M. Chauzat *et al.*, 2009; Wan *et al.*, 2018). This phenomenon has been observed with some fungicides which increases the toxicity of pyrethroids (Pilling *et al.*, 1995; Pilling & Jepson, 1993), and of some neonicotinoids (Iwasa *et al.*, 2004) to the honeybee. Furthermore, GLY, as well as the herbicide formulation containing GLY, can affect the intestinal microbiota of bees, leading to dysbiosis and increased susceptibility to bacterial infection (Motta *et al.*, 2020, 2022). GLY exposure has been found to decrease the expression of genes encoding antimicrobial peptides and inhibit melanization, which are important components of the bee's innate immune system (Vázquez *et al.*, 2018). Additionally, GLY exposure may disrupt the beneficial intestinal microbiota of bees, potentially affecting bee health and their effectiveness as pollinators (Motta *et al.*, 2018). The effects of GLY on bee health and intestinal microbiota may vary depending on individual and colony susceptibility (Helander *et al.*, 2018, 2023)

Risk assessment for consumers to contaminated pollen

According to our results, a high pollen consumption in southeastern Mexico could lead to a daily ingestion of 0.005 μg GLY/kg bodyweight. No sample in our study exceeded the toxicological reference values. Rubio *et al.* (2014) found GLY residues in 70% of honey samples from countries that permitted the cultivation of genetically modified organisms, compared to only 21% in those that did not. In our study, we identified GLY residues in 100% of the pollen samples, indicating that contamination by this herbicide extends beyond honey, aligning with findings reported in soybeans, cereals, and ice cream (IARC, 2017; Kolakowski *et al.*, 2020; Rubio *et al.*, 2014; Vicini *et al.*, 2021). While tolerance levels for GLY and its metabolites have been established in various foods (Code of Federal Regulations., 2018), none have been set for pollen and honey. Mexico lacks federal monitoring programs for GLY, making it challenging to estimate the extent of food contamination in the country. In contrast, countries like the USA include GLY in their annual pesticide residue-monitoring program, detecting it in various commodities (FDA, 2017). The Canadian Food Inspection Agency reported GLY in food, with a presence in 29.7% of 3,188 analyzed food samples in 2015-2016.

GLY is recognized as a public health risk, prompting global concern and social action, especially since it has entered the human food chain (Mills *et al.*, 2017). Recent research indicates an increase in the prevalence and average concentration of GLY in human urine between 1993 and 2016 (Philipp Schledorn, 2014). Possible mechanisms underlying GLY toxicity in mammals have been described in recent studies (Mensah *et al.*, 2015). The 2016 report from the International Agency for Research on Cancer of the World Health Organization (IARC, 2017) summarizes scientific data, and based on that report, the state of California in the USA listed GLY as known to cause cancer, requiring products to be labeled accordingly (California Environmental Protection Agency, 2017). Moreover, other studies have reported that the toxicological effects of GLY depend on the type of cells, chemical composition, as well as the magnitude and time of exposure (Agostini *et al.*, 2020). This includes neurological effects (Martinez & Al-Ahmad, 2019), damage to the immune system (Santovito *et al.*, 2018), effects on human embryonic and placental cells (Benachour & Séralini, 2009; Richard *et al.*, 2005) and decreased sperm motility, viability, and mitochondrial activity (Nerozzi *et al.*, 2020). It has also been noted that even at concentrations below toxic levels ($<1\ \mu$g/L), GLY can reduce testosterone production by 35% and disrupt estrogen-regulated genes, promoting breast tumor growth (Hokanson *et al.*, 2007). This situation is concerning, particularly since the Soconusco region has shown GLY residues in various matrices for human consumption (honey and well water). Although the concentrations of GLY reported in our studies did not appear to pose a high risk, a recent increase in the incidence of kidney problems, reproductive issues, cancer, and leukemia in the region has been reported, potentially associated with the unregulated use of this herbicide (Rivera-luna *et al.*, 2014).

CONCLUSIONS

The detection of GLY in pollen collected by honeybees raises concerns about potential contamination in food supplies for both humans and bees. Nevertheless, despite the presence of GLY residues in all samples analyzed in this study, the reported concentrations do not seem to pose any apparent risk to the health of humans and bees, as per our calculations and considering the LD50. However, given the widespread distribution of GLY in the region, it is imperative to underscore the importance of conducting long-term studies to comprehend the potential chronic effects of the pesticide on all species in the area.

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Infestation of Cattle with the Tick *Amblyomma mixtum* in the States with the Highest Cattle Inventory in Mexico

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ABSTRACT

Objective: The *Amblyomma mixtum* tick is one of the main parasites affecting cattle in Mexico. Epidemiological records of *A. mixtum* in leading states in the national cattle industry, such as Jalisco, Chiapas, Michoacán, Tabasco, and Veracruz, are outdated since this tick species was previously classified as *A. cajennense*. The objective was to update the records of the *A. mixtum* tick in the states of Jalisco, Chiapas, Michoacán, Tabasco, and Veracruz, as well as the main ixodicide molecules used for its control.

Methodology: From March 2022 to July 2023, ticks were collected from cattle in 46 bovine production units (BPUs) distributed in the states mentioned above. Identification was performed using standardized taxonomic keys. Scanning electron microscopy was performed on specimens corresponding to *A. mixtum*.

Results: Out of a total of 619 specimens of the genus *Amblyomma* from 22 PBUs, it was confirmed that 100% correspond to the species *A. mixtum*. Amitraz, an ixodicide molecule belonging to the chemical class of amidines, was used in 63.2% of the BPUs where the presence of *A. mixtum* was also reported.

Conclusions: This research confirms cattle infestation with *A. mixtum* in states with the highest cattle production in Mexico. Further studies with a focus on resistance and extension are required to prolong the usefulness of available tools, including ixodicides, for integrated control of *A. mixtum* infestations where this tick infests cattle in Mexico.

Keywords: Livestock, *Amblyomma mixtum*, distribution, acaricides, amitraz.

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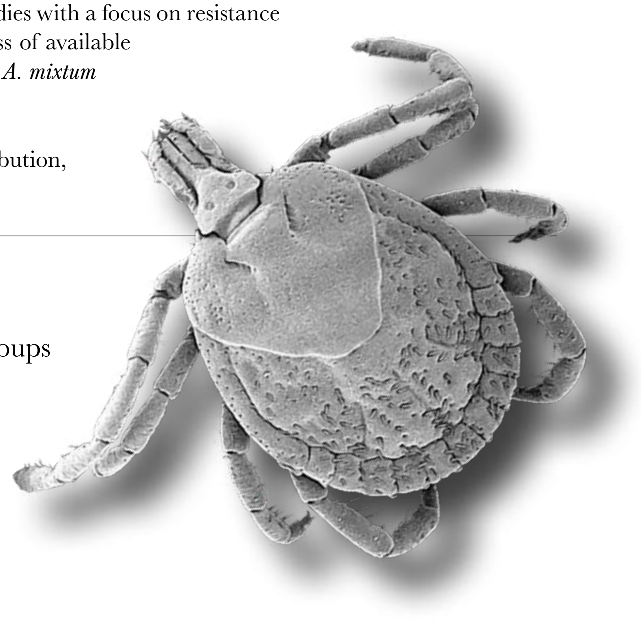
INTRODUCTION

Ticks are one of the most important groups of ectoparasites that affect cattle globally (Pérez de León *et al.*, 2020). In addition to feeding off the blood of their host, due to their hematophagous habit,



several species of ticks are vectors of pathogens that can cause high morbidity and mortality in cattle, as well as in other domestic animal species, wildlife and humans (de la Fuente *et al.*, 2023). Ticks belong to the Phylum Arthropoda, Class Arachnida, Order Acarina and within it, the suborder Ixodoidea; the family Ixodidea derives from this, which is divided in four: Ixodidae, Argasidae, Nuttalliellidae and Deinocrotonidae (Polanco-Echeverri and Ríos-Osorio, 2016; Peñalver *et al.*, 2017). Around 109 tick species have been reported in Mexico (Guglielmone *et al.*, 2010; Pérez *et al.*, 2014); from these, *Amblyomma mixtum* is considered in veterinary medicine as one of the species of highest economic impact for the livestock industry due to the decrease in cattle health and productivity in Mexico that it causes (Almazán *et al.*, 2018).

The genus *Amblyomma* is found in the subdivision Ixodidae and classified as the third largest (Rivera-Páez *et al.*, 2016), from which half of the species classified in it are located in the American continent (Guglielmone and Nava, 2006). Likewise, *A. mixtum* was originally described in 1844 (Koch, 1844), although the species was classified as *A. cajennense* (Beati *et al.*, 2013). Recent studies based on morphology, genetics and reproduction confirmed that *A. mixtum* is a species that is part of the *A. cajennense* complex (Beati *et al.*, 2013; Nava *et al.*, 2014). *A. mixtum* is a tick whose biological cycle has three hosts and in addition has the widest range of distribution, from the south of Texas to Ecuador (Aguilar-Domínguez *et al.*, 2021). Although cattle infestations highlight its importance in veterinary medicine, the larvae, nymphaea, and adults of *A. mixtum* can infest horses, dogs, several wildlife species such a white-tailed deer, and humans in the Neotropical region (Rodríguez-Vivas *et al.*, 2016; Aguilar-Domínguez *et al.*, 2019).

The cattle population in Mexico in 2022 was around 36.6 million heads including meat and dairy cattle (Consulta SIAP, 2022). Out of the 32 states in Mexico, the presence of *A. mixtum* has been reported in 16 states (Nava *et al.*, 2014; Castillo-Martínez *et al.*, 2020). However, information is necessary about cattle infestation of the main livestock producing states in the country, among them Veracruz, Jalisco, Michoacán, Tabasco, and Chiapas (Juárez and Salas-González, 2023).

It is also necessary to understand that ixodicides are used to treat cattle infested with ticks that can include *A. mixtum*. The use of these molecules is the most common practice to control ticks in cattle in Mexico. Chemical ixodicides are used primarily to control the *Rhipicephalus microplus* tick, which is considered as the most important tick for cattle that can be co-infested with *A. mixtum* in tropical and subtropical areas of the country where these two species of ticks coexist (Rodríguez-Vivas *et al.*, 2017; Almazán *et al.*, 2018).

The objective of this study was to update the records on cattle infestation with *A. mixtum*, as well as the main molecules of ixodicides used for their control in the states of Jalisco, Chiapas, Michoacán, Tabasco, and Veracruz.

MATERIALS AND METHODS

Sampling sites

Simple convenience sampling was carried out in a total of 46 Bovine Production Units (BPUs) distributed in the states of Chiapas, Jalisco, Michoacán, Tabasco and Veracruz, in the period from March 2022 to July 2023. These states, in addition to having the largest populations of cattle, have geographic locations favorable for the development of the biological cycle of *A. mixtum*, because they are completely or partially within the Neotropical region of the country (Morrone, 2005). When sampling, the location of the BPUs was established based on the methodology described by Aguilar-Domínguez *et al.* (2021), where the potential distribution of this species of ticks in the states previously mentioned is indicated (Table 1).

Collection of specimens and data of the BPU/Host

In each BPU, cattle were inspected from head to tail against the hair to locate the specimens and with the help of entomological pliers, light tractions from the top down were carried out to remove them (CDC, NCEZID, 2018). Each tick was conserved individually in ethanol at 70% v/v. In addition to the collection, a survey was applied by BPU to gather data on the control of ectoparasites, including the use of acaricides to control infestation with ticks, and the presence of one or more domestic or wild species in contact with the cattle. The coordinates of the sampling points were taken through the implementation of GPSmap GARMIN® (Table 1). The ticks collected were transported to the Parasitology Laboratory, located in the Diagnostics Unit of the Torreón del Molino Ranch from the Veterinary Medicine and Zootechnics School of Universidad Veracruzana. The ticks were processed in the laboratory according to the methodology described by Aguilar-Domínguez *et al.* (2019).

Morphological identification and electron microscopy

The identification of specimens was conducted through morphological taxonomic keys established by Guzmán-Cornejo *et al.* (2011) for *Amblyomma cajennense* and by Nava *et al.* (2014) for *Amblyomma mixtum*. With the aim of describing the main morphological structures, ticks identified as *A. mixtum* and after determining through inspection that the specimen was complete, were selected for the processing of scanning electron microscopy, following the modified methodology from Corwin *et al.* (1979) and Dixon *et al.* (2000). For this purpose, cleaning with extra-fine Dumont® pliers and brushes was carried out and they were placed in ethanol at 70% v/v; and the sonic process was continued, where they are placed in a Cole-Parmer® 8848 ultrasonic cleaner for 5 min with 2 repetitions.

Dehydration was conducted through gradual ethanol in 80 and 90% for 90 minutes in each concentration and 3 changes in absolute ethanol for 30 minutes in each change. After this cycle ends, they are placed in xylene at a temperature of 40 °C for 24 hours. Then, they were transferred in absolute ethanol for 30 minutes with three repetitions. To dry the material, a critical point drier was used of the brand Quorum® model K850 using CO_2. After this phase is completed, the dry ticks were placed on aluminum slides adhered with double-face carbon conductive tape, to cover the specimens with gold using a Quorum® model Q150R S metal ionizer. To finish, the specimens were analyzed with the scanning electron microscope of field emission FEI Quanta 250 FEG.

Table 1. Location of sampling sites by state and municipality.

State	Municipality	Locality	Geolocalization	Altitude MASL
Chiapas	Tapachula	Oro Verde	14° 50’ 31.2” N, 92° 20’ 58.3” W	0
		Corlai	14° 52’ 25.6” N, 92° 21’ 46.4” W	0
		Tapachula	14° 54’ 22.0” N, 92° 17’ 42.1” W	0
	Mapastepec	Adolfo López Mateos	15° 26’ 48.7” N, 92° 59’ 56.5” W	0
		Dos Pasajes	15° 28’ 57.1” N, 93° 03’ 44.5” W	0
		La Trinidad	15° 32’ 31.6” N, 92° 59’ 56.6” W	0
	Pijijiapan	Puente Margaritas	15° 35’ 37.0” N, 93° 02’ 41.0” W	0
		La Herradura	15° 35’ 48.1” N, 93° 04’ 35.1” W	0
		Gabriel Toledo	15° 34’ 57.2” N, 93° 10’ 13.9” W	0
		Las Carmelitas	15° 36’ 04.1” N, 93° 10’ 41.0” W	0
		Caña Brava	15° 38’ 34.1” N, 93° 10’ 39.6” W	0
		Pijijiapan	15° 42’ 16.0” N, 93° 13’ 38.9” W	0
	Tonalá	Agua Prieta	16° 00’ 59.5” N, 93° 37’ 46.6” W	0
	Palenque	Palenque	17° 32’ 17.9” N, 91° 58’ 21.0” W	0
Jalisco	Cihuatlán	Emiliano Zapata	19° 17’ 36.7” N, 104° 42’ 50.4” W	0
	La Huerta	El Progreso	19° 19’ 31.4” N, 104° 49’ 11.8” W	0
	Concepción de Buenos Aires	Concepción de Buenos Aires	19° 59’ 55.4” N, 103° 15’ 50.3” W	0
	Encarnación de Díaz	Concepción de Buenos Aires	20° 00’ 39.5” N, 103° 15’ 18.1” W	0
		La Cuadra	21° 31’ 39.7” N, 102° 11’ 29.5” W	0
Michoacán	Lázaro Cárdenas	Lázaro Cárdenas	17° 59’ 01.1” N, 102° 14’ 23.2” W	0
		Buenos Aires	18° 02’ 12.9” N, 102° 16’ 46.2” W	0
		Playa Azul	17° 59’ 20.7” N, 102° 22’ 56.8” W	0
		El Habillal	18° 01’ 22.0” N, 102° 21’ 40.6” W	0
		El Habillal	17° 59’ 34.7” N, 102° 22’ 53.7” W	0
	Arteaga	Arteaga	18° 20’ 31.1” N, 102° 17’ 27.4” W	0
		Arteaga	18° 21’ 08.2” N, 102° 17’ 15.9” W	0
	Aquila	El Aguacate	18° 29’ 54.4” N, 103° 17’ 48.2” W	0
	Tuxpan	El Malacate	19° 32’ 44.9” N, 100° 28’ 40.4” W	0
	Morelia	Cañada de Buena Vista	19° 33’ 15.6” N, 101° 15’ 12.0” W	0
		Santiago Undameo	19° 35’ 25.9” N, 101° 15’ 28.0” W	0
		Santiago Undameo	19° 35’ 34.4” N, 101° 15’ 30.6” W	0
	Zamora	Romeo de Guzmán	20° 02’ 29.7” N, 102° 14’ 55.4” W	0
	Cuto de la Esperanza	Cuto de la Esperanza	19° 43’ 43.0” N, 101° 20’ 31.8” W	0
Tabasco	Balancán	El Tornillo	17° 50’ 37.6” N, 91° 31’ 00.6” W	25.5
	Tacotalpa	Puente de Piedra	17° 35’ 33.2” N, 92° 37’ 10.9” W	0
	Huimanguillo	Ocuapan	17° 49’ 49.4” N, 93° 30’ 08.2” W	0
Veracruz	Cosoleacaque	Calzadas	18° 07’ 49.0” N, 94° 31’ 36.0” W	0
	San Juan Evangelista	Rancho Azteca	17° 39’ 30.2” N, 94° 58’ 00.1” W	0
	Juan Rodríguez Clara	Perseveranza	17° 56’ 19.1” N, 95° 11’ 28.1” W	0
	Coatzacoalcos	Matilla de Conejo	18° 04’ 11.3” N, 95° 15’ 29.7” W	0
		Matilla de Conejo	18° 03’ 49.3” N, 95° 16’ 22.3” W	0
		Matilla de Conejo	18° 10’ 24.4” N, 94° 15’ 56.0” W	0
	Isla	El Tigre	17° 56’ 09.2” N, 95° 21’ 42.0” W	0
	Túxpam de Rodríguez Cano	Guillermo Prieto	18° 10’ 24.4” N, 94° 15’ 56.0” W	0
		San Isidro	18° 04’ 52.1” N, 95° 32’ 02.6” W	0
		Lindavista	20° 48’ 58.6” N, 97° 14’ 20.2” W	0
	Manlio Fabio Altamirano	Mata Loma	19° 08’ 14.2” N, 96° 18’ 00.7” W	0
	Tlapacoyan	San Francisco	20° 02’ 08.0” N, 97° 06’ 22.7” W	0

Statistical analysis

Descriptive statistics were conducted to obtain the frequency and percentage of *A. mixtum*, according to their taxonomic identification and to the molecule used. Likewise, the sampling sites were georeferenced to perform a detailed map with the information gathered through the Maptive GIS Mapping Software.

RESULTS AND DISCUSSION

In 22 BPUs of the states of Chiapas, Jalisco, Michoacán, Tabasco and Veracruz, a total of 619 *Amblyomma mixtum* ticks were collected from cattle (Figure 1).

Table 2 presents the distribution of *A. mixtum* ticks per state. Veracruz was the state where the largest number of *A. mixtum* ticks was collected (n=428), followed by Tabasco and Jalisco with 75 and 46, respectively.

Figures 2 and 3 present the main characteristics of *A. mixtum* through scanning electron microscopy. An ornamental shield1 is observed, the complete marginal furrow limiting all the festoons[2] as well as the presence of keratinous structures (chitin tubercles or mamelons) present dorsally to the festoons[3]. The complete spatulate hypostome can also be seen with dental formula 3/3[4], and the base of the sub-rectangular chapter with short cornu[5]. The

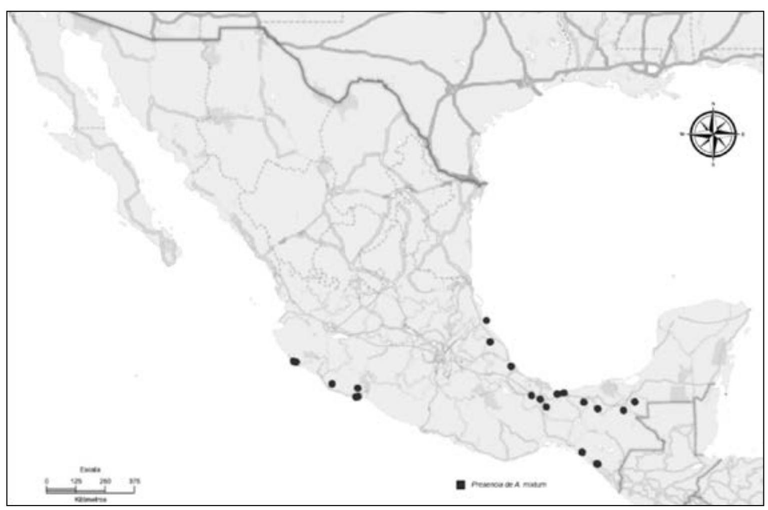


Figure 1. Sampling sites where the presence of *A. mixtum* was identified.

Table 2. Classification of *A. mixtum* ticks according to the genus and the state sampled.

State	Number of *A. mixtum*	(%)	Female (number)	(%)	Male (number)	(%)
Veracruz	428	69.1	240	70.8	188	69.6
Michoacán	45	7.3	29	8.6	16	5.9
Tabasco	75	12.1	30	8.8	45	16.7
Chiapas	25	4.1	16	4.7	9	3.3
Jalisco	46	7.4	24	7.1	12	4.5
Total	619	100	339	54.8	270	43.6

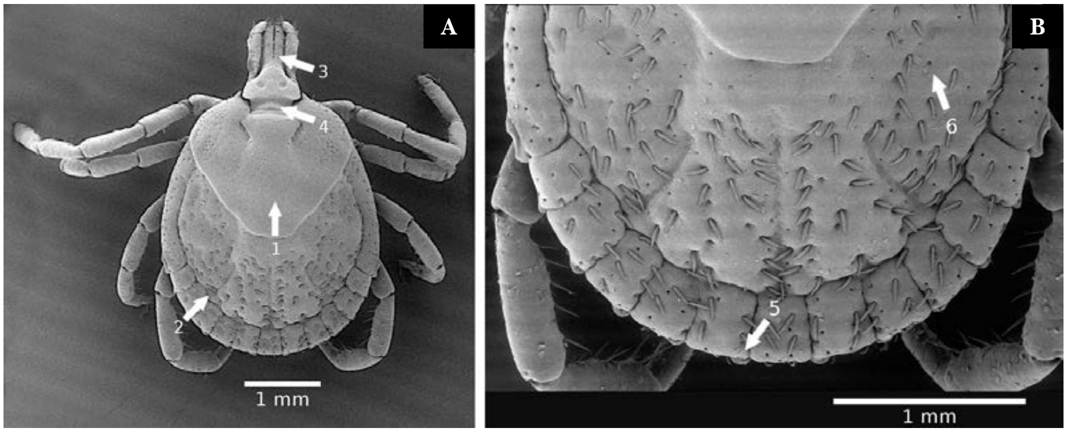


Figure 2. Dorsal view of an *Amblyomma mixtum* female.

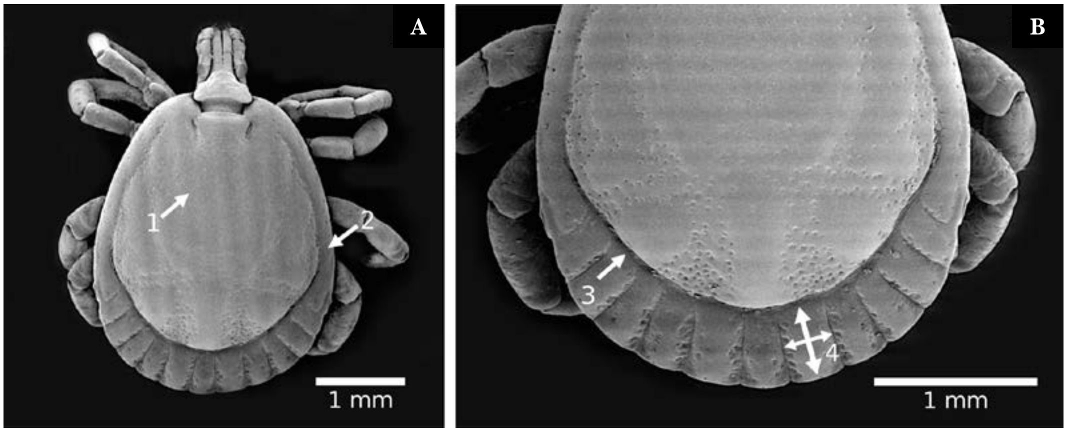


Figure 3. Dorsal view of an *Amblyomma mixtum* male.

oval idiosome with long gnatosome, as well as deep punctuations distributed throughout the body and with greater depth in the lateral fields[6].

The ornamental shield is observed with brown spots limited by whitish spots[1]. Complete furrow limited by festoons, deep to the IV coxa[2] followed by a line of pores or points that continue to the eyes[3]. Festoons that are wider rather than long[4]. Regarding the use of ixodicides in the BPUs, it was seen that tick control was carried out through the application of chemical products. The molecules used belong to the group of the Amidines, Organophosphates, Macrocyclic lactones (ML) and Pyrethroids (Table 3).

Amidines are the most frequently used acaricide in the BPUs (63.6%), followed by organophosphates (27.2%). Macrocyclic lactones and pyrethroids are the least used molecules in the BPUs, and the use of pyrethroids was found in the states of Michoacán and Chiapas, while macrocyclic lactones were only reported for the state of Chiapas. It is important to mention that this information is based on the frequency of the *A. mixtum* tick in the herds sampled.

Table 3. Molecules used in the BPUs where the presence *A. mixtum* was recorded.

Molecule	State	BPU where they are used	(%)
Amidines	Michoacán	14	63.6
	Jalisco		
	Veracruz		
	Tabasco		
	Chiapas		
Organophosphates	Jalisco	6	27.2
	Tabasco		
Macrocyclic lactones	Chiapas	2	9.1
Pyrethroids	Michoacán, Chiapas	2	9.1
Total		22	100

This study confirms the presence of *A. mixtum* in the states of Chiapas, Michoacán and Jalisco, which is consistent with what is reported for the state of Veracruz by Aguilar-Domínguez *et al.* (2019, 2021). This information updates and increases the knowledge of the distribution of *A. mixtum* in Mexico, in relation to the restructuring of the taxonomy of this species (Nava *et al.*, 2014), which generated a breach of outdating for more than 50% of the national territory. The information reported here about the infestation of cattle in the states with highest livestock production in the country suggests that the infestation of cattle with *A. mixtum* can be more prevalent than what is estimated. The frequency was 50.4% which agrees with what was reported by Ulloa-Ramones and Ulloa-Ramones (2021) and by Noda *et al.* (2015), who reported a frequency of 50% and 67%, respectively. It is likely that this increase of the mean corresponds to the displacement of the ecological niche of *R. microplus*, indicating that resistance problems are probably happening or it could be due to the need for management in extension work to improve the handling with ixodicide products that are commercially available for the use of livestock producers in Mexico (Juache-Villagrana *et al.*, 2023; Espinoza *et al.*, 2021; Alonso-Díaz *et al.*, 2013). These results highlight the need for more detailed epidemiological studies to determine the effectiveness in the use of acaricides to treat cattle co-infested with *A. mixtum* and *R. microplus* (Higa *et al.*, 2020), and to estimate the economic impact of *A. mixtum* in the livestock industry of Mexico.

Concerning the use of ixodicides in the BPUs, in this study it was seen that tick control is done through the application of chemical products. Several classes of acaricides are part of the veterinary products used as ectoparasiticides in 63.6% of the BPUs where the presence of *A. mixtum* is reported. Products based on amidines, including amitraz as representative of this class of acaricide, can be effective in the control of ticks and other ectoparasites in animals. However, its indiscriminate use has resulted in populations of *A. mixtum* that are resistant to amitraz (Alonso-Díaz *et al.* 2013). Resistance to amitraz of up to 100% has been reported in *A. mixtum* where a triggering factor of this extreme phenotype could be the more frequent infestation with larvae, nymphaea, and adults of this same species in livestock where the signaling for treatment was the detection of *R. microplus* (Higa *et al.*, 2019).

Organophosphates, another class of ixodicides, have been used in Mexico since the 1980s, and they can still control ectoparasites where resistance is not prevalent (Sharma *et al.*, 2019). They act by interfering with their nervous system and have shown efficacy against various insects and mites (Bernal-González *et al.*, 2023). The frequency of use according to the BPUs where the presence of *A. mixtum* is reported was 27.2%, and it is only used in Jalisco and Tabasco. This indicates scarce use in the leading livestock production states, even when acaricide effectiveness is reported within the genus *Amblyomma* (Natala *et al.*, 2005).

Macrocyclic lactones, which include compounds such as ivermectin and other molecules (some in prolonged action products), are used as ectoparasiticides to treat livestock (Rodríguez-Vivas *et al.*, 2014). These veterinary products are effective against intestinal worms, mites, and other parasites. According to the frequency of *A. mixtum*, their use was reported only in the state of Chiapas. However, there is no literature inquiring about the resistance of these molecules in populations of *A. mixtum* in Mexico, in contrast with what was reported for *R. microplus*.

Pyrethroids, synthetic byproducts of natural pyretrins present in certain flowers (Lara Lafargue *et al.*, 2018), are widely used in insecticides and in products directed to pest control, ticks, and other insects (Díaz and Vallejo., 2013). Veterinary products in the market include flumethrin and cypermethrin as the most common pyrethroids in products used during the sampling period where the presence of *A. mixtum* was reported and it must be pointed out that its use was only reported in two BPUs. Resistance was reported in larvae of *A. mixtum* (Higa *et al.*, 2020). According to the data gathered, this group is the one that is least attributed the property of resistance and according to the information collected in the sampling, it is also the one that is least used. In some cases the problem of resistance to acaricides is so acute that there is a need to resort to the combination of many classes of acaricides, with the purpose of taking advantage of different modes of action and to broaden the range of action against the ticks that parasite cattle in Mexico (Rodríguez-Vivas *et al.* 2018). Additional research is required to develop and implement integrated management tools that mitigate the evolution of resistance to acaricides in populations of *A. mixtum* through their geographic range that affect the health and productivity of cattle herds in Mexico.

CONCLUSION

The presence of *Amblyomma mixtum* was confirmed in the leading livestock producing states in southeastern Mexico. The information obtained on control measures for *A. mixtum* in infested cattle, primarily in the use of various acaricide chemicals, generates alert for the risk of development of resistance to the classes of acaricides used by the producer. This situation highlights the need for additional studies with an approach on resistance and extension work to prolong the usefulness of the tools available, including acaricides, to control infestations with *A. mixtum* where this tick infests cattle in Mexico.

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COMPLIANCE WITH ETHICAL STANDARDS

This project was approved on April 12, 2023, by the Bioethics and Animal Welfare Commission of the School of Veterinary Medicine and Zootechnics in Universidad Veracruzana.

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Colegio de Postgraduados

Productive and Ruminal Microbiological Behavior of Sheep Fed with Two Levels of Dehydrated Orange Residue

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ABSTRACT
Objective: To evaluate the productive and ruminal microbiological behavior of sheep fed with two levels of dehydrated orange residue (DOR).
Design/methodology/approach: Thirty Dorper×Katahdin crossbred male sheep with an average live weight of 22±1.0 kg were distributed in a completely randomized design, with three treatments and ten repetitions. The distribution of treatments was as follows: T1=diet with 0% DOR (control), T2=diet with 15% DOR, and T3=diet with 30% DOR. The variables evaluated were daily weight gain, dry matter intake, feed conversion, ruminal pH, and microbiological analysis.
Results: There were no significant differences ($p>0.05$) in the productive variables due to the inclusion of DOR in the diet. Similarly, the concentration of ruminal microorganisms did not present significant differences between treatments.
Study limitations/implications: The study did not include an economic analysis that would demonstrate a reduction in production costs by decreasing the inclusion of maize in the diet.
Conclusions: Maize grain is one of the most commonly used cereals as an energy source in sheep feeding; however, it can be replaced by DOR up to 30% without affecting the productive and ruminal microbiological variables in fattening sheep.

Keywords: by-product, bacterium, citric, ovine

INTRODUCTION

In Mexico, the sheep inventory totals 8,766,678 heads, which are developed in both extensive and intensive systems (SIAP, 2023). In the latter, sheep are generally fattened

with grain-based diets (Nuncio-Ochoa *et al*., 2001). In recent years, the price of grains has increased, resulting in higher production costs. Consequently, research needs to focus on finding new alternatives that partially replace the energy sources in diets for sheep feeding.

There is a wide variety of agro-industrial by-products used in ruminant feeding, such as maize cob and pine sawdust (Guerra-Medina *et al*., 2014; Pérez-Sato *et al*., 2020), bakery waste (Escorza-Montoya *et al*., 2019), and agave bagasse (Guerra-Medina *et al*., 2015). These by-products exhibit significant variability in their chemical composition, which is why it is necessary to know them before their incorporation into diets. There is a wide variety of agro-industrial by-products used in ruminant feeding, such as maize cob and pine sawdust (Guerra-Medina *et al*., 2014; Pérez-Sato *et al*., 2020), bakery waste (Escorza-Montoya *et al*., 2019), and agave bagasse (Guerra-Medina *et al*., 2015). These by-products exhibit significant variability in their chemical composition, which is the reason why a thorough understanding before their incorporation into diets is needed. Orange residue (*Citrus sinensis* L.) is a by-product of the juice industry composed mainly of peel, pulp, seeds, and a small portion of juice (Espinoza-Zamora *et al*., 2019; Rincón *et al*., 2005). Its chemical composition includes crude protein (10%), crude fiber (14.92%), ether extract (3.20%), nitrogen-free extract (64.51%), and ash (3.30%). Additionally, it serves as a suitable source of degradable carbohydrate fractions as an energy source (2.67 Mcal of ME), making it an excellent alternative in ruminant feeding (Bampidis & Robinson, 2006; Cabrera-Núñez *et al*., 2020; Calsamiglia *et al*., 2016).

Given that orange residue offers an alternative as an energy source in ruminant feeding, it is important to study whether productive and microbiological variables are unaffected by its inclusion. Therefore, the objective was to evaluate the productive and microbiological behavior of sheep fed with two levels of dehydrated orange residue, partially substituting maize grain (*Zea mays* L.) as an energy source.

MATERIALS AND METHODS

Location of the experiment

The experiment was conducted at the sheep module facilities of the Faculty of Agricultural and Livestock Sciences of the Benemérita Universidad Autónoma de Puebla, Mexico (19° 83' 84.08" N and 97° 48' 55.81 O), at an altitude of 1330 meters above sea level (INEGI, 2017).

Diets and Experimental Treatments

The diets (Table 1) were formulated for male sheep with an average weight of 22±1.0 kg and an expected daily weight gain of 250 g d^{-1}, according to the nutritional requirements for sheep (NRC, 2007). They provided 15% crude protein and 2900 Mcal ME kg^{-1} dry matter (DM) of metabolizable energy.

The experimental treatments were as follows:

- T1 = diet with 0% dehydrated orange residue (DOR) (Control)
- T2 = diet with 15% DOR
- T3 = diet with 30% DOR

Table 1. Ingredients and composition of the experimental diets (%).

Ingredients \ Variable	Treatments		
	T1	**T2**	**T3**
Soybean meal	17.0	17.0	17.0
Urea	1.0	1.0	1.0
Crushed corn	59.0	44.0	29.0
Minerals	1.0	1.0	1.0
Molasses	5.5	5.5	5.5
Dehydrated orange residue	0.0	15.0	30.0
Alfalfa hay	15.0	15.0	15.0
Calcium carbonate	1.5	1.5	1.5

Animals, Housing, and Feeding

Thirty sheep (Dorper×Kathahdin crossbreeds) were used, with 10 animals randomly assigned to each treatment group and housed in individual pens measuring 1.2 m^2. Feed was provided ad libitum in the morning (09:00) and afternoon (16:00 h) for 60 days. A 10-day adaptation period was given, during which the animals were also dewormed and given vitamin supplements (Pérez-Sato *et al.*, 2020).

Evaluated Variables

Dry Matter Intake (DMI)

To calculate DMI, the food offered to the 30 sheep was weighed daily, and 24 hours later, the refused feed was weighed using a portable digital balance with a capacity of 3200 g and a sensitivity of 0.2 g. DMI was calculated by the difference in weight between the offered and refused food weights, divided by the 60 days of the experimental phase, and reported in g DM day^{-1} (Pérez-Sato *et al.*, 2020).

Daily Weight Gain (DWG)

Each of the 30 sheep was weighed in the morning (08:00) using a hanging digital scale with a weighing capacity of 300 kg and weighing precision of 100 g, before offering feed, both at the beginning and end of the evaluation period. Daily Weight Gain (DWG) was calculated as the difference between the final and the initial weight, reported in g animal d^{-1} (Pérez-Sato *et al.*, 2020).

Feed Conversion Ratio (FCR)

The feed conversion ratio was calculated by dividing the Dry Matter Intake (DMI) by the Daily Weight Gain (DWG) individually for each of the 30 sheep (Pérez-Sato *et al.*, 2020).

Ruminal pH

Approximately 100 mL of ruminal fluid was collected using an esophageal probe 4 hours after feeding, at days 30 and 60 of the experiment. The ruminal fluid was filtered, and pH was measured using a portable pH meter (ORION, model SA 210, USA) calibrated at two points with pH buffer solutions (4.0 and 7.0, respectively) (Ley-de Coss *et al.*, 2016).

Microbiological Analysis

The concentration of total bacteria (TB), cellulolytic bacteria (CB), and protozoa was determined at two time points (30 and 60 days after the start of the experiment). The TB concentration was determined by direct counting using a Petroff-Hausser counting chamber (Hauser Scientific, USA) on a LAUKA-HKS12 contrast microscope (total magnification 100X). The total concentration of bacteria per mL of ruminal fluid was calculated as the product of the mean number of cells counted in a volume of $0.05\times0.5\times0.2$ mm by 2×10^7 (Ley-de Coss *et al.*, 2016). The CB concentration was estimated using the most probable number (MPN) technique (Harrigan and McCance, 1966) after incubating rumen fluid in 13×100 mm culture tubes (in triplicate) containing anaerobic liquid medium prepared according to Hungate (1969) and Cobos *et al.* (2002). Positive growth was indicated by the degradation of Whatman No. 541 paper after 10 days of incubation at 38.5 °C. The concentration of protozoa per milliliter of ruminal fluid was determined using a Neubauer chamber (bright line, Marienfeld, USA).

Experimental Design and Statistical Analysis

A completely randomized design with three treatments and ten replications per treatment was employed. The results were analyzed using the general lineal model (GLM) procedure (SAS, 2006), and treatment means were compared using Tukey's test ($p\leq0.05$).

RESULTS AND DISCUSSION

The diets were formulated aiming for a daily weight gain (DWG) of 250-300 g day^{-1} according to the nutritional requirements for intact male sheep (NRC, 2000). It can be observed from Table 2 that the DWG in the current experiment did not show significant differences and fell within the expected range regardless of the level of inclusion of dehydrated orange residue (DOR). This suggests that up to 30% DOR can be used in fattening sheep diets without significantly affecting DWG, which was also reflected in the final weight.

Lower results (238.4 and 209.2 g) were found when including 15% and 30% of fresh orange residue (FOR) in sheep diets, replacing sorghum grain (Villanueva *et al.*, 2013). This difference is likely due to higher moisture content in FOR, as increasing its quantity decreases dry matter intake, thereby affecting DWG. Similar findings were reported by Luzardo *et al.*, 2021, who noted that increasing fresh citrus pulp from 0% to 30% reduced dry matter intake but not DWG (1.580 and 1.480 kg day^{-1}). Authors such as Sharif *et al.* (2018) found no differences when including 10%, 20%, 30%, and 40% of dehydrated citrus pulp in sheep diets, concluding that up to 40% utilization is feasible without impacting productive parameters. Likewise, different inclusions (10, 20 and 30%) of dehydrated orange peel have been used in diets for goats, which increases milk fat percentage (Hernández-Meléndez *et al.*, 2015).

Regarding dry matter intake (DMI), no significant differences ($p>0.05$) were observed for any of the inclusions (15% and 30%) compared to the control treatment (0%). This indicates that the use of this by-product in sheep diets is advisable without affecting DMI. There are studies where by-products from the timber industry (such as pine sawdust) have been used as unconventional fiber sources in sheep diets, reporting a decrease in DMI, possibly due to the presence of turpentine, which is part of the resin and has a pungent taste (Pérez-Sato *et al.*, 2020).

In contrast, residues from the juice industry contain 82% total digestible nutrients and 6.7% crude protein (NRC, 2000). Additionally, they are highly palatable, which is why they are used as a source of energy to replace cereals in the ruminant feeding (Al Khawajah, 2003; Crickenberger, 1991).

Authors such as Sharif *et al.* (2018) report a DMI of 1.39 and 1.41 kg day^{-1} with the inclusion of 30% and 40%, respectively, of dehydrated citrus pulp in sheep, which are similar to the DMI observed in the present study. Similarly, Taniguchi *et al.* (1999) mentioned that they did not observe significant differences in feed intake when steers were fed diets containing different levels of dehydrated citrus pulp. Different results were obtained when 15%, 20%, 25%, and 30% fresh orange residue were included in lamb diets, where a significant decrease ($p<0.05$) in DMI was observed as the inclusion percentages increased (1.35, 1.22, 1.07, and 0.97 kg day^{-1}). These differences are again attributed to the high moisture content of the fresh residue, which limits DMI (Villanueva *et al.*, 2013).

Feed conversion ratio (FCR) (Table 2) was not significantly affected (4.60, 5.59, and 5.97) as the inclusion percentage of DOR increased (0%, 15%, and 30% for T1, T2, and T3 respectively). These results differ from those reported by Villanueva *et al.* (2013), who found significant differences in FCR (5.20, 4.80, 4.30, and 3.80) in lambs as the inclusion of fresh orange residue (FOR) increased (15%, 20%, 25%, and 30%) as a replacement for sorghum in the diet. A similar behavior was observed by Luzardo *et al.* (2021), who recorded a significant improvement in FCR (7.51, 7.37, 6.98) when increasing the inclusion of fresh citrus pulp from 0% to 30% in the diet of fattening steers. This is attributed to the fact that FOR contains fractions of highly degradable soluble carbohydrates in the rumen, which facilitates their utilization by ruminal bacteria (Espinoza-Zamora *et al.*, 2019).

Table 2. Productive variables of sheep fed with dehydrated orange residue.

Variable	Treatments			
	T1	**T2**	**T3**	**SEM**
Initial weight (kg)	23.25a†	23.25a	22.50a	3.96
Final weight (kg)	40.26a	37.96a	38.06a	2.64
Average daily gain (g d^{-1})	283.33a	245.17a	250.00a	34.92
Daily dry matter intake (g d^{-1})	1.50a	1.26a	1.41a	0.21
Feed to gain ratio	4.60a	5.59a	5.97a	0.87
Ruminal pH	6.43a	6.35a	6.12a	0.35

†=means with the same letters within each column do not differ statistically (Tukey, $p\leq0.05$). T1=diet with 15% dehydrated orange residue (DOR), T2=diet with 30% DOR, T3=diet with 0% DOR (Control), and SEM=standard error of the mean.

The consumption of easily fermentable carbohydrates increases lactic acid production, thereby decreasing ruminal pH value. However, in the present experiment, no significant differences in pH were observed across treatments (Table 2). Luzardo *et al.*, 2021 mention that inclusions of 30% of fresh citrus pulp in diets of fattening steers do not affect rumen pH (6.8) Similarly, Cruz *et al.* (2019) reported that pH remained unaffected (6.8±0.12) when dairy cows were provided with 2 kg animal^{-1} of orange peel silage.

The pH is a determinant in the fluctuations and concentrations of microorganisms in the rumen, as a low pH affects these concentrations. Since no significant differences in pH were observed with the inclusion of dehydrated orange residue (Table 2), the concentration of total and cellulolytic bacteria was not affected in the present experiment and falls within the range reported in the literature (107 to 1010 cells per milliliter of ruminal fluid) (Table 3). Additionally, the fiber present in this type of by-product is highly digestible, promoting favorable conditions for the activity of ruminal microorganisms, increasing acetic acid production, and causing moderate changes in ruminal pH value (Belibasakis and Tsirgogianni, 1996; Villarreal *et al.*, 2006). Similarly, the concentration of protozoa showed no significant differences and remained within normal concentrations.

Table 3. Concentration of total bacteria, cellulolytic bacteria, and protozoa per mL of ruminal fluid.

Period*	T1	T2	T3
Total bacteria (10^{10} bacteria mL^{-1})			
1	10.2	11.3	13.3
2	15.1	14.4	16.8
Cellulolytic (10^{7} bacteria mL^{-1})			
1	4.27	3.3	4.3
2	5.2	5.2	5.3
Protozoa (10^{4} protozoa mL^{-1})			
1	3.58	2.38	2.39
2	5.2	4.2	3.3

CONCLUSIONS

Maize grain is one of the most used cereals as an energy source in sheep feeding. However, it can be replaced by DOR up to 30% without affecting the productive and ruminal microbiological variables in fattening sheep.

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Colegio de Postgraduados

Physicochemical characterization of exoskeleton-based shrimp waste meal obtained by dehydration processes

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ABSTRACT
Objective: To characterize the physicochemical and nutritional compounds of meal made from shrimp (*Farfantepenaeus aztecus* L.) exoskeleton, obtained through hot air dehydration processes, in order to value new products and applications, according to their nutritional components.
Design/Methodology/Approach: Shrimp producers from Alvarado, Veracruz, were interviewed using a quantitative approach with a descriptive scope. Likewise, shrimp (*Farfantepenaeus aztecus* L.) shells were dehydrated and powdered to transform them into meal. The proximate chemical composition was analyzed to determine its nutritional composition and its macro and micronutrients, as well as to identify the deficiencies or excesses of certain nutrients. The general public and local ranchers were surveyed and interviewed to establish the opinions, preferences, and behaviors of consumers regarding meal by-products, in order to evaluate their market acceptance.
Results: In Alvarado, Veracruz, 2.5 tons of shrimp shell are wasted. The meal obtained from these shells has a high protein (45.40%) and mineral content, which includes Ca (11.17%), Na (6.86%), Fe (237 mg/Kg^{-1}), and Zn (77 mg/Kg^{-1}). Therefore, this nutrient content guarantees the quality of the protein input that is acquired and the food that is provided.
Study Limitations/Implications: Access to outdated statistical information, resulting from the COVID-19 pandemic, hindered the collection of relevant data. Likewise, the lack of interest of the producers in monitoring the project and the limited participation and knowledge of the workers represented a significant challenge to the progress of the study.
Findings/Conclusions: The meal under study contained nutritional elements, making it a product with high nutritional quality that could add value to shrimp waste and that has potential applications in the food industry, in the production of fertilizers, and in the formulation of balanced feed for animals. Consequently, it would contribute to the reduction of pollution in the Alvarado lagoon system.

Keywords: Shrimp, aquaculture residue, hot air drying, nutritional value.

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INTRODUCTION

The United Nations Food and Agriculture Organization (FAO) considers Mexico as the second-best shrimp fishing and aquaculture producer in Latin America (CONAPESCA, 2024). According to the Comisión Nacional de Acuacultura y Pesca (CONAPESCA,

Image by Enric Caujapé at Pixabay

2024), the preliminary national statistical information for 2023 (obtained from the harvest notifications of the shrimp aquaculture sector) indicates that a >192,600 t production was achieved, resulting in an economic value of >$19,800 million Mexican pesos.

Shrimp holds the third place in fishing production in Mexico in terms of volume; however, its value puts it in first place, with a mean annual growth rate of 4.56% during the last 10 years. Likewise, Mexico is ranked fourth in terms of fishing species, behind the United States of America, China, and Japan (CONAPESCA, 2021).

Freshwater shrimp production reached more than 30,200 tons, equivalent to more than $2.2 billion Mexican pesos. Preliminary official statistics indicate that Sinaloa recorded more than 8,400 t of deep-sea shrimp and more than 14,300 t of shrimp caught by small boats (CONAPESCA, 2024). Regarding the use large and small boats, other states with great producing potential include: Campeche, >1,040 t and >800 t; Veracruz, >400 t and 700 t; Oaxaca, >370 t and 400 t; Nayarit, >48 and >1,900 t; and Chiapas, with >212 t and 500 t (CONAPESCA, 2024).

Sequential shrimping in the state of Veracruz takes advantage of the biological cycle of the shrimp (4-6 months). This system allows fishers to catch both juveniles in coastal lagoons (artisanal fishing) and adults in the deep-sea (industrial fishing) (IMIPAS, 2018). In particular, freshwater fishermen carry out estuary shrimping in the main lagoons of Veracruz (*e.g.*, Sistema Lagunar de Alvarado, Pueblo Viejo, La Costa, Tamiahua, and Chila) and deep-sea shrimping in the northern and southern areas of the Veracruz coast, where the predominant species is the brown shrimp (*Farfantepenaeus aztecus* L.) (IMIPAS, 2018).

Fishing and aquaculture significantly contribute to the volume of agri-food waste generated by society; therefore, communities must participate in the change towards a circular economy (Veronesi-Burch, Rigaud, Binet, and Barthélemy, 2019; CONAHCYT, 2020). Although shrimp is a widely consumed crustacean, not all its parts are edible and the head, tail, and exoskeleton (body shell) —which account for 45-60% of its total weight— are usually discarded (Pattanaik, 2020). Based on consumer demand, inadequate waste management in the shrimp industry is a problem that affects not only the Alvarado region, but also many other coastal regions around the world (FAO 2022).

Shrimp waste contains large amounts of proteins, lipids, chitin, and carotenoids, such as astaxanthin (Pattanaik, 2020). Shrimp shell meal is a by-product of this waste, which, as it is a rich source of protein and minerals, can be used as an ingredient in animal feed and, due to its content of nitrogen and other nutrients that are beneficial for plants, can be used as an organic fertilizer (Universidad Autónoma del Estado de México, 2018). Processing meal from dehydrated shrimp shells is an interesting initiative that seeks to take advantage of a resource that would otherwise be wasted. Shrimp waste (shell) is transformed into high value-added products, such as chitin, chitosan, amino, sugars, proteins, and pigments, which are used in the cosmetic, food, agricultural, and pharmaceutical industries (Salas-Ovilla, Gálvez-López, and Rosas-Quijano, 2017).

Shrimp is a source of waste for the Alvarado area, due to the high concentration fishmongers dedicated to the retail sale and national trade of shrimp pulp. Hundreds of kilos of shrimp are cooked daily and the exoskeleton is discarded; therefore, the objective of this study was to characterize the chemical and nutritional compounds of shrimp shell meal, valuing new products and applications, according to their nutritional components.

MATERIALS AND METHODS

This research used a quantitative approach with a descriptive scope. Documentary and field information sources were used to determine the amount of waste generated and the final destination of the shrimp shell, through direct interviews with the 12 shrimp producers in the Alvarado area. The waste was transformed into shrimp shell meal and its proximate chemical composition and micronutrients were analyzed. Structured surveys were applied to a sample of 600 people and 5 local ranchers were interviewed to describe and explore the opinions, preferences, and behaviors of consumers in relation to shrimp meal and its various uses. A detailed understanding was achieved, from beginning to end, of the acceptance of the product in the market. The research team proposes that this organic product that can be used as fertilizer, seasoning, or the basis for a balanced diet for livestock, therefore reusing a resource that was considered waste.

Figure 1 shows the amount of shrimp processed weekly. The results were obtained from the interviews carried out with the producers. The smallest producer processes less than 500 kg (Pesqueros de La Trocha) and the largest national distributor and seller in the entire area handles 3,000 kg of processed shrimp (Productos Majo).

The production of shrimp pulp in the municipality of Alvarado is concentrated in 12 establishments that, in average, process approximately 9,640 kg per week, which means that around 2,410 kg of shrimp exoskeletons could be used to produce meal. These quantities were recorded during the mid-season (May-June) and increase by up to 20% in the high season (November-December and March-April). However, the said supply will depend on various factors, such as availability, increased demand, and market prices.

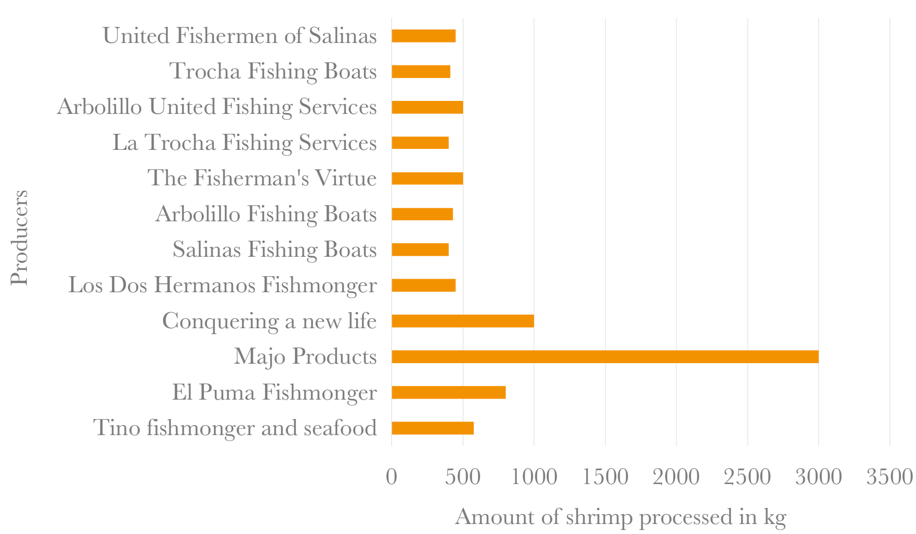

Figure 1. Kilograms of processed shrimp (cooked and peeled) per producer.

Figure 2 shows the amount of shrimp shells that each producer discards per week. The wasted volume ranges from 100 kg to more than 700 kg, which constitutes a source of contamination.

According to the data collected through the survey, 100% of shrimp producers throw all their shrimp waste into the Alvarado lagoon. They process over 9,640 kg every week, 25% of which is made of exoskeletons. Only the exoskeleton was considered for this study, because local fishmongers usually receive headless shrimps. In conclusion, more than 2.5 tons of exoskeleton are discarded into the lagoon system each week (Figure 3).

Production of shrimp shell meal

For this research, 6,500 kg of shrimp exoskeleton were collected from the same producers that had been interviewed. In order to determine the capacity of the production process and the result of the final product, the Majo fishmonger, located on Francisco I. Madero,

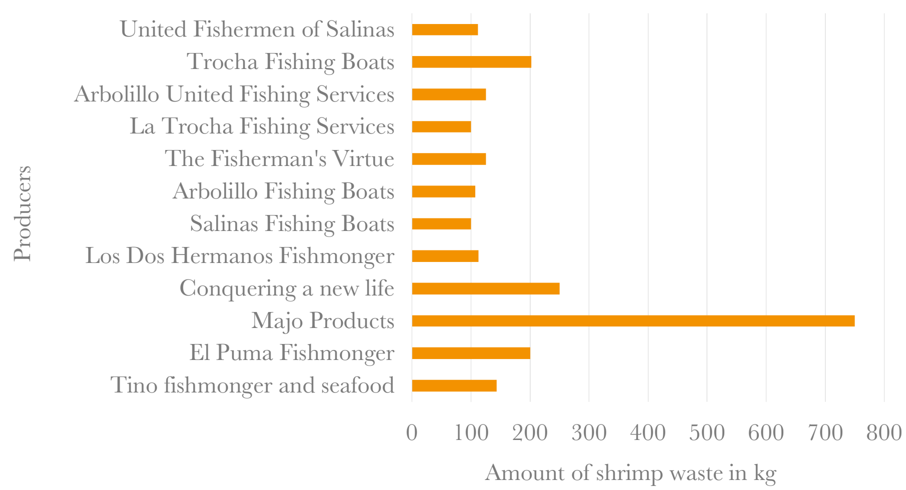

Figure 2. Kilograms of shrimp exoskeleton that producers discard per week.

Figure 3. Shrimp exoskeletons.

Colonia Centro, Alvarado, Veracruz was chosen as the supplier of the raw materials for this study. The shrimp shells were directly collected from the waste containers of the shrimp marketing companies, inspected to eliminate any residues or impurities, cooked at 90 °C for 10 minutes, and processed in a MIGSA FD-1 hot air electric dehydrator, at 65 °C for 5 hours, following a similar procedure to that described by Moncada-Guamán (2011). The abovementioned parameters were analyzed with a 2k factorial design, studying four factors with two levels, resulting in a total of 16 tests with three replications. The shells were subsequently pulverized in a Yf3-1 grain mill and sieved with a YC200 stainless steel electric vibrating sieve. Figure 4 shows the flow diagram of the production process used to obtain meal.

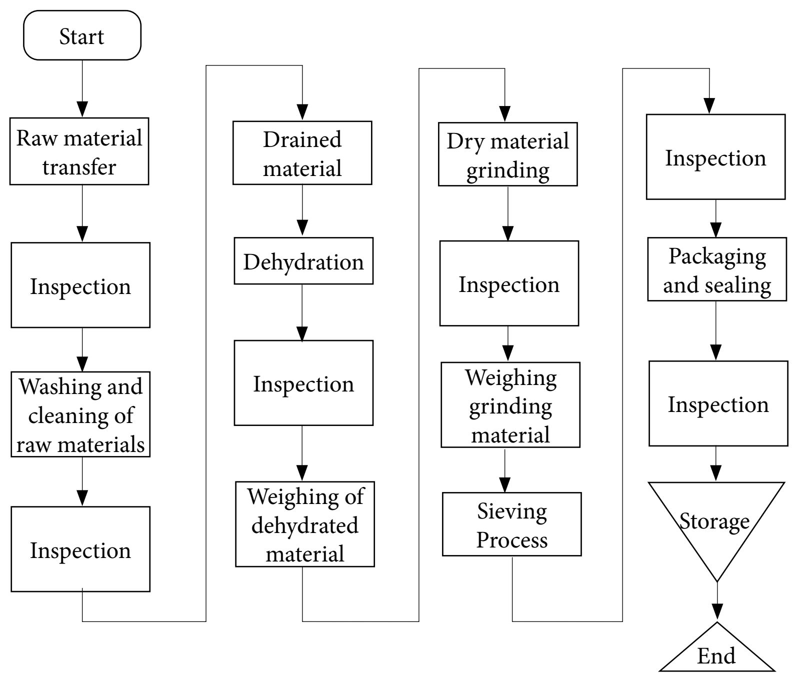

Figure 4. Operation Flow Diagram.

Figure 5. Shrimp shell meal.

Proximate chemical analysis

A proximate chemical analysis of the shrimp shell meal was carried out according to the methods developed by AOAC: AOAC 925.10 (moisture), AOAC 923.03 (ash), AOAC 920.39 (ether extract), AOAC 920.87 (proteins), and AOAC 878.10 (fiber). The purpose of this procedure was to obtain relevant information about the nutritional composition of meal, as well as its potential applications in the production of fertilizers, balanced animal feed, or in the food industry. This analysis can also help to identify possible contaminants or impurities in shrimp shell meal (AOAC, 2019).

Analysis of micronutrients in shrimp shell meal

Macro and micronutrients were analyzed to obtain the concentrations of potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), sulfur (S), boron (B), and phosphorus (P), through wet digestion with HNO_3-$HClO_4$ (Sauceda, 2013) and quantification by a plasma optical emission spectroscopy (ICP-OES) in a Perkin Elmer spectrophotometer. Micronutrient analysis can help to identify deficiencies or excesses of certain nutrients. This procedure can help to adjust the formulation of balanced feeds or to improve the nutritional quality of foods made with meal.

RESULTS AND DISCUSSION

Proximate chemical and micronutrient analysis

Table 1 shows the results of the proximate chemical analysis of shrimp meal used to determine the quality of the raw material. A 45.40% protein content was observed. Due to its cost, it is the most important nutrient in the diet in a commercial operation. An appropriate evaluation allows producers to control the quality of both the protein inputs that are acquired and the feed that is supplied. Additionally, this content could be compared with the protein values reported by Khan *et al.* (2013) and Liu *et al.* (2023), in similar material from the thoracic and abdominal exoskeleton of the shrimp, with 47.48% and 48.65% protein values, respectively.

Data represent the mean of three independent dimensions ±SD. However, according to Liu *et al.* (2021), the maximum value for the thorax and tail exoskeleton was 11.30%, and 8.81%, respectively, in heads for the species *Macrobrachium rosenbergii*. These results are similar to the findings of Wu *et al.* (2021), who recorded protein values of 10.32% for the species *Penaeus vannamei* in heads. The brown shrimp (*Farfantepenaeus aztecus* L.) raw

Table 1. Proximate chemical analysis of shrimp meal (g/100g).

	g/100g
Humidity	6.1±0.09
Protein	45.40±0.97
Fat	10.74±0.48
Ashes	5.93±0.26
Carbohydrates + fiber	31.83

material used in this research would provide better nutritional capacities, if it were included in a food formulation, because the crude protein of the by-products consists of 70% to 80% of myogenic fibronectin and 20% to 30% of sarcoplasmic protein, which increases the nutritional value of seafood protein (Halim, Yusof, and Sarbon, 2016).

Additionally, the ether extract or amount of fat was determined; the resulting percentage (10.74%) is consistent with the feeding regimes (Yi *et al.*, 2015), since the shrimp from which the exoskeleton was extracted was grown in farms. Nevertheless, this value may be subject to seasonal variations or physiological differences (Gordon and Roberts, 1977; Kinsella, 1988), as well as other physicochemical factors —*i.e.*, salinity, water temperature, and food availability (Cahú *et al.*, 2012). Likewise, this raw material had 6.1% moisture, which suggests that this meal can have a long shelf-life, depending on the type of container used for storage. Regarding the mineral content (ash), a 5.93% result was obtained, a similar percentage to that obtained by Liu *et al.* (2023) for different species, reflecting, to a certain extent, the content of inorganic compounds in biological samples, proof that the high mineral content of shrimp exoskeleton.

In another sense, the 31.83%carbohydrate content makes it the second largest compound of the meal, only after proteins. Crude shrimp fiber consists mainly of chitin, a high-molecular-weight linear polymer of N-acetyl-D-glucosamine (N acetyl-2-amino-2-deoxy-D-glucopyranose) units linked by β-D bonds ($1\rightarrow4$), which exist mainly in the shell of shrimp and are the second most abundant natural polymer on Earth after cellulose (Younes *et al.*, 2014). However, chitin —a white, hard, inelastic substance, and a nitrogenous polysaccharide— is the main source of surface pollution in coastal areas (Gupta and Ravi Kumar, 2000). As a highly nitrogenous compound, it is mainly used as a chelate, although, as a natural polymer, it has excellent properties, such as biocompatibility, biodegradability, non-toxicity, adsorption, etc. (Muzzarelli, 1973).

Therefore, this meal can be used as raw material to obtain chitin and its derivative chitosan. This biomaterial has been used to manufacture coating films that have been successfully used to preserve seafood, extending its shelf-life and improving its quality, as a consequence of its antimicrobial and antioxidant effects (Chang, Wu, and Tsai 2018; Kontominas *et al.*, 2021). This phenomemon would represent an economic advantage by adding value to a product that, under normal conditions, would be discarded.

MINERAL ANALYSIS

Table 2 shows the results of the macro and micronutrient analysis of shrimp shell meal. The shrimp exoskeleton was found to be rich in Ca, Na, Fe, and Zn. As expected, they had a considerable concentration of P (≈3.26%), because this element is one of the main

Table 2. Analysis of the macro and micronutrients of meal made from the exoskeleton of shrimps camarón (*Farfantepenaeus aztecus* L.).

N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn	B
%						mg Kg^{-1}				
6.86	3.26	0.06	11.17	0.71	0.70	13	237	17	77	9

ingredients of the structures of crustaceans. Likewise, combined with ca, P plays a major role in the formation and strengthening of the tissues of crustaceans and shellfish.

These results were contrasted with findings of Balogun and Akegbejo-Samsons (1992), who determined that *Parapenaeopsis atlántica* had a Ca>Na>P relationship, just like *Farfantepenaeus aztecus* L did in this study. In their turn, Brito *et al*. (2020) reported that Ca is the main macroelement present in shrimp meal made from heads and thorax exoskeleton.

The most abundant microelement in shrimp (*Farfantepenaeus aztecus* L.) meal was Fe (237 mg/Kg), followed by Zn and Mn with 77 mg/kg and 17 mg/kg, respectively. These results are similar to those reported by Brito *et al*. (2020) and Lee *et al*. (2017).

In general, the data about the main composition and minerals found in shrimp meal show a nutritious food biomaterial that, through a bioconversion process, can be used as the base for other materials (*e.g*., chitin and chitosan), adding value to the shrimp waste from of the Alvarado lagoon system, in Veracruz.

CONCLUSIONS

This study highlights the importance of exploiting the shrimp exoskeleton waste and of establishing a process to obtain this product, potentially contributing to a significant degree to the reduction of pollution in the Alvarado lagoon system and to the sustainable use of marine resources. The study also points out the need to fully explore the potential applications of this meal and its acceptance in the market, potentially providing an economic benefit to local producers and benefiting the environment.

From an environmental point of view, the composition of the shrimp exoskeleton is a contamination risk. Shrimp (*Farfantepenaeus aztecus* L.) meal from the Alvarado lagoon system proved to be a product of high nutritional quality, due to its quantity of nutritional elements, as well as to its amount of high-quality and low-cost protein. In addition, the reduction of humidity prevents microbial growth and a homogeneous particle size facilitates the logistics of its handling. Some by-products had a clear potential: their nutritional contribution could be used in the food area. Shrimp meal hasa high-quality protein that could be exploited in the health care and nutrition sector or as fertilizer or feed for livestock. This added value could be an economic benefit for local producers, as well as beneficial for the environment.

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Colegio de Postgraduados

Microbiological quality of marketed fish and shrimp in San Luis Mextepec in the State of Mexico, Mexico

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ABSTRACT

Objective: To analyze the common and widely marketed fishing products in the popular area of San Luis Mextepec, in the state of Mexico belonging to the Mexican Republic to determine the hazards and potential risks to health from consumption of those products.

Design/methodology/approach: Samples of fish fillet and whole shrimp were collected from available fish shops in the popular aquatic food marketing area every week, for a month. The evaluation of their microbiological quality was performed through test aerobic mesophiles, total coliforms, *Salmonella*, fungi, and yeasts.

Results: The analysis of aerobic mesophiles in fish and shrimp indicated that they did not exceed the permissible limits of the health standard, while coliforms in fish and shrimp 50% exceeded the permissible limit in 100% and 50% of the samples respectively. For fungi in fish and shrimp they presented counts that ranged between 8 and 2150 CFU/g, while the yeast values ranged between 95 and 1010 CFU/g. Finally, in the analysis of *Salmonella*, 50% of fish and shrimp samples tested positive for the presence of the pathogen, exceeding the limit established by health standards and indicating a health risk for consumers.

Limitations on study/implications: This study should be replicated at another time of the year since the type and degree of contamination in fish and shrimp can vary, influencing microbiological hazards and risk to the health of consumers.

Findings/conclusions: The microbiological analysis of marketed fish and shrimp indicated the presence of microbiological contamination that influences their quality and safety, becoming a hazard and public health risk.

Keywords: Quality, pathogens, foodborne diseases, food safety, fish, public health.

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INTRODUCTION

Fish is a food rich in vitamins, minerals, lipids (polyunsaturated) and proteins. For the above, fish is considered an important part of human nutrition, where the Food

and Agriculture Organization (FAO) has recommended its consumption twice or more times a week (de Oliveira & Amancio, 2012; Murillo *et al.*, 2023). For definition purposes, the term fish refers to fish, crustaceans, mollusks, among other members of aquatic environments intended for human consumption (de Paiva Soares & Gonçalves, 2012; de Oliveira & Amancio, 2012). As a food, fish is an important part of cultural traditions around the world, in addition to being among the most commercialized foods, and being fundamental in global and local economic aspects (Murillo *et al.*, 2023). Despite its nutritional benefits, fish tends to be very susceptible to deterioration and contamination due to autolysis, oxidation and microbiological activity, reducing its shelf life, and becoming a risk of disease for consumers (de Paiva Soares & Gonçalves, 2012). Foodborne diseases are currently an important public health problem worldwide due to the extent of their impact (de Paiva Soares & Gonçalves, 2012; Torrens *et al.*, 2015) and negative economic and social impact, being the most vulnerable populations the elderly, children and the immunocompromised, as well as the population living in high levels of poverty and unhealthiness (Torrens et al., 2015). Fish and related products have been identified as high-risk food implicated in various outbreaks around the world, where the main contaminating agents being microorganisms which are the main causes of contamination due to inadequate handling, conservation conditions and practices (Olea *et al.*, 2012; López *et al.*, 2013; Espinosa *et al.*, 2014). In Mexico, gastrointestinal diseases due to the consumption of microbiologically contaminated foods are also one of the main public health problems, with the low socioeconomic levels of the population being the most affected (Hernández *et al.*, 2011). Therefore, this study focuses on the analysis of common and widely marketed fishing products at San Luis Mextepec, in the state of Mexico, which is part of the Mexican Republic, to determine the hazards and potential risks to the health's public from consumption of these products.

MATERIALS AND METHODS

Area and sample collection

The fish samples consisted of carp (*Cyprinus carpio communis*) in fresh fillet presentation and whole frozen shrimp (*Farfantepenaeus brevirostris*) that were collected randomly from two available fish shop (A and B) in the central area of San Luis Mextepec, where the popular sale of foods of aquatic origin takes place. The samples collected consisted of 2 fillets (150 g) and 2 whole shrimp (603 g) per fish shop per week for 4 weeks between the months of June and July 2023, for a total of 32 samples.

Methodology

The samples were collected following the NOM-109-SSA1-1994 procedure and transported in aseptic and cold conditions to the laboratory of the Universidad Autónoma Metropolitana, Lerma Unit, for analysis. Once in the laboratory, the samples were subjected to the analysis of total aerobic mesophiles (AM) (NOM-092-SSA1-1994), total coliforms (TC) (NOM-113-SSA1-1994), *Salmonella* spp. (NOM-114-SSA1-1994), fungi (F) and yeasts (Y) (NOM-111-SSA1-1994). The studies were done in triplicate.

Analysis of data

The data analysis was performed using the Microsoft Excel spreadsheet software for Windows version 15 (2013).

RESULTS AND DISCUSSION

Aerobic mesophiles (AM) and total coliforms (TC)

The carp fillet samples from fish shop A, during their second week of study, obtained the highest values for AM ranging between 26,400 and 25,500 CFU/g, while for fish shop B the highest proportions were found in samples collected in the third week of the study, ranging between 22100 and 22600 CFU/g, where no one exceeded the permitted health limit. Meanwhile, for TC, all carp fillet samples exceeded the sanitary limit, with the highest proportions in samples from fish shops having values between 9300 and 18450 CFU/g in the second week of the study. For shrimp, the highest proportions of AM found were 16,400 and 14,500 CFU/g in samples from week 2 of the study from fishery B, although without exceeding the sanitary limit. Finally, for TC in shrimp, 50% of the samples exceeded the sanitary limit, being the samples from fish shop B, in week 1 (W), with the highest proportions obtained ranging from 2450 to 5300 CFU/g (Table 1).

AM are microbiological indicators of food quality, where high counts indicate contaminated raw materials or unsatisfactory processing treatments, inadequate time/

Table 1. Microbiological analysis of AM and TC in carp samples (*Cyprinus carpio communis*) in fresh fillet presentation and frozen whole shrimp (*Farfantepenaeus brevirostris*).

FS	W	C	AM CFU/g	TC CFU/g	S	AM CFU/g	TC CFU/g	MSAM CFU/g*▲	MSC CFU/g+
A	1	1	13800±282	2320±254	1	2250±353	275±35	1×10^7	1×10^3
		2	15050±777	4000±707	2	2500±282	750±70		
	2	1	26400±1979	18450±2192	1	2400±141	2375±106		
		2	25500±707	9300±989	2	2050±212	1350±70		
	3	1	21600±989	13900±1555	1	4800±282	1400±141		
		2	22150±212	9350±919	2	5450±353	1150±212		
	4	1	13950±1060	5350±919	1	1750±212	550±70		
		2	14850±212	4050±1343	2	2002±144	450±70		
B	1	1	13450±550	2550±450	1	9050±950	2450±50		
		2	13550±650	1600±400	2	7600±400	5300±200		
	2	1	20700±2700	9650±350	1	16400±600	2160±40		
		2	21000±2400	9300±700	2	14500±500	2065±35		
	3	1	22100±1100	12450±550	1	2400±200	730±20		
		2	22600±600	8800±1200	2	2800±200	665±45		
	4	1	11300±500	4200±800	1	5000±1000	675±75		
		2	11050±750	2700±500	2	6500±1500	325±25		

FS: Fish shop. W: Study week C: Carp sample. S: Shrimp sample. AM: aerobic mesophiles, TC: total coliforms, CFU: Colony-Forming Unit. MSAM: Microbiological specification for aerobic mesophiles, MSC: Microbiological specification for coliforms. *NOM-027-SSA1-1993. ▲ ICMSF. +BOE-A-1991-2073.

temperature conditions during storage. It should also be noted that the presence in a high proportion may indicate that there were favorable conditions for the multiplication of possible mesophilic pathogenic microorganisms of human or animal origin (Obregón & Zambrano, 2017). Coliforms are Gram-negative bacteria, whose presence in food can come from an environmental origin or from fecal contamination. Their presence is considered an indicator of inadequate cleaning operations, unsanitary conditions, or contamination during or after processing stages, failures in process conditions such as temperature management or critical control points (NOM-113-SSA1-1994). On the other hand, a high presence of coliforms can lead to the monitoring of pathogens. It has been pointed out that they are indicators of fecal contamination and the possible presence of viral or bacterial pathogens (Barrera-Escorcia *et al.*, 2013). In related studies Quintero *et al.* (2012) reported that through the analysis of AM in fresh fillet of the species *Mugil cephalus*, *Scomberomorus sierra* and *Coryphaena hippurus*, sold in two different points Mazatlán, Sinaloa, Mexico, presented an average quality range of 5.2 to 5.9 $\log_{10}$ CFU/g, which was within the maximum permissible sanitary limit for marketing. Morales *et al.* (2004) in a microbiological study that involved different microorganisms, including total coliforms from the skin or surface of *O. niloticus* produced in the northern area of Costa Rica intended for human consumption, reported that 74% of the samples analyzed had a proportion $\geq 10^3$ CFU/g, reflecting poor hygiene in their production, high contamination of the waters of origin and involving a risk to public health.

Fungi (F) and yeast (Y)

In carp fillet, the highest proportions of fungi were found during week 4 in samples from both fish shops A and B with values that ranged between 2100 and 2150 CFU/g; meanwhile, for Y, the highest proportions found throughout the study were in samples from fish shop B during week 1 (W), having values of 2450 and 2260 CFU/g. For shrimp, the proportion of F in samples ranged between 8 and 101 CFU/g, while for Y the values were between 125 and 1010 CFU/g throughout the study (Table 2).

The determination of fungal organisms in food allows establishing an indicator of inadequate sanitary conditions and practices in raw materials, production and storage processes, in addition to health risks due to foods contaminated by the potential presence of mycotoxins (NOM-111-SSA1-1994; Centeno & Rodríguez, 2005). Currently, official Mexican standards do not have microbiological limits for fungi and yeasts in frozen fish fillets or shrimp. In related studies Centeno & Rodríguez (2005) reported average counts of F and Y in *Scomberomorus* spp., and *Merluccius* spp., of 1.9×103 CFU/g and 2.0 x 102 CFU/g respectively, both marketed in markets Sucre, Venezuela.

Salmonella

The analysis of carp and shrimp fillet samples from fish shops A and B indicated that 50% tested positive for *Salmonella* spp., presence, exceeding the limit established by health standards in food, and indicating a risk to the health of consumers (Table 3), especially if consumed in a raw state as in typical culinary preparations of the country as the ceviche.

Table 2. Microbiological analysis of F and Y in samples of fresh fillet carp (*Cyprinus carpio communis*) and whole frozen shrimp (*Farfantepenaeus brevirostris*).

FS	W	C	F CFU/g	Y CFU/g	S	F CFU/g	Y CFU/g
A	1	1	8±3	113±17	1	95±7	225±35
		2	20±4	115±21	2	90±14	212±17
	2	1	250±70	325±35	1	93±10	450±70
		2	105±7	317±24	2	96±6	350±70
	3	1	125±35	110±14	1	15±7	235±49
		2	112±17	105±7	2	8±3	290±14
	4	1	2150±70	102±3	1	95±7	215±21
		2	2100±141	190±14	2	90±14	222±31
B	1	1	1125±25	2450±50	1	95±5	550±50
		2	725±25	2260±40	2	97±3	650±50
	2	1	225±25	190±10	1	96±5	1010±110
		2	325±25	290±10	2	95±5	230±30
	3	1	125±25	95±5	1	45±5	125±25
		2	110±10	105±5	2	9±1	240±40
	4	1	2150±50	103±3	1	101±1	325±25
		2	2125±75	190±10	2	95±5	315±15

FS: Fish shop. W: Study week. C: Carp sample. S: Shrimp sample. F: Fungi, Y: Yeasts, CFU: Colony-Forming Unit.

Table 3. Microbiological analysis of *Salmonella* spp., in samples of carp (*Cyprinus carpio communis*) in fresh fillet presentation and whole frozen shrimp (*Farfantepenaeus brevirostris*).

FS	W	C	*Salmonella* spp. Presence/ Absence in 25g.	S	*Salmonella* spp. Presence/ Absence in 25g.	Microbiological specification for fish and products *△ ▲ δ
A	1	1	Absence	1	Absence	Absence in 25g.
		2	Absence	2	Absence	
	2	1	Absence	1	Presence	
		2	Absence	2	Presence	
	3	1	Presence	1	Presence	
		2	Presence	2	Presence	
	4	1	Presence	1	Presence	
		2	Presence	2	Presence	
B	1	1	Absence	1	Presence	
		2	Absence	2	Presence	
	2	1	Absence	1	Absence	
		2	Absence	2	Absence	
	3	1	Presence	1	Absence	
		2	Presence	2	Absence	
	4	1	Presence	1	Absence	
		2	Presence	2	Absence	

FS: Fish shop. W: Study week. C: Carp sample. S: Shrimp sample. *NOM-027-SSA1-1993, △ NOM-242-SSA1-2009, ▲ NTE-INEN 183-2013, δ'BOE-A-1991-20734.

Salmonella spp., is a bacteria considered one of the zoonotic agents responsible for numerous cases of foodborne illnesses, including fish, around the world (Arias & Buelga, 2005; Espinosa *et al*., 2014), mainly from waters contaminated with fecal matter and associated with inadequate hygiene conditions and practices during the processing, conservation, or post-capture handling of fish (Arias & Buelga, 2005). In this study, the variation in the qualitative and quantitative microbiological analysis found in fish and shrimp may be associated with changes in hygienic handling conditions, post-capture product temperature control and marketing in sampling fisheries during the study period. In a related study Cedeño *et al*. (2021) reported that the marketed tuna (*Thunnus alalunga*), sold at the municipal market of Chone, Ecuador, had a presence of *Salmonella* of 100% in the samples analyzed, considering them as risk to the health of consumers.

CONCLUSIONS

The evaluation of fish and shrimp sold in the area indicated the presence of microbiological contamination that may influence their quality and safety, becoming a hazard and risk to the health of consumers. In the microbiological analysis, the proportion of AM in 100% of the samples was below the limit of the health standard. For TC in fish, 100% of the samples and 50% of the shrimp samples were above the permissible sanitary limit, while *Salmonella* spp., was found in 50% of the fish and shrimp samples, exceeding the permissible sanitary limit and being considered a risk to the health of consumers. As a recommendation, it is essential to reinforce training in the hygienic handling of fish in post-capture and marketing conditions to reduce the microbial load and risk of food-borne diseases.

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Colegio de Postgraduados

Horses an important specie in charreria, its management and welfare

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ABSTRACT
Objective: Describe the management practices provided to charrería horses in the state of Guerrero, Mexico and relate them to animal welfare.
Design/methodology/approach: Sixty-four owners of charrería horses were interviewed and 10% underwent an evaluation according to the protocol of the Welfare Quality®
Results: The most used breeds are Creole, Quarter Horse, Aztec, Spanish and Arabian. The starting age in charrería is two to four years. Hoof shoeing is done every 8 to 12 weeks in 54.3%. The diet is based on forage and commercial feed (82.8%). Deworming is every six months (60.9%), vaccinated against tetanus, rabies, and influenza. The most frequent diseases are digestive and locomotor, skin and respiratory. The stereotypes were rocking, nodding, kicking doors, chewing wood, and walking in circles. Abundant body condition ranged from good to obese in the animals. The majority had white hairs as an indication of injuries caused by the harnesses.
Limitations on study/implications: The lack of knowledge of the owners of horses dedicated to charro sport, to relate the five freedoms of well-being that must be given to every animal to improve their sporting performance, has caused problems in the animal's life quality.
Findings/conclusions: The findings observed in the horses indicate that the welfare conditions were affected, which led to the development of vices, poor body condition, sick animals, and skin lesions in the animals.

Keywords: Horses, management, sport, behaviors, well-being.

INTRODUCTION

Mexico has a large population of equines worldwide, according to the Food and Agriculture Organization of the United Nations (FAO) (2021), it ranks first in horse

population with 12,955,040. specimens, fifth place in donkey population (3,284,347 specimens), and first place in mules with 3,287,994 specimens; These animals are used for various activities such as agricultural work and daily tasks, or as sport and recreation. Since Spanish colonization, the equid has played an important role in the history and culture of Mexico (Vázquez *et al.*, 2017). The need for these animals for transportation, loading, drafting, and handling of livestock has been disappearing in more developed places due to the introduction of new technologies. However, among people with a cultural heritage linked to horses, it continued as a means of coexistence through equestrian competitions, including charrería (Mota *et al.*, 2016). Charrería is a representative practice of Mexico, traditional due to its history and culture; dedicated to managing livestock with the help of horses (UNESCO, 2016). Unlike other sporting activities, it takes two individuals working together to be successful in the activity to be carried out, which is why the human-horse relationship is a fundamental aspect. However, in this interaction, human beings consciously or unconsciously neglect the basic principles of well-being that their animals must enjoy, to be free from suffering (Whay *et al.*, 2015). The World Organization for Animal Health (OIE) considers that an animal is in a state of well-being when it is free of disease, comfortable and well fed, can express normal behavior, and does not suffer pain, fear, or stress (Sanmartin *et al.*, 2015). Recently, welfare assessment models have been adapted for equids in order to measure and manage their quality of life; these can be used in working animals and in equestrian sports disciplines (Sommerville *et al.*, 2018). In the state of Guerrero, many families use horses for work, transportation, fun and even for sport. Despite this, there are no studies that objectively describe the welfare conditions of these animals due to the management they receive. For this reason, the objective of the study was to describe the management practices given to charrería equids in the state of Guerrero and determine their relationship with animal welfare.

MATERIALS AND METODS

Description of study area

The study was carried out in the state of Guerrero, which is located in the south of the Mexican Republic in the tropical zone, between 16° 18' and 18° 48' north latitude and 98° 03' and 102° 12' west longitude. It borders to the north with the states of Mexico, Morelos, Puebla and Michoacán; to the south, with the Pacific Ocean; to the east with Puebla and Oaxaca; and to the west with Michoacán and the Pacific.

Study design and analysis

The sample size was 384 surveys determined by the equation described by Rojas (2013) for studies in large populations:

$$n = \frac{Z^2 p.q}{E^2}$$

where, Z (confidence level) of 95%, p and q (variability of the phenomenon to be studied) of 50% and E (precision level) of 5%.

The sample was distributed into subsamples (n=64) by strata formed by the activity carried out by the horses in the state of Guerrero (1. Agricultural work, 2. Charrería, 3. Dancing, 4. Racing, 5. Walking, 6 Tourist work). To describe zootechnical management and its relationship with animal welfare, 64 owners of charrería equids were surveyed. For the interviews, a survey was used with closed questions on general aspects, basic management, feeding, housing and sanitary management of the animals. In addition, 10% of the surveyed population underwent an evaluation in accordance with the Welfare Quality® protocol for evaluating well-being in equines. The evaluation consisted of a physical or direct inspection of the animals and an indirect inspection, through the animals' environment; considering the four principles of well-being (Good Food, Good Housing, Good Health, and Good Behavior) based on the five freedoms (1. Free from thirst and hunger, 2. Free from discomfort, 3. Free from pain, injury, or illness, 4. Free to express normal behavior and 5. Free from fear and distress). The data of the variables included in the survey were analyzed by descriptive statistics for the preparation of tables and figures in the Microsoft Word Excel program.

RESULTS AND DISCUSSION

Of a total of 159 equids raised by 64 owners, the equid used for the equestrian activity of charrería is the horse (*Equus caballus*). The preferred breeds for the charro competition and festival are the Creole (horse native to the region that has evolved by natural selection), quarter horse, Aztec, Spanish and Arabian (Table 1). The exclusive identification of horses (*E. caballus*) as the only species used for charrería is attributed to the fact that although mules (*E. mulus*) are animals with adequate strength and resistance to carry out this type of activities, the General Official Regulations for Charro Competitions 2018-2020 of the Mexican Charrería Federation (2019), specifies that only the use of the horse is allowed for these competitions. Regarding the breeds used, the results coincide with those reported by Vázquez *et al.* (2017) who mentioned that the Creole horse is the predominant breed in Mexico due to its importance in agriculture and the Mexican equestrian tradition, although there is also a great variety of horses of various breeds such as Aztec, quarter mile, Spain thoroughbred, Lusitanians and Arabs, among others.

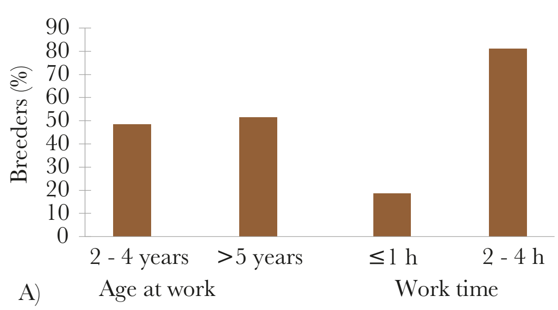
All horses are handled during competition and/or training with leather harnesses, mandatory material for the Mexican Charrería Federation (2019); However, according to the OIE Terrestrial Animal Health Code (2018), the harnesses used for the management

Table 1. Preferred breeds for Charrería activities in the state of Guerrero.

Breeds	Owner (n=64)	
	Number	%
Creole	32	50
Aztec	21	32.8
Mile quarter	24	37.5
Spain	3	4.6
Arabs	1	1.5

of equids must be of an adequate size and material to provide comfort during work without risks of pain or injury; Therefore, it is not a guarantee that they are free of discomfort or injuries due to poor adjustment or use of them. The age at which equids begin the charrería activity is early (2 to 4 years) in 48.4% of the animals, with long workdays between two-to-four-day hours in 81.2% of the horses, and only the 18.7% of owners work their animals between 30 minutes and 1 hour (Figure 1A). The recommended age to start working a horse is after five years of age, which is when the bone growth plates have completely closed, and the risk of development problems and future locomotor alterations decreases (Baxter, 2011). Furthermore, Dixon (2002) complements that at that age the horse's teeth are complete, and the use of bits and bits does not cause discomfort or injuries to the oral cavity. The hours that horses dedicate to their activity differ from what was reported by Mariscal and Córdoba (2015) in draft horses, in which the animals worked 6 to 8 hours a day; however, Mota *et al.* (2016) mentioned that during the charra lucks, the horses manifested physiological changes that altered respiratory exchange, acid-base, mineral and energy balance; as well as increased blood lactate and hematocrit levels, causing muscle fatigue and dehydration.

It is advisable to trim, and shoe hoofs every 4 to 6 weeks, because anatomically the hoof has an approximate growth rate of 1 cm per month (Obregón and Ramos 2011). In our study, most owners (54.3%) do not carry out this activity with the appropriate frequency, they do it every 8, 10 and after every 12 weeks; only 45.3% do what is recommended by these authors (Figure 1B). It is important to consider that equids that are shod with a longer interval may present locomotor alterations by not having natural wear due to impediment of the horseshoe, as well as excessive wear by not having it (Schade *et al.*, 2013). This implies that owners must periodically attend to the structure and integrity of their animals' hooves because excessive wear or growth can affect the balance and/or normal locomotion of the equids. All horses destined for charro sport were housed in stables, and of these, 45.3% are housed with manure bedding produced by the animals themselves, and to a lesser extent earth, shavings, and sand bedding are used, respectively (Figure 2A). However, the bedding material preferred by owners is not recommended because it becomes a dusty material that can cause respiratory conditions, in addition to providing a suitable environment for pathogens; On the other hand, straw is the ideal material, since it keeps the horse busy preventing unwanted behaviors (Kwiatkowska *et al.*, 2016). According to Ruet *et al.*



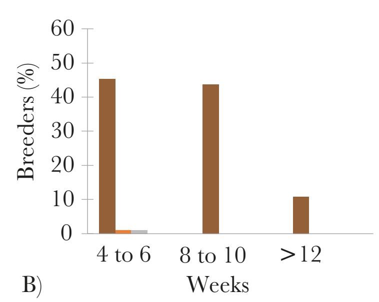


Figure 1. Start of charro work, time dedicated to equestrian activity (A) and frequency of hoof trimming and shoeing (B) of Charreria horses.

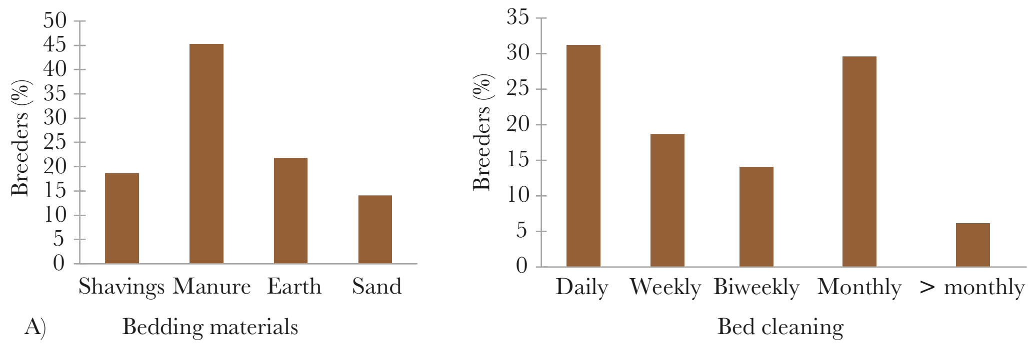


Figure 2. Materials and cleaning frequency of Charrería equid beds.

(2019) any bedding material is suitable if it provides a hygienic condition and a stable microclimate so that equids have a comfortable rest. In this study, a good proportion of horses receive bedding cleaning daily (31.2%), and about 62.4% receive cleaning between one to four weeks, and only 6.2% receive cleaning after one month, which can extend up to two months (Figure 2B).

Approximately 32.8% of the horses used for charra activity are raised in isolation, avoiding coexistence and interaction with other equids of this or a different species and a smaller proportion, 20.3% of the horses, are even isolated from contact with other animal species (bovines, sheep, goats among others) (Figure 3A). Having social contact with other equids is ideal because living together gives security to each individual and promotes freedom to express the behavior of their species (Zuluaga *et al.*, 2018). On the other hand, McBane (2008) and Robledo-Reyes *et al.* (2019) described that the interaction with animals of different species as happens in the study at least allows individuals to focus on their surroundings, although it limits the expression of their natural behavior.

In nutritional management, the majority of charrería horses are fed forage and commercial feed (82.8%), and in a smaller proportion (14.06%) they are fed with forage and homemade waste and only 3.1% receive only forage (Figure 3B); The diet was received by the horses in two portions and three portions daily in 60.9 and 37.5% respectively and in up to four portions in 1.5% of the animals (Figure 3C). Access to water was free in 96.8% of the horses and in 3.1% it was rationed in three doses during the day, in 21.8% of the cases the water was supplied with automatic drinkers and in the rest of the animals it was supplied in traditional drinkers (Figure 3D). The diet was at the discretion of the owners without considering age, physiological state, body weight, zootechnical activity, as recommended by the NRC (2007) for the equine species, which could cause deficiencies or excesses that affect the health and welfare of the animals. The feeding frequency was inadequate in most cases, considering that the food is kept in the stomach for a period of approximately 2 to 6 hours, stimulating the amount of saliva necessary to maintain the pH balance to protect the stomach from heartburn caused by the continuous secretion of hydrochloric acid (Merrit and Julliand, 2013; Weyenberg *et al.*, 2006). Luthersson and Nadeau (2013) reported that horses in confinement tended to spend more time without eating food, which decreased protection against gastric acidity and increased the risk of developing various

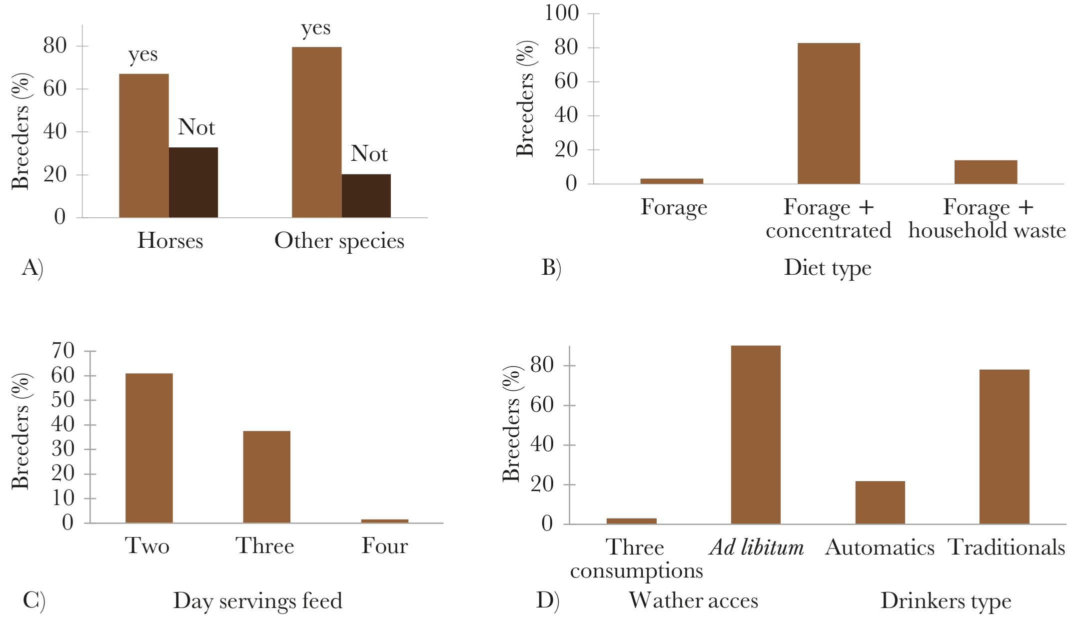


Figure 3. Social interaction (A), type and frequency of feeding (B and C) and access to drinking water (D) of charrería sport horses.

gastrointestinal problems; Proper management for these animals is to allow them free access to forage to produce a continuous flow of saliva and keep the stomach protected most of the day. Nyman and Dahlborn (2001) reported that horses prefer to consume water in a traditional bucket than in an automatic waterer, in addition to the fact that in these the daily consumption is unknown and water intake may decrease, causing serious clinical disorders.

In preventive medicine, the majority of charrería horses (60.9%) were internally dewormed every six months and less frequently every year and quarterly, but it is important to emphasize that a small proportion 3.1% of owners do not deworm their horses (Table 2). Kusmina *et al.* (2016) recommend deworming 3 to 4 times per year, as they reported a significant reduction in parasites compared to a deworming frequency of 2 or fewer times per year; However, it is worth mentioning that parasite control is due to many management factors. Furthermore, parasites reduce the productive efficiency and the activity carried out by animals, which is why deworming is of great importance as preventive medicine; However, inadequate management can increase anthelmintic resistance levels (Nielsen, 2012). Very few owners (17.1%) implemented vaccination against viral and/or bacterial diseases such as tetanus, rabies, and influenza (Table 2) and less than half request veterinary attention when their become ill animals (48.4%). These results differ from those reported by Márquez *et al.* (2010) where all owners provided veterinary care to their horses and carried out an adequate vaccination and deworming program.

The diseases that frequently affected horses were of the digestive and locomotor systems, followed by the integumentary, respiratory, eye and genitourinary systems (Table 3), attributed to poor nutritional and health management, as well as to the impacts and locomotor contractions caused by this equestrian activity. These data were similar to those

Table 2. Vaccination implemented by owners of charrería equids.

Variables	Owner (n= 64)	
	Number	%
Deworming		
Every 3 months	6	9.3
Every 6 months	39	60.9
Annual	17	26.5
Not deworming	2	3.1
Veterinary services	31	48.4
Vaccines		
Tetanus	11	17.1
Rabies	3	4.6
Influenza	2	3.1
Encephalitis	0	0
Rhinopneumonitis	0	0

Table 3. Most frequent conditions in charrería horses.

System	Conditions	Owner (n = 64)	
		Number	%
a) Diseases			
• Digestive	Abdominal pain, Diarrhea, Mouth ulcers, dental anomalies	59	92.1
• Locomotor	Limping, Deformities	59	92.1
• Integumentary	Wounds, Inflammations, Scabs, Pruritus	53	82.8
• Respiratory	Runny nose, Cough	50	78.1
• Eye	Wounds, Discharge, Ulcers	29	45.3
• Genitourinary	Abortions, placental retention, anestrus, dysuria	7	10.9
b) Annual period with a higher incidence of sick animals			
• Spring-Summer		32	50.0
• Autumn-Winter		23	35.9
• Occasional		9	14.1
c) Stereotypies in charreria horses			
• Balanced		14	21.8
• Head not		11	17.18
• Kick doors		10	15.6
• Wood chewing		9	14.06
• Circles walk		7	10.9

reported by Regan *et al.* (2105) who identified locomotor and respiratory conditions in working equids and differ from what was reported by Marquez *et al.* (2010) who presented skin conditions as the main problem due to improper use of harnesses. The year period with the highest incidence of disease animals occurs in autumn-winter (50%), and the lowest in spring-summer (14.06%), 35.9% of the owners answered that their animals get sick at any time of the year (Table 3). Nelson (2004) mentions that during winter low temperatures stress animals, increasing their susceptibility to getting sick, and the lack of feed availability with the necessary nutrients decreases the immune function of the organism, because the immune system needs energy to deal with pathogens; likewise, short days physiologically affect several factors of the immune system.

The most frequently presented stereotypies in the study were rocking, followed by nodding the head, kicking doors, chewing wood, and circle walking (Table 3). Which can be attributed to several factors, Lesimple *et al.* (2019) related the abnormal behavior of horses to restricted housing space and poor social contact according to their needs as a species. Likewise, the same authors reported that the stereotypies observed were a consequence of the type of restriction that the horse experienced.

Physical inspection of animals

On a scale from 0 (very thin) to 5 (Very obese), the body condition (BC) of the horses evaluated was good (3/5) in 33.3% of the animals, obese (4/5) in 33.3 %, moderate (2/5) in 16.6% and Poor (1/5) in 16.6%, with a shiny (66.6%) and matte coat condition (33.3%) (Figure 4A). These results can be attributed to the nutritional management provided by the owners without considering what is recommended by the NRC (2007) according to the activity carried out by the horses. In the case of obese equids (CC4/5), excess consumption of digestible energy above what is required produces excessive accumulation of adipose tissue. In contrast, poor body condition is due to the loss of adipose and muscle mass due to energy consumption below the animals' requirements (Carter and Dugdale, 2013). 16.6% with poor body condition (1/5) had abnormal wear of incisors, which according to Welfare Quality® (2011) this wear will affect food consumption.

In the housing, the living space of the equids was adequate in 66.6% and inadequate in 33.3% of the cases (Figure 4B). McBane (2008) considered that an accommodation must have a living space of at least 4 m^2, thereby providing ease of movement and comfort of rest. Dalla *et al.* (2016) mentioned that very small accommodation considerably decreased the horse's ability to move freely, affected comfortable rest and social contact with other individuals. In addition, they acquired abnormal behaviors by depriving them of being able to express normal behavior according to their species, which affected the welfare quality (Ruet *et al.*, 2019).

In the inspection of the horse's integrity, white hairs were identified more frequently in the region of the withers and back, in the lower part of the legs (hock, carpus, shank, fetlock and pastern) and in the hindquarters (rump and thighs), and less frequently in the head and shoulders (Table 5). White hairs grew from non-pigmented skin because of loss of melanocytes, caused by skin injury (Stachurska and Phaff, 2012). According to Dalla *et al.* (2014) the presence of white hairs indicated an injury that occurred in the past, which could have been a consequence of the type and intensity of the work, due to the harnesses used, the presence of ectoparasites or aggressive social interactions.

The wounds presented were mostly in the region of the back and withers, hindquarters, lower part of the legs, snout, and head (Table 5). All the wounds presented were type 1

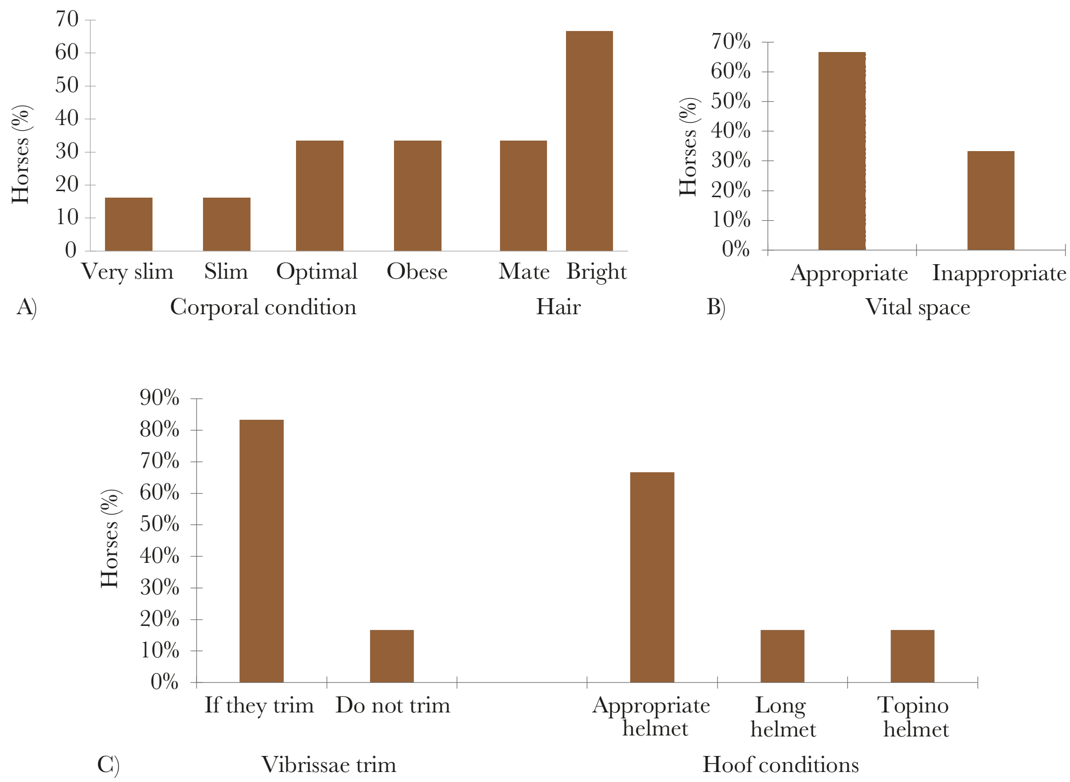


Figure 4. Corporal condition, vital space, and hoof condition in charreria horses.

(hair loss, stain, or scar) and type 3 (Superficial wound, scrape) according to the Welfare Quality® protocol (2011). Classifying the pain degree in the dorsal muscles into none (0), a little (1) and a lot (2), 50% of the animals showed a pain level of 1 in the region of the withers, and 16.6% a degree pain of 2 in the regions of the withers, back and loin (Table 5), attributing it to the improper use of harnesses according to the OIE (2018).

Most owners of the equids evaluated (83.3%) trim their animals' vibrissae from the ears and whiskers (Figure 4C) which, according to Yllera *et al.* (2016) is an inadequate practice, since they are sensory elements that serve as protection and guidance to explore their territory, making up for the lack of vision in their blind spots. The condition of the hooves was adequate in most of the equids (66.6%), 16.6% had long hooves and the other 16.6% had a topine hoof (Figure 4C). These abnormal conditions can present problems with locomotion and conformation of equids according to Baxter (2011).

In the human-horse relationship, 50% were alert and the other half were neutral; no animal was aggressive or avoided being manipulated, which indicates that there is a good interaction between the owner and his animal. A good human-equine relationship is essential to reduce negative states of animal welfare. This relationship is influenced by the interaction and trust that the equine feels in the presence of the human (Dalla *et al.*, 2015). The stereotypies observed were rocking, head nodding and wood chewing in 50% of the evaluated population (Table 4). These abnormal behaviors are attributed to the previously described by Lesimple *et al.* (2019). Similarly, Ruet *et al.* (2019) mentions

Table 4. Stereotypies prevalence presented in charreria horses.

Stereotypies	Owner (n=64)	
Survey	Number	%
Physical Inspection		
Swinging	2	33.3
Head nod	1	16.6
Wood chewing	2	33.3

Table 5. Physical integrity of charreria horses.

	Owners (n=6)	
	Number	%
White hairs		
Cross and back	3	50
Hindquarters	2	33.3
Lower legs	2	33.3
Head and shoulder	1	16.6
Injuries		
Cross and back	4	66.6
Hindquarters	3	50
Lower legs	2	33.3
Head and shoulder	2	33.3
Pain in back muscles		
Cross	3	50
Back	1	16.6
Loin	1	16.6
Rump	0	0

that accommodation in inappropriate places can cause stereotypies, poor human-horse relationships, and stress; harming welfare.

CONCLUSIONS

Charrería horses are of great cultural and sporting importance for Mexico since they represent the traditional identity of Mexicans. In the state of Guerrero there are a great variety of equids that carry out this type of activity, but not all of them receive adequate management to provide them with a good life quality. A wide variety of management practices are inadequate, compromising in some way the well-being of the animals.

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Colegio de Postgraduados

Phenotypic analysis of mezcal agaves from the Central Valleys of Oaxaca

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ABSTRACT

Objective: Mezcal agaves in the state of Oaxaca have a high economic value due to the demand for mezcal production; therefore, the purpose of this work was to assess and highlight the importance of morphological diversity within and among cultivated Agave species.

Design/Methodology/Approach: A completely randomized experimental design with 25 treatments (populations) and 11 replicates (individuals) was implemented. The plant (individual) was the experimental unit and 19 morphological descriptors proposed by the National Seed Inspection and Certification Service (SNICS) were assessed. A total of 275 individuals of the *Agave angustifolia*, *Agave karwinskii*, *Agave marmorata*, *Agave rhodacantha*, *Agave potatorum*, *Agave seemanniana*, and *Agave nussaviorum* species were assessed using a multivariate analysis to determine their phenotypic variability and existing relationships.

Results: The dendrograms for the Q-mode and R-mode were obtained by means of a cluster analysis, forming 4 groups based on the average linkage generated from the standardized BDM of the sampled species of the genus *Agave*. Four groups were formed using the k-means clustering method, in accordance with field observations and a review of the taxonomic bibliography. The first two principal components (PC) accounted for 66.4% of the total variation, according to the principal component analysis (PCA). For PC1 and PC2, the variables with the highest contribution were those related to leaf shape (Fh), size of the lateral spine (FEL), number of leaves (Nh), plant height (H), uniformity in the size of the lateral spines (UTE), and terminal spine shape (FET).

Study Limitations/Implications: A comprehensive study requires taxonomic keys to identify species, subspecies, and even varieties of *Agave*. Additionally, molecular characterization is essential to understand the variability and phylogenetic relationships of these populations, subject to a phylogenetic analysis.

Findings/Conclusions: Multivariate analysis techniques revealed that three species showed high phenotypic variability in the maturation stage under cultivation conditions. The *A. potatorum* and *A. karwinskii* species had a greater intra-population phenotypic variability, with significant differences within the same species. *Agave marmorata* showed no intra- or inter-population variability. Leaf texture (Txh) was the only variable that explained the variation within its group. This is a tall species whose diameter is larger than in the other species. The variables of the Mexican (*Agave rhodacantha*) group showed low correlation, as their behavior was highly dispersed. The variables obtained in the field from this group of populations must be meticulously assessed to identify the degree of correlation between the variables and to confirm the behavior of this group.

Key words: Morphological diversity, multivariate analysis, hierarchical clustering, clustering, k-means clustering.

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INTRODUCTION

Mexico's agave diversity is unevenly distributed, despite the presence of native and cultivated populations in most of the country. In some areas, *Agave* can be very diverse. For example, up to 20 species coexist in the Tehuacán-Cuicatlán region (García-Mendoza, 2002, 2004, 2011). Meanwhile, the state of Oaxaca is home to the largest number of *Agave* species (43) (Mora *et al.*, 2011; León *et al.*, 2013). Other states with a significant number of *Agave* species include Jalisco (38), as well as Hidalgo, Veracruz, and San Luis Potosí (32 each) (Villaseñor, 2016).

Currently, agaves within the genus *Agave* are an economically important product, mainly due to their industrial characteristics, and are consequently in high demand. Therefore, generating scientific information is of vital importance to support their use and conservation.

Data analysis and morphological and molecular characterization studies are tools that help to efficiently manage accessions within a germplasm bank (Barrera-Guzmán *et al.*, 2020). The analysis of genetic variation between and within populations is fundamental for their conservation and genetic improvement. It provides estimators of the extent of available genetic variation, contributes to germplasm monitoring, and predicts potential genetic gains (Moreno-González and Cubero, 1993). A multivariate analysis can be conducted for this purpose, enabling the comprehensive study of the attributes or characteristics of the elements of a given population. Furthermore, it provides the opportunity to quantify the intensity of the influence or association of independent variables in a specific mathematical model or to use them as a starting point to research a given phenomenon on a dependent variable (Garbanzo and Navarro, 2016). Consequently, the objective of the present study was to expand the knowledge about the genetic diversity of genus *Agave*, through the implementation of a robust multivariate analysis.

MATERIAL AND METHODS

Location of the study area

The study area is in the areas of Central Valleys of Oaxaca where *Agave* is cultivated. Sampling was conducted in the municipalities listed in Table 1, which are recognized for the sowing and production of mezcal.

Experimental design

A completely randomized experimental design was used with 25 treatments (populations) and 11 replicates (individuals). The experimental unit consisted of one mature individual from each population, defining maturity as the presence or proximity of floral scape or a minimum age of five years (SNICS, 2014). Twenty-five samples were collected at different time points over a 5-month period.

Plant species used in the study

A phenotypic analysis was conducted on populations of seven species of *Agave* that formed the experimental unit in cultivated conditions. As shown in Table 1, locations were selected based on the existence of cultivated agave populations in the area.

Table 1. Identification and description of sampled agave populations.

P	Specimen ID	Ni/P	District	Crop location	Geographic coordinate	Alt.
1	*A_angustifolia*	11	Ejutla	San Agustín Amatengo	16° 29' 59" N, 96° 46' 36" O	1380
2	*A_angustifolia*	11	Ejutla	Agua del espino	16° 35' 25" N, 96° 48' 31" O	1422
3	*A_angustifolia*	11	Ocotlán	Santa Catarina minas	16° 47' 23" N, 96° 36' 16" O	1630
4	*A_angustifolia*	11	Ocotlán	San Dionisio	16° 44' 55" N, 96° 40' 11" O	1530
5	*A_angustifolia*	11	Ocotlán	San Dionisio	16° 29' 59" N, 96° 46' 36" O	1500
6	*A_karwinskii*	11	Ejutla	San Agustín Amatengo	16° 30' 59" N 96° 46' 32" O	1440
7	*A_karwinskii*	11	Ejutla	Agua del espino	16° 35' 45" N, 96° 48' 45" O	1457
8	*A_karwinskii*	11	Ejutla	La compañía	16° 35' 40" N, 96° 48' 49" O	1380
9	*A_karwinskii*	11	Ocotlán	Santa Catarina minas	16° 47' 5" N, 96° 36' 55" O	1560
10	*A_karwinskii*	11	Ocotlán	Santa Catarina minas	16° 47' 5" N, 96° 36' 55" O	1562
11	*A_seemanniana*	11	Ocotlán	San Dionisio	16° 44' 57" N, 96° 40' 5" O	1530
12	*A_potatorum*	11	Ocotlán	Santa Catarina minas	16° 46' 41" N, 96° 36' 58" O	1630
13	*A_nussaviorum*	11	Tlacolula	San Dionisio Ocotepec	16° 48' N, 96° 24" O	1670
14	*A_seemanniana*	11	Tlacolula	San Baltazar	16° 46' N, 96° 29' O	1548
15	*A_potatorum*	11	Tlacolula	San Dionisio	16° 48' N, 96° 24' O	1670
16	*A_rhodacantha*	11	Ocotlán	Santa Catarina minas	16° 47' 5" N, 96° 36' 55" O	1630
17	*A_rhodacantha*	11	Ocotlán	Santa Catarina minas	16° 47' 5" N, 96° 36' 55" O	1630
18	*A_rhodacantha*	11	Tlacolula	San Baltazar Guelavila	16° 46' N, 96° 29' O	1548
19	*A_rhodacantha*	11	Tlacolula	San Dionisio	16° 48' N, 96° 24' O	1670
20	*A_rhodacantha*	11	Tlacolula	San Baltazar Guelavila	16° 46' N, 96° 29' O	1548
21	*A_marmorata*	11	Ocotlán	Santiago Apóstol	16° 47' 58" N, 96° 42' 56" O	1440
22	*A_marmorata*	11	Ocotlán	Santa Ana Zegache	16° 48' 42" N, 96° 43' 48" O	1480
23	*A_marmorata*	11	Tlacolula	San Baltazar Guelavila	16° 46' N, 96° 29' O	1548
24	*A_marmorata*	11	Tlacolula	Tlacolula	16° 43' N, 96° 33' O	1600
25	*A_marmorata*	11	Tlacolula	Matatlán	16° 51' 48" N, 96° 22' 58" O	1718

P: population; Alt: altitude (meters above sea level); Ni/P: number of individuals/population.

Morphological variables

A descriptive analysis of 25 populations under cultivation conditions was conducted and the basic data matrix (BDM) was integrated. The study data consisted of 19 morphological variables, 10 of which were quantitative (QN) and 9 were qualitative (QL) (Table 2). These variables were recorded for the 275 SU (Study Units) and were obtained from the *Guía técnica para la descripción varietal de Agave* (Technical Guide for the Varietal Description of Agave) handbook developed by the National Seed Inspection and Certification Service (SNICS). The whole multivariate analysis was based on the differences between the SUs. Only 19 out of the 32 variables assessed in the technical guide provided an acceptable explanation, according to Barrientos (2019).

Data were collected using a flexometer, a tape measure, a ruler, and a vernier scale. A GPS was used to determine the coordinates and altitude of the sampled sites.

Table 2. Quantitative (QN) and qualitative (QL) variables assessed for the phenotypic analysis of agaves from the Central Valleys of Oaxaca.

Quantitative (QN) characteristics	Variable ID
Plant: height	H
Plant: rosette diameter	DIAM
Plant: number of leaves	Nh
Leaf: wide	Ah
Leaf: length	Lh
Leaf: size of the lateral spine in 10 cm	Tel
Leaf: number of lateral spines in 10 cm	Nel
Leaf: distance between the lateral spines	Del
Leaf: length of the terminal spine	Let
Plant: flowering initiation cycle	CIF
Qualitative (QL) characteristics	
Leaf: terminal spine shape	FET
Leaf: color intensity	Ich
Steam: visibility	VT
Leaf: shape	Fh
Leaf: texture	Txh
Leaf: shape of lateral spines	FEL
Leaf: profile of the lateral spine	PEL
Leaf: uniformity in the size of the lateral spines	UTE
Plant: growth habit	HC

Statistical analysis

Cluster analysis includes a set of techniques that are available during the installation of the R Project software (RStudio, 2021.09.0.0).

Q-mode and R-mode hierarchical clustering

Following Palacio *et al.* (2020) and based on a BDM (basic data matrix), the hclust () function and the method argument were used to determine the optimal clustering method to be applied. Additionally, the Pearson correlation coefficient was estimated, and a correlation matrix was generated using the RStudio statistical package.

The cophenetic matrix was calculated using the cophenetic function (), based on the new similarity matrix (SM). Based on these data, the Pearson correlation between the cophenetic matrix and the original SM was calculated, using the cor () function and the Pearson argument. This procedure was repeated with Ward's method (simple, average, and complete linkages). Table 3 shows the cophenetic correlation values obtained with the application of the different methods. The average linkage method was applied and the groups that included the study units (SUs) were formed using the Factoextra graphics package.

Table 3. Values of the cophenetic correlation coefficients (CCC) for Ward's methods (simple, complete, and average linkages), based on the BDM of the species of the genus *Agave*.

Linkage technique	CCC
Ward's Method	0.755528
Simple Linkage	0.870786
Average Linkage	0.887935
Complete Linkage	0.835104

The distortion of a linkage technique calculated by means of the Pearson correlation is deemed suitable when it has a ≥0.8 value. Since the highest value was obtained for the average linkage (0.887), this method was employed for the development of dendrograms.

The number of groups was determined using Figure 1 as a reference, which was generated using arguments from the standardized BDM and the fviz_nbclust() function of the Factoextra package, under the hierarchical clustering parameters. This image identifies four groups as the optimal minimum. From group 4 onwards, an abrupt decrease in intra-group variation was observed. Finally, the corresponding dendrogram was developed using the fviz_dend() function. To obtain a dendrogram in which the variables are clustered instead of the SU, the matrix is simply transposed (R-mode hierarchical clustering).

k-means clustering

This analysis was conducted using a classification provided by a hierarchical algorithm, in which the k-means clustering method was used without specifying the centers of the clusters. In the case of unknown centers, the k-means clustering method begins with a division of the data set into (x) randomly configured groups. Subsequently, the method seeks to improve this initial classification by reassigning the elements to the centroid of the nearest cluster or group, in order to reduce the average distance between each element of a group and its centroid (de la Fuente, 2011).

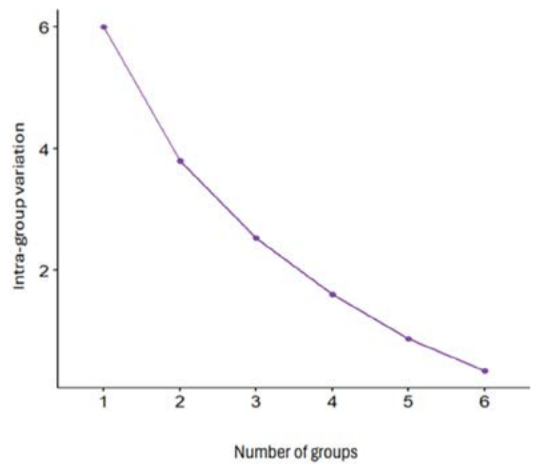

Figure 1. Number of groups *vs.* intra-group variation obtained with the function fvz_nbclust() on the hierarchical clustering of the *Agave* BDM.

Figure 2 shows the result of the *a posteriori* determination of the optimal number of groups applying the function fviz_nbclust() to the k-means clustering method. This figure illustrates the relationship between the number of clusters versus the sum of squares (intra-cluster variation), concluding that the optimal number of clusters is four.

The software selects the configuration with the lowest intra-cluster variation. Once the BDM has been standardized, the output results in a matrix (cluster means) whose values are the average of each standardized variable per group and the percentage of variation explained by the cluster (within-cluster sum of squares by cluster) and calculated as the variation between groups (between-SS) over the total variation (total-SS). In this case, this variation was calculated using the following formula:

$$\frac{between\_SS}{total\_SS} = 73.3\%$$

A principal component analysis was applied to visualize the k-means clustering result and to reduce the number of dimensions. The values of the first components were then used to create the graph (Kassambara and Mundt, 2017).

RESULTS AND DISCUSSION

Dendrogram of the hierarchical clustering (Q-mode)

The phased structure shown in Figure 3A suggests that the individuals under analysis exhibit significant differences between each SU.

The dendrogram resulting from the hierarchical clustering (Q-mode) —based on the average linkage generated from the standardized BDM of the species of the genus *Agave*— comprised four groups (Figure 3A). The first group in the dendrogram (from the bottom to the top) was formed at a distance of 1.2 with populations of the *Agave potatorum* (Figures 3A and 4E), *Agave seemanniana* (Figures 3A and 4F), and *Agave nussaviorum* species (Figures 3A and 4D). The second group was established at a distance of 1.4 with Tepeztate (*Agave marmorata*) populations (Figures 3A and 4A), with a low level of similarity to the rest of the

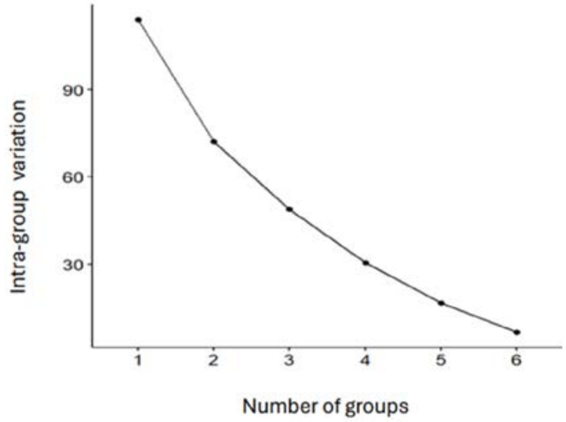

Figure 2. Number of groups *vs.* intra-group variation on the k-means clustering method applied to the *Agave* BDM.

groups. The third group was integrated at distance of 1.3 with populations of the *Agave karwinskii* species, commonly known in the region of Central Valleys of Oaxaca, including as Tobasiche, Kuish, and Marteño (Figures 3A, 4G, 4H, and 4I). The fourth group was structured at a distance of 0.80, with populations of the *Agave angustifolia* (Figures 3A and 4B) and *Agave rhodacantha* (Figures 3A and 4C) species. The dendrogram in Figure 3A indicates that the closest species (in phenotypic terms) are *A. angustifolia* and *A. rhodacantha*, while the most remote are *A. marmorata* and *A. karwinski*.

Dendrogram of relationships between variables (R-mode)

Figure 3B illustrates the relationships between the variables assessed in the dendrogram. Four groups were subsequently identified. The first group was formed at a distance close to 1.0 by the Nel, PEL, FET, UTE, Let, Tel, and Fh variables. In this group, the related variables were Nel (number of lateral spines) and PEL (profile of the lateral spine), in addition to FET and UTE, which belong to the lateral spine shape pattern and have a uniform lateral spine size. Furthermore, the Let, Tel and Fh variables demonstrated a high correlation between lateral spine size and leaf shape. The second group was formed at a distance of 0.70 by HC (growth habit), Nh (number of leaves), and VT (stem visibility). These variables were found to be highly related, since stem visibility implies that agave plants grow upwards and have a greater number of leaves. The third group was constituted at a distance of 0.95 by the Del (distance between the lateral spines), H (plant height), DIAM (rosette diameter), and Lh (leaf length) variables, based on which, leaf diameter and leaf length were observed to be highly related. Finally, a fourth group was formed at distance of 1.2, based on FEL (shape of lateral spines), CIF (flowering initiation cycle), Txh (leaf texture), Ah (leaf width), and lch (color intensity). The most closely related variables were DIAM (rosette diameter) and Lh (leaf length), suggesting that the greater the leaf length, the greater the rosette diameter.

The variable that distinguishes *Agave marmorata* from the other species is its rough leaf texture. For *Agave potatorum*, *Agave nussaviorum*, and *Agave semanniana*, the variable with the

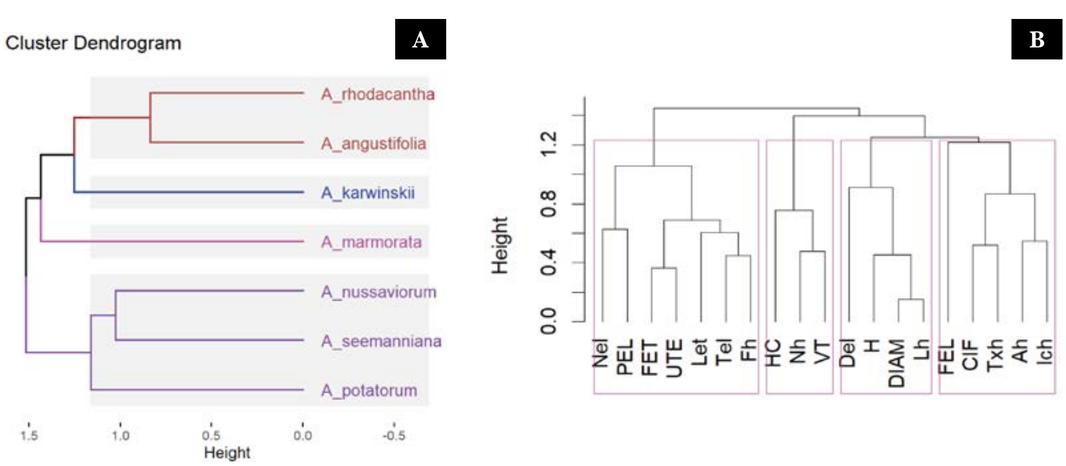

Figure 3. Dendrogram A: hierarchical clustering (Q-mode). Dendrogram B: (R-mode) based on the average linkage. Generated from the standardized BDM of the species of the genus *Agave*.

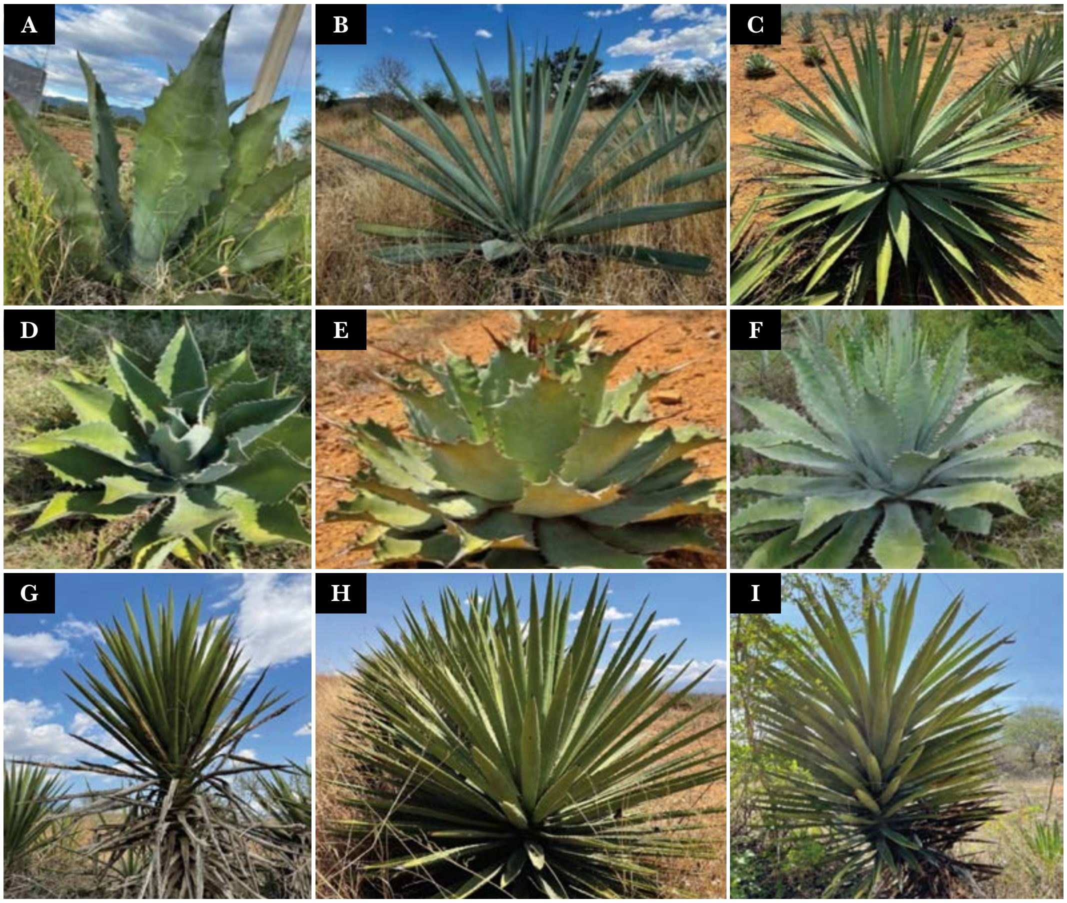

Figure 4. Phenotypes of the different species. A: Tepeztate (*Agave marmorata*, B: Espadín (*Agave angustifolia*), C: Mexican (*Agave rhodacantha*), potatorum group, D: *Agave nussaviorum*, E: *Agave potatorum*, F: *Agave seemanniana*, Karwinskii group, *Agave karwinskii*, G: Kuish, H: Barril, and I: Marteño.

highest correlation value was FET (terminal spine shape). These three species have curved terminal spines. The variables that distinguished *Agave karwinskii* were its growth habit and the number of leaves. In the case of the Mexican (*Agave rhodacantha*) group, the constituting variables were not correlated. However, these populations share a certain morphological similarity with the Espadín (*Agave angustifolia*) complex.

Hierarchical clustering analyses are useful tools for grouping agave specimens according to the similarities or dissimilarities of their morphological variables (Castro, 2010). In this study, quantitative and qualitative characteristics were assessed and morphological relationships among *Agave* species were identified. According to Rodríguez-Garay *et al.* (2009), additional genetic analysis would provide further information about the variation among populations and species.

K-means clustering

The K-means clustering analysis showed the integration of four distinct groups (Figure 5). The most distantly related group was constituted exclusively of the Tepeztate (*Agave marmorata*) species. A second group was formed with the Espadín (*Agave angustifolia*)

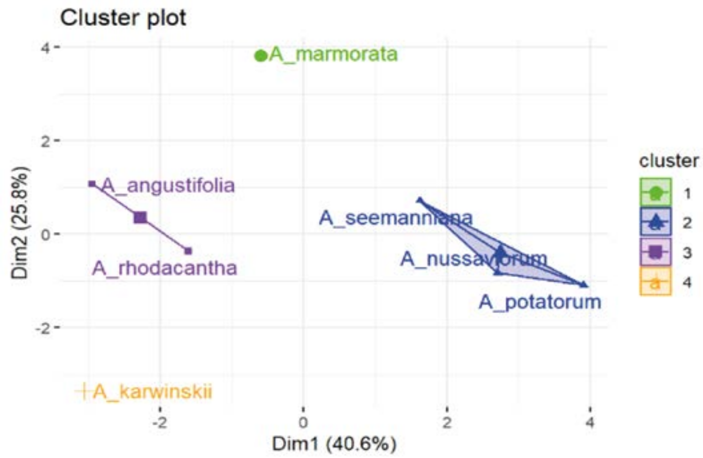

Figure 5. Groups of related *Agave* species, according to a k-means analysis using a principal component analysis. Percentages on the axes indicate the variation per component.

and Mexican (*Agave rhodacantha*) species. The third group was comprised of the *Agave seemanniana*, Agave potatorum, and *Agave nussaviorum* species, from the Tóbala group. Finally, the fourth group was integrated with *Agave karwinskii*.

The analysis of the assessed variables facilitated the determination of some morphological characteristics by means of which the populations and species of *Agave* will be differentiated. As stated by Narez-Jiménez *et al.* (2014), the demand for raw material for mezcal production has increased in recent years, making *Agave* spp. cultivars a valuable genetic resource that should be studied to improve their use and conservation.

CONCLUSIONS

The Q-mode hierarchical clustering analysis indicated that the closest *Agave* species studied are *Agave angustifolia* and *Agave rhodacantha*. In contrast, the R-mode hierarchical clustering analysis showed the relationship, selective value, and variation patterns of the characteristics assessed among the species. According to the K-means analysis, *Agave marmorata* was the least related species and had the lowest morphological variability; its rough leaf texture was defined as the differential variable of this species. The *Agave potatorum* and *Agave karwinskii* species recorded the greatest phenotypic variability. The populations of the Espadín (*Agave angustifolia*) group did not show morphological variability. Regarding *Agave potatorum*, *Agave nussaviorum*, and *Agave semanniana*, the FET (terminal spine shape) variable showed a high correlation value. The three species in question have curved terminal spines. The characteristic variables of *Agave karwinskii* are its growth habit and number of leaves. The variables of the Mexican (*Agave rhodacantha*) group showed no correlation, even though these populations share certain morphological similarities with the Espadín (*Agave angustifolia*) complex.

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Colegio de Postgraduados

Marafalfa grass (*Pennisetum purpureum* Schum) and mucuna (*Stizolobium pruriens* L. Medik) silage in a semi-confined sheep system in southeastern Mexico

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ABSTRACT

Objective: The objective of this study was to evaluate an alternative for feeding with silage to limit the decrease in productivity in dry seasons for sheep producers.

Design/methodology/approach: Ensilage was made with marafalfa grass (*Pennisetum purpureum* Schum) and endemic mucuna (*Stizolobium pruriens* (L.) Medik). The feeding method evaluated consisted of a semi-confined system, allowing the sheep to graze for a period of five hours per day, to later supplement it with the proposed silage. Studies of bromatological parameters were carried out with the three proposed silage treatments, and the weight of the sheep was measured at the beginning, during and at the end of fattening.

Results: The results suggest that the *P. purpureum* silo demonstrates significant weight gain, which is why it is proposed as an alternative for dry seasons.

Limitations on the study/implications: In the state of Campeche, sheep production is carried out under extensive direct grazing systems on grasslands, with scarce technology and low productivity. In the dry season, the productivity of these systems suffers a significant loss, due to limitations.

Findings/conclusions: Ensilage without the addition of mucuna (*S. pruriens*) obtained better results in sheep growth and this may be due to palatability. It is suggested to include a drying process prior to adding the mucuna (*S. pruriens*).

Keywords: Dry season, grazing, extensive livestock, bromatological analysis, tropical livestock production.

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INTRODUCTION

Sheep livestock production in tropical and subtropical regions is conducted under grazing of pasturelands or meadows, since they are sources of feed that entail lower costs for producers, and the types of feeding systems used are the extensive, intensive and semi-confined method (Ranieri *et al.*, 2015; Poli *et al.*, 2020). In broad regions of Mexico, the

Image by Hans at Pixabay

productivity of grazing systems for sheep tends to decrease during the months of March to June (dry period) due to the low productivity of native grasses, significantly affecting the profits of producers (Bobadilla-Soto *et al.*, 2021); it is necessary to resort to the use of hay pasture to complement the diet of the animals in this stage of the year through a semi-confined system (Chávez-Espinoza *et al.*, 2022).

In the state of Campeche, sheep production has had a constant growth during the last 15 years, reaching a state herd of around 200,000 heads (SIAP, 2020). However, the conditions of climate and state vegetation generate problems, such as productive limitations by stages of the year (Candelaria-Martínez *et al.*, 2015), parasite problems in the sheep (Flota-Bañuelos *et al.*, 2019, 2023), and bad selection of grasses for fodder (Pérez, 2017). In this sense, the need to explore alternatives to improve the diet of these animals emerges, not only to combat low productivity during the dry period, but also to limit some of the problems mentioned.

The use of hay pasture to supplement the diet of sheep presents an interesting area of opportunity, since many of the grasses used constitute weeds in other regional agrosystems (Santana *et al.*, 2020). In this sense, Ramírez and Pérez (2006) state that the maralfalfa grass (*Pennisetum* spp.) is of great interest for sheep livestock feed in the tropics, since it generates a good and high biomass production for producers including important characteristics for ensilage in times of scarcity (Ramírez and Pérez, 2006). Maralfalfa grass (*P. purpureum*) has high productivity (Chiquini-Medina *et al.*, 2019), and nutritional values that fulfill the requirements of sheep (Clavero and Razz, 2009; González-Garduño *et al.*, 2011). The objective of this study consisted in evaluating the adequate supplementation of maralfalfa grass (*P. purpureum*) enriched with mucuna (*S. pruriens*) to supplement the sheep with higher protein contribution, in a semi-confined system, and to evaluate the yield in the animal's growth, in Campeche, Mexico; the hypothesis consisted in that the higher protein content contributed by mucuna will increase the weight gain without affecting the amount consumed by the sheep.

MATERIALS AND METHODS

Location of the experimental site

The experiment was carried out in the "La Unión" Agricultural Ranch, located in the agriculture and livestock production colony "Miguel Alemán" Xcampeu, Campeche (19° 47' 53" N; 90° 23' 49" W), located on Km 19 of the Campeche-Holpechén federal highway, with detour to ejido San Antonio Bobolá.

Conducting the experiment

The collection of mucuna (*S. pruriens*) was carried out in different sites of the state of Campeche, because it is common to find it in barren lands and in the ejido San Antonio Bobolá. The cut was made before it had pod and began to dry. The mucuna (*S. pruriens*) collected was ground in a 2000a multi-functional high-speed mill located in the laboratory of the Instituto Tecnológico de Chiná. Cutting the grass *P. purpureum* was done at 90 days with an approximate height of 170 cm, to later be chopped and ensilaged with 10% for T2 and 20% for T3 of mucuna (*S. pruriens*) flour, leaving them to store in a lapse of 21 days. Each bag of silo had an approximate weight of 15 kg, calculating the consumption for one week of feed for the sheep. Confinement pens size 4×4 m were used, which were cleaned adjusting the feeding and drinking troughs.

The selection of sheep was carried out visually with the objective of having a homogeneous weight of the animals. The sheep were dewormed with the anthelmintic Paradex® 10%, at a dose of 0.5 mL/10 kg of live weight orally using disposable syringes of 5 mL. The animals had one week of adaptation, for seven days they were taken out to graze in a period of five hours daily and then their feed was supplemented with the silo. After the week of adaptation, the animals were taken out to graze in a fixed schedule of 8:00 to 13:00 h. During this lapse, cleaning of the pens was done taking out the excrement and urine; likewise, a change in drinking water and cleaning of the feeding trough was carried out. The research was conducted for a period of 6 weeks. During this time in the morning (8:00 am), the sheep were weighed every 7 days and the amount of silo that was given to them was increased once they gained weight and therefore increased the amount that they were fed.

Treatments and experimental design

The weight gain in growing sheep was evaluated using six repetitions per treatment in an experimental design of complete random blocks. In total there were 18 sheep with genetics of the breeds Black Belly and Pelibuey, which were weighed and divided for each treatment to have a total of 6 animals. Three treatments were considered, branding each animal with five different colors to be able to identify what treatment they belonged to. The white and black belts were used for the control treatment fed with *P. purpureum* silage without mucuna (*S. pruriens*) (T1). The black belts were used for the treatment of feed with *P. purpureum* + 10% *S. pruriens* (T2) silage, and the white belts for the treatment with *P. purpureum* + 20% *S. pruriens* (T3) silage.

Bromatological analyses

The bromatological analyses were carried out in the Instituto Tecnológico de Conkal, Yucatán, inside the animal nutrition laboratory. Three silage samples were taken which corresponded to the formulas evaluated in the treatments. The analyses that were conducted on each sample were the following: neutral detergent fiber and acid detergent fiber with the technique of paper bag filter/ANKON, raw protein with the micro-Kjeldahl technique (NOM-F-90-S-1978) (DOF, 1979), ash with the calcination method (NOM-F-066-S-1978) (DOF, 1978), and ethereal extract with the Soxhlet method (NMX-F-615-NORMEX-2018) (DOF, 2019).

Statistical analyses

To evaluate the significant differences, the data obtained were analyzed through Analysis of Variance and Tukey's means comparison test at 5% $\alpha=0.05$, through the use of the statistical package SAS (Statistical Analysis System) (SAS, 2004).

RESULTS AND DISCUSSION

The results from the bromatological analysis carried out with the three types of feed pointed out that ensilage with marafalfa grass (*P. purpureum*) and the addition of 20% mucuna (*S. pruriens*) presented a higher content of raw protein (8.37%) together with a high percentage of ash (3.84%); the treatment of maralfalfa (*P. purpureum*) with addition of 10% mucuna (*S. pruriens*) presented higher content of ash (4.02%), in addition to 6.91% of raw protein. Finally, the maralfalfa (*P. purpureum*) silage without addition presented lower percentage of protein and ash (3.94 and 3.26%, respectively), but with slightly higher percentages of detergent and acid fiber (Table 1).

In the treatments with maralfalfa (*P. purpureum*) with addition of mucuma (Table 1), similar percentages of proteins were obtained to those reported by other authors with corn ensilage commonly used (Galina *et al.*, 2008; Herrera *et al.*, 2015); however, the percentages were lower than the corn silages added with other grasses with protein percentages higher than 10%, such as those added with alfalfa (Aguirre *et al.*, 2016), or buffel grass (Barros and Silva *et al.*, 2022). Under the conditions of study, although the silages evaluated presented lower protein, they were used as complement to grazing and not as sole source of feed. In the first week of feeding, all the sheep presented observable weight loss with the three treatments, attributed to the period of adaptation of the animals to the conditions of study (González-Garduño *et al.*, 2011; Barros e Silva *et al.*, 2022). When the tests were finished, it was seen that the weight gains were constant week after week in each treatment (Figure 1).

The treatment that showed the best response of sheep weight gain was maralfalfa (*P. purpureum*) silo without addition, followed by the silo with *P. purpureum* + 10% of *S. pruriens*. These results were attributed to the better palatability of the maralfalfa grass (*P. purpureum*). The addition of fresh mucuna (*S. pruriens*) to the ensilage has a fermentation process that affected the consumption of silage per animal and from this the lower weight gain per animal. Maralfalfa (*P. purpureum*) is a soft grass, highly sweet and palatable (González-Garduño *et al.*, 2011; Sevilla-Panchano, 2011), which suggests a higher consumption of silage (Figure 2).

The results obtained showed that the best treatment to obtain sheep weight gain during the dry season is the *P. purpureum* hay pasture silage, which is why the inclusion of mucuna (*S.*

Table 1. Nutritional content of the maralfalfa grass (*P. purpureum*) alone and with the addition of mucuna (*S. pruriens*) and (*S. pruriens*) at 10% and 20%.

Nutritional content	Silage type		
	***Pennisetum purpureum* + 10% de *Stizolobium pruriens* (T1)**	***Pennisetum purpureum* + 20% de *Stizolobium pruriens* (T2)**	***Pennisetum purpureum* (Tc)**
Acid detergent fiber (%)	35.83	41.00	41.81
Neutral detergent fiber (%)	65.80	64.86	66.56
Crude protein (%)	6.91	8.37	3.94
Dry biomass (%)	72.04	70.00	83.31
Ashes (%)	4.02	3.84	3.26
Ethereal extract (%)	1.11	0.75	0.37

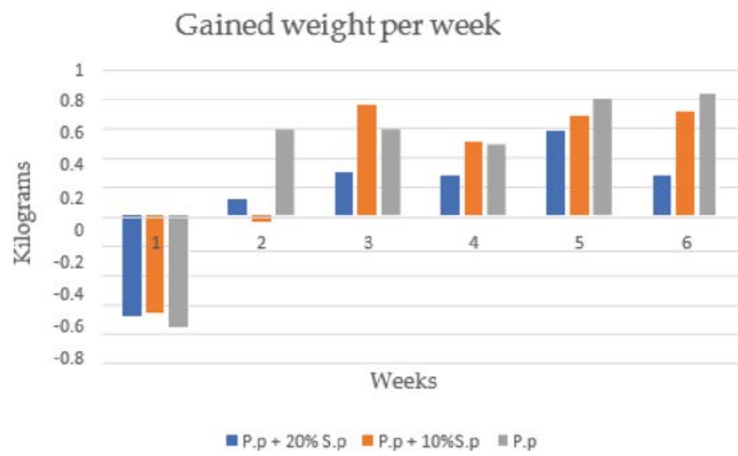

Figure 1. Average individual weight gain trend of the sheep subjected to the three treatments of maralfalfa grass (*P. purpureum*) each week of feeding.

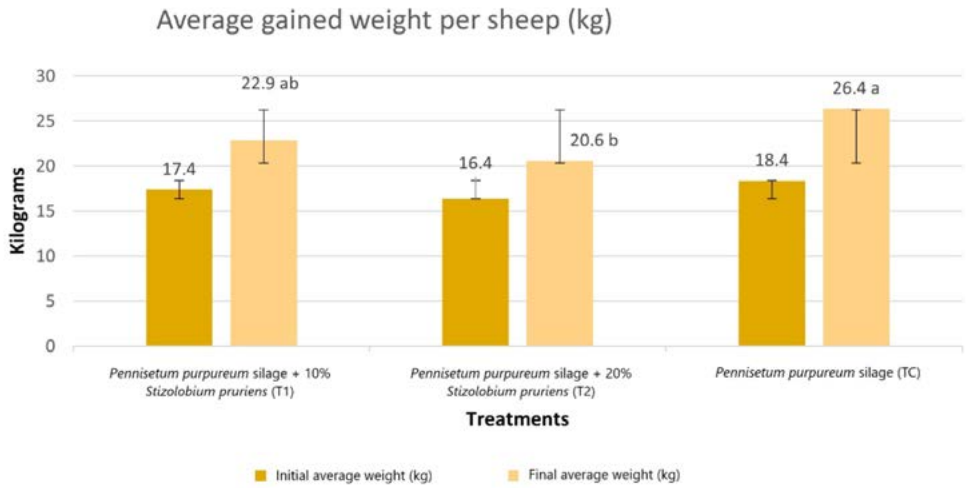

Figure 2. Average weight gain per sheep, as a result of the type of silage. Treatments with the same letter are statistically equal (Tukey, α=0.05).
DMS =3.202

pruriens) to increase the protein content may be unnecessary. The possible low palatability of the silage with mucuna (*S. pruriens*) can be due to the lack of drying and grinding of the legume to avoid a fermentation process during the ensilage with maralfalfa (*P. purpureum*) and its change in flavor. In this regard, Santos Silva *et al.* (2023) mentioned the importance of an adequate drying process in the silages high in nitrogen, since there is an effect on the palatability in tropical sheep with an influence in the decrease of consumption.

In general, the increase in daily weight of treatments Tc (193.1g) and T1(107.56 g) were within an acceptable range reported by different authors (Aguirre *et al.*, 2016; Silva *et al.*, 2021; Barros e Silva *et al.*, 2022; Santos Silva *et al.*, 2023), while not for T2 (53.35 g). Daily average gains between 100 and 150 grams are considered profitable in sheep fattening (Poli *et al.*, 2020; Chávez-Espinoza *et al.*, 2022). In this study, the maralfalfa (*P. purpureum*) silage promoted a better daily weight gain yield when compared to corn (Aguirre *et al.*,

2016) or alfalfa (Reséndiz *et al.*, 2013) silage, which agrees with the previous publications such as Ramírez and Pérez (2006) and González-Garduño *et al.* (2011).

Despite having a significant gain in treatments with the use of *P. purpureum* silage without additive in semi-confined systems for sheep in Campeche, it is recommended to evaluate a drying process and more adequate implementation of mucuna (*S. pruriens*) to increase the percentage of raw protein in the silage, without affecting the palatability.

CONCLUSIONS

Ensilage made with maralfalfa (*P. purpureum*) without mucuna (*S. pruriens*) additive showed a better weight gain yield in the semi-confined grazing system in sheep. The study suggests including a drying process prior to the addition of mucuna (*S. pruriens*) to increase the protein content offered to the sheep without compromising the palatability that could decrease the consumption, since the fermentation process is compromised with the presence of green legumes.

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Methane in Dairy Farms in Aguascalientes: Corn Silage

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ABSTRACT

Objective: To evaluate the potential methane gas production from corn silages (CS) intended for Holstein cattle in dairy farms in the state of Aguascalientes (Ags), Mexico.

Design/Methodology/Approach: Methane (CH_4) is one of the greenhouse gases, and worldwide plans and actions are being developed to monitor, control, and reduce their environmental impact. In Mexico, methane emissions from livestock are equivalent to 10.1% of CO_2 equivalent are recorded. CS samples were collected from six municipalities in Ags, representing a total of 18 dairy farms. The *in vitro* gas production technique was used to determine methane gas production, employing a nested mixed model to compare variables between municipalities using residual maximum likelihood method.

Results: The average methane production in CS was 29.3 mL/gDM. The Ags municipality showed significantly higher methane production (35.9 mL/gDM, $p<0.05$), while San Francisco de los Romo (SFR) displayed the lowest production (21.5 mL/gDM, $p<0.05$). In the state of Aguascalientes, CS-derived CH_4 production was projected at approximately 2,884 metric tons (MT) annually.

Study Limitations/Implications: There were no identified limitations in the study.

Findings/Conclusions: The potential CH_4 gas production derived from CS projected in the study represented 0.103% of what was reported by INEGyCEI in 2019.

Keywords: Environmental impact, greenhouse gas, forages.

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INTRODUCTION

Mexico is one of the 125 countries that signed the Global Methane Pledge (CH_4) to reduce methane emissions by 30% from 2020 levels by 2030, which could decrease global temperatures by 0.2 °C by 2050 (www.globalmethanepledge.org). According to the National Institute of Ecology and Climate Change (INECC in Spanish) through the National Inventory of Greenhouse Gases and Compounds Emissions (INEGyCEI in Spanish) of 2018, methane (CH_4) accounted for 20.3% of the total gas emissions, of which 50.7% is attributed to livestock, of this, 75.72% is due to enteric fermentations and 24.27% to manure management.

Image by Cally Lawson at Pixabay

In the digestive process of the rumen, there is a microbial ecosystem that facilitates fermentative digestion, within this ecosystem, there are groups of bacteria that can be defined or classified according to the substrate they ferment (Klein, 2014).

There are methanogenic bacteria that can vary depending on the diet and the geographical location of the host (Hook *et al.*, 2010). In the ruminal environment, three groups can be mentioned based on the pathway through which methanogens produce CH_4: the hydrogenotrophic, methylotrophic, and acetoclastic pathways (Extension Circular, 1996; Lambie *et al.*, 2015). In a general stoichiometry of volatile fatty acids (VFAs), acetic acid has a higher proportion relative to propionic acid, as seen in animals that receive a forage-rich diet (Madigan *et al.*, 2015). This would suggest that acetic acid is one of the main pathways for CH_4 production; however, methanogenesis is the primary way to remove or stabilize ruminal H_2. Therefore, as the amount of H_2 increases, methanogenesis would be further triggered (Yuan *et al.*, 2019).

Forages with better fiber quality can eventually reduce CH_4 emissions (Hassanat *et al.*, 2017), or when plants are in early vegetative stages (Brask *et al.*, 2013). Cabezas-García *et al.* (2017) found an increase in CH_4 production when using forages with lower starch content. Hatew *et al.* (2016) found a direct reduction of 7.9% in total CH_4 production when using corn silage (*Zea mays* L.) that reached 40% DM maturity.

Due to the wide range of results presented in various studies using different strategies to mitigate methane production, an alternative for determining potential CH_4 production levels of different ingredients is the *in vitro* gas production technique (Macome *et al.*, 2017; Miranda-Romero *et al.*, 2020). This technique has been used to evaluate and monitor changes in the quality of ingredients and diets used for ruminants, detecting changes derived from nutrients ranging from macro to microelements such as minerals (Sandoval-González *et al.*, 2016). Based on the above, the potential methane gas production of corn silages (CS) intended for Holstein cattle in dairy farms in the state of Aguascalientes, Mexico, was evaluated.

MATERIALS AND METHODS

Area of Study

The study was conducted in six municipalities of Aguascalientes State, Mexico, in the year 2021. A total of 18 dairy farms were included in the study, with a total of 11,904 animals. Of these, 51.47% (6,128) were lactating cows, 8.97% (1,068) were dry cows, and 39.55% were replacement animals from newborns to pre-first-calving. The projected volume of corn silage required annually for consumption in the sampled dairy farms was 77.8 thousand metric tons (MT).

Corn Silage Sampling

Samples of corn silage (CS) were collected from 18 dairy farms across the following municipalities: Aguascalientes (AGS), El Llano (ELL), Pabellón de Arteaga (PAR), Rincón de Romos (RR), San Francisco de los Romo (SFR), and Tepezalá (TPZ). From each dairy farm, 0.5 kg of CS was taken from 10 different points on the silo face, and then mixed, and a final 1.0 kg sample was obtained for each ranch. Subsequently, 200 g of each sample was taken and dried in a forced-air oven at 65 °C for 48 hours to determine partial dry matter. The samples were ground using a Wiley mill with a 2 mm sieve. Four subsamples of 0.5 g each were weighed and placed into individually identified amber glass bottles with a capacity of 120 mL each.

Nutritional Content Analysis of Corn Silage

Bromatological analyses of CS were conducted, dry matter, protein (Prot), ether extract (EE), and ashes (Ash) using the AOAC (1990) method. Neutral detergent fiber (NDF) was determined by the technique of Van Soest *et al.* (1991). These records were documented in an Excel® spreadsheet database.

In Vitro Gas Production Technique

Gas determination was conducted at the Food and Forage Analysis Laboratory of the Technological Institute El Llano, Aguascalientes. The *in vitro* gas production technique cited by Miranda-Romero *et al.* (2020) was employed for this purpose.

Extraction of CH_4, CO_2, and Global Warming Potential Index (GWPI)

For the estimation of CH_4, CO_2, and other minor gas production, values were expressed as mL/g DM. The GWPI was calculated using the formula cited by Martínez-Hernández *et al.* (2019) with values for CH_4 and CO_2, expressed as mL CO_2 eq gDM.

$$GWPI\left(mL\ \mathrm{CO_2}\ eq\ gDM\right) = \mathrm{CO_2}\left(mL/g\right) + \mathrm{CH_4}\left(mL/g\right) * 23$$

The readings were taken every 6 hours over a total period of 24 hours, and these records were entered into an Excel® spreadsheet database.

Production of Gas Fractions

For the quantification of different carbohydrate fractions, the technique of production of gas fractions was used (Miranda-Romero *et al.*, 2020). In this technique, GP8 represents gas production in the first 8 hours and corresponds to simple sugars, GP24 encompasses gas production accumulated between 8 and 24 hours, representing starches, and GP72 is the gas production accumulated between 24 and 72 hours, representing cellulose content, values were expressed in mL/g DM (Jiménez-Santiago *et al.*, 2019).

Degradation at 24 and 72 hours

For the *in vitro* degradation at 24 and 72 h, the technique adapted from Theodorou *et al.* (1994) was used. The samples used for CH_4 gas production and gas fractions were dried in a forced-air oven at 65 °C for 48 h after the process, to determine degradation at 24 and 72 h.

Statistical Analysis

A nested mixed-effect model was employed with "n" ranches nested within "r" localities and the variable "t" representing time within "n" ranches, to compare study variables across municipalities.

Statistical Model

$$Y_{ijkl} = \mu + Ra\left(L_j\right)_i + A_k + L_l + (A \times L)_{kj} + \varepsilon_{ijkl}$$

Y_{ijkl} represents the response variables such as Methane, Prot (Protein), NDF (Neutral Detergent Fiber), EE (Ether Extract), Ash (Ash), NFC (Non-fiber carbohydrates), *In vitro* Degradability at 24 and 72 hrs, GP8, GP24, GP72. μ=is the overall mean of the experiment. $Ra\left(L_j\right)_i$ = the effect of the *i*-th ranch within the *j*-th locality. A_k=the effect of the *k*-th time. L_l=is the effect of the *l*-th locality. $(A \times L)_{kj}$ = is the interaction effect of the *k*-th year and the *l*-th locality. ε_{ijkl}=represents the experimental error effect.

In the mixed model, the Type IIIA model as described by Sanni and Ukaegbu (2012) was used, with the locality variable considered as fixed and the ranch and time variables as random. Two types of analyses were conducted to compare the results: ANOVA and Maximum Likelihood Residual methods. Pairwise mean comparisons were performed using the Student's T-test ($p<0.05$). The analyses were conducted using R-Studio® version 2022.02.3., with the libraries readxl, agricolae, ggplot2, tidyverse, tseries, nlme, sjPlot, nortest, quadprog, emmeans, MuMIn, and sjstats (www.R-project.org).

RESULTS AND DISCUSSION

Nutritional Content of Corn Silage

The protein (Prot) content was significantly higher in El Llano (ELL) compared to the municipalities of PAR, RR, and SFR (+1.9, +1.8, +1.8; $p<0.05$) respectively. The silages from PAR, SFR, and TPZ had the highest content of NFC (40.5%, 38.1%, and 37.6% respectively). The municipality of ELL had a higher ash content than PAR (+2.07%; $p<0.05$). The neutral detergent fiber (NDF) content was statistically higher in the municipalities of ELL and RR (48.5% and 48.4% respectively) compared to PAR (44.5%, $p<0.05$). There were no significant differences in the contents of ether extract (EE), acid detergent fiber (ADF), and lignin (Lig) among the municipalities (Table 1).

CH_4, CO_2 and GWPI production and of Corn Silages

Methane (CH_4) production in corn silage was significantly higher in AGS compared to SFR, ELL, TPZ, and PAR (+14.4, +11.1, +9.9, and +8.2, $p<0.05$) respectively, and RR was higher than SFR (+7.3, $p<0.05$). CO_2 production did not differ significantly among municipalities. The Global Warming Potential (GWPI) production was higher in AGS compared to SFR (+327, $p<0.05$) (Table 2).

The approximate potential volume of CH_4 and CO_2 derived from the total volume of corn silage required annually by the dairy farms was 664,230 thousand liters of CH_4 per MT DM Tot and 2,993,788 thousand liters of CO_2 per MT DM Tot (Table 3).

Production of gas fractions PG8, PG24, and PG72

The AGS municipality had significantly lower GP-8 production of gas fractions (mL/gDM) than ELL and RR (−25.0, $p<0.05$; −23.1, $p<0.05$) respectively, although

Table 1. Average nutritional values (%) per municipality of corn silages sampled in 2021.

Municipality	Corn silage (%) 2021							
	PDM	**Prot**	**NDF**	**NFC**	**EE**	**Ash**	**ADF**	**Lig**
Aguascalientes	26.4	8.7 ^{ab}	47.1^{ab}	36.4 ^{ns}	1.80^{ns}	7.10 ^{ab}	26.7^{ns}	5.4^{ns}
El Llano	28.9	9.8 ^{a}	48.5^{a}	33.2 ^{ns}	1.66^{ns}	8.08 ^{a}	27.0^{ns}	4.9^{ns}
Pabellón de Arteaga	31.6	7.9 ^{b}	44.5^{b}	40.5 ^{ns}	2.03^{ns}	6.01 ^{b}	25.3^{ns}	4.8^{ns}
Rincón de Romos	29.6	8.0 ^{b}	48.4^{a}	35.4 ^{ns}	1.58^{ns}	7.47 ^{ab}	27.6^{ns}	5.6^{ns}
San Francisco de los Romo	26.8	8.0 ^{b}	45.4^{ab}	38.1 ^{ns}	1.62^{ns}	7.74 ^{ab}	26.3^{ns}	5.5^{ns}
Tepezalá	27.4	8.5 ^{ab}	46.0^{ab}	37.6 ^{ns}	1.88^{ns}	7.07 ^{ab}	25.6^{ns}	5.5^{ns}
Average	28.4	8.5	46.7	36.9	1.76	7.20	26.4	5.3
Coefficient of Variation (CV)		0.16	0.12	0.17	0.23	0.15	0.13	0.16
R^2		0.26	0.09	0.16	0.17	0.35	0.07	0.19

^{abc} Values with different letters in the same column are different ($p<0.05$). ns=not significant, CV=Coefficient of Variation, R^2=Coefficient of Determination, PDM=Partial Dry Matter, Prot=Protein, NDF=Neutral Detergent Fiber, NFC=Non-Fiber Carbohydrates, EE=Ether Extract, Ash=Ashes, ADF=Acid Detergent Fiber, Lig=Lignin.

Table 2. Methane and carbon dioxide production (mL/gDM) and Global Warming Potential Index expressed in CO_2 eqgDM of corn silages sampled by municipality in 2021.

Municipality	CH_4 mL/gDM	CO_2 mL/gDM	GWPI mL CO_2 eq g^1MS
Aguascalientes	35.9 ^{a}	119.7 ^{ns}	945 ^{a}
El Llano	24.8 ^{bc}	125.9 ^{ns}	696 ^{ab}
Pabellón de Arteaga	27.7 ^{bc}	127.5 ^{ns}	765 ^{ab}
Rincón de Romos	28.8 ^{ab}	120.5 ^{ns}	782 ^{ab}
San Francisco de los Romo	21.5 ^{c}	124.0 ^{ns}	618 ^{b}
Tepezalá	26.4 ^{bc}	144.7 ^{ns}	751 ^{ab}
Average	27.6	127.0	759.5
CV	0.147	0.045	0.123
R^2C	0.247	0.112	0.26
*LogLiK	−121.49	162.42	−223.14
**REML p=	0.052	0.919	0.041

^{abc} Values with different letters in the same column are different ($p<0.05$). ns=not significant, CV=Coefficient of Variation, R^2C=Conditional Coefficient of Determination, *LogLik=Likelihood coefficient. **REML p=p-value of the Residual Maximum Likelihood method. CH_4=Methane, CO_2=Carbon dioxide, GWPI=Global Warming Potential Index. gDM=grams dry matter, eq=equivalent, mL=milliliter.

production of gas fractions in PAR and SFR was lower than ELL (−15.0 and −16.3) respectively, it was not significant. Regarding GP24 gas fraction production (mL/gDM), AGS had lower content than ELL and RR (−27.3 and −22.6 $p<0.05$) respectively. GP72 gas production did not show a statistically significant difference between municipalities (Table 4).

Degradability at 24 and 72 hours

The 24 h degradability (DG24) averaged 45.1% across all municipalities, with AGS being significantly lower than ELL (−5.1, $p<0.05$) and PAR (−5.63, $p<0.05$). The 72 h

Table 3. Total DM tons required per year by sampled dairy farms and their potential methane and carbon dioxide production per municipality derived from corn silage.

Municipality	MT Tot DM	Lt CH_4/ MT Tot DM (K)	Lt CO_2/ MT Tot DM (K)
Aguascalientes	4,146	151,261	485,468
El Llano	2,281	55,218	296,670
Pabellón de Arteaga	5,496	150,813	723,053
Rincón de Romos	1,943	54,029	248,673
San Francisco de los Romo	4,256	101,263	511,690
Tepezalá	4,543	151,646	728,236
Total	22,665	664,230	2,993,788

TM DM Tot=Total dry matter tons, Lt CH_4/TM DM Tot (K)=Thousand liters of methane per ton of total dry matter, Lt CO_2/TM DM Tot (K)=Thousand liters of carbon dioxide per ton of total dry matter.

Table 4. Average of gas fractions production at 8, 24, and 72 hours from sampled corn silage in 2021.

Municipality	GP-8	GP-24	GP-72
	mLg^{-1} DM^{-1}		
Aguascalientes	83^{b}	159^{b}	157^{ns}
El Llano	108^{a}	186^{a}	148^{ns}
Pabellón de Arteaga	93^{ab}	169^{ab}	144^{ns}
Rincón de Romos	106^{a}	182^{a}	150^{ns}
San Francisco de los Romo	92^{ab}	183^{a}	142^{ns}
Tepezalá	100^{ab}	183^{a}	148^{ns}
Coefficient of variation (CV)	0.075	0.085	0.108
R^2C	0.308	0.221	0.221
*LogLiK	−149.65	−149.5	−154.11
**REML p=	0.176	0.086	0.867

^{abc}Values with different letters in the same column are different ($p<0.05$). ns=not significant. CV=Coefficient of variation, R^2C=Conditional determination coefficient, *LogLik=likelihood coefficient. **REML p=p-value of residual maximum likelihood method, GP-8=gas fraction production at 8 hrs, GP24=gas fraction production at 24 hrs, GP72=gas fraction production at 72 hrs. gDM=grams per dry matter, eq=equivalent, mL=milliliter.

degradability (DG72) averaged 66.3%, with no significant differences found between municipalities (Table 5).

Correlation of variables in the production of CH_4, CO_2, and GWP Index

The production of CH_4 in corn silages was negatively affected by GP24 and DG24 but positively affected by DG72 ($p=0.0000134$, $p=0.000307$, and $p=0.000108$; $R^2=56.01$), respectively (Table 6).

The NDF content (p=0.000228, R2=39.19) had a negative effect on CO2 production (Table 7).

The variables GP24 and DG24 had a negative effect, and DG72 had a highly significant positive effect on GWPI when expressed as mL CO_2eq/gDM ($p=0.0000127$,

Table 5. Means of Degradability at 24 and 72 hours per Municipality of Corn Silage Sampled in 2021.

Municipality	DG24 %	DG72 %
Aguascalientes	41.8[b]	65.6[ns]
El Llano	46.9[a]	68.6[ns]
Pabellón de Arteaga	47.4[a]	68.8[ns]
Rincón de Romos	44.1[ab]	65.3[ns]
San Francisco de los Romo	45.2[ab]	63.5[ns]
Tepezalá	45.5[ab]	65.7[ns]
Coefficient of variation (CV)	0.067	0.065
R^2C	0.234	0.199
*LogLiK	131.67	−99.4
**REML p=	0.217	0.115

[abc] Means with different letters in the same column are significantly different ($p<0.05$). ns=not significant. CV=Coefficient of variation, R^2C=Conditional coefficient of determination, *LogLik=likelihood coefficient. **REML p=p-value from the residual maximum likelihood method. DG24=24-hour degradability, DG72=72-hour degradability.

Table 6. Model by variable selection with the highest correlation to CH_4 production in corn silages.

Model CH_4	R^2 Adjusted
$Y=31.822-0.261GP24+0.092GP72-1.049DG24+1.133DG72+\varepsilon_{ijkl}$	0.56
$Y=31.822-0.261GP24+0.092GP72-1.049DG24$	0.33
$Y=31.822-0.261GP24-1.049DG24$	0.29
$Y=31.822-0.261GP24+1.133DG72$	0.25
$Y=31.822-0.261GP24$	0.20
$Y=31.822+1.133DG72$	0.10

Y=response variable, GP24=gas fraction production between 8 and 24 h, GP72=gas fraction production between 24 and 72 h, DG24=degradation at 24 h, DG72=degradation at 72 h.

Table 7. Model by variable selection with the highest correlation to CO_2 production in corn silages.

Model CO_2	R^2 Adjusted
$Y=140.864-2.10NDF+0.234GP24+0.891DG24+\varepsilon_{ijkl}$	0.39
$Y=140.864-2.10NDF$	0.35
$Y=140.864+0.891DG24$	0.06
$Y=140.864+0.234GP24$	0.01

Y=response variable, NDF=neutral detergent fiber, GP24=gas fraction production between 8 and 24 hours, DG24=degradation at 24 hours.

$p=0.000492$, and $p=0.0000872$; $R^2=56.34$), respectively. GP72 showed a positive trend ($p=0.06$) (Table 8).

Nutritional Content of Corn Silage

The content of protein (Prot), neutral detergent fiber (NDF), and acid detergent fiber (ADF) in corn silages fall within the ranges typical for corn silage reported by Dairy One

Table 8. Model by variable selection with the highest correlation to GWPI production in corn silage.

Model GWPI	R^2 Ajusted
$Y=759.789-5.722GP24+2.178GP72-22.007DG24+25.215DG72+\varepsilon_{ijkl}$	0.56
$Y=759.789-5.722GP24-22.007DG24+25.215DG72$	0.48
$Y=759.789-5.722GP24+25.215DG72$	0.28
$Y=759.789-22.007DG24+25.215DG72$	0.28
$Y=759.789-5.722GP24$	0.21
$Y=759.789+25.215DG72$	0.11

Y=response variable, GP24=gas fraction production between 8 and 24 h, GP72=gas fraction production between 24 and 72 h, DG24=degradation at 24 h, DG72=degradation at 72 h.

(revised in September 2023), except in the municipality of ELL with 9.8% protein. It has been observed that the use of nitrogen fertilizers in corn silage leads to increased protein content (Soto *et al.*, 2004). Conversely, when manure is used as fertilizer, which is a common practice in Aguascalientes to minimize the use of chemical fertilizers, there has been an increase in protein yield per hectare. However, this response is not linear, and there is no difference in the protein:DM ratio with varying levels of manure inclusion per hectare (Ortíz-Díaz *et al.*, 2022).

The average content of non-fiber carbohydrates (NFC) found in the municipalities is within the limits reported by Dairy One (2023), albeit it is just above the lower range. However, the content of ether extract (EE) in the different municipalities is 46% lower than that reported by Dairy One (2023). This discrepancy may be related to the lower limits of NFC content, as NFC largely represents the carbohydrate content of corn.

CH_4, CO_2 and GWPI production of Corn Silages

Estimating the weighted average volume of CH_4 production derived from the fermentation of CS among the total active animals in the sampled dairy farms, an average annual production of 55.8 m^3 CH_4 per animal was observed. Based on the census reported by SIAP (2021) for the state of Aguascalientes, this equates to 2,884 metric tons (MT) of CH_4 annually.

The GP24 fraction, identified by Miranda-Romero *et al.* (2020) as starches, had a negative effect on the CH_4 production of corn silage, consistent with findings by Hatew *et al.* (2016). Increasing starch content in silage with higher maturity or rapidly fermenting starches (Hatew *et al.*, 2014) lowers ruminal pH due to starch fermentation, which has been found to decrease protozoa population over time, thereby reducing CH_4 production.

Starch fermentation favors bacteria that produce propionate (Hook *et al.*, 2011; Benchaar *et al.*, 2014). However, increasing starch concentration within the first days can alter the rumen bacterial population (Neubauer *et al.*, 2018).

GP72, as indicated by Miranda-Romero *et al.* (2020), representing cellulose within slowly fermenting carbohydrates, was positively associated with CH_4 production, as mentioned by Hatew *et al.* (2014). Cellulose fermentation pathway leads to volatile fatty acids (VFA), CO_2, and CH_4 production (Madigan *et al.*, 2015), resulting in an expected scenario (Danielsson *et al.*, 2017). Although the NDF content in the current study was lower (46.7% *vs.* 51.91%, respectively) compared to findings by Kara (2015), the average CH_4 production was similar (27.6 *vs.* 25.9 mL/g DM, respectively). This contrast highlights the results sometimes associated with the 24-hour incubation period, where despite insufficient time for NDF fermentation, greater amounts of CH_4 can be produced (Pirondini *et al.*, 2012).

CONCLUSIONS

Under the conditions of the present study, the effect found in the production of gas fractions representing cellulose, and the very marked negative effect that starch content had on methane production derived from corn silages, it is important to consider determining the carbohydrate content using the *in vitro* fermentation technique, this would provide greater precision in estimating the potential production of methane and CO_2 gases from corn silages.

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Colegio de Postgraduados

Profitability analysis of the use of a variable dosing system for the differentiated application of fertilizer in corn crops

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ABSTRACT
Objective: a profitability comparison was made between corn production with the use of a variable doser and a conventional fertilizer doser. The variable doser for the differentiated application of fertilizer was built at CENEMA of INIFAP.
An experimental plot was established, applying the recommendations established in the technological package for grain corn production, by INIFAP, in the State of Mexico.
With the variable doser, it was verified that the fertilizer needs of the soil required 11.5% less than that supplied by a constant application doser. The yield obtained was 5.6% higher than in the conventional one. The B/C benefit/cost ratio of corn production with the variable doser was 1.60, while with the conventional doser it was 1.44, for both cases the profitability is positive, with a difference of $0.16 cents.
The profitability with the use of the variable doser was higher than with the conventional doser.

Keywords: Precision agriculture, profitability, benefit-cost.

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INTRODUCTION

The pursuit of maximizing agricultural production has led to the indiscriminate use of chemical fertilizers, which does not always translate into higher yields and leads to an increase in production costs (Audelo and Irizar, 2012). The adverse effects of excessive fertilizer use include nutrient losses in the environment, water pollution, and the eutrophication of freshwater systems and coastal areas [Food and Agriculture Organization (FAO, 2022)]. One of the current challenges is to continue maximizing production, and sustainably optimizing the use of resources (Martínez, 2008).

Agricultural mechanization in the agricultural production process constitutes a fundamental pathway for the further development of agriculture and the satisfaction of demands for agricultural products (Ávila *et al.*, 2019). Conventional agriculture considers soil conditions as homogeneous and applies the same amount of inputs, such as fertilizers.

Image by Andrew Martin at Pixabay

This increases costs and raises the risks of environmental contamination (Santillán and Rentería, 2018). Precision Agriculture (PA) is a set of techniques aimed at optimizing the use of agricultural inputs (seeds and agrochemicals) based on the quantification of spatial and temporal variability in agricultural production (Bongiovanni *et al.*, 2006; Santillán and Rentería, 2018). PA can reduce the use of agricultural inputs released into the environment by up to 90%. Its use depends on information technologies, where communication between devices is one of the most important tools (Santillán and Rentería, 2018). In Mexico, there is no fertilizer with a variable rate doser, that is, equipment that distributes fertilizer according to specific soil needs (Santillán and Rentería, 2018).

In the market, fertilizer spreaders with conventional dosers are distributed, which apply fertilizer homogeneously (Audelo and Ayala, 2023). The National Center for Agricultural Machinery Standardization (CENEMA) of the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP) designed, built, and evaluated a variable rate doser for the differentiated application of fertilizer that adapts to a row crop planter. The evaluation included functional and economic aspects and was carried out considering corn cultivation, given its importance in Mexico. The objective of this research was to conduct a comparative study of profitability between corn production using a conventional doser and using a variable rate doser for differentiated fertilizer application, aiming to observe optimization of the costs with the use of this doser and to determine its profitability for the producer.

MATERIALS AND METHODS

Conventional and Variable Seeding Machine

For the profitability estimation, data obtained from the assessment of the functionality of a variable rate doser and those from a conventional doser were utilized. These assessments were conducted in an experimental corn plot at the Experimental Field of Valle de México, Texcoco, State of Mexico. In this evaluation, a 0L Magnus 400 seed planter from the Dobladense brand was employed, equipped with both a conventional doser and a variable rate doser. The variable rate doser includes a sensor capable of assessing the nutritional requirements of the surface before applying the fertilizer.

Figure 1. Installation of the variable doser in a seeding machine, 2023.

Experimental set up and profitability calculation

Establishment of the experiment and profitability calculation

The preparation of the land began in April, the sowing took place on May 12th and the harvest on November 16th and 17th, 2023. It was sown in rows of 55 m, with a distance of 0.8 m between them and 0.1 between plants.

Due to the configuration of the seeding machine, three rows were planted using the constant doser unit and three rows using the designed doser, making 5 repetitions of each of the sowings. Table 1 shows the amounts of fertilizer used by each doser. In the conventional one, the technical recommendations established in the technological package for the production of corn published by the INIFAP in the State of Mexico (INIFAP, 2021) were used and in the variable rate doser, the quantities were applied according to the information obtained by the sensor that was used.

To quantify the costs of cultivation, a record was kept. Prices inherent to the inputs were used: seed, agrochemical products such as fertilizers and insecticides, wages, and the rent of machinery used in land preparation, cultural labor, irrigation, and harvesting.

To determine profitability, algebraic expressions based on economic theory were used (Krugman and Wells, 2006; Samuelson and Nordhaus, 2009):

$$CT=P_x X$$

Where: CT=Total production cost, P_x=Price of input or activity, and X=Activity or input.

Total income per hectare is obtained by multiplying the crop yield by its market price. The algebraic expression is:

$$IT=P_y Y$$

Where: IT=Total income (\$ ha^{-1}), Py=Market price of crop Y (\$ Mg^{-1}) and Y=Crop yield (Mg ha^{-1}).

The market price that was used to calculate the income was the guarantee price reported by the Ministry of Agriculture and Rural Development (SADER, 2023).

A cost-benefit analysis (C/B) was conducted to evaluate profitability. According to the C/B analysis, the project will be profitable if the C/B ratio is greater than unity.

Table 1. Fertilizer used by doser.

Product	Quantity Used (kg ha^{-1})		
	Urea	Diammonium phosphate	Potassium chloride
Conventional Doser	361.0	217.66	160.30
Variable Rate Doser	323.77	172.48	180.09

RESULTS AND DISCUSSION

During the corn production cycle, it was verified that the variable rate doser applied up to 11.5% (Figure 2) less fertilizer than the conventional (constant) doser. This variation is based on soil fertilizer requirements since the variable doser used a georeferenced data sensor, which allows nutritional requirements to be evaluated. According to the Institute for Diversification and Energy Saving (IDAE, 2010), variable rate fertilizer distribution requires prior acquisition of data on soil fertility and/or crop status through the use of systems that are based on real-time sensors aboard mobile equipment that lead to immediate responses, measuring soil fertility. Similarly, Santillano *et al.* (2013) mention that the efficiency of fertilizer use could be improved, thus enhancing the sustainability of agricultural production, through the use of sensors.

The variable rate doser contributed to a 11.5% reduction in fertilizer usage. According to Carvajal and Mera (2010), indiscriminate use of chemical fertilizers does not guarantee increased yields in production; on the opposite, it has caused losses in soil productivity where incorrect agricultural practices are carried out, leading to degradation of the soil's biological, physical, and chemical properties. Santillano *et al.* (2013) mentions that through the use of sensor technology, it is possible to obtain substantial savings in fertilization costs, contribute to increasing profitability for producers and reduce environmental impacts by avoiding the application of unnecessary fertilizers. The negative effects of synthetic fertilizer use on the environment are indisputable. Chemicals found in fertilizers, such as nitrates and phosphates, contaminate aquifers and surface water bodies (Orozco and Valverde, 2012). Orozco *et al.* (2016) mention that low fertility of soils has been affected by the excessive use of chemical fertilizers, resulting in high pollution rates, increased compaction and salinity, decreased organic matter, and a decrease in soil microbiology, negatively impacting yield and quality.

One of the challenges faced in agricultural production is to continue maximizing production, while optimizing available resources, since not only the expenditure is excessive, but the contamination that it generates is greater. According to Pazos *et al.* (2016), nitrogen fertilizer added to crops causes physiological changes in plants, which affect the

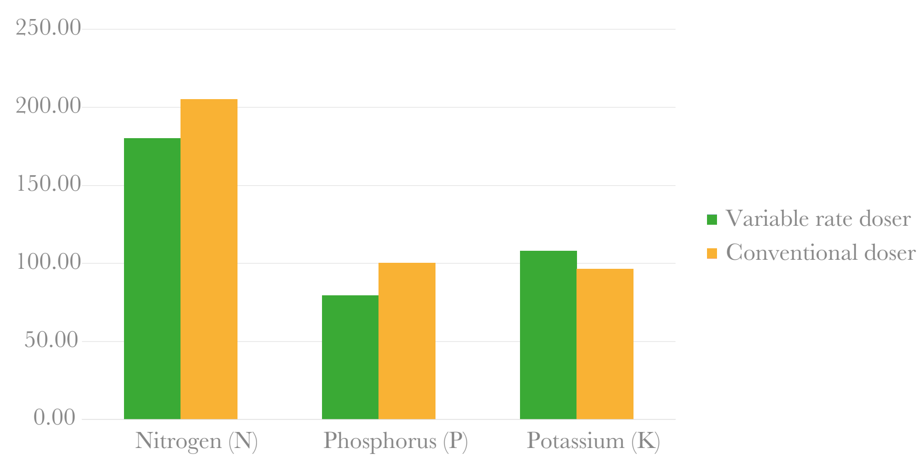

Figure 2. Fertilizer dosage used by each doser (kg/ha).

establishment of beneficial microorganisms. Therefore, the proper use of fertilizer becomes essential. Figure 3 shows the yields obtained with the use of the variable rate doser and the conventional doser. On average, this increased by 5.60%, that is, there was a higher yield where the variable rate doser was used.

Table 2 shows the costs and the benefit-cost relationship, as an indicator of the profitability of the crop. These data were obtained during the corn production cycle in both plots. It is observed that the cost-benefit ratio shows a difference of 0.16. This means that when the variable rate doser is used, $0.60 MXN are earned for every peso invested, while with the conventional doser, $0.44 MXN are earned. This indicates that it is 11.1% more profitable. This means that costs are saved, and the necessary nutrients are applied to the soil.

The results indicate that the use of the variable rate doser allows for cost savings. Costs were reduced by 10.4%, while yield increased by 5.6%. This difference in profitability

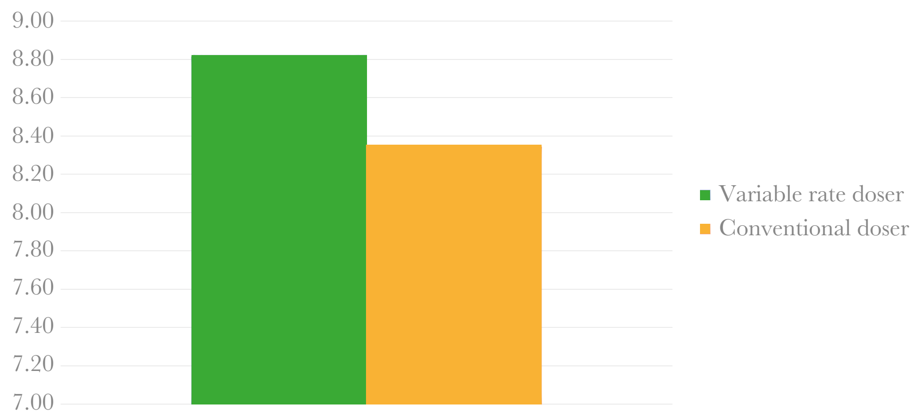

Figure 3. Yield obtained in both plots.

Table 2. Corn profitability, with variable rate doser, with prices.

Concept ($)	Cultivation using Variable rate doser	Cultivation using Conventional doser
Land preparation	1,600.00	1,600.00
Sowing	800.00	800.00
Agricultural tasks	8,744.44	8,744.44
Inputs	21,683.66	23,394.90
Harvest	4,972.22	4,972.22
Total cost ha^{-1}	37,800.32	39,511.57
Yield ($Mg\ ha^{-1}$)	8.82	8.35
Price (Mg^{-1})	6,805.00	6,805.00
Revenue ha^{-1}	60,020.10	56,821.75
Utility ha^{-1}	22,219.78	17,310.18
Cost Mg^{-1}	4,285.75	4,731.92
Utility Mg^{-1}	2,519.25	2,073.08
Cost-Benefit ratio	1.60	1.44

benefits those producers with small production areas and impacts the profitability of the crop.

CONCLUSIONS

A comparative study of profitability was conducted between corn production using a conventional doser and using a variable rate doser for differentiated fertilizer application. Corn production using the variable rate doser proves to be more profitable compared to the conventional method. Differentiated fertilization allowed reduced production costs without affecting yield, since it considers the specific nutritional conditions of the soil and applies the precise amount of fertilizer. The use of this type of doser represents progress in the application of Precision Agriculture, benefiting small-scale producers.

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Colegio de Postgraduados

Global Trends in Blue Carbon Research in Mangroves

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ABSTRACT

Objective: To identify the central themes and research trends on blue carbon in mangroves through a bibliometric analysis of the existing global scientific literature.

Design/Methodology/Approach: A total of 1,128 scientific documents from the period 1986-2023 were analyzed, obtained from the Scopus database. The Bibliometrix package in R Studio and VOSviewer were used for processing.

Results: The results, encompassing 4,602 authors, 94 countries, 64 research areas, and 346 journals, indicated an exponential growth (R^2=0.99) in mangrove carbon research. The most productive author, country, research area, and journal were Lovelock, L. C., the United States of America, environmental sciences, and Science of The Total Environment, respectively. The studies focused on four thematic clusters: carbon storage, sedimentation, carbon dynamics, and climate change and anthropogenic impact. Topics related to blue carbon, carbon stores, climate change, restoration, and remote sensing are currently of significant interest to the scientific community.

Study Limitations/Implications: While Scopus covers a vast number of peer-reviewed journals, it may omit some relevant research on the topic. Although the main research themes were identified, more in-depth information on each of them is still needed.

Findings/Conclusions: Four globally relevant themes in blue carbon research in mangroves were identified, providing a roadmap for researchers to strategically direct efforts and funding in future scientific investigations.

Keywords: Carbon stores, sediments, carbon dynamics, climate change, anthropogenic impact.

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INTRODUCTION

Since the second half of the last century, Lugo & Snedaker (1974) pointed out that mangroves are wetlands composed of halophytic plants that develop along the tropical and subtropical intertidal zones of the planet. These ecosystems have a significant capacity to capture and store carbon dioxide (CO_2) from the atmosphere (Duarte de Paula Costa & Macreadie, 2022), thus playing a crucial role in climate change mitigation and adaptation (Macreadie *et al.*, 2021).

The organic carbon (C) captured and stored by mangroves is termed "blue carbon," a concept introduced to underscore the significant contribution of ocean and coastal ecosystems with vegetation to carbon capture (Nellemann *et al.*, 2009). Global interest from various stakeholders in blue carbon has spurred the scientific community to address a breadth of uncertainties surrounding carbon science in mangroves (Macreadie *et al.*, 2019). While some important topics have been well-documented in abundant scientific literature (Duarte de Paula Costa & Macreadie, 2022; Yin *et al.*, 2023), there are still areas that require greater attention.

Bibliometrics is considered one of the most effective tools for the quantitative and qualitative analysis of academic literature. Through statistical methods based on computer science, bibliometrics allows exploration of the current state of scientific research (De Moya-Anegón *et al.*, 2007). This analysis also helps identify gaps and trends to strategically guide efforts and budgets for scientific research (Chen, 2017). Therefore, the objective of this study was to identify the central themes and research trends on blue carbon in mangroves through bibliometric analysis of the existing global scientific literature.

MATERIALS AND METHODS

The document search was conducted on September 6, 2023, using Scopus, which is widely recognized for its authority and importance in the international scientific community. An advanced search strategy with Boolean operators was employed, using the following equation: TITLE-ABS-KEY ("mangrove" OR "mangroves" AND "carbon sink" OR "carbon storage" OR "carbon sequest" OR "blue carbon" OR "carbon burial" OR "carbon pool" OR "carbon stock" OR "carbon accumulation" OR "carbon drawdown" OR "carbon cycl"). Additionally, a filter was applied for document type, selecting only research and review articles.

For the processing and analysis of the documents, eight indicators were utilized: number of documents, language, historical scientific production, research area, geographical distribution, most productive authors, most cited articles, and most productive journals. To perform a quantitative and statistical analysis of these indicators, the Bibliometrix package in RStudio was employed (Aria & Cuccurullo, 2017).

Cluster analysis was conducted to identify thematic groups in the current state of research using the keyword co-occurrence technique in VOSviewer (van Eck & Waltman, 2010). Additionally, to identify current topics of interest among researchers, an overlaid visualization of the co-occurrence network was utilized. Keyword co-occurrence included all terms from titles, abstracts, and keywords of the documents, with a minimum occurrence threshold set at 14 to facilitate network visualization.

RESULTS AND DISCUSSION

A total of 1,281 documents related to blue carbon in mangroves were recorded (1,172 research articles and 109 reviews) over a period of 37 years (1986-2023), with the majority published in English (1,241). Historical scientific production shows exponential growth (R^2=0.99, Figure 1) in the number of publications. Publication trends can be categorized into three distinct periods.

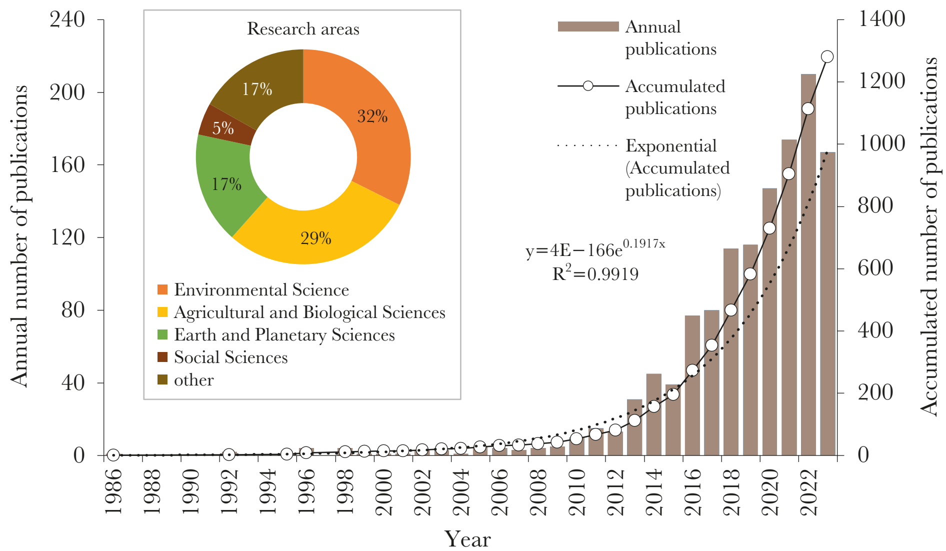

Figure 1. Historical global scientific production and research areas on carbon in mangroves during the period 1986-2023.

Firstly, the exploratory phase (1986-2008) saw a maximum of five documents published per year. Secondly, the phase of steady growth (2009-2014) began after Nellemann *et al.* (2009) first introduced the term "blue carbon" to the literature. Lastly, the period of rapid development (2015-2023) which started following the 21st United Nations Climate Change Conference and the Paris Agreement, events that led to the official recognition of the role of blue carbon ecosystems in climate change mitigation and adaptation (Zhong *et al.*, 2023).

Studies were classified into 64 research areas related to the topic of interest, demonstrating that blue carbon science in mangroves is a multidisciplinary field, similar to findings reported by Jiang *et al.* (2022). The most popular research areas were environmental sciences and agricultural and biological sciences, accounting for over 60% of the scientific production (Figure 1). Social sciences showed significant growth, ranking fourth, highlighting the importance of including mangroves in national greenhouse gas inventories (Friess, 2023) and their connection with rural communities (Herrera-Silveira *et al.*, 2020). Moreover, there is a need to enhance research in the field of economics to reduce uncertainty in carbon valuation (Macreadie *et al.*, 2019).

Regarding geographical distribution, a total of 94 countries were identified as involved in research on this topic (Figure 2). More than 50% of the total publications were jointly produced by the three most productive nations: United States of America (348), Australia (284), and China (242). Indonesia, United Kingdom, and Brazil followed in the ranking. Mexico ranked 11th with 61 documents.

This distribution responds to two main reasons: the level of economic development of the country and the extent of mangrove coverage they have. Thus, developed countries like the United States of America, China, and the United Kingdom, which have little to no mangrove coverage, benefited from their strong economic base facilitating high scientific output (Yin *et al.*, 2023). Conversely, the extensive mangrove ecosystems in Indonesia,

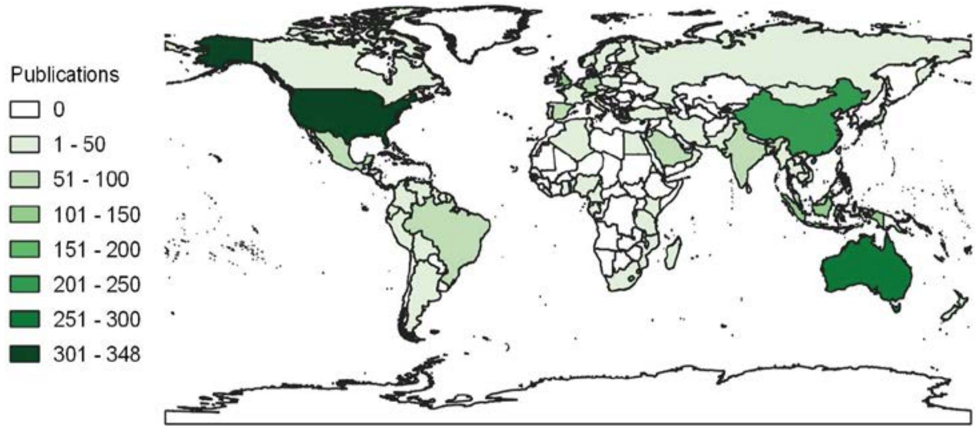

Figure 2. Countries that published on carbon in mangroves during the period 1986-2023.

Australia, and Brazil —ranked first, second, and third globally in terms of area— explain their high number of publications (Bunting *et al*., 2018). In contrast, Mexico ranks fourth in mangrove area but studies on this topic are considered scarce, indicating a need for more assessments to promote climate change adaptation, in Mexico and other developing countries rich in blue carbon (Herrera-Silveira *et al*., 2020).

Out of the 4,602 authors involved in the research, the top ten most productive in terms of number of publications come from five different countries (Table 1). Six of these authors are affiliated with institutions in Australia, the second most productive country globally. The strong performance of Australian researchers in blue carbon science was also noted by Jiang *et al*. (2022).

Within the top ten most cited articles, the primary one addresses methodology for estimating tree carbon in tropical forests (Table 2). Only two of these articles exclusively studied mangroves, while the others evaluated them alongside other coastal ecosystems. Moreover, four out of the ten were published before the term "blue carbon" was first introduced in the literature (2009), including the most cited and influential article in blue carbon science (Duarte de Paula Costa & Macreadie, 2022).

Table 1. Authors who produced the highest number of publications on carbon in mangroves during the 1986-2023 period.

Number	Authors	Affiliation	Publications	Citations
1	Lovelock, C. E.	The University of Queensland, Australia	51	2117
2	Sanders, C. J.	Southern Cross University, Australia	48	1020
3	Duarte, C. M.	King Abdullah University, Arabia Saudí	39	2307
4	Macreadie, P. I.	Deakin University, Australia	35	909
5	Friess, D. A.	Tulane University, United States of America	33	1169
6	Santos, I. R.	Southern Cross University, Australia	32	770
7	Serrano, O.	Spanish National Research Council, Spain	25	704
8	Maher, D. T.	Southern Cross University, Australia	24	686
9	Rogers, K.	University of Wollongong, Australia	23	712
10	Murdiyarso, D.	IPB University, Indonesia	21	1214

Table 2. Most cited articles in research on blue carbon in mangroves during the 1986-2023 period.

Number	Article	Authors	Citations
1	Tree allometry and improved estimation of carbon stocks and balance in tropical forests	Chave *et al.* (2005)	2139
2	A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO_2	Mcleod *et al.* (2011)	2051
3	Mangroves among the most carbon-rich forests in the tropics	Donato *et al.* (2011)	1765
4	Global carbon sequestration in tidal, saline wetland soils	Chmura *et al.* (2003)	1178
5	Seagrass ecosystems as a globally significant carbon stock	Fourqurean *et al.* (2012)	1159
6	The role of coastal plant communities for climate change mitigation and adaptation	Duarte *et al.* (2013)	1151
7	Major role of marine vegetation on the oceanic carbon cycle	Duarte *et al.* (2005)	962
8	Estimating Global "Blue Carbon" Emissions from Conversion and Degradation of Vegetated Coastal Ecosystems	Pendleton *et al.* (2012)	958
9	Carbon cycling and storage in mangrove forests	Alongi (2014)	862
10	Carbon and carbonate metabolism in coastal aquatic ecosystem	Gattuso *et al.* (1998)	804

The ten journals (out of a total of 346) with the highest scientific impact related to blue carbon in mangroves represented 25.9% of the total production (Table 3). The research landscape was dominated by Elsevier (four journals) and Springer (two journals), while other publishers had only one journal each in the top 10 list. Science of The Total Environment and Estuarine, Coastal and Shelf Science were the most productive, similar to findings highlighted by Jiang *et al.* (2022).

Regarding the current state of blue carbon science in mangroves, from a total of 6,520 keywords and 275 terms retained through co-occurrence, four clusters of research were identified (Figure 3):

Table 3. Most productive journals in blue carbon research in mangroves during the period 1986-2023.

Number	Journal	Impact factor	Quartile	Publications
1	Science of The Total Environment (Elsevier)	9.8	Q1	67
2	Estuarine, Coastal and Shelf Science (Elsevier)	2.8	Q1	53
3	Forests (MDPI)	2.9	Q1	35
4	Global Change Biology (Wiley Online Library)	11.6	Q1	31
5	Frontiers in Marine Science (Frontiers)	3.7	Q1	29
6	Wetlands (Springer)	2.0	Q2	25
7	Regional Studies in Marine Science (Elsevier)	2.1	Q2	24
8	Wetlands Ecology and Management (Springer)	1.8	Q2	24
9	Forest Ecology and Management (Elsevier)	3.7	Q1	23
10	Biodiversitas (Society for Indonesian Biodiversity)	1.5	Q3	21

I) Carbon storage (red cluster). This thematic group comprises the largest number of studies, primarily related to the estimation of carbon stocks in tree biomass and soil components across different regions worldwide (Akhand *et al.*, 2023). Long-term accumulation rates are reported to a lesser extent due to their difficulty in obtaining, yet Adame *et al.* (2015) emphasize their critical role in carbon payment programs. Globally, approximate values between 856 and 1,023 Mg ha^{-1} of total ecosystem carbon reservoirs are noted (Alongi, 2014; Kauffman *et al.*, 2020). Remote sensing is also addressed in this cluster as an emerging technological opportunity to enhance the accuracy of carbon measurement and monitoring in mangroves (Araya-Lopez *et al.*, 2023).

II) Sedimentation (green cluster). Sediment in mangroves accounts for between 70 and 85% of the total ecosystem carbon stocks (Beloto *et al.*, 2023), making it the focus of the majority of publications analyzed in this group. Recent studies in this cluster have implemented stable isotopes to effectively distinguish the origin of organic matter in sediments (Tang *et al.*, 2023). As noted by Huang *et al.* (2023), mangrove sediments derive from a variety of terrestrial, limnetic, and marine sources, including leaf litter and mangrove roots, algae, microalgae, and phytoplankton. On the other hand, some studies assess the impact of sea level rise on sediment accretion and its potential effect on carbon reservoirs (Saintilan *et al.*, 2023).

III) Carbon dynamics (blue cluster). An important theme in this cluster is the study of vertical and horizontal carbon fluxes in mangroves to understand their complex cycle. Studies highlight the significance of lateral exchange of dissolved and particulate organic carbon between mangroves and other coastal ecosystems (Lu *et al.*, 2023; Ray *et al.*, 2021). Regarding greenhouse gas flux measurements, in addition to carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) have been addressed, though they remain limited in many regions (Sugiana *et al.*, 2023). Multiple factors control carbon flux in these ecosystems, prominently biotic factors (Zhao *et al.*, 2023), as well as spatial-temporal and physical factors (Nie *et al.*, 2023).

IV) Climate change and anthropogenic impact (yellow cluster). This group addresses the study of carbon in mangroves as a nature-based solution to mitigate climate change (Zeng *et al.*, 2021), a topic of significant interest in achieving the global goal of limiting the increase in global temperature to no more than 1.5 °C by 2035. Relevant studies include those evaluating the impact of mangrove loss and land use change on CO_2 emissions to the atmosphere (Adame *et al.*, 2021; Das *et al.*, 2023), as well as those focusing on the conservation and restoration of these wetlands as a measure for carbon sequestration (Ray *et al.*, 2023; Sharma *et al.*, 2023). Additionally, the effects of climate change on future carbon stocks are analyzed through modeling approaches (Chatting *et al.*, 2022).

Regarding current research interests, the overlay map of the co-occurrence network depicts them in yellow circles (Figure 4). Notably among these are blue carbon and carbon stocks, which, despite being extensively studied, still have regions of the world where such research is scarce (Herrera-Silveira *et al.*, 2020). Other recent topics of interest include

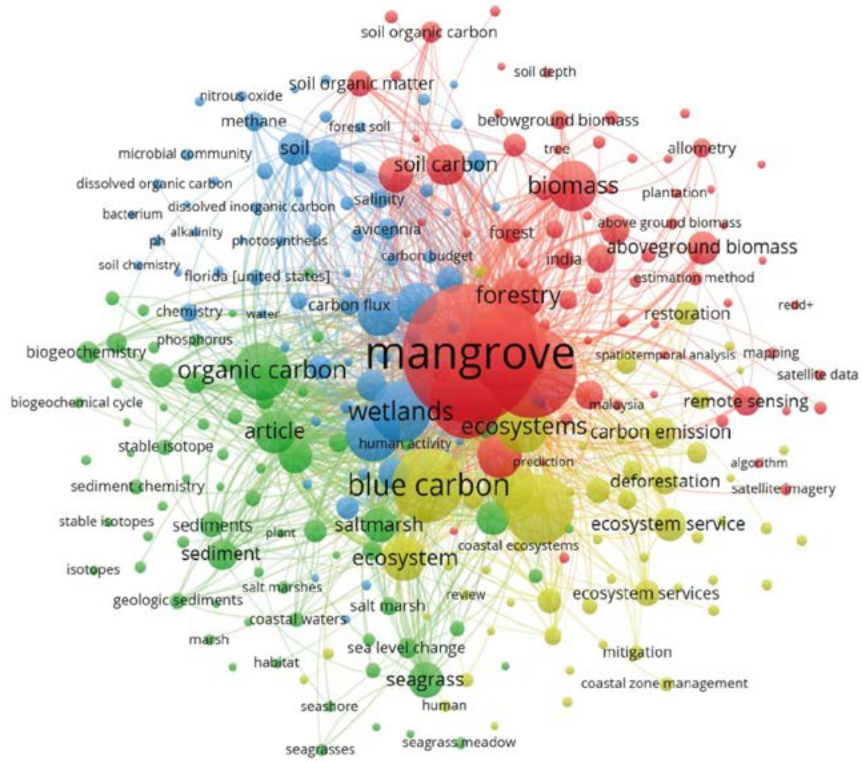

Figure 3. Co-occurrence network map based on articles related to carbon in mangroves during the period 1986-2023.

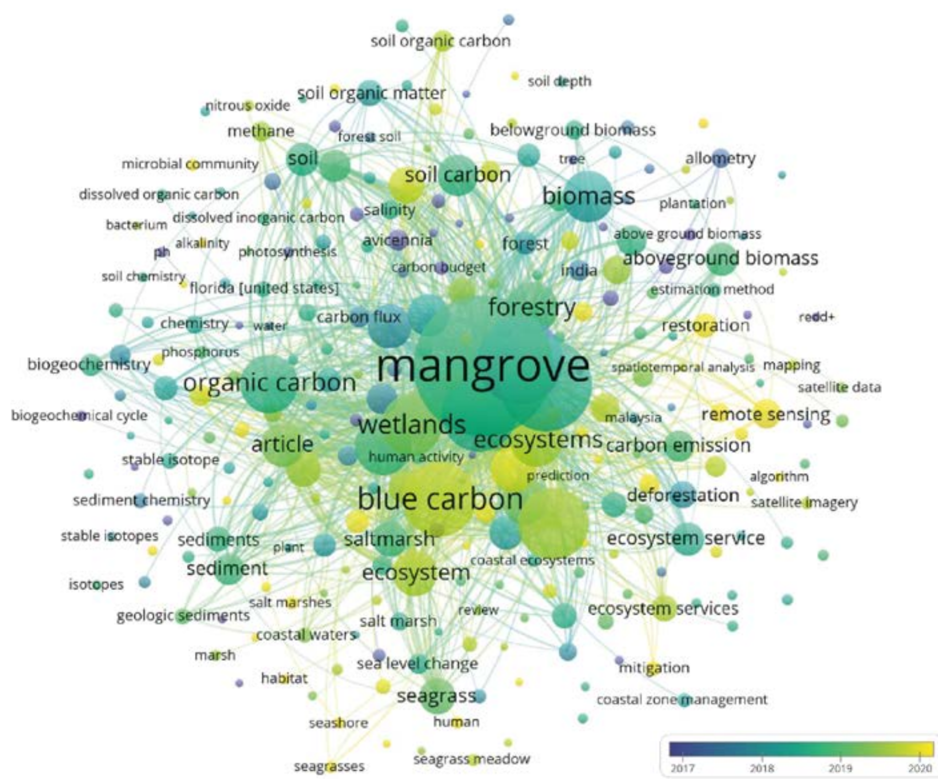

Figure 4. Overlay map of the co-occurrence network based on articles related to carbon in mangroves during the period 1986-2023.

climate change, greenhouse gas emissions, sea level rise, eutrophication, nutrients, restoration, conservation, biodiversity, microbial community, and remote sensing. Future research is expected to focus on addressing these topics.

CONCLUSIONS

The study successfully identified four key thematic trends in global research on blue carbon in mangroves, which can serve as a roadmap for researchers to strategically allocate efforts and funding in the future. Significant research has focused on carbon stock estimations, yet there is a need to increase studies providing insights into capture rates. Future research should target regions with scarce data to promote conservation and restoration efforts for these ecosystems. This study also highlights the importance of addressing research gaps related to the sociopolitical aspects of blue carbon science in these wetlands.

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Clonal micropropagation and *ex-situ* conservation of *Rhyncholaelia digbyana* (Lindley) Schltr

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ABSTRACT

Objective: To obtain protocols for clonal micropropagation, crop planning, and *in vitro* conservation of *Rhyncholaelia digbyana* (Lindley) Schltr.

Methodology: The effects of the Kundson C basal medium and benzylaminopurine concentration were evaluated for clonal micropropagation. The treatment with the greatest number of shoots formed per apex was selected for crop planning. Experiments were conducted to determine the effect of basal medium Murashige and Skoog concentration at 2.2 gL^{-1} and 4.4 gL^{-1}; sorbitol, mannitol, and sucrose at 1, 2, and 3% on slow growth.

Results: The best treatment for clonal micropropagation and crop planning was identified as 21.60 gL^{-1} Knudson C with 8.80 μM benzylaminopurine. This treatment resulted in uniform-sized shoots produced. The multiplication process can yield 10,240 seedlings in 12 months. Slow growth was achieved using Murashige and Skoog basal media at 2.2 gL^{-1} with 1% mannitol.

Implications: More experiments must be conducted to determine the best shoot induction conditions and improve resource efficiency.

Conclusions: These findings represent the first report on micropropagation and *ex-situ* conservation to preserve germplasm for this species as an important resource for the floriculture industry.

Keywords: mannitol, minimum growth, orchids conservation, plant tissue culture, sorbitol.

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INTRODUCTION

Orchids are highlighted in floriculture, with a global revenue of more than 400 million US dollars (Kanlayavattanakul *et al.*, 2018). Further, orchids have ethnopharmacological activities (Kaur *et al.*, 2022). Conventionally, orchid propagation is 2 to 3 years. The availability of prized commercial orchids is limited due to the paucity of plant materials. Plant tissue culture is crucial in ensuring a supply of raw materials for the floriculture industry (Bhattacharyya *et al.*, 2023; Cazar *et al.*, 2023). Plant tissue culture of orchids has been achieved with different focus. For example, propagation from asymbiotic seed germination of orchids such as *Dracula felix* (Luer), *Thrichocentrum stramineum*, *Thunia marshalliana*, and *Dendrobium densiflorum* (Quijia-Lamiña *et al.*, 2023; Ramos-Ortiz *et al.*, 2020; Pongener & Ranjan Deb, 2019). Clonal micropropagation from shoot tips culture of *Brassavola nodosa* and *Doritis pulcherrima* (Xu *et al.*, 2022; Mondal *et al.*, 2013). On the other hand, clonal micropropagation from Transverse thin cell layer (t-TCL) segments of *Malaxis acuminata* (Bhattacharyya *et al.*, 2022). Clonal micropropagation from apices has been evaluated in species such as *Delonix regia*, *Origanum scabrum*, *Abelmoschus esculentus*, and *Trichilia pallida*, using basal media such as Murashige and Skoog basal media (MS) (Alexopoulos *et al.*, 2023; de Souza *et al.*, 2021; de Oliveira Costa *et al.*, 2020). In orchids, MS is the most used basal media for micropropagation (Ahmadi *et al.*, 2023; Xu *et al.*, 2022; Bhattacharyya *et al.*, 2022;); as well as Knudson C (Pathak *et al.*, 2022; Ramos-Ortiz *et al.*, 2020), and Vacin and Went (Quijia-Lamiña *et al.*, 2023; Sunitibala & Neelashree, 2018). For clonal micropropagation, cytokinins such as benzilamkinetinynopurine (BAP), kinetin, and zeatin are plant growth regulators (PGRs) that induce cell division, generation of shoots, initiation, and elongation of roots. Orchid micropropagation requires a crop planning if the purpose is to market them. This planning involves organizing time, materials, labor, and space to produce the number of plants needed according to the market demand in a stipulated time. On the other hand, the Orchidaceae family faces threats due to the disturbed integrity of the ecosystems (Fonge *et al.*, 2019; Debnath & Kumaria, 2023). Although *in situ* conservation is essential for preserving plant species and maintaining genetic variability, it poses risks, such as invasion by pests, diseases, and natural disasters. Thus, *ex-situ* conservation is indicated for the conservation (Generoso *et al.*, 2023). *Ex-situ* conservation is achieved through seed banks, a relatively low-cost method for preserving the genetic diversity of many individuals. An alternative to reduce labor and time spent is to induce slow plant growth to reduce the turnover frequency (Chappell *et al.*, 2020; Generoso *et al.*, 2023). Slow or minimum growth is a biotechnological tool for medium-term plant germplasm conservation under *in vitro* conditions, reduces the metabolic activity, *i.e.*, the growth rate of *in vitro* cultures, by maintaining them on a modified growth medium or in altered culture conditions (Chauhan *et al.*, 2019). Physical factors chemical substances for supplementation of culture media can be applied to conserve plants by the slow-growth method (Mayo-Mosqueda *et al.*, 2022; El-Hawaz *et al.*, 2019). These factors are evaluated individually or combined. In tissue culture media, slow growth using chemical substances is induced using sorbitol, sucrose, or mannitol. Mannitol is a sugar alcohol that causes osmotic stress by reducing plant water uptake, resulting in slower plant growth in tissue culture media (Chappell *et al.*, 2020). Mannitol has been evaluated for slow growth in *Morus alba*, *Vanilla planifolia*, *Saccharum* spp., and *Xanthosoma* spp. (Espinosa-Reyes *et al.*, 2021; Bello-Bello *et al.*, 2015; Bello-Bello *et al.*, 2014; Rayas *et al.*, 2013). Sorbitol is also an osmoregulatory agent used to slow the growth of bananas, potatoes, and Asparagus (Singh *et al.*, 2021; Muñoz *et al.*, 2019; Thakur *et al.*, 2015;). Sucrose, another osmolyte, has also been evaluated for in vitro conservation through minimal growth in species such as *Morus alba*, *Dioscorea alata*, and *Dioscorea rotundata* (Espinosa-Reyes *et al.*, 2021; Díaz *et al.*, 2015). *Rhyncholaelia digbyana* (Lindl.) Schltr is distributed in southern Mexico, Guatemala, Honduras, and Costa Rica. It is an epiphytic plant that produces white or greenish, aromatic, long-lasting flowers. This species has the largest flower of the orchids found in the Yucatan Peninsula and has economic importance in hybridization processes (Wrigth *et al.*, 2017; Sánchez-Martínez *et al.*, 2002). Hence, this research aims to obtain clonal micropropagation, crop planning, and the conditions for conserving *in vitro* plants of *Rhyncholaelia digbyana*.

MATERIALS AND METHODS

Plant material

Fully closed *Rhycncholaelia digbyana* from five capsules fully closed were collected from experimental Field El Tormento, Campeche, Mexico, at the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP). The methods for disinfection of capsules and asymbiotic germination of plants are described by (López Puc & Herrera Cool, 2022).

Clonal micropropagation from apices

For *in vitro* establishment, the apices were extracted from *Rhyncholaelia digbyana in vitro* plants of Bank germplasm of orchids of Research Center of Jalisco (CIATEJ). The apex size of 1 cm^2 was taken and placed separately according to treatment. The factors evaluated were the basal medium's concentration Knudson C (KC) and the BAP concentration, with two levels of each factor and one central. Point. The central point was the intermediate level of each factor. The dependent variables were the number of shoots per apex, shoot length, number of leaves, and leaf length. Five repetitions per treatment were performed, and the experimental unit was one explant per flask. All treatments were supplemented with 15% coconut water and gel rite at 0.23% w/v. The apices were sown in clonal propagation treatments for 90 days. Conditions of the photoperiod room were 23±2 °C, 16/8 (light/darkness). The pictures of shoot development from the apex were captured using a Nikon stereoscopic microscope (zoom 4, observation of 640 LP/mm).

Crop planning

A plan was devised for the large-scale propagation of *Rhyncholaelia digbyana* plants through micropropagation. The most effective treatment for inducing the highest number of shoots per apex was selected. To produce around 10,000 plants, the number of propagation cycles required was calculated. The initial explants used were apices from *in vitro* seedlings (cycle 1), then at 3, 6, 9, and 12 months of culture, plants obtained were multiplied (cycles 2-4) subcultures in the same multiplication medium. During each cycle, the number of shoots developed was counted to estimate the multiplication rate of shoots per cycle.

Slow growth for *in vitro* conservation

Slow growth experiments were conducted using 2 cm plantlets from asymbiotic germination and established in a factorial experiment of 2×2^3 for conservation. The three factors were the concentration of basal medium (2.2 gL^{-1} and 4.4 gL^{-1}), type of carbon source (sorbitol, mannitol, and sucrose), and carbon source concentration (1, 2, and 3% w/v). Two controls evaluated were MS at 2.2 gL^{-1} and 4.4 gL^{-1}) without a carbon source. All treatments were supplemented with Gelrite® SIGMA. Treatments with 2.2 gL^{-1} of MS basal medium were solidified with 3.1 gL^{-1} of Gelrite®, and treatments with 4.4 gL^{-1} MS were solidified with 2.2 gL^{-1} of Gelrite®. The media pH was adjusted to 5.7 before sterilization by autoclave at 121 °C for 15 minutes. Five plantlets per treatment were cultured in 150×25 mm tubes. After being cultured in a conservation medium for six months, variables such as plantlet growth and root growth in millimeters and new shoots and leaves were evaluated.

The independent contribution of each variable on the induction of shoots and slow growth was analyzed by analyzing each response variable. Significant differences were calculated according to the LSD test at the 5% significance level using Stat graphics® Centurion XVI statistical software. Data were presented as means ± standard error.

RESULTS AND DISCUSSION

Clonal micropropagation from apices

This study presents the clonal propagation protocol by inducing *Rhyncholaelia digbyana* shoots from apices as explants. The induction of shoots from apices as an explant is possible because apical dominance is inhibited by the action of cytokinins, promoting the activation of axillary buds (de Souza *et al.*, 2021). An advantage of clonal propagation is that it provides greater genetic stability since meristematic activation is less favorable to somaclonal variation events (de Oliveira Costa *et al.*, 2020). In this study, clonal micropropagation was induced using Knudson C. This basal media is among the most used for micropropagation of orchids. It has been used in propagation from leaf explants of *Vanda cristata*. (Pathak *et al.*, 2022), as well as shoot tip culture by *Doritis pulcherrima* Lindl. (Mondal *et al.*, 2013).

The induction of shoots was significantly greater in the treatments with the highest concentrations of BAP (T3, T4, and T5). The highest average number of shoots was obtained using 21.60 gL^{-1} of KC with 8.80 μM BAP (T5) that showed a maximum multiplication rate (4.83±1.16, Table 1) like different authors (de Souza *et al.*, 2021) obtained between 5 and 7 shoots per explant by adding 2.0 μM BA to the MS medium. A similar response was obtained by Ram *et al.* (2014), where the maximum average shoot multiplication was 4.92 shoots per explant by adding 2.2 μM of BAP to the MS medium. It is considered an acceptable multiplication rate in an intermediate range. The Pareto chart standardized showed that BAP concentration had a significant effect on the variables of the number of shoots and leaves (Figure 1A and Figure 2A, respectively). This agrees with what was obtained by De Souza (2020), where the addition of BAP to the culture medium promoted the formation of axillary shoots from apices in *Trichilia pallida*. However, BAP concentration did not significantly affect shoot and leaf length (Figure 1B and Figure 2B, respectively). Regarding shoot length, lower concentrations of KC produced longer shoots. A significant effect of KC concentration was found for the variable of shoot length (Figure 1B) and number of new leaves (Figure 2A) at lower concentrations; these results can be related to the growth habit characteristics of epiphytic orchids, presenting nutritional minimal requirements (Mamani Sánchez *et al.*, 2022). While for shoot production and leaf length, there was no significant effect (Figure 1A and Figure 2B).

On the other hand, the number and length of leaves, T1 (17.80 gL^{-1} KC, 4.40 μM BAP), was the treatment that showed the highest number of leaves compared to the other treatments. (Table 1). T5 presented the highest number of new shoots and shoot length

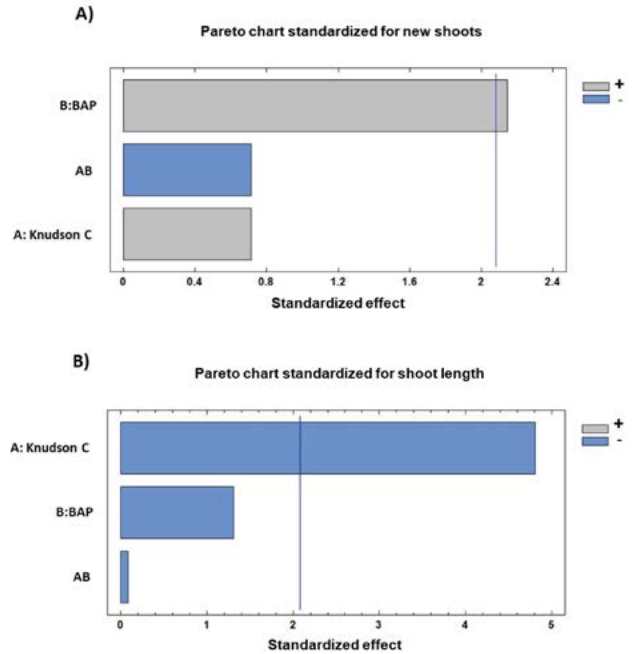

Figure 1. Main effects of benzylaminopurine (BAP) and Knudson C basal medium on the number of new shoots, shoot length during clonal micropropagation of *Rhyncholaelia digbyana* (Lindl.) Schltr.

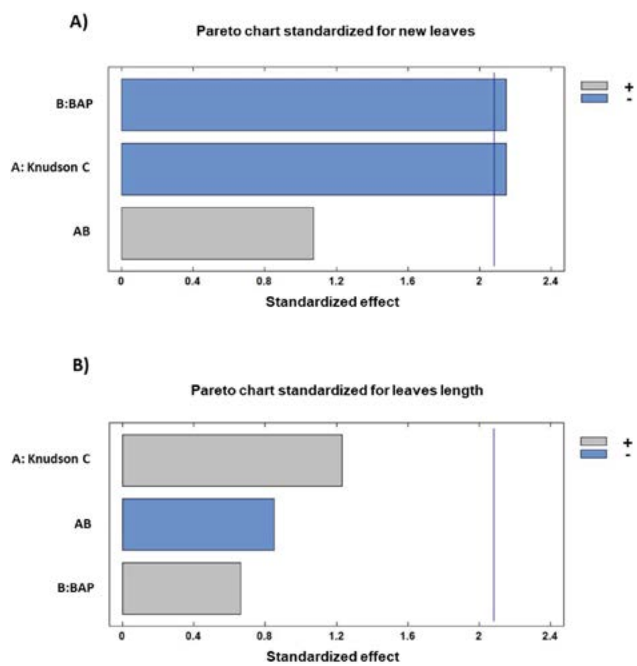

Figure 2. Main effects of BAP and the Knudson C basal medium on the number and length of leaves during clonal micropropagation of *Rhyncholaelia digbyana* (Lindl.) Schltr.

and was the third treatment with the largest shoots. Regarding the number of leaves, it was the second treatment with the highest number of leaves, so it was selected as the best treatment due to the morphology of the shoots with uniform size and the highest number of shoots produced.

Table 1. Clonal propagation from the apices of *Rhyncholaelia digbyana* (Lindley) Schlter.

Treatments	KC (gL^{-1})	BAP (μM)	Number of Shoots	Shoot length of the new shoots (mm)	Number of leaves	Leaves length (mm)
T1	17.80	4.40	3.33 ± 0.51^{a}	24.83 ± 4.30^{b}	3.83 ± 0.40^{b}	13.0 ± 1.89^{c}
T2	28.40	4.40	3.33 ± 1.75^{a}	14.83 ± 7.35^{b}	2.66 ± 1.36^{a}	9.33 ± 1.96^{a}
T3	17.80	12.0	4.33 ± 1.03^{ab}	19.83 ± 5.63^{ab}	3.00 ± 0.63^{ab}	11.0 ± 4.09^{ab}
T4	28.40	12.0	4.66 ± 0.81^{ab}	17.50 ± 3.50^{a}	3.33 ± 0.81^{ab}	8.50 ± 0.83^{a}
T5	21.60	8.80	4.83 ± 1.16^{b}	19.00 ± 3.68^{ab}	3.50 ± 0.54^{ab}	9.83 ± 1.47^{a}

Different letters indicate significant statistical differences according to the Tukey test ($p \leq 0.05$). KC: Knudson C Basal Medium, BAP: 6 benzylaminopurine.

Crop planning

The plan to produce approximately 10,000 seedlings of *Rhyncholaelia digbyana* was carried out, considering that T5 produces an average of 4.8 shoots. For planning, 40 apex explants from 40 mother plants were used, each apex being planted individually in a jar with its respective culture medium for the development of shoots. At the end of the cycle, 160 average seedlings were obtained; in turn, the apices were obtained from these seedlings. Subsequently, the formation of shoots was induced again. The multiplication process is repeated until 10,240 seedlings are obtained from 2,560 induction flasks (Table 2).

Slow growth for *in vitro* conservation

The slow growth of *Rhyncholaelia digbyana* was achieved using mannitol at 1% like potatoes (Chappell *et al*., 2020); However, on *Morus alba*, mannitol reduced growth but did not improve survival and physical condition of the explants during their *in vitro* conservation (Espinosa-Reyes *et al*., 2021). There is evidence that depending on the response of the specific genotypes to media containing carbon sources, in *Catasetum integerrimum*, *Castanea* spp., slow growth can be obtained using sucrose (López Puc & Herrera Cool, 2022; Gomes *et al*., 2021) the sorbitol was the best option for *Epidendrum chlorocorymbos* and *Vanilla planifolia* conservation (Lopez-Puc, 2013; Divakaran *et al*., 2006). The sorbitol allowed more development of all variables' growth evaluated in *Rhyncholaelia digbyana*. Hence, sorbitol can be an adequate way to allow development after a slow growth period. MS medium at 2.2 and 4.4 gL^{-1} allows the slow growth of *Rhyncholaelia digbyana* plantlets, while in *Vanilla planifolia*, MS concentration was 1.5 gL^{-1} (Divakaran *et al*., 2006). MS

Table 2. Crop plan for the production plants from apices of *Rhyncholaelia digbyana* (Lindley) Schlter with 1×4 rate multiplication of seedling.

Cycle number	Vegetal material source	Glass containers**	Plants obtained	Time (months)
1	40*	40	160	3
2	160	160	640	6
3	640	2560	2560	9
4	2560	2560	10240	12

* Seedlings (Initial source of apex explant). ** apex per glass container with 30 mL of 21.60 gL^{-1} Knudson C and 8.80 μM BAP.

at 2.2 gL^{-1} was used to conserve *Arnica montana* and Epindendroideae orchids (Petrova *et al.*, 2021; Menezes-Sá *et al.*, 2019). These results are relevant because there is scarce information about the *in vitro* propagation of *Rhyncholaelia digbyana*, so developing a clonal propagation protocol can be used for large-scale plant production. On the other hand, the slow growth protocol established will allow germplasm preservation, saving resources and replanting time.

The treatment with the lowest plant growth was SL5 (2.2 gL^{-1} of MS, 2% Sucrose); T5 had no significant difference with SL1-T4 and SL6-T12 treatments. Despite T5 being the best treatment for slow growth, the plant morphology was disproportionate since it had greater root growth in a proportion of 1 to 6 (2.75 mm of plant growth and 16.75 mm of root growth). In SL6 (2.2 gL^{-1} MS, 3% Sucrose), an average plant growth of 3.75 was observed, with 11 new leaves. However, in SL5 and SL6, the sucrose darkens the growing medium culture. Whereas SL10 (2.2 of gL^{-1} MS, 1% mannitol), an average of 5.25 mm of plant and root growth was obtained, 0.25 and 0.75 shoots and roots, respectively (Table 3), so this treatment was selected for the conservation of *Rhyncholaelia digbyana*. During a slow growth period, the carbon source had a significant effect on the variable for new shoots formed (Figure 3A), plant growth (Figure 3B), and new leaves formed (Figure 3C). The effect of the carbon source and MS strength had a significant effect on root growth variable (Figure 3D).

Table 3. Seedlings development of *Rhyncholaelia digbyana* (Lindley) Schlter in slow growth conditions.

Treatments	MS (gL^{-1})	Carbon source (%)		Plant growth (mm)	Root growth (mm)	New shoots	New leaves
SL1	4.4	Sucrose	10	5.50 ± 3.3^{abc}	1.00 ± 0.8^{a}	1.00 ± 0.1^{ab}	9.00 ± 2.1^{abcd}
SL2	4.4		20	3.75 ± 3.8^{ab}	0.50 ± 1.0^{a}	0.50 ± 0.1^{a}	5.25 ± 1.7^{abc}
SL3	4.4		30	3.5 ± 2.3^{ab}	0.75 ± 0.9^{a}	0.75 ± 0.1^{ab}	6.5 ± 2.4^{abc}
SL4	2.2		10	4.0 ± 0.8^{ab}	14.75 ± 3.5^{ab}	0.50 ± 0.1^{a}	2.00 ± 0.7^{ab}
SL5	2.2		20	2.7 ± 0.9^{a}	16.75 ± 5.0^{ab}	0.25 ± 0.1^{a}	0.75 ± 0.2^{a}
SL6	2.2		30	3.7 ± 2.7^{ab}	11.00 ± 4.9^{ab}	1.25 ± 0.2^{abc}	0.75 ± 0.2^{a}
SL7	4.4	Mannitol	10	7.0 ± 2.8^{abcd}	14.75 ± 7.0^{ab}	0.25 ± 0.1^{a}	3.00 ± 1.1^{ab}
SL8	4.4		20	5.00 ± 6.2^{ab}	11.25 ± 3.9^{ab}	0.00 ± 0.0^{a}	1.50 ± 0.6^{ab}
SL9	4.4		30	6.50 ± 3.6^{abcd}	10.75 ± 8.3^{ab}	0.25 ± 0.1^{a}	2.00 ± 0.4^{ab}
SL10	2.2		10	5.25 ± 1.3^{ab}	5.25 ± 5.1^{ab}	0.25 ± 0.1^{a}	0.75 ± 0.1^{a}
SL11	2.2		20	5.25 ± 1.5^{ab}	15.75 ± 4.8^{ab}	0.25 ± 0.0^{a}	1.25 ± 0.2^{ab}
SL12	2.2		30	5.25 ± 1.0^{ab}	11.75 ± 2.3^{ab}	0.00 ± 0.0^{a}	1.50 ± 0.2^{ab}
SL13	4.4	sorbitol	10	10.75 ± 2.4^{cde}	15.25 ± 3.7^{ab}	4.00 ± 0.2^{bcd}	18.25 ± 5.4^{cde}
SL14	4.4		20	8.75 ± 2.9^{bcde}	12.00 ± 2.4^{ab}	5.25 ± 0.5^{d}	23.50 ± 5.5^{e}
SL15	4.4		30	8.25 ± 2.5^{bcde}	9.25 ± 2.0^{ab}	4.50 ± 0.3^{cd}	23.25 ± 6.9^{e}
SL16	2.2		10	12.75 ± 3.6^{e}	43.25 ± 11.0^{c}	5.75 ± 0.2^{d}	25.25 ± 3.3^{e}
SL17	2.2		20	11.5 ± 3.8^{de}	28.75 ± 9.1^{bc}	5.00 ± 0.2^{d}	21.25 ± 4.3^{de}
SL18	2.2		30	8.25 ± 2.9^{bcde}	21.25 ± 7.1^{abc}	3.00 ± 0.3^{abcd}	14.25 ± 2.7^{bcde}

Different letters indicate significant statistical differences according to the Tukey test ($P \leq 0.05$). MS: Murashige and Skoog basal medium.

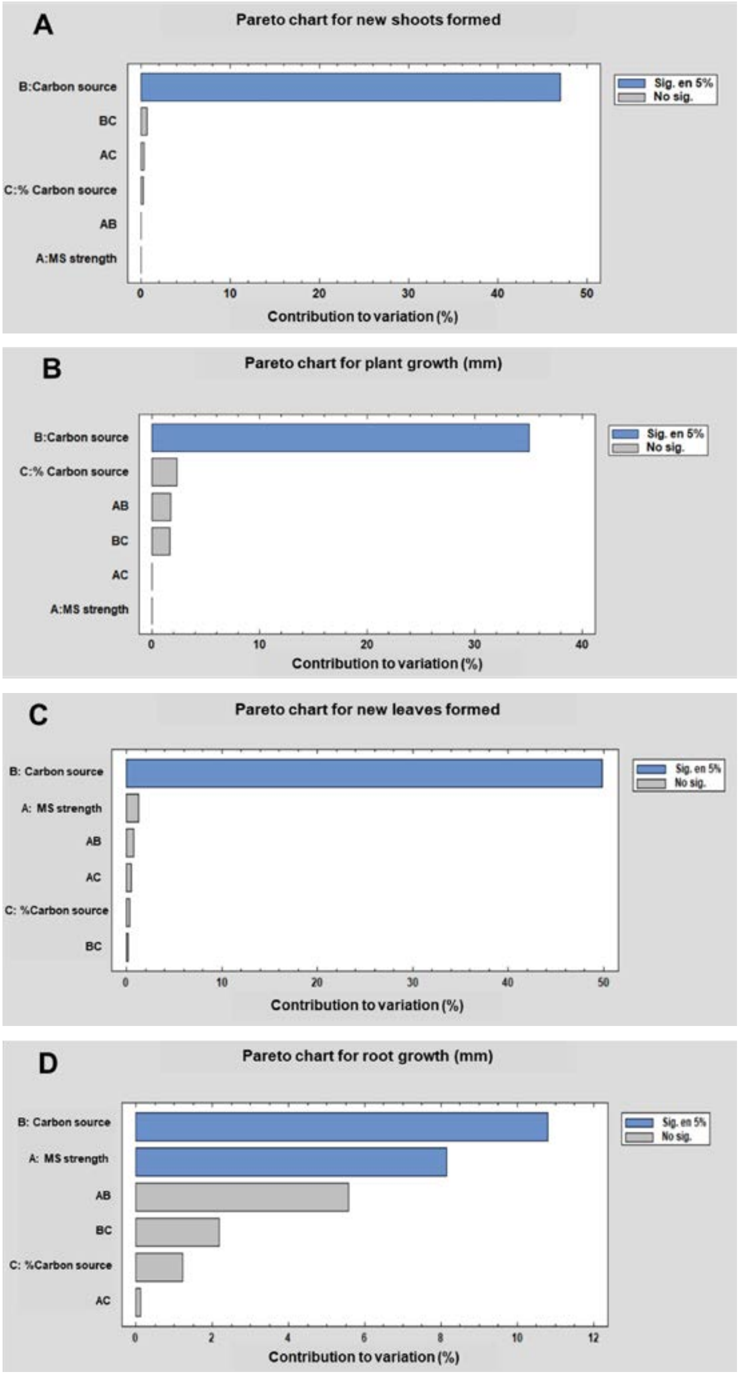

Figure 3. The Pareto charts for plant and root growth, and the number of shoots and leaves formed during the slow growth period of *Rhyncholaelia digbyana* (Lindley) Schlter.

CONCLUSION

This work represents the first report on micropropagation and *ex-situ* conservation to preserve germplasm for this species as an important resource for the floriculture industry.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Economic and Productive Impact of the Implementation and Use of Agricultural Irrigation in the State of Tabasco, Mexico

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ABSTRACT

Objective: Three support programs for agricultural irrigation in 8 municipalities in the State of Tabasco were evaluated for a total area of 972.4 ha with 7 crops per municipality. One production cycle was used for the evaluation of the irrigation units in the State of Tabasco.

Design/methodology/approach: The software "System for the Evaluation of Irrigation Units" SISEVUR 3.0 was used for the integration and evaluation of a) general operation of infrastructure; b) producer satisfaction with the infrastructure; c) aspects of improvement in the quantity and quality of production; d) benefits of irrigation on agricultural production and suggestions and opinions of producers regarding hydro-agricultural programs e). The economic/financial evaluation.

Results: The crops that responded best to the application of irrigation were: 1) forage corn, with an increase in production of 140.7%; 2) lemon with 97.98%; 3. banana with 58.6%, and 4 sugar cane with 41%.

Limitations on study/implications: Data collection required several visits to the producer to improve the collection of reliable data. However, there is a margin of error that could not be quantified due to the particularities of the producers and the work.

Findings/conclusions: Bananas and citrus improved production quality and product maintenance throughout the year, favoring supply and demand commitments in the domestic and international markets.

Keywords: irrigation, water efficiency, evapotranspiration, agricultural productivity.

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INTRODUCTION

The State of Tabasco, in Mexico, experiences abundant rainfall with an average of 2,543.8 mm*year^{-1} (Agenda Técnica Agrícola Tabasco, 2015). Although these rains might suggest that water needs for crops are satisfied, the temporal distribution is not optimal for ideal agricultural development despite being one of the wettest regions in the country (CONAGUA, 2014). Excess moisture is observed between June and December, while water deficits occur from February to May, negatively impacting production. Consequently, an increasing number of farmers are turning to irrigation systems as a solution (Mendoza-Hernández *et al.*, 2021).

State of Tabasco ranks first in terms of surface water resources availability in the country, as the Usumacinta and Grijalva rivers, which run through the region, transport more than 116 billion m^3*year^{-1} (Velázquez, 1994). However, this water resource does not fully benefit agricultural activity in Tabasco, which occupies an area of 223,089 hectares, composed of 111,574 ha of perennial crops and 111,515 ha of annual crops, the majority of which rely on rainfall (INEGI, 2017). Currently, only 3% of this agricultural land is equipped with irrigation systems, which approximately amounts to 6,692 ha (SEDAFOP, 2023).

Rainfall in State of Tabasco does not exhibit a homogeneous distribution throughout the year. During the dry season, precipitation falls below 100 mm/month, leading to a water deficit (Bamber *et al.*, 2012) that reduces productivity, especially in sensitive crops such as bananas (Martínez-Varona, 2013). To ensure optimal moisture conditions, the use of supplementary irrigation becomes indispensable. This plays a fundamental role in plantation management, contributing to maintaining fruit quality and productivity throughout the year (Castillo, 2005). Additionally, it has enabled farmers to venture into international markets (Cigales & Pérez, 2011).

With the aim of economically evaluating and financially assessing the Federal Government programs related to irrigation, this study utilized the 'Irrigation Units Evaluation System' (SISEVUR 3.0), provided by CONAGUA. These economic evaluations and financial assessments are essential for examining the efficiency, effectiveness, quality, and social impact of agricultural investment projects, in accordance with Article 26 of the Federal Expenditure Budget in Mexico.

MATERIALS AND METHODS

The irrigation programs assessed in this study were the Efficient Use of Water and Electric Energy Program (UEAEE-2008), the Full Use of Hydroagricultural Infrastructure Program (UPIH-2008), and the Modernization and Technification of Irrigation Units Program (MOTUR 2011 and 2012), which are federal government programs supported by the Ministry of Energy (2015) and CONAGUA (2014) (Mexican Official Standard NOM-006-2015). These programs were economically and financially evaluated using the 'Irrigation Units Evaluation System' (SISEVUR 3.0).

The study was conducted in eight municipalities in the State of Tabasco, selected based on their importance in terms of the number of irrigation works. The evaluated municipalities were Huimanguillo with 10 works (433.4 ha), Cárdenas with eight (211 ha), Teapa with six (149 ha), Balancán with two (90 ha), Cunduacán with two (43 ha), and the municipality of Centro with one (16 ha). A total of 29 irrigation works were evaluated out of a total of 31. The benefited crops, in order of importance according to the number of hectares, were sugarcane (*Saccharum officinarum* L.) with 253 ha, forage and grain maize (*Zea mays* L.) with 236.4 ha, lemon (*Citrus limon* (L.) Osbeck) with 220 ha, banana (*Musa paradisiaca* L.) with 175 ha, cut forage grass (*Panicum maximum* Jacq.) with 48 ha, pineapple (*Ananas comosus* (L.) Merr.) with 30 ha, and vegetables with 10 ha. In total, an area of 972.4 ha was evaluated.

The analysis of the irrigation program data (UPIH, UEAEE, and MOTUR for the years 2011 and 2012) was conducted in two stages: a desk stage and a field stage. The desk stage involved reviewing the 31 technical files containing information regarding: general data of the beneficiary producers, plot data (surface area and crop), type of hydroagricultural work performed (type of irrigation system installed), data on hydraulic utilization, concessioned volume, and data on the percentages of economic participation for the works of the state and federal governments, as well as the beneficiary producer. This information was recorded in an Excel spreadsheet for subsequent analysis.

The field stage consisted of verifying the condition of the installed irrigation infrastructure, general functioning of the irrigation system, crops benefited by irrigation, crop production data, depth of irrigation requirements, hydraulic expenditure, pumping hours, volume of water used, gross and net irrigation depth applied, irrigation efficiency, planted and harvested area, production value, and, importantly, beneficiary producers were asked about the attention received from government agencies to obtain support and their satisfaction upon completion and delivery of the irrigation work. Field information was obtained through the application of a structured questionnaire in three sections: a) characterization of the beneficiary producer; b) technical and operational data of the irrigation work; and c) production (harvested tons and selling price). All information was captured in the computer program 'Irrigation Units Evaluation System' (SISEVUR 3.0). With the collected data, the following indicators were calculated: 1) Gross Land Production (GLP) before and after irrigation, 2) Net Land Production (NLP) before and after irrigation, 3) Gross and net water production after irrigation, 4) Economic water efficiency, and 5) Water applied vs production ratio. The main objective was to determine the incremental difference in agricultural production due to irrigation use. To evaluate the production value at real prices in the 'after' scenario of irrigation use, the production value obtained 'before' irrigation use was updated using the monthly inflation rate issued by INEGI in Mexico. In the study, the Cost-Benefit Analysis method was used to evaluate the financial viability of irrigation programs. This method is based on quantifying benefits and costs in monetary terms, although in practice it is not always possible to quantify all benefits and costs accurately (Dupuit, 1844). To compare the values of gross (GLP) and net land productivity (NLP) before and after irrigation for sugarcane (CA), forage maize (MF), citrus (C), and banana (P), the Student's t-test for the mean of two paired samples was used with a significance level of 5% (alpha=0.05).

RESULTS AND DISCUSSION

The total investment in the 31 projects amounted to 35.17 million pesos (mp). The contribution from the three sectors (federal government, state government, and beneficiary producers) was as follows: the National Water Commission contributed 14.27 mp, the government of the State of Tabasco contributed 11.59 mp, and the beneficiary users contributed 9.31 mp.

The majority of the beneficiaries of these supports had a favorable opinion regarding the degree of satisfaction with the results of the programs (maximum=23/27), and regarding the management time of a project (fast=26/28), that is, from the initiation of procedures until the achievement of resources and signing of the commitment letter through the publication of results, as well as the opportunity of the available economic resources (excellent=22/27): which indicates that the programs are well-founded, have all the necessary elements for their operation, have clear rules, as well as well-established timelines, and the availability of resources was in a timely manner.

The opinions and suggestions of the beneficiaries agreed on the need for every farmer with irrigation works to be provided with a Training Course in irrigation management, to be offered technical assistance, and for the irrigation programs to be continued. They also expressed the need to simplify procedures to access financial support and to make them accessible to small landowners. This aligns with Aguillón's recommendations (2020) on the importance of providing training and technical assistance to producers for adequate irrigation management, as well as simplifying procedures to facilitate access to support, especially for small farmers.

An essential aspect was their opinion, when they stated that these programs have allowed them to improve productivity and the quality of production (70%), and that the above allows them to ensure production (30%). Pérez-Magaña *et al.* (2019) found that local agricultural practices such as crop diversification and water reuse also contribute to ensuring production, which could complement the strategies of producers in the State of Tabasco.

It was also observed that the volumes granted by CONAGUA were much higher than those used by the farmer, which meant that the use of water was lower than initially estimated and that the water was being used appropriately (Figure 1). Palacios-Vélez and Escobar-Villagrán (2016) mention that it is necessary to promote a culture of payment for water service and reduce subsidies to encourage more efficient management of the resource, which could be relevant considering the low use of water volumes concessioned in the State of Tabasco.

To carry out the financial evaluation of irrigation programs, it is necessary to assess the amount of the investment, as aforementioned. Figures 2 and 3 compare the Gross Land Productivity (GLP) and the Net Land Productivity (NLP), respectively, comparing before and after irrigation. It can be seen that for both productivity the increases of the "after" profits are consistent for the crops (SC) Sugarcane; (FM) Forage maize; (C) Citrus fruits and (B) Banana.

Nevertheless, profits are higher in banana and citrus cultivation, since, according to producers, the use of irrigation resulted in larger fruits, particularly in the case of lime. This statement is corroborated by the findings of Medellín-Azuara *et al.* (2010). Consequently, it is concluded that irrigation serves as an excellent catalyst for banana cultivation or that the crop responds favorably to the application of irrigation.

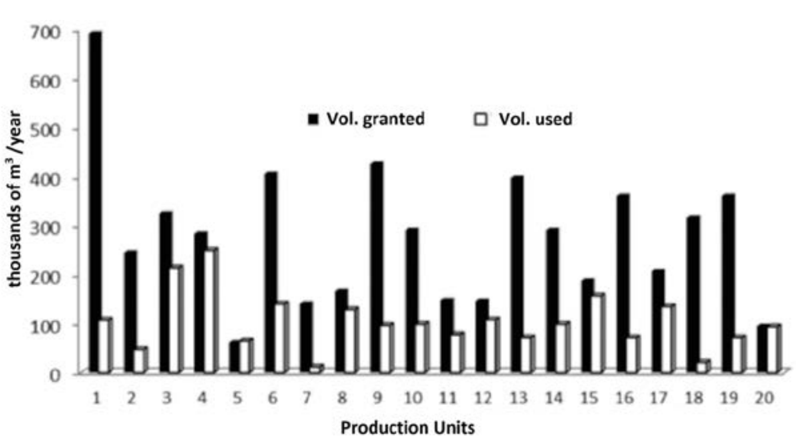

Figure 1. Behavior of water volumes granted *vs.* used.

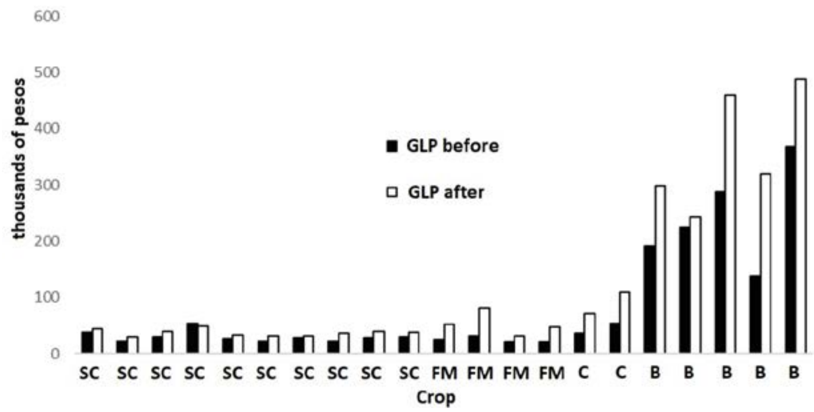

Figure 2. Gross Land Productivity in thousands of pesos (GLP), before and after irrigation, for sugarcane (SC); forage maize (FM), citrus (C) and banana (B).

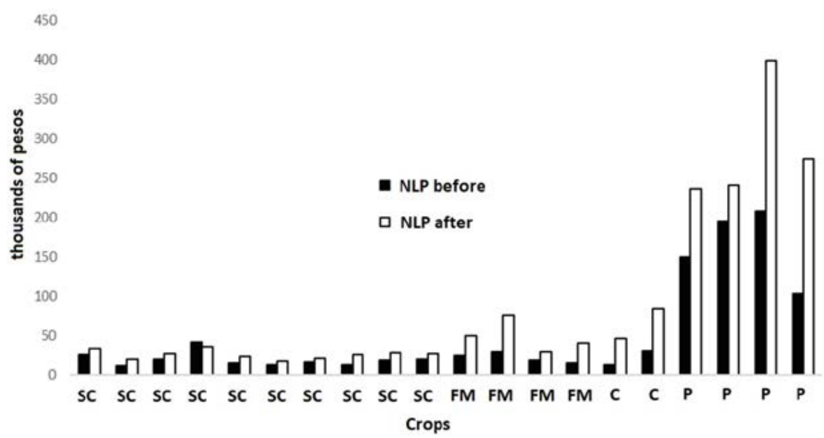

Figure 3. Net Land Productivity in thousands of pesos (NLP), before and after irrigation, for sugarcane (SC), forage maize (FM), citrus (C), and banana (B).

This production behavior is consistent with that reported by Colimba *et al.* (2021). The methodological approach and productivity and economic efficiency indicators developed by Villalobos-Cano *et al.* (2020) could be useful for further analyzing the impact of irrigation on the productivity and profitability of different crops in the State of Tabasco.

The aforementioned is confirmed by applying a Student's t-test, where significant differences were observed in the earnings before and after the application of irrigation (Table 1). These results for banana and lime trees can be attributed to the experience in irrigation management that producers of these crops already have in the region, as mentioned by Santacruz and Santacruz (2020). Irrigation management is a central factor that ensures applied water reaches the soil and plants, resulting in a favorable crop response. Hence, the importance of subjecting different irrigation methods to constant hydraulic evaluation. For the other crops, there were no significant differences, but this does not imply that they do not respond to irrigation, but possibly due to the limited experience of the producers, who had never used irrigation before (Table 1). Pérez-Magaña *et al.* (2019) emphasize the importance of social organization and customary rules among producers

Table 1. T-test for means of two paired samples. Gross Primary Productivity (GPP) and Net Primary Productivity (NPP) of land in banana cultivation.

	GPP		**NPP**	
	Variable 1	**Variable 2**	**Variable 1**	**Variable 2**
Mean	242	362	164	288
Variance	7962	11347	2288	5787
Observations	5	5	4	4
CCP	0.791		0.452	
DHM	0		0	
Degrees of freedom	4		3	
t Statistic	−4.100		−3.568	
P(T≤t) one-tail	0.00743		0.019	
t Critical one-tail	2.132		2.353	
P(T≤t) two-tail	0.0149		0.038	
t Critical two-tail	2.776		0.038	

CCP: Coeficiente de correlación de Pearson, DHM: Diferencia hipotética de la media.

for collective water management and irrigation infrastructure, which could be relevant for strengthening the adoption and proper management of irrigation systems in the State of Tabasco.

From the field data collected regarding the production of crops benefited by irrigation, it can be seen in Table 2 that the average production values increased for all crops. When the Student's t-test was evaluated, a value of $p \leq 0.05$ was obtained, so it was concluded that there were noteworthy differences between crop yields before and after irrigation.

It is worth mentioning that in addition to the increases recorded in production volumes (Table 3), the quality of production was also improved (Medellín-Azuara *et al*., 2010). As mentioned, (Yedra *et al*., 2016), irrigation is an auxiliary factor in production, which favors the plant and fruit vegetative development; and ensures quality in agricultural products, especially for export markets.

In this study, this could be confirmed, especially in the case of banana and lime trees, which allowed producers to increase their participation in the international market. As mentioned by Vidal *et al*. (2021) and Castelán-Estrada *et al*. (2021), banana is considered in State of Tabasco as one of the main drivers of the region's economy, since around half a million boxes are exported monthly to the United States, Asia, Europe, and Russia,

Table 2. Average value of gross land production (AVGLP) and Average value of net land production (AVNLP), in thousands of pesos per year.

Crop	**Before irrigation**		**After irrigation**	
	AVGLP	**AVNLP**	**VPPBT**	**VPPNT**
Sugarcane	30.4	37.5	19.5	25.7
Forraje maiz	24.8	52.8	21.6	65.5
Citrus	45.7	90.6	21.2	65.5
Banana	242.4	362.2	164.2	287.7

mainly. Furthermore, this allowed ensuring production volumes, a fundamental aspect in the international market. The quality of production could be determined considering the export volumes that were reported, in the case of lime trees up to 15%, and in the case of bananas up to 27.5% of the total production (data retrieved from producers).

The production costs of crops, ordered from lowest to highest investment in crop establishment are as follows: forage grass ($15,000*ha^{-1}), secondly, forage maize ($35,000*ha^{-1}), followed by bananas ($52,000*ha^{-1}), and finally, pineapples ($150,000*ha^{-1}). The production costs with irrigation increased on average by 38%; however, the profits also increased. For example, gross land productivity (GLP) increased by 60%, and net land productivity increased by 87%.

The authors Palacios-Vélez and Escobar-Villagrán (2016) address important topics such as the challenges of aquifer overexploitation, the methods to evaluate it, and management recommendations, in addition to techniques to update well censuses, the establishment of technical committees, and implementing artificial recharge projects. These aspects were not considered in this study; however, they are relevant for a more sustainable management of water sources used for irrigation. Other aspects to be delved into in similar studies, according to Ibarra Aguillón (2020), include public investment in irrigation programs, requested irrigation systems, and water savings achieved, which could complement the financial and water impact analysis of the evaluated programs.

Based on the information obtained regarding the economic and productive impact of agricultural irrigation in the State of Tabasco, several implications, solutions, and proposals can be identified that could be considered by government agencies in the state in order to improve and maximize the benefits of irrigation in agriculture.

Firstly, the study clearly demonstrates that the implementation of irrigation systems has had a significant impact on increasing the productivity and quality of crops, especially in the case of bananas, citrus fruits and sugar cane. This implies that the State government should continue investing in agricultural irrigation support programs and expanding their coverage to benefit more farmers. It is crucial to simplify procedures and processes so that small-scale farmers can easily access financial support.

Furthermore, the study also points out the importance of providing training and technical assistance to farmers in proper irrigation management. Therefore, it is suggested that the government of the State of Tabasco strengthen agricultural extension programs and provide specific training courses on efficient irrigation techniques and water management. This would help farmers optimize the use of water resources and maximize the benefits of their irrigation systems.

Table 3. Increases in crop production with the irrigation use.

Crop	Without irrigation	With irrigation	Increase %
	Tons/ha		
Forage Maize	27.0	65.0	140.7
Lemon	24.5	48.5	58.5
Sugarcane	61.7	87.0	41.0
Pineapple	80.0	110.0	37.5

Although the issue of insecurity has not been addressed in the article, some consulted farmers mentioned insecurity and the theft of irrigation equipment. It is essential for the State government to implement security measures and surveillance in agricultural areas where these systems have been installed. This could include collaboration with local authorities and the community to establish protection and reporting mechanisms against criminal acts that affect farmers.

Finally, considering the potential of banana and citrus crops to access international markets thanks to irrigation, it is recommended that the government promote association between farmers, research centers and entrepreneurs, and also provide support in marketing and exportation of these products.

The study on the impact of agricultural irrigation in the State of Tabasco offers valuable lessons and opportunities for the state government to take concrete actions for the benefit of farmers and rural development. Through continuous investment in irrigation programs, technical training, simplification of procedures, field security, and support in marketing, the State of Tabasco can maximize the potential of its agriculture and improve the quality of life of its producers.

CONCLUSIONS

The use of irrigation technology has represented a significant advancement in agriculture, increasing crop production and quality, which in turn has allowed continuous production and facilitated access to international markets. A 42% increase in sugarcane production was reported after adopting irrigation systems, accompanied by a notable increase in net productivity, reaching in some cases more than 70%. This advance translates into an increase in income and profitability, especially in crops such as bananas, which have managed to penetrate international markets and obtain higher prices. The economic benefits obtained motivate farmers to invest in advanced technology, which improves their quality of life. However, they face challenges such as insecurity, highlighting the need for irrigation training for more efficient management of water and economic resources, and to mitigate security problems.

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Morphological identification and characterization of the formation of floral primordium in *Vanilla planifolia* (Orchidaceae)

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ABSTRACT

Objective: To morphologically identify and characterize the formation of floral primordium and the individual flower development in *Vanilla planifolia* Jacks Ex. Andrews.

Design/Methodology/Approach: Inflorescence primordia and young inflorescences in different development stages were sampled from the stem internodes of the following positions: basal (11-15), middle (6-10), and distal (1-5). Four samples were taken from each stem position from February to May, with five repetitions per sampling date. Observations and characterization were made with a stereo microscope. The study site was located at Rancho Xanathtlan, in Barriles, municipality of Gutiérrez Zamora, Veracruz.

Results: The development of the *V. planifolia* raceme is described in seven phases: (I) differentiated meristem of the floral inflorescence; (II) appearance of the third bract; (III) initiation of the racemes formation; (IV) elongation of the floral primordium; (V) development and growth of the individual floral primordium in the acropetal direction, (VI) anthesis of the inflorescence in the acropetal direction; and (VII) complete flowering of the raceme. The development of the buds in the raceme is described in five stages from the appearance of the third bract in the acropetal direction.

Study Limitations/Implications: The biochemical processes and the interaction of environmental aspects on the floral development of *V. planifolia* pose questions that remain unanswered.

Findings/Conclusions: The first aspects of floral formation within the inflorescence of *Vanilla planifolia* were determined, along with its floral phenology.

Keywords: Floral growth, floral phenology, inflorescences, orchids.

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INTRODUCTION

Orchidaceae is one of the largest plant families on the planet, with approximately 28,500 recognized species (Novotná *et al.*, 2023). Given the commercial importance of their flowers, the *Cypripedium*, *Cattleya*, *Cymbidium*, *Dendrobium*, *Miltoniopsis*, *Phalaenopsis*, and *Zygopetalum* are considered valuable genera (López and Runkle, 2005). For their part, the ripe fruits of *Vanilla planifolia* have a high agricultural value (Lima-Morales

et al., 2021) and it is currently the second most expensive spice in the world after saffron (Bramel and Frey, 2021).

According to the FAO (2021), global vanilla production amounted to 8,000 tons until 2019. However, its price has decreased between 60 and 70% compared to the maximum prices obtained from 2017 to 2018 (Bramel and Frey, 2021). In Mexico, the yield of vanilla production has decreased over the past 25 years (Rocha-Flores *et al.*, 2018; Santillán *et al.*, 2018), as a result of its discontinuous annual flowering subject to environmental conditions over the years (Parada-Molina *et al.*, 2022), as well as premature fruit drop (Hernández-Miranda *et al.*, 2020).

V. planifolia yield is associated with vegetative and reproductive aspects, such as the number of shoots, flowers, and fruits (Rocha-Flores *et al.*, 2018). In that sense, studying floral phenology is essential, because it plays a substantial role in the production of vanilla fruits.

Studies about the anatomy of orchids are scarce, even if vascular patterns are fundamental for the correct development of the fruit (Gamboa-Gaitán, 2015). Consequently, the shape, development, and growth of inflorescences (floral raceme) usually have a great influence on floral production and crop yields (Ju *et al.*, 2012). For example, genus *Magnolia* has a wide floral morphological variation within the floral primordium in the basal, middle, and distal positions of each plant, as well as in the species that make up the genus (Gutiérrez-Lozano *et al.*, 2021). Likewise, wild orchids, such as *V. planifolia*, experience an irregular flowering between years and between different plant positions (Pfeifer *et al.*, 2006), resulting in low flowering.

Although vanilla is a very important species and a product of high culinary value (Borbolla-Pérez *et al.*, 2016; Bramel and Frey, 2021), the formation and development of the inflorescence during the transition to floral shoots within the different positions of the plant have not been described. Nevertheless, some components of its reproductive biology have been analyzed. This study aims to morphologically identify and characterize the formation of the inflorescence primordium and the individual development of the flowers in the inflorescence of *Vanilla planifolia*. The hypothesis was that, if a relationship was found between the morphological and anatomical changes of the *Vanilla planifolia* species, from the differentiated floral bud to the floral opening, the flowering process of the species could be understood.

MATERIALS AND METHODS

Experimental site

The study site was located at Rancho Xanathtlan, in the town of Barriles, municipality of Gutiérrez Zamora, Veracruz, Mexico (Figure 1). Table 1 shows the characteristics of the study area.

Sampling and definition of morphological variables

To morphologically identify and characterize the formation of inflorescence primordium and the individual development of *Vanilla planifolia* flowers, three inflorescences were collected per plant section. Different development stages of the

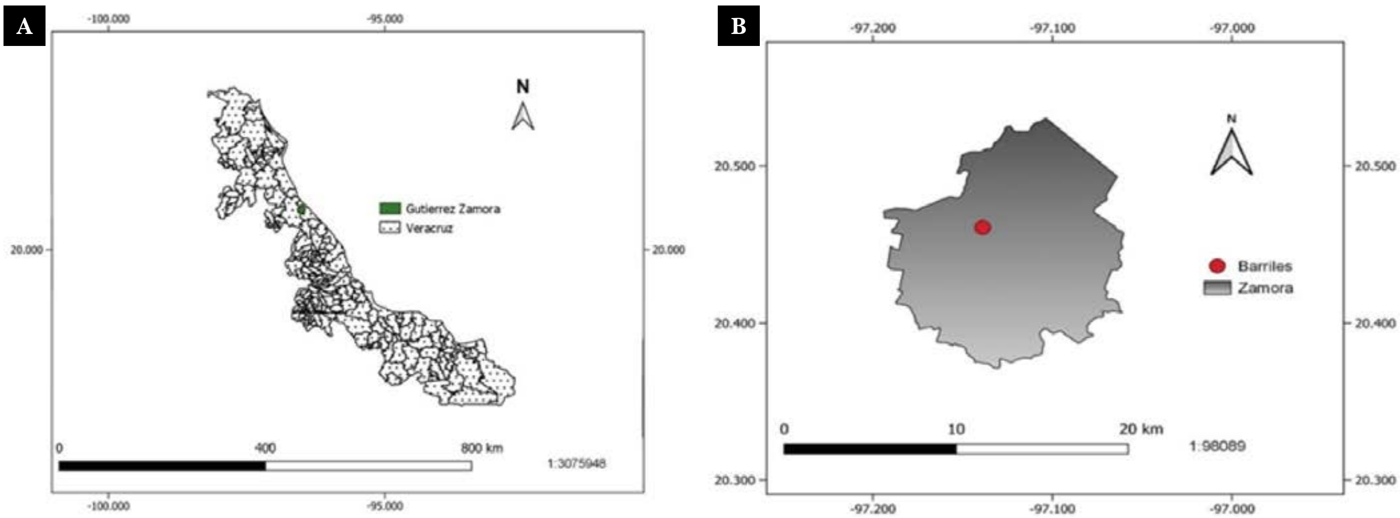

Figure 1. Location of the study site. A) Municipality of Gutiérrez Zamora, in Veracruz, Mexico B) Town of Barriles, in the municipality of Gutiérrez Zamora.

Table 1. Characterization of the *V. planifolia* agroecosystem in the study site.

Characteristics	Agroecosystem of the study site
Altitude	20 masl
Weather	Cw, temperate with summer rainfall
Precipitation	1200 -1500 mm
Soil type	Regosol
Predominant vegetation	Medium sub evergreen tropical rainforest

Source: INEGI (2022).

individual floral primordium were identified and they were classified based on the following morphological traits: appearance of the inflorescence bud, appearance of bracts, elongation of the rachis, and appearance and growth of individual floral primordium and anthesis. Monthly samplings were carried out to identify the morphological formation of the primordium, at the beginning of flowering: February 18, March 18, and April 19. Subsequently, before the floral opening, samples were taken every 15 days (May 4 and May 13, 2022), in order to observe the individual development of the flowers. The plant material was obtained from three different positions of the stem: basal (11-15 internodes), middle (6-10 internodes), and distal (1-5 internodes). A total of 5 repetitions were recorded for each plant section.

The tissues were placed in a FAA fixative solution (10% formaldehyde; 50% ethanol; 5% acetic acid; 35% water), and then rinsed and placed in a GAA solution (25% glycerol; 50% ethanol; 25% distilled water) for preservation. The length of individual floral primordia inside the floral cluster was measured with a digital vernier and their morphology was analyzed with a stereo microscope (Leica Microsystem Vertrieb GmbH, Wetzlar, Germany) and a digital camera (Rodríguez-Rojas *et al.*, 2021).

Scanning Electron Microscopy (SEM)

A modified version of the protocols proposed by Ruzin (1999) was used for this procedure. Individual flower buds, fixed and preserved in GAA, were sectioned in the median plane to study their internal structures. The medium fragments were postfixed in osmium tetroxide, 2% aqueous, and subsequently washed with distilled water and dehydrated in graded ethanols (50%, 70%, 96%, 100%, 100%). Subsequently, the tissues were dehydrated with a Samdri®-780A critical point dryer (Tousimis, MD, USA) and coated with gold/palladium with a Desk IV metallizer (Denton Vacuum, NJ, USA). Observations were performed on a JSM 6390 Scanning Electron Microscope (Jeol, Japan) at the Unidad de Microscopía Electrónica of the Colegio de Postgraduados.

Morphological and anatomical evaluation

According to Feng *et al.* (2021), different phases of floral development can be identified in relation to morphological and anatomical changes of each genus and species. Wei *et al.* (2010) described the development of a single flower of the genus *Phalaenopsis* and identified seven development phases: 1) initial differentiation, 2) differentiation of the inflorescence primordium, 3) differentiation of the flower primordium, 4) differentiation of the sepal primordium, 5) differentiation of the petal primordium, 6) differentiation of the column, and 7) development of the pollinium.

RESULTS AND DISCUSSION

Based on the morphological measurements and the references described by Feng *et al.* (2021), seven development phases were identified in the floral raceme of *V. planifolia*, from the differentiated floral bud to its floral anthesis: (I) formation of the differentiated inflorescence meristem, (II) appearance of the third bract, (III) initiation of the formation of the floral raceme, (IV) elongation of the floral primordium, (V) development and growth of the floral primordium in an acropetal direction, (VI) beginning of the acropetal floral anthesis, and (VII) complete flowering of the raceme (Figure 2).

From phase IV of the development of the floral raceme, each of the floral primordium (buttons) of the inflorescence (floral raceme) can be clearly identified, due to their acropetal development (from the base to the apex) (Figure 3).

In this floral branch, the floral primordia were classified into five development stages according to their size. In order of appearance within the floral cluster, they were ordered from the youngest (apical) to the most developed (basal) (Figure 3).

In the *Lantana camara* species the inflorescences on the floral branches have an acropetal development. Since they are arranged in a spiral phyllotaxy from the axis, the most developed inflorescences are found in the basal part, while the inflorescences in the apical part are still in bud (Caroprese *et al.*, 2011). This type of acropetal development with spiral phyllotaxy was similar to the one recorded in *V. planifolia*. Ontogenic events occur for all species; therefore, it is important to know where and when certain genes are activated, as well as the self-organized dynamics that are generated from their interactions (Álvarez and Roces 2002).

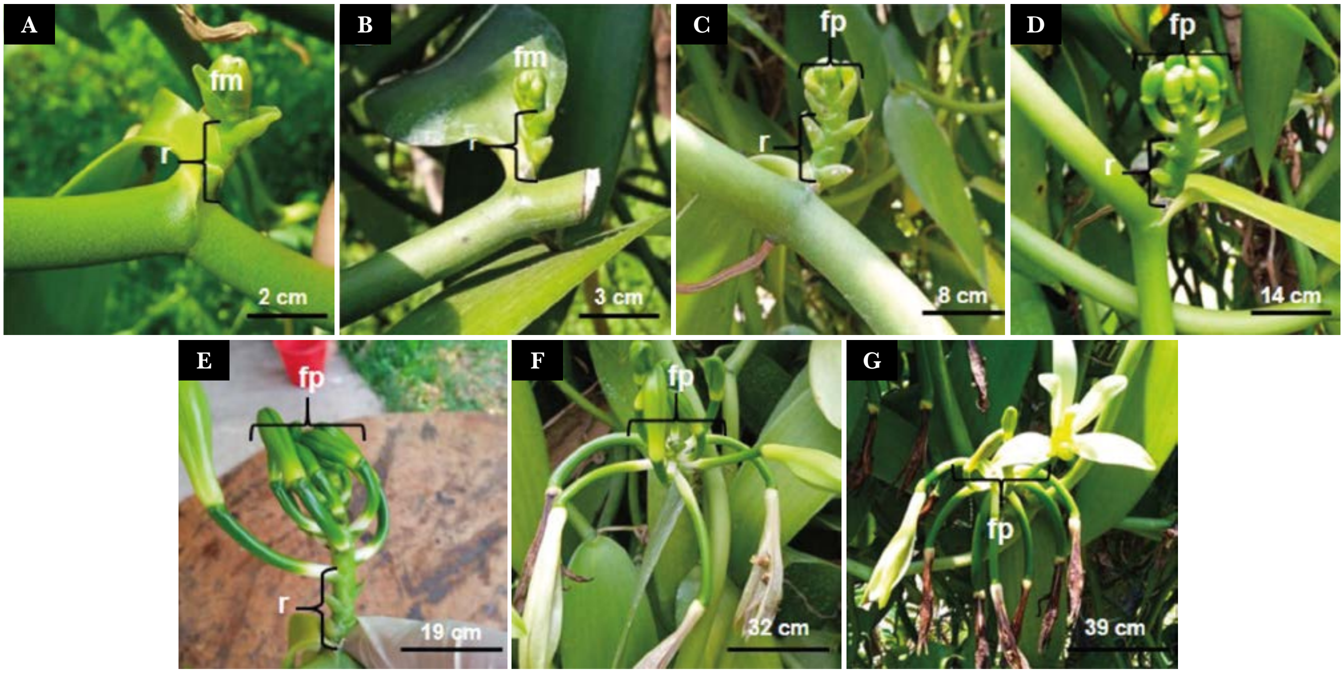

Figure 2. Development of the inflorescence (floral raceme) in *Vanilla planifolia*, from the differentiation of the flowering shoot to complete anthesis of the raceme. A. Phase I, recorded on February 18, 2022; B. Phase II, recorded on March 18, 2022; C. Phase III, recorded on March 18, 2022; D. Phase IV, recorded on April 19, 2022; E. Phase V, recorded on May 4, 2022; F. Phase VI, recorded on May 4, 2022; and G. Phase VII, recorded on May 13, 2022. fm=floral meristem; r=rachis; fp=floral primordium.

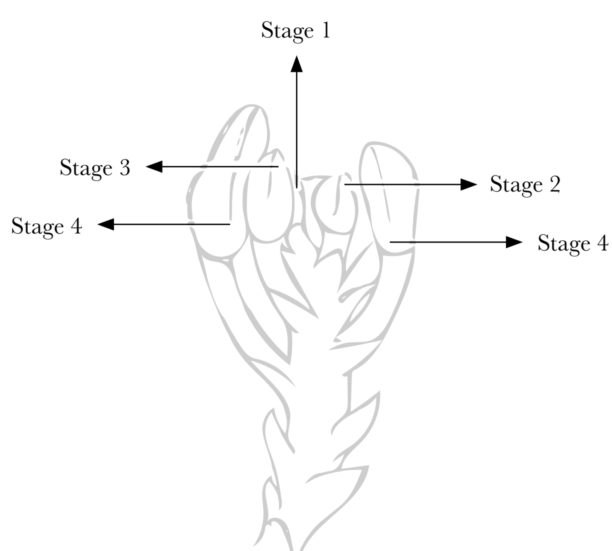

Figure 3. Representation of the acropetal formation (from the base to the apex) of floral primordium within the floral raceme of *Vanilla planifolia*.

Figures 4 and 5 describe the developmental stages observed in the first basal bud of *V. planifolia* inflorescence sampled on March 18 and the structural changes occurring over time showing morphological changes and associated dates. Detailed observations of morphological changes were recorded on five key dates, from March 18 to May 13; Stage

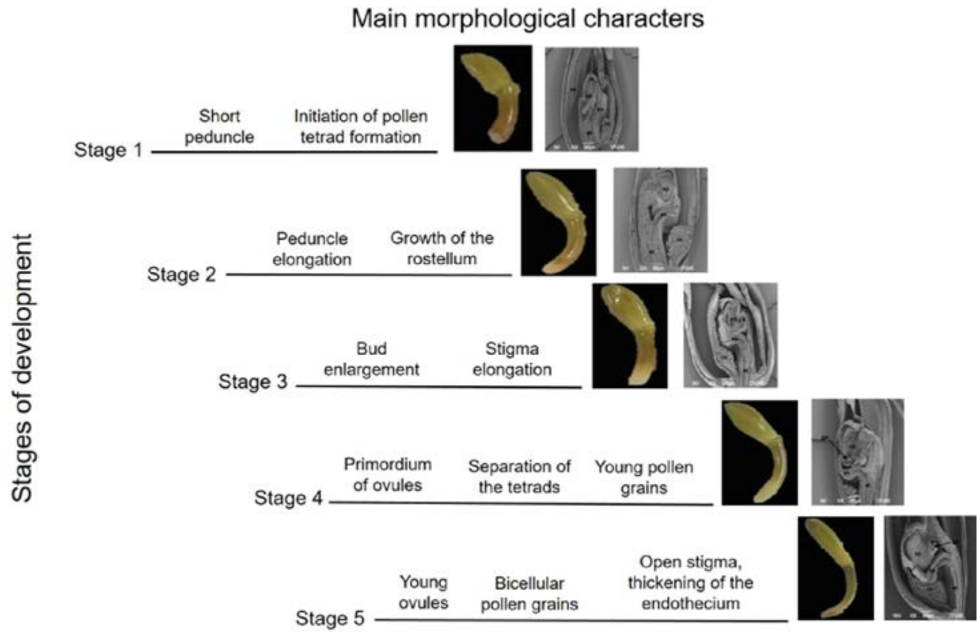

Figure 4. Flower bud development from the appearance of the first bract on the inflorescence of *V. planifolia*. Structural characters that characterize five stages of development. Stage 1 corresponds to the first flower bud of Phase III with dimensions of 6 mm long by 5.3 mm wide.

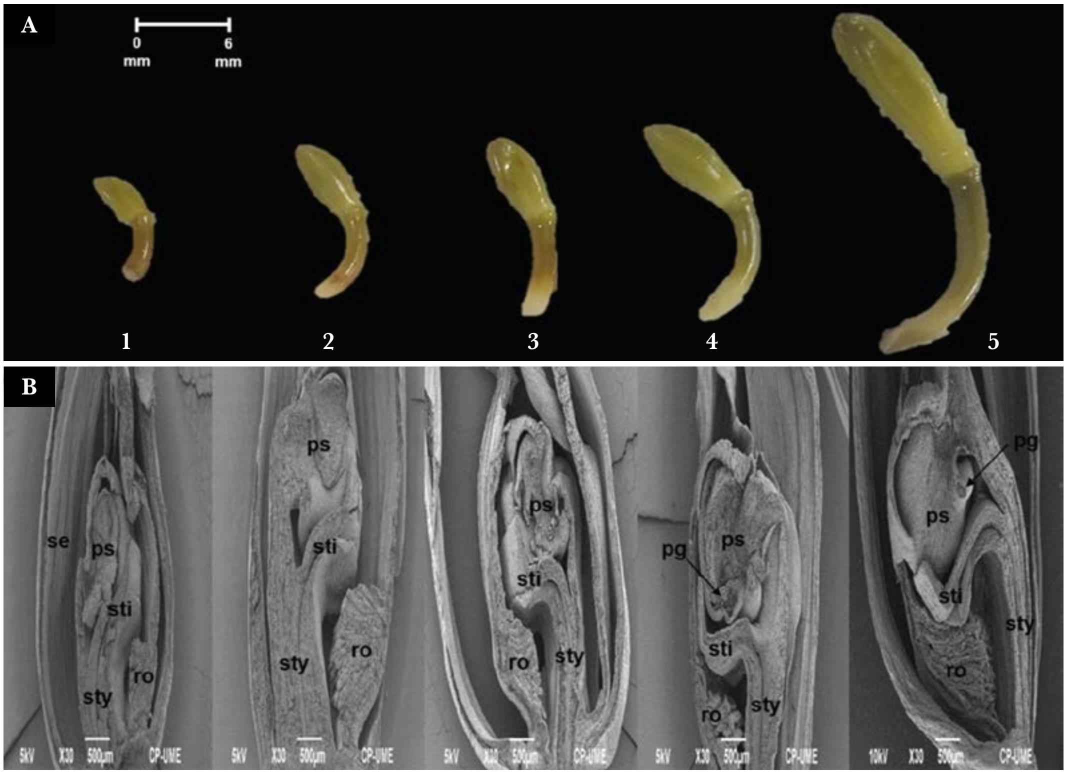

Figure 5. Morphology and structure of the floral buds at different stages of development in the raceme of *V. planifolia*. A. External morphology of the 5 stages of development: 1. Stage 1, 3.51-6.50 mm; 2. Stage 2, 6.51-9.50 mm; 3. Stage 3, 9.51-12.50 mm; 4. Stage 4, 12.51-16.50 mm; 5. Stage 5, 16.51-20.50 mm. B. Scanning microscopy of the microstructure of the development stages described in Figure 4. sti=stigma; sty=style; pg=pollen grains; ro=rostelo; se=sepal; ps=pollen sac.

1; the flower bud has a short peduncle (ovary+accessory tissue) and the pollen tetrads begin to form on the pollinium. Stage 2; the elongation of the peduncle and the growth of the rostellum were the crucial indicators of this stage. Stage 3; the enlargement of the floral bud and the elongation of the stigma were highlighted. Stage 4; it is characterized by the beginning of the ovule emergence from the placenta, and the disappearance of the callose and the separation of the tetrads, leaving the young pollen grains free, contained in an average floral bud of 12.51 mm. Stage 5; this is the stage prior to anthesis, characterized by a pronounced elongation of the peduncle containing the ovary, the ovules are still young, but the pollen grains are bicellular and the endothecium shows thickened walls. The thickening of the endothecium is the preparation for flower anthesis. Also at this stage the stigma is split in two, showing glandular cells at the internal epidermis. It is evident that the development of the ovules is late with respect to the development of the pollen grains, which mature first (Figure 5A-B). But these observations provide the basis for future studies of the ovule and embryo sac.

The orchid *Laelia anceps* has similar developmental sequence characteristics to those reported in this research; therefore, the growth and floral development variables were similar to each other with regard to the section within the plant (Sánchez-Vidaña *et al.*, 2018). As mentioned by Gutiérrez-Lozano *et al.* (2021), this phenomenon may be caused, to a large extent, by the external factors found in the milieu of the species; therefore, as long as the plant has the necessary conditions to carry out the floral transition within all plant positions, a similar growth and development of floral meristem within the plant will be recorded (Aguilar-Delgado *et al.*, 2018).

Thus, during flowering, *V. planifolia* shows that the growth and development of the floral meristems is more intense in the distal section of the stem, since in this position the meristems are more exposed to external factors, such as light and temperature (Sánchez-Vidaña *et al.*, 2018).

However, many questions remained unanswered. Although this study is very similar to the work of Caroprese *et al.* (2011), Aguilar-Delgado *et al.* (2018), and Gutiérrez-Lozano *et al.* (2021), most of the documented information corresponds to plants of specific growth. Therefore, species of indeterminate growth (*e.g.*, planifolia and the genus *Vanilla*) may have similar floral characteristics to species of determinate growth and fruit trees.

CONCLUSIONS

This research shows the first aspects of the development of floral buds within the floral raceme of *Vanilla planifolia*. Important characteristics about the flowering of the species were observed in each development stage. This is a major discovery in the otherwise scarcely studied flowering of the Orchidaceae family.

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Update on the terrestrial orchid flora of the Tacana volcano and close area, Chiapas, Mexico

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ABSTRACT

Objective: To assess the number of terrestrial orchid species on the Tacaná volcano, Chiapas, and to empirically observe distribution/elevation patterns and ecological conditions.

Design/Methodology/Approach: Random line transect sampling, over two years, for a total of 8 sites, each starting from the vicinity of rural villages. Samples were deposited in the CICY herbarium and analysed using dichotomous keys and field photographs. New records were compared with the GBIF data distribution.

Results: 52 different taxa have been identified. 24 species are new records for the Soconusco region, where the Tacaná volcano is located. In this way, the Soconusco region becomes the richest Mexican region for the number of orchid species, joining a total of 351 species.

Study Limitations/Implications: Building an accurate prediction model based on environmental and topographic variables could suggest microsites within the Tacaná Park that we have not visited for practical and technical reasons.

Findings/Conclusions: The total number of taxa in the Soconusco region increases to 351. Terrestrial orchids are not usually studied in depth in tropical places, but they are also important in the ecological balance of the natural site. A specific inventory could show more richness of tropical ecosystems.

Keywords: checklist, new botanical records, Orchidaceae, protected area, Soconusco.

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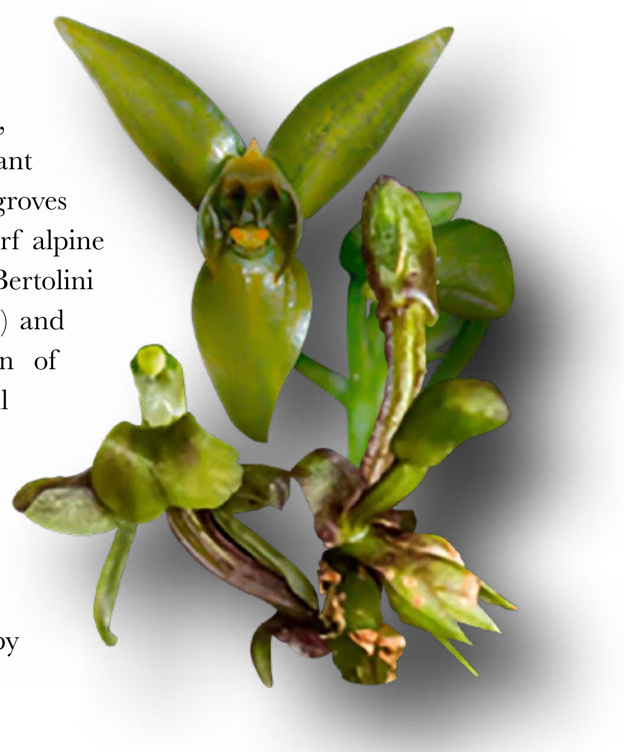
INTRODUCTION

The Soconusco region of Chiapas, located on the Pacific slope of southeastern Mexico, is home to highly fertile soils and an important diversity of ecosystems, ranging from mangroves on the coast to tropical, temperate and dwarf alpine forests on the Tacaná volcano (4,092 m) (Bertolini *et al.*, 2016b). According to Rzedowski (1978) and Breedlove (1981), Soconusco has vegetation of tropical evergreen forest, mountain mesophyll forest, coniferous forest, *Quercus* forest and grasslands, and moor at the top. In addition, it has two priority terrestrial regions (Spanish acronym: RTP): El Triunfo-La Encrucijada-Palo Blanco (RTP 133) and Tacaná-Boquerón (RTP 135), set up by



the National Commission for the Use and Conservation of Biodiversity (CONABIO), and three biosphere reserves: Volcán Tacaná, La Encrucijada and El Triunfo, catalogued by the National Commission of Protected Areas (CONANP) (Arriaga *et al.*, 2000; Damon, 2013). The orographic-environmental heterogeneity of the area is suitable for a wide variety of ecosystems-ecological niches that allow for an important biodiversity. Soconusco, part of the Sierra Madre de Chiapas (SMC), has been the subject of research for about eight decades, with explorations by renowned botanists such as Matuda (1950a, 1950b), Miranda (1953) and Breedlove (1981, 1986). Recently, the SMC's floristic knowledge has been enhanced by botanists from various national institutions, with a focus on protected areas such as El Triunfo (Long y Heath, 1991; Williams, 1991; López-Molina, 2000; Pérez-Farrera, 2004; Pérez-Farrera y Miceli-Méndez, 2004; Pérez-Farrera *et al.*, 2012; Martínez-Meléndez *et al.*, 2008, 2009), La Frailesca (Bachem & Rojas, 1994) and La Sepultura (Castillo, 1996; Reyes-García, 2008).

In this sense, floristic knowledge is incomplete for areas such as the orographic system formed by Tacaná volcano and Cerro Boquerón, referred to here as the Tacaná-Boquerón region (RTB), which forms part of the Mesoamerican-Mexican Biological Corridor (CBMM). This corridor extends from the area of the great volcanoes of Guatemala and enters Mexico through the Sierra Madre de Chiapas, providing an important link between the reserves of the Tacaná volcano, El Triunfo, La Sepultura and La Encrucijada; la Selva del Ocote also maintains the unity of the protected natural areas of northeastern Chiapas, which extend into Guatemala through the Petén (CONABIO, 2024). The corridor allows the integration, continuity and conservation of biological and ecological processes of a Nearctic and Neotropical biota, as well as elements that probably originated in Mesoamerica (Mittermeier *et al.*, 1999). The RTB represents an environmental gradient (50 km long) from sea level on the Pacific coast to the summit of the Tacaná volcano, with variations in soil types, climates, plant communities and agro-ecosystems, and therefore a high biological richness, which is a priority for conservation in Mexico (Arriaga *et al.*, 2000). However, the lowland and intermediate areas of the RTB are affected by severe deforestation, intensive and extensive agricultural and livestock farming practices, increasing human population, poor road construction techniques and the effects of climate change (Arriaga *et al.*, 2000; Soto Arenas *et al.*, 2007a, 2007b; Challenger *et al.*, 2010). In addition, their highly fragmented habitats, with little continuity between forest remnants and loss of transition zones, affect the local flora and fauna of the areas concerned, increasing possible local extinctions (personal observation).

The Orchidaceae are one of the most charismatic groups of the Chiapas flora due to their great biological and ecological diversity. It has an estimated richness of 723 species (Beutelspacher and Moreno, 2018), more than half of the richness reported for Mexico, which is approximately 1,200-1,300 species (Hágsater *et al.*, 2005; Villaseñor, 2016). Chiapas occupies the first places of orchid diversity in Mexico (Damon, 2010) and Soconusco contributes an important part of its diversity: its geographical location is strategic for the connectivity of biodiversity of North and South America. Floristic studies of orchids in Chiapas are concentrated in protected areas such as La Lacandona (Soto Arenas, 1994), Montebello (Cabrera-Chacón, 2000; Soto Arenas, 2001), El Cañón del

Sumidero (Miceli *et al.*, 2009; Espinosa-Jiménez *et al.*, 2011), El Triunfo (Pérez-Farrera y Miceli-Méndez, 2004; Martínez-Meléndez *et al.*, 2009, 2011; Martínez-Camilo *et al.*, 2012) and la Selva del Ocote (Miceli, 2002; Moreno-Molina, 2010).

The RTB and adjacent areas have ideal abiotic conditions (such as diverse forest types) for Orchidaceae, considering the high presence of orchids (325 species) in the region, positioning it as the second richest in Mexico for Orchidaceae. (Solano *et al.*, 2016), second only to the area of El Momón-Las Margaritas-Montebello (Chiapas) with 333 especies (Soto-Arenas, 2001). Two new records have recently been reported for the site, ***Svenkoeltzia congestifolia*** (Orchidaceae) (Bertolini *et al.*, 2016a) and *Lockhartia hercodonta* (Orchidaceae) (Martínez-Meléndez *et al.*, 2017), total of 327 species for the RTB.

The following paper updates the knowledge of the terrestrial orchids of the Tacaná volcano. The scarce or non-existent information on terrestrial orchids from some parts of the area shows the great challenge of locating and identifying them, perhaps because most of them are tiny and seasonal.

MATERIALS AND METHODS

In 2016 and 2017, 102 specimens of terrestrial orchids were collected in the Tacana Volcano biosphere reserve. [licences SEMARNAT and CONANP y *Reserva de la Biosfera volcán Tacana*; files: SGPA/DGVS/04089/15, SGPA/DGGFS/712/4131/15 and REBIVTA /032/2015). 8 sites were sampled, four per year, using 1-3 random transects 5-8 km long and 4 m wide. 68 surveys were carried out, each lasting 4-6 hours. The 8 sites were located with the rural communities Peloponeso and Finca Perú Paris (municipality of Tapachula); Agua Caliente, Benito Juárez "El Plan", Milán and Santa María La Vega (municipality of Cacahoatán); Los Alpes and Chiquihuites (municipality of Unión Juárez) (Figure 1).

During the field trips, the habitat and its conservation were empirically assessed for future analysis. Collected samples were herbarised at the ECO-TA-H herbarium (Ecosur, Tapachula), 2 flowers per samples were preserved in a 90% alcohol solution of benzoic acid and glycerol. Blooms were photographed using a Sony alpha 37® camera and specimens were georeferenced using a Garmin GPS, Oregon 650®. The specimens were deposited in the CICY herbarium (*Centro de Investigación Científica de Yucatán, A.C.*).

RESULTS AND DISCUSSION

The 102 herbarium specimens were classified into 52 species of geophytic orchids (Table 1; Figure 2).

The altitudinal data show a higher frequency of species between 1,500 and 2,000 m a.s.l. with just over 30 species. On the other hand, between 2,000 and 2,500 m a.s.l. there were 20 species (Figure 3).

The highest number of specimens collected came from the villages of the municipality of Cacahoatán, with four different villages visited. The lowest number of specimens collected came from the municipality of Tapachula (Figure 4).

Habitats with the highest number of samples were from Tropical montane cloud forest (TMCF), coffee plantation (CP) and secondary forest (SF). Mood (M) and Pine forest (PF) were the poorest (Figure 5).

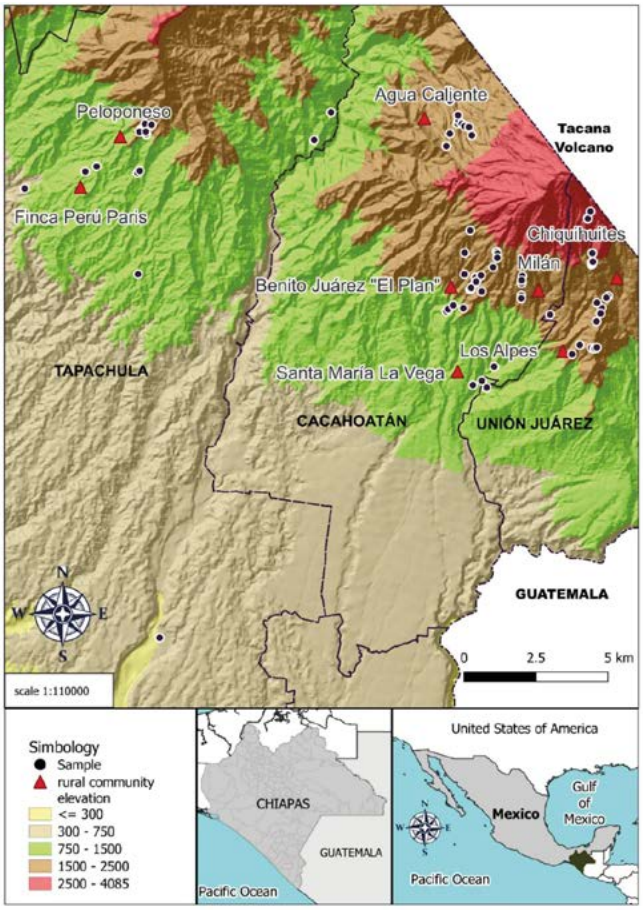


Figure 1. The location of sampled specimens: Tacaná volcano and the close area.

The empirical observations made on the state of conservation suggest that there is a degree of disturbance due to the anthropic impact of settlements and associated productive agricultural activities. The site we consider the best conserved is Agua Caliente, followed by Benito Juárez el Plan (both in Cacahoatán), and the least conserved is Chiquihuites (Unión Juárez), because we have observed microsites with high levels of disturbance and soil erosion due to logging.

These results show that ten of the records are novel for the state of Chiapas compared to previous estimates (Villaseñor *et al.*, 2016). In addition, 24 records are new for the study area compared with Solano *et al.* (2016). Underestimation of sampling in areas of low conservation interest could be a source of bias in the process of global biodiversity consensus, in our case for Orchidaceae in Mexico and Chiapas. These results highlight the importance of continuing to explore the various forests of the country, despite the fact that they are not priority areas for conservation or hard to explore. In addition, future botanical explorations and even detailed herbarium research, including sampling in areas of little interest as well as areas never explored, are likely to add to our knowledge of the country's floristic biodiversity with new records or the discovery of new species (ex.: Martínez-Camilo *et al.*, 2019).

Table 1. Taxa of terrestrial orchids found on Tacaná volcano and close areas. *TDF=Tropical dry forest; TMCF: Tropical montane cloud forest; CP=Coffee plantation, PF=Pine forest; M=Moor; FL=Fallow land; SF=Secondary forest. Distribution report for the area (DRA): NRS=new records versus Solano *et al*. (2016), NRV=new records versus both Solano *et al*. (2016) and Villaseñor (2016), Conservation status (+) according with NOM-059-SEMARNAT-2010 (SEMARNAT, 2010). Voucher CICY herbarium (VCH): number of samples (collector: V. Bertolini).

Taxa	Municipality	Elevation (m)	Habitat	VHC	DRA
Aulosepalum hemichreum (Lindl.) Garay	Tapachula	1107	TDF*	*10*	
Aulosepalum hemichreum (Lindl.) Garay	Unión Juárez	1815	TMCF*	*173*	
Bletia purpurata A. Rich. & Galeotti	Cacahoatán	1803	Road	*121*	
Bletia purpurea (Lam.) A.DC.	Cacahoatán	1107	TDF	*11*	
Cranichis apiculata Lindl.	Tapachula	1606	TMCF	*154*	NRS
Cranichis apiculata Lindl.	Cacahoatán	2031	TMCF	*162*	
Cranichis muscosa Sw.	Cacahoatán	898	CP*	*226*	
Cranichis notata Dressler	Cacahoatán	965	CP	*232*	NRV
Cranichis revoluta Hamer & Garay	Cacahoatán	1781	TDF	*118*	NRS
Cranichis wageneri Rchb.f	Cacahoatán	1803	TMCF	*120*	
Cranichis wageneri Rchb.f	Cacahoatán	1433	CP	*149*	
Cranichis wageneri Rchb.f	Cacahoatán	2133	TMCF	*168*	
Cranichis wageneri Rchb.f	Cacahoatán	1462	TMCF	*233*	
Cyclopogon comosus (Rchb. f.) Burns-Bal. & E.W. Greenw.	Cacahoatán	1749	SF*	*23*	NRS
Cyclopogon comosus (Rchb. f.) Burns-Bal. & E.W. Greenw.	Tapachula	1513	SF	*151*	
Cyclopogon comosus (Rchb. f.) Burns-Bal. & E.W. Greenw.	Unión Juárez	2340	TMCF	*203*	
Cyclopogon cranichoides (Griseb.) Schltr.	Unión Juárez	2570	TMCF	*17*	
Cyclopogon elatus (Sw.) Schltr.	Unión Juárez	3103	M*	*228*	NRS
Cyclopogon luteo-albus (A. Rich. & Galeotti) Schltr.	Cacahoatán	1703	SF	*19*	NRS
Cyclopogon luteo-albus (A. Rich. & Galeotti) Schltr.	Unión Juárez	1654	TMCF	*94*	
Cyclopogon luteo-albus (A. Rich. & Galeotti) Schltr.	Tapachula	1606	TMCF	*184*	
Cyclopogon luteo-albus (A. Rich. & Galeotti) Schltr.	Cacahoatán	1462	CP	*192*	
Cyclopogon miradorensis Schltr.	Cacahoatán	1983	TMCF	*25*	NRV
Cyclopogon miradorensis Schltr.	Unión Juárez	1639	TMCF	*29*	
Cyclopogon miradorensis Schltr.	Unión Juárez	1811	TMCF	*42*	
Cyclopogon miradorensis Schltr.	Tapachula	1513	SF	*151*	
Cyclopogon papilio Szlach.	Cacahoatán	1704	TMCF	*135*	
Cyclopogon papilio Szlach.	Cacahoatán	2020	TMCF	*166*	
Cyclopogon papilio Szlach.	Cacahoatán	1704	TMCF	*134*	
Cyclopogon prasophyllum (Rchb. f.) Schltr.	Cacahoatán	1785	SF	*24*	
Cyclopogon saccatus (Rchb. f.) Burns-Bal. & E.W. Greenw.	Cacahoatán	1719	SF	*222*	NRS
Epidendrum radicans Pav. ex Lindl.	Unión Juárez	1856	Road	*63*	
Epidendrum ramosum Jacq.	Unión Juárez	1856	CP	*56*	
Epidendrum verrucosum Sw.	Cacahoatán	1769	TDF	*117*	
Funkiella rubrocallosa (BL Rob. & Greenm.) Salazar & Soto Arenas	Unión Juárez	3159	PF*	*230*	NRV
Goodyera dolabripetala (Ames) Schltr.	Cacahoatán	2438	TMCF	*82*	NRS
Goodyera major Ames & Correll	Unión Juárez	1812	TMCF	*106*	NRV

Table 1. Continues...

Taxa	Municipality	Elevation (m)	Habitat	VHC	DRA
Goodyera striata Rchb.f	Cacahoatán	2328	TMCF	*132*	
Goodyera striata Rchb.f	Cacahoatán	2127	TMCF	*223*	
Goodyera striata Rchb.f	Unión Juárez	2441	TMCF	*227*	
Govenia superba (Lex.) Lindl.	Tapachula	1488	TMCF	*71*	
Govenia superba (Lex.) Lindl.	Cacahoatán	2250	TMCF	*74*	
Govenia superba (Lex.) Lindl.	Tapachula	1488	Road	*98*	
Habenaria distans Griseb	Cacahoatán	1759	TDF	*116*	NRS
Habenaria distans Griseb	Unión Juárez	1818	SF	*142*	
Habenaria distans Griseb	Unión Juárez	2449	CP	*144*	
Habenaria distans Griseb	Cacahoatán	1433	SF	*155*	
Habenaria distans Griseb	Cacahoatán	2031	SF	*165*	
Habenaria distans Griseb	Tapachula	1513	FL	*182*	
Habenaria distans Griseb	Cacahoatán	2127	TMCF	*224*	
Habenaria distans Griseb	Cacahoatán	1462	TMCF	*231*	
Habenaria distans Griseb	Cacahoatán	1462	TMCF	*233*	
Habenaria eustachya Rchb. F	Cacahoatán	2328	SF	*136*	
Habenaria eustachya Rchb. F	Unión Juárez	1818	TMCF	*138*	
Habenaria eustachya Rchb. F	Cacahoatán	1433	CP	*150*	
Habenaria eustachya Rchb. F	Cacahoatán	1885	CP	*157*	
Habenaria eustachya Rchb. F	Cacahoatán	2060	SF	*161*	
Habenaria eustachya Rchb. F	Cacahoatán	1440	CP	*179*	
Habenaria eustachya Rchb. F	Tapachula	890	SF	*189*	
Habenaria eustachya Rchb. F	Tapachula	1088	SF	*221*	
Kreodanthus casillasii R. González	Tapachula	1624	CP	*153*	NRS
Liparis arnoglossophylla (Rchb. f.) Hemsl.	Unión Juárez	2378	TMCF	*215*	NRV
Liparis cordiformis C. Schweinf.	Unión Juárez	2332	TMCF	*212b*	NRS
Liparis fantastica Ames & C. Schweinf	Unión Juárez	2332	TMCF	*212*	NRV
Liparis nervosa (Thunb.) Lindl	Cacahoatán	1462	SF	*225*	NRS
Malaxis histionantha (Otto) Garay & Dunst.	Tapachula	1282	FL	*66*	NRV
Malaxis lepanthiflora (Schltr.) Ames	Tapachula	2062	TMCF	*211*	
Malaxis lepanthiflora (Schltr.) Ames	Cacahoatán	2097	TMCF	*217*	
Malaxis macrostachya (Lex.) Kuntze	Cacahoatán	1882	TMCF	*124*	
Malaxis maianthemifolia Schltdl. & Cham.	Cacahoatán	1882	TMCF	*123*	NRV
Malaxis pandurata (Schltr.) Ames (+)	Unión Juárez	1935	TMCF	*61*	
Malaxis parthonii C. Morren	Cacahoatán	1746	TMCF	*78*	NRV
Malaxis parthonii C. Morren	Cacahoatán	2132	TMCF	*81*	
Malaxis parthonii C. Morren	Cacahoatán	1774	SF	*216*	
Malaxis parthonii C. Morren	Cacahoatán	1549	SF	*47*	
Malaxis parthonii C. Morren	Unión Juárez	1823	TMCF	*88*	
Malaxis parthonii C. Morren	Unión Juárez	2025	TMCF	*92*	

Table 1. Continues...

Taxa	Municipality	Elevation (m)	Habitat	VHC	DRA
Malaxis parthonii C. Morren	Unión Juárez	2004	TMCF	*93*	
Malaxis parthonii C. Morren	Unión Juárez	2004	TMCF	*95*	
Malaxis parthonii C. Morren	Unión Juárez	2004	TMCF	*96*	
Malaxis soulei LO Williams	Tapachula	1566	TMCF	*100*	NRS
Malaxis soulei LO Williams	Cacahoatán	2060	TMCF	*163*	
Microchilus lunifer (Schltr.) Ormerod	Cacahoatán	2056	TMCF	*167*	
Oeceoclades maculata Lindl.	Tapachula	1257	CP	*64*	
Pelexia funckiana (A. Rich. & Galeotti) Schltr	Cacahoatán	1705	TMCF	*176*	
Pelexia funckiana (A. Rich. & Galeotti) Schltr	Cacahoatán	1780	TMCF	*191*	
Pelexia funckiana (A. Rich. & Galeotti) Schltr	Cacahoatán	1661	TMCF	*201*	
Platythelys maculata (Hook.) Garay	Cacahoatán	1691	TMCF	*126*	
Platythelys maculata (Hook.) Garay	Unión Juárez	1744	TMCF	*84*	
Platythelys maculata (Hook.) Garay	Tapachula	1663	FL	*102*	
Platythelys maculata (Hook.) Garay	Cacahoatán	1433	CP	*148*	
Platythelys maculata (Hook.) Garay	Unión Juárez	973	CP	*214*	
Platythelys venustula (Ames) Garay	Tapachula	1624	CP	*152*	
Ponthieva triloba Schltr.	Cacahoatán	2328	TMCF	*131*	
Ponthieva tuerckheimii Schltr.	Cacahoatán	1704	TMCF	*110*	
Prescottia stachyodes (Sw.) Lindl.	Cacahoatán	2328	TMCF	*133*	
Prescottia stachyodes (Sw.) Lindl.	Unión Juárez	2449	CP	*145*	
Prescottia stachyodes (Sw.) Lindl.	Cacahoatán	1990	TMCF	*170*	
Psilochilus macrophyllus (Lindl.) Ames	Tapachula	1642	TMCF	*4*	NRS
Rhynchostele bictoniensis (Bateman) Soto Arenas & Salazar	Cacahoatán	2031	TDF	*164*	
Sacoila lanceolata (Aubl.) Garay	Tapachula	326	Urban street	*46*	
Sacoila lanceolata (Aubl.) Garay	Cacahoatán	947	Urban street	*206*	
Sarcoglottis cerina (Lindl.) PN Don (+)	Cacahoatán	1788	TDF	*196*	
Sobralia rogersiana Christenson	Cacahoatán	1780	TDF	*53*	NRS
Sobralia rogersiana Christenson	Tapachula	1488	TMCF / CP	*72*	NRS
Triphora trianthophoros (Sw.) Rydb.	Cacahoatán	1885	TMCF	*112*	NRV
Triphora trianthophoros (Sw.) Rydb.	Cacahoatán	1882	TMCF	*122*	

Between 1,500 and 2,500 m.a.s.l., the greatest diversity of specimens was obtained in terms of individuals collected (Table 1), as well as species diversity. At that elevation we can find the TMCF, the ecological niche with highest biodiversity in México and priority of conservation policy (CONABIO, 2024; González-Espinosa *et al.*, 2016, Ochoa-Ochoa *et al.*, 2010; Ochoa-Ochoa *et al.*, 2021). High altitude and mountain conditions appear to favour an increase in species diversity, and it is also suggested that these are conditions that have had a positive effect on species diversification in the past compared to other ecosystem types (Givnish *et al.*, 2016).

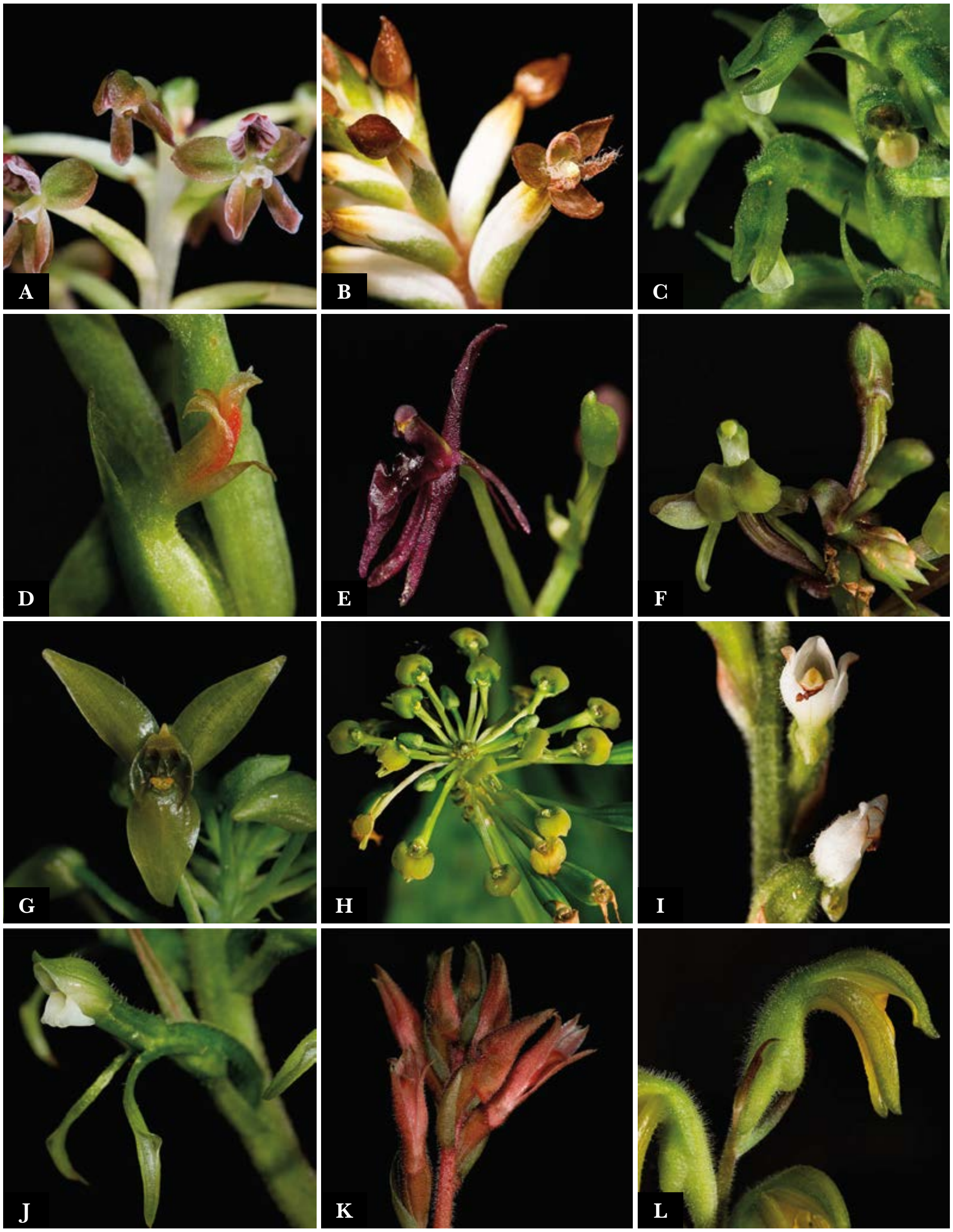


Figure 2. Example of microphotography of some specimens of terrestrial orchid of Tacaná. A) *Cranichis apiculata* Lindl; B) *Cranichis wageneri* Rchb.f; C) *Cyclopogon luteo-albus* (A. Rich. & Galeotti) Schltr; D) *Funkiella rubrocallosa* (BL Rob. & Greenm.) Salazar & Soto Arenas; E) *Liparis cordiformis* C. Schweinf; F) *Liparis nervosa* (Thunb.) Lindl; G) *Malaxis lepanthiflora* (Schltr.) Ames; H) *Malaxis parthonii* C. Morren; I) *Microchilus lunifer* (Schltr.) Ormerod; J) *Pelexia funckiana* (A. Rich. & Galeotti) Schltr; K) *Sacoila lanceolata* (Aubl.) Garay; L) *Sarcoglottis cerina* (Lindl.) PN Don (photographer: M.Sc. Eduardo Rafael Chamé Vázquez).

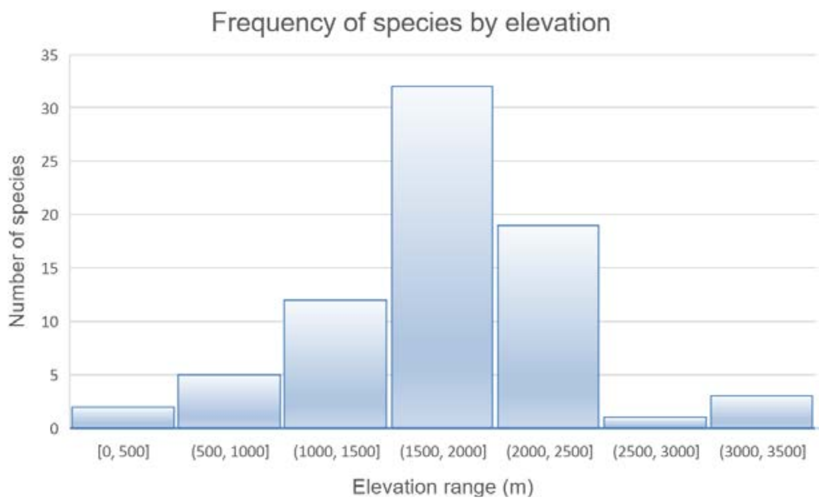


Figure 3. Frequency of species by elevation.

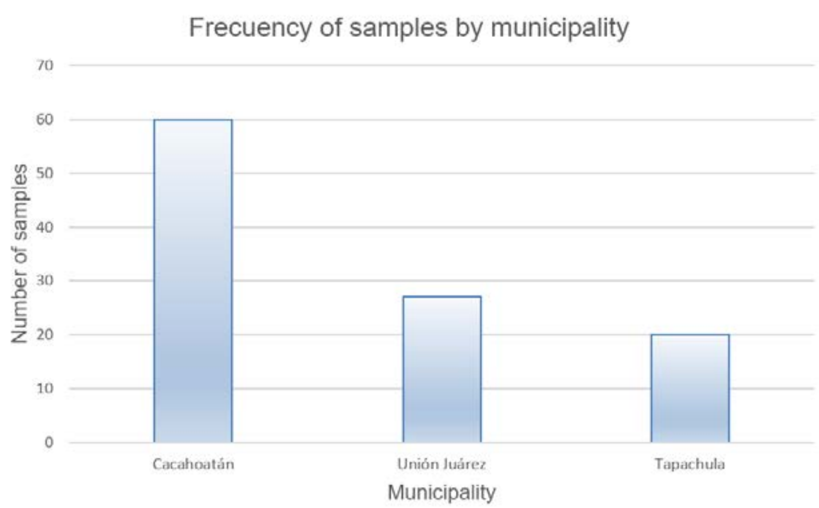


Figure 4. Municipality and number of samples.

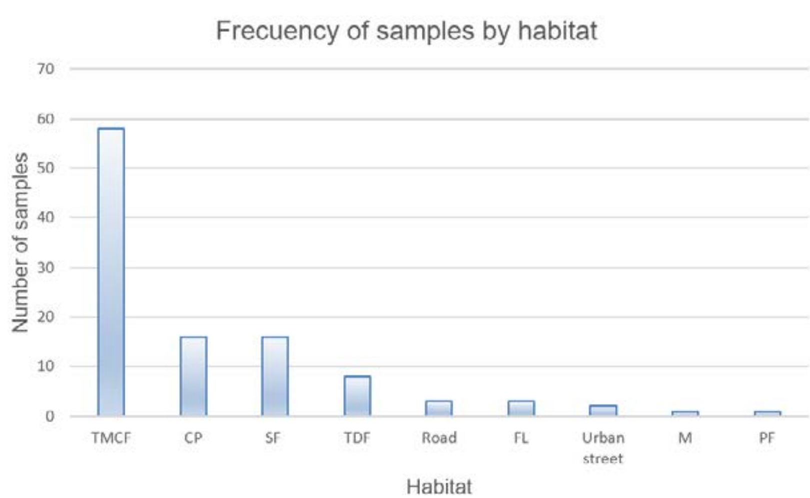


Figure 5. Different habitats and number of samples found.

Figure 6. *Psilochilus macrophyllus* (Lindl.) Ames *in situ*. Left picture shows a plant in bloom, the right one a plant with capsule.

Although fragmented, observations (*e.g.* increased vegetation cover, isolation from urban sprawl) suggest that there is still a high level of forest conservation. The conservation status of the sampled habitats lacks formal conservation studies and should therefore be prioritised for a systematic and timely assessment of scientific and certainly social interest (*e.g.* the important contribution of ecosystem services provided by forests) to provide objective and representative data to determine the conservation status of the flora.

Malaxis pandurata (Schltr.) Ames and *Sarcoglottis cerina* (Lindl.) PN Don are listed as priorities according to the NOM059-2010 (SEMARNAT, 2010). The data from this study position the RTP 135 Tacaná-Boquerón as the Mexican region with the highest number of Orchidaceae species in Mexico, followed by El Momón-Las Margaritas-Montebello with 333 species. These results suggest a total of 351 orchid species for this region of Mexico, which should be maintained as a high conservation priority. The orchid diversity is likely to increase with future explorations into less accessible areas of the Tacaná. For example, for Peru, for many years (Tamayo-Cen *et al.*, 2020), the genus Encyclia was only considered present in the country with eight species; however, during the last explorations, its diversity has risen to 13, of which three are new reports for the country and two are new entities in the process of publication (Tamayo-Cen *et al.* in press; Ocupa-Horna *et al.*, 2024). Finally, we recommend that researchers include a multidisciplinary approach in future floristic lists (when it is possible), which include as much information as possible from each collection, allowing these data to be useful in future research, such as conservation status analyses. Even the possibility of making calculations such as phylogenetic or taxonomic diversity should be included to provide different metrics-evidence that fit the needs of particular areas for conservation decision-making.

CONCLUSIONS

The terrestrial priority region 135 Tacaná-Boquerón represents an important source of biodiversity for Mexico. On this occasion we determined that the orchid record for this site is still incomplete, in reference to the terrestrial elements of this family. This is probably the case for many sites in Mexico. A careful scheme of field trips during different seasons of the year could detect new records providing further knowledge of Mexican biodiversity. With these latest findings, the Tacaná region reaches a total of 351 taxa of orchid species equal to taxonomic units.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Productive Characteristics, Nesting Substrates, and Colonies of the *Escamolera* Ant (*Liometopum apiculatum* M.) in Zacatecas, Mexico

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ABSTRACT

Objective: The objective of this study was to connect measurements, weights, and production of *escamoles* with nesting substrates, foraging paths, nest types, and colony sizes of the *escamolera* ant.
Design/Methodology/Approach: The data about nests, colonies, and larvae were gathered during morning and evening field walks, with the support of escamoles harvesters. The basic statistics of the data were estimated (N=59 nests/colonies) and analyzed with the Kruskal-Wallis H test. In addition, the Mann-Whitney U test was used to determine the differences per nest type.
Results: The highest production of escamoles was recorded in the *Prosopis laevigata* substrate (x=551.08 g/N=1), while the lowest production was recorded in the *Echinocereus stramineus* substrate (x=228.31 g/N=4). The length and width of the larvae (N=1,100 larvae) were similar in all the substrates. The weight of the larvae varied from 0.09 g, in the *Prosopis levigata* substrate, to 0.16 g, in the dry palm (*Yucca* spp.) substrate; therefore, 11,111 and 6,250 larvae are required, respectively, to obtain 1 kg of escamoles.
Study Limitations/Implications: The information of this study is limited to a single harvesting region.
Findings/Conclusions: The low escamoles production indicates that its harvesting must comply with a regulatory framework and a better organization, in order to guarantee the continuous presence of *Liometopum apiculatum* colonies.

Keywords: Conservation, edible insects, habitat, invertebrates, arid zones.

INTRODUCTION

Mexico has an exceptional natural wealth; however, only a fraction of all its native species has been studied. Biological diversity diminishes constantly throughout time (Martínez-Meyer *et al.*, 2014), despite the governmental policies aimed to stop environmental and wildlife degradation (Rosas-Rosas *et al.*, 2018).

Image by Cyril at Pixabay

The threats posed to biodiversity directly cause the extinction of species and change based on their taxonomic group. The major threats and causes include habitat destruction, pollution, overexploitation, introduction of exotic species, and recently climate change (Martínez-Meyer *et al*., 2014).

The *escamolera* ant (*Liometopum apiculatum* Mayr.) can be found in a wide variety of weathers and soils of 24 Mexican states. *L. apiculatum* is a thermophilic, monogynous, polyandrous, and omnivorous ant, with a low biological vulnerability (Berumen-Jiménez *et al*., 2021). The escamolera ant is ecologically and socioeconomically important for Zacatecas, Mexico (Rafael- Valdez *et al*., 2014; Briones-Santoyo *et al*., 2022). One kilogram of its larvae (escamoles) costs up to USD $30; consequently, they are harvested and sold without an appropriate control throughout the region.

As a result of its economic importance, the colonies of *escamolera* ants are overexploited (Briones-Santoyo *et al*., 2022) and their habitats are overgrazed. In addition, there are few studies about this species. Nevertheless, their nesting substrate (Rafael-Valdez *et al*., 2014), the use of their habitat (Cruz-Labana *et al*., 2014), their long-range foraging (Rafael-Valdez *et al*., 2014), the density of the nests (Cruz-Labana *et al*., 2019; Hernández-Roldan *et al*., 2017), the nutritional content (Cruz-Labana *et al*., 2018), and their conservation state (Berumen-Jiménez *et al*., 2021) have been studied.

Entomophagy is widely known and has substantially increased in Mexico and all over the world. Insects are sold in different portions and shapes in markets, street markets, and restaurants, as well as by domestic and international companies (Ramos-Elourdy *et al*., 2006).

The larvae and pupae of *Liometopum apiculatum* are appreciated for their taste and nutritional value. Currently, the demand from restaurants, high prices, and other local factors endanger the escamolera ant populations (Berumen-Jiménez *et al*., 2021). Although *L. apiculatum* is an important economic resource for rural communities in the arid and semi-arid regions of Mexico, this species faces problems such as overgrazed pasturelands, a lack of regulations for the management of its habitats (Hernández-Roldan *et al*., 2017), and the harvesting of its larvae.

Therefore, the objective of this study was to connect the sizes, weights, and production of *escamoles* with the nesting substrate, the foraging path, and the type and size of the *escamolera* ant (*Liometopum apiculatum* Mayr.) nests in Pánfilo Natera, Zacatecas.

MATERIALS AND METHODS

Study Area

The study was carried out in the General Pánfilo Natera municipality (Pámanes colony), Zacatecas, Mexico, during the 2022 *escamoles* harvesting season (March and April). Pánfilo Natera is located in the southeast of the state and borders with the municipalities of Trancoso and Guadalupe (north), Villa González Ortega (south), Ojocaliente (west), and Salinas and Villas de Ramos in San Luis Potosí (east). The study area is located 48 km to the southeast of the city of Zacatecas, at 22° 40’ 00” N, 102° 07’ 00” W, and 2,100 m.a.s.l. (INEGI, 2010).

Data Gathering

The data (8 variables) were obtained during morning (7 am to 1 pm) and evening (4 pm to 7 pm) field walks, with the help of escamoles harvesters. During these hours, soil temperature does not damage the ants.

The nesting substrate and number of foraging paths per colony were visually determined when the nest was found. In addition, the nests were classified as "new nest" or "disturbed nest (previously harvested)" during the larvae collection. The location of the nests was registered using a GARMIN ETREX GPS.

To determine the *escamoles* production per nest, each nest was dug, the plant material was removed from the nest, and the larvae were collected. Although sieves were used to remove plant material, soil, rocks, and other organic waste from the larvae, some impurities were still present. The larvae harvested from the nests in the field were weighted with a Silverline digital scale; afterwards, they were washed and the rest of the impurities were removed. The impurity percentage (60%) per nest was determined with the weight recorded in the field (larvae and impurities) and the weight recorded from clean *escamoles*. This value was used to determine the actual production of escamoles in subsequent nests. The size of the colony (small, medium, and large) was visually determined once the nests were opened and the quantity of ants was observed. Once the harvesting was completed, the nests were covered and protected with plant material (dry leaves, prickly pear, and palm).

The production of *escamoles* was estimated for each nest. Twenty larvae were collected per nest and were placed in Petri dishes; subsequently, they were frozen until they were analyzed. The weight (g), length (mm), and width (mm) of the samples were recorded. Weight was determined using an ADAM Nimbus® analytical balance, while the size of the samples was determined with a TRUPER® Vernier digital caliper. The data gathered in the field included: colony number, substrate number, nest size, nest type, number of foraging paths, nesting substrate, and production per nest. Microsoft® Excel was used to develop a database in the lab.

Statistical Analysis

The database consisted of dependent and independent variables. The dependent variables were escamoles production per nest and per substrate (g), as well as length (mm), width (mm), and weight (g) of the *escamoles*. Meanwhile, the independent variables were nesting substrate, colony size, number of foraging paths, and nest type (new nest or disturbed nest).

The data were verified with the Kolmogorov-Smirnov H test (normality) and Levene's test (homogeneity of variances).

The data analysis determined the basic statistics and contrasts of the variables; in addition, a multivariate clusting was conducted. The tables include the calculation of the mean of the data and the standard deviation $(\bar{x} \pm \mathrm{SD})$. The contrasts were used to compare the nesting substrate, the colony size, and the number of foraging paths. The Kruskal-Wallis H test was carried out to establish possible differences. Afterwards, the post hoc test of the Tukey method was used to specify the differences; values $<\alpha(\alpha 0.05)$ indicate significant statistical differences.

The *escamoles* production and larvae size per nest type (new or disturbed nest) were analyzed with a Mann-Whitney U test. The comparisons were explained by the mean and its standard error $(\bar{x} \pm \mathrm{SE})$.

The optimal number of clusters was calculated using the fviz_nbclust function of the factoextra package of RStudio. Subsequently, the k-means clustering was used for the multivariate analysis of four centroids. The data were standardized, using the Ward's method and the Euclidian distance. The contrasts were based on a significance level of $p<0.05$. All the data were analyzed using the Statistica 7.0 and Project Management Framework 6.3.1 (R Project) statistical packages and the graphs were developed using the SigmaPlot 10.0 software.

RESULTS AND DISCUSSION

This research analyzed data from 59 nests: 6 new nests and 53 disturbed nests. In addition, 8 nesting substrates were analyzed. The nesting substrates chosen by the ant colonies were prickly pear (*Opuntia ficus-indica*; 10 colonies), maguey (*Agave salmiana*; 16 colonies), palm (*Yucca filifera*; 15 colonies), strawberry cactus (*Echinocereus stramineus*; 4 colonies), mezote (dry prickly pear; 3 colonies) dry palm (2 colonies), mesquite (*Prosopis laevigata*; 1 colony), and bare soil (8 colonies). Out of the 59 ant nests, 29 were large colonies, 22 were medium colonies, and 8 were small colonies.

Regarding the foraging paths, 3 colonies had 2 paths, 23 colonies had 3 paths, 24 colonies had 4 paths, and 8 colonies had 6 paths.

Nesting Substrate

Maguey was the chosen substrate of *L. apiculatum*, followed by palm. This choice can change depending on the harvesting area. In Villa González Ortega, the escamolera ant nests are mainly located in maguey and palm also (Rafael-Valdez *et al.*, 2017).

The highest production of escamoles was recorded in the mesquite, prickly pear, and maguey substrates, while the lowest production was recorded in the strawberry cactus substrate (Table 1). According to Figueroa-Sandoval *et al.* (2018), the prickly pear substrate yielded a high volume of escamoles, while the maguey substrate recorded a low production. The nesting substrates are fundamental in the production of escamoles: they provide potential foraging areas and thermal protection (Juárez-Sandoval *et al.*, 2010). However, longevity, size, and nest type are also important factors that can change production and, therefore, must be taken into account.

The width and length of the escamoles were very similar in each substrate; nevertheless, the heaviest escamoles were found in the dry palm substrate and in bare soil (Table 1).

The Kruskal-Wallis H test established differences between the 8 nesting substrates. The following results were recorded: production ($KW7, 1180=40.36$, $p<0.01$; Figure 1A); length ($KW7, 1180=40.36$, $p<0.01$; Figure 1B); width ($KW7, 1180=43.07661$, $p<0.01$; Figure 1C), and weight ($KW7, 1180=62.47$, $p<0.01$; Figure 1D).

Table 1. Production, size, and weight of escamoles per nesting substrate, based on the mean and its standard deviation $(\bar{x} \pm \text{SD})$, in Pánfilo Natera, Zacatecas.

Nesting substrate	Production (g)		Length (mm)		Width (mm)		Weight (g)	
	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.
Strawberry cactus (*Echinocereus stramineus*)	282.31	186.96	8.56	0.92	4.72	0.43	0.12	0.03
Maguey (*Agave salmiana*)	431.04	280.29	8.87	1.22	4.64	0.57	0.13	0.04
Prickly pear (*Opuntia ficus indica*)	451.37	245.50	8.89	1.23	4.75	0.53	0.14	0.03
Mezote (Dry prickly pear)	380.02	330.66	8.57	1.32	4.60	0.50	0.14	0.04
Mesquite (*Prosopis laevigata*)	551.08	0.00	8.37	0.70	4.24	0.29	0.09	0.01
Palm (*Yucca* spp.)	350.32	298.05	8.46	1.23	4.59	0.56	0.14	0.04
Dry palm	351.02	263.62	8.57	1.45	4.39	0.48	0.16	0.07
Bare soil	334.34	239.66	8.80	0.99	4.70	0.44	0.15	0.04

±D.S.=standard deviation.

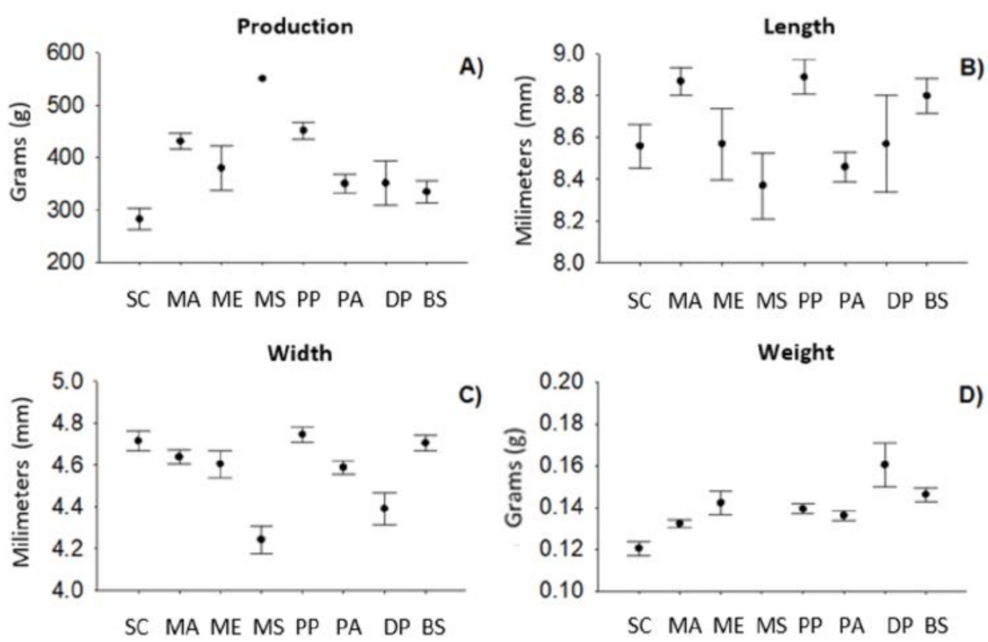

Figure 1. Comparison of eight nesting substrates based on production (A), length (B), width (C), and weight (D) of escamoles, as well as the mean ± standard error $(\bar{x} \pm \text{SE})$. SC: strawberry cactus, MA: maguey, ME: mezote, MS: mesquite, PP: prickly pear, PA: palm, DP: dry palm, and BS: bare soil.

Colony Size

Understanding the variables that better explain the presence of the escamolera ant is fundamental to improve the management of the colonies and the habitats; consequently, the size of the nests and the colonies must be determined (Jofré and Medina, 2012; Figueroa-Sandoval *et al.*, 2018). The *escamoles* production and weight are directly proportional to the size of the colony, because larger colonies have a higher average production (Table 2); however, the colonies had very similar sizes. According to Hoey-Chamberlain *et al.* (2013), a colony can house up to 250,000 ants and, consequently, an excessive population growth can impact the quality of the nest, reducing its productivity (Figueroa-Sandoval *et al*, 2018).

The Kruskal-Wallis H test established differences between the size of the colonies, regarding production (KW2, 1180=279.17, p<0.01; Figure 2A), length (KW2, 1180=63.82, p<0.01; Figure 2B), width (KW2, 1180=41.17, p<0.01; Figure 2C) and weight (KW2, 1180=78.32, p<0.01; Figure 2D).

Table 2. Production, size, and weight of the escamoles, per colony size, based on the mean and its standard deviation $(\bar{x} \pm \text{SD})$, in Pánfilo Natera, Zacatecas.

Colony size	Production (g)		Length (mm)		Width (mm)		Weight (g)	
	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.
Small	119.83	76.44	8.09	1.33	4.45	0.54	0.12	0.05
Medium	361.06	242.25	8.63	1.10	4.58	0.47	0.13	0.03
Large	485.43	277.21	8.93	1.16	4.74	0.55	0.15	0.04

±D.S.=standard deviation.

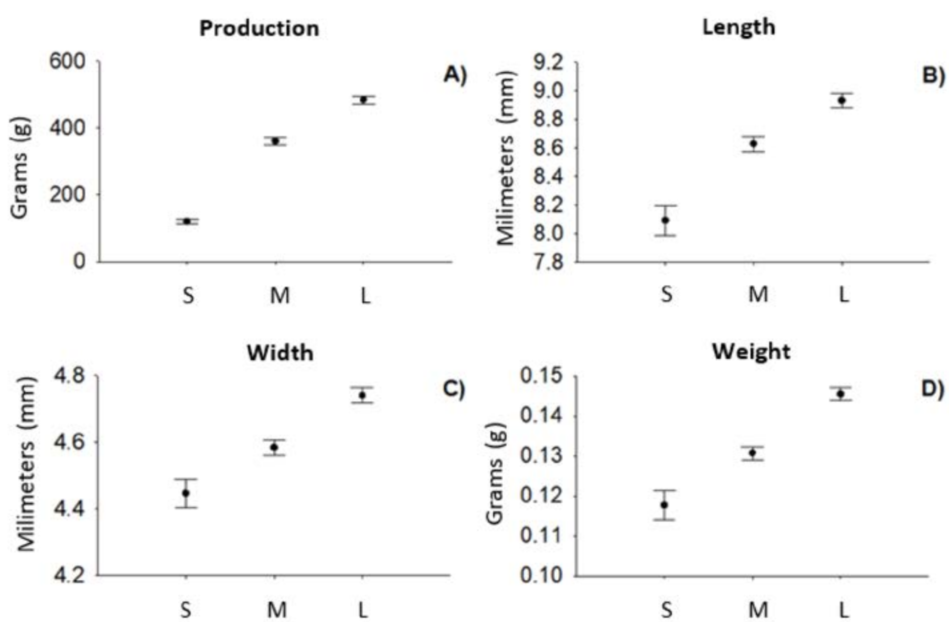

Figure 2. Contrast of the colony size of the *escamolera* ant: small (S), medium (M), and large (L), based on the mean ± standard error $(\bar{x} \pm \text{SE})$.

Foraging Paths

The colonies with four and five paths recorded the highest production, while the colony with six foraging paths registered the lowest production (Table 3). Usually, *escamolera* ant colonies have three, four, or five paths (Rafael-Valdez *et al*., 2017). The colony with six foraging paths recorded the highest *escamol* weight, followed by the colony with five paths (Table 3). Based on the method described by Rafael-Valdez *et al*. (2019), the following results were obtained: a foraging area of 607.25-2,467.2 m^2 and an average straight distance from the nest to the foraging substrate of 24.3±11.36 m.

The Kruskal-Wallis H test established differences between the number of paths per colony, regarding escamoles production ($KW4, 1180=76.75$, $p<0.01$; Figure 3A), length ($KW4, 1180=38.94$, $p<0.01$; Figure 3B), width ($KW4, 1180=17.24$, $p<0.01$; Figure 3C), and weight ($KW4, 1180=56.97$, $p<0.01$; Figure 3D).

Table 3. Production, size, and weight of the escamoles per number of paths, based on the mean and its standard deviation $(\bar{x} \pm SD)$, in Pánfilo Natera, Zacatecas.

Number of paths	Production (g)		Length (mm)		Width (mm)		Weight (gr)	
	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.
2	352.49	332.32	7.77	1.38	4.34	0.64	0.10	0.04
3	328.40	235.95	8.75	1.13	4.64	0.52	0.14	0.04
4	455.06	294.38	8.74	1.12	4.66	0.54	0.13	0.04
5	412.04	248.95	8.88	1.37	4.70	0.49	0.15	0.04
6	150.97	0.00	8.10	1.14	4.58	0.36	0.16	0.06

± D.S.=standard deviation.

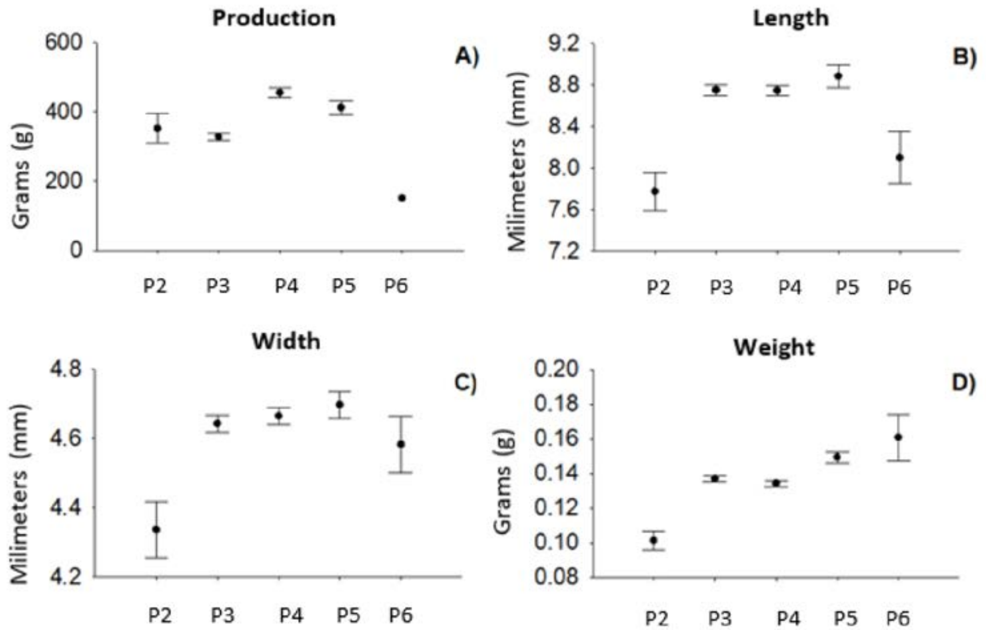

Figure 3. Contrast of the foraging paths of the *escamolera* ant colonies, based on the mean ± standard error $(\bar{x} \pm SE)$. P2: 2 foraging paths, P3: 3 foraging paths, P4: 4 foraging paths, P5: 5 foraging paths, and P6: 6 foraging paths. The *post hoc* test of the Tukey method was used for the comparison and values $<\alpha (\alpha=0.05)$ were considered significant.

Nest Type

New nests recorded the highest *escamoles* production. The escamol sizes and weights were very similar between new and disturbed nests (Table 4). Figueroa-Sandoval *et al*. (2018) evaluated 77 nests in four localities of the Potosino-Zacatecano plateau: 61 nests were disturbed, while 16 were conserved. The conserved colonies recorded a higher escamoles production (543.1 g) than the disturbed colonies (169.2 g). The study concluded that the new nests —*i.e.*, nests that had never been dug— had a higher *escamoles* production.

The comparison between types of nest established differences in *escamoles* production ($U_{1,\ 1180}$=52 000.00, $p<0.01$; Figure 4A). However, there were no differences regarding escamol length ($U_{1,\ 1180}$=63 059.00, $p<0.88$; Figure 4B), width ($U_{1,\ 1180}$=62 304, $p<0.71$; Figure 4C), and weight ($U_{1,\ 1180}$=61 416.50, $p<0.54$; Figure 4D).

Table 4. Production, size, and weight of the escamoles per nest type, based on the mean and its standard deviation $(\bar{x} \pm \mathrm{SD})$, in Pánfilo Natera, Zacatecas.

Nest type	Production (g)		Length (mm)		Width (mm)		Weight (g)	
	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.	Mean	± D.S.
New nest	459.01	268.62	8.74	1.02	4.62	0.48	0.14	0.03
Disturbed nest	381.61	273.62	8.70	1.22	4.64	0.54	0.14	0.04

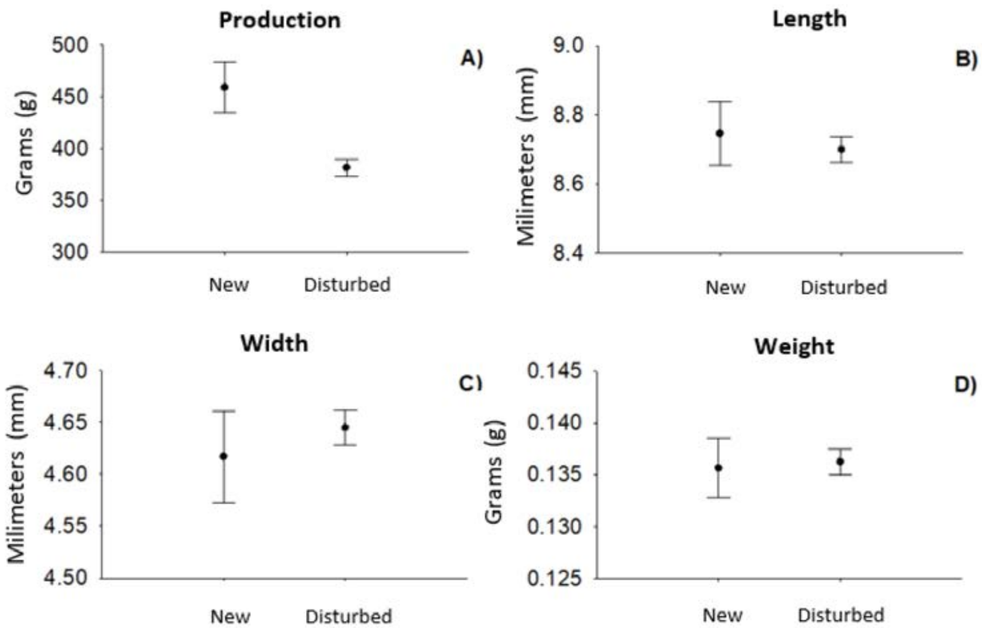

Figure 4. Nest type contrast (new and disturbed nests). The *post hoc* test of the Tukey method was used for the comparison and values $<\alpha(\alpha=0.05)$ were considered significant.

Clustering

Cluster 1 (Figure 5) (n=316 *escamoles*). This group included 8 nesting substrates; however, most of the samples (53.8%) were collected from the maguey substrate (MA) and

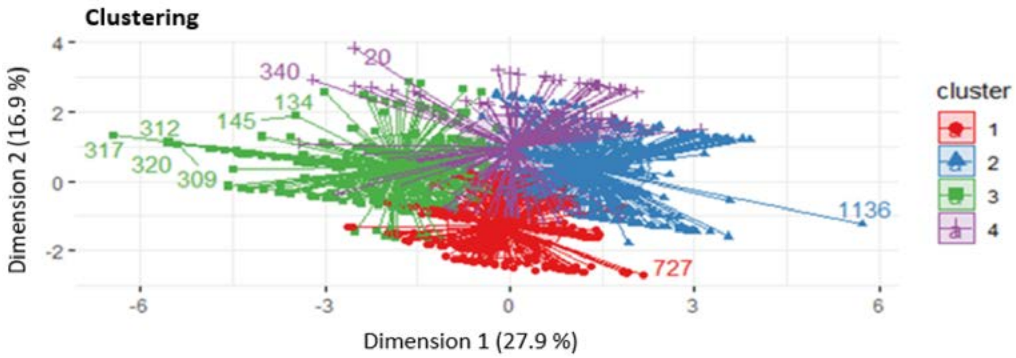

Figure 5. K-means clustering of the 4 groups. Cluster 1 (red, n=316), cluster 2 (blue, n=438), cluster 3 (green, n=306), cluster 4 (purple, n=120).

the bare soil (BS), while the lowest number of samples (0.3%) was found in the mezote (ME) substrate. Colonies with 2 foraging paths are not included in this group. Eighty-eight percent of the samples were taken from colonies with 3 and 4 foraging paths. All the nests in this group had been disturbed nest. Likewise, 77.5% of the subjects were located in medium-sized colonies. This group recorded the lowest average production of the 4 groups (244.37 g) and the minimum production (31.14 g) (Table 5).

Cluster 2 (Figure 5) (n=438 *escamoles*). This group included 7 nesting substrates; mesquite (MS) was not included in this group. The Maguey (MA) and prickly pear (PP) substrates accounted for 54.1% of the total. Only colonies with 2, 3, 4, and 5 foraging paths were found in this area. The colonies with 3 and 4 foraging paths recorded the highest number of individuals (76.6%). This group only included disturbed nests and excluded small colonies. The colonies were divided as follows: large (94%) and medium (6%). This group recorded the highest escamoles production among the 4 groups (1,271.4 g) (Table 5).

Cluster 3 (n=306 escamoles). This group recorded the lowest length (7.38 mm), width (4.10 mm), and weight (0.09 g) mean. In addition, it recorded the second maximum production (975.5 g). The highest incidence was obtained by the larger colonies (40%), all of which were disturbed nests. The colonies in this group included all the types of foraging paths (2, 3, 4, 5, and 6 paths) (Table 5). All (8) substrates were available in this area; however, the palm (PA) substrate was significantly different, because it reached 35.29% of the total, followed by prickly pear (PP) with 16.66% and maguey (MA) with 13.72%.

Cluster 4 (n=120 escamoles). This group recorded the lowest number of individuals. All the colonies included in this group were new colonies. Medium-sized colonies accounted for 50% of the total. In addition, the colonies of this group had the lowest number of foraging paths (3, 4, and 5). Nevertheless, there is an important contrast between the foraging paths, because 66.66% of the individuals were found in colonies with 4 foraging paths (Table 5). The nesting substrate of this group recorded the lowest incidence: 60.0, 16.7, and 33.3% of maguey (MA), mezote (ME), and palm (PA), respectively.

Table 5. Comparison of the groups determined using the K-means clustering, based on the dependent *escamoles* variables (production, length, width, and weight), and the group size, the mean, the standard error, and the maximum and minimum values.

Clustering	Dependent variables	Group size (n)	Mean	Standard error	Minimum	Maximum
Cluster 1	Production (g)	316	244.37	±9.47	31.14	746.73
	Length (mm)	316	9.00	±0.04	5.84	12.13
	Width (mm)	316	4.75	±0.02	3.57	5.77
	Weight (g)	316	0.14	±0.00	0.07	0.24
Cluster 2	Production (g)	438	541.73	±13.81	131.34	1271.41
	Length (mm)	438	9.40	±0.04	6.79	11.93
	Width (mm)	438	4.95	±0.02	3.82	9.70
	Weight (g)	438	0.16	±0.00	0.10	0.26
Cluster 3	Production (g)	306	294.14	±12.38	41.30	975.56
	Length (mm)	306	7.39	±0.06	1.54	9.84
	Width (mm)	306	4.11	±0.02	2.53	5.13
	Weight (g)	306	0.10	±0.00	0.03	0.19
Cluster 4	Production (g)	120	459.01	±24.52	67.02	843.54
	Length (mm)	120	8.74	±0.09	5.83	12.12
	Width (mm)	120	4.62	±0.04	3.09	5.60
	Weight (g)	120	0.14	±0.00	0.05	0.19

CONCLUSIONS

The highest *escamoles* production was recorded by the larger nests and in the mesquite substrate. Compared with colonies with other number of foraging paths, the colonies with four foraging paths recorded the best production. In addition, new nests produced more *escamoles* than disturbed nests.

The highest *escamol* individual weight (0.16 g) was recorded in the dry palm substrate —*i.e.*, 1 kg of escamoles is equal to 6,250 larvae (princesses and drones). The lowest escamol weight was recorded in the mezquite substrate (0.09 g); based on that result, 1 kg is equal to 11,111 larvae.

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