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AGRO PRODUCTIVIDAD

AP

Paula Poinsettia

(*Euphorbia pulcherrima* Willd. ex Klotzsch)

indoor variety

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Contenido

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Contacto principal
Jorge Cadena Iñiguez
Guerrero 9, esquina avenida Hidalgo, C.P. 56220, San Luis Huexotla, Texcoco, Estado de México.
agroproductividadeditor@gmail.com

Contacto de soporte
Soporte
5959284703
agroproductividadsoporte@gmail.com

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Habitat characteristics of the Montezuma quail (*Cyrtonyx montezumae* Vigors 1830) in Durango, Mexico

Guzmán-Girón, Diana F.[1]; Ugalde-Lezama, Saúl[2*]; Martínez-Montoya, Juan F.[1]; Tarango-Arámbula, Luis A.[1]; Hernández, Fidel[3]; Olmos-Oropeza, Genaro[1]

[1] Colegio de Postgraduados Campus San Luis Potosí. Salinas de Hidalgo, San Luis Potosí, México. C. P. 78622.
[2] Universidad Autónoma Chapingo. Texcoco, Estado de México, México. C. P. 56230.
[3] Texas A&M University-Kingsville, United States of America. C. P. 78363.
* Correspondence: biologo_ugalde@hotmail.com

ABSTRACT

Objective. To characterize the habitat of the Montezuma quail (*Cyrtonyx montezumae*) in Rancho Chapultepec, Durango, Mexico.

Design/Methodology/Approach. From February to October 2020, fieldwork was carried out to search for Montezuma quail sites using transects and sounds. Subsequently each site-of-use was characterized; for each of them three random sites were located. Intercept lines (or Canfield), embedded frames, and nearest neighbour techniques were used. Recorded data were analysed using Principal component analysis (PCA); Frequency of observation (FO); Kruskal-Wallis; and Poisson regression (PR).

Results. The PCA showed values for sites of use=48, non-use=43, and both=36 of the present variability in vegetation-habitat variables. FO showed values per site of use=6.25%; non-use=2.08% and both=1.56%; and per species in sites of use=3.57, non-use=3.85 and both=3.33%. Kruskal-Wallis exhibited significant differences in richness and abundance and similarity in diversity. Poisson regression (PR) showed the effect of some vegetation-habitat variables on the Montezuma quail.

Limitations /Implications of the study. The monitoring period evaluated only comprised a short space of time. It is suggested to extend the sampling time, allowing to visualize the population fluctuation regarding this variable.

Findings/Conclusions: It was possible to determine that there is an association between the variables of the vegetation-habitat with the presence of *C. montezumae*. Vegetation type is the variable that determines the incidence of this species.

Keywords: birds, quail, scrubland, herbaceous vegetation, grassland.

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INTRODUCTION

The grasslands of arid and semi-arid areas of Mexico comprise approximately 128.3 million ha; they are sustenance for domestic livestock and wildlife (Jurado-Guerra *et al.*, 2004). However, this type of vegetation faces varying degrees of deterioration, affecting bird communities, modifying their richness, diversity and reducing their population size.

The Montezuma quail (*Cyrtonyx montezumae* Vigors 1830) has ecological importance in forest, arid and semi-arid environments (Sánchez *et al.*, 2011). These environments are

found in the state of Durango, where 28.73% of its land extension is occupied by the Valley Region with the presence of natural grasslands (González *et al.*, 2007). Rancho Chapultepec is located in those valleys, an arid region where there is evidence of the natural distribution of this quail.

This study characterizes the habitat of the Montezuma quail in a representative area of the Valles de Durango region, within the Rancho Chapultepec, through the following objectives: to characterize the habitat of the Montezuma quail in terms of Beta diversity by types of use and non-use in Rancho Chapultepec, Durango Mexico; and to determine the level of association for variables of vegetation–habitat with the presence of C. montezumae at sites of use and non-use in Rancho Chapultepec, Durango Mexico.

MATERIALS AND METHODS

This study was conducted in Rancho Chapultepec, a small property (category of private land tenure) located in the municipality of Durango, Mexico, whose main building is at the geographical coordinates 24° 14' 37" N and 104° 25' 56.75" W. This small property has an area of 5034 ha, mainly intended for extensive livestock.

Search for the Montezuma quail

The fieldwork began in February and ended in October 2020; paddocks were surveyed to know where there could be quail Moctezuma (Hernández *et al.*, 2006). For the location of the quail, quail sounds were reproduced (playback) from both sides of the transect, at 50 m taking the path as the center. The vocalizations of the species were reproduced (Vázquez-Pérez *et al.*, 2009) from both female and male; obtained from the library specialized in bird sounds (https://www.xeno-canto.org/). When the song was answered, coordinates were recorded (altitude, length, and altitude) with a GARMIN MAP 64s GPS (Bristow and Ockenfels, 2004). The site was marked for later location and characterization.

Characterization of quail sighting sites

Each site of use was characterized; for each one three points were located at random. In each site the habitat was characterized; four sampling methods were used: a) intercept line or Canfield, b) 4×4 m plots, c) 1×1 m plots, and d) nearest neighbor.

a) Intercept or Canfield lines; to know the coverage of each component considered (Canfield, 1941; Bristow and Ockenfels, 2004).
b) On the 4×4 m plot, data on scrubland species were recorded.
c) A plot of 1×1 m was sampled. In this plot, information on herbaceous plant species was collected.
d) Nearest neighbor. It was used to obtain data on tree species that quails use as an escape option.

Richness, abundance and diversity of plant species

In order to know the variables of vegetation and habitat, that explained the greater variance in the characterization of the sites, a principal component analysis (PCA) was applied in three axes. This analysis was performed with R version 3.6.1 (Fox, 2005).

The frequency of observation (FO) and relative abundance index (RAI) at sites and species levels were determined. To evaluate the possible association between vegetation-habitat variables and the frequency of quails recorded in sites, a Poisson regression analysis (ARP) was applied, using the Stepwise polynomial-variable selection procedure, the fit of the models was assessed by the minimum Akaike criterion (AIC; Akaike, 1969) in R-version 3.6.1.

In order to establish possible statistically significant differences, the results of the estimators Jacknife 1 (Richness), relative abundance index (RAI) and Shannon-Wiener (Diversity) obtained for sites of use vs. non-use, were analyzed by the non-parametric Kruskal-wallis test. This due to not fulfilling the assumptions for parametric statistics; such analyses were carried out in Xlstat v. 2021.4.

RESULTS AND DISCUSSION

It was observed that the first three axes (components) explained in use=48; non-use=43 and both=36 of the total present variation. Six important variables were recorded, which compose and describe the variability of vegetation-habitat (Table 1).

FO: indicates average values per site for use=6.25%; non-use=2.08% and both=1.56%; and per species in sites of use=3.57, non-use=3.85; and both 3.33%. The relative abundance index suggests mean values of RAI=0.06; 0.02 and; 0.02, respectively (Figure 1).

Poisson regression indicated that the best-fitted model has values of AIC=54.48; AIC=77.37 and; AIC=144.22, which determined the best adjusted models for each case of analysis, exhibiting 1; 11 and; 18 variables (vegetation-habitat), respectively (Table 2).

Kruskal-Wallis results for the estimators Jacknife 1 (Richness); Relative abundance index (RAI) and; Shannon-Wiener (Diversity) obtained for sites in condition of use *vs.* non-use, allowed to show that there are significant differences ($p=-0.007$; $p\leq0.0001$) in richness and abundance; however, diversity appears to be similar ($p=-0.21$) (Figure 2).

The trends showed by this study (richness) are consistent with those reported by Bristow and Ockenfels (2002; 2004; 2006) and Hernández *et al.* (2006) who evaluated the habitat of

Table 1. Main components that explain in three axes the proportion of the accumulated variance out of 100% of the total present variation that compose and determine the presence of the Montezuma quail.

Variables studied	Component 1	Component 2	Component 3
Grasses	−0.04269102	−0.30848731	0.17848068
Herbaceous (%)	0.31925713	−0.05725741	−0.04342608
Grasses (%)	0.10888704	−0.18769812	−0.16302592
Frangula microphylla	−0.09201525	−0.30588082	0.09435311
Prosopis sp.	−0.2898917	0.0983107	−0.12683786
Tree height	−0.29929015	−0.05612058	−0.00141829

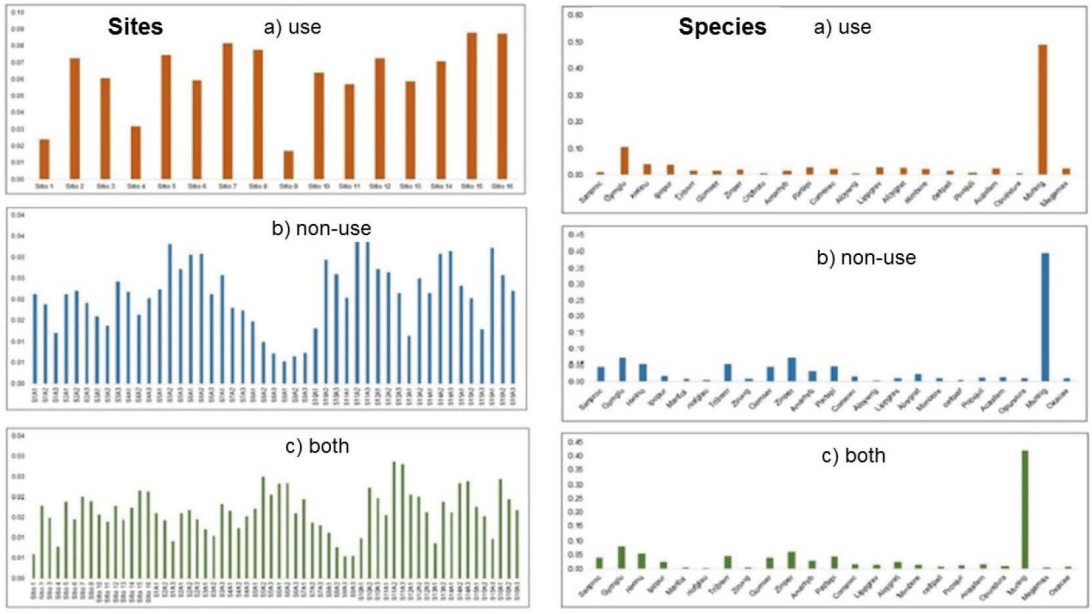

Figure 1. Relative abundance index (RAI) for sites and by species for sites in A) use; B) non-use; C) both conditions, in Rancho Chapultec, Durango, Mexico.

the Montezuma and Scaly quails in the Chihuahuan Desert, evergreen forest and desertic grassland, respectively. Those authors analyzed the macrohabitats at feeding sites and random sites, finding richness values of 23% and 29% using the Simpson estimator; the diversity and uniformity using the sum test were 6.5, 0.3 and 7.6, 0.3, respectively; thus, they found a similar richness between sites.

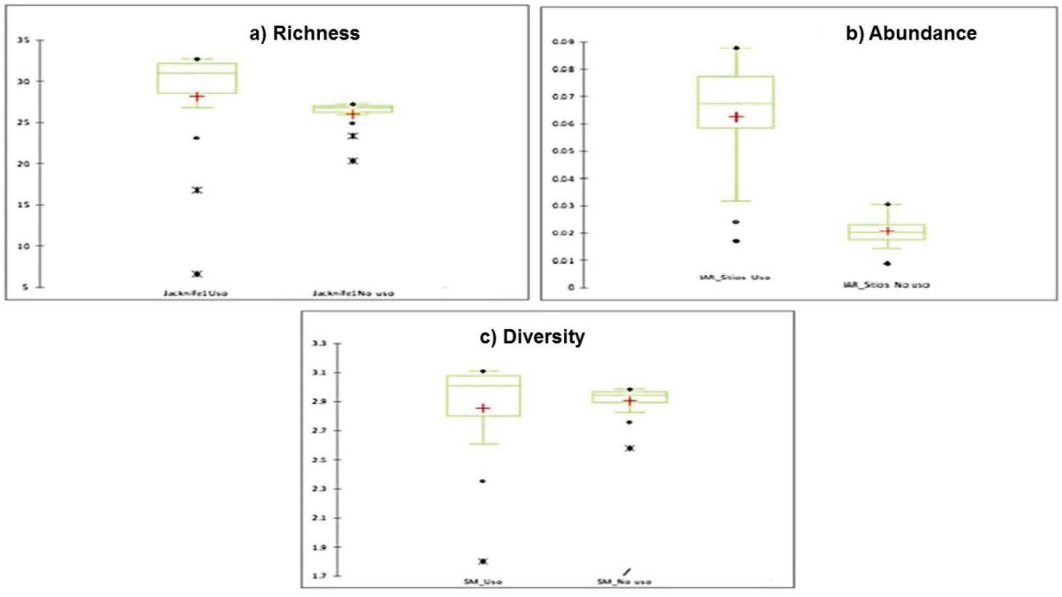

Figure 2. Kruskal-Wallis Box plots for Jacknife 1 estimator (richness); estimated for sites in condition of use vs. non-use, in Rancho Chapultec, Durango, Mexico.

Table 2. Poisson regression analyses using GLM models to determine the degree of association between vegetation-habitat variables and quail frequency recorded at sites of use; non-use and both conditions, in Rancho Chapultec, Durango, Mexico.

	Estimate	Std. Error	z value	Pr(>|z|)
Sites of use				
Intercept	0.70825	0.19607	3.612	0.000304 ***
Tree coverage	0.10173	0.05024	2.025	0.042855 *
Non-Use Sites				
Intercept	−3.7592	1.1788	−3.189	0.00143 **
Shrub cover	−1.6545	0.2614	−6.329	2.47e-10 ***
Grass coverage	8.3511	1.4616	5.714	1.11e-08 ***
Herbaceous coverage	−21.7551	4.2393	−5.132	2.87e-07 ***
Tree height	3.9074	0.759	5.148	2.63e-07 ***
Herbaceous height	29.772	4.7813	6.227	4.76e-10 ***
Shade	7.6237	1.5497	4.919	8.68e-07 ***
Grasses	−1.4625	0.2743	−5.331	9.77e-08 ***
Tree coverage	−0.7872	0.1682	−4.679	2.88e-06 ***
Tree distance	−2.9307	0.5422	−5.405	6.47e-08 ***
Distance to the *Frangula microphylla*	15.5066	3.0894	5.019	5.19e-07 ***
Both conditions				
Intercept	−0.23099	0.55686	−0.415	0.678279
Shrub cover	−0.43953	0.08602	−5.11	3.23E-07***
Grass coverage	0.48209	0.14786	3.26	0.001112 **
Herbaceous coverage	3.23777	0.74715	4.334	1.468E-05 ***
Tree height	0.7023	0.3091	2.272	0.023083 *
Gramineous height	−4.40945	1.0647	−4.141	3.451E-05 ***
Shrubbery	0.81744	0.17783	4.597	4.292E-06 ***
Shade	−1.19331	0.23928	−4.987	6.13E-07 ***
Basal area	−3.54666	1.73088	−2.049	0.040457 *
Tree coverage	0.20334	0.0787	2.584	0.009777 **
Shadow / *Dalea bicolor*	4.86159	1.18131	4.115	3.865E-05 ***
Frangula microphylla	3.10164	0.81646	3.799	0.000145 ***
Herbaceous	−0.03723	0.0096	−3.878	0.000105 ***
Coverage area	0.08506	0.02331	3.649	0.000264 ***
Tree distance	1.05221	0.26991	3.898	9.684E-05 ***
Distance to the *Frangula microphylla*	−3.1632	0.63032	−5.018	5.21E-07 ***
Distance to the *Vachellia* sp.	1.02205	0.23052	4.434	9.262E-06 ***
Distance to the *Prosopis* sp.	−1.76188	0.37391	−4.712	2.452E-06 ***
Distance to the *Opuntia* sp.	−0.48184	0.22017	−2.188	0.028635 *

Significant at *** p≤0.001; ** p≤0.01; * p≤0.05.

However, the index used herein (this study) differs from that used in those other studies; which agree in reporting a similar richness between conditions, especially in grassland. This assumes that *Cyrtonyx montezumae* seems to use similar ecological conditions in the composition of plant species, particularly those that compose the lower stratum (grasses and grains) in this region of Mexico.

Thus, this research supports the idea that the Montezuma quail behaves similarly at the stratum level in the use of habitat in this type of systems. It is imperative to note that this study was carried out in a scrubland, which should be noted included sites that sustain diverse plant communities such as Spiny hackberry (*Celtis pallida*), prickly pear cactus (*Opuntia durangensis*), the twisted or Schaffner's acacia (*Vachellia shaffneri*) and smooth mesquite (*Prosopis laevigata*). Within this vegetation type, the herbaceous stratum dominant in cover was an association of Deer grass (*Muhlenbergia rigens*). All these species are within their natural distribution in the study area.

This does not agree with what was reported by Leopold and McCabe (1957) who described the Montezuma quail as a species of bird that preferentially inhabits pine-oak forests. Those authors indicated tough, that quails also tend to use the understory; particularly in forests with secondary succession, which turn out to be a more favorable habitat for this bird species. However, these assertions are notably in contrast with Tapia *et al.* (2002) and Garza (2007) who mentioned that this species is associated, in the vertical vegetation profile, with the community structure of pine, oak, juniper and oak forests. Cork (2017) partially supported this, after evaluating the habitat selection and spatial ecology of the Montezuma quail in the mountains of New Mexico, indicating that the selection of feeding sites is a function of the height and average distance of the nearest trees.

This latter statement supports part of the trends recorded in this study, where we recorded such a phenomenon, tough it was associated to a greater extent with herbaceous communities (grassland), which suggests the relevance of trees closer to grassland areas as cover spots for escape and refuge from possible predators. Nevertheless, the activity recorded in this research, especially on the sites in codition of use, seems to be more related to the lower stratum of the evaluated plant structure.

CONCLUSIONS

Habitat characterization by sites in condition of use and non-use was achieved in regard to Betha diversity; through interpreting richness, abundance and diversity as complementary parameters in the contrast between sites.

It was possible to determine that there is an association between variables of the vegetation-habitat with the presence of *Cyrtonyx montezumae*; we accomplished identifying that such an association occurs in greater proportion with the vegetation variables.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Chemical composition of twelve accessions of *Moringa oleifera* Lam. grown in Mexico

Ruíz-Hernández, Rafael[1*]; Hernández-Rodríguez, Martha[2]; Pérez-Vázquez, Arturo[3]; Cruz-Monterrosa, Rosy G.[1]; Rayas-Amor, Adolfo A.[1*]

[1] Departamento de Ciencias de la Alimentación. División de Ciencias Biológicas y de la Salud. Universidad Autónoma Metropolitana Unidad Lerma. Av. de las Garzas 10. Col. El Panteón, Lerma de Villada, Estado de México. C. P. 52005.

[2] Postgrado de Recursos Genéticos y Productividad-Genética, Colegio de Postgraduados, Campus Montecillo. Municipio de Texcoco, Estado de México, México. C.P. 56230

[3] Colegio de Posgraduados, Campus Veracruz. Predio Tepetates, Manlio F. Altamirano, Veracruz. Carretera Xalapa-Veracruz km 88.5, CP. 91700.

* Correspondence: r.ruiz@correo.ler.uam.mx; a.rayas@correo.ler.uam.mx

ABSTRACT

Objective: The objective was to evaluate the growth and proximal analysis of twelve accessions of *Moringa oleifera* Lam. grown in Mexico.

Design/Methodology/Approach: The seeds were collected in Veracruz, Oaxaca, Guerrero, Chiapas, and Yucatan. The seeds were sown in a nursery and transplanted in the field in a completely randomized block experimental design. Height, basal diameter, and the number of branches were recorded, and leaves were collected for proximal analysis determination.

Results: Significant statistical differences ($P<0.05$) were identified among the accessions based on tree height, basal diameter, number of branches, moisture content, ash, protein, and fat contents.

Study limitations/implications: Accessions with high growth rates and nutritional characteristics can be selected to establish low-cost food banks.

Result/Finding/Conclusion: The accessions from Chiapas (C1 and C2) were superior to the others in tree height, basal diameter and number of branches, protein, and fat contents.

Keywords: moringa, protein, lipids, genetic improvement.

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INTRODUCTION

Moringa oleifera Lam. is a fast-growing, drought-resistant tree distributed in tropical, subtropical, arid, and semi-arid zones (Owon *et al.*, 2021). This plant species thrive under medium rainfall conditions, 20 to 30 °C, medium and low fertility soils, and good drainage (Mendieta-Araica *et al.* 2013). Due to its great adaptability, high carbon sequestration capacity, and tolerance to environmental changes, it represents an alternative for food production in the face of climate change scenarios (Gedefaw, 2015). The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) identified and promoted the potential of moringa to address issues related to food and the prevention of various types of diseases (Shil, 2021). The leaves are consumed in Ethiopia, Nigeria,

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India, Malawi, East Africa, and Ghana when combined with traditional foods. Moringa leaves have a higher protein content than other foods and are recommended to address malnutrition problems (Daba, 2016). The range of protein in dried moringa leaves can range from 10.74 to 32.06%, and within this protein are the amino acids valine, threonine, isoleucine, lysine, methionine, phenylalanine, histidine, leucine, arginine, glycine, alanine, serine, aspartic, glutamine, tyrosine, cysteine and proline (Mbailao *et al.*, 2014; Valdez-Solana *et al.*, 2015). They also contain vitamins (A, B1, B2, B6, B12, C, and E) and minerals such as Ca, K, S, P, Mg, K, Na, Zn, Cu, Mn, Fe (Stevens et al., 2021).

In Mexico, moringa is distributed in 14 states and is used for various purposes, mainly food and medicinal, due to its high nutritional content (Olson and Alvaro-Cárdenas, 2016). Studies have been conducted on its nutritional composition by collecting materials in different geographical regions. The results show variations in protein, ash, lipid, and moisture content of the evaluated accessions (Jongrungruangchok *et al.*, 2010; Valdez-Solana *et al.*, 2015; Lamidi *et al.*, 2017). However, the difference in nutritional content may be influenced by soil type, climate, altitude, and type of material evaluated (Bopape-Mabapa *et al.*, 2020). Therefore, the evaluation under homogeneous conditions clarifies the quantitative variation of the Proximal analysis of moringa accessions with greater precision. Identifying materials with higher nutritional content allows the selection of highly nutritious ones and will facilitate the establishment of food banks. Therefore, the objective of this study was to evaluate the Proximal analysis of 12 *Moringa oleifera* Lam. accessions grown in Mexico.

MATERIALS AND METHODS

Biological material

Moringa populations were identified, and seeds were collected in the states of Chiapas, Guerrero, Oaxaca, Veracruz, and Yucatan. Table 1 and Figure 1 show the geographic locations of the accessions evaluated. The research was carried out at the Colegio de Postgraduados, Campus Veracruz, geographically located at 19.27556 N latitude, 96.27556 W longitude at an altitude of 16 masl, with a warm sub-humid climate (AW0) and a mean annual rainfall of 1000 mm.

Sowing and experimental design. Seeds were sown in a substrate composed of soil, manure compost, and sand (5:4:1) under nursery conditions. Two months after sowing, the seedlings were transplanted in a completely randomized block design with five blocks and five replications. The distance between plants and rows was 3 m (Ruiz *et al.*, 2021). Weeds were eliminated when they exceeded 20 cm in height, and regular irrigation was applied. The physicochemical characteristics of the soil of the experimental area were texture, clay loam, sand (37.35%), silt (25.76%), clay (36.89%), pH (6.25), nitrate (0.0 mg L^{-1}), nitrite (0.0 mg L^{-1}), P (2.5 mg L^{-1}), K (21 mg L^{-1}) and 2.58% organic matter (Reyes, 2018).

Data collection: Three-year-old trees were selected for quantitative data collection. Height was measured with a flexometer from the base of the trees to their apex. The basal diameter was measured at ground level with a vernier, and the number of branches was obtained by counting.

Table 1. Geographical location of *M. oleifera* Lam. accessions grown in different states of Mexico.

Accession	State	Municipality	Community	Longitude	Latitude
C1	Chiapas	Tuzantán	Villa Hidalgo	−92.374722	15.108056
C2	Chiapas	Tuxtla Gutiérrez	Colonia La Salle	−93.0868889	16.7429444
G2	Guerrero	Acapulco de Juárez	Parrotillas	−99.6155837	16.8787834
G4	Guerrero	Tecpán de Galeana	Mitla	−99.8934352	16.8789425
O1	Oaxaca	Santa Cruz Xoxocotlán	San Juan Bautista	−96.7280556	16.9791667
O2	Oaxaca	Santa María Huatulco	La Herradura	−96.3658333	15.7772222
O3	Oaxaca	Mariscala de Juárez	Guadalupe la Huertilla	−98.1088889	17.8513889
V1	Veracruz	Soledad de Doblado	El Progreso	−96.4022719	19.0818742
V4	Veracruz	Misantla	Santa Cruz Hidalgo	−96.8628092	19.9555656
V1	Veracruz	Soledad de Doblado	El Progreso	−96.4022719	19.0818742
Y3	Yucatán	Peto	Teshan	−88.62125	20.1486389
Y4	Yucatán	Baca	Felipe Carrillo Puerto	−89.6070099	20.9954688

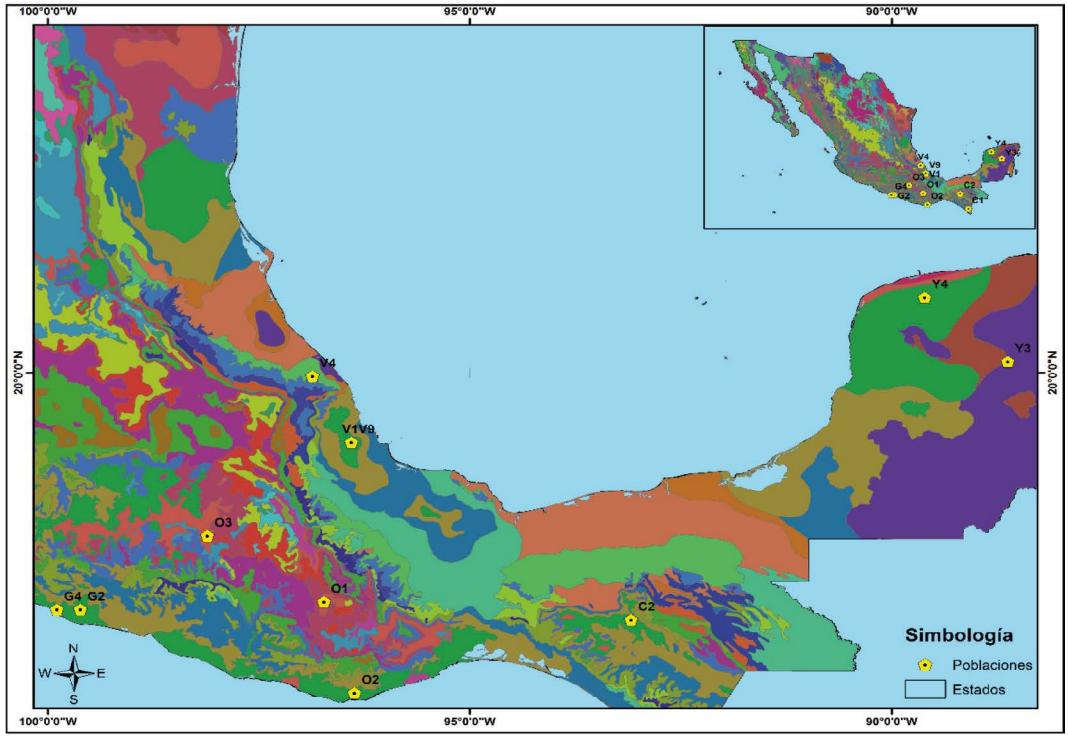

Figure 1. Geographical location of the *M. oleifera* accessions.

Sample collection: 3 kg of mature and healthy leaves were collected from each population. Leaflets were separated from the central rachis, and those with yellowing were removed. Leaves were dehydrated in a forced-air oven at 50 °C for 30 h. Leaves were sprayed with a spray solution. The leaves were pulverized with a plant tissue mill and sieved on a 1 mm mesh. Moringa leaf meal was stored in airtight bags at 20 °C until analysis.

Proximal analysis: moisture, ash, protein, fat, and fiber contents in dried moringa leaves were determined using AOAC (1990) methodology, and samples were analyzed in triplicate.

Moisture determination: Porcelain crucibles were brought to constant weight. One gram of moringa meal was added to the crucible and allowed to dry in an oven at 105 °C until a constant weight was reached. The moisture content was determined under the following equation.

$$Humidity\ (\%) = \frac{Initial\ weight - final\ weight}{sample\ weight} \times 100$$

Ash determination: It was determined through the gravimetric method AOAC 923.03. Porcelain crucibles were brought to constant weight. One gram of moringa meal for each sample was added to the crucible. The samples were burned until carbonization with a Bunsen burner. Subsequently, the crucibles were placed in a flask at 550 °C for five h. The samples were brought to constant weight and room temperature. The crucibles were weighed with the grayish-white ashes. The determination was carried out in triplicate. The total ash content was calculated with the following equation.

$$Ash\ (\%) = \frac{ash\ weight}{sample\ weight} \times 100$$

Protein determination: it was determined by the Kjeldahl method. One gram of moringa meal was added to each Kjeldahl flask, and 0.5 g of $CuSO_4$, 10 g of K_2SO_4, and 25 mL of concentrated H_2SO_4 were added. The tubes were placed in the digesters and heated until a light green, transparent color was reached. They were allowed to cool to room temperature. Sixty mL of 40% (v/v) NaOH was slowly added to each tube and placed in the distiller. The distillate was collected in a 250 mL flask, 50 mL of 0.1 N HCl, and three drops of methyl red indicator. The distillation was concluded when a volume of 100 mL was obtained. Subsequently, the distillate was titrated with NaOH. The protein content was calculated from the total nitrogen content using a factor of 6.25 with the following equation.

$$N(\%) = \frac{(V_B - V_M) \cdot N \cdot Meq}{PM} \times 100$$

$$P(\%) = (N\%)(6.25)$$

Where: V_B=ml of NaOH spent on the blank; V_M=ml of NaOH spent on the problem; N=Normality of NaOH; Meq=Milliequivalent of N; PM=Sample weight; N(%)=Nitrogen; P (%)=Total protein.

Determination of total fat (lipids): 250 ml flasks were brought to constant weight. Three g of moringa flour were placed in each cartridge. The Soxhlet-type extractors were installed, and the cartridges were introduced. 125 ml of hexane (solvent) was added to each sample, and the siphoning effect was performed for five h from the first heating. The total fat content was determined with the following equation:

$$Fat\ (\%) = \frac{Grams\ of\ residue\ in\ flask}{(Weight\ of\ sample\ in\ grams)} \times 100$$

Statistical analysis

The data obtained were recorded in an Excel spreadsheet, version 2016®. Analysis of variance (ANOVA) was performed, and Tukey's test ($p<0.05$) was used to determine the differences between height, basal diameter, number of branches, and Proximal analysis of moringa meal. Correlation analysis of the variables was also performed. Statistical tests were performed with InfoStat software version 2016.

RESULTS AND DISCUSSION

Height, basal diameter, and number of branches

Significant statistical differences ($p<0.05$) were observed in the height of the evaluated accessions. The accessions with the lowest and highest heights were V9 with 3.82 m and C1 with 5.95 m. Ruiz *et al.* (2021) mentioned differences in the height of the evaluated accessions. Accession C1 represents the material with the most significant ($P<0.05$) growth during the three years of development. This genetic material presented more remarkable development under climatic conditions of the sub-humid tropics. Therefore, it can be used for the establishment of protein banks.

The basal diameter of the evaluated accessions showed a progressive development. The statistical test determined significant differences ($P<0.05$) in the basal diameter of the moringa accessions. Accession Y3 presented the most significant ($P<0.05$) diameter growth with a value of 11.30 cm. The lowest basal diameter growth was 7.89 and corresponded to accession O2. De Swaef *et al.* (2015) mentioned that the diameter size of trees is a significant indicator for studies related to drought resistance. Moreover, those accessions with a larger diameter can resist or tolerate this abiotic stress. The number of tree branches indicates the number of leaves and fruits an accession can produce.

In this study, the number of branches fluctuated from 11 to 31 among the accessions. This variation was statistically significant ($p<0.05$), and those materials with a higher number of branches can be selected for fruit and seed production.

Proximal analysis

Table 3 shows the proximate content of 12 moringa accessions. Significant statistical differences ($P<0.05$) were obtained in moisture, ash, protein, and fat content in dried moringa leaves.

Table 2. Height, basal diameter, and the number of branches of the accessions of *M. oleifera* Lam.

Accession	Height±S.D. (m)	Basal Diameter±S.D. (cm)	Number of Branches±S.D.
C1	5.95 ± 0.16^{e}	10.05 ± 0.33^{cde}	19.00 ± 1.29^{bc}
C2	5.18 ± 0.16^{cde}	9.00 ± 0.33^{abc}	24.00 ± 1.29^{c}
G2	4.48 ± 0.16^{abc}	9.67 ± 0.33^{cde}	14.67 ± 1.29^{ab}
G4	4.90 ± 0.16^{bcd}	9.03 ± 0.33^{abc}	18.67 ± 1.29^{bc}
O1	5.40 ± 0.16^{de}	7.70 ± 0.33^{a}	10.50 ± 1.29^{a}
O2	4.30 ± 0.16^{ab}	7.89 ± 0.33^{ab}	13.00 ± 1.29^{ab}
O3	5.41 ± 0.16^{de}	10.13 ± 0.33^{cde}	18.50 ± 1.29^{bc}
V1	4.80 ± 0.16^{bcd}	8.93 ± 0.33^{abc}	16.00 ± 1.29^{ab}
V4	4.94 ± 0.16^{bcd}	10.83 ± 0.33^{de}	31.00 ± 1.29^{d}
V9	3.82 ± 0.16^{a}	7.43 ± 0.33^{a}	11.67 ± 1.29^{a}
Y3	5.42 ± 0.16^{de}	11.30 ± 0.33^{e}	19.00 ± 1.29^{bc}
Y4	4.30 ± 0.16^{ab}	9.40 ± 0.33^{bcd}	15.33 ± 1.29^{ab}

Table 3. Proximal analysis (%) of 100 g of dried leaves of *M. oleifera* accessions from different states of Mexico.

Accession	Hum±S.D.	Ash±S.D.	Protein±S.D.	Fat±S.D.
C1	7.02 ± 0.17^{bc}	8.99 ± 0.10^{cde}	22.13 ± 0.65^{ef}	5.43 ± 0.14^{c}
C2	8.6 ± 0.17^{e}	10.86 ± 0.10^{h}	20.23 ± 0.65^{def}	6.80 ± 0.14^{d}
G2	6.9 ± 0.17^{bc}	8.59 ± 0.10^{bc}	16.60 ± 0.65^{abc}	5.69 ± 0.14^{c}
G4	5.8 ± 0.17^{a}	8.11 ± 0.10^{b}	20.70 ± 0.65^{def}	3.65 ± 0.14^{b}
O1	6.54 ± 0.17^{ab}	9.33 ± 0.10^{efg}	16.73 ± 0.65^{abc}	5.15 ± 0.14^{c}
O2	6.63 ± 0.17^{ab}	8.71 ± 0.10^{cd}	18.73 ± 0.65^{bcd}	4.00 ± 0.14^{b}
O3	7.73 ± 0.17^{cde}	9.63 ± 0.10^{fg}	14.13 ± 0.65^{a}	3.35 ± 0.14^{ab}
V1	8.00 ± 0.17^{de}	8.93 ± 0.10^{cde}	23.50 ± 0.65^{f}	5.05 ± 0.14^{c}
V4	6.76 ± 0.17^{b}	8.55 ± 0.10^{bc}	14.57 ± 0.65^{a}	3.47 ± 0.14^{ab}
V9	6.75 ± 0.17^{b}	9.17 ± 0.10^{def}	19.50 ± 0.65^{cde}	5.01 ± 0.14^{c}
Y3	7.31 ± 0.17^{bcd}	9.70 ± 0.10^{g}	13.67 ± 0.65^{a}	3.52 ± 0.14^{b}
Y4	7.10 ± 0.17^{bc}	7.53 ± 0.10^{a}	15.50 ± 0.65^{ab}	2.78 ± 0.14^{a}
Average	7.09	9.01	18.00	4.49

Humidity

Significant statistical differences ($P<0.05$) were obtained in the moisture content in the dry leaves of the 12 moringa accessions. The minimum and maximum values were 5.8 and 8.6%, corresponding to accessions G4 and C2. The values obtained in this research are like the 6.94 to 8.67% reported by Pérez-Ángel *et al.* (2020). Higher than the 5.7% of Díaz-Fuentes, (2019), 3.34 and 3.06 of Valdez-Solana *et al.* (2015), 4.64% of Chan-Matú *et al.* (2020), and 7.50 of Chelliah *et al.* (2017) and lower than the 22.08% of Mbailao *et al.* (2014). High moisture contents decrease the quality and shelf-life period of dried leaves.

Moisture content in dried leaves depends on dehydration conditions (shade, direct light, and air stoves) and storage materials.

Ash

The range of ashes identified fluctuated from 7.53 to 10.86% among the accessions. These values showed significant statistical differences ($P<0.05$) in ash content in dried moringa leaves under subtropical climate conditions. Pérez-Ángel *et al.* (2020) reported 7.98 to 9.62% when evaluating moringa genotypes in Mexico. The values of 7.22% of Mbailao *et al.* (2014) and 9.3% of Díaz-Fuentes (2019) were also observed. Higher the 3.00 of Chelliah *et al.* (2017), 3.35% of Chan-Matú *et al.* (2020) and lower than 11% of ash Shih *et al.* (2011), 11.18% of Valdez-Solana *et al.* (2015) and 14.60% of ash Sanchez-Machado *et al.* (2010). Accession C2 presented 10.86% ash in dry leaves, and according to Valdez-Solana *et al.* (2015), values similar to 11% ash in dry leaves indicate high mineral content. The ash content represents a valuable source of minerals that can be used in human and animal nutrition and positively favor the organism's functions (Owo *et al.*, 2021). High ash content indicates the plant's ability to absorb minerals from the soil and channel them to the leaves (Guzman *et al.*, 2015). Another factor that can influence ash content in dry leaves is the year's season due to the translocation of minerals by temperature effect (Shih *et al.*, 2011). The macro- and micronutrients contained in moringa leaves could be used to address problems related to malnutrition and obesity in developing countries. Furthermore, in the case of animals, it can serve to minimize mineral deficiency (Miten *et al.*, 2017).

Lipids

Lipid content in leaves represents intense energy in food, and in the evaluated accessions, a range from 2.78 to 6.80% was observed for Y4 and C2 materials, respectively. This variation was statistically significant ($P<0.05$), representing a selection criterion for materials with higher lipid content. The range of values obtained in this research is similar to the 2.94% of Mbailao *et al.* (2014), 4.96% Sánchez-Machado *et al.* (2010), and 5.7 of Díaz-Fuentes, (2019), higher than the 1.90% of Chelliah *et al.* (2017) and lower than the 7.75 of (Shih *et al.*, 2011), 9.17 (Pérez-Ángel *et al.*, 2020) and 10.21 lipids (Valdez-Solana *et al.*, 2015). Lipids in moringa leaves represent an essential source of fatty acids such as myristic, palmitic, palmitoleic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic, eicosenoic, behenic, lignoceric (Fejér *et al.*, 2019).

Protein

Higher than 10.74 and 11.48% (Valdez-Solana *et al.*, 2015) and similar to 22.05% of Chan-Matú *et al.* (2020), 22.42% of Sánchez-Machado *et al.* (2010) and 22.8% Owon *et al.* (2021). Although some accessions exceeded 20% protein, they were lower than the 24.90-30.36% of Pérez-Ángel *et al.* (2020), 25.29% of Shih *et al.* (2011), 26.9% of Díaz-Fuentes, (2019) and 32.06% of Mbailao *et al.* (2014). Fejér *et al.* (2019) and Owon *et al.* (2021) reported 22.8% protein in dried moringa leaves. They identified amino acids such as tryptophan, valine, threonine, isoleucine, lysine, methionine, phenylalanine, histidine,

leucine, arginine, glycine, alanine, serine, aspartic, glutamine, tyrosine, cysteine and proline which are necessary for the proper functioning of the organism.

This work identified a considerable variation in the protein content of dried moringa leaves under homogeneous edaphoclimatic conditions. Therefore, the variation identified can only be attributed to the genetic constitution of each accession; this is in agreement with Miten *et al.* (2017), who identified differences in Proximal analysis when evaluating the accessions: green, reddish green, red and aromatic green present in Indonesia. The latter authors reported that the aromatic green accession presented higher protein and amino acid content when compared with the other accessions.

Dried moringa leaves represent a source of protein for people in developing countries who do not have access to nutritious and low-cost food (Mbailao *et al.*, 2014). Moringa leaves are an alternative for food fortification and enrichment or food supplements (Lamidi *et al.*, 2017). Materials with higher protein content can produce high-quality forage and facilitate animal meat production in areas where high-quality feed is deficient (Méndez *et al.*, 2018).

Correlation analysis

The Pearson correlation matrix between the evaluated variables is presented in Table 4. A positive correlation was found between basal diameter and the number of branches (r=0.68). A negative correlation was identified between basal diameter and protein content (r=−0.46).

CONCLUSIONS

Moringa accessions present an essential nutritional content that can be used to establish food banks with high nutritional content and little agronomic management. Evaluating the Proximal analysis of moringa accessions allows for taking advantage of the nutritional properties of the cultivars present in Mexico. The identification of elite materials facilitates the development of programs focused on selecting and reproducing this multipurpose species.

Table 4. Correlation matrix of variables recorded for 12 accessions of *M. oleifera* from different states of Mexico.

	Height	Diameter	Branch Number	Humidity	Ash	Protein	Fat
Height	1						
Diameter	0.51	1					
Branch number	0.36	0.68	1				
Humidity	0.23	0.21	0.26	1			
Ash	0.42	0.02	0.17	0.66	1		
Protein	−0.03	−0.46	−0.17	0.09	0.04	1	
Fat	0.13	−0.34	−0.09	0.39	0.58	0.56	1

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Dynamics of agricultural practices in corn (*Zea mays* L.) cultivation in the municipality of Jilotepec, Estado de Mexico

Cruz-Rangel, Sara[1]; Camacho-Villasana, Yolanda[2]; Jiménez-Guzmán, Judith[3]; Arce-Vázquez, María B.[3]; García-Garibay, José M.[3]; Pérez-Ruiz, Rigoberto Vicencio[3*]

[1] Universidad Autónoma Metropolitana, Unidad Lerma. Licenciatura en Ciencia y Tecnología de Alimentos. División de Ciencias Biológicas y de la Salud. Av. de las Garzas No. 10, Col. El Panteón, Municipio Lerma de Villada, Estado de México, C.P. 52005.

[2] Universidad Nacional Autónoma de México Departamento de Genética Molecular, Instituto de Fisiología Celular, México C.P. 04510.

[3] Universidad Autónoma Metropolitana Unidad Lerma. Departamento de Ciencias de la Alimentación. División de Ciencias Biológicas y de la Salud. Av. de las Garzas 10. Col. El Panteón, Lerma de Villada, Estado de México. C. P. 52005.

* Correspondence: rv.perez@correo.ler.uam.mx

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ABSTRACT

Objective: Determine which agricultural practices are currently used by corn (*Zea mays* L.) growers in the municipality of Jilotepec, Estado de México.

Design/methodology/approach: An individual survey composed of both open and close-ended questions was applied to 93 corn producers from the municipality of Jilotepec, Estado de México, in order to obtain information about the planting practices they carry out. From there, 10 producers were selected for on-site monitoring their plots.

Results: Maize growers in Jilotepec, Estado de México, are conscious of the genetic and cultural richness of native maize. However, the agricultural practices they use for their production are centered on the conventional agricultural model using chemical compounds obtained by chemical synthesis and a part is focused on monoculture production.

Study limitations/implications: The applied survey consisted of 13 questions, and the main limitation was the fact that it was not an extensive questionnaire because it is hard for people to devote a lot of time to this kind of polls; it is known that few questions generate more accurate information, while detailed polls have high levels of inaccuracy.

Findings/Conclusions: The results of this study showed that most of the producers are dedicated to growing native corn with a single crop per year and do not use any agroecological practices for their production or only very limited ones; they are also convinced that it is important to preserve native corn as it is a wealth for the Mexican people.

Keywords: Corn, native, culture, practices

Image by Whisker Flowers at Pixabay

INTRODUCTION

The corn (*Zea mays*) was cultivated by Mexican ancestors between 7,000 and 10,000 years ago, originated in the Tehuacán valley of Puebla (México) and in caves near the Balsas River (Zizumbo-Villareal & Colunga-García, 2010; Ramos-Madrigal *et al.*, 2016). Its ancestor is the teosintle or teosinte (Stitzer & Ross-Ibarra, 2018). The ancient settlers of Mexico through observation and expertise managed to generate several breeds with different qualities, adapted to produce in different geographic regions of the country in a diversity of climates and different altitudes above sea level (Araus *et al.*, 2012).

Nowadays there are 64 breeds in Mexico, 59 are exclusive of this country and five have been found also in other regions of the world, these breeds constitute a genetic and cultural richness (Bellon *et al.*, 2018; CONABIO, 2020). Each one of these breeds has different characteristics that make them appropriate for the elaboration of a great variety of dishes, some breeds are better for tamales elaboration such as toluqueño and the chalqueño breeds, others are better for totopos elaboration such as the bolita, the Nal-Tel and the zapalote chico breeds, others are ideal for the atoles elaboration such as the conical breed and some others are appreciated for elaboration of more sophisticated foods such as the pozole which uses cacahuacintle breed (Turrent *et al.*, 2012). The above are examples of the versatility that this crop offers in the elaboration of flours in Mexico. In addition, as result of crosses between existing breeds, several varieties or hybrid corn have been generated and are now cultivated in other regions of the world (Turrent *et al.*, 2012). For this reason, native corn has an immense and an invaluable potential for the varieties that in the future can be obtained from crosses of currently existing breed (CONABIO, 2020).

On the other hand, they not only provide a food source for humans, but also for animals, which in addition of eating their grains, also feed on the stubbles in such a way that the plant can be better used to produce forage, a source of animal feed (Boschini & Amador, 2000).

Maize has been such an important food source for the Mexican people, during the domestication of this plant as well as other crops such as beans, chili, squash, purslane, tomatoes and many others, the first Mesoamerican populations began to become sedentary and settled in specific places where they could control the growing of various crops that would allow them to subsist and so on (Zizumbo-Villareal & Colunga-García, 2010; Aguilar *et al.*, 2019). Therefore, agricultural systems began to be developed where corn was the main crop for its production, however, along this cereal, other species were cultivated, so that polyculture was established since ancient times, so the "milpa" was a system that has been managed over time, however this type of agricultural system has experienced several changes through time (Aguilar *et al.*, 2019).

When the green revolution arrived, which aimed at producing more food, caused a drastic change in the way cultivation was traditionally practiced in our country in terms of the strategies employed to keep the plants healthy and disease-free (Aguilar *et al.*, 2019). These strategies mainly promoted the use of vast tracts of land for the cultivation of a single type of plant (monoculture), and in order to increase production, it is common to use chemical fertilizers and insecticides (Martínez, 2009).

This leads to soil degradation and erosion, the loss of forests and jungles, pest resistance, and so on, which fails at protecting the environment and biodiversity (Martínez, 2009). In contrast, in Mexico exist several ways to produce food in an eco-friendly way, these practices are called agroecological, however, this type of practices are not all new (Sans, 2007). Traditionally, besides corn, other plants such as beans, squash, chili, as well as medical plants and flowers, were grown at the same time. In this system, organic fertilizers and composts are used *in situ*, and for pest control, plants or products with insecticidal or repellent properties are also used (Aguilar *et al.*, 2019).

These practices are environmentally sustainable, in other words, they contribute to the preservation of the environment and biodiversity because they are not aggressive which is not the case of insecticides or chemical fertilizers used in the cultivation of crops. Due to the increasing demand to produce more food and globalization, several practices from the conventional agricultural model have penetrated the traditional way of production in Mexico, giving way to a more sustainable form of agriculture that has significant environmental impacts (Nichols *et al.*, 2015; Sans, 2007; Martínez, 2009).

MATERIALS AND METHODS

Sample size: This study was carried out in the municipality of Jilotepec, a name derived from the náhuatl, xilotl (jilote, meaning tender cob) and tepetl meaning hill, which can be translated as "on the hill of the xilotes" or the hill of the tender ears of corn. This municipality is known for being a large corn production area in the Estado de México. The municipality has an area of 586.53 km^2 and the population consisted of 87,671 habitants in 2020, according to INEGI 2020 data.

A personal survey was applied to 93 producers in this municipality in order to obtain information about the habits and customs of corn cultivation in this part of Estado de México. After the information obtained was analyzed, 10 growers were selected to follow up *in situ* on the type of agricultural practices they carry out during production in order to photographically record the development of their fields.

Application: In order to determine the type of agricultural practices employed by corn growers in Jilotepec, a survey was conducted to find out if they are using conventional farming practices, meaning that they prefer single-crop production and use synthetic fertilizers to prevent insect pests, or whether they use agro-ecological practices that include organic composts and fertilizers which are environmentally friendlier, as well as the types of seeds they grow, whether native or hybrid. The survey consisted of thirteen questions about the crops production, whether it was for self-consumption, third-party sales or mixed production. Also, the exact location where the plot or cropland was established, in order to set up a database. As well as the number of sowings per year and the expected planting dates if they were devoted to sowing in rainy season or if they had an artificial irrigation system that could help them in case there was a delay in the rainfall system.

RESULTS AND DISCUSSION

This study was carried out in order to obtain information about different aspects related to corn cultivation in the municipality of Jilotepec de Molina Enríquez in the Estado de México (Figure 1A).

For the purpose of knowing the current situation about the type of practices used for corn production, the present study was performed between april-august 2022, initially a survey was applied to 93 producers consisting of closed and open-ended questions to have the possibility of collecting additional information that the producers might share and to allow the management of additional data. Afterwards, from the results obtained, 10 plots were selected to be followed up directly in the field until the biological material (cobs) were obtained.

After analyzing the results, most of the farmers (96.7%) obtained one harvest per year, while only 3.3% obtained two harvests per year (Figure 1B). These results suggest that most of the farmers obtain the harvest taking advantage of the rainy season because of the greater water availability and it does not generate additional costs derived from the use of systems for extraction and distribution. These activities help to keep low costs during production; farmers prefer to use these resources as supplies that are considered important to ensure their production, which are further discussed in the next section.

Another interesting feature is the way of designing the milpa, most of the farmers imitate the traditional agricultural system, which is based only on planting corn, so the monoculture is the strategy used by 60.2% of the producers, Figure 1C. The rest of the farmers (39.8%) are dedicated to multi-crop production, in addition to corn, they grow squash, chili, beans, sunflower, oats, wheat, barley and grass, in an interspersed arrangement in the plots and rotating the areas in use.

Although less than half of the growers are inclined to cultivate in the polyculture mode, a good part of them imitates the traditional way of cultivating, incorporating other vegetables

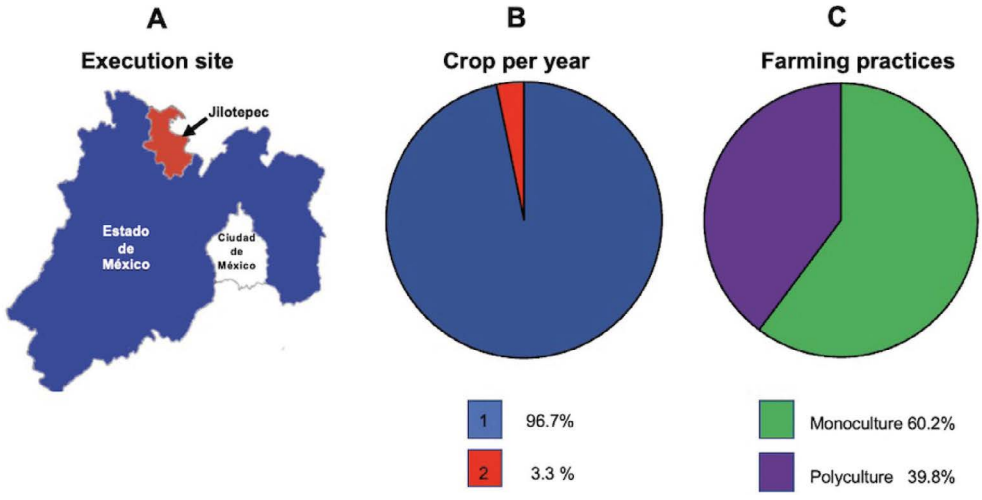

Figure 1. Study site of this work and types od agricultural practices. (A) Location of the municipality of Jilotepec in the State of Mexico. (B) Number of plantings per year carried out by producers in Jilotepec, 1 indicated in blue and 2 indicated in red. Type of planting carried put by producers, monoculture (green) and polyculture in purple.

for production. These results suggests that this type of practice, which has ancestral origins, is not entirely lost. Therefore, it is relevant that polyculture continues to be a conserved method. Because, in the same way as corn, it can preserve other plant species, besides the fact that it is produced in a more ecological way, without depleting the soil and favoring diversity.

In order to find out if farmers grow corn for self-consumption or for sale to third parties, they were asked about the area of land dedicated to this activity. The results obtained strongly suggest that the farmers interviewed are in the range of small to medium producers because the extensions of land dedicated to corn production oscillate between 0.5-5 hectares (Figure 2) and only a few of them have more land dedicated to this activity with over 10 hectares while only 1 producer reported having more than 40 hectares dedicated to corn cultivation.

Additionally, it was documented that the municipality of Jilotepec grows different corn seeds; native and hybrid varieties, both have a significant impact on the producers' preferences.

Half of the growers raise exclusively native corn (Figure 3), even though hybrid varieties tend to give higher yields per hectare, growers are aware that native corn has qualities that make it unique for production, even though in some cases the yield is lower than improved varieties. A quarter of the farmers plant only hybrid varieties and the remaining 24.7% plant native corn in combination with hybrid varieties.

Moreover, 48.4% of the producers in Jilotepec plant native corn breeds, as can be observed in Figure 3A, this percentage was readjusted as 100% to obtain a relative value regarding native breeds planted in this municipality. The results obtained show that the most sown race is the chalqueño (53.3%), followed by the cacahuacintle (23.4%), while the yellow mountain is sown by 10% of the producers, the coscomatepec race is sown by 6.5% of the producers and the negrito is the race that is sown the least, with 3.4% (Figure 3B). In the same proportion, it was found that there are producers who plant native breeds, but they could not clearly state which breeds they are. The five breeds that are planted in the municipality of Jilotepec represent 8.4% of the 59 native breeds that are registered in our

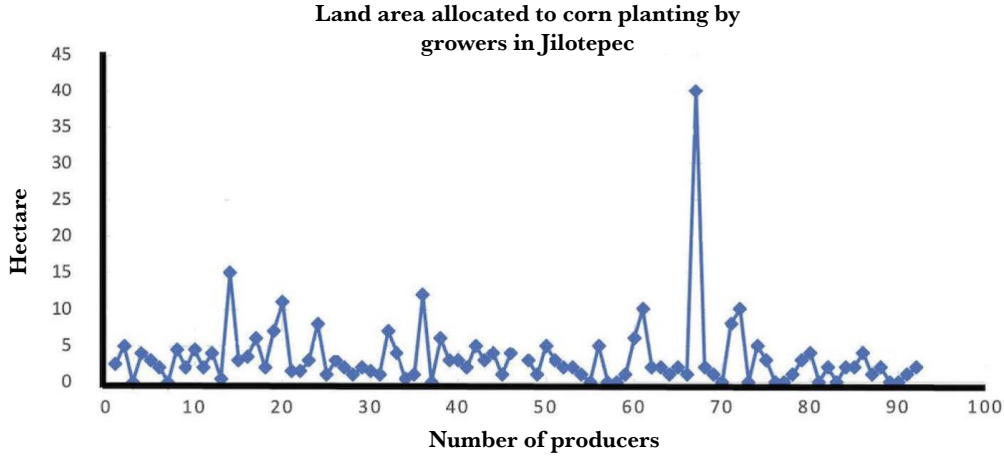

Figure 2. Number of growers and corn planting area in Jilotepec. The majority produce on 5 hectares or less.

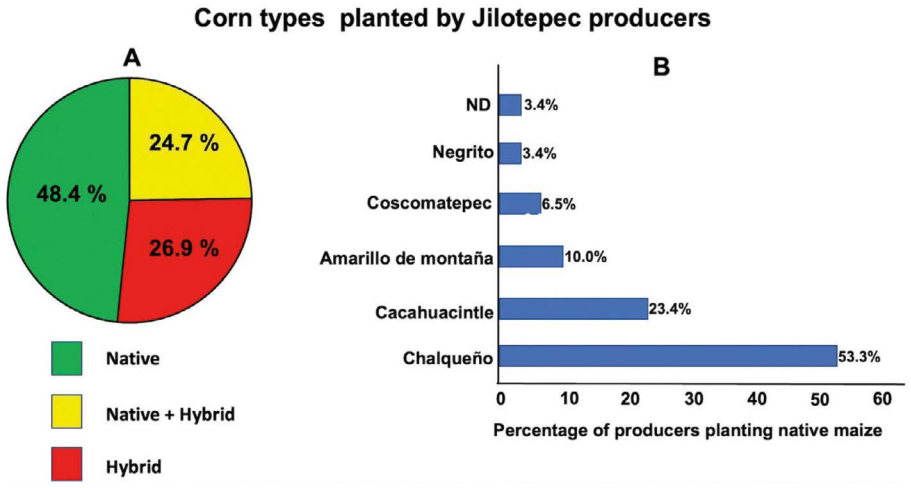

Figure 3. Types of corn seeds planted by growers in Jilotepec. (A) green=native, red=hybrid and yellow=native and hybrid. Figure B is the percentage of producers who planted only native corn as a subgroup of 48.4%. These data was readjusted to be the 100%.

country, so this part of the Estado de México is contributing to these breeds preservation. The harvest of the different breeds continues because small producers, which allows this genetic heritage to remain present for the benefit of future generations, all over Mexico and the rest of the world.

The results suggest that, despite the producers of Jilotepec prefer native seeds for production, it is also a matter of fact that hybrid varieties have become more popular, and it is noteworthy that hybrid varieties may soon displace native maize, which would be a significant loss due to the biological and cultural bastion it represents for both the country and the whole world. Such a change in the type of corn that is planted is possibly caused by experienced producers who refuse to opt for a different type of seed and usually choose the ones they have been collecting for a long time, possibly preserved, and provided by their parents and grandparents. Alternatively, companies dedicated to the production and sale of hybrid maize may provide technical support and demonstrations to growers, so that the higher yield per hectare may be a motivating factor that allows some of them to make changes towards this type of seed they must purchase, leaving aside the native seeds and adopting those generated by traditional breeding. Among the modified seeds used in the municipality of Jilotepec, it is observed an ample range of brands (Table 1), such as Asgrow, Ceres, Hartz Seeds, Bayer-Monsanto, Corteva-Agriscience.

Most of the growers recognize which hybrid they are handling from these brands. However, others do not recognize them at all and just identify the seeds according to the brand name. It is relevant to point out that they are also using seeds developed by the public sector. One of the hybrids used is HIT-7, which was developed by the Instituto de Investigación y Capacitación Agropecuaria, Acuícola y Forestal (ICAMEX) for high valleys and whose grains are white crystalline. A further hybrid used is H52, developed by the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP),

Table 1. Hybrid corn seed used.

Hybrid	Business/Institution
Albatros	Asgrow
Cenzontle	Asgrow
Cherokee	Asgrow
Cimarrón	Asgrow
Faizan	Asgrow
Niebla	Ceres
Z-25	Hartz Seeds
Z-60	Hartz Seeds
Hit-7	ICAMEX
H-52	INIFAP
unrecognized	Asgrow
unrecognized	Bayer-Monsanto
unrecognized	Corteva Agriscience

Brands and hybrids varieties used by Jilotepec's producers.

adapted for the central highlands of Mexico, a material that has been widely tested and registered in the Catalog of Feasible Varieties since 2006.

To determine whether additional supplies are used in corn production to achieve optimal yields, we asked which compounds, such as fertilizers, herbicides and pesticides, were used to increase yields. The results clearly suggest that producers, despite planting very small-scale plots (of about ¼ hectare), are using various chemical compounds. Slightly more than half of the farmers use urea and diammonium phosphate, 51.6%.

In addition to fertilizers, 16.1% of the producers use both herbicides and insecticides to avoid the risk of weeds, and to minimize and eliminate pests that could hinder the

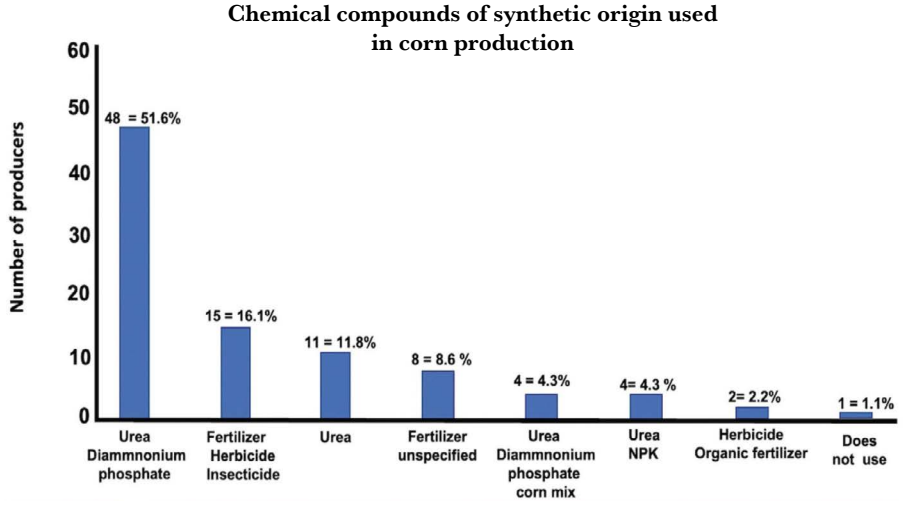

Figure 4. Commercial compounds obtained by chemical synthesis that are used in the municipality of Jilotepec for the development and growth of corn plants. They were applied alone or in combination, as indicated for slash.

normal development of the crop by severely affecting yields per hectare. On the other hand, 8.7% of the producers mentioned using chemical fertilizers to obtain favorable harvests, however, they could not specify what type of fertilizers they use in the handling of their crops. A smaller percentage of producers (4.3%) use a combination of different formulations to improve yields, *i.e.*, some use urea, diammonium phosphate and corn blend fertilizer (composed of total nitrogen, urea, nitrogen, total sulfur, boron, silicon and zinc). Another group of producers also uses urea and nitrogen,phosphorus and potassium (NPK).

Surprisingly, a remarkably low percentage reported the use of organic fertilizers, such as composts based on plant residues and animal excrements, though not exclusively because chemical compounds are also used. Only one producer mentioned not using any type of chemical compound to favor the yield of his plot. As can be seen, the use of chemical compounds is widespread among producers in Jilotepec, Estado de México, possibly because these compounds can be used to manage weeds and pests more efficiently, because the use of fertilizers results in more vigorous plants, which are able to assimilate more energy due to a healthy foliage development that results in higher yields. This can be observed at a glance since the visited plots contained healthy plants, which not only was noticed after germination since a photographic record of the selected plots was made, figure 5A-F, are examples of how these plots devoted to corn production look like, and when monitoring them, healthy maize was seen to have reached successful growth, this can be seen in figure 5A-C, with are plots under monoculture. In figure 5D-F while something similar occurs in the plots under polyculture production.

However, this kind of farming practices are not environmentally friendly, even if the compound applications are not spread over very large areas, we must not lose sight of the effect they have on these fields.

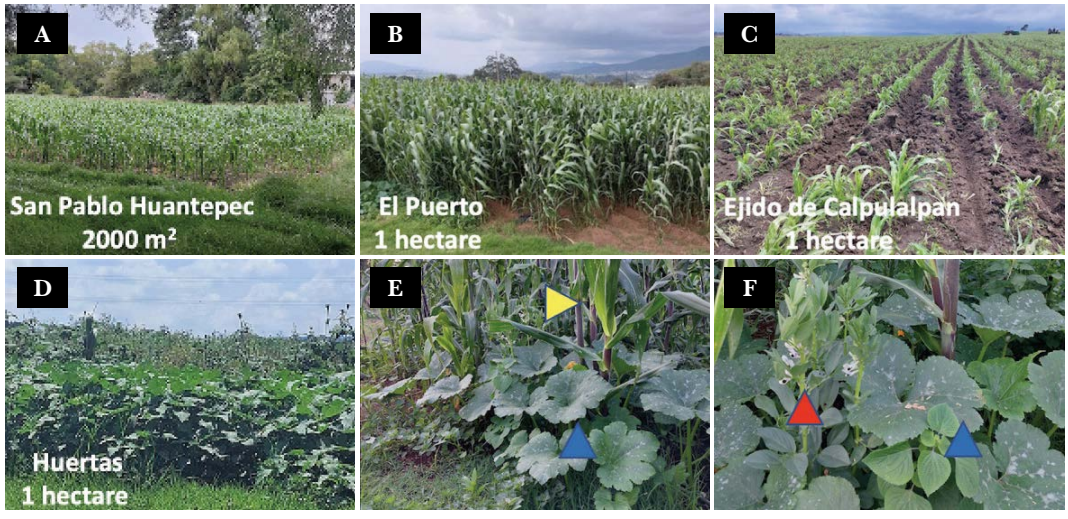

Figure 5. Plot types found in Jilotepec. A, B and C, are plots with different extension used only for maize monoculture. Plot dedicated to polyculture (D). In addition to maize (yellow triangle) could be associated with pumpkins, blue triangle (E) and broad bean, red triangle (F). E and F are close up of D.

CONCLUSIONS

The obtained results in this paper suggest that although there are several companies dedicated to certified corn seed selling that offer higher yields per hectare than native seeds, most corn producers in Jilotepec prefer native seeds, which contributes to maintain the heritage of the diverse corn breeds that are adapted to the high valleys of Mexico.

Even though the milpa was originated in pre-Hispanic Mexico, it has suffered changes in the way it is planted. One of the biggest differences is that polyculture is disappearing among Jilotepec's producers, since less than half prefer this mode of production, and the most widely used monoculture as a conventional agricultural model. In addition, the organic fertilizers to promote growth and plant development, such as the use of organic plant compounds to prevent insect pests, have been replaced by synthetic compounds, which means that traditional agricultural practices that are environmentally friendly are also gradually disappearing among the small producers in the municipality of Jilotepec, which basically use harvested crops for their own consumption and rarely for sale to third parties.

Finally, although the Jilotepec producers are not strongly implementing agroecological methods in corn cultivation, it can be mentioned that these methods are partially present in the productive activity as they favor the native corn cultivation, lean towards polyculture and do not use artificial irrigation systems, unfortunately, the use of chemical compounds is still prevalent in the plots' management.

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AGRO PRODUCTIVIDAD

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Encapsulation of bioactive compounds of food interest: applications, current advances, challenges, and opportunities

Fabela-Morón, Miriam F.[1*]; Pérez-Ruíz, Rigoberto V.[1]; Ruíz-Hernández, Rafael[1]; Arce-Vázquez, María B.[1]; Aguilar-Toalá, José E.[1]; Jiménez -Guzmán, Judith[1]; García-Garibay, José M.[1]

[1] Universidad Autónoma Metropolitana, Unidad Lerma. Departamento de Ciencias de la Alimentación. Av. de las Garzas No. 10, Col. El Panteón, Municipio Lerma de Villada, Estado de México, México. C.P. 52005.

* Correspondence: mf.fabela@correo.ler.uam.mx; qa.miriamfabela@gmail.com

ABSTRACT

Objective: The encapsulation of bioactive compounds of food interest provide protection against ambiental factors and degradation reactions. Therefore, the encapsulation of these compounds, was studied and analyzed considering the applications, current advances, challenges, and opportunities on the topic.

Design/methodology/approach: Wall materials, bioactive compounds of food interest, encapsulation methods, applications, current advances, challenges, and opportunities in encapsulation of bioactive compounds were explored, described, and discussed considering the principal literature on the topic, and scientific databases were used for the bibliographic research.

Results: Encapsulation process is a novel technology that allows the increasing the stability of aromas, flavors, pigments, and microorganisms, beside of improve the sensory, physical chemical and functional properties, quality, and the extend the shelf-life.

Limitations on study/implications: Foods contain bioactive compounds that are susceptible to oxidation and degradation, which can reduce their quality and shelf life. To preserve these compounds, is important to develop other encapsulation systems considering alternative wall materials from different sources that can be applied under different process conditions from laboratory, pilot to industrial scale.

Findings/conclusions: Encapsulation process provide protection to bioactive compounds enhancing the sensory, physical chemical and functional properties, quality, and extend the shelf-life considering the integral and sustainable use of agricultural products.

Keywords: encapsulation, bioactive compounds, food interest, applications

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INTRODUCTION

Food systems are susceptible to degradation making them unacceptable for its consumption and shelf-life. Bioactive compounds from foods require to be protected to extend the shelf-life, to improve its physical chemical, and sensorial properties, quality and stability in food science and technology. Exist an increasing interest in the development of food products containing natural compounds with antioxidant activity and other valuable properties. However, due to their structure and nature certain bioactive compounds such as polyphenols, flavonoids, carotenoids, among others, are not stable when are incorporated in food systems. The stability and shelf-life of bioactive compounds increase when are protected against chemical and physical factors prior to their application. In this sense, encapsulation process provides protection due to its potential for stabilization

and controlled release of sensitive bioactive compounds, increasing the bio accessibility of bioactive molecules during digestion, and raising the water solubility of bioactive ingredients of food interest in different applications (Hcini *et al.*, 2021; Jayaprakash *et al.*, 2023; Komijani *et al.*, 2022).

Encapsulation technology

Encapsulation process is the technology by which a material or mixture of materials is entrapped with another material in the form of micro- and/or nanostructures through entrapment of a bioactive core with another substance called wall materials to provide protection to bioactive compounds as flavors, colorants, among others (Finney *et al.*, 2002; Jayaprakash *et al.*, 2023). This technology is widely used in the pharmaceutical, chemical, cosmetic and food industries. In the food industry, is used to encapsulates oils, fats, aromatic compounds, oleoresins, vitamins, minerals, colorants, flavors, antioxidants, and enzymes (Madene *et al.*, 2006). The material inside the microcapsule is called "core", "inner phase" or "filler", while the wall is referred to as "shell", "coating", "wall material" or "membrane". Wall material does not react with the encapsulated compound (Gharsallaoui *et al.*, 2007).

Wall materials as encapsulant agents

Several food grade biopolymers have been used as wall materials in encapsulation of bioactive compounds, such as gum arabic, alginates, carrageenan, mesquite gum, proteins (milk or whey proteins, gelatin, soy, pea), maltodextrins with different dextrose equivalents, waxes and their blends (Mohan *et al.*, 2015; Wandrey *et al.*, 2010; Yang & McClements, 2013).

Physical chemical properties such as solubility, molecular weight, glass transition, crystallinity, diffusivity, film formation and emulsion properties, compatibility with the food product, dissolution release, among others, are very important to select the encapsulant agents (Madene *et al.*, 2006). Carbohydrates such as starches, corn syrup solids and maltodextrins are frequently employed as encapsulating agents. These materials exhibit low viscosities at high solids concentrations and good solubility (Da Costa *et al.*, 2012; Madene *et al.*, 2006), due to reduced interfacial properties of these materials are employed with mixtures of proteins or gums (Jayasundera *et al.*, 2011; Vélez-Erazo *et al.*, 2021). Starch and starch-based ingredients (modified starches, maltodextrins and cyclodextrins) act as carrier agents for flavor encapsulation, fat replacers and emulsion stabilizers (Falcão *et al.*, 2022; Madene *et al.*, 2006). Maltodextrin is a polysaccharide obtained by partial hydrolysis of corn, potato, or rice flour by means of acids or enzymes. Its chemical composition consists of D-glucose units linked with bonds (1-4) and with a low number of bonds (1-6) in random position. It has been determined that Maltodextrin with 10-20 DE are more effective for microencapsulation (Šturm *et al.*, 2019; Vidović *et al.*, 2014). Pectin is a polymer that produces stable emulsions due to its emulsifying properties related to the protein residues present with the pectin chains and to its chemical composition characterized by a high content of acetyl groups (Braccini & Pérez, 2001; Popoola-Akinola *et al.*, 2022). Gums and thickeners decrease sweetness in encapsulation process (Madene *et al.*, 2006), being used

in encapsulation for their emulsion-stabilizing and film-forming properties; the most used is gum arabic (Krishnan *et al.*, 2005). Gum arabic is a branched heteropolysaccharide consisting of D-glucoronic acid, L-rhamnose, D-galactose and L-arabinose (F. C. da Silva *et al.*, 2013). This material has emulsifying properties due to the presence of arabinogalactan structure (Archaina *et al.*, 2019; Mcnamee *et al.*, 1998). Proteins as natural encapsulant matrix have functional properties (solubility, film formation, ability to interact with water, emulsion formation and stability properties), proteins play a role as potential ingredients for the development of novel encapsulant systems. In recent years, protein-based carrier agents like whey protein and soy protein isolate are reported to be more effective and to produce higher product yields even when used at low concentrations (Jayasundera *et al.*, 2011; Nesterenko *et al.*, 2013).

Chitosan is other wall material derived from chitin, that can be found in the cell wall of fungi, exoskeleton of arthropods, and crustaceans. Is environmentally friendly, non-toxic, and has excellent antimicrobial and fil-forming properties to be used in encapsulation process (Ashfaq *et al.*, 2022; Chausali *et al.*, 2022; Ré, 2006). Hydrogels and nanocarriers, also can be used as natural carrier agents for encapsulation as three-dimensional (3D) colloidal systems contained of physically or chemically bonded linear or branched polymer chains that can retain and absorb considerable amounts of water or bioactive compounds owing to hydrophilic features in the substitutional gaps between chains with amide ($-CONH-$), carboxyl ($-COOH$), hydroxyl ($-OH$), and sulfonic acid ($-SO_3H$) inside chains (Do *et al.*, 2022). Principal coating materials that can be used in hydrogels are polysaccharides, proteins, and lipids (Amiri *et al.*, 2022; Wen *et al.*, 2022). On the other hand, when polymers are used in mixtures of two or more encapsulating agents, the encapsulate structure can exhibit excellent physical characteristics and properties. The synergistic effect resulting from the mixture of carbohydrates, gums and proteins offers a significant application in bioactive compounds encapsulation, cost reduction in price and to create new macro, micro and nanostructures (Carneiro *et al.*, 2013; Du *et al.*, 2014; V. M. Silva *et al.*, 2014). For example, mixtures of whey proteins with maltodextrin, corn syrup and lactose; soy proteins with maltodextrin; sodium caseinate with lactose; whey protein concentrates with maltodextrin, increase the retention of volatile compounds and the efficiency of encapsulation of food oils by spray-drying (Rodea-González *et al.*, 2012).

Bioactive compounds of food interest

Among the bioactive compounds of food interest to encapsulate, stand out ingredients that provide flavor, color, beside of antioxidants, polyunsaturated fatty acids, polyphenols, etc. Table 1 indicates the principal bioactive compounds of food interest to be encapsulated:

Flavors. Flavors whether liquid or solid, are a complex mixture of volatile substances and labile components, susceptible to oxidation, chemical interactions, or volatilization and consequently affect sensory perception. To minimize damage, microencapsulation is used to trap liquid flavor substances: essential oils, oleoresins, aroma blends and acidulants. In this regard, research has been conducted to evaluate the wall material composition and operational conditions in relation to the retention and control of the release of encapsulated flavors (Buffo *et al.*, 2002; Goula & Adamopoulos, 2010). Enzymes. One of

Table 1. Principal bioactive compounds of food interest

Bioactive compounds	Source	Application	References
Bioactive peptides	Spirulina	Peptide based foods.	(Ovando *et al.*, 2018)
Phenolic compounds	Agroindustrial waste of mango	Food products with antioxidant activity	(Castro-Vargas *et al.*, 2019)
Antioxidants, phenolics, flavonoids carotenoids, anthocyanins, pectin	Tropical fruit, agri-food by-products	In functional foods and nutraceuticals	(Cádiz-Gurrea *et al.*, 2020; Gullón *et al.*, 2020)
Aminoacids, phenolics	Beans (*Phaseolus vulgaris*), (*Phaseolus lunatus*), (*Phaseolus coccineus*)	Development of functional foods with nutraceutical potential.	(Alcázar-Valle *et al.*, 2021)
Polyphenols	*Citrus pomace*	As nutraceuticals in functional foods and beverages	(Caballero *et al.*, 2021)
Phenolics, pectin, oils	Pomegranate fruit biowastes	Industrial application	(El-Shamy & Farag, 2021)
Vitamins, carotenoids, polyphenols	Kiwi fruit, byproducts	Development of novel valuable foods	(Sanz *et al.*, 2021)
Phenolics, oils	Olive byproducts	Foods packaging systems.	(Khwaldia *et al.*, 2022)
Carotenoids, pigments	Agrowastes from fruits, vegetables	Red, orange, and yelow colorants in food industry	(Cassani *et al.*, 2022)
Unsaturated fatty acids, amino acids, carotenoids, chlorophylls, phycocyanins, phenolic compounds	Spirulina	For use as colorants, antioxidants and functional ingredients in food products.	(Bortolini *et al.*, 2022)
Capsaicinoids, phenolics, pigments	Habanero chili pepper, Bell pepper wastes	Ingredient in food products.	(Fabela-Morón *et al.*, 2020; Razola-Díaz *et al.*, 2022)
Anthocyanins, ellagic acid, tocopherols, tannins, polyphenols	Jabuticaba (*Myrciaria* spp. or *Plinia* spp.)	As a food supplement in beverages, bakery products, and biodegradable film	(Fernandes *et al.*, 2022)
Flavonols, phenols and anthocyanins	Berries agroindustrial waste	Development of protective films.	(Romero *et al.*, 2022)
Amino acids, phenolic, flavonoids compounds	*Moringa oleifera* Lam.	Agroindustrial and food products uses.	(Ruiz-Hernandez *et al.*, 2022)
Antioxidants	Legumes	Chemopreventive agents in foods	(Sánchez-Chino *et al.*, 2022)

Source: own elaboration.

the main advantages of enzymes encapsulation by spray drying is the short times of this process. Due to the thermosensitivity of enzymes, the duration of this process is used to microencapsulate without causing severe damage to the enzyme structure. In addition, the incorporation of proteins as encapsulant agents increases porosity, surface flexibility, mechanical strength, and water evaporation (Jayaprakash *et al.*, 2023; Wen *et al.*, 2022).

Antioxidants. In food industry, antioxidant compounds have become of great interest due to their potential benefits as important nutraceuticals ingredients, because they have a protective effect on oxidative process (Fang & Bhandari, 2010).

Polyphenols. The consumption of polyphenols (anthocyanins, catechins, tannins and flavonoids) reduces cardiovascular damage and the risk of cancer. The effectiveness of these compounds depends on the stability, bioactivity, and bioavailability of the active compound. Due to their sensitivity to environmental factors such as light, heat and oxygen, encapsulation is an effective process for their preservation (Bakowska-Barczak & Kolodziejczyk, 2011; Koop *et al.*, 2022)to a large extent, due to its content of bioactive nutrients and their importance as dietary antioxidants. There is a growing demand for delivery of antioxidants through functional foods with the related challenge of protecting their bioactivity during food processing and subsequent passage through the gastrointestinal tract. This study focuses on the evaluation of concentration of bioactive compounds in black currant berries (*Ribes nigrum* L.).

Carotenoids. Composed of the hydrophobic carbon chain and are defined as natural pigments synthesized by plants, algae, bacteria, and fungi sources, which are related to the yellow, orange, and red colors (Luana Carvalho de Queiroz *et al.*, 2022).

Polyunsaturated fatty acids. Encapsulation of polyunsaturated fatty acids as omega-3 and omega-6 fatty acids, is commonly used in food fortification for their considerable health benefits to preserve their characteristics such as odor, flavor and oxidative stability (Carneiro *et al.*, 2013; Khwaldia *et al.*, 2022; Quintanilla-Carvajal *et al.*, 2010).

Encapsulation methods

There are different encapsulation methods that can be used to encapsulate bioactive compounds of food interest, which are indicated in Figure 1:

Spray drying. Is an encapsulation method that provides maximum protection of the encapsulated compounds in dried and stable form (Fang & Bhandari, 2010), improving flavor, aroma, stability, nutritional value and appearance, and allowing the complete release of the bioactive ingredient from wall material (Adamiec & Kalemba, 2006; Krishnan *et al.*, 2005). These process achieves microparticles or microcapsules with particular characteristics and properties (Burgain *et al.*, 2011).

Spray chilling. Is a variant of spray drying, which consists of cooling or freezing, where the material to be encapsulated is mixed with the carrier and atomized by means of nebulization of the emulsion or suspension containing the wall material and the solid or liquid active substance (sensitive pigments, flavors, and aromas, antioxidants, and natural preservatives) in cold air (Figueiredo *et al.*, 2022; Yáñez *et al.*, 2002). The coatings usually used are vegetable oils in the case of cooling spraying or hydrogenated vegetable oil for freeze spraying; thus, heat-sensitive liquids and materials that are not soluble in conventional solvents can be encapsulated.

Freeze drying. Is the widely used method for the encapsulation of thermosensitive substances, because minimizes thermal degradation reactions for carotenoids encapsulation (Šeregelj *et al.*, 2021). Coacervation. involves the separation from solution of colloid particles which then agglomerate into separate, liquid phase called coacervate, the core material

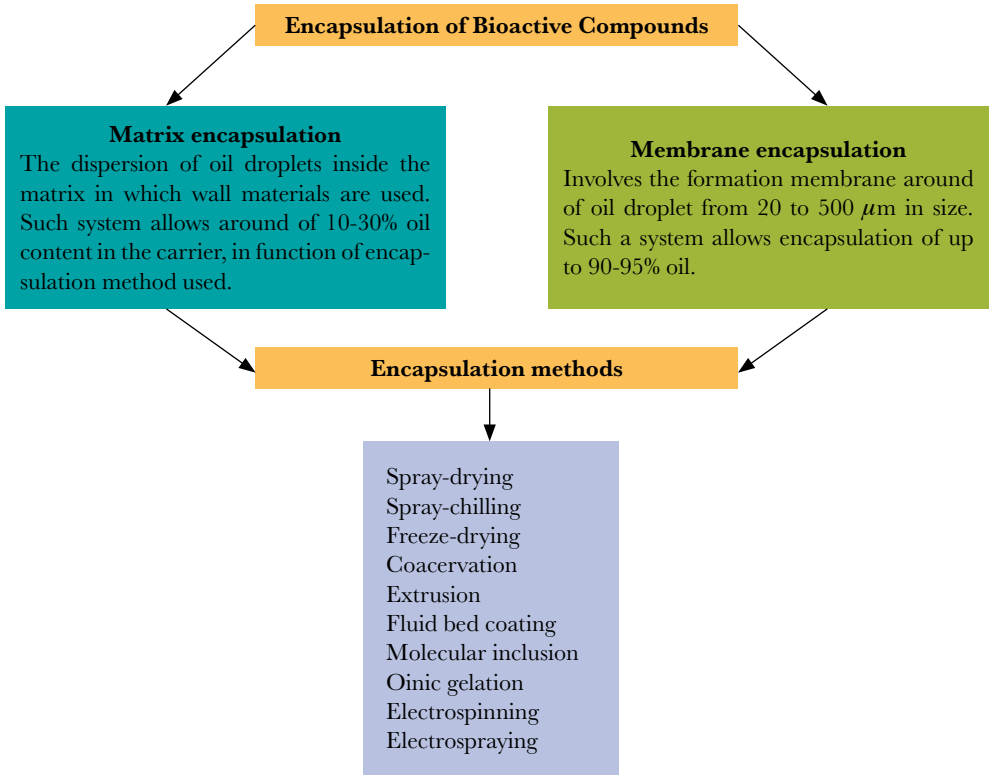

Figure 1. Classification of encapsulation methods. Source: own elaboration.

used must be compatible with the receiver polymer and be insoluble or partially soluble in the coacervation medium. Simple coacervation involves only one type of polymer with the addition of strongly hydrophilic agents to the colloidal solution. For complex coacervation, two or more kind of oppositely charged polymers are used to protect bioactive compounds (Madene *et al.*, 2006; Ré, 2006).

Extrusion. is based on the gelation of an anionic polysaccharide, when in contact with calcium or any other multivalent ion, immobilizing microorganisms or bioactive compounds. Alginate, k-carrageenan and whey proteins can be used to obtain capsules (beads) by this method.

Fluid bed coating. Consists of suspending solid particles in air at high velocity in a temperature and humidity controlled chamber, where the wall material is atomized with hot air to encapsulate bioactive ingredients using hydrogenated vegetable oils, stearins, fatty acids, emulsifiers, waxes, starches, gums and maltodextrins (Yáñez *et al.*, 2002).

Molecular inclusion. Encapsulation of compounds is performed using cyclodextrins and maltodextrin as biopolymer matrix (Yáñez *et al.*, 2002).

Ionic gelation. Process that encapsulates by means of extrusion dripping, is an efficient and low-cost method that does not require specialized equipment, high temperature, or organic solvents, making it appropriate for hydrophobic or hydrophilic compounds using calcium alginates (Arriola *et al.*, 2019).

Immobilized cell technology. In this process, the material to encapsulate as probiotics, enzymes, is trapped throughout the matrix, but not necessarily inside it by

extrusion or emulsification. Involves the entrapment of living cells in spherical gel beads and was initially designed to improve continuous fermentation processes, especially in the dairy industry, as well as to enhance cell protection against undesirable conditions (Frakolaki *et al.*, 2021).

Electrospraying. Is used in food and nutraceutical applications, based on electrohydrodynamic process using high voltage electrical field without heating, offers high product quality, encapsulation efficiency, short drying time and economical as novel alternative method (Jayaprakash *et al.*, 2023). Electrostatic spray drying has recently been introduced as novel process, which uses electrostatic charge technology, and achieve low drying temperatures, higher encapsulation efficiency, and reduced bioactive compounds degradation (Jayaprakash *et al.*, 2023).

Applications and current advances in encapsulation of bioactive compounds

Table 2 shows the applications and current advances of encapsulated bioactive compounds using different wall materials. The advantages of encapsulating these compounds are related to retard auto-oxidation, increase stability, enhance flavor, mask the bitter taste of lipidic substances, and to reduce the risk of oxidation.

Challenges and opportunities in the encapsulation of bioactive compounds of food interest

Meat and seafood products contain compounds like polyunsaturated fatty acids and fat-soluble vitamins that are susceptible to oxidation, which can reduce their quality and shelf-life. Therefore, there is interest in developing antioxidant packaging materials to preserve these sea products as part of principal challenges in encapsulation technology for their preservation. Respect to fruits and vegetables, have been protected with nanostructured wall materials, alginate-based films, cellulose nanocrystals, and polylactic acid/gelatin/polylactic acid multilayer films, among others. Consequently, in food products is being of interest the development of another encapsulant systems to improve their protection, properties, and shelf-life considering the different alternatives that encapsulation methods and wall materials provide.

In addition, the development of encapsulation process suitable for different applications in the food industry has some challenges and opportunities to be investigated and improved, which need to be addressed in the future research of encapsulation of bioactive compounds.

The current development of novel encapsulation systems is mainly focused on the preparation step in the laboratory, and most of the research developed does not take into consideration the cost and production conditions process. Also, other challenges and opportunities in the encapsulation of bioactive compounds include the exploration of alternative wall materials from different sources that can be used in different foods stuffs and food matrices to improve the performance in the creation of microparticles. Therefore, is necessary to extend the research developing of manufacturing processes to encapsulate bioactive compounds with different wall materials and selecting the kind of encapsulation method from pilot-scale to industrial scale. Additionally, is important to evaluate and establish the possible effects of encapsulant agents and process conditions

Table 2. Applications of bioactive compounds encapsulated.

Bioactive compounds	Wall materials	Encapsulation method	Application	References
Aqueous extract of pink-fleshed guava fruit	Maltodextrin, gum arabic, and their mixtures.	Spray-drying	Product that can be incorporated into different food products in powder form.	(Osorio *et al.*, 2011)
Paprika oleorresin	Modified starch Capsul®	Spray-drying	Carotenoids, antioxidants protection.	(Rascón *et al.*, 2015)
Phenolic and antioxidant compounds from Roselle (*Hibiscus sabdariffa* L.)	Maltodextrin, gum arabic	Spray-drying	Instant beverage powder to extend shelf-life.	(Archaina *et al.*, 2019)
Non-dewaxed Propolis and antioxidants from honey	Maltodextrin, gum arabic, inulin	Freeze-drying Spray-drying	Water-dispersible propolis powder and phenols protection.	(Šturm *et al.*, 2019)
Antioxidants from Bottle Gourd (*Lagenaria siceraria*) juice	Maltodextrin, whey protein isolate, soy protein isolate	Spray-drying	Beverage powder to extend shelf-life and bioactive compounds protection.	(Bhat *et al.*, 2021)
Antioxidants from food sources	Gelatin, whey, soy proteins, cellulose, starch, chitosan, alginate, lipids, beeswax, palm wax, fats, and oils.	Nanotechnology process	Active food materials as nanoparticles, nanofibers, nanocrystals, and nanoemulsions with antioxidants properties.	(Cheng *et al.*, 2022)
Phenolic compounds	Tunisian Rosemary (*Rosmarinus officinalis* L.) Extracts	Coprecipitation	Nanoparticles for use in food tecnology.	(Hcini *et al.*, 2021)
Capsicum oleoresin	Gum arabic, modified corn starch (EMCAP™), modified malt (MALT)	Spray-drying	*Capsicum oleoresin* protection with capsaicin retention, and antioxidant activities.	(da Silva Anthero *et al.*, 2022)
Asthaxantin	Carrageenan, chitosan, lupin protein isolate	Spray-drying	Functional ingredient in Foods.	(Morales *et al.*, 2021)
Cinnamon esential oil	Sodium alginate, chitosan powder,	Droplet-based millifluidic technique	Food engineering.	(Farahmand, Emadzadeh, *et al.*, 2022)
Probiotics	Prebiotics matrices	Immobilized cell technology, Extrusion, Spray-drying, Freeze-drying	For food products to enhance microbial balance.	(Frakolaki *et al.*, 2021)
Lycopene	Basil gum, zein	Electrospinning	Functional nanofibers.	(Komijani *et al.*, 2022)
Probiotics	Low molecular chitosan powders	Ionic gelation	Probiotics protection and controlled release.	(Farahmand, Ghorani *et al.*, 2022)
Phenolics, probiotics	Chitosan, fructans, whey protein, alginate, gelatin, oils	Electrospraying, spray-drying	Use in food products.	(Jayaprakash *et al.*, 2023)

Source: own elaboration.

on sensory, physical chemical, and tecno-functional properties, human health, and the environmental impact in a sustainable perspective (Cassani *et al.*, 2022; Cheng *et al.*, 2022; Figueiredo *et al.*, 2022; Šeregelj *et al.*, 2021).

CONCLUSIONS

Encapsulation process is a novel technology with many advantages for its applications in a food science and technology, that allows the increasing the stability of aromas, flavors, pigments, and microorganisms, cover undesirable odors and flavors, allow controlled release, and increase the bioavailability of bioactive compounds, with health benefits for consumers. This process has avoided the development of wall materials for different uses in food science and technology. The physical and chemical properties of wall materials can improve the antioxidant and functional properties of active ingredients encapsulated, thus inhibiting the oxidative and degradation reactions in foods. Consequently, encapsulation processes of bioactive compounds can improve the sensory, physical chemical and functional properties, beside of quality, in addition to extend the shelf-life considering the integral and sustainable use of agricultural products within the applications, challenges and opportunities to improve the encapsulation of bioactive compounds of food interest from laboratory, pilot to industrial scale.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Presence of pesticides in edible insects: Risk to human health. The case of Mexico

Hernández-León, Karla P.[1]; Aguilar-Toalá, José E.[2]; Díaz-Ramírez, Mayra[2], Cruz-Monterrosa, Rosy G.[2*]

[1] Universidad Autónoma Metropolitana, Unidad Lerma Licenciatura en Ciencia y Tecnología de Alimentos. División de Ciencias Biológicas y de la Salud.
[2] Universidad Autónoma Metropolitana, Unidad Lerma Departamento de Ciencias de la Alimentación. División de Ciencias Biológicas y de la Salud.
* Correspondence: r.cruz@correo.ler.uam.mx

ABSTRACT

Objective: To perform a literature review of the presence of pesticides in edible insects, main pesticides used in Mexico and to discuss the potential risk of contaminated edible insects for human consumption.

Design/methodology/approach: Concise analysis of the main research topics related with the impact of pesticides on insects, through a wide review of specialized journals on insects' field.

Results: The majority of edible insects are considered as a common plague in some crop varieties, causing a decrease in their production yield. As a result, farmers use mainly chemical insecticides to control this plague. Besides, farmers use also chemical herbicides and fungicides to control weeds and fungi. However, those pesticides have a negative impact on edible insects because they can be contaminated. These contaminated edible insects can be collected from different crop varieties for their use as food.

Limitations on study/implications: To conduct further research to identify and determine the pesticide levels in edible insects consumed in Mexico.

Findings/conclusions: Edible insects may represent a potential risk to human health, especially when insects are wild harvested because can be contaminated with pesticides, particularly insecticides, herbicides, and fungicides.

Keywords: Edible insects, pesticides, hazardous, health risk.

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INTRODUCTION

Mexico is one of the countries with the highest reported number and variety of edible insects (Baiano, 2020). The majority of edible insects are considered as a common plague in diverse crops because ravaging leaves, flowers, and fruits, and even can destroy a whole plant when they come in massive numbers (Gross, 2021; McLaughlin & Dearden, 2019). However, it is also recognized that some insect species have important roles in the agro-ecosystem such as pollination service, nutrient cycling, soil formation, decomposition, and water purification (van der Sluijs, 2020). In Mexico, it has been reported that insects can affect to staple food crops such as maize (*Zea mays* L.), alfalfa (*Medicago sativa* L.), coffee (*Coffea arabica* L.), wheat (*Triticum aestivum* L.), tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), and bean (*Phaseolus vulgaris* L.), to name a few (Zelaya-Molina *et al.*, 2022). In addition, other problems such as weeds and fungi damage crops (Anamika Sharma, Jha, & Reddy, 2018) harming agricultural activity, which lowers crop yield that impact the economy of farmers.

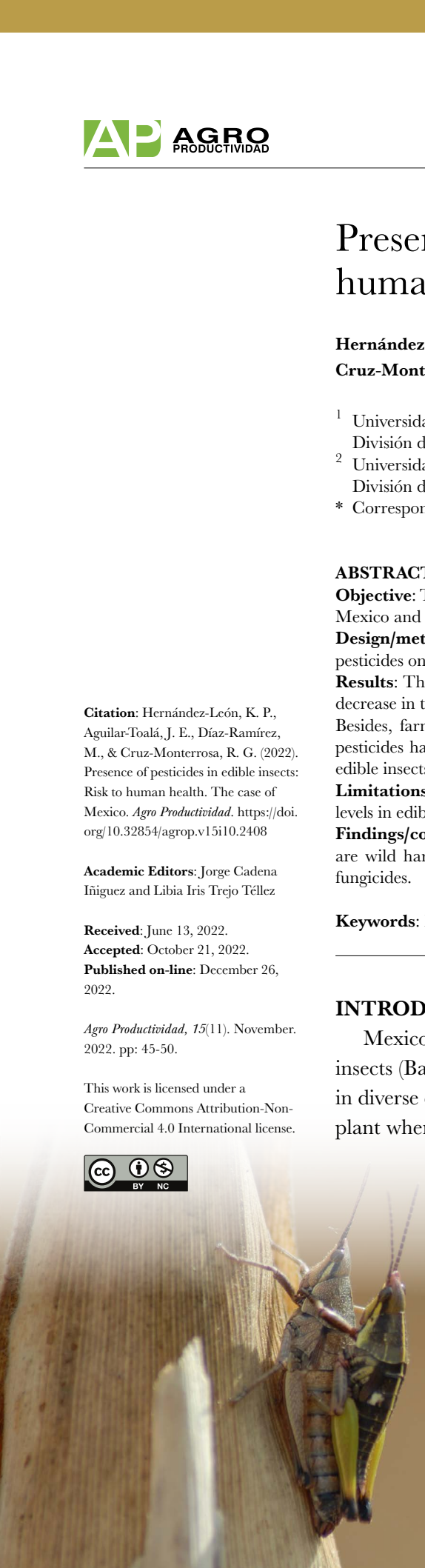

Image by Alvaro González at Pixabay

Thus, pesticides are widely used in agriculture for helping in the control of those pests, and increases food production (McLaughlin & Dearden, 2019; Ndakidemi, Mtei, & Ndakidemi, 2016). It is known that pesticides impact non-target species, which is why not only insecticides have a direct impact on insects but also herbicides and fungicides might have a direct or indirect effect on them (Sánchez-Bayo, 2021). The effect of pesticides on edible insects includes the affectation on their survival of a range of different life cycle stages, reductions in their reproductive capacity, and cause direct mortality (Ndakidemi *et al.*, 2016). Besides, it has been reported that some insects are resistant/development resistant or are not affected by some pesticides because they are not the target; therefore, they survive and result in contaminated insects because carry pesticide residues (Calvo-Agudo, Tooker, Dicke, & Tena, 2022). Also, edible insects can be contaminated because fed on plants that accumulate pesticides (Houbraken *et al.*, 2016). Thus, the consumption of contaminated edible insects collected from crops can represent a potential risk to human health. Various types of health problems have been associated with the exposition to pesticide residues present in some food stuffs, including cancer, diabetes mellitus, respiratory disorders, neurological disorders, reproductive syndromes, and oxidative stress (Rani *et al.*, 2021). The exposure to pesticides through the consumption of contaminated edible insects can be considered accidental or non-occupational, generally low-level and long-term exposure, even though it is difficult to directly relate the exposure to pesticides and their hazardous effects (Sabarwal, Kumar, & Singh, 2018). Thus, the severity of those health problems is related to the pesticide bioaccumulation in human tissues, damage occasioned, detoxifying system activity, as well as antioxidant and immune responses of the consumers (Lushchak, Matviishyn, Husak, Storey, & Storey, 2018; Sabarwal *et al.*, 2018).

The above is relevant because in Mexico the insect consumption dates from pre-Hispanic times being an important component of current culture, gastronomy, and staple diet (Baiano, 2020; Imathiu, 2020). Accordingly, the consumption of edible insects in Mexico could represent a risk to human health due to the presence of pesticides. To the best of our knowledge, there are only a few reports summarizing findings on the presence of pesticides in edible insects. Hence, this review contributes with new and novel information regarding the presence of insecticides, herbicides, and fungicides in edible insects, the potential risk to human health of the consumption of contaminated insects, and the situation of Mexico related to the contamination of edible insects by pesticides.

Presence of pesticides in edible insects

Few studies have identified the pesticides in edible insects consumed in different regions of the world. The majority of the studies were screening-type and do not determine the pesticide levels. In a recent study, De Paepe *et al.* (2019) identified the herbicide isoproturon (<1 μg kg^{-1}) in grasshopper (*Locusta migratoria*), whereas house cricket (*Acheta domesticus*), yellow mealworm (*Tenebrio molitor*), and black soldier fly (*Hermetia illucens*) do not showed residues of this herbicide. Likewise, Poma *et al.* (2017) found that some edible insects and insect-based foods commercialized in Belgium showed presence of some insecticides (*e.g.*, affinine class, empenthrine, methoprene, pirimiphos-methyl),

herbicides (*e.g.*, class of chlorbufam, difenzoquate, class morfamquate, tributylphosphate), and fungicides (*e.g.*, azoxystrobine, cycloheximide, tributylphosphate). However, the main limitation of this study was the screening method used with a qualitative approach. Thus, farmed insects could potentially accumulate chemicals from their substrate. Similarly, Brühl *et al.* (2021) investigated the direct pesticide exposure of flying insects in different areas in Germany. While the authors do not identify taxonomically the wild insect species that were collected, they detected 47 pesticide residues and insect samples were on average contaminated with ca. 17 pesticides. Residues of the herbicides metolachlor-S, prosulfocarb, and terbuthylazine, and the fungicides azoxystrobin and fuopyram were recorded in all insect samples. Besides, the insecticide thiacloprid was identified in most samples.

Likewise, it has been reported that edible Locust, short-horned grasshoppers in the family Acrididae, captured in Kuwait showed pesticides residues such as sumithion (740.6 μg kg^{-1}) and malathion (49.2 μg kg^{-1}), which are organophosphorus type, as well as chlorinated type such as benzene hexachloride (3 μg kg^{-1}), lindane (2.2 μg kg^{-1}), and aldrin (6.2 μg kg^{-1}). Likewise, Calatayud-Vernich, Calatayud, Simó, and Picó (2018) reported that honey bees (*Apis mellifera*) and their bee-derived products (*e.g.*, fresh stored pollen and beeswax) showed the presence of some pesticides including miticides (*e.g.*, coumaphos, fluvalinate, 2,4-dimethylphenylformamide) and insecticides (*e.g.*, chlorpyrifos, chlorfenvinphos). Also, in few samples of pollen and bees showed the presence of prohibited pesticides in the European Union such as dichlofenthion, carbendazim, and fenitrothion. On the other hand, also has been demonstrated that insects used as animal feed showed pesticides residues. Charlton *et al.* (2015) reported residues of the insecticide chlorpyrifos (800 μg kg^{-1}) in house fly (*Musca domestica*) and piperonyl butoxide (200 μg kg^{-1}) in blue bottle (*Calliphora vomitoria*).

While some authors mentioned that the chemical safety of insects in relation to pesticide contamination can be considered high (De Paepe *et al.*, 2019). The toxicological significance of the presence of pesticides in diverse edible insect should not be overlooked. For example, excessive residues of pesticides type organophosphates were found in grasshoppers/locusts in Kuwait and Arabia representing a human risk to consumers (Saeed, Dagga, & Saraf, 1993; van der Spiegel, Noordam, & van der Fels-Klerx, 2013).

The case of Mexico

To the best of our knowledge, there are no reports about the presence of pesticides in edible insects consumed in Mexico. However, in Mexico the application of pesticides is excessive, even are applied some products that are prohibited or not allowed in other countries. For example, according to the Food and Agriculture Organization of the United Nations (FAO), during 2013-2017 the average use of pesticides was estimates in 2 kg/ha (Calderon *et al.*, 2022), peaking in 2014 at 3.85 kg ha^{-1} (López-Gálvez, Wagoner, Beamer, de Zapien, & Rosales, 2018). Besides, although Mexico participates in different international agreements dealing with pesticides, continues to be using pesticides prohibited in other countries such as paraquat, endosulfan, lindane, methyl bromide, parathion, and malathion (Anket Sharma *et al.*, 2019).

In Mexico, the Secretariat of Agriculture and Rural Development (SADER, for its acronym in Spanish) regulates and establishes the maximum residue limit (MRL) for pesticides in foods through the Official Mexican Standard NOM-082-SAG-FITO/SSA1-2017. Additionally, the Federal Commission for Protection against Health Risks (COFEPRIS, for its acronym in Spanish) and the Secretariat of Environment and Natural Resources (SEMARNAT, for its acronym in Spanish) regulate the use of pesticides in Mexico. However, for edible insects and insect-derived products there are no regulations and no specific limits on their pesticide residue content, because edible insects are not considered as food in Mexican legislation (van der Spiegel *et al.*, 2013). Besides, among Latin American countries, Mexico has been placed among the countries with the highest rates of diseases related to pesticide exposure among farmworkers (Payán-Rentería *et al.*, 2012).

On the other hand, Table 1 described the most used pesticides in Mexico, which have been classified as dangerous at different levels (Bejarano González, 2017).

According to their degree of toxicity, pesticides with toxicity categories I and II are classified as extremely and highly hazardous, respectively, which can cause death in the case of ingestion, either if they are inhaled or if they come into contact with skin (Bejarano González, 2017; Githinji, Mwaura, & Wamalwa, 2019). On the other hand, the categories III and IV are considered as moderately and slightly hazardous, which are considered toxics in the case of ingestion (Bejarano González, 2017; Githinji *et al.*, 2019). Thus, it is very likely that edible insect wild harvested from crops in Mexico to be contaminated with the pesticides described in Table 1. Consequently, the consumption of edible insects could represent a risk to human health. However, to the best of our knowledge, there are no studies addressing the impact of pesticides on edible insects consumed in Mexico. Nevertheless, the scientific evidence about the presence of pesticides in edible insects consumed in other countries around the world, supporting the fact that the consumption of edible insects in Mexico may have a negative impact on consumers.

Table 1. Most commonly used pesticides in Mexico.

Active ingredient	Type	Classification	Toxicity category
Parathion	Insecticide	Organophosphates	II
Chlorpyrifos	Insecticide	Organophosphates	III
Cypermethrin	Insecticide	Pyrethroid	III
Malathion	Insecticide	Organophosphates	IV
Permethrin	Insecticide	Pyrethroid	IV
Mancozeb	Fungicide	Carbamate	IV
Chlorothalonil	Fungicide	Chloronitriles	IV
Glyphosate	Herbicide	Phosphonomethyl-glycine	IV
Atrazine	Herbicide	Triazine	IV
Deltamethrin	Insecticide	Pyrethroid	III

Bejarano González (2017). The list was ordered from more to less used.

CONCLUSIONS

Edible insects may represent a potential risk to human health, especially when insects are wild harvested because can be contaminated with pesticides, particularly insecticides, herbicides, and fungicides. It is too necessary to estimate the dietary risk of pesticides through the consumption of contaminated edible insects, mainly in regions of Mexico where the consumption of edible insects is greatest. Therefore, it is evident the need to pursue more studies aimed to reveal pesticide levels in edible insects consumed in different regions of Mexico, as well as to determine the possible health effects on the consumers due to exposure to pesticides through edible insects' consumption.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

State of the art of poecilid fish (Pisces: Poeciliidae) in México

Hernández-López, Marcos[1], Lango- Reynoso, Fabiola[1], Castañeda-Chávez, María R.[1], Montoya-Mendoza, Jesus[1], Castellanos-Onorio, Olaya[2], Diaz-González, Mario[2], Martínez-Cárdenas, Leonardo[3*]

[1] Tecnológico Nacional de México, Instituto Tecnológico de Boca del Río, Carretera Veracruz-Córdoba Km 12, A. P. 68. Boca del Río, Veracruz, México. C.P. 94290.
[2] Tecnológico Nacional de México, Instituto Tecnológico de Veracruz, Av. Miguel Ángel de Quevedo 2779, Formando Hogar, Veracruz, México. C.P. 91897.
[3] Universidad Autónoma de Nayarit Dirección postal: Ciudad de la Cultura Amado Nervo s/n. Tepic, Nayarit. Mexico. C.P. 63190.
* Correspondence: leonardo_martinez@uan.edu.mx

ABSTRACT
Objective: To present a review about the knowledge of the fish of the family poecilidade in Mexico.
Design/methodology/approach: The present review was carried out through the search and bibliographic compilation of literature, as well as in the consultation of articles in different databases, for its subsequent analysis.
Results: Mexico has a great diversity of poeciliids with 105 species registered, 64 are endemic, most are in some category of protection, distributed throughout the national territory, mainly in the Usumacinta River basin. In this area, their reproductive biology and feeding have been studied, which vary according to the species. Their culture in Mexico, focuses on the commercialization of ornamental species both native and exotic.
Limitations on study/implications: The poecilid fishes are a well study group; however, the information about the mexican species is scattered and in different areas are limited.
Findings/conclusions: Mexico is the country with the greatest diversity of poecilid fish worldwide, most of which are in some category of protection, and are a group well distributed throughout the national territory, with potential as environmental indicators and toxicological studies.

Keywords: Fish, Bioindicator, Life stories, Native species, invasive species, Diversity.

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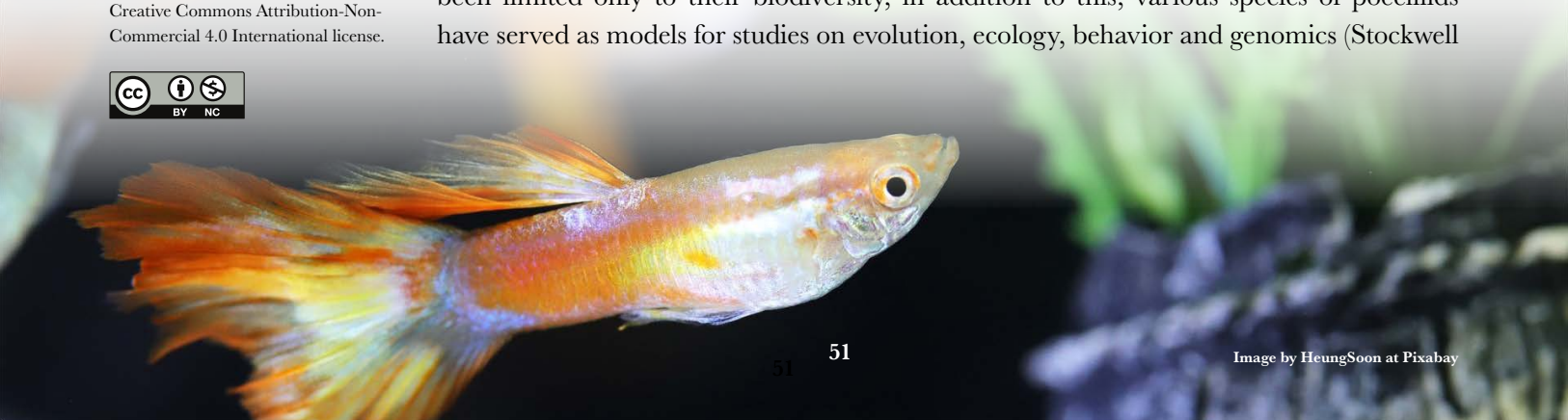
INTRODUCTION

Fish are the most diverse group of vertebrates on the planet, and they have a great variety, high endemism, as well as ecological and economic value (Espinosa-Pérez, 2014). The poeciliids present a tropical distribution, in the American continent from the United States United to Argentina (Gómez-Márquez *et al.*, 1999), the Poeciliidae family is of great importance, both ecologically and economically due to its commercialization as ornamental fish (Miller *et al.*, 2009), In recent years the study of poeciliids has not been limited only to their biodiversity, in addition to this, various species of poeciliids have served as models for studies on evolution, ecology, behavior and genomics (Stockwell



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& Henkanaththegedara, 2011), and more recently they have been used in studies of biomonitoring and bioaccumulation of toxins in aquatic habitats where they inhabit, due to their wide diversity and easy adaptation to captivity (Trujillo-Jimenez & Toledo, 2007; Ramírez-Ayala *et al.*, 2020).

MATERIALS AND METHOD

The selection of bibliographic material was carried out by searching in different databases and scientific search engines data from the National Fish Collection of the Institute of Biology of the National Autonomous University of Mexico (UNAM), the Global Biodiversity Information Facility (GBIF), the IUCN Red List of Species and the official Mexican standard for environmental protection and native species (NOM-059-SEMARNAT-2010) in its last update (2019), as well as digital repositories from different universities and book chapters. To delimit the information found in the different sources, was, considering those that refer to poeciliids species with natural distribution and introduced within the national territory (Mexico).

RESULTS AND DISCUSSION

Biodiversity and conservation

According to the data obtained, 105 species of fish belonging to the Poeciliidae family are distributed throughout the Mexican territory, which represents 30.17% of the 348 species of Poeciliids registered worldwide, this species richness is higher than the last data published for this family with 81 species described for Mexico (Miller *et al.*, 2009). For this reason, the poeciliids continue to be the group of fish with the highest number of species in Mexico, followed by the cyprinids with 76 species and the cichlids with 48 species (Miller *et al.*, 2009). The Poeciliidae family in Mexico it is represented by 14 genera, highlighting the following: *Xiphophorus*, *Gambusia* and *Poeciliopisis*, for presenting the largest number of species with 25, 24 and 22 species respectively. Among this genus, *Xiphophorus*, occupies an important place, since in Mexico there are 89.28% of the 28 species described worldwide (Albornoz-Garzón & Villa-Navarro, 2017). In Mexico, from the total registered species, more than half (54.28%) are in some category of protection, either by international or national organizations, some species such as: *Priapella bonita*, *Xiphophorus couchianus* and *Xiphophorus meyeri*. are considered extinct by international organizations while in Mexico they are considered in danger of extinction (SEMARNAT, 2010) From the 64 endemic Mexican poeciliids more than half (60.93%) are considered under some category of conservation (Jelks *et al.*, 2008). In Mexico 10 exotic species, became invasive in many water bodies in the country, displacing native species, such as: *Poecilia reticulata*, while *Pseudoxiphophorus bimaculatus*, *Xiphophorus helleri* and *Poeciliopsis infans* although they are Mexican, have been introduced into bodies of water where they are not native, becoming invasive (Torres-Orozco & Pérez, 2009).

Distribution

The Poeciliidae family in Mexico, inhabits fresh waters and some brackish waters (Gómez-Márquez *et al.*, 1999). This can be associated with the fact that in a good part of

the Mexican territory the two great continental biogeographic zones of America converge: the nearctic and neotropical regions (Torres-Orozco & Pérez, 2009). The poeciliids are distributed throughout the entire national territory; however, the Usumacinta ichthyofaunistic region stands out for being one of the most studied, within this region there is many endemisms (Tobler *et al.*, 2008; Miller *et al.*, 2009). A relevant fact about this province is that large poecilid species live there, being the largest of them *Belonesox belizanus* family that can measure up to 20 cm in standard length (SL) while *Poecilia catemaconis* and *Poeciliopsis catemaco* occupy the second and fourth place respectively (Escalera, 2011; Pires *et al.*, 2011; García-Villar *et al.*, 2019).

Feeding

Although some members of the Poecillidae family have been kept in captivity and their diet is known, few studies on their biology have been carried out under natural conditions with native species (Trujillo-Jiménez & Toledo, 2007), Within this category, the Mexican species feed mainly on invertebrates, particularly insects, others are omnivores, detritivores, planctophages or carnivores such as *Belonesox belizanus*, which feeds on fish (Greven & Brenner, 2008; Sánchez & Regil, 2011).

Reproductive aspects and life stories

Reproductive aspects of some native species such as *Poeciliopsis occidentalis* have been studied, finding that females born in places with less food availability reach sexual maturity in one year (Constantz, 1979). *P. bimaculatus* has also been studied, whose total length at sexual maturity was determined, 27 mm in males and 40 mm in females, a fertility of 7-79 embryos at the time of analysis, and it was determined that females live more than two years, while males only one (Olinger *et al.*, 2016). Reproductive aspects of *Xiphophorus* species, such as *X. pygmaeus*, *X. multilenatus* and *X. nigrensis*, have been studied, it has been reported that the size of the litter depends on the size of the female and the time of year without presenting superfetation, unlike other species such as *Poeciliopsis gracilis*, which do present this pattern (Morris & Ryan, 1992; Frías, 2015).

Culture

In Mexico, the production and culture of poeciliids has focused mostly on exotic species, with *P. reticulata* being the most cultivated, mainly for ornamental purposes, followed by native species: *P. sphenops*, *X. helleri* and *X. maculatus*, in their ornamental varieties, (Maya *et al.*, 2007; Scotto, 2020).

Species such as *P. velifera*, *P. maylandi* and *B. belizanus* have also been bred for conservation and insect control purposes, while *P. velifera* and *P. shenops* are cultivated in their wild form as food for fish with commercial importance (Rodríguez *et al.*, 2020).

Diseases

The study of diseases of the native poeciliids of Mexico is limited and has been focused on their parasites, both external and internal, as for ectoparasites the lerneosis, a disease caused by copepods, is a great problem in crops of ornamental poecilids, as it causes

reproductive damage in the internal and external characteristics, causing death (Maya *et al.*, 2007). The most studied group of internal parasites are the helminths, of which there are 46 species that parasitize native Mexican poecilids, and to a lesser extent the monogeneous, being *P. mexicana* the host with the most species of registered helminths (24 species), the role of exotic species as vectors of parasites has also been studied, affecting native poecilids, which are especially susceptible due to geographic restriction (Razo-Mendivil *et al.*, 2013; Salgado-Maldonado *et al.*, 2020).

Bioindicators

In recent years, various species of mexican poeciliids have been object of toxicological studies, due to the relatively easy maintenance and little space required, highlighting *X. helleri* and *Cnesterodon decemmaculatus*, in pesticide toxicity studies and *G. yucatana* who has been used in the toxicity evaluation presented by various sun blockers. (Hernández, 2019; Rámirez-Ayala *et al.*, 2020; Pérez-López *et al.*, 2020). Native poeciliids have been used as bioindicators in various studies: to assess the water quality in the ecosystem, to determine environmental flows, define protection zones within nature reserves (Torres-Bugarín *et al.*, 2007), as well as insect biomonitoring tools based on their stomach content, to determine biotic integrity indices (Carbajal-Becerra *et al.*, 2020).

CONCLUSIONS

Mexico is a country with a great diversity of poeciliid species, in which its endemisms and native species stand out, there is little biological information about them, while production and cultivation are focused on species of commercial interest for aquaculture; However, in recent years, research has been carried out in which they are used as biological indicators to monitor contamination in water bodies, a field in which they have great potential in Mexico. The present work synthesizes information on Mexican species, being able to serve as a base for future investigations.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Evaluation of chromosome organization and microtubule arrangement in goat (*Capra aegragrus*) oocytes after vitrification, *in vitro* maturation and fertilization, and early embryo development

González-Silvestry, Fátima B.[1]; Chirino, Yolanda I.[2]; Delgado-Buenrostro, Norma L.[2]; Medina-Reyes, Estefany I.[3]; Kjelland, Michael E.[4,5]; Parra-Forero, Lyda Y.[6,7]; Hernández-Ochoa, Isabel[7]; López-Baños, Benito[1]; Delgado-Tiburcio, Guadalupe A.[8]; Romo, Salvador[1*]

[1] Universidad Nacional Autónoma de México, Departamento de Ciencias Pecuarias, Facultad de Estudios Superiores Cuautitlán, , Cuautitlán Izcalli, Estado de México, México. C.P. 54714.
[2] Universidad Nacional Autónoma de México, Laboratorio de Carcinogénesis y Toxicología, Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Tlalnepantla, Estado de México, México. C.P. 54090.
[3] Universidad Nacional Autónoma de México, Departamento de Biología, Facultad de Química, Ciudad de México, México. C.P. 04510.
[4] Conservation, Genetics & Biotech, LLC, Valley City, ND 58072, USA.
[5] Mayville State University, Mayville, ND, USA. ZIP 58257.
[6] Universidad Autónoma Metropolitana, Departamento de Producción Agrícola y Animal. Unidad Xochimilco, Ciudad de México, México. C.P. 04960.
[7] Instituto Politécnico Nacional (CINVESTAV), Departamento de Toxicología, Centro de Investigación y de Estudios Avanzados del. Ciudad de México, México. C.P. 04960.
[8] Colegio de Postgraduados, Campus Montecillo, km 36.5 Carretera México-Texcoco, Montecillo, Texcoco, Estado de México. CP. 56264.
* Autor para correspondencia: sromo_99@gmail.com

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ABSTRACT

Objective: Evaluate the use of Ethylene Glycol (EG), Dimethyl Sulfoxide (DMSO), Sucrose and Fetal Bovine Serum (FBS) as cryoprotectants and their effect on the organization of chromosomes and the arrangement of microtubules, during the vitrification process in goat oocytes matured *in vitro* and in the development of preimplantation embryos produced *in vitro*.

Design/methodology/approach: *In vitro* matured oocytes were divided into 3 groups (control group, cryoprotectant exposed group, vitrified group). A mixture of 15% EG, 15% DMSO, 0.4 M sucrose and 20% FBS was used for the vitrification using the Cryotop device. *In vitro* matured oocytes were warmed and afterwards each group was divided into two more groups. Both groups were subjected to immunofluorescence, the first group to observe the damage produced to the chromosomes and microtubules and the second group to observe the effect on the *in vitro* embryo development.

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Results: The combined use of 15% EG, 15% DMSO, 0.4 M Sucrose and 20% FBS during vitrification did not prevent cryoinjuries in goat oocytes and *in vitro* produced embryos, since embryo development was disrupted before the blastocyst stage by stopping cleavage at the morula stage. This disruption was associated with chromosome decondensation and the absence of a microtubule network, thereby hindering chromosomal segregation.
Limitations on study/implications: The effect of conventional cryoprotectants on chromosomes and microtubules arrangement on vitrified goat oocytes and *in vitro* embryo production.
Findings/conclusions: The combined use of 15% EG, 15% DMSO, 0.4 M sucrose and 20% FBS as vitrification cryoprotectants did not prevent cryoinjuries in caprine oocytes and did not improve caprine embryo development *in vitro*.

Keywords: Caprine, cryopreservation, *in vitro* embryo production, microtubules, oocytes.

INTRODUCTION

Oocyte cryopreservation is an artificial reproductive technology widely used to preserve fertility in different species. In addition, oocyte cryopreservation can be used for the preservation of species in danger of extinction and the improvement of species and breeds intended for meat, milk or wool production. Vitrification, can be used to preserve gametes and embryos at different stages of maturation, causing less damage to the cells, compared to freezing, due to the formation of ice crystals that have been correlated with a decrease in the fertilization rate, which could affect the development of the embryo and its implantation (Yurchuk *et al.*, 2018). The vitrification method has different levels of success among oocytes of different species. In fact, the oocytes show different tolerances to the cryoprotectants used in the vitrification procedures (Sudiman *et al.*, 2019). With regard to oocytes, the most reported alterations during cryopreservation are interruptions of the tubulin network, meiotic spindle disorganization, abnormal chromosomal distributions and a reduced *in vitro* fertilization rate (Tamura *et al.*, 2013, Lei *et al.*, 2014, Serra *et al.*, 2020), probably caused by the increase in aneuploidy (Buderatska *et al.*, 2020, Dviri *et al.*, 2021), genomic alterations that entail structural (Gao *et al.*, 2018, Wasielak-Politowska *et al.*, 2022) and biochemical changes (Ren *et al.*, 2019, Tsuiko *et al.*, 2019) that compromise cell viability even to the point of cell death (Balboula *et al.*, 2020, Vining *et al.*, 2021).

The composition of cryopreservation solutions plays a crucial role in the outcome of cell preservation between different species. Notably, goat oocytes are particularly susceptible to cell damage during vitrification (Youm *et al.*, 2014, Marques *et al.*, 2018). At present, there is no foolproof mix of successful chemicals for vitrification and there is still a need to clarify the optimal cryoprotective composition for goat oocyte preservation. Some compounds such as Dimethyl Sulfoxide (DMSO) and Fetal Bovine Serum (FBS) are regularly used in preservation media (Moawad *et al.*, 2012, Guo *et al.*, 2017) in different species (Fernández-Reyes *et al.*, 2012, Arcarons *et al.*, 2016), however, the proportion of these varies greatly in the literature, some of them associate it with toxicity at high concentrations (Awan *et al.*, 2020). The objective of the present study was to evaluate the effect of the combined use of Ethylene Glycol, DMSO, Sucrose and FBS to goat oocytes matured *in vitro* and to preimplantation embryos developed *in vitro*, concerning the organization of chromosomes and the arrangement of microtubules, during the vitrification process with the Cryotop device.

MATERIALS AND METHODS

Goat oocyte collection and in vitro maturation

Goat ovaries were obtained from a meat processing plant, from adult females. The ovaries were transported to the laboratory at 35 °C in isotonic saline solution (0.9% NaCl) supplemented with 100 IU/mL Penicillin and 0.1 mg Streptomycin (Sigma Aldrich, St. Louis, MO, USA). After 2 hours of transport, the ovaries were washed with isotonic saline solution and the cumulus-oocyte complexes (COC) were recovered from the ovarian follicles by follicular puncture using the technique reported by Chaves *et al.* in 2017, follicles with diameters between 2 and 8 mm were aspirated and then deposited in drops of saline physiological solution to select only COCs with uniform cytoplasm and more than 3 layers of cumulus cells, which were deposited in the *in vitro* maturation medium.

Groups of 30-35 COC were incubated in Petri dishes with drops of 100 μL of maturation medium (TCM (Tissue Culture Medium, In vitro S.A., México), supplemented with 0.3% BSA (Albumin Bovine Serum, Sigma Aldrich, USA), 5 μL/mL Follicle Stimulating Hormone (FSH; Sigma Aldrich, USA), 5 μL/mL Luteinizing Hormone (LH, Sigma Aldrich, USA), 0.32 mM Pyruvic Acid (Sigma Aldrich, USA), 10 μL/mL Penicillin-Streptomycin (P/E; Sigma Aldrich, USA)) (Izquierdo *et al.*, 2002) and then covered with mineral oil (Sigma Aldrich, USA). The samples were incubated at 38.5 °C in an atmosphere of 5% CO_2 in 95% air, with 95% humidity, for a total time of 27 h.

Oocytes vitrification and warming

Vitrification of *in vitro* cultured oocytes was performed by using a Cryotop device (Kuwayama *et al.*, 2005; Kuwayama, 2007; Liang *et al.*, 2012). First, oocytes were randomly divided into three groups: a) control (untreated oocytes), b) exposed (non-vitrified oocytes in cryoprotectant medium) and c) vitrified (vitrified cryoprotected oocytes). Exposed and vitrified oocytes were washed with 500 μL TL- HEPES buffer (In vitro S.A, México) and transferred into 300 μL holding medium (TCM-199 with Hepes; In Vitro S.A, México) supplemented with 20% FBS (38 °C for 5 min) (Gibco, USA) (Morató *et al.*, 2008; Purohit *et al.*, 2012). Next, groups of four oocytes were placed into droplets with 5 μL of equilibrated solution, a mixture of holding medium and 7.5% EG (Sigma Aldrich, USA), 7.5% DMSO (Sigma Aldrich, USA), at 38 °C for 9 min. Finally, oocytes were added immediately to the vitrification solution consisting of holding medium with 15% EG, 15% DMSO and 0.4 M sucrose (Sigma Aldrich, USA) for 1 min at 38 °C (Begin *et al.*, 2003; Morató & Mogas, 2014).

Only the vitrified group of oocytes was plunged into the liquid nitrogen (during 2 h). For warming, the tip of the Cryotop device was submerged in 3 mL of base medium supplemented with 0.5 M sucrose for 1 min at 38 °C. Then, the oocytes were recovered and washed for 3 min in four well dishes containing a 0.3, 0.25 and 0.125 M sucrose, to remove the cryoprotectants, followed by washing oocytes with holding medium and then incubated at 38 °C to complete 27 h of total incubation in maturation medium (Morató & Mogas, 2014).

In vitro fertilization and embryo development analysis

Before fertilization, 20 to 30 oocytes were washed and incubated with 100 μL of *in vitro* fertilization medium (Vitrogen, Brazil). Frozen-thawed buck semen in 0.25 mL straws was used in the experiment. A higher concentration of motile spermatozoa was obtained using a discontinuous Percoll (Sigma Aldrich, USA) density gradient (45:90). The centrifugation of semen was carried out for 20 min at 360 g, and viable spermatozoa were located primarily in the sperm pellet at the bottom of the gradient. For capacitation, the motile spermatozoa were suspended in fertilization medium to achieve a final concentration of (1×10^6 cells/mL) and incubated at 38.5 °C with 95% humidity and 5% CO_2 in air for 30 min. Both, spermatozoa and COC were incubated with fertilization medium for 18 h at 38.5 °C (Albarracín *et al.*, 2005).

After fertilization (Day 0), the presumptive zygotes were washed with PBS and denuded by gentle pipetting with embryo culture medium (Vitrogen, Cravinhos, SP, Brazil). Then, a maximum of 10 embryos were incubated in a drop with 10 μl embryo culture medium, 5 drops per dish and covered with 3 mL mineral oil for 7 d at 38.5 °C with 95% humidity and an atmosphere of 5% CO_2 in air. Cleavage stage rate was evaluated at 48 h post-fertilization, embryos with more than 6 cells were evaluated at 72 h post-fertilization, and the morulae stage rate was evaluated at 96 h, blastocyst stage rate was evaluated on days 5 to 7 post fertilization.

Oocyte immunostaining

The COC were disaggregated by pipetting in order to obtain the oocytes. Then, the oocytes were fixed with 2% paraformaldehyde for 72 h, permeabilized with 1% Triton X-100 (v/v) in PBS at 37 °C for 25 min and blocked with 1% BSA at room temperature for 15 min. Next the oocytes were incubated with anti-–tubulin coupled to Alexa-Fluor 488 (dilution 1:250; Thermo-Fisher, USA) at 4 °C overnight. Afterwards, the oocytes were washed three times with PBS and stained with DAPI (Sigma Aldrich, USA). Finally, 10 oocytes were placed on poly-L-lysine-treated slides and preserved with PBS:Glycerol (1:3). The images were obtained by a confocal microscope (Leica SP8, Wetzlar, Germany) at 63X magnification. The chromosome and microtubule criteria were based on the previous report by Albarracín *et al.* (2005). Briefly, normal spindle morphology was considered symmetrical, barrel-shaped, lacking astral microtubules and a metaphase plate diameter longer than the pole-pole distance. Spindle structure was regarded as disorganized if there was microtubule disruption, partial or total disorganization, while the absent classification equated to a complete lack of microtubules. For chromosomes, the standard organization was classified as chromosomes arranged on a compact metaphase plate at the equator of the structure. Chromosomes were classified as dispersed if they were disorganized (chromosomes misaligned at the metaphase plate) aberrant if not structured as a standard chromosome, and decondensed if a prominent less condensed appearance was observed (Albarracín *et al.*, 2005).

Statistical analysis

A two-way analysis of variance (ANOVA) was used to examine differences in chromosomes and microtubule classifications. One-way ANOVA was used to analyze differences for all the remaining variables. The ANOVA analysis was followed by a Bonferroni test for differences between means. The data are presented as mean ± standard error. Statistical significance was set at $\alpha=0.01$. All data analyses were performed using the GraphPad Software, version 6.

RESULTS

A total of 304 oocytes were evaluated for immunostaining. Both, the chromosomal organization and the spindle morphology were observed in all of the groups.

Chromosomal organization and microtubule arrangement in goat oocytes after vitrification

The chromosomes and microtubules were well organized in the control group forming a well-defined meiotic spindle; however, they were disorganized in both the exposed and Cryotop vitrified oocytes ($p<0.01$) (Figure 1A. Representative micrographs). The evaluation of *in vitro* matured oocytes presenting different chromosomal organization patterns showed 49.8% with normal chromosomes, 33.15% with dispersed chromosomes and 17.01% with decondensed chromosomes in the control group, this distribution was similar for exposed oocytes (51.09%, 28.47% and 20.43% with normal, dispersed and decondensed chromosomes, respectively) with no statistical differences compared to the control group. Vitrified oocytes did not show a statistical difference in the numbers of normal and dispersed chromosomes (39.6% and 23.99%, respectively) but had a significant increase of 42.36% decondensed chromosomes ($p<0.01$) (Figure 1B. Percentage of normal, dispersed and decondensed chromosomes). The evaluation of microtubule organization of the *in vitro* matured oocytes after vitrification resulted in 45.2% with the typical microtubule network, 33.59% with disorganized microtubules and 21.17% with absent microtubules in the control group. Exposed oocytes had no statistical difference compared to the control group, *i.e.*, 26.15% normal microtubules (and 34.73% disorganized microtubules but the percentage of absent microtubules was statistically different (39.12%) ($p<0.01$). The vitrified oocyte group had 20.99% normal microtubules, significantly lower than the control group ($p<0.01$), and 46.22% with absent microtubules, which was also statistically different from the control group. Notably, there were no statistical differences concerning percentages of disorganized microtubules for any groups (Figure 1C. Percentages of normal, disorganized and absent microtubule oocytes).

Embryo development after goat vitrified oocyte fertilization

The embryo development 48-h post-fertilization was followed (Figure 2. Embryo development). The cleavage in oocytes was significantly reduced ($p<0.01$) from 54.7% in the control group to 36.6% in exposed and to 21.3% in vitrified oocytes (Figure 2B. Cleavage stage). In addition, the +6 cells stage was also reduced from 36.3% in the control group

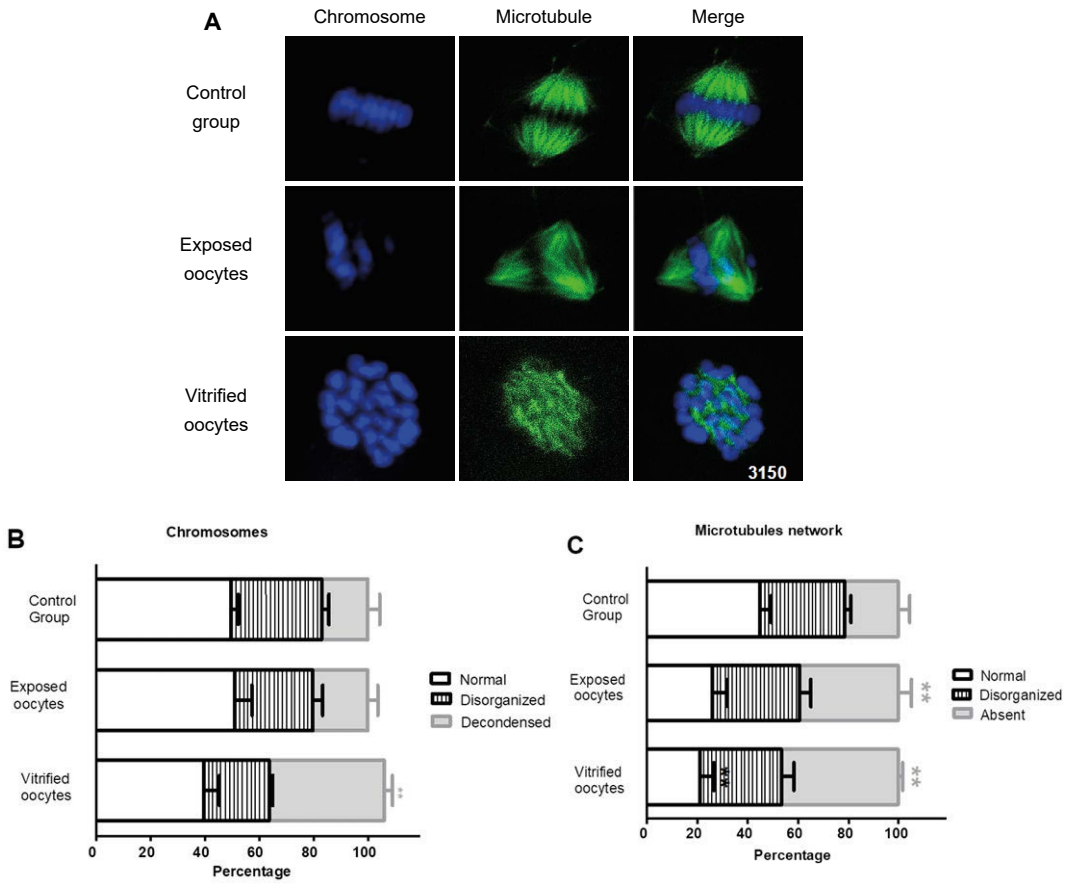

Figure 1. Oocyte vitrification increases metaphase plate disorganization and disrupts microtubule formation. A) Representative micrographs of the evaluated oocytes of the control and vitrified group, the DNA of the chromosomes are stained with DAPI (blue color) and beta-tubulin in the microtubules (green color). A) shows the barrel-shaped formation without the presence of chromosomes in any microtubule, a pattern that was found in a higher percentage in the control group (A1). While different patterns were found in the vitrified oocytes, they show an erratic arrangement (A2) and scattered decondensed chromosomes as seen in the last panel (A3). B) Percentage of normal chromosomes, dispersed chromosomes and decondensed chromosomes of each group. C) Percentages of normal, disorganized and absent microtubule oocytes in the group. Data show media ± standard error of three independent experiments. **p<0.01 *vs.* untreated oocytes.

to 11.2% and 6.6% in non-vitrified cryoprotected oocytes, respectively (Figure 2C. +6 cells stage) The morulae stage was also affected, and it was reduced from 19.2% in control group to 0.7% and 0.1% in exposed and vitrified oocytes, respectively (Figure 2D. Morulae stage) The blastocyst stage was only achieved in the control group, since no embryos were observed at this stage using exposed and vitrified oocytes (Figure 2E. Blastocyst stage).

Discussion

The rationale for animal gamete cryopreservation lies not only in preventing the extinction of many different species, but other motivations are also relevant including economic reasons, cultural identity, social role, environmental importance and scientific purposes for the benefit of humans (Kukovics, 2016). The scientific literature demonstrates a higher degree of knowledge in oocyte cryopreservation for livestock such as cow, pigs,

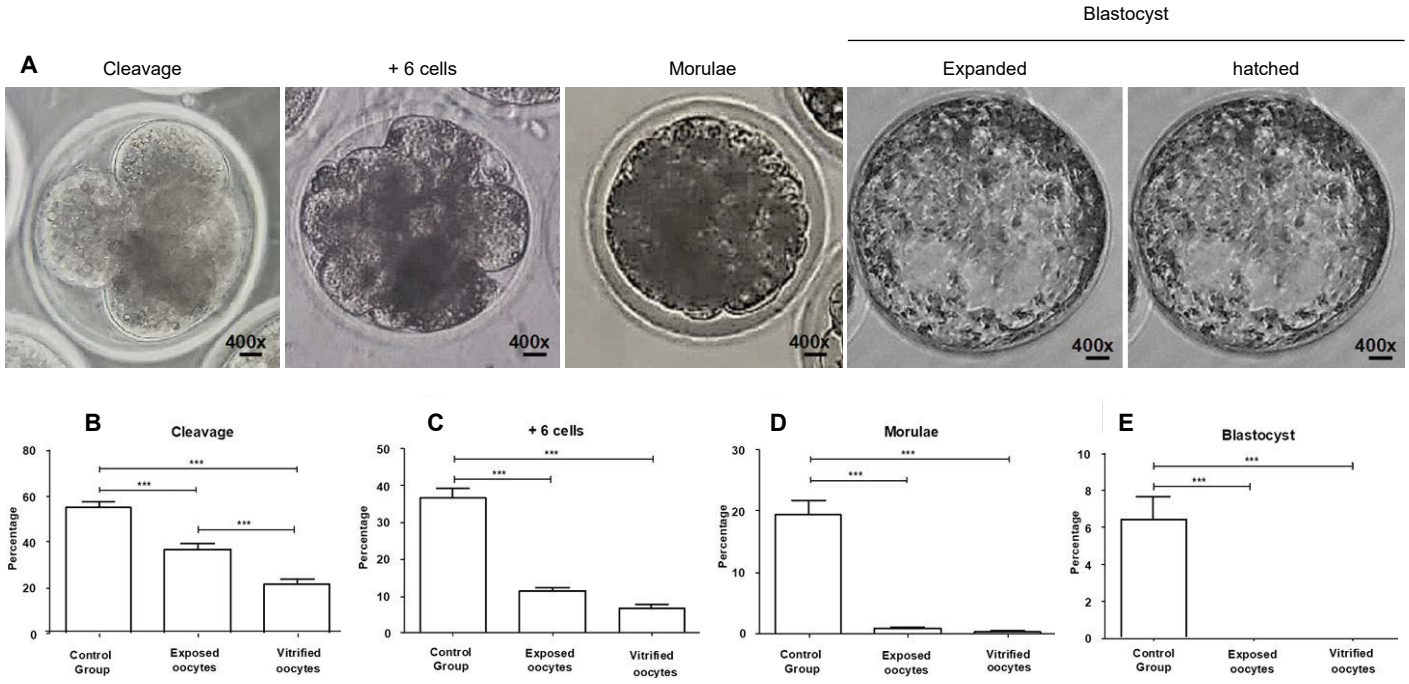

Figure 2. Embryo development was affected in exposed oocytes and vitrified oocytes. A) Representative images of embryo stages after *in vitro* fertilization by inverted bright field microscopy. Embryo development at B) cleavage stage (48 h post-fertilization), C) +6 cells stage (72 h post-fertilization), D) morulae stage (96 h post-fertilization) and E) blastocyst stage (5 days post fertilization) as quantified in the control group, exposed and vitrified goat oocytes. Data show media ± standard error of three independent experiments. ***$p<0.01$ *vs.* control group.

sheep, and buffalo than for goat oocytes (Casillas *et al.*, 2015; Kukovics., 2016, Chaves *et al.*, 2017).

In general, the failure in oocyte cryopreservation is related to the injury caused by cryoprotectants, with DMSO being one of them. In terms of cryopreservation, the reduction of DMSO has already been assessed. The use of 16% DMSO has been tested in other oocytes such as porcine oocytes (Casillas *et al.*, 2015) and 15% in bovine oocytes (Arcarons *et al.*, 2016) while the usage of 20% DMSO commonly used during cryopreservation, has not been successful for goat embryo development (Begin *et al.*, 2003; Srirattana *et al.*, 2013, Quan *et al.*, 2014). Based on this, the DMSO was decreased to 15% but unfortunately, did not improve the rate of goat embryo development.

We also tested the 20% FBS addition to the cryoprotectant mixture since proteins contained in the FBS confer cell protection. Indeed, some other studies in livestock such as pigs showed that 20% FBS was successfully used in the cryoprotectant mixture (Fernández-Reyes *et al.*, 2012) but as demonstrated in this study, no benefit was found concerning goat oocyte cryopreservation.

The causes of goat embryo development failure during cryopreservation and vitrification have not been thoroughly investigated, but the alterations in chromosome segregation and microtubule networks are reported for human oocytes during cryopreservation (Bromfield *et al.*, 2009). There is also evidence of cytoskeleton degeneration of cattle oocytes cryopreserved by liquid nitrogen (Guo *et al.*, 2017). However, literature related to goat oocyte cryopreservation has not found a highly successful cryopreservation method.

Perhaps a description of cellular mechanism of embryo development failure during the current cryopreservation methods is needed first. We demonstrated that the use of 15% DMSO and the addition of 20% FBS did not improve embryo development, and this effect was associated with chromosomal defects and microtubule network disruption. Although, the result is not disappointing either, according to Wu *et al.*, (2020) the use of 35% EG and 20% FBS, can result in a good cleaved embryo percentage, *i.e.*, 64.9% of cleaved embryos was relatively high. The two variables mentioned above, are crucial for embryo development. As it has been demonstrated, the oocyte meiotic spindle is responsible for chromosome segregation and this spindle depends on the microtubule network, and together they are a predictive marker of blastocyst ploidy in humans (Tilia *et al.*, 2020).

In summary, the use of EG, the decreased percentage of DMSO and the addition of FBS did not improve the cryoprotectant mixture used for vitrification. Indeed, this modification probably was insufficient for preventing the ice-crystal formation during the subzero reached temperatures, which cause cryoinjuries (Yurchuk *et al.*, 2018).

Regarding the cryoprotectant composition, an alternative to test in future research is DMSO between 5% and 10% and such a concentration decrease might improve embryo development. Another alternative is to replace 20% FBS with lyophilized albumin between 0.4% and 4% as has previously been tested in goat oocytes (Purohit *et al.*, 2012).

CONCLUSIONS

The cryoprotectants and the cryopreservation by vitrification methods used in this study disrupted the goat embryo development. This effect was associated with disruption of DNA integrity, as was seen by alterations in chromosome structure and also, by disruption in the microtubule network. However, vitrified cryoprotected oocytes exhibited a higher degree of alterations in terms of oocyte cleavage, +6 cells stage at 72 h post-fertilization, decondensation of chromosomes and absence of a microtubule network compared with exposed oocytes.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Physiological development of red anthurium (*Anthurium andreanum* Linden) var. Tropical in three *in vitro* culture systems

López-Páez, Fátima Karina 1; Galindo-Tovar, María Elena 1; Murguía-González, Joaquín 1; Bulbarela-Marini, Javier Emanuel 1; Castañeda-Castro, Odón 2; Pastelín-Solano, Miriam Cristina 2*

[1] Universidad Veracruzana. Facultad de Ciencias Biológicas y Agropecuarias. Camino Peñuela-Amatlán de los Reyes S/N, C.P. 94945 Teléfono 2717166129.
[2] Universidad Veracruzana. Facultad de Ciencias Químicas. Prolongación de Oriente 6 No. 1009. Orizaba, Veracruz. México. C.P. 94340. Teléfono y Fax: 2727240120
* Correspondence: mpastelin@uv.mx

ABSTRACT

Objective: To evaluate the physiological development of red *Anthurium andreanum* L. var. Tropical in three *in vitro* culture systems: semi-solid, partial immersion and RITA® bioreactor.

Design/methodology/approach: A completely randomized design with three treatments, semi-solid medium, partial immersion and RITA® bioreactor, and four repetitions each was used. Vitroplants of anthurium were selected with a size of 0.5 cm from the stem to the highest leaf, with three leaves in each specimen. Morphometric, chlorophyll content and hormone content analyses were carried out after 60 days of sowing. Analysis of variance and means comparison tests were performed on the data obtained through Kruskal-Wallis and Tukey, respectively, using the statistical software R-STUDIO.

Results: The highest shoot rate and root length were obtained in partial immersion; however, the number of leaves, shoots and root multiplication did not show differences with the RITA® bioreactors. The highest concentration of chlorophylls and indole acetic acid was observed when using RITA® bioreactors.

Study limitations/implications: The results are favorable for the in vitro production of anthurium, although the use of RITA® bioreactors for commercial production is a high cost in the initial investment.

Findings/conclusions: With the results obtained, it is considered that the RITA® bioreactors obtained the best results for the production of anthurium, followed by the partial immersion system. This is due to the liquid medium and better gas exchange, which favors the development of plants.

Keywords: *Anthurium andreanum* L.; chlorophyll; phytohormones; immersion systems.

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INTRODUCTION

The bract of the anthurium plant is highly valued in the ornamental market, due to its beauty and vase life; in addition, the leaves are also marketed as foliage (Mireles-Ordaz *et al.*, 2015). The flower stems reach prices of $25 (SNIIM, 2019). The optimal temperature for the development of this plant is 20 to 35 °C and relative moisture between 70 and 80%. Its production requires for the climate requirements of light,

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height, temperature, etc., to be fulfilled (Gayosso-Rodríguez *et al.*, 2013). In Mexico, the central region of the state of Veracruz has the soil-climate conditions that are apt for its cultivation (García-Alonso *et al.*, 2014).

Traditionally, anthurium is propagated by seed and stem segmentation; however, these techniques are slow and present high genetic variability (Teixeira da Silva *et al.*, 2015). This translates into marketing problems, and therefore the tools for *in vitro* culture are advisable, which allow obtaining specimens in less time, which are genetically homogeneous, free of disease; these tools also allow controlling growth speed and tissue regeneration (Rangel-Estrada *et al.*, 2018). The semi-solid medium is effective, basic and provides support to the tissues; however, higher production has been reported in various species when using liquid medium, due to the contact that is maintained between the medium and the explants, which makes the absorption of nutrients more efficient (Ontaneda *et al.*, 2020).

Among the plant tissue cultivation systems, temporary immersion systems outperform those mentioned. Since they are semi-automatized, they decrease the maintenance costs and renew the gaseous atmosphere avoiding the accumulation of toxic gases within the system (Rosales *et al.*, 2018); in addition, a higher contact between the cultivation medium and the plant, good gas exchange, as well as the system's automatization increase the multiplication rates. Among these systems, the Automatized Temporary Immersion Recipients, RITA® (for its initials in Spanish, *Recipientes de Immersión Temporal Automatizado*), represent a technological advancement that allows an improvement in plant tissue culture (Alamilla-Magaña *et al.*, 2019); however, the results of the use of TIS are limited by factors such as the composition of the culture medium, which is reflected in the amount of nutrients that the plant can absorb affecting its quality (Vilchez & Albany, 2014).

The atmosphere created in the temporary immersion systems promotes physiological processes such as photosynthesis, respiration, chlorophyll synthesis, and stomata functioning (Ramírez -Mosqueda *et al.*, 2019); therefore, it is important to analyze the concentration of chlorophyll in plant tissues (Marín-Garza *et al.*, 2018). In addition, phytohormones have mechanisms of action capable of triggering effects and physiological changes; however, in in vitro culture the plants do not produce them in sufficient amount, which is why they must be aggregated to the culture medium (Elías & Padrón, 2020). Nevertheless, the concentration and absorption by the plant tissue can generate both an improvement in micropropagation and alterations in tissues such as de-differentiation between them, and this is why knowing the content of absorption by the plant tissue allows understanding its interaction and the response that it generates (Aguilar *et al.*, 2019).

To make more efficient the use of *in vitro* tissue culture techniques, especially the use of immersion systems, the objective of this study was to evaluate the physiological development of *A. andreanum* micropropagated in three culture systems: semi-solid medium (SS), partial immersion (PI), and in RITA® bioreactors.

MATERIALS AND METHODS

This research study was conducted in the LADISER (Laboratory of Teaching, Research and Services - *Laboratorio de Docencia, Investigación y Servicios*) of Plant Biotechnology and

Cryobiology at the School of Chemical Sciences, in Orizaba, Veracruz, which depends on Universidad Veracruzana.

Morphometric characteristics were measured (number of shoots, size of shoots, size of root in cm, number of leaves and number of roots), content of chlorophylls (mg/g^{-1} PF) and phytohormones (mg L^{-1}) in leaves from anthurium vitroplants. A completely random design with four repetitions was used; the treatments were PI systems, RITA® and semi-solid medium. With the data obtained, a Kruskal-Wallis one-way analysis of variance was conducted and Tukey's means comparison tests through the minimum significant difference ($P \leq 0.05$), using the R-STUDIO statistical software version 2019 for Windows.

Vitroplants of *A. andreanum* were micropropagated in MS culture medium (Murashige & Skoog, 1962) supplemented with 1mg L^{-1} of 6-Benzylaminopurine (BAP), 30 g L^{-1} of sucrose. For the semi-solid medium, 3 g L^{-1} of phytagel were used, while the pH of the medium was adjusted to 5.7 with a potentiometer Brand HANNA, and sterilized at 121 °C during 15 min in a vertical autoclave brand EVAR. The plants were incubated at 24±2 °C with a photoperiod of 16 h light and 8 h darkness. The light intensity was 36 μmol m^{-2} s^{-1}, supplied by white fluorescent lamps.

Vitroplants were micropropagated in MS medium, and with a size of 3.5 cm, three leaves and no roots were sown in the different systems. For the semi-solid and partial immersion mediums, four glass containers were used with capacity of 250 mL to which 25 mL of culture medium were added. Four explants were placed in each container. In four RITA® temporary immersion systems, 200 mL of culture medium were added and four plants per bioreactor. These were connected to an air system which came from a compressor brand Adir with a maximum entry flow of 5.89 with pressure of 29 psi, and the air entered to the bioreactors through a Midisart® 2000 filter of 0.2 μm (PTFE and polypropylene). The immersions in TIS were carried out in intervals of four hours, six immersions per day, which had a lapse of two minutes, according to what was described by del Rivero-Bautista *et al.* (2004) for the *in vitro* propagation of anthurium.

After 60 days the morphometric development of the anthurium vitroplants was analyzed through the number of shoots, leaves, roots, length of shoots and roots, using graph paper. The quantification of chlorophylls was done following the methodology described by Harborne (1973) in a spectrophotometer (Thermo Scientific®, Genesys 10S UV-VIS) at 645 and 665 nm of absorbance.

The hormonal analysis was carried out with the technique described by Pan (2010) using leaf samples, through HPLC (Agilent Techonologies 1200 serie).

RESULTS AND DISCUSSION

Morphometric analysis

For the analysis of the morphometric data (number of shoots, number of leaves, number of roots, size of shoots and size of root), a Kruskal Wallis non-parametric test and analysis of variance were conducted, as well as a means comparison through the t-student test ($P \leq 0.05$). Significant statistical differences were obtained for the morphometric development of anthurium cultivated in the different systems according to the Kruskal

Wallis test, regarding the systems which consist in a liquid medium compared to the treatment with semi-solid medium.

The development of plants is very plastic, which allows them to react to the environmental changes in size, morphology, etc. (Escaso Santos *et al.*, 2011). The treatment with the highest values for length of shoots was partial immersion. However, for number of shoots, number of leaves and number of roots, the RITA® and PI systems were statistically equal. No differences were observed between treatments for root length (Table 1).

The use of the liquid medium in the PI and RITA® systems favored the morphometric development of anthurium vitroplants. These results agree with what was reported by García *et al.* (2015), who mention that a higher rate of explant development was obtained in the systems where a liquid medium was used because they allow a higher contact of the explants with the culture medium, allowing a better absorption of nutrients and maximizing their development. In addition, the temporary immersion system allows supplying the culture medium to the explants by time lapses, and this allows the renovation of the gaseous atmosphere avoiding the hyper hydricity in the plant tissue with it, as well as the accumulation of toxic gases within the systems.

Chlorophyll content

When it comes to chlorophyll content, significant statistical differences were found in the three systems. The highest concentrations of chlorophylls a, b and total were observed in the RITA® bioreactor (Table 2).

Table 1. Morphometric development of seedlings of *Anthurium andreanum* cultivated *in vitro* for 60 days, in three systems.

System	Sprouts (number)	Sprouts (cm)	Leaves (number)	Root (number)	Root (cm)
SS	1.81b	2.59b	6.19b	1.40b	3.05b
IP	2.93a	3.28a	9.25a	2.11a	4.06a
RITA®	3.19a	2.40b	10.25a	1.98a	3.90ab

SS: semi-solid medium, IP: partial immersion and RITA® bioreactor. Different letters in the columns indicate significant statistical differences between treatments by Kruskal Wallis test for ($P \leq 0.05$).

Table 2. Effect of the different *in vitro* cultivation systems in the concentration (mg/g^{-1} PF) of chlorophylls a, b and total in anthurium plants cultivated for 60 days.

System	Chlorophylls (mg g^{-1} PF)		Total
	a	b	
SS	0.38±0.06c	0.14±0.02c	0.46±0.071c
IP	0.58±0.05b	0.21±0.017b	0.80±0.07b
RITA®	0.97±0.04a	0.35±0.01a	1.34±0.06a
DMSH/HSD	0.196	0.070	0.24

SS: semi-solid medium, IP: partial immersion and RITA® bioreactor, DMSH=minimum significant honest difference, different letters in columns indicate significant statistical differences between treatments ($P \leq 0.05$).

According to the means comparison, the highest leaf content of chlorophylls a, b and total (0.97±0.04, 0.35±0.01, 1.34±0.06 mg g^{-1} PF respectively) was obtained when using the RITA® systems, compared to other treatments, and this is because the conditions of moisture and gas exchange inside the systems foster the synthesis of chlorophylls and, with that, photosynthesis. These results agree with what was reported by Ramírez-Mosqueda *et al.* (2019) who reported different contents of chlorophyll in different bioreactors of temporary immersion.

In addition, the chlorophyll content probably contributes to *in vitro* growth and the adaptation to an autotroph environment (Martins *et al.*, 2015).

Content of phytohormones

Phytohormones, in addition to their important role in the regulation of growth and development, are related to mechanisms that allow the plant to respond to the changes to which they are subjected. After 60 days of culture of *in vitro* anthurium seedlings, significant statistical differences were obtained in the content of kinetin (KIN), abscisic acid (ABA) and indole acetic acid (AIA) in the leaf tissue. The highest contents for KIN and ABA were observed in the semi-solid medium, of 24.98±0.92 mg L^{-1} and 13.55±0.42 mg L^{-1}, respectively. Meanwhile, for AIA the highest result was obtained in the RITA® bioreactor, of 24.33±1.64 mg L^{-1}.

The different treatments had significant statistical differences according to the means comparison obtained for KIN (24.98±0.92) and ABA (13.55±0.42) in the semi-solid culture medium, which contrast with the lowest morphological results obtained in this system. The development of the plant is influenced by the coordination of positive and negative regulators. Cytokinins, although they stimulate cell division, seem to do it at low levels in the root (Escaso Santos *et al.*, 2011). On the other hand, ABA was generally an antagonist and growth inhibitor, which explains the results obtained. Meanwhile, AIA is related to cell division, growth and differentiation, favoring the growth of roots, shoots and stems (Azcón-Bieto and Talón, 2003); this explains the higher statistical result obtained for the RITA® bioreactor (24.33±1.64). These results agree with what was reported by Aguilar Jiménez & Rodríguez De la O (2018), who described that the presence of AIA had a favorable effect in the *in vitro* growth and development of agave shoots and roots.

Table 3. Content of KIN, ABA, and AIA in leaf tissue of anthurium seedlings grown *in vitro* for 60 days.

	Phytohormones (mg L^{-1})		
System	**Kinetin**	**ABA**	**AIA**
SS	24.98±0.92 a	13.55±0.42 a	10.58±0.45 b
IP	2.82±0.06 c	1.55±0.016 c	3.86±0.01 c
RITA®	7.58±1.40 b	9.17±0.04 b	24.33±1.64 a
DMSH/HSD	4.2	1.05	4.26

SS: semi-solid medium, IP: partial immersion and RITA® bioreactor, DMSH=honest least significant difference, different letters in the columns indicate statistically significant differences between treatments (P≤0.05).

Likewise, Flores-Mora *et al.* (2015) describe that there are advantages in temporary immersion systems compared to the other tissue culture systems that have an impact on the response in the absorption of plant hormones making it faster due to the atmosphere created inside the systems and the type of immersion.

CONCLUSIONS

The morphometric development of anthurium vitroplants var. Tropical was favored using partial immersion systems and the RITA® bioreactor where the highest number of leaves, number and size of shoots were seen. The higher content of chlorophylls a, b and total, as well as indole acetic acid in the anthurium leaf tissue was favored when using those bioreactors in comparison to other treatments; meanwhile, the absorption of kinetin and abscisic acid was favored by the semi-solid medium. According to the results obtained, the RITA® system was considered to be the most viable option for *in vitro* culture of anthurium, since as a whole the characteristics studied will foster the best development of plants and, with that, the most desirable characteristics for their production.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Polyhydroxyalkanoates from pear (*Pyrus communis* L) waste by *Bacillus subtilis*

Hernández-Manjarrez Cynthia Estefania[1]; Cortés-Sánchez Alejandro De Jesús[2]; Fabela-Morón, Miriam F.[3]; Rayas-Amor Adolfo A.[3]; Díaz-Ramírez Mayra[3*]

[1,3] Universidad Autónoma Metropolitana, Unidad Lerma, Departamento de Ciencias de la Alimentación. División de Ciencias Biológicas y de la Salud, Lerma de Villada, Estado de México, México.

[2] Consejo Nacional de Ciencia y Tecnología (CONACyT). Departamento de Ciencias de la Alimentación, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana, Unidad Lerma. Lerma de Villada, Estado de México, México

* Correspondence: m.diaz@correo.ler.uam.mx

ABSTRACT

Objective: Analyze the production of polyhydroxyalkanoates (PHA) by *Bacillus subtilis* sp., *subtilis* in submerged culture using pear residues as a carbon source.

Design/methodology/approach: The culture consisted of pear residue flour concentrations of 5 and 15% for 72 h. Reducing sugars, biomass, pH, protein, and pHA extraction and quantification were analyzed during submerging cultivation of *Bacillus subtilis* sp., *subtilis.*

Results: The results showed that *Bacillus subtilis* sp., *subtilis* can grow and generate PHA, having its maximum proportion in PHA extract of 0.094 g/L at 72h of culture, at a substrate concentration of 5%.

Study limitations/implications: More studies related to the optimization of culture conditions for PHA production are required and prior treatment of pear waste flour will be considered.

Findings/Conclusions: The synthesis of these biopolymers is important to promote their large-scale production using agro-industrial waste, thus contributing to reduce the environmental impact.

Keywords: *Bacillus*, pear, biodegradable plastic, polyhydroxyalkanoates.

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INTRODUCTION

Plastics are called, conventionally, those polymeric products of the petrochemical industry, which are widely used at an industrial level, becoming the basis of most common consumer products due to their elasticity and flexibility properties, easy molding, and resistance (Lemos and Mina, 2015). Nevertheless, these compounds can last before, during and after their use in nature for long periods, due to their chemical properties, making them recalcitrant materials and resistant to biodegradation, accumulating and negatively affecting the environment (González-García *et al.*, 2013). For several years, bioplastics have emerged as potentially substitute materials for petroleum-derived plastics, which can be produced from renewable, biodegradable, and environmentally compatible sources, being the polyhydroxyalkanoates (PHA) among the bioplastics studied (Lemos and Mina, 2015).

Image by Alicja at Pixabay

PHAs are polymers of hydroxyalkanoic acids that different microorganisms, such as Gram positive and negative bacteria; they synthesize and accumulate intracellularly as reserve material under adverse culture conditions, to later use it as a carbon and energy source (González-García *et al.*, 2013). They have physical characteristics like those of petroleum-derived plastics, but they can be degraded to carbon dioxide and water under aerobic conditions or to methane in anaerobic conditions in the environment (Lemos and Mina, 2015). For several years, studies on these bio-compounds have focused on the search for substrates, as well as economic production and extraction strategies that allow them to be considered substitutes for chemical plastics, widely spread in the market, and of a polluting nature (Lemos and Mina, 2015). During the processes of production, industrialization and consumption of food, waste and losses are generated, which constitute a worldwide problem, and where the fruit sector (apple, pear, orange, pineapple, etc.) is the most affected (Cervilla *et al.*, 2019) generating air, soil, and water pollution, as well as pests and disease vectors. It has been proposed through various investigations around the world that agro-industrial fruit residues can be exploited and used, through microbial activity, to obtain different value-added products such as biofuels, enzymes, plant growth stimulators, formulation of prebiotics for animal feed (Costa *et al.*, 2009; Serrat *et al.*, 2016). Therefore, the objective of this research is to analyze the production of polyhydroxyalkanoates (PHA) by *Bacillus subtilis* sp., *subtilis* from agro-industrial pear residues (*Pyrus communis* L) in submerged culture.

MATERIALS AND METHODS

Materials. Agro-industrial residue of pear (*Pyrus communis* L) was obtained from the supply center of the city of Toluca, State of Mexico, Mexico. All reagents used were reagent grade.

Pear waste flour. Once the pear (*Pyrus communis* L) waste was collected, it was washed and disinfected, and stored frozen at −18 °C. After, the pear residues were ground in a blender (Oster, México) and was subsequently dried in an oven (Thermo Scientific, USA) at 60 °C/6 days; after, the dry mixture was placed in a blade mill (High-speed multifunction Grinder-Maya 70 -300) and sieved to obtain fine particles. Finally, the sample was stored in a desiccator until use (Aguirre *et al.*, 2018).

Biological material propagation and conservation. The bacteria were inoculated in Tryptic Soy Broth and incubated at 37 °C/24h. Subsequently, 0.5 mL of culture in tubes in Tryptic Soy Broth and 0.5 mL of previously sterilized 50% glycerol were transferred to sterile vials, with an operating volume of 1.5 mL; the content was homogenized and stored at −80 °C.

Starter culture. The starter culture was prepared by adding, at room temperature, the contents of a vial with *B. subtilis* sp., *subtilis* stored at −80 °C, to a flask with culture medium corresponding to Tryptic Soy Broth and incubated at 37 °C/24h.

Preparation of culture medium for PHA production. The culture medium to produce PHA was prepared from the one reported by Du *et al.* (2001), modified with the incorporation of 2 different concentrations of pear waste flour (5% and 15%). Each culture medium was sterilized at 121 °C/15 min prior to use.

Submerged cultivation. 250 mL Erlenmeyer flasks, with an operating volume of 100 mL of culture medium, were used for PHA production. Submerged culture conditions were 35 °C/200 rpm/72h using a shaking incubator (Thermo Scientific, Mod. MAXQ 4000). The pH was adjusted to 7 prior to adding the starter culture of *Bacillus subtilis* sp., *subtilis*, corresponding to 2% of the operation volume, and samples were taken every 24 h for 3 days for the analysis of biomass, pH, reducing sugars, protein, extraction, and PHA quantification.

Reducing sugars; 5 mL of sample were placed in conical tubes and centrifuged at 5000 rpm/15 min; then, the supernatant was separated, and 3 mL of 3,5-dinitro salicylic reagent (Sigma-Aldrich) were added; afterwards, the mixture was heated to 90 °C between 5-15 min and 1 mL of 40% sodium potassium tartrate was added; finally, the reading at 575nm was recorded in a spectrophotometer (Shimadzu UV spectrophotometer UV-1800) (Bello *et al.*, 2006).

Biomass determination. For biomass determination, 10 mL of the medium were placed in conical tubes and centrifuged at 4400 rpm for 10 min at 25 °C. The supernatant was separated for subsequent analyses and the pellet was washed with distilled water, shaken, and centrifuged 3 times. The sediment was placed in aluminum pans at constant weight (90 °C/24 h) and oven-dried at 105 °C/24 h. Biomass was determined by weight difference (Harris, 2001).

pH determination. The pH determination was performed directly using a potentiometer (Thermo Scientific, Orion Star A211).

Protein determination. To quantify the protein, a sample was taken from the supernatant of the biomass determination. This sample was centrifuged at 4400 rpm/10 min and 10 μL were placed in a 96-well microplate and 100 μL of Bradford reagent and the absorbance at 595 nm was recorded in a spectrophotometer (Thermo Scientific, Multiskan GO) (Lozano *et al.*, 2011).

PHA extraction and quantification. The extraction and quantification of PHA consisted of taking 10 mL of culture and placing it in test tubes at constant weight, centrifuging at 4700 rpm for 20 min and discarding the supernatant; then, adding 3 mL of concentrated NaClO to the sediment and allowing it to settle at 37 °C/2h. After that, it is centrifuged again at 4700 rpm/20 min and the pellet obtained is suspended in $CHCl_3$; finally, it is placed in the oven at 60 °C/15 h for the volatilization of the solvent. For quantification, the sample is cooled and the PHA amount was determined by weight difference (Grados, 2020).

Analysis of results. The experiments were done in triplicate. MS-Excel 2014 software was used for data analysis.

RESULTS AND DISCUSSION

Parameters evaluated during submerged culture

Reducing sugars. Figure 1 shows the percentage of consumption of reducing sugars by the microorganism at the end of the bioprocess. The highest consumption of reducing sugars was in concentrations of 5% of the substrate with 89.9% and, finally, 64.8% of consumption of sugars in concentrations of 15% of substrate, identifying that in the lower

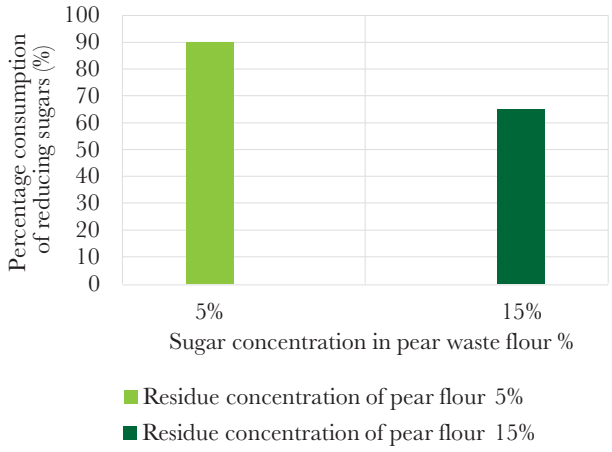

Figure 1. Percentage of reducing sugars consumption at the end of the submerged culture of *Bacillus subtilis* sp., *subtilis* to two different concentrations of pear waste flour (*Pyrus communis* L).

concentration of substrate, the consumption of reducing sugars by the microorganism was greater for the growth, production and accumulation of PHA.

The difference in carbon source consumption may be due to adaptation to culture conditions and substrate proportion by the microbial strain. The carbohydrates supplied by the pear waste flour are the main source of carbon in the culture medium, so the microorganism during the culture establishes a balance between the generated bacteria and non-viable cells. The remaining bacteria tend to consume the source of carbon for survival and generate reserve compounds such as PHA. Growth conditions have been reported to influence PHA formation (Jia *et al.*, 2013), where the metabolic process to produce short-chain PHA is through derivatives of acetyl-CoA. The carbon source is initially converted into thioesters of coenzyme A, with the activity of enzymes 3-ketothiolase (acetyl-CoA acetyltransferase, acetoacetyl-CoA reductase, hydroxybutyryl-CoA dehydrogenase, and poly(3-hydroxybutyrate) synthetase (Akiyama *et al.*, 2003).

Biomass determination. Figure 2 shows the highest growth of *Bacillus subtilis* sp., *subtilis* using the 2 different concentrations of pear waste flour, at 24h of cultivation, at a concentration of 5% pear waste flour with 41±3 g/L to subsequently decrease to 15.5 g/L at 72h. Meanwhile, there was a concentration of 15% of pear residue flour at 24h, which presented an adaptation phase in the first 24h of cultivation, with a decrease in biomass with respect to the initial value to later increase, reaching a maximum at 48h with 25±3g/L.

In related studies, Anjali *et al.* (2014) reported *B. subtilis* AMN1 in culture supplemented with sugarcane molasses from 10 to 100% as carbon source at 30 °C and 150 rpm for 48 h biomass yield dry weight of 2.09 at 1.07 g/100 mL. Meanwhile, Sanabria and Sarmiento (2017), and using potato starch residues at a concentration of 10 and 15g/L for the growth of *B. subtilis* at 35 °C, 15.75 rad/s, pH 7 for 72 h, reported a biomass yield of 0.01 to 0.07 g/ml, at a substrate concentration of 10g/L and from 0.06 to 0.07 g/ml of biomass at a concentration of 15g/L of substrate. The biomass proportions obtained in this study are lower than those reported in related studies. However, it should be noted that the

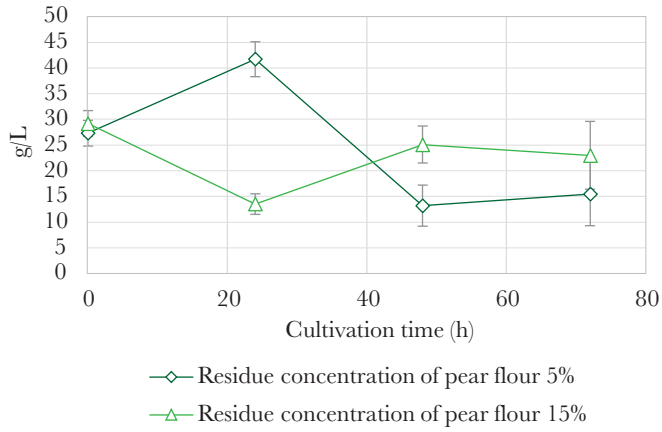

Figure 2. Biomass (g/L) during the growth of *Bacillus subtilis* sp., *subtillis* in submerged culture with different concentrations of pear waste flour (*Pyrus communis* L).

culture conditions (temperature, time, agitation, inoculum, type of carbon and nitrogen source, and treatment) influence the growth and production of PHA by microorganisms (Mohapatra *et al*., 2017).

pH determination. pH is an important factor that must be evaluated during microbial growth in culture. Microorganisms, in the growth phase, assimilate the carbon source consisting mainly of carbohydrates, generate and release various acidic metabolites into the medium, causing a change in the pH of the medium. In the present study, there was a decrease in pH in the 2 study concentrations, where a concentration of 5% of pear waste flour had the greatest decrease with pH 6.27 at 24h and later had an increase to pH 6.9 at 72h; at the same time, at a concentration of 15% it had its lowest decrease at 48h with pH 6.35 to later rise to 6.67 at 72h (Figure 3). The largest decreases in pH at a concentration of 5% at 24h and 15% at 48h are related to the highest proportions of biomass obtained in similar culture times (Figure 2).

Protein determination. Figure 4 shows the protein concentration during the growth of *Bacillus subtillis* sp., *subtillis*, using pear waste flour as substrate. The highest proportions

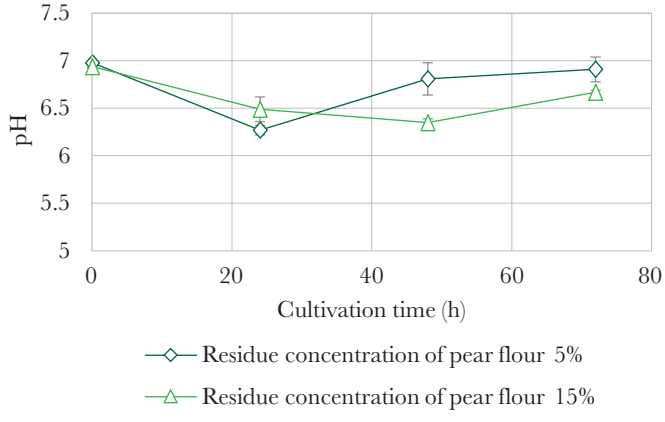

Figure 3. pH of the submerged culture of *Bacillus subtilis* sp., *subtillis* in different concentrations of pear waste flour (*Pyrus communis* L).

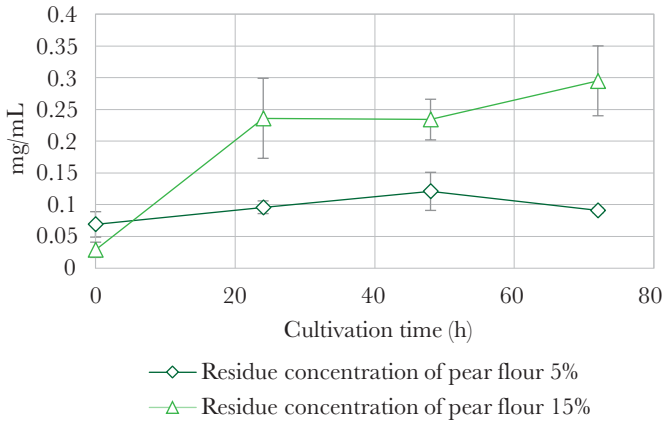

Figure 4. Protein (mg/mL) in submerged culture of *Bacillus subtilis* sp., *subtilis* at different concentrations of pear waste flour (*Pyrus communis* L).

were presented at concentrations of 15% with 0.23 and 0.29 mg/mL at 48 and 72h of culture, followed by 0.096 and 0.121 mg/mL at 24 and 48h, respectively, for the 5% concentration of flour from residues of pear. The protein content in the culture medium may be related to the assimilation of the carbon source for microbial growth and the synthesis of enzymes in the metabolism of the carbon source. *Bacillus* species can secrete a variety of extracellular enzymes, including α-amylases and proteases. Besides, *Bacillus* can use polysaccharides and polypeptides for cell growth and accumulation of PHA directly (Tsuge *et al.*, 2015).

Extraction and quantification of PHA. In the extraction and quantification of PHA during cultivation, there was an increase with respect to time at a substrate concentration of 5% pear residue flour, reaching its maximum at 72 h with 0.094 g/L. Meanwhile, at a concentration of 15%, it presented its maximum proportion of PHA extracted with 0.039 g/L at 24 h to subsequently decrease to 0.023 g/L at 72 h (Figure 5). Between the concentrations of 5 and 15% of pear residue flour, the highest proportion of PHA extracted was at 24 h for a concentration of 5% with 0.094g/L of PHA.

In related studies, Sanabria and Sarmiento (2017), reported the production of PHA by *B. subtilis* in submerged culture for 72 h using potato starch as substrate without prior

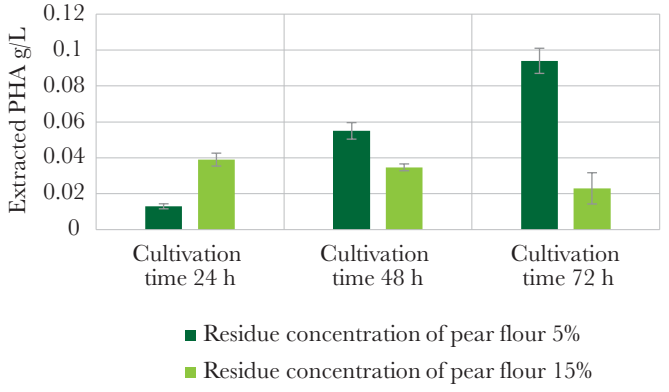

Figure 5. PHA production (g/L) during the growth of *Bacillus subtilis* sp., subtilis in submerged culture from pear waste flour (*Pyrus communis* L) at different concentrations.

hydrolysis treatment of the substrate at concentrations of 5 and 15 g/L, obtaining PHA extracts using extraction method with NaCl, NaOH and ethanol at 24 and 72h from 0.006 to 0.058 g, respectively, in substrate concentration of 5 g/L and 0.43 and 0.12g of PHA at 24 and 72h, respectively, for substrate concentration of 15g/L.

Likewise, the accumulation of intracellular PHA during the submerged culture indicated the tendency to increase as the culture time elapsed at a concentration of 5% substrate, having its maximum of 0.6% at 72h, while at a concentration of 15% substrate the trend was inverse, decreasing during the culture, reaching a minimum of 0.1% at 72h of culture. Between both concentrations of 5 and 15% of substrate, the highest percentage of PHA accumulation occurred at 5% at 72h of culture with 0.6% (Figure 6).

Most bacteria can accumulate PHA to around 30-50% dry cell weight, while *Ralstonia eutropha*, a known PHA producer, is able to accumulate PHA to more than 90% dry cell weight (Tsuge *et al.*, 2015). Several, but not all, species of the genus *Bacillus* sp., can accumulate PHA under unbalanced growth conditions. The species reported as native PHA producers are *B. amyloliquefaciens*, *B. anthracis*, *B. aryabhattai*, *B. badius*, *B. brevis*, *B. cereus*, *B. circulans*, *B. coagulans*, *B. firmus*, *B. flexus*, *B. laterosporus*, *B. lentus*, *B. licheniformis*, *B. macerans*, *B. megaterium*, *B. mycoides*, *B. odyssey*, *B. pasteurii*, *B. pumilus*, *B. sphaericus*, *B. subtilis*, *B. thuringiensis*, and some isolates of unidentified species (Tsuge *et al.*, 2015).

Bacillus spp. can synthesize PHA in the stationary and exponential growth phases. The accumulation of PHA may, or may not, be associated with growth compared to other producing microorganisms; therefore, when it is not associated with growth, the synthesis occurs in the stationary phase of growth with limitation of N, P, Mg and oxygen and excess carbon source, unlike the production of PHA associated with growth that takes place in balanced conditions (Mohapatra *et al.*, 2017).

For species of the genus *Bacillus* spp., PHA accumulation percentages ranging between 6.43 and 48.2% are generally reported (Santimano *et al.*, 2009). A wide range of cheap carbon sources assimilable by *Bacillus* strains useful to produce PHA has been reported, such as oligosaccharides from soybean molasses, sugarcane molasses, sugarcane bagasse, date syrup, palm oil and fruit bunch hydrolyzate (Tsuge *et al.*, 2015). In this study, accumulation

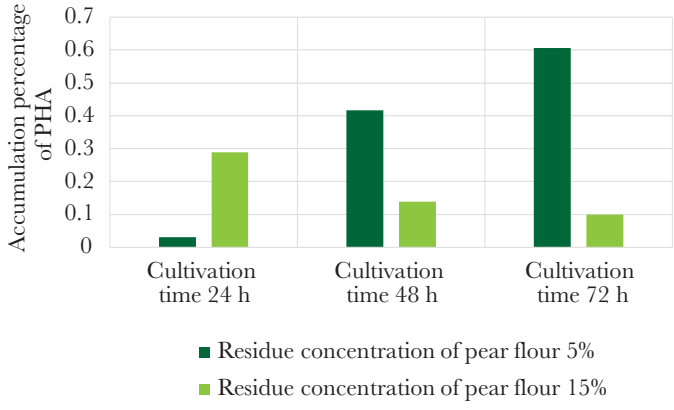

Figure 6. Percentage of accumulation of PHA by *Bacillus subtilis* sp., *subtilis* in submerged culture from pear waste flour (*Pyrus communis* L) at different concentrations.

percentages lower than those reported by other researchers were achieved. It should be noted that for the production of PHA, the use of economic carbon sources has been proposed, including agro-industrial residues (of fibrous and cellulosic composition) and it has been reported that a hydrolysis treatment prior to the culture with microorganisms (which was not the case in this study) for the production of PHA favors the availability and release of metabolizable sugars, and therefore the production of PHA (Rojas *et al.*, 2016) even with respect to common substrates such as starch in strains such as *Bacillus* sp. (Santimano *et al.*, 2009).

CONCLUSIONS

The growth and production of PHA by *B. subtilis* sp., *subtilis* was present in the two concentrations of pear waste flour (*Pyrus communis* L). The use of pear waste flour (*Pyrus communis* L) may help reduce the cost of the substrate and in turn provide value to the waste generated by the fruit industry. However, further studies are required focused on prior treatment of pear waste flour for its use as a carbon source, as well as the optimization of culture conditions to improve production, PHA extraction and characterization.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of sugar substitution by aguamiel on the physicochemical quality of pear jam (*Pyrus communis* L.)

Ramírez-Peñaloza Salma[1]; Pérez-Ruiz, Rigoberto V.[2]; Ruiz-Hernández, Rafael[2]; Aguilar-Toalá, José E.[2]; Fabela-Morón, Miriam F.[2]; Díaz-Ramírez Mayra[1*]

[1] Universidad Autónoma Metropolitana, Unidad Lerma, Licenciatura en Ciencia y Tecnología de Alimentos, Departamento de Ciencias de la Alimentación. División de Ciencias Biológicas y de la Salud.
[2] Universidad Autónoma Metropolitana, Unidad Lerma, Departamento de Ciencias de la Alimentación. División de Ciencias Biológicas y de la Salud, Lerma de Villada, Estado de México, México.
* Correspondence: m.diaz@correo.ler.uam.mx

ABSTRACT

Objective: Analyze the effect of the sugar substitution by dehydrated aguamiel on the physicochemical quality of pear jam (*Pyrus communis* L.).

Design/methodology/approach: Different levels of sugar substitution by dehydrated aguamiel were analyzed (0, 25 and 50%). Physicochemical parameters on pear jam as color, pH, acidity, density, consistency and soluble solids were evaluated.

Results: Results showed that the physicochemical and color characteristics of the pear jam was changed by the substitution of sugar by dehydrated aguamiel.

Study limitations/implications: More studies related to sensorial analysis of the pear jam and technological functions of dehydrated aguamiel are required.

Findings/Conclusions: Pear in advanced stage of maturity could be considered as a good ingredient in jam formulation. Dehydrated aguamiel was used as an alternative sweetener in jam.

Keywords: Aguamiel, pear jam, quality, sweetener

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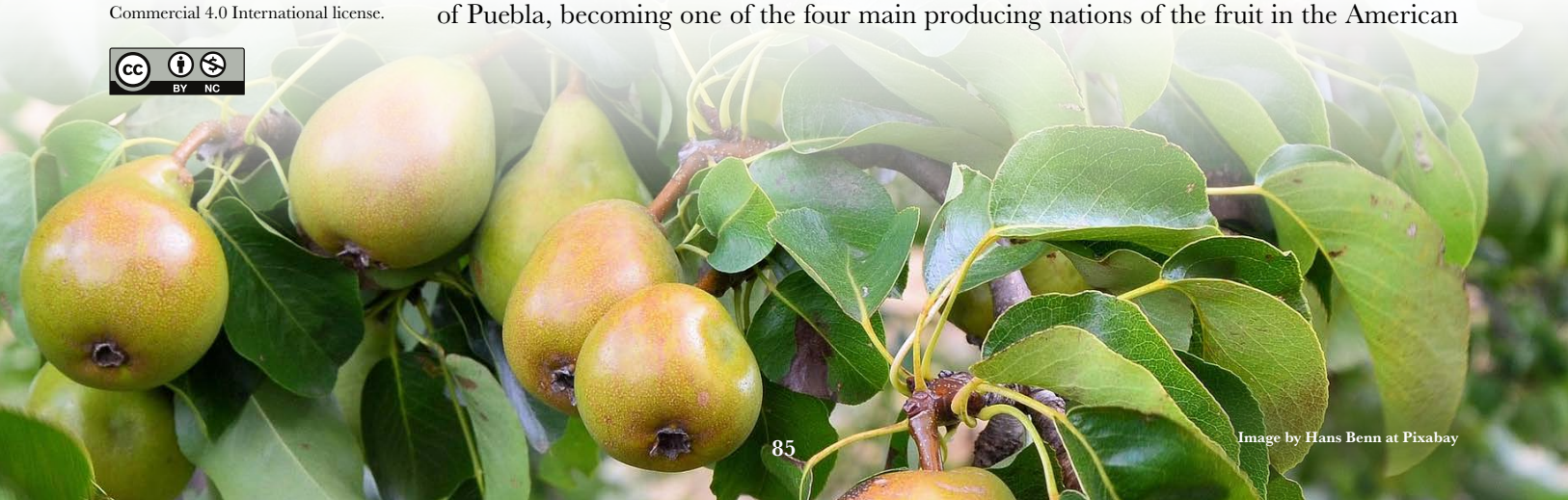
INTRODUCTION

The pear (*Pyrus communis* L.) is a fruit that is native to regions of Eastern Europe, China, Central Asia, Western Asia. It grows in a mild temperate zone and its cultivation is very old; it is classified as a *Pyrus* species of the Rosaceae family of the Rosales group (Kalkisim *et al*., 2018). China is one of the nations that contributes two thirds of the world pear production, being a little more than 25 million per year (Kalkisim *et al*., 2018). Mexico produces approximately 26,000 tons of pear annually, where the main producer is the state of Puebla, becoming one of the four main producing nations of the fruit in the American



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continent (SADER, 2020). It is estimated that the edible portion of the pear is 88 g per every 100 g of fresh product (FEN, 2022). Its maturation velocity is very high, so its incorporation in different foods should be promoted. Among the uses of the pear are the production of cider, liqueurs, jams, juices, jellies, preserves, among others (SADER, 2020).

On the other hand, aguamiel is a liquid that is obtained from the Agavaceae family, it has a sweet smell and taste (Ramírez-Cuellar *et al.*, 2018), and it has a translucent and yellowish appearance; its composition is water and sugars of which glucose, fructose and sucrose predominate, it also contains proteins, and mineral salts (Ramírez-Cuellar *et al.*, 2018). Aguamiel has nutritional and physicochemical properties that give it a prebiotic food category (Ramírez-Cuellar *et al.*, 2018), as well as being a source of compounds with antioxidant activity (Romero-López *et al.*, 2015). This liquid has been used as an ingredient in products as drinks (Changoluisa Maigua, 2020) and nectars (Bautista, 2006), but it can be incorporated in products derived from fruits such as jam. Therefore, the aim of this work was analyze the effect of the sugar substitution by dehydrated aguamiel on the physicochemical quality of pear jam.

MATERIALS AND METHODS

Materials. Pear (*Pyrus communis* L.) was obtained from the supply center of the city of Toluca, State of Mexico, Mexico. Aguamiel was obtained from local producers of Texcaltitlan, Estado de México. Sugar (Zulka, Zucarmex, México), water (Bonafont, México), pectin (Aglupectin®, Italy) and citric acid (Fermont, México) were used to jam elaboration. All reagents used were reagent grade.

Physicochemical evaluation of pear and its pulp. Color (Chroma meter CR-41, Konica Minolta), hardness (Model CT3 10, Brookfield), total soluble solids (Máster refractometer, Atago), titratable acidity (Parra *et al.*, 1998), and moisture determination (OHAUS, Model MB-27) were evaluated.

Aguamiel dehydration. Aguamiel was put into an aluminum recipient and was heated at 92 °C during 3 h.

Jam elaboration. 150 mL of water was put in an aluminum recipient, and it was heated at 70 °C, then, pear, sugar, dehydrated aguamiel, pectin and citric acid were added according the formulations of Table 1, and were mixed during 15 min. The jam was cooled at room temperature and was kept in a glass container in refrigeration until its analysis.

Table 1. Formulations of pear jam at different level of sugar substitution by dehydrated aguamiel (DA).

Formulation	Control (0%)	25%	50%
Pear (g)	500	500	500
Sugar* (g)	250	187.5	125
DA (g)	0	78.13	156
Water (mL)	150	150	150
Pectin (g)	8	8	8
Citric acid (g)	3	3	3

*Sugar substitution was according to the sugar content (100%).

Physicochemical analysis of aguamiel. Total soluble solids (Máster refractometer, Atago, Japón), pH (Muñiz *et al.*, 2020), titratable acidity (López. 2017), and color (Chroma meter CR-410, Konica Minolta, Chiyoda, Japón) were evaluated in fresh and dehydrated aguamiel.

Physicochemical analysis of pear jams. Total soluble solids (Máster refractometer, Atago, Japón), pH, density (Elcometer, Gardco Paul N. Gardner Company Inc, USA), consistency (Bostwick Consistometer, CSC Scientific Company, INC, USA), titratable acidity (López. 2017), and color (Chroma meter CR-410, Konica Minolta, Chiyoda, Japón) of pear jams were analyzed.

Analysis of results. The experiments were done in triplicate. MS-Excel 2014 software was used for data analysis.

RESULTS AND DISCUSSION

Physicochemical evaluation of pear and its pulp. Table 2 shows the results of the analyzes carried out on the pear, which were color, hardness, soluble solids, titratable acidity, and moisture.

The color analysis of the evaluated pear residues presented values of L of 29.259, a* of 5.63 and b* of 10.72 with a tendency to black, red, and yellow. This is due to the high state of maturity that it presents, as well as a decrease in chlorophyll and the appearance of carotenoids, where the green color tends to decrease as the state of maturity advances, presenting the yellow color (Poveda, 2015).

The hardness or firmness is an important property in the quality, acceptance of the fruit by consumers and storage (López-Camelo, 2003). This property is a function of the harvest or harvest time and storage temperature, related to the external color of the fruit (López-Camelo, 2003; Infoagro, 2022). The hardness value of the pear residues was 7.98±0.22 N (0.8137 kgf), a value commonly associated with softening due to the state of advanced maturity that they show. The firmness and texture of fruits change due to the hydrolysis of starches and pectin, reduction of their fiber content and cell wall degradation processes (López-Camelo, 2003; Infoagro, 2022). In related studies Moggia *et al.* (2005) reported, for pears, an average of 7.8 kgf of postharvest firmness and 5.3 and 5.7 kgf after one month. Meanwhile, Parra *et al.* (1998), reported an initial firmness value of 13 kgf and a decrease to 7.17 kgf after 14 days.

Soluble solids in fruits are composed of sugars, salts, acids, and other water-soluble compounds that are part of the juice, being sugars and the organic acids present inside the fruit the most abundant, increasing as the maturity stage progresses derived from the hydrolysis of structural polysaccharides such as starch, cell wall pectin, accumulating glucose, fructose and sucrose, so they are related to the firmness and color of the fruit,

Table 2. Characterization of pear and its pulp (*Pyrus communis* L).

Color			Hardness (N)	Soluble Solids %	Titratable Acidity %	Moisture %
L	a	b				
29.259	5.63	10.72	7.98±0.22	11.87±0.06	0.49±0.019	82.92±0.014

being pointed out as quality indicators for consumers in these foods (Kader 1999; Yanes, 2018). The percentage of soluble solids in the pear was 11.87±0.06; this value ranges vary from researcher to researcher. Poveda (2005) reported values of 12.83% at advanced maturity; Parra *et al.* (1998) reported values of 6.04% at the beginning of harvest up to 12.7% as the state of maturity progresses.

The fruits acidity is the result of organic acids and sugar precursors present; it is estimated a general tendency to decrease the acidity during the maturation of various fruits, in addition to the biochemical and sensory importance of organic acids, which lies in the fact that they help in flavor, in a typical relationship between sugars and acids in different fruits (del Pilar Pinzón *et al.*, 2007). The titratable acidity of pear residues was 0.4913±0.019%, which is related to values reported by Chiquillanqui (2014) with 0.48% and Kalkisim *et al.* (2017) from 0.2 to 0.59% depending on the variety of the fruit in the locality; it should be noted that the acidity value is low due to the development of the stage of maturity in which the sugar content increases and the malic acid content tends to decrease.

Finally, the moisture content of the pear was 82.92±0.014%, being water the main component. In related studies, Kadam (1995) indicates a humidity value of 87.7% and Kalkisim *et al.* (2018) reports from 63.51 to 88.25% for pear (*P. communis*). The moisture content in fruits and foods is important since it is related to texture, firmness, as well as conservation and processing processes that contribute to quality and safety (Chuquillanqui, 2014).

Physicochemical characterization of fresh and dehydrated aguamiel. Table 3 shows the results of the physicochemical analysis of aguamiel. A higher soluble solids value of dehydrated (80±0.01) than fresh (10.75±0.35) in aguamiel is observed due to the water evaporation during the heating process. According to Palafox *et al.* (2017), the value of soluble solids in fresh aguamiel from Nanacamilpa, Tlaxcala was 12.1±1.4, thus, the value in this study was lower because, it depends on the place and temporality when it was obtained; and in the case of dehydrated aguamiel it depends on the dehydration process. The pH value of the fresh aguamiel was 6.65, which is according to the NMX-V-022-1972 and to Palafox *et al.* (2017) who indicate a range between 6.6 and 7.5 and a value of 6.0±0.4, respectively; after heating the pH value decreased, probably because of the proton exposition after molecular movement and heating. Acidity value of fresh aguamiel was 0.59±0.01, which was lower than the value of the NMX-V-022-1972 and it value also decreased after heating, probably due to acids degradation. On the other hand, the results of color show a black and red color tendency and a reduction of yellow color, these values are associated with a non-enzymatic darkening or the Maillard and caramelization reactions which happened during heating process.

Table 3. Physicochemical characteristics of fresh and dehydrated aguamiel.

Aguamiel	Soluble solids	pH	Acidity (%)	Color		
				L	a	b
Fresh	10.75±0.35	6.65±0.07	0.59±0.01	60.09±0.60	0.79±0.02	12.14±0.32
Dehydrated	80±0.01	4.77±0.01	0.11±0.01	40.73±0.04	6.98±0.04	5.93±0.04

Physicochemical quality of pear jams. The results of the physicochemical characteristics of the different pear jams with and without aguamiel are shown in Table 4. Regarding the soluble solids for the 0% formulation, the value was 51.5±0.7, for 25% it was 43.25±0.35, and for 50% of 46.25±0.35. These results show that the higher the amount of dehydrated aguamiel, the lower the number of soluble solids in the sample. These results are explained because, despite the aguamiel went through a dehydration process, there is still a quantity of water that interferes with the total soluble solids content of the pear jam.

The pH results of the control formulation (0%) (Table 4), showed a higher value (3.805±0.007) compared to that indicated in the COVENIN Standard, which can range between 3.0-3.3; in the case of the other formulations of 25% and 50%, the pH is higher, this due to the presence of dehydrated aguamiel that has a pH of 4.77 (Table 3) so that the pH increases with respect to the increase in the amount of dehydrated aguamiel in the jam.

The density in the jam is a parameter that relates the mass per unit volume of the product. This parameter varies by the type and amount of ingredients in the formulation; at 0% it showed a value of 1.218±0.003 cm^3/mL while at 25% it was 1.186±0.027 cm^3/mL and at 50% it was 1.189±0.024, in both formulations the density decreased.

The consistency is a parameter that is very commonly evaluated in jams; this parameter is an indicator of the gel formation during the cooking of the ingredients. The results obtained show that the jam had a longer run in the consistometer, 0% (2.75±0.35), 25% (6.25±0.07) and 50% (6.4±0.5), which indicates that as the substitution of dehydrated aguamiel increased, the consistency of the jam decreased. The consistency is related to the density which also decreased; this is explained because sugar has an important technological function in the formation of the gel and its texture in such a way that its decrease negatively affects these properties.

The acidity result was different in the formulation of 50% (0.038±0.077) with respect to the formulation of 0% (0.060±0.004) and 25% (0.060±0.004). Kerstupp (2010), who analyze xoconostle jam (*Opuntia joconostle*), shows an acidity percentage of 0.04%, indicating that the 50% formulation has a result like the one presented in this work.

Finally, Table 5 shows the color results of the jams. The value of L decreases as the sugar decreases, but also as the content of dehydrated aguamiel increases, so the values found are because aguamiel had a very dark hue (Table 4) because of heating to which underwent by caramelization of sugars. The a* parameter was positive in all cases, so

Table 4. Physicochemical characterization of pear jams with sugar substitution by dehydrated aguamiel.

Parameter	0%	25%	50%
% Soluble solids	51.5±0.7	43.25±0.35	46.25±0.35
pH	3.805±0.007	4.265±0.021	4.72±0.05
Density	1.218±0.003	1.186±0.027	1.189±0.024
Consistency	2.75±0.35	6.25±0.07	6.40±0.5
Acidity (%)	0.060±0.004	0.060±0.004	0.038±0.077

Table 5. Color of pear jams with sugar substitution by dehydrated aguamiel.

Parameter	0%	25%	50%
L	37.266±0.054	33.91±0.04	29.766±0.060
a	5.71±0.06	9.446±0.045	8.062±0.048
b	10.468±0.093	10.453±0.028	5.944±0.024

the jams have red tones, these values being higher as the concentrated aguamiel content increases. Finally, regarding the b* values, these were positive, showing a tendency towards yellow, but in this case, as the sugar decreases and the concentrated aguamiel increases, the yellow hue decreases.

In general, it was observed that the color was modified mainly by the addition of dehydrated aguamiel, making it darker and tending to red.

CONCLUSIONS

Physicochemical characterization of the pear and its pulp showed an advanced stage of maturity of the fruit, however it could be considered as a good ingredient in jam formulation. Substitution of sugar by dehydrated aguamiel modified the physicochemical characteristics and color of jam pear, therefore more studies about technological function of dehydrated aguamiel are necessary to considered it as an alternative a sweetener in foods. Also, more studies related to sensorial analysis of the pear jam are necessary to conclude about the characterization of the pear jam.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Evaluation of the botanical composition of kikuyu and fescue grasslands associated with white clover during two seasons in the high valleys of Mexico

Zamora-Juárez, Yessica G.[1]; Plata-Reyes, Dalia A.[1*]; Marín-Santana, María N.[1]; López-González, F.[1]; Hernández-Mendo, O.[2]; Martínez-García, Carlos G.[1]; Arriaga-Jordán Carlos, M.[1]

[1] Universidad Autónoma del Estado de México. Instituto de Ciencias Agropecuarias y Rurales Campus El Cerrillo, El Cerrillo Piedras Blancas, Toluca, Estado de México, México. C. P. 50090. Tels.: 722 296 5552/722 180 6124/722 180 6136 ext. 6500.

[2] Colegio de Postgraduados Campus Montecillo. Programa de Ganadería, Texcoco, México. C. P. 56264. Tel. 55 5804 5900.

* Correspondence: dplatar144@alumno.uaemex.mx

ABSTRACT

Objective. To evaluate the botanical composition of grasslands of kikuyu (*Cenchrus clandestinus*) compared to tall fescue (*Lolium arundinaceum* cv. Cajun II), each one in association with white clover (*Trifolium repens* cv. Ladino), in two independent experiments conducted during two seasons, autumn 2018 and winter 2019.

Methodology. Two independent experiments under small-scale milk production system (SMPS) were established in the municipality of Aculco, State of Mexico, during autumn 2018 and winter 2019. The botanical composition of grasslands under intensive continuous grazing by breeding cows was evaluated. One grassland planted with tall fescue cv. Cajun II and the other invaded by kikuyu; each grassland was associated with white clover cv. Ladino. The botanical composition of both experiments was analyzed using a complete randomized experimental design.

Results. The kikuyu grassland recorded significant differences ($p<0.05$) with a higher proportion of forage during the winter 2018. Whereas the tall fescue cv. Cajun II grassland recorded a proportion of forage ($p<0.05$) higher than its proportion of dead tissue during autumn 2019.

Study Implications: The study of the botanical composition of mixed grasslands destined for livestock grazing allows to identify, propose and define strategies for forage production facing agroclimatic and management conditions in order to generate a better and higher forage yield.

Conclusions: The proportion of kikuyu was higher than that of tall fescue cv. Cajun II during the two seasons and years evaluated. This highlights the adaptability of kikuyu grass under agroecological conditions such as the absence of rains and high temperatures, coupled with the high stocking densities of the milk production systems in the study region.

Keywords: *Cenchrus clandestinus*, winter, *Lolium arundinaceum*, autumn, *Trifolium repens*.

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INTRODUCTION

Small-scale milk production systems (SMPS) are characterized by employing family labor; allocating an area of 6.25 ha (hectares) of land for farming activities; and generating income using regional forage resources. This latter is an aspect that has a favorable impact by increasing economic sustainability (Próspero-Bernal *et al.*, 2017).

SMPSs have a potential for development towards sustainable models based on the improvement of their production processes through the management of their feeding strategies. Such as the implementation of grazing, intensive and continuously, in grasslands which are the basis of livestock feeding (Fadul-Pacheco *et al.*, 2013; Camacho-Vera *et al.*, 2017; Plata-Reyes *et al.*, 2018).

In the State of Mexico during 2019-2020, the reported area destined for agriculture and pastures was 933 586 ha of which 17 210 ha are mostly represented by areas that changed from agricultural crops to grasslands (SIAP, 2021) and represent an important forage source destined for feeding dairy cattle. The botanical composition is highly variable and susceptible to management and agroclimatic effects, especially in the oldest established grasslands (Sowers *et al.*, 2019).

Therefore, several authors agree on the need to evaluate the different species and varieties of grasses and legumes, in order to obtain knowledge that allows to better face the challenges of seasonal livestock production. Through the inclusion of grasslands characterized by their yield for grazing, adaptation attributes, availability, quality and resistance to different agroecological and management conditions under SMPS (Arango-Gaviria *et al.*, 2017; Marín-Santana *et al.*, 2020; Muciño-Álvarez *et al.*, 2021; Plata-Reyes *et al.*, 2021).

Due to its great adaptation capacity and wide geographical distribution, the kikuyu (*Cenchrus clandestinus* Hochst. ex Chiov Morrone formerly *Pennisetum clandestinum*) has developed in humid subtropical zones and environments different from its natural habitat (Arango-Gaviria *et al.*, 2017), since being a C4 species, it has a greater resistance to high temperatures and to the reduction or absence of rainfall. Its optimum growth temperature ranges between 18-30 °C and it is susceptible to long frost periods. Due to its aggressive growth behavior even in unfavorable agroecological conditions, it shows survival potential; thus, it has been considered a non desirable species (Fraser *et al.*, 2017; Benvenutti *et al.*, 2020).

Whereas the tall fescue (*Lolium arundinaceum* [Schreb] Darbysh formerly *Festuca arundinacea*) cv. Cajun II is a cold-season perennial species recognized for its growth in autumn under favorable temperature and humidity conditions, remaining green for winter grazing after frosts, but its vegetative regrowth decreases during the summer due to stress caused by high temperatures and absence of rainfall (Lee *et al.*, 2013).

The objective of this study was to evaluate the botanical composition of one kikuyu grassland (*Cenchrus clandestinus*) compared to another of tall fescue (*Lolium arundinaceum* cv. Cajun II), each one associated with white clover (*Trifolium repens* cv. Ladino), in two independent experiments carried out during two seasons, autumn 2018 and winter 2019.

MATERIALS AND METHODS

The experiments were conducted in production units (UP) of two collaborating farmers (who jointly manage their UP as one); they follow the rural participatory research guidelines for livestock technology development (Conroy, 2005). Those UP where the experiment was implemented are in Aculco de Espinoza (20° 00' a 20° 17' N, and 99° 40' a 100° 00' W) State of Mexico. Aculco has an area of 453.3 km^2, an altitude of 2440 m and a temperate sub-humid climate; average temperature of 13.2 °C, average annual rainfall of 700 mm,

presence of frost from October to February, a rainy season from May to October and a dry season from November to April (Celis-Álvarez *et al.*, 2017).

Experimental specifications

In the field, under the specifications of rural participatory research, two experiments were established independently for 42 d, divided into three experimental periods 14 d each. As it is shown in Figure 1. Experiment 1 (Experiment 1): conducted from September 1 to October 12, 2018. Experiment 2 (Experiment 2): conducted from February 15 to March 29, 2019.

Grasslands

Two grasslands of 1.0 ha each were evaluated, delimited with electric fence, both were grazed by four cows for 14 d (experimental period).

Experiment 1. Kikuyu grass (*Cenchrus clandestinus*), naturally established since 2013 by invasion on a plot of untilled land; average grassland height during the experiment, 5.33 cm±0.47.

Experiment 2. Tall fescue (*Lolium arundinaceum* cv. Cajun II) free of endophytes, that was seeded on February 8, 2018; average grassland height during the experiment, 1.87 cm±0.00.

During the time of the experiment, the tall fescue cv. Cajun II grassland was notably invaded by kikuyu. Also, in both experiments each grassland was associated with white clover (*Trifolium repens* cv. Ladino). The seed dose per hectare of tall fescue cv. Cajun II was 22 kg and 3 kg of white clover seed cv. Ladino. It was fertilized at the time of planting with 60 kg of N, 80 kg of P and 60 kg of K per hectare.

The grasslands of the two experiments were fertilized with 50 kg of N approximately every 28±7 d and with 80 kg of P and 60 kg of K per hectare every six months. The results of production, forage chemical composition and animal production were reported in previous studies by Marín-Santana *et al.* (2020) and Plata-Reyes *et al.* (2021).

Treatments

The treatments evaluated were:

EXP1=*Cenchrus clandestinus*+*Trifolium repens* cv. Ladino (KY+TB)
EXP2=*Lolium arundinaceum* cv. Cajun II+*Cenchrus clandestinus*+*Trifolium repens* cv. Ladino (CJ+KY+TB)

Period 1	Period 2	Period 3
September 01 to September 14	September 15 to September 28	September 29 to October 12

Experiment 1 (Fall 2018)

Period 1	Period 2	Period 3
February 15 to March 01	March 02 to May 16	March 17 to March 29

Experiment 2 (Winter 2019)

Figure 1. Distribution of the experimental periods.

Evapotranspiration

The estimation of potential evapotranspiration is important as an indicator in water use with agricultural, ecological, and other activities that require planning. To calculate the monthly potential evapotranspiration, the model described by Segura-Castruita and Ortiz-Solorio (2017) was used from maximum-minimum temperatures and altitude recorded for both experiments.

Botanical composition of grasslands

It was determined, from five subsamples per grassland and experimental period of 50 g each, cut randomly with scissors at ground level delimiting the cutting area with a metal quadrant of 0.5×0.5 m, the cuts were made during three experimental periods with a frequency of 14 d. Subsequently, the samples were separated manually in the laboratory, depending on the species present, grasses (Kikuyu or tall fescue cv. Cajun II), white clover, living, dead and other components (wild plants); samples were dried in a forced-air stove at 55 °C up to constant weight for 48 h and results were expressed as g per 100 g DM (Plata-Reyes *et al.*, 2021).

Statistical analysis

The experimental design was of repeated measures in time according to the following model of analysis of variance (Gutiérrez and Manrique, 1996, Kaps and Lamberson, 2004):

$$Y_{ijk} = \mu + \tau_i + \delta_{ij} + \rho_k + (\tau\rho)_{ik} + \varepsilon_{ijk}$$

where: Y_{ijk}=response variable; μ=overall mean; τ_i=treatment effect; δ_{ij}=error associated with experimental units; ρ_k=period effect; $(\tau\rho)_{ik}$=interaction between period and treatment; and ε_{ijk}=errors associated with periods.

The analysis of variance and Tukey test were performed on all variables (all were normally distributed) to identify significant differences ($p<0.05$) between the type of prairie, time of year and period. The results were analyzed with Minitab statistical software (v.14, Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

Figure 2 shows the temperature, minimum, average and maximum recorded during experiment 1. A lower temperature is observed compared to the winter 2019 (Figure 3).

Table 1 presents the climatic conditions during the experiments. Rainfall was only recorded at the beginning of the first period of experiment 1 with 10.7 mm (Figure 2). Whereas the estimated potential evapotranspiration was lower in the third period of autumn 2018 (Figure 4), and higher in the second period of winter 2019 (Figure 5). This was the result of precipitation disruption, limited irrigation management, and high stocking density on grazing (Plata-Reyes *et al.*, 2021). In addition to decreasing annual rainfall in the

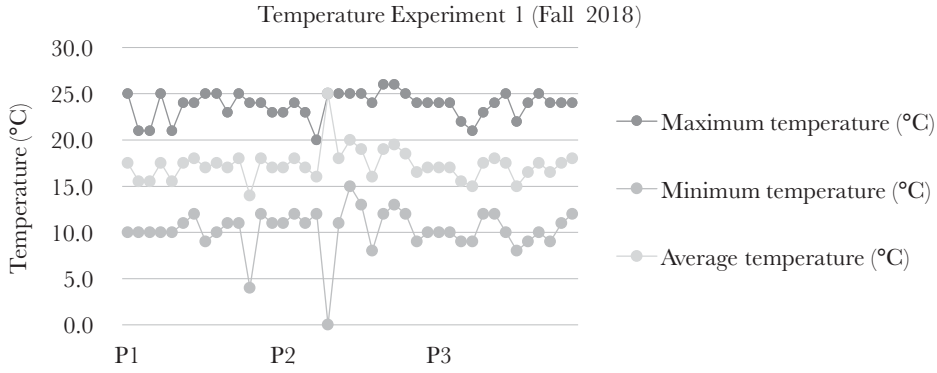

Figure 2. Maximum, average, and minimum temperatures recorded during experiment 1.

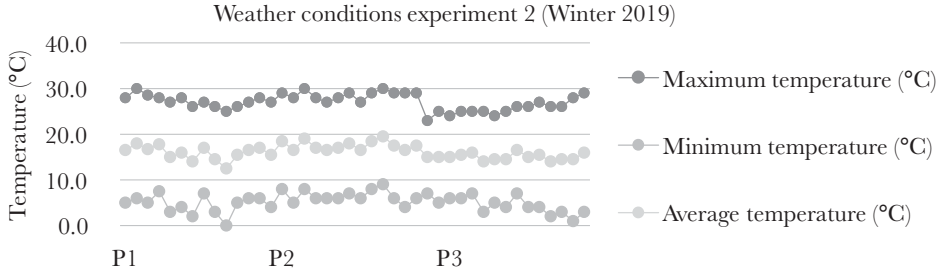

Figure 3. Maximum, average, and minimum temperatures recorded during experiment 2.

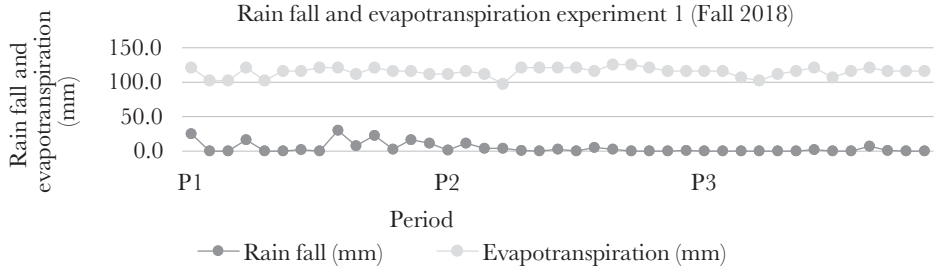

Figure 4. Evapotranspiration and rainfall recorded during experiment 1.

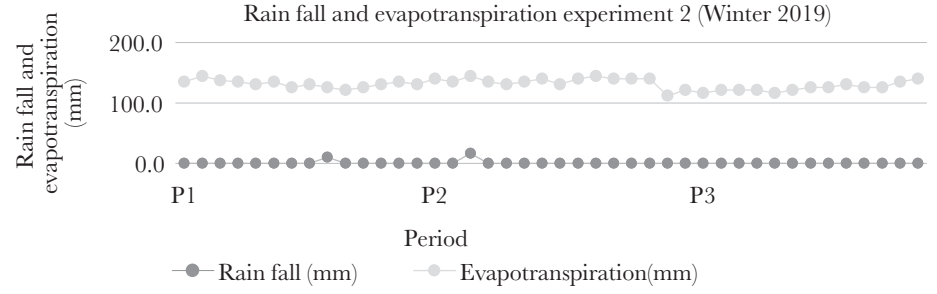

Figure 5. Evapotranspiration and rainfall recorded during experiment 2.

area, and the water available to producers for irrigation, derived from a water catchment-dam. Such water is distributed locally during the dry season but is limited (López-González *et al.*, 2020).

Botanical composition between grasslands

Significant differences ($p<0.05$) were recorded between grasslands for the botanical composition of kikuyu, tall fescue, white clover, and the proportion of dead tissue, as shown in Table 2. Both treatments presented a similar production of living tissue depending on their contribution to botanical composition without significant differences ($p>0.05$). Significant differences ($p<0.05$) were recorded in terms of the proportion of grass in each of the grasslands. Kikuyu performed best in both grasslands, because it is a very competitive species with easy growth. Despite the high stocking density and limited irrigation which are characteristics of SMPS in the study area.

On the other hand, white clover also recorded significant differences ($p<0.05$) and a greater presence in the KY+TB grassland, which could have influenced a lower proportion of dead tissue, probably due to the complementarity between species because of the grass-legume combination (Muciño-Álvarez *et al.*, 2021; Plata-Reyes *et al.*, 2021). In contrast with the values in the CJ+KY+TB grassland, with a higher mean ($p<0.05$) statistically significant, as a function of the proportion of dead tissue. Finally, wild plants neither showed significant differences ($p>0.05$) in terms of their contribution on each grassland.

Botanical composition by season

By season, all components showed significant differences ($p<0.05$), except white clover ($p>0.05$), as shown in Table 3. It is observed that kikuyu grass had a significant greater contribution ($p<0.05$) during the winter compared to tall fescue. Regardless thermal tolerance ranges between 7-38 °C (Fraser *et al.*, 2017), within the 42 d of duration of experiment 2, 33 d were measured with temperatures below 7 °C (Figure 2). Greater resilience and resilience were observed in kikuyu grass that contributed directly to adaptability through its latency capacity (Marais, 2001; Bell *et al.*, 2013; García *et al.*, 2014).

Table 1. Weather conditions recorded during experiments.

Experiment	Period	Precipitation (mm)	Maximum temperature (°C)	Temperature Minimun (°C)	Temperature Average (°C)	Evapotranspiration (mm)
Exp1	P1	10.7	23.7	10.0	16.9	115.1
	P2	3.1	24.1	11.6	17.9	117.1
	P3	0.8	23.6	10.1	16.8	114.4
Exp2	P1	0.7	27.1	4.6	15.9	131.0
	P2	1.1	28.1	6.4	17.3	135.8
	P3	0.0	25.8	4.3	15.0	124.8

Exp1 = *Cenchrus clandestinus* + *Trifolium repens* cv. Ladino (KY+TB)
Exp2 = *Lolium arundinaceum* cv. Cajun II + *Cenchrus clandestinus* + *Trifolium repens* cv. Ladino (CJ+KY+TB)

Table 2. Botanical composition results by grassland (g per 100g DM).

Component	Experiment	Praire	Mean	SEM	Value of P
Kikuyo	Exp1	KY+TB	43.9^{b}	8.3***	0.000
	Exp2	CJ+KY+TB	22.4^{a}		
Tall Fescue cv. Cajun II	Exp1	KY+TB	0.0^{a}	17.0***	0.000
	Exp2	CJ+KY+TB	44.1^{b}		
White clover cv. Ladino	Exp1	KY+TB	22.8^{b}	6.3***	0.000
	Exp2	CJ+KY+TB	6.4^{a}		
Dead	Exp1	KY+TB	25.5^{a}	4.8**	0.002
	Exp2	CJ+KY+TB	37.7^{b}		
Live	Exp1	KY+TB	63.4^{a}	1.6^{NS}	0.208
	Exp2	CJ+KY+TB	59.1^{a}		
Other	Exp1	KY+TB	7.9^{a}	0.8^{NS}	0.592
	Exp2	CJ+KY+TB	9.0^{a}		

Exp1=*Cenchrus clandestinus*+*Trifolium repens* cv. Ladino (KY+TB);
Exp2=*Lolium arundinaceum* cv. Cajun II+*Cenchrus clandestinus*+*Trifolium repens* cv. Ladino (CJ+KY+TB); SEM=standard error of the mean; NS=p>0.05; *=p<0.05; **=p<0.01; ***=p<0.001; ^{abc}=different letters indicate significant differences between grasslands.

This capacity of kikuyu grass shown during this study is worth noticing; despite it is a tropical species, its growth does not stop in conditions of low temperatures. Meanwhile, tall fescue also registered a significantly higher proportion (p<0.05) during the autumn of 2018, where an average 8.21 mm rainfall was reported, compared to the 2.91 mm average rainfall in the winter 2019 with average temperatures between 16.8-17.9 °C. This agrees

Table 3. Botanical composition (g per 100g DM) by season (2018-2019).

Component	Season of the year	Experiment	Mean	SEM	Value of P
Kikuyo	Autumn 2018	Exp1	23.2^{a}	7.7***	0.000
	Winter 2019	Exp2	43.1^{b}		
Tall Fescue cv. Cajun II	Autumn 2018	Exp1	28.1^{b}	4.7*	0.023
	Winter 2019	Exp2	15.9^{a}		
White clover cv. Ladino	Autumn 2018	Exp1	13.9^{a}	0.5^{NS}	0.585
	Winter 2019	Exp2	15.3^{a}		
Dead	Autumn 2018	Exp1	53.8^{b}	12.2***	0.000
	Winter 2019	Exp2	9.4^{a}		
Live	Autumn 2018	Exp1	31.8^{a}	22.7***	0.000
	Winter 2019	Exp2	90.7^{b}		
Other	Autumn 2018	Exp1	0.6^{a}	6.1***	0.000
	Winter 2019	Exp2	16.3^{b}		

Exp1 (Otoño 2018)=*Cenchrus clandestinus*+*Trifolium repens* cv. Ladino (KY+TB); Exp2 (Invierno 2019)=*Lolium arundinaceum* cv. Cajun II+*Cenchrus clandestinus*+*Trifolium repens* cv. Ladino (CJ+KY+TB); SEM=standard error of the mean; NS=p>0.05; *=p<0.05; **=p<0.01; ***=p<0.001; ^{abc}=different letters indicate significant differences between grasslands.

with Muciño-Álvarez *et al.* (2021), who mentioned that tall fescue improved its growth during the fall. Regardless, the contribution of tall fescue was like that of kikuyu during the autumn probably because the tropical grass tolerates better the water deficit and adverse conditions of the study region (Figure 2).

Botanical composition by experimental period

Table 4 presents the botanical composition recorded during the experiments. Kikuyu grass was the component with the highest presence (33%) in each of the experimental periods, tall fescue accounted for 22% and white clover 15%, reflecting the higher proportion of grasses and legumes over wild plants and dead tissue. These are favorable aspects that have an impact on the nutritional quality of forage in grasslands under continuous grazing (Edwards *et al.*, 2011; Claffey *et al.*, 2020).

The proportion recorded of living tissue showed significant differences ($p<0.05$); it can be observed how it decreased during the experimental periods, the proportion of dead tissue per period was significant ($p<0.05$) (Table 4). According to what was reported by Muciño-Álvarez *et al.* (2021), the combination of grasses and legumes allows complementarity between species, resulting in constant forage production. In this regard, Lowe *et al.* (2009) mentioned that the number of species established in a mixed grassland allows each of the species to be properly established.

Table 4. Botanical composition (g 100 g^{-1} DM) of both grasslands by experimental period.

Variable	Period	Mean	SEM	Value of P
Kikuyo	P1	28.9[a]	2.15[NS]	0.476
	P2	34.6[a]		
	P3	35.9[a]		
Tall Fescue cv. Cajun II	P1	23.3[a]	3.16[NS]	0.085
	P2	14.2[a]		
	P3	28.6[a]		
White clover cv. Ladino	P1	15.8[a]	1.34[NS]	0.752
	P2	13.5[a]		
	P3	14.5[a]		
Dead	P1	29.4[a]	6.03**	0.003
	P2	24.8[a]		
	P3	40.6[b]		
Live	P1	61.9[a]	2.29**	0.003
	P2	68.6[a]		
	P3	53.2[b]		
Other	P1	7.7[a]	2.97[NS]	0.174
	P2	11.3[a]		
	P3	6.3[a]		

SEM=standard error of the mean; NS=$p>0.05$; *=$p<0.05$; **=$p<0.01$; ***=$p<0.001$; [abc]=different letters indicate significant differences between grasslands.

CONCLUSIONS

The performance of kikuyu grass in the experiments during the two seasons of the year is highlighted, which is according to its reported agronomic characteristics. Thus, it has a better survival potential compared to the agronomic management carried out by the producers of the area. This management is characterized by a high stocking density and little availability of irrigation water. The proportion of white clover may have influenced forage growth in the kikuyu grassland due to the complementarity between species, which is a result of the combination between grasses and legumes.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Anaerobic co-digestion of bovine manure and residual sludge from tilapia fish (*Oreochromis niloticus*) breeding ponds

Campos-Montiel, Rafael[1]; Medina-Pérez Gabriela[1*]; Afanador-Barajas, Laura[2]; Ibarra-Sánchez, Claudia[3]; Shona Prince[1]; Pérez-Ríos, Sergio[1]; Hernández-Niño, Jairo[4]

[1] Institute of Agricultural Sciences, Autonomous University of the State of Hidalgo, Hidalgo 3600, Mexico.
[2] Natural Sciences Department and Engineering and Sciences Faculty, Universidad Central, Bogotá 110311, Colombia.
[3] Department of Microbiology, National School of Biological Sciences, National Polytechnic Institute, Mexico City, Mexico.
[4] Environmental Biology Program at Unitropico (International University of the American Tropic), Yopal, Casanare, Colombia.
* Correspondence: gabriela_medina@uaeh.edu.mx

ABSTRACT

Objetive: Tilapia production was 4.2 million tons in 2016, and almost half of the production came from aquaculture. At the same time, organic waste from breeding increases as the demand for tilapia production. An alternative to using such waste is the production of energy and organic fertilizers. The use of a co-substrate can help to achieve the moisture content necessary to feed the digester. The aim of this study was to determine the effect of the anaerobic co-digestion of bovine manure with residual sludge from tilapia fish breeding ponds in the production of sludge.

Methodology: Methane and CO_2 production, chemical oxygen demand (COD), total solids (TS), total fixed solids (TFS), total volatile solids (TVS), volatile fatty acids (VFA), total nitrogen (TN), total phosphorus (TP) content, and microbiological parameters (fecal coliforms and salmonella) during composting were determined. The organic fertilizer obtained was evaluated by a germination and seedling growth assay.

Results: The results of this study showed that the mixture of bovine manure and residual sludge from tilapia fish breeding ponds (1:1) produced high methane and low CO_2 in the composting process compared to the when these raw materials were composted individually.

Conclusions: Alfalfa germination and seedling growth were significantly boosted by the application of sludge from the mixture of bovine manure with residual sludge from tilapia fish breeding ponds.

Keywords: *Oreochromis niloticus*; composting; germination; sludge; methane.

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INTRODUCTION

The growing demand for energy and food for a growing population has led to the depletion of conventional sources of resources. Alternative technologies have the potential to generate sustainable economies by transforming organic material (livestock manure, agricultural residues, etc.) into biofuels and biofertilizers that help conserve resources and protect the environment[1]. Anaerobic digestion (AD) has become a viable alternative to mitigate the problems caused by organic wastes, such as odor, the large volumes generated,

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the loss of essential nutrients, possibly decreasing pollution[2]. In the anaerobic digestion process, microorganisms consume substrate, either manure or other organic material, resulting in biogas (a mixture of methane and carbon dioxide), and a non-pathogenic fertilizer, which is rich in organic material, humus, nitrogen, phosphorous and potassium, as the end products[3]. Regarding anaerobic codigestion, this is a process that improve the anaerobic digestion performance, optimizing the production of biogas. The world fish production in 2017 was 172.6 million tons. In 2016, the world tilapia production was 4.2 million tons, and almost half of the production came from aquaculture[4]. The increased demand for tilapia is reflected in increased waste that is generated during this process. Economically feasible ways to utilize this waste is to produce biogas and biofertilizers. The wastewater from tilapia breeding ponds after production mainly comprises feces, unconsumed food and bacterial biomass[5]. The conventional production system also produces residual sludge resulting from natural sedimentation. Alternatives have been proposed for the use of the wastewater: to irrigate and fertilize plants, and to be treated for reuse in greenhouses and hydroponic systems [6-11]. The production of fuels such as biogas, using mixtures of livestock manure and aquaculture waste [12-14] could be a clean, renewable, low-cost source of energy, which could replace conventional energy sources. The aim of this study was to determine the effect of the anaerobic co-digestion of manure with residual sludge from tilapia fish (*Oreochromis niloticus*, Linnaeus, 1758) breeding ponds in the production of solid residue. The potential of these solid residue to fertilize was also determined.

MATERIALS AND METHODS

Location of the experiment

The experimental phase of this work was carried out in the Comprehensive Agro-Food Exploitation Laboratory of the Institute of Agricultural Sciences (ICAP, acronym in Spanish) of the Autonomous University of the State of Hidalgo (UAEH, acronym in Spanish).

Obtaining raw material for the experiment

Fresh bovine manure was used, which was provided by the UAEH Ranch, it was kept cold 6 °C until starting the test. The organic solid residue was obtained from an aquaculture farm located in Tezontepec de Aldama, Hidalgo. Fresh residual mud was collected from ponds where tilapia is bred. Before the incubation of the mixtures, wastes in an isolated way were characterized. Proximal composition of residual sludge from Tilapia breeding ponds (RST) was total solids (%) 2.8±0.61, available phosphorus (mg mL^{-1}) 65.04±15.09, calcium (mg mL^{-1}) 595.75±13.7, potassium (mg mL^{-1}) 43.11±6.33, pH 5.61±0.30. Fresh bovine manure (BM) composition was 25.07±5.5 total solids (%), moisture (%) 72.89±12.11, C/N 16.85 pH 6.01±0.45.

Setting up of the experiment and Anaerobic digestion tests

The digestion tests were carried out in 75 mL glass jars at 35±2 °C. In each digester RST and BM were used as inoculum and substrate, both were mixed before being added

to the digesters. To induce an anaerobic environment, each digester was flushed for 5 min (280 mL/min) with inert gas (N_2). Three composting treatments were prepared as followed: a) BM only; b) RST only and c) BMRSM, a 1:1 (V/V) BM and RST mixture. Each treatment had seven replicates, and samples were collected in triplicate on each sampling day (0, 30, 60 and 90 days). The total soluble solids (organic solids) were adjusted to 15%. The composting process was carried out in incubation at 3 5±2 °C for 90 days until solid residue was formed. The initial parameter to determine the samples was the total solids, a balance between the inoculum and the substrate is considered crucial to achieve an optimal production of methane and biogas [15]. The moisture content established was 85%, recommended by [16]. The range of solids used in all tests did not require adding water to reduce the solids loading. Methane and CO_2 production, physicochemical and microbiological parameters during composting were determined. The solid residue obtained was then applied to the biological germination assay, which was subsequently evaluated.

Physicochemical and microbiological parameters during composting

On day 0, 30, 60 and 90 of the incubation, two jars from each treatment were selected at random to determined digestion. The organic samples collected were frozen until characterization. For the characterization and monitoring of the composted organic material samples, the chemical oxygen demand (COD), total solids (TS), total fixed solids (TFS), total volatile solids (TVS), the total organic phosphorus (TP) and nitrogen (TN) content. All the analyses were performed in triplicate.

The total nitrogen content was determined by the Kjendahl method, 0.5 g of sample was weighed on nitrogen-free paper, previously tared. After the sample was placed in the bottom of the Kjendahl flask, 2 g of acceleration mixture and 10 to 15 mL of concentrated sulfuric acid were added later. Then it was placed in the digester flask where it was heated until its complete oxidation points where the mixture turned transparent light green. After the digestion was completed, when the flask was cooled, 200 mL of distilled water was added to dissolve completely. The distillation apparatus was prepared, at the outlet of the refrigerant, a glass tube was adapted which remained immersed in 75 mL of 4% boric acid contained in a 500 mL erlenmeyer flask added with a few drops of wesslow indicator, then 5 mL of 40% NaOH was added to the kjendahl flask, slowly stratifying for each ml of sulfuric acid added during digestion, plus 10 mL of excess due to the possible carbonation of the soda. The distillation was carried out in a kjendahl apparatus, after recovering approximately 250 mL to ensure that all the ammonia has passed. It was titrated with 0.1N HCl solution (turn from green to violet).

For measurement of total calcium and potassium in the samples, 5 g of the samples were digested with 20 mL of concentrated HNO_3 and 10 mL of $HClO_4$, at 170 °C on a hot plate, until the fume changed to white. After cooling, the digested sample was filtered and diluted in the volumetric flask to 100 mL with distilled water. The solution was analyzed for Ca and K by using an atomic absorption spectrometer. For phosphorus determination, the solution was agitated with activated charcoal for 1.0 h and filtered to remove the color. Phosphorus in the solution was analyzed by a molybdate blue colorimetric method. For

extractable Ca, K, Mg and P determination, 5.0 g sample was weighed. A 20 mL mixture of 0.05 mol/L HCl and 0.0075 mol L^{-1} H_2SO_4 was added. After shaking (5 min), the suspension was passed through a filter paper and the filtrate was diluted in the volumetric flask to 100 mL with distilled water. Then, Ca, K, Mg, and P in the solution were analyzed as previously described following the methods described in Standard Methods for the Examination of Water & Wastewater [17]. For the pH measurement, a Thermo Orion pH meter, calibrated with buffer solutions with pH 4 and 7, was used. The sample was shaken for several minutes to obtain a homogeneous mixture, the electrode was introduced, and the reading was recorded once stabilized.

Determination of volatile fatty acids (VFA) by Capillary Electrophoresis

The determination of VFA was carried out in a capillary electrophoresis equipment (Coulter Beckman) with a 47 cm × 75 μm D.I. X 375 μm DE capillary, and an injection length detection of 40 cm. Nitrogen pressure in the capillary was 0.5 psi. The separation was carried out at constant capillary temperature and the UV detector reading was set at 214 nm. The buffer used was a solution of 10mM Benzoic Acid (C_6H_5-COOH), 10 mM Histidine and 1 mM Tetradecyl Trimethyl Ammonium Bromide (TTAB) and adjusting the pH to 6 with 1 M NaOH. A mixture of 50 mg kg^{-1} acetic acid, 50 mg kg^{-1} propionic and 50 mg kg^{-1} butyric acid (1:1:1) was used as a control. The type of VFA was identified based on the migration times of the areas of the chromatograms, and the calculations were performed depending on the concentrations of each of the components of the control [18].

Measuring biogas production

Biogas was collected dairy and measured by water displacement method. Biogas samples were examined by gas chromatography. The gas production was evaluated weekly during incubation. Methane gas was measured using a Perkin Elmer gas chromatograph. A 30 m long Elite Plot-Q capillary column (DVB Plot column) was used. Nitrogen was used as a carrier gas. The injection volume was 0.5 μL. The temperature of the detector was 200 °C, that of the injector 150 °C and that of the column 50 °C [19].

Detection of pathogenic bacteria during the composting process

Multiple tube fermentation tests were used to determine total and fecal coliforms according to the method used by [17]. Salmonella was determined as described by [17] and [20].

Alfalfa (*Medicago sativa* var San Miguel) seed germination assay with solid final residue

The experiment was conducted in green house conditions. Alfalfa seed germination and seedling emergence, sown in biosolids obtained from anaerobic digestion of substrates (BM, RST, BMRSM), were evaluated. Alfalfa seeds were sown in a 100 mm pots containing a sandy soil. The test pots were fertilized with 20 mL of the solid final residue while no solid final residue was added to the control pots. There were three replicates of each treatment.

Seedling emergence rates were recorded. After emergence, only 2 Alfalfa seedlings were kept in each pot. Alfalfa seedlings were watered once weekly. After 20 days, plant height as well as fresh and dry plant weights were measured.

Data analysis

A completely randomized experimental design was used, and a one-way analysis of variance was conducted by performing an analysis in triplicate (n=3). When there were differences of $P<0.05$, the Tukey mean comparison test was used. All data were analyzed using the NCSS 2001, version 5, software (Wireframe Graphics, Kaysville, UT, USA). All experiments were performed in triplicate.

RESULTS AND DISCUSSIONS

Anaerobic co-digestion is an alternative with important results, since it can improve gas production in the anaerobic digestion of solid waste. In this study, this method was used, because the lack of water during fermentation is a limitation, by itself the RST does not have the characteristics of richness of organic material, however it provides the system with high humidity due to the origin of this waste; the mineral richness of RST could activate the enzymatic systems of the microorganisms present in the BM, which functions at the same time as substrate and inoculum for fermentation The two wastes used to carry out the anaerobic co-digestion experiment for the production of biogas and a waste intended to be used as a fertilizer presented relatively high contents of TS and VS, which makes them usable waste in this type of process [21]. Both BM and RSTB residues present different proximal compositions, it is known that the organic fraction of bovine manure is rich in microorganisms [22], on the other hand, the residual sludge from tilapia ponds is rich in micronutrient contents which are found in assimilable forms. 3.1. Chemical Oxygen Demand (COD)

The change in COD in the treatments during the anaerobic digestion is shown in Table 1. This measurement was made as a follow-up to the activity during fermentation. The decrease of COD in the samples at the end of the experiment suggest degradation of organic matter during the composting process. These results coincide with those obtained by [23] in a study on anaerobic digestion; COD reduction was 28.2% after 10 days at temperature of 25 °C in an anaerobic reactor. The variations in COD in the treatments may be related to the chemical characteristics of the organic matter of each sample; degradation of these particles increase the COD [24].

Methane and carbon dioxide production

Figure 1 shows the results regarding the production of methane and CO_2. These results correspond to methanogenesis phase. The highest methane and the lowest CO_2 production were obtained in the BMRSM treatment up to day 60 with about 70% methane produced, but only 43% and 2% methane produced in BM and RST respectively. Methane production depends on the hydrolysis phase: soluble organic matter is produced during hydrolysis, which serves as the substrate in methanogenesis [25]. The formation of soluble organic matter and later methanogenesis were favored in samples from the BMRSM treatment.

Table 1. Chemical oxygen demand (COD) and pH time evolution (90-day trial) during the bio digestion process of bovine manure, residual sludge from tilapia fish aquaculture breeding ponds and 1:1 mixture of both.

Treatment	Time (days)							
	0		30		60		90	
	pH	COD (mg L^{-1})	pH	COD (mg L^{-1})	pH	COD (mg L^{-1})	pH	COD (mg L^{-1})
BM	5.92 ± 0.31^{aA}	90007 ± 310^{dC}	7.30 ± 0.13^{bA}	83795 ± 233^{cC}	7.35 ± 0.12^{bA}	80716 ± 222^{bC}	7.23 ± 0.11^{bA}	76079 ± 173^{aC}
RST	7.26 ± 0.12^{bC}	59193 ± 255^{dB}	7.34 ± 0.18^{bA}	5560 ± 210^{cB}	7.52 ± 0.18^{bA}	48067 ± 197^{bB}	7.75 ± 0.19^{bB}	42606 ± 202^{aB}
BMMR	6.7 ± 0.15^{aB}	51013 ± 184^{dA}	7.49 ± 0.12^{bA}	44853 ± 196^{cA}	7.56 ± 0.11^{bA}	40393 ± 183^{bA}	7.6 ± 0.16^{bB}	28973 ± 192^{aA}

The results are expressed as means standard deviation. Lowercase letters in the same row indicate significant differences ($p<0.05$) among treatments on different analysis day. Different capital letters in the same column indicate significant differences ($p<0.05$) among each treatment on the same analysis days.

The values are like those reported by 1 and 2 that showed results from several experiments, where modifications were made to some factors such as hydraulic retention time, load, type of substrate, pH, among others, obtaining biogas production with methane concentrations between 33-69%. After the bio-oxidative phase, the CO_2 concentration remained stable until the end of the process. This could have occurred because the easily biodegradable compounds were metabolized during the first stage of the process [26] additionally the low content of water content in the reactor results in a rapid accumulation of volatile fatty acids, especially for easily digestible raw materials that hinder the activity of methanogenic bacteria, leading to low biogas production [21].

Volatile Fatty Acids (VFA), Total Solids (TS), Total Volatile Solid (TVS), and Total Fixed Solids (TFS)

Table 2 shows the TS, TVS and TFS content in the different samples. Table 2 shows that of the TS, most were quantified as SV. The initial values of TS and VS of the wastes and the mixtures could be considered as high contents, therefore, its use is attractive for anaerobic

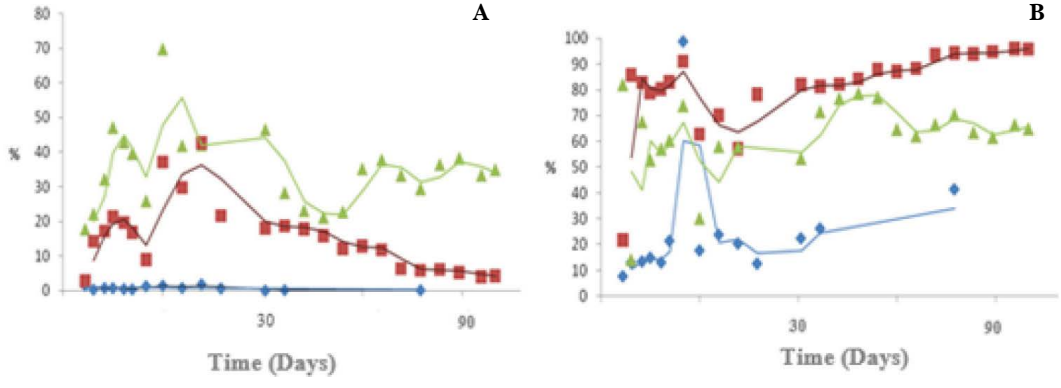

Figure 1. Percentage of (A) methane and B) CO_2 from anaerobic digestion BM, bovine manure (■), RST (residual sludge from tilapia fish Breeding ponds (◆), and BMMR mixture 1:1 BM-RST (▲). Data are the mean of seven jars×three experiments×three measurements by each day of collection. Whole experiment lasted 90 days.

Table 2. Evolution of total solids (TS), total fixed solids (TFS), and total volatile solids (VTS), volatile fatty acids (VFA) and percentages of macronutrients (N, P, C, C/N) during the anaerobic digestion process of substrates.

Time (days)	VFA			Solids			Biosolid composition			
	AA	AP	AB	TS	TFS	VTS	N	P	C	C/N
	$mg{\cdot}L^{-1}$			$g{\cdot}L^{-1}$			%			
BM										
0	2770 ± 320^{b}	960 ± 88^{c}	119.3 ± 21	9.32 ± 0.22^{c}	2.38 ± 0.07^{a}	6.94 ± 0.12^{d}	0.25 ± 0.02^{b}	0.13 ± 0.04^{a}	4.02 ± 0.04^{d}	16.1 ± 0.11^{c}
30	2808 ± 220^{b}	854 ± 71^{c}	ND	8.75 ± 0.13^{b}	3.16 ± 0.09^{b}	5.59 ± 0.20^{c}	0.24 ± 0.03^{ab}	0.12 ± 0.07^{a}	3.24 ± 0.06^{c}	13.5 ± 0.05^{b}
60	2456 ± 174^{ab}	617 ± 53^{b}	ND	8.54 ± 0.25^{ab}	4.13 ± 0.05^{c}	4.41 ± 0.24^{b}	0.22 ± 0.01^{a}	0.12 ± 0.02^{a}	2.56 ± 0.07^{b}	11.6 ± 0.10^{ab}
90	1560 ± 135^{a}	432 ± 31^{a}	ND	8.23 ± 0.23^{a}	4.29 ± 0.06^{c}	3.94 ± 0.15^{a}	0.20 ± 0.03^{a}	0.11 ± 0.09^{a}	2.12 ± 0.10^{a}	10.6 ± 0.09^{a}
RST										
0	420 ± 63^{c}	370 ± 39^{b}	ND	4.43 ± 0.11^{b}	3.78 ± 0.22^{a}	0.65 ± 0.07^{b}	0.06 ± 0.009^{c}	0.04 ± 0.009^{b}	0.38 ± 0.01^{b}	6.3 ± 0.01^{b}
30	$199\pm.35^{b}$	127 ± 38^{a}	ND	4.15 ± 0.12^{a}	3.81 ± 0.15^{a}	0.34 ± 0.11^{a}	0.05 ± 0.003^{bc}	0.03 ± 0.001^{ab}	0.21 ± 0.09^{ab}	4.2 ± 0.08^{a}
60	55 ± 12^{a}	ND	ND	4.10 ± 0.19^{a}	3.85 ± 0.19^{a}	0.25 ± 0.09^{a}	0.04 ± 0.003^{b}	0.03 ± 0.003^{ab}	0.15 ± 0.08^{a}	3.8 ± 0.02^{a}
90	ND	ND	ND	4.05 ± 0.1^{a}	3.84 ± 0.13^{a}	0.21 ± 0.08^{a}	0.03 ± 0.004^{a}	0.02 ± 0.001^{a}	0.13 ± 0.01^{a}	4.3 ± 0.03^{a}
BMMR										
0	11755 ± 21^{d}	434 ± 57^{b}	ND	7.89 ± 0.17^{d}	4.08 ± 0.19^{a}	3.81 ± 0.08^{d}	0.15 ± 0.02^{a}	0.09 ± 0.001^{b}	2.21 ± 0.01^{d}	14.7 ± 0.18^{d}
30	859 ± 96^{c}	165 ± 12^{a}	ND	7.01 ± 0.23^{c}	4.67 ± 0.18^{b}	2.34 ± 0.12^{c}	0.12 ± 0.03^{a}	0.08 ± 0.008^{ab}	1.32 ± 0.03^{c}	11.0 ± 0.13^{c}
60	659 ± 68^{b}	ND	ND	6.35 ± 0.16^{b}	4.77 ± 0.17^{b}	1.58 ± 0.15^{b}	0.11 ± 0.04^{a}	0.07 ± 0.002^{a}	0.91 ± 0.09^{b}	8.3 ± 0.25^{b}
90	318 ± 17^{a}	ND	ND	6.02 ± 0.14^{a}	4.78 ± 0.09^{b}	1.24 ± 0.19^{a}	0.13 ± 0.03^{a}	0.06 ± 0.007^{a}	0.75 ± 0.05^{a}	5.8 ± 0.19^{a}

BM Bovine manure only, RST tilapia breeding ponds residual sludge, MBMR 1:1 mixture of MB and RST, TS-Total solids, TFS-Total fixed solids, VTS-Volatile total solids, VFA-Volatile fatty acids, AA- Acetic acid, AP-Propionic acid, AB-Butyric acid, ND- No detected, N (nitrogen), P (phosphorus), C (carbon), C/N (Carbon/nitrogen relation). Data are the mean of seven jars × three experiments × three measurements by each day of collection. Whole experiment lasted 90 days. [a, b, c] Different letters in columns means statistically different values by Tukey test ($P<0.05$).

co-digestion tests for biogas production [27]. The BM samples had higher concentrations of TS, TVS and TFS compared to other treatments. The high concentration of TS in BM samples was a consequence of TVS and TFS accumulation.

According to [1] TS concentration was 15.6% and 59.5% in bovine and llama manures, respectively, while TVS concentrations were 82.8% and 74.4%. In a previous study, the characteristics of anaerobic digestion of pig manure from different growth stages were investigated. According to growth stage, batch experiments were performed using gestating sow manure (GSM), swine nursery with post-weaned piglet manure (SNM), growing fattening manure (GFM) and mixed manure (MM) as substrates at four substrate concentrations (40, 50, 65 and 80 gVS/L) under mesophilic conditions, the volatile fatty acids/total inorganic carbon (VFA/TIC) ratio increased from 0.10 to 0.89 when loading increased from 40 to 80 gVS/L for GFM [28]. At the beginning of this study, TS was 9.32% in BM, 4.43% in RST and 7.89% in BMRSM. However, the amount TS at the end of the study decreased due to the decomposition of organic matter, which was transformed into methane and CO_2.

According to previous reports, the accumulation of VFA caused a decrease in pH [25]. This contrasts with our results; the pH during digestion remained practically unchanged. An anaerobic digestion process that is carried out efficiently should have a concentration of VFA less than 2000 mg/L [29]. It is normally assumed that part of the hydrolyzed organic matter is converted to VFA and if the concentration of VFA is not high enough to exceed the buffer capacity of the system, the pH remains unchanged, subsequently allowing for methanogenesis[30]. Acetic and propionic acid could be quantified; their concentration increases in the acid-genesis stage then decreases during methanogenesis.

Nitrogen content during digestion

The nitrogen content in BM reported as the minimum necessary for the incorporation into the cell structure of methanogenic bacteria is 0.6% [1]. Conversely, an excess of nitrogen in the substrate leads to the formation of ammonia and lead to decreased methane production since ammonia can alter the pH. The soluble forms of nitrogen are immediately assimilated [1] and [2] the insoluble forms are solubilized before being use by microorganisms during hydrolysis and fermentation [31]. Methanogenic activity decreases with increasing nitrogen concentration[32]. The nitrogen concentration in the BMRSM treatment was highly stable throughout the phases; meanwhile, nitrogen concentration was 20% higher in BM than in BMRSM and RST (Table 2), both having TN content similar to those reported by [1]. In a study carried out by [26] using horse manure mixed with garden waste as substrate, nitrogen content decreased during the first 90 days of the digestion process.

Phosphorus

Phosphorus must be conserved until the end of the co-digestion process since it is an essential nutrient in organic fertilizers. The phosphorus concentration in the BM treatment was 50% higher compared to the BMRSM treatment and around 90% higher

than the RST treatment. The percentage of phosphorus remained practically unchanged throughout the digestion process, being 1.22% in BM, 0.10% in RST and around 0.43% in BMRSM (Table 2).

Carbon content and carbon/nitrogen relation

The carbon content and the relation with nitrogen are important factors in soil since both are crucial elements for the survival of microorganisms. For example, bacteria are known to require 25 to 30 times more carbon than nitrogen. In the assimilation process of this nitrogen compounds part of the C is oxidized to CO_2, so that the concentration of C in the reactor decreases when bacteria recover the nitrogen, then the digestion can continue, but the overall process will be much slower than if the organic material used had a more adequate C/N ratio. If the ratio is low, the C will be depleted before the N, causing the fermentation process to stop and later the material will lose the remaining N [30]. In the case of [26] they found that the C/N ratio showed a slight increase during the first few days of the digestion process, followed by a decrease from 36.7 to 25.8 over a period of 190 days. According to [28] the inhibition of ammonia nitrogen (AN) is a common issue in anaerobic digestion of animal manure and carbon-nitrogen ratio (C/N) is important for the activity of anaerobic microorganisms and performance of anaerobic digestion process, in fact low C/N ratio has the potential risk of leading to ammonia nitrogen inhibition. In this study, the C/N ratio during the co-digestion process showed a significant decrease at the end of the process as reported in another research. Low C/Nm ratio of bovine manure facilitates the balancing of the C/N ratio during fermentation in bioreactors [33].

Microbiological analysis

As can be seen in Table 3, RST had a higher fecal coliform (CF) content compared to BM at the beginning of the composting process. The amount of *Salmonella* detected in all the residues at the beginning was greater than 100 CFU/g. The anaerobic digestion resulted in a 99% elimination of both pathogenic microorganisms. However, composting tilapia residues was very effective in the reduction of fecal coliforms and *Salmonella*. The other two treatments were inefficient in the total inactivation of these bacteria during the co-digestion process. Temperature has been found to be the principal factor that

Table 3. Microbiological profile of the substrates during anaerobic digestion.

Time (days)	BM		RST		BMMR	
	Salmonella	**Faecal coliforms**	***Salmonella***	**Faecal coliforms**	***Salmonella***	**Faecal coliforms**
	CFU/100mL					
0	$11\times10^{3}\pm563^{b}$	$18\times10^{6}\pm773^{d}$	320±245	$21\times10^{4}\pm670^{c}$	$23\times10^{2}\pm321^{b}$	$97\times10^{4}\pm7890^{d}$
30	$40\times10^{2}\pm329^{a}$	$74\times10^{5}\pm512^{c}$	ND	$11\times10^{3}\pm463^{b}$	310 ± 78^{a}	$19\times10^{3}\pm328^{c}$
60	$35\times10^{2}\pm477^{a}$	$30\times10^{4}\pm396^{b}$	ND	$77\times10^{2}\pm378^{a}$	ND	$54\times10^{2}\pm345^{b}$
90	ND	$21\times10^{3}\pm129^{a}$	ND	ND	ND	$11\times10^{2}\pm129^{a}$

BM Bovine manure only, RST tilapia breeding ponds residual sludge, MBMR 1:1 mixture of MB and RST. CFU/mL, colony forming units by milliliter. Data are the mean of seven jars × three experiments × three measurements by each day of collection. Whole experiment lasted 180 days.

determines the inactivation of pathogens during anaerobic digestion [31]. In a study by [32] they determined that an effective reduction of *E. coli* in anaerobic digestion of bovine manure required approximately 60 days at 25 °C and 34 days at 37 °C. Similarly, [34] studied anaerobic co-digestion of swine manure. They observed a significant reduction of pathogens as like *Salmonella enterica* and *Escherichia coli* at 24 °C or 37 °C for 30 days. In contrast, [35] found that after mesophilic anaerobic digestion of dairy manure, pathogens were not completely eliminated and can pose a biosecurity risk. Understanding pathogens behavior in the transformation of manure residues is important to minimize the possible transmission of hazardous microorganisms in crops [36].

Evaluation of the solid residue obtained

The effects of adding sludges from the different treatments to soils on Alfalfa seed germination and growth are presented in Table 4. The samples with sludges showed low seed germination rates, which is probably attributed to the decrease in oxygen tension due to the addition of the solid organic matter. No negative effects were observed regarding the growth parameters of Alfalfa. The height of the plants was significantly higher when BM was applied. It is probable that the microbial community in these samples can promote plant growth [37]. There were no significant differences between the fresh and dry weight of the plants grown in soil to which BM and BMRSM sludges were added. Ash content was higher in plants grown in soil with BMRSM than with RST. The length of the roots was longer when RST was applied compared to the control. Alfalfa germination and seedling growth was significantly improved by the application of sludges [38].

CONCLUSIONS

The results of this study show that using the mixture of bovine manure and residual sludge from tilapia fish breeding ponds (1: 1) yielded a higher production of methane and a lower production of CO_2, which occurred during the first 60 days of the co-digestion process. At the end of the co-digestion process, the stabilization of organic matter was achieved. There was a reduction of more than 99% in the content of pathogenic bacteria in the sludges formed at the end of the co-digestion process.

Table 4. Evaluation of different substrates after anaerobic digestion, on germination of alfalfa (*Medicago sativa* var San Miguel) growth for 20 days.

Treatments	SE	PH (cm)	FW (g)	DW (g)	Ash (mg)	RL (cm
Control	1.04 ± 0.24^{a}	3.16 ± 0.22^{ab}	2.08 ± 0.37^{a}	1.06 ± 0.38^{a}	1.37 ± 0.33^{ab}	1.21 ± 0.16^{b}
BM	0.842 ± 0.15^{a}	2.48 ± 0.19^{a}	3.35 ± 0.25^{b}	2.15 ± 0.36^{b}	1.71 ± 0.24^{b}	0.75 ± 0.26^{a}
RST	0.774 ± 0.19^{a}	3.47 ± 0.17^{b}	2.11 ± 0.56^{a}	1.03 ± 0.23^{a}	1.54 ± 0.18^{b}	1.17 ± 0.30^{b}
BMMR	0.832 ± 0.23^{a}	2.54 ± 0.26^{a}	3.42 ± 0.46^{b}	1.37 ± 0.16^{a}	1.11 ± 0.27^{a}	0.67 ± 0.22^{a}

BM Bovine manure only, RST tilapia breeding ponds residual sludge, MBMR 1:1 mixture of MB and RST. Seedling emergence (plants per day), % Plant height (cm) Fresh weight (g) Dry weight (g), Root length (cm). Data are the mean of 90 plants (three experiments × three measurements) by each treatment. Whole experiment lasted 20 days. $^{a, b, c}$ Different letters mean statistically different values by Tukey test ($P<0.05$).

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Weed control with small ruminants: Exploratory evaluation on *Senecio inaequidens*

López-González, Felipe[1]; Sánchez-Valdés, Jair Jesús[1*]; de Jesús-Sebastián, Omar[2]; Sainz-Ramírez, Aurora[1]; Martínez-García, Carlos Galdino[1]; Arriaga-Jordán, Carlos Manuel[1]

[1] Instituto de Ciencias Agropecuarias y Rurales.
[2] Facultad de Medicina Veterinaria y Zootecnia.
Universidad Autónoma del Estado de México, Campus UAEM El Cerrillo. El Cerrillo Piedras Blancas, Toluca, Estado de México, México, C.P. 50090.
* Correspondence: jaircio2000@yahoo.com.mx

ABSTRACT

Objective: Carry out an exploratory evaluation of sheep and goats grazing in a temperate pasture invaded by *Senecio inaequidens*, as a means of biological control of weeds in small-scale dairy systems (SSDS).

Methodology: Crude protein (CP), neutral detergent fibre (NDF), and acid detergent fibre (ADF) were determined on pasture and *Senecio* samples. The effect of grazing by sheep and goats on the pasture was assessed by means of the height, density and soil cover of *Senecio*, and also sward height; and the live weight of the animals, daily weight gain and body condition score were recorded. Statistical analysis was by a 2×2 factorial design, with species (sheep or goat) and two grazing intensities at 28.3 or 50.3 m^2/animal per day.

Results: height, coverage and density of *Senecio* did not show significant differences ($P>0.05$), neither did sward height. The chemical composition of the pasture did not show statistically significant differences for the assessed periods either. Animal weight, body condition and daily weight gain did not show significant differences among the evaluated treatments ($P>0.05$).

Implications: This is the first report evaluating sheep and goat grazing to control of *Senecio* invasion in temperate pasture in central Mexico. In addition, knowing the chemical quality of *Senecio* will allow decisions to be made for supplementation in grazing systems with sheep or goats.

Conclusions: The grazing of sheep and goats reduced the number of *Senecio* plants in the assessed pasture, which indicates the possibility of controlling this weed by grazing sheep or goats over longer-term grazing.

Keywords: *Senecio inaequidens*; weed control; grazing; sheep; goats.

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INTRODUCTION

Cow feed in small-scale dairy systems (SSDS) is based on pastures cultivated for grazing, avoiding cut and carry (Pincay-Figueroa *et al.*, 2016); however, such pastures face the invasion of species that are considered weeds, which directly compete for space and nutrients, making the pasture less persistent, thereby the animals have less forage available to feed on (Gardner *et al.*, 2006).

Image by Alexander Lesnitsky at Pixabay

One such invasive species is *Senecio inaequidens* DC, which comes from North Africa. It contains pyrrolizidine alkaloids that are toxic for livestock and human beings (Scherber *et al.*, 2003). The way it was introduced into Mexico is not known, though it has become a great problem for cultivated pastures. In the municipality of Amealco, state of Queretaro, Mexico, it is an extremely abundant plant; although it was collected for the first time in 1990, the local population has known it for more than 50 years (Rzedowski *et al.*, 2003). To control or eliminate invasive species, different physical measures are undertaken such as controlled slash and burn, chemical or rudimentary biologic controls with herbicides and insects, as well as grazing management (Firn *et al.*, 2013). Measures to combat *Senecio* include herbicide application and goat and sheep grazing (Rzedowski *et al.*, 2003).

Different animal species have different grazing behaviours. Sheep and cows prefer grazing on herbaceous, whereas goats prefer more mature growths such as ears and also graze woody species such as heathers and gorses, among others (Osoro *et al.*, 2000); goats as compared to other ruminants are more tolerant to tannins and alkaloids, and prefer shrub by plants when herbaceous species are in dry vegetative rest (Azócar, 1987).

In dairy production systems, attempts have been made to control *Senecio* invasions, so far however, this plant has neither been eradicated nor controlled. Although in South America (Brazil, Argentina and Uruguay) there are works on the control of this weed, in Mexico few are the works reported on this species. In this way, the goal of the present work was to carry out an exploratory evaluation by means of setting up sheep and goat grazing, in a temperate pasture invaded by *Senecio inaequidens*, as a means of biological control of weeds in SSDS, and determine the chemical composition of *Senecio* and pasture.

MATERIALS AND METHODS

Experimental site

The study was carried out in January and February 2020, in a small-scale dairy farm in the municipality of Aculco (20° 06' N and 99° 50' W), at an altitude of 2440 m. Climate is temperate subhumid, with minimal temperatures below zero in winter, and mean maximum temperatures of 24 °C, and around 800 mm rainfall (Plata-Reyes *et al.*, 2021).

In December 2018, a pasture of 7844 m^2 was established with ryegrass, associated with red clover and white clover, at a seeding rate of 30 kg ryegrass, 8 kg/hared clover, and 3 kg/ha white clover. Fertilization at seeding was 160 kg/ha diammonium phosphate, 120 kg/ha potassium chloride; plus, 50 kg urea/ha were applied every four weeks. About a year after establishing the pasture, it was invaded by *Senecio inaequidens*, nowadays it has almost disappeared due to the presence of this invasive plant.

The animals used were 4 sheep and 4 crossbreed goats, 2 years of age, weighing between 20 and 30 kg, with a body condition (BC) score of 2.6 for sheep and 2.5 for goats.

Sheep and goats were tethered and had access to a grazing surface of pasture and *S. inaequidens* (28.27 m^2 and 50.26 m^2, respectively). The following treatments were evaluated: T1=goat+50.26 m^2; T2=sheep+50.26 m^2; T3=goat+28.27 m^2; T4=sheep+28.27 m^2; animals were not supplemented and had *ad libitum* access to water on buckets for each one.

Pasture variables

Senecio inaequidens density in pasture was measure using a 0.5×0.5 m quadrant (0.25 m^2), estimating the number of plants/m^2 according to Ibarra-Flores *et al.* (1999), 4 measurements were taken for each sub-pasture. Sward height was according to Hodgson (1994), measurements were taken following a "W" pattern every 20 steps, covering the total area of each sub-pasture. To measure the height of *S. inaequidens*, the Santa Cruz method for the height of the key species was resorted to (Borrelli and Oliva, 2001), over a transect in each sub-pasture, every 10 steps, the first *Senecio* plant closest to the foot in front was located and three plants were counted; these were evaluated with a 1-metre rule, measuring from the ground up to the leaf modal level, when it was noticed that the plant had been eaten unevenly, the height of long and short leaves was averaged.

S. inaequidens coverage was with the point method (Levy and Madden, 1933), drawing a transect with a 50-metre rope at random in each sub-pasture. The rope was divided in 5 groups of 10 points each, all separated 1.0 m from one another, plants in touch with the points above were counted, considering that if a point did not touch a plant, it was not counted, but was assessed. Coverage was evaluated with the percentage of the points of touch of *Senecio* plant in relation with the total points.

Simulated grazing samples, weighing about 50.0 g DM, were taken every day the pasture was measured; as well, samples of *S. inaequidens* were obtained, taking notice of what parts of the plants were eaten by sheep or goats.

Animal variables

Live weight (kg) and body condition (5-point scale) were registered every 8 days, at the end of each evaluation period; moreover, to evaluate daily weight gain, a linear regression was applied.

Chemical analyses of forages

The samples were dried in a forced draft oven at 60 °C, up to constant weight to ascertain dry matter (DM). Nitrogen in samples was by the Kjeldahl method (AOAC, 1990); the results were multiplied by a factor of 6.25 (AFRC, 1993) to calculate crude protein (CP) content. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were by ANKOM following Van Soest *et al.* (1991). *In vitro* dry matter digestibility (IVDMD) for the pasture and *S. inaequidens* was according to the equation by Jerenyama and García, (2004): $IVDMD = 88.9 - (0.779 * ADF\%)$.

Statistical analysis

A 2×2 factorial design was utilised (Lawal, 2014) with the following mathematic model:

$$Y_{ijk} = \mu + B_i + T_j + BT_{ij} + e_{ijk}$$

Where: Y_{ijk}=response variable; μ=overall mean; B_i=effect of animal species, T_j=effect of forage availability; BT_{ij}=effect of the interaction between animal species and forage availability; e_{ijk}=experimental error. With these data, a variance analysis was run with

Minitab V 14 (2003); in case of significant differences between treatments, a Tukey test was applied at a significance level ($P<0.05$). Descriptive statistics was used to report crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), and *in vitro* dry matter digestibility (IVDMD).

RESULTS AND DISCUSSION

Table 1 shows results for live weight and body condition. No significant differences were noticed ($P>0.05$) among treatments for daily weight gain. Although no significant differences are observed in the variable of daily weight gain, a 20% more weight gain is observed in goats compared to sheep, which is also reflected in the weight of the animals, as well same in body condition.

Live weight for sheep and goats (Table 1), which is higher for goats, indicates that the hardiness of goats helps them to consume not very palatable foods such as *Senecio*. Santra and Karim, (2001) found that goats are more efficient to digest cellular walls in comparison with sheep. These results are in line with Wilson and Mulham (1980), who reported higher weight gain by goats versus sheep in mixed grazing in arid forest, dominated by belah (*Casuarina cristata*) coverage and other trees, shrubs, and herbage.

Animals had the same body condition during the experiment, which suggest that pasture and *Senecio* covered the requirements of these animals. These results diverge from García *et al*. (2005), for sheep and goat mixed grazing in heathlands-shrublands, burnt beforehand, and previously grazed by sheep, in Asturias, Spain, 2004, the authors observed BC losses of 0.54 for sheep, while 0.66 for goats.

Considering the condition of the pasture infested by *S. inaequidens*, the fact that sheep and goats had gained a little weight may be because the pasture they grazed had good

Table 1. Live weight and body condition of sheep and goats over all the experimental periods.

Surface (m^2)	Animal	Weight (kg)	Mean	SEM	P-value
28.27	Sheep	28.80	29.72	1.85	0.25
50.26	Sheep	28.75			
28.27	Goat	30.85			
50.26	Goat	30.05			
Body condition (1-5)					
28.27	Sheep	2.60	2.63	0.19	0.78
50.26	Sheep	2.55			
28.27	Goat	2.75			
50.26	Goat	2.65			
DWG (kg)					
28.27	Sheep	0.028	0.030	0.004	0.62
50.26	Sheep	0.026			
28.27	Goat	0.033			
50.26	Goat	0.035			

SEM: Standard error of the mean; BC: Body condition; DWG: Daily weight gain; * Interaction between animals and surface was not representative.

Table 2. Variables of the pasture invaded by *Senecio inaequidens*.

Surface (m^2)	Animal	Height of Senecio	Mean	SEM_{as}	P value
28.27	Sheep	28.27	28.72	1.85	0.25
50.26	Sheep	29.17			
28.27	Goat	31.63	31.01		
50.26	Goat	30.40			
SEM_S		2.78			
Density					
28.27	Sheep	16.56	16.99	2.42	0.64
50.26	Sheep	17.43			
28.27	Goat	16.25	15.90		
50.26	Goat	15.56			
SEM_S		3.96			
Coverage					
28.27	Sheep	48.75	47.99	2.21	0.88
50.26	Sheep	47.23			
28.27	Goat	44.26	44.39		
50.26	Goat	44.52			
SEM_S		1.41			
Sward height					
28.27	Sheep	1.88	1.81	0.52	0.33
50.26	Sheep	1.75			
28.27	Goat	2.04	2.06		
50.26	Goat	2.08			
SEM_S		0.77			

SEMas=Standard error of the mean for animal species; SEMS=Standard error of the mean for grazing surface; *Interaction between animals and surface was not representative.

quality, adding to good dry matter digestibility, as noticed in Table 3; furthermore, *Senecio* contains tannins, which in moderate amounts help to better use the proteins in feed. In this regard, Bonilla-Valverde *et al.* (2017) found an increase in the daily weight gain of sheep by adding tannin extract to the feed.

Table 2 shows results for variables of the pasture invaded by *S. inaequidens*. The height, density and coverage of *Senecio* did not show significant differences among treatments, interaction was not significant either ($P>0.05$). The height of the *Senecio* was higher on the surface of the goats, without presenting significant differences ($P>0.05$) with respect to the surface of the sheep. However, there was less Senecio coverage on the surface where goats grazed, compared to sheep, although without significant differences ($P>0.05$). The height of the pasture was greater in the area grazed by goats compared to the area grazed by sheep ($P>0.05$).

The height of *Senecio* is a marker for the consumption of the animals; a considerable height is noticed, which may point out that the animals preferred pasture over *Senecio*. The

mean of the four treatments at the end of the experiment is within the range from 15 to 70 cm in height mentioned by Rzedowski *et al.* (2003). *Senecio inaequidens* has a great spread capacity (CONABIO, 2016), which makes this plant have good density in the pasture under assessment (16.86 plants/m^2), concurring with Sindel and Coleman (2012), who report that one plant of *Senecio madagascariensis* might produce from a few to hundreds of new plants when cows graze the pastures, since they scatter the *Senecio* seeds; this situation was also noticed in this experiment. Besides, it was observed that wind is a factor that contributes to the spread of the plant. This relates with the density of the plants present in the pasture on average. Added to the grazing pressure of the animals, it causes the *Senecio* to disperse quickly and there are risks of intoxication in the animals (Rissi *et al.* 2007).

As for the coverage of *Senecio* at the end of the experiment (Table 2), it covers about 50% of 1.0 m^2, which indicates that the amount of *Senecio* plants in the pasture was higher than what animals were able to consume, adding to this, the duration of the experiment was relatively short. The coverage results of this investigation were lower than Jáuregui *et al.* (2009), who evaluated the coverage of heather, a weed similar to *S. inaequidens*, after the 3-year experiment heather coverage was 36.2% in pastures grazed by sheep, and 31.8% in pastures grazed by goats, which suggests that time and liberty to graze influence on the species coverage. In an experiment with sheep to control *Senecio*, Bandarra *et al.* (2012) found a drop from 1622 Senecio plants on a 2700-square-metre surface to 0 plants/m^2; this is because these authors' experiment lasted about three years.

In this experiment, sward height is below Hodgson's (1994) recommendation, *i.e.*, sward height must be between 5.0 and 8.0 cm so that animals graze well and their voluntary consumption is not restricted. Short sward height may be due to the competence between the gramineous plant with *Senecio*; this competence depends on the dynamics of the plants under study and on the grazing of goats, which help to control shrubby and woody species (Jáuregui *et al.*, 2009).

The chemical characteristics of pasture and *Senecio inaequidens* are displayed in Table 3. The pasture CP content over the assessment period was 160.2 g/kg DM; NDF was 446.4 g/kg DM, ADF 216.8 g/kg DM and IVDMD 720.05 g/kg DM.

There is an inverse relationship between CP and the number of structural carbohydrates (Table 3); this may be because the contents of CP and structural carbohydrates change

Table 3. Chemical composition pasture and Senecio inaequidens (g kg^{-1} DM) grazed by sheep and goats.

Period	Pasture			IVDMD	Senecio inaequidens			IVDMD
	CP	NDF	ADF		CP	NDF	ADF	
1	162.3	422.6	210.2	725.2	78.4	762.4	432.5	552.1
2	156.5	446.2	218.4	718.8	78.6	766.2	423.7	558.5
3	163.4	454.6	216.2	720.5	77.4	778.4	428.4	555.2
4	158.6	462.4	222.4	715.7	76.4	782.6	438.2	547.6
Mean	160.2	446.4	216.8	720.05	77.7	772.4	430.7	553.3
SD	3.21	17.22	5.09	34.3	1.01	9.63	6.16	41.5

CP: Crude protein; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; IVDMD: *In vitro* dry matter digestibility; SD: Standard deviation.

over time and such changes are attributed to the ripeness of the plant and the increase in stem ratio (Ammar *et al.*, 2004). CP in pasture is different from Martínez-Hernández *et al.* (2019), who report 102.9 g/kg DM, and from Plata-Pérez *et al.* (2020), for pastures cultivated with annual ryegrass, winter vetch and common vetch (99.3 g kg^{-1} DM).

The chemical quality of *S. inaequidens* (Table 3) is lower than that of tropical grasses. CP content over the assessment period was 77.7±1.01 g kg^{-1} DM; NDF was 772.4 g kg^{-1} DM, ADF 430.7 g kg^{-1} DM and IVDMD 553.3 g kg^{-1} DM. The above suggests that as a forage *Senecio* has poor quality (López-González *et al.*, 2020), in addition to the toxicant agents it has (pyrrolizidine alkaloids), which may severely harm liver, mainly in cows; *Senecio* is a grave problem for pastures in dairy production systems.

CONCLUSIONS

Sheep and goat grazing reduced the number of individuals of *Senecio inaequidens* in the evaluated pasture without noticing significant differences. Assessing the effects over longer periods of time is necessary.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Artisanal extraction of native chili seeds (*Capsicum* sp.)

Crescencio de la Cruz Castillo Aguilar[1], Domingo Coh Méndez[1], Eugenio Carrillo[1], Ricardo Antonio Chiquini Medina[2*]

[1] Colegio de Postgraduados, Campus Campeche. Carretera Haltunchén-Edzná km 17.5, C. P. 24400 Sihochac, Champotón, Campeche, México.
[2] Instituto Tecnológico de Chiná. Calle 11 S/N entre 22 y 28 Colonia Centro, 24520 Chiná, Campeche, México.
* Correspondence: ricardochiquini@yahoo.com.mx

ABSTRACT

Objective: To establish a methodology for the artisanal extraction and benefit of chili pepper seed from native and wild chili pepper varieties.

Design/Methodology/Approach: From 2016 to 2019, nine chili pepper ecotypes were evaluated. The ecotypes include: habanero (*Capsicum chinense* Jacq); Ixcat ik, Bobo, Sweet, Maax, Bolita, Green (with red ripening), green (with yellow ripening), all of which belong to the *Capsicum annuum*; and Pico Paloma (*Capsicum frutescens*). The aim of the evaluations was to determine the morphological characteristics and the degree of genetic diversity. The extraction and exploitation method of the seeds was also evaluated. Cutting knives were used to extract the seeds. The seeds were dried in the open air, sheltered in closed and ventilated places. Both glass and plastic containers were used to store the seeds; the jars were lined with kitchen foil.

Results: Two seed extraction procedures were used. Medium and large ecotype fruits were subject to circular and longitudinal cuts. For their part, small fruits were macerated. In order to dry the seeds, they were placed on metal trays or in perforated plastic baskets. The trays or bags were placed in ventilated and cool places (<40 °C) for 8 to 10 days. Afterwards, they were stored at 5 °C with low moisture and without light.

Findings/Conclusions: The proposed methodology to obtain and exploit seeds in humid tropic conditions will support the conservation of the *Capsicum* resource, in the state of Campeche and the Yucatan Peninsula.

Keywords: Extraction, artisanal, native varieties of chili, wild chili peppers.

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INTRODUCTION

The Yucatan Peninsula is an important region for the diversification of chili (*Capsicum* spp.) species (Morales-Soriano *et al.*, 2018); consequently, their conservation is fundamental, given the economic and cultural importance that chili has for the rural communities. Native and wild chili species can be found in rural areas. In many backyards and *milpas* of the Yucatan Peninsula, there are wild chili peppers populations. The seeds of these chili plants have been spread by birds, sometimes from the environment, sometimes from other backyards and *milpas*, and they grow without human intervention. These plants are tended and used by local farmers, who harvest their fruits (Jardon-Borbolla, 2017).

Image by Ursula at Pixabay

Given the importance of the *Capsicum* genera conservation as a native regional resource, the seed must be considered as a basic link in the production and reproduction of native species. Additionally, it plays a key role in the conservation of genetic resources. Commercially speaking, although introduced seeds are used, many producers in rural communities sow their own chili plants, extracting the seeds of the fruits of plants grown in small plots or backyards. Regarding wild chili species, producers extract the seeds from the fruits they harvest.

Therefore, the objective of this study was to provide chili producers with knowledge that allows them to improve how they select and conserve seeds. This study includes recommendations that can be applied by the producers based on the evaluations carried out in the research.

MATERIALS AND METHODS

From 2016 to 2019, nine chili pepper ecotypes were morphologically evaluated. The varieties were: habanero (*Capsicum chinense* Jacq); Ixcat ik, Bobo, Sweet, Maax, Bolita, Green (with red ripening), and green (with yellow ripening), all of which belong to the *Capsicum annuum* variety; and Pico Paloma (*Capsicum frutescens*). They were collected in the rural areas of Calkiní, Campeche, Champotón, and Palizada. Additional to the morphological evaluation, an artisanal seed extraction method was established, taking into account the color and size of the fruits, as well as the extraction, drying, and storage of the seeds. The experimental design used in the study of the chili ecotype morphologies consisted of random blocks, with three repetitions, 1.5-m long furrows, and 0.25 m between plants. Taking into account the abovementioned arrangement, seed samples were collected from 10 plants per repetition. The seed was extracted from the best fruits, which were chosen according to their size and color. Crop management was carried out according to the recommendations proposed by Soria *et al.* (2002).

RESULTS AND DISCUSSION

The results of the research carried out in four sowing cycles in order to evaluate the morphology and genetic diversity of native and wild chilis, allowed us to determine the artisanal seed extraction method in the humid tropic. This method took into account the different fruit selection stages: seed extraction, seed drying and exploitation, and seed storage.

Selecting fruit for seed extraction

The artisanal extraction of the seed begins with the selection of ripening fruits from healthy and pest-free plants. This selection is carried out using both native varieties grown by the farmers and wild chili varieties.

The results showed that the best fruits based on their germination capacity (90%) were those that reached their physical maturity. Red chilies prevailed, although the final coloring included yellow, orange, brown, and red shades, among others. These results matched the findings of Ayala-Villegas *et al.* (2014), who obtained 80% gemination using ancho pepper and árbol chili. Preferably, the fruits must have the same size, color,

Figure 1. Chili (*Capsicum* sp.) ecotypes evaluated. From left to right and from top to bottom: Habanero (*Capsicum chinense* Jacq), Ixcat ik, Bobo, green (with red ripening), Sweet, Maax, green (with yellow ripening), Pico de paloma, and Bolita.

shape, and number of loci. They can also be selected by their pungency level (Reveles-Hernández *et al.*, 2013).

In the small plots where the fruits were selected for seed extraction, the best fruits belong to the second cut, when the plant is still young and strong. They were selected taking into account the physical and physiological characteristics of the seeds. In order to obtain the fruits and extract the seeds, selecting complete competition, healthy, non-flattened, pest- and disease-free plants is recommended. This selection was carried out according to the Reveles-Hernández *et al.* (2013) methodology.

The fruits of wild chili plants are usually collected taking into account their red coloring or ripening color. The fruits were gathered from plants with similar characteristics to plants in production plots. The characteristics of the seed (*e.g.*, size, weight, and germination percentage of a given ecotype) change depending on the harvesting region or area. This matches the findings of Carrillo *et al.* (2009), who concluded that the characteristics of the seed of water chili change according to the gathering place.

Seed extraction

In the case of habanero chili and sweet chili, a longitudinal and circular cut must be made along the base of the fruit. Using this technique, the seeds will remain attached to the placenta and they will easily come off. Subsequently, the seeds can either be kept in the fruit and extracted once they are dry or they can be extracted immediately. Therefore,

the most appropriate method for fruits with an elongated shape in which the placenta runs along the fruit is a longitudinal cut.

For small chilies such as Maax, Pico de Paloma, or Bolita, the fruits are placed in a container with water and macerated with a solid object. They must be treated carefully, trying not to harm the seed. Another way of extracting the seed is to place a certain number of fruits and water in a blender and blend the mixture for very short periods, at the lowest possible speed. Subsequently, the content must be poured into a container, where the seed extraction will begin. The pulp with which the seed is mixed can be removed washing the mixture with water. The waste pulp and seed will become a supernatant that can be removed with a small sieve. The useful seeds will remain in the bottom of the container.

Drying and exploitation of the seeds

Once the seeds were extracted and washed, they were left in a sieve to drain. Subsequently, they were dried for 8-10 days, following the recommendations of Gaviola (2020). They suggest placing the seeds in metallic meshes or big sieves that allow air circulation. The seeds should be placed in a dry, shaded, and ventilated place, at less than 40 °C; these recommendations are different from those of Gaviola (2020), who recommended drying the seeds directly in the sun in dry weather regions. Under the humid tropical weather

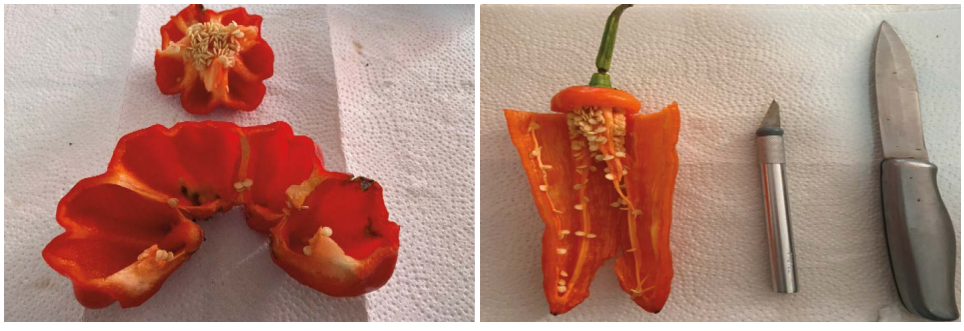

Figure 2. Extraction of seeds from large *Capsicum annum* fruits. Left: sweet chili. Right: Ixcat ik chili.

Figure 3. Extraction of seeds from small-sized fruits Maax (*Capsicum annuum*).

conditions of this study, drying the seeds in the sun damaged the embryo and, consequently, the germination capacity was lost. In addition, the seeds must be constantly shaken during the drying process to promote an even loss of humidity, avoiding fungi diseases. This procedure confirms the findings of Reveles *et al.* (2013). Common fans at low speed can be used to accelerate the drying process, as proposed by Gaviola (2020).

In order to avoid the appearance of fungi during the storage of the seeds, Captan 50 WP (fungicide) can be applied in a 1.0 g L^{-1} water dose. Other treatments such as 5 mL^{-1} water of Vitavax 200F® (Carrbonin and Thiran) or 5.0 g L^{-1} of Interguzan (Cabañas and Galindo, 2004) can also be used. Under rural conditions, a good practice to avoid diseases is to mix the seeds of larger chilies with ash, which reduces the humidity in the seeds. Once the seeds were subjected to these treatments, they were left to dry at room temperature for three days. Subsequently, they were placed in plastic or glass containers, covered with kitchen foil (Reveles-Hernández *et al.*, 2013). Glass containers are the preferred option for this process.

Seed storage

After the seeds were dried, they were put into glass jars and kept in refrigeration at 5 °C, with relatively low humidity (Colley *et al.*, 2010) and scarce light. We used plastic and glass jars to store the seeds. The results were positive, due to their hermetic seal; however, aluminum cans and bags can also be used to store the seeds. According to this research, the containers must be covered with kitchen foil to avoid light during the storing stage; this technique favors the viability of the seed for longer periods (3 to 5 years) as mentioned by Berke (2001).

Figure 4. Pico Paloma (*Capsicum frutescens*), Rosita habanero (*Capsicum chinense* Jacq), Bolita, and Green (*Capsicum annuum*) seeds after the drying process.

Inadequate storing conditions can cause a reduction in the seed quality, as a result of the loss of its germination capacity, the reduction of its longevity, or its exposure to antagonistic pathogens (Steiner, 2012). The humidity content of the seed was not measured at the moment of its storage; however, in order to extend its viability, a 5% humidity is recommended (Tay, 2004).

CONCLUSIONS

The proposed methodology for the extraction and exploitation of seeds, under humid tropic conditions, will help greenhouse growers and a great number of chili producers in the state of Campeche and the Yucatan Peninsula. It will contribute to the conservation, reproduction, and production of the native chili ecotypes.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Use of tropical macrophytes in wastewater treatment

Lara-Acosta, Marimar[1]; Lango-Reynoso, Fabiola[1*]; Castañeda-Chávez María del Refugio[1]

[1] Tecnológico Nacional de México/Instituto Tecnológico de Boca del Río, Carretera Veracruz-Córdoba km 12, Boca del Río, Veracruz, México, C. P. 94290.

* Correspondence: fabiolalango@bdelrio.tecnm.mx

ABSTRACT

Objective: To evaluate the adaptation process of ornamental tropical macrophytes irrigated with wastewater, through physiological measurements before and after planting in a tropical constructed wetland (CW).

Design/Methodology/Approach: Three fractions were evaluated with 50%, 75%, and 100% wastewater and natural water (blank) in 0.5×2.0 m fiberglass containers. The following species were placed in the containers during 40 days: *Strelitzia reginae*, *Alpinia purpurata*, *Canna indica*, *Xanthosoma robustum*, *Cyperus papyrus*, *Pistia stratiotes*, *Spathiphyllum wallisii*, *Ruellia brittoniana*, *Pasto pennisetum*, *Solenostemon scutellarioides*, *Iresine herbstii*, *Lantana camara*, *Duranta eracta* golden, and *Asparagus densiflorus*. Subsequently, the individuals —including *Heliconia psittacorum* and *Iris germanica*— were planted and evaluated in a CW after the adaptation period, in order to replace the macrophyte lost during the said period. The following physiological variables were measured: survival percentage, stem thickness, number of flowers, chlorophyll index, and biomass (as a growth variable).

Results: During the first stage (containers), only 11 out of the 14 initial species survived (78.5%), which allowed us to establish which plants had the highest survival capacity in high concentrations of pollutants. These results determined the priority with which these would be planted in the CW.

Study Limitations/Implications: Significant physiological differences were observed ($p \leq 0.005$) in all CW species. *Canna indica*, *Xanthosoma robustum*, *Ruellia brittoniana*, *Alpinia purpurata*, *Cyperus papyrus*, and *Heliconia psittacorum* recorded better adaptation.

Findings/Conclusions: The macrophytes studied show great adaptation as phytoremediative plants in tropical CW systems; however, their physiological development is different.

Keywords: Adaptation, macrophyte, purification, wastewater, constructed wetland.

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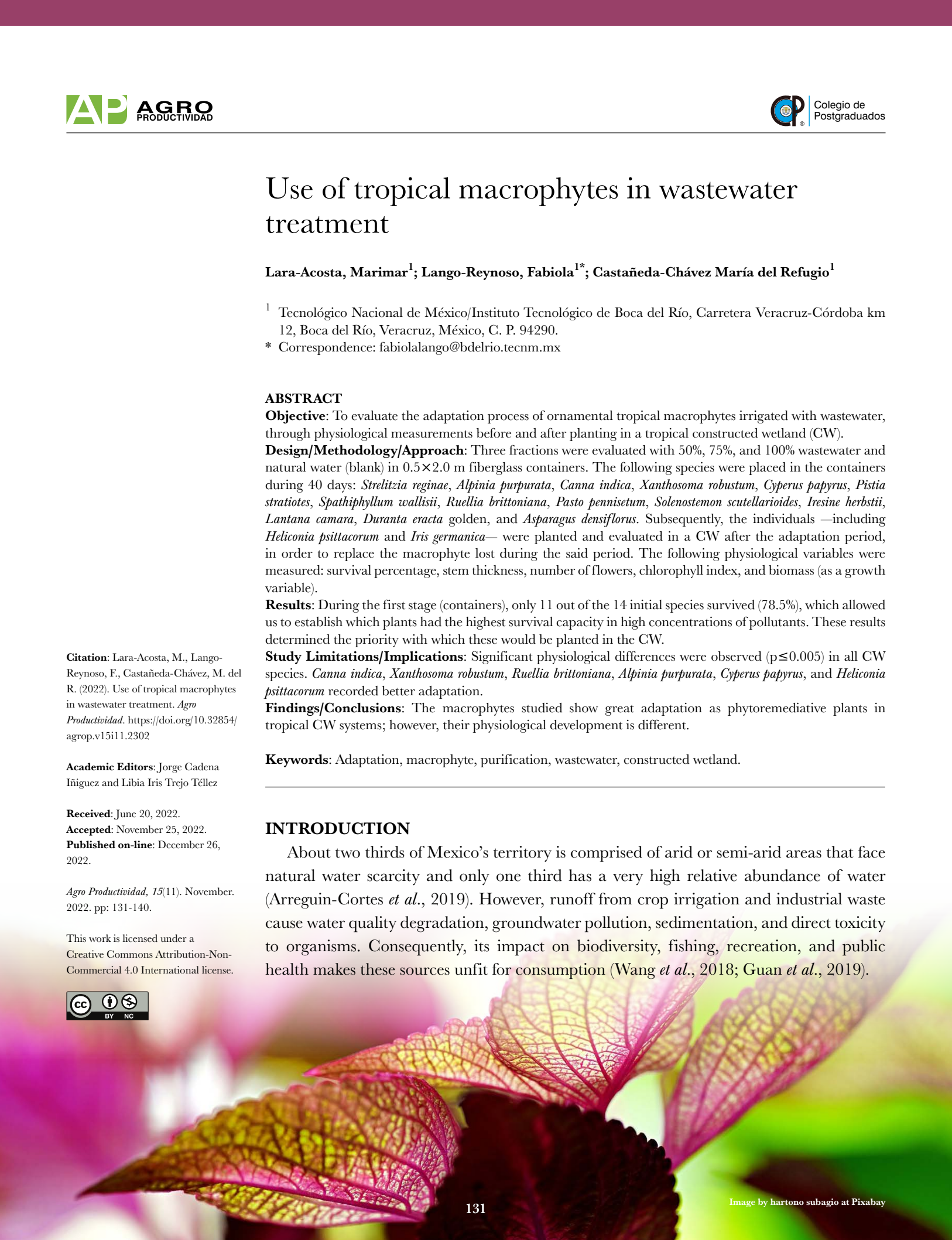
INTRODUCTION

About two thirds of Mexico's territory is comprised of arid or semi-arid areas that face natural water scarcity and only one third has a very high relative abundance of water (Arreguin-Cortes *et al*., 2019). However, runoff from crop irrigation and industrial waste cause water quality degradation, groundwater pollution, sedimentation, and direct toxicity to organisms. Consequently, its impact on biodiversity, fishing, recreation, and public health makes these sources unfit for consumption (Wang *et al*., 2018; Guan *et al*., 2019).



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There are many wastewater treatment technologies that seem to be highly efficient; however, they require higher energy consumption and release more carbon emissions, depending on their scale (Chang *et al.*, 2017; Landa-Cansigno *et al.*, 2020). Therefore, constructed wetlands (CW) are a viable and environmentally-friendly alternative for water treatment. CWs consist mainly of shallow ponds in which plant species adapted to aquatic life are implanted and in which purification is based on natural microbiological, biological, physical, and chemical processes. Likewise, their implementation and development are low-cost and they are easy to operate (Li *et al.*, 2018; Liu *et al.*, 2019). Terrestrial ornamental plants are important in these systems, since they provide the main source of oxygen, through a process that occurs in the root zone, known as radial oxygen loss (Sandoval *et al.*, 2019b).

Macrophytes are a wide and varied group of plants that is used for wastewater treatment. In a natural water body, they can be found in different types of growth, along the depth gradient that extends from the edge to the deepest parts (Maine *et al.*, 2009; 2013). In constructed wetlands (CW), an attempt to replicate this type of gradient is made by planting the different types of macrophytes according to how they grow (Figure 1).

The macrophytes in the CW have a thermoregulatory effect that promotes a variety of biological and chemical processes. They increase the filtering effect and porosity throughout the root distribution, they capture and store some essential nutrients in their tissues, and they act as a purification reaction, improving the diversity of the process in the rhizosphere (Türker *et al.*, 2016). One of the main limitations for the development of a plant is the lack of oxygen. Its transportation in an aqueous system is 10,000 times slower than in a porous medium. Therefore, in flooded systems, many plants die due to the lack of oxygen and consequently generate a wide range of responses to stress. The most important adaptation of macrophytes in these conditions is the development of the aerenchyma, a tissue characterized by a large number of air channels that transport oxygen from the aerial part of the plant to the roots (Moreno-Casasola and Waner, 2009; Alarcón-Herrera

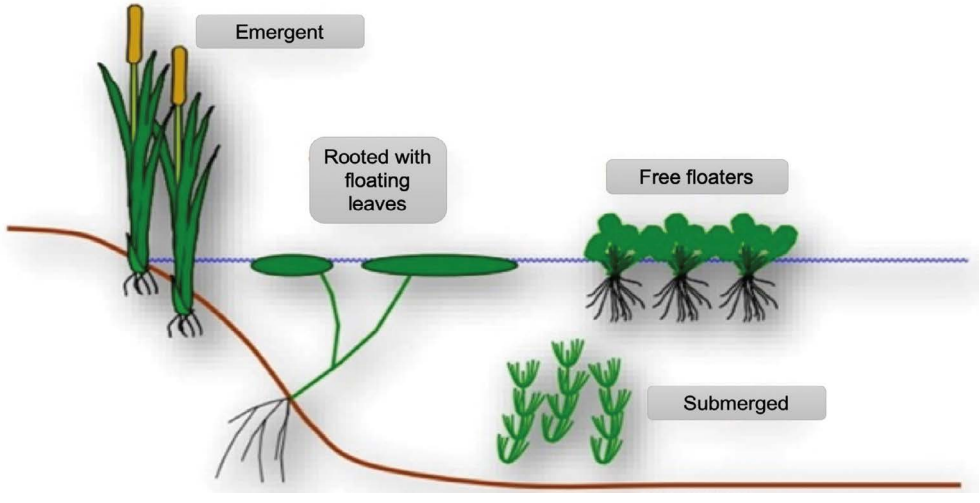

Figure 1. Diagram that represents the different macrophyte life forms (Alarcón-Herrera *et al.*, 2018).

et al., 2018). The uptake rate of plants is also limited by their net productivity (growth rate) and the nutrient concentrations in the tissues. In addition, age greatly influences the physiological activity of plants, especially their roots (Valipour *et al.*, 2015).

Generally, the plants used for CWs in tropical and intertropical areas are macrophytes typical of natural wetlands, such as: *Phragmites australis* and species of the genera *Typha*, *Scirpus*, and *Cyperus* (Sandoval *et al.*, 2019b). However, ornamental vegetation is a promising alternative in CW systems, due to its aesthetic and commercial value, and other aggregates related to biodiversity and ecosystem services. Among the species most frequently used to remove pollutants from CWs, *Canna*, *Iris*, *Spathiphyllum blandum*, *Heliconia*, and *Zantedeschia* stand out (Tejeda *et al.*, 2015; Sandoval *et al.*, 2019; Mateo *et al.*, 2020). These species are highly efficient for the elimination of suspended solids, total phosphorus, ammoniacal nitrogen, and fecal coliforms (Mateo *et al.*, 2020) and contribute to the reusability of wastewater for irrigation purposes. Growth is an indicative parameter of physiological health, carbon assimilation, and adaptation. Consequently, if a plant is stressed by an external agent, long-term symptoms will manifest themselves as lower growth and lower production of leaves, flowers, and stem (Pagter *et al.*, 2005; Heynes-Silerio *et al.*, 2017). The objective of this work was to evaluate the adaptation of ornamental tropical macrophytes to different wastewater concentrations, through physiological measurements. These macrophytes are to be planted in a tropical CW in the central zone of the Gulf of Mexico.

MATERIALS AND METHODS

The study was carried out in the municipality of Boca del Río, Veracruz, Mexico, within the facilities of the Tecnológico Nacional de México/Instituto Tecnológico de Boca del Río (TecNM/ITBoca). The municipality is located in the central coastal zone of the state, at 19° 07' N and 96° 06' W, and an altitude of 10 m (Figure 2).

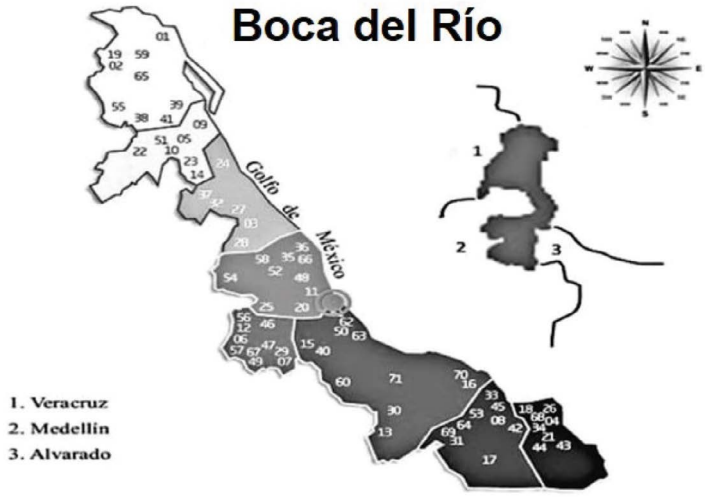

Figure 2. Geographical location of the municipality of Boca del Río, Veracruz and its surroundings (modified from the Municipal Information System, 2016).

First stage of the experiment

Selection of the ornamental macrophytes

The macrophytes selection was based on the following criteria: 1) easy adaptation, 2) resistance to weathering agents, and 3) commercial interest (Zurita *et al.*, 2006, 2009; Hadad *et al.*, 2007; Maine *et al.*, 2009; 2013; Sandoval-Herazo *et al.*, 2016; Marín-Muñiz, 2017).

The plants were purchased from a local nursery within the municipality of Veracruz. Endemic plants from tropical climates were selected, based on the floristic list of the state of Veracruz (Gutiérrez-Báez *et al.*, 2017). Complete young individuals between 15 and 20 cm high (including flowers) were selected for later adaptation (Table 1).

Macrophyte adaptation

After making holes in their envelopes, the acquired plants were placed in 0.5×2.0 m fiberglass containers filled with water from the natural environment for 10 d to avoid stress. Four water concentrations were prepared: three fractions of wastewater from the ITBoca (50%, 75%, and 100%) and a blank. The wastewater fractions were analyzed before proceeding to the next stage. Plants of each species were placed in each of the concentrations in order to adapt them to the new medium, where they remained for 40 d, maintaining a 15 cm water sheet (Table 1). This adaptation process was carried out from October to December 2019, through the manual insertion of water.

To evaluate the behavior of the selected macrophyte in the flooded systems, the stem thickness was measured with a 0-150 mm Vernier caliper and the chlorophyll index was measured using a SPAD 502 Plus. The survival percentage and the number of flowers were likewise estimated.

Table 1. Selected macrophytes in the adaptation process.

Species		Number of individuals collected per fraction of wastewater			
Common name	**Scientific name**	**75%**	**100%**	**50%**	**B**
Platanillo	*Canna indica*	5	5	4	1
Bird of paradise	*Strelitzia reginae*	5	5	4	1
Hawaiian	*Alpinia purpurata*	5	5	4	1
Elegant blade	*Xanthosoma robustum*	5	5	3	1
Papyrus	*Cyperus papyrus*	3	3	2	1
Moses Cradle	*Spathiphyllum wallisii*	26	27	26	1
Coleos	*Solenostemon scutellarioides*	5	5	4	1
Pennisetum grass	*Pennisetum purpureum*	3	3	3	1
Amaranth	*Iresine herbstii*	15	15	14	1
Lantana	*Lantana camara*	10	10	9	1
Duranta	*Duranta erecta golden*	15	15	14	1
Mexican Petunia	*Ruellia brittoniana*	5	5	4	1
Asparagus	*Asparagus densiflorus*	1	1	1	0
Water lettuce	*Pistia stratiotes*	15	15	15	30

Percentage of the concentration of wastewater taken from the ITBoca sump; concentration at 50%, concentration at 75%, concentration at 100%, B; blank with natural water.

Second stage of the experiment

Based on Valles and Alarcón (2014), after the macrophytes had been placed in the acclimatization ponds for 40 d, the second stage began.

Description of the constructed wetland

The second stage of the experiment was developed in a CW with horizontal subsurface flow; water enters the system from a 2,500-L tank that acts as a settling tank. The system operates with a 2-d hydraulic retention time, constantly supplying the system with 31.5 m^3 d^{-1}. It is made up of 7 cells as a treatment train filled with three different substrates: 1) stone material with a 70% porous surface; 2) inert material with 80% porosity (Sandoval *et al*., 2019a), and 3) calcareous material.

Stocking and evaluation of the macrophyte in the constructed wetland

For this system, the plants used were individuals and suckers that survived the previous adaptation (Table 2). *Heliconia psittacorum* and *Iris germanica* are used to replace the macrophyte lost during the adaptation period. It has a 40-cm growth length. Plants of the same species were cultivated in polycultures, 1 m apart from each other and at a 30-50 cm depth in each cell. The cells were kept outdoors under the normal environmental conditions of the state of Veracruz and afterwards were watered with wastewater from ITBoca.

To determine the influence of the CW in the development of the plant, the temperature was evaluated using an INI-T A12T digital hydrometer and the pH with pH-Fix 4.5-10 reactive strips. Five months later, when the plants were well established in each cell, the number of flowers, stem thickness, indirect chlorophyll, and biomass were measured (Table 2).

The information obtained was subject to an analysis of variance, using the Minitab Version 19 statistical software. A randomized block design and a Tukey test were used to detect significant statistical differences ($p \leq 0.005$) between the physiology of the macrophyte (confidence: 95%).

RESULTS AND DISCUSSION

In the 50%, 75%, and 100% wastewater concentrations, it was possible to observe which of the 14 macrophytes had the greatest ability to survive high pollutant concentrations.

Table 2. Macrophytes that survived the adaptation period, planted in the CW.

Common name	Scientific name	Common name	Scientific name
Strelitzia reginae	Bird of paradise	*Pistia stratiotes*	Water lettuce
Canna indica	Platanillo	*Iris germanica*	Iris
Alpinia purpurata	Hawaiian	*Spathiphyllum wallisi*	Moses Cradle
Heliconia psittacorum	Pennisetum grass	*Pennisetum purpureum*	Pennisetum grass
Xanthosoma robustum	Elegant blade	*Ruellia brittoniana*	Mexican Petunia
Cyperus papyrus	Papyrus		

Consequently, the order in which they would be planted (Figure 3) was determined, based on which plants had the greatest functionality as a phytoremediative plant in CW systems. These results are different from those obtained by Gallegos-Rodríguez *et al.* (2018).

In most cases (including ours), the adaptation of the vegetation is carried out in functioning CWs, in a one-month period (Marín-Acosta *et al.*, 2016). However, according to Valles and Alarcón (2014), this period could vary.

The values of the chemical characteristics of the wastewater used are summarized in Table 3.

Physiological behavior of the macrophyte during the adaptation period

During the adaptation period, only 11 out of the 14 initial species (78.5%) survived. Plant survival percentage for 50%, 75%, and 100% wastewater fraction ranged from 28-100%, 26-100%, and 20-100% respectively. However, during this adaptation period, *Alpinia purpurata*, *Spathiphyllum wallisii*, *Cana indica*, *Xanthosoma robustum*, and *Cyperus papyrus*

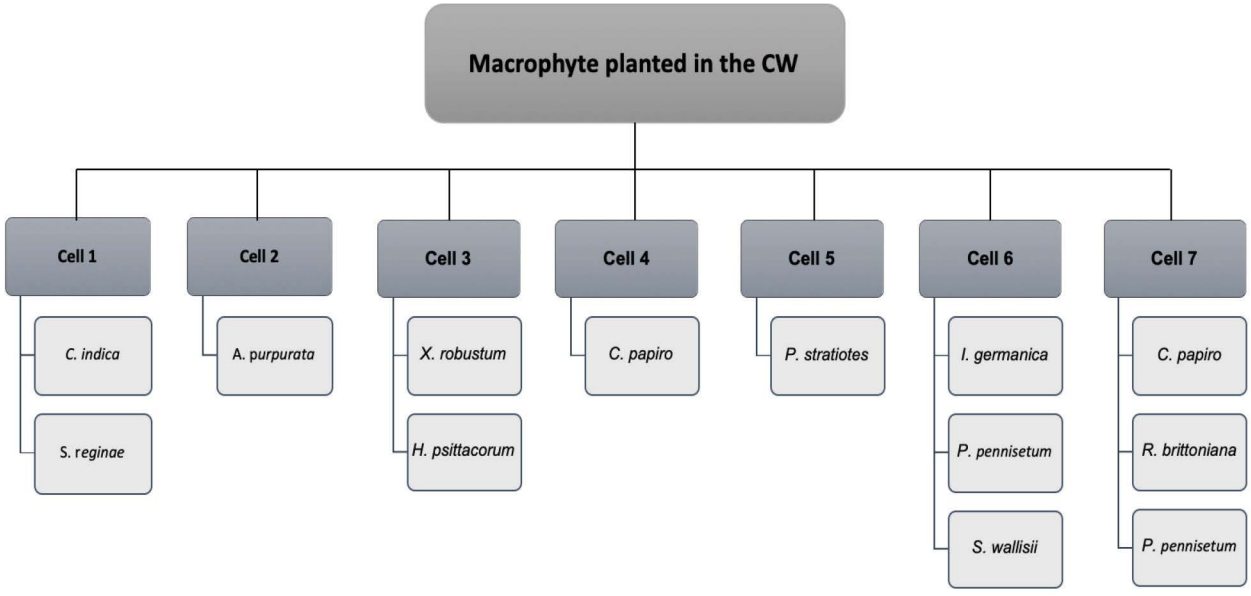

Figure 3. Order in which the macrophytes were seeded in each of the constructed wetland (CW) cells.

Table 3. Characteristics of the TecNM/ITBoca wastewater.

Parameter	Value (mg L^{-1})
Biochemical oxygen demand (BOD)	219.00
Chemical oxygen demand (DQO)	482.15
Total phosphorus (PT)	4.79
Total nitrogen Kjeldahl (NTK)	0.75
Total suspended solids (SST)	75.00
Total dissolved solids (SDT)	845.00
pH	7.90

grew faster and produced a large number of suckers, which indicates that they are more capable of inducing adaptation mechanisms in flooded environments. Most of the plants were healthy, except for the lost vegetation such as *Solenostemon scutellarioides*, *Asparagus densiflorus*, *Lantana camara*, *Iresine herbstti*, and *Duranta erecta* golden, which after a few days showed a less defined state of health.

Among the flowering species, *Spathiphyllum wallisii* produced more flowers (>7 flowers) than *Alpinia purpurata* (≈3-5 flowers) and *Strelitzia reginae* (0 flowers). Although the latter flower is used in artificial wetland systems with domestic waters, it did not develop or flower in any of the wastewater fractions (Arias-Martínez *et al*., 2010). The coloration of the species showed a direct relationship both with the photosynthetic process carried out during the experiment and with the chlorophyll index, based on which it was compared with the level of pollution of the university's 50, 75, and 100% wastewater fractions. The chlorophyll index was only analyzed in seven species, since species with small leaves could not be read in the infrared, showing a higher chlorophyll concentration at a 75% wastewater fraction in *Ruellia brittoniana*, *Canna indica*, and *Strelitzia reginae* with 51-65, 42-47, and 52-61 SPAD units, respectively. Therefore, planting in the system is recommended after an adaptation period of 40 days.

Physiological behavior of the macrophyte after planting in the constructed wetland

Stem thickness and number of flowers. Regarding the physiological responses of the stem, higher growth rates have been recorded in the first third of the length of the CW in the flow direction than in the rest of the stem, possibly because most of the organic matter and nutrients are consumed in this length (Peña-Salamanca *et al*., 2013). For this CW, the stem thickness between the same species showed faster growth in the first cells (1, 2, and 3), with significant differences ($p \leq 0.005$) in all species. The *Canna indica* and *Xanthosoma robustum* species are the most representative, with stem thickness of 44.4 and 63.5 mm, respectively, during the 5 months in the CW (Figure 4).

Regarding the number of flowers, *Spathiphyllum wallisii* was the species that changed the most during the adaptation period to the CW system, showing that the conditions in which it was exposed were not favorable. Meanwhile, *Strelitzia reginae* did not produce any flowers, because it was part of a polyculture system, along with *Canna indica*, which showed great development, producing up to 5 flowers per plant (Figure 4).

Chlorophyll index. The fluorescence emission of chlorophyll in plants is a sensitive test of the photosynthetic efficiency of the plant and reflects long-term disturbances in the photosynthetic apparatus. Therefore, the chlorophyll of the vegetation in a wetland can be considered a physiological indicator of plant metabolism and participates in the transformation of the different nutrients in the system (Pérez-Asseff *et al*., 2007). For this CW system, all species recorded a >20 chlorophyll index ($p \leq 0.05$). The plants with more representative values were: *Ruellia brittoniana*, *Strelitzia reginae*, *Canna indica*, *Alpinia purpurata*, *Heliconia psittacorum*, and *Xanthosoma robustum*. This is a sign of a high acclimatization capacity regarding the pollution conditions to which they were subjected during the study (Figure 5).

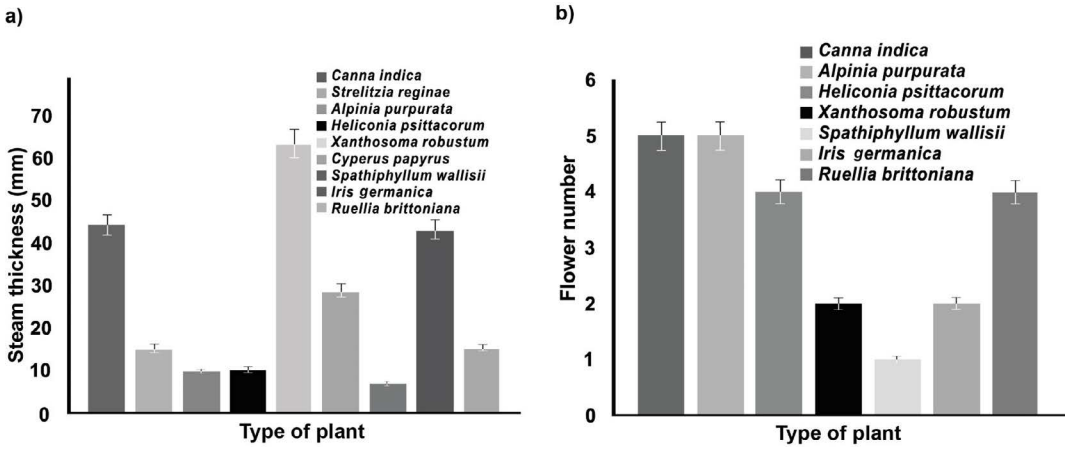

Figure 4. Stem thickness a) and number of flowers b) of the plants in the activated constructed wetland (CW) system.

Biomass. If the root system of the plants has a low development, the soluble or easily assimilated products found in the gradient (height of the substrate) are not absorbed (Ramírez-Cadavid, 2018). Biomass production is shown in three different sections of the plants (root, area, and body). The following species have significantly different values: *C. indica*, *S. reginae*, *H. psittacorum*, *X. robustum*, and *C. papyrus*. These species are suitable for CWs, due to their rapid growth and adaptation. The greatest amount of biomass remains concentrated in the root and the body of the plant, with the exception of *R. brittoniana* and *I. germanica*, where its highest concentration was recorded in the aerial part (6.23 and 12.93 g, respectively). *P. pennisetum* had the lowest biomass production in the three sections (1.51 in the body, 1.2 in the area, and 4.5 g in the root) (Figure 5).

CONCLUSION

Although most of the ornamental macrophytes species survived during the adaptation period, which indicates their capacity to enter flooding mechanisms, each one had different

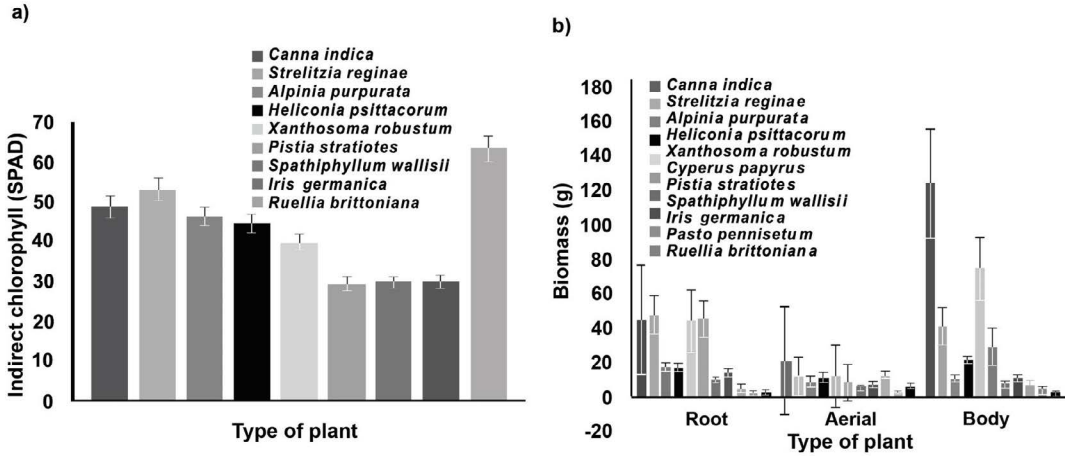

Figure 5. Indirect chlorophyll a) and biomass b) of the plants in the activated constructed wetland (CW) system.

physiological development. Our study obtained information that will serve as the basis for further research about CW systems in the tropical and subtropical areas of the central zone of the Gulf of Mexico.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

The chita, handicrafts in vegetable fibers

Carrasco Soria, Juana[1]; Sánchez Quintanar Concepción[1]; Figueroa Rodríguez Óscar L.[1], Castañeda Mendoza Arturo[2]; Jiménez Velázquez Mercedes A.[1]

[1] Colegio de Postgraduados Campus Montecillo, km. 36.5 carretera México-Texcoco. Montecillo, Texcoco, Estado de México, C.P. 56230.
[2] Universidad Intercultural del Estado de Puebla, Calle Principal a Lipuntahuaca S/N, Lipuntahuaca, Huehuetla, Puebla, C.P. 73475.
* Correspondence: carrasco.juana@colpos.mx

ABSTRACT

Objective: Identify the vegetable fibers and activities carried out in the production of basketry, in particular the chita, as the most significant craft of the community of San Miguel Tenango (SMT), in Zacatlán, Puebla.

Disign/methodology/approach: A phenomenological study oriented to the artisan production of San Miguel Tenango was carried out, through documentary research, observation and interviews with artisans and key informants. The value chain approach was used to understand the process, the inputs and the actors involved in the elaboration of the chita, the main handicraft in vegetable fibers in the study site.

Results: The vegetable fibers used in the elaboration of traditional crafts were identified, the "chita" was distinguished as the most significant. The links of the value chain in production were recognized, as well as the perception that artisans have about their work.

Study limitations/implications: Most of the people dedicated to basketry do not recognize themselves as artisans, moreover, they are not organized or adequately account for the cost of their work.

Findings/conclusions: The plant species used in the elaboration of handicrafts are easily accessible for their use; although discrimination against native (ethnic) peoples and the use of traditional materials persists. They are biodegradable, highly resistant, flexible and can be an ecological option in the face of the indiscriminate use of synthetic materials.

Keywords: Handicrafts, Indigenous Peoples, vegetable fibers, Zacatlán, chita, value chain.

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INTRODUCTION

In the Sierra Norte of the State of Puebla, Mexico, there are various Indigenous People, among their cultural richness they preserve a link with nature that provides them with various inputs. In the community of San Miguel Tenango, in Zacatlán, there are plant species that grow wild, with which utensils are made that, in addition to being used by the community, have become a valued merchandise for tourism. In this municipality, tourism has been a trigger for local development, involving local actors with various economic activities, as well as institutions that interact with each other, having a positive impact (Figueroa, 2017); however, the benefits are not reaching the entire population.

One of the objectives of rural development is to improve the quality of life of the people, and for this, strategies are required to avoid the migration of the inhabitants, improving their income at the local level, in order to maintain local employment, reduce migration and poverty. Work with vegetable fibers has ancient roots in Mexico; however, in this type of crafts there is no value chain approach, which could help in having a dynamic analysis and a holistic vision, since it analyzes activities, people and actions, because culturally the producer only deals with the production stage without taking into account other activities such as marketing, market analysis, negotiation processes and strategic planning, necessary to be successful in the market (Figueroa, 2012). The art of weaving palm leaves (*Yucca filifera*), wicker sticks (*Salix viminalis*), reeds (*Phragmites australis*), jonote (*Heliocarpus appendiculatus*), and vines (*Mikania glomerata*), among others, arises as a necessity where the natural environment offers whatever is used (Anonymous, 2019). Based on the above, the objective was to identify the activities carried out during the production of handicrafts with vegetable fibers, applying the value chain approach in the chita, in order to learn the perception that artisans have of their trade and those that they have of themselves.

MATERIALS AND METHODS

Study site

San Miguel Tenango is located southeast of the municipal capital of Zacatlán, to reach the town you have to travel 15.3 km of road through the "Barranca de los Jilgueros", (López, 1998) at an altitude of 1993 masl, latitude and longitude 19.9062483, −97.9395337, its climate is humid temperate, with rain all year round. It has a population of 1,323 inhabitants, 708 women and 615 men, with 94.86% Indigenous Population, 64.02% of the population speak an indigenous language and only 3.25% do not speak Spanish, INEGI (2020). The main economic activities are agriculture and commerce (INAFRED, 2020). However, as of April 2011, with the appointment of Zacatlán as a "magical town", the services sector has become another of the main activities of the municipality and an opportunity for more people to supplement their income with the preparation and sale of handicrafts.

Intervention method

It is a case study focused on the artisans dedicated to the elaboration of basketry from San Miguel Tenango, in Zacatlán, Puebla, Mexico. A phenomenological method was chosen, since it requires the description that people make about the experiences lived around a situation or event, in order to capture the fact or phenomenon as close as possible to reality. The observation technique was used, interviewing artisans and people considered key because of their knowledge of the community and artisan work. The chain approach is appropriate for carrying out diagnoses that are useful for development projects that make an organization efficient in the market (Figueroa, 2012). Additionally, the links of the value chain were identified (Porter, 1986) in order to determine competitive advantage from the set of activities in the processes, creating value in the products and competing with similar products in the market (Quintero, 2016).

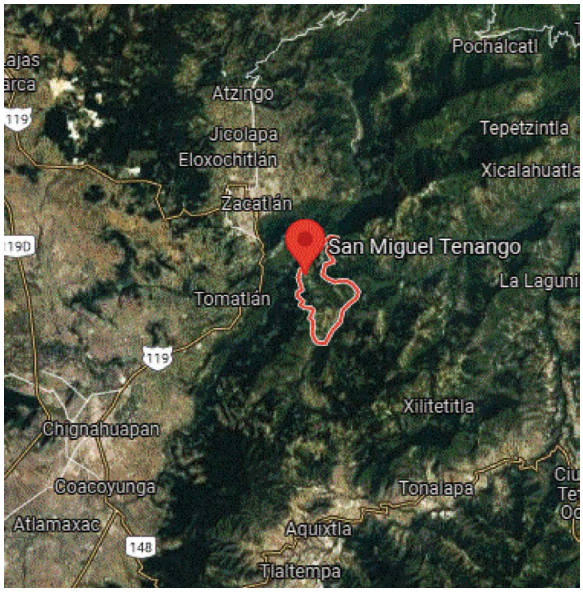

Figure 1. Satelital map of San Miguel Tenango. Source: Google, date november, 2021.

Variables and statistical analysis

Interviews were conducted with 10 key informants (five men and five women), mostly City Council managers and some of the most outstanding SMT artisans, as well as 10 Tenango artisans dedicated to basketry (eight women and two men), who contributed with: characteristics in general terms of the community, matlahuacal (*Cornus excelsa*), ixtle (*Agave* sp.) and palm (*Yucca filifera*) were identified as the main fibers used in basketry. The study variables that were identified were: family and crafts, cultural identity, organization and production, marketing, economy and tourism, and socioeconomic effects of craft work. Transcription, concentration, categorization and coding were carried out for the analysis of the interviews, through the Excel program statistical tools were applied in order to obtain data such as: average, percentage, mean, median, maximum, minimum; Dynamic tables were also prepared to organize, analyze and interpret the information collected, achieving the elaboration of the chita value chain.

RESULTS AND DISCUSSION

Community Overview

The municipality of Zacatlán has large extensions of coniferous forests and mountain cloud forest; where the matlahuacal (*Cornus excelsa*), ixtle (*Agave* sp.) and palm (*Yucca filifera*) are found, among other plant species, in their natural state (Iracheta, 2010). The craft called chita, also known as huacal, is made from the extracted vegetable fibers. According to the Great Nahuatl Dictionary of the National Autonomous University of Mexico (2012), the chita is an object of pre-Hispanic origin, originally called "chitatli" that appears in the Acolhuacan and Telleriano Codes. In San Miguel Tenango the "chita" is part of the traditional clothing of men and women, and unlike other regions where shawls are used to transport infants, in this region the chitas are mainly used for this purpose, although they

are also a load implement to carry fruit, harvest corn, or transport other products in their daily lives. Over time, artisans have used these fibers to make new products, adapting to the needs and tastes of local and visiting buyers. Table 1 was prepared with data provided by the artisans and documentary information, it describes the plant species from which the fibers used in basketry are extracted.

Chita or huacal value chain

Figures 1, 2, 3 and 4 indicate the brief links in the "chita" handicraft value chain, in order to make it more simplified and to be able to visualize the interrelationship between the various links.

Table 1. Vegetable fibers from which chitas are made.

Matlahuacal	Ixtle	Palm
Scientific name: (*Cornus excelsa* H.B.K.) Family Cornaceae, Common name: olive, wild jasmine, etc. (Peredo, 2020) in Zacatlán it is known as matlahuacal. It grows in humid canyons and bordering some streams in oak and pine forests. It is distributed in Guatemala, Honduras, Panama and in a large part of Mexico (Calderón, 2001). Uses: helps control erosion, infiltrates rainwater, improves soil, honey species, ornamental and basketry.	Extracted from the Maguey Tlaximalmitl (maguey to scrape) *Agave* sp. Small maguey that grows on the slopes of the hills of San Miguel Tenango. Deforestation, droughts and fires that arise every year are reducing the population of this species. Its main use is the extraction of the ixtle, from which ties, loins for pack animals, among other utensils can be made.	It can be "hat palm", yucca or izote (also called quisiote) (*Yucca filifera*) grows wild on the slopes of the hills, it is used fresh either green (outer part) or white (internal part), it is common that it is used as "live posts" to delimit the cultivation lands in this region.

Source: Own elaboration with research data.

Figure 2. Links in the chita value chain.

Figure 3. Sample of chita crafts based on vegetable fibers.

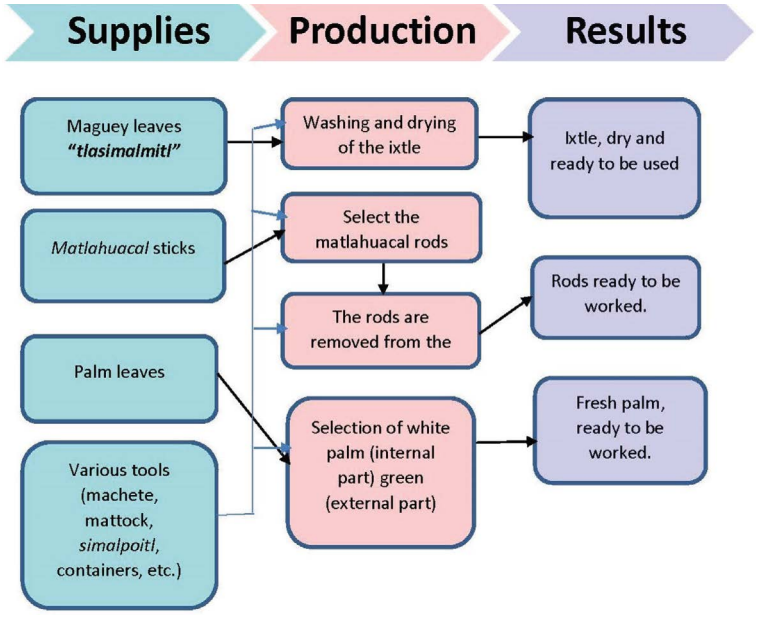

Figure 4. Brief description of the vegetable fibers for the elaboration of the final product.

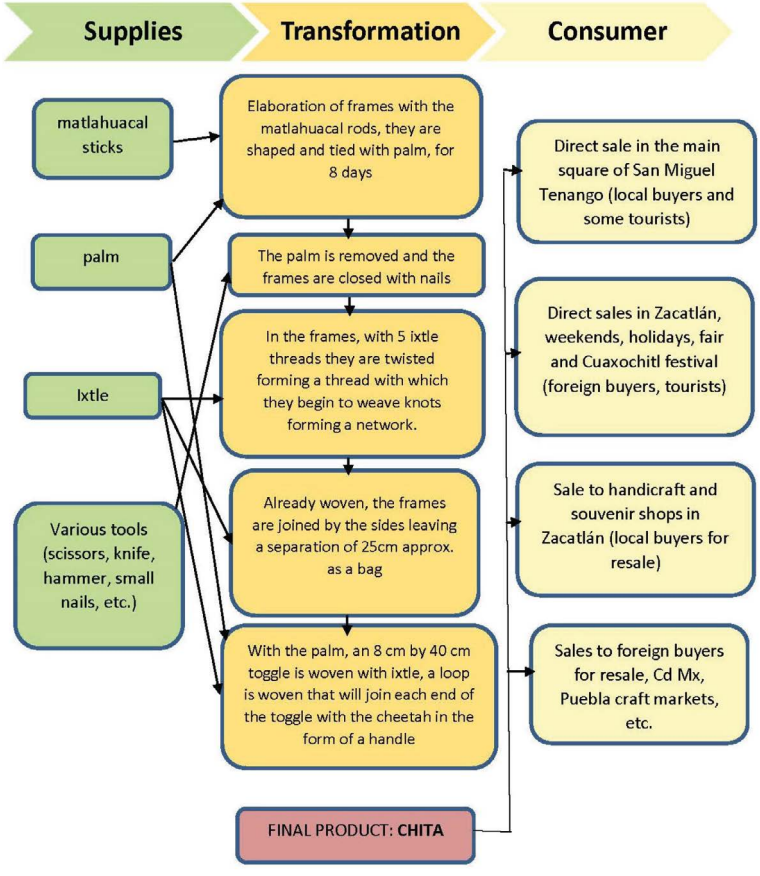

Figure 5. Links in the value chain of "cheetah" handicrafts based on vegetable fibers.

The actors in the chita value chain are artisans, jarcierías, tlapalerías, artisan shops, local buyers, foreigners, resellers (Figure 4).

Production costs of a standard size chita

The preparation begins with a kg of ixtle at a cost of $5.14 USD, 300 g of palm at a cost of $1.54 USD, as well as free sticks, nails at $0.15 USD, and a processing time of 15 days, added to the marketing event, which includes transportation to the point of sale for $1.85 USD, adding a final total of the chita that ranges between $12.86 USD and $18.00 USD (Prices in the month of October 2021).

Ditrect sales are made in the streets of the center of Zacatlán, where they do not have a fixed place and sometimes they are also charged for occupying a space, sometimes they are discriminated by the artisans of the municipal head, sometimes, the municipal Tourism Directorate invites them to occupy a stand at significant events such as the "Cuaxochitl" festival or the "Apple Fair" among others. Some artisans from Tenango concur in five semi-formal artisan organizations that bear the names: Sihuamej Ma Xochil Tlatzomani, Macehual Zihuatzitzin Matiquitini, Tojtla Tojtojmi tlajkitini, Yolotl and Xochitl, two of these share a local artisan shop in the lower part of the auxiliar presidency of San Miguel Tenango; however, and according to what was expressed by the artisans, they need to be supported through government institutions, so that the artisans themselves recognize themselves as artisans.

It is important to specify that a productive chain, by consuming what is produced locally, generates development, jobs and possibly inhibits the desire of the population to migrate in search of sustenance, improving living conditions in general. For the artisans, making and selling their chitas is a symbol of pride and satisfaction, the transmission of knowledge mostly occurs in childhood, within the family nucleus, strengthening their identity ties with their community; however, Tenango artisans do not have an efficient organization, they usually work in isolation, and lack communication and cooperation networks that allow them to access governmental and non-governmental programs that could strengthen their customs and the use of traditional materials, such as vegetable fibers.

Regarding whether Tenango artisans consider that handicrafts can become their main economic activity, 80% said yes and only 20% said no, mentioning that this would be viable only if tourism increases, taking advantage of the popularity of Zacatlán as a tourist destination and spreading not only the municipal capital but its other localities, thus attracting more customers and increasing sales.

The vegetable fibers to make the chita are still easily accessible; however, it would be advisable to encourage their propagation since, in addition to their artisanal use, they may have other purposes. The findings set the tone for more people in the academic field to become interested in the study of traditional vegetable fibers. It is necessary to carry out field inventories, and it is advisable to use conventional methods to quantify and evaluate the existing resources in the area, as well as their properties and uses in various areas. It is suggested to carry out workshops aimed to artisans, in order to propose strategies such as the value chain approach, make visible the importance of their work, highlight the importance of the interrelationships of the various actors involved in the processes, as well

as the possible advantages to which they can access. Provision of trainning is necessary, also a decent space where they can present their crafts and where they are not discriminated by other artisans, in this way they would achieve better marketing.

CONCLUSIONS

From the phenomenological point of view, the different perspectives of Tenango artisans and the perception that other key people have of artisan work were known, agreeing on the lag that still permeates the living conditions of Indigenous Peoples.

The production of handicrafts is important for Tenango families that are dedicated to it, through the elaboration of the value chain it is noted that the main advantages that SMT artisans have are the vegetable fibers that are in a free natural state, their vast knowledge of handling the materials used, as well as their attachment to their traditions and cultural identity.

Handicrafts are an opportunity for the inhabitants of the Indigenous People to generate new opportunities for development, self-esteem and strengthening of tourism.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Goat production systems of the central highlands of Veracruz, Mexico

Emmanuel de Jesús Ramírez-Rivera[1]; Gregorio Hernández Salinas[1]; Eusebio Ortega-Jiménez[2]; Adán Cabal-Prieto[3]; José Andrés Herrera Corredor[4]; Paloma Villalvazo-Ceballos[2]; Pablo Díaz-Rivera[2]

[1] Tecnológico Nacional de México-Instituto Tecnológico de Zongolica. Dirección postal: Km 4 Carretera a la Compañía S/N, Tepetitlanapa, Zongolica, Zongolica, Veracruz, México. C.P. 95005.
[2] Colegio de Postgraduados Campus Veracruz Dirección postal: Km. 88.5 Carretera Xalapa-Veracruz. Predio Tepetates, Manlio Fabio Altamirano, Veracruz, México. C.P. 91690.
[3] Tecnológico Nacional de México-Instituto Tecnológico de Huatusco. Dirección postal: Huatusco, Veracruz, México. C.P. 94106.
[4] Colegio de Postgraduados-Campus Córdoba. Dirección postal: Km 348 Carretera Córdoba-Veracruz, Amatlán de los Reyes, Veracruz, México. C.P. 94946.
* Correspondence author: pablod@colpos.mx

ABSTRACT

Objective: This study was to characterize the goat production units of the central mountain area of the state of Veracruz, Mexico.

Disign/methodology/approach: A multiple case study was carried out taking into consideration socioeconomic aspects, type of forage, goat feeding, reproduction, improvement, and health.

Results: The results showed that goat and cheese production account for 50-100% of the producers' income. The forages grown and the type of goat feed used in the production units of the mountain area are: King grass, CT-115, maralfalfa, giant star grass, alfalfa, and corn. Animal reproduction is carried out by controlled mating and births take place at the beginning and end of each year. Flocks do not exceed 150 heads; the predominant breeds are Saanen and Alpine. The average daily production of milk ranges from 16 to 90 L, with a production of 0.75-3 L milk animal^{-1} day^{-1}.

Study limitations/implications: None

Findings/conclusions: The activity is family-based and involves cultivation, animal management, and milk and artisanal cheese production. Further studies are required to confirm individual milk production.

Keywords: Multiple case study, problematic, milk production, artisanal cheeses.

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INTRODUCTION

Goat production allows poor rural populations to achieve economic development (Escareño-Sánchez *et al.*, 2011). There are more than 909 million goats worldwide; the largest inventories are held by China (150 million) and India (154 million) (Bidot-Fernández, 2017). Ninety-five percent of the goat population are used in doble-purpose production (meat-milk), while the remaining is mainly used to obtain milk (Bidot-Fernández, 2017).

Image by Alexa at Pixabay

In Mexico, the heterogeneity of the goat production systems (GPS) depends on the production system type; the most common systems in Mexico are the extensive and stall-feeding systems. The Servicio de Información Agroalimentaria y Pesquera (SIAP, 2021) reported 8,830,720 goat heads. The states of Puebla, Oaxaca, San Luís Potosí, Zacatecas, and Guerrero have the largest goat inventories. There are 159,527 goats in Veracruz and they are mainly concentrated in the municipalities of Coatepec, Coacoatzintla, Perote, Tatatila, and Xico (SIAP, 2021). However, the presence of brucellosis (Martínez-Herrera *et al.*, 2010) and the areas that favor the development of goat production systems (Ramírez-Rivera *et al.*, 2017) have been the only subjects studied in the area. There are not study cases that determine the operability of these GPSs in Veracruz. Therefore, the objective of this research is to characterize the GPSs of the central mountain region of Veracruz.

MATERIALS AND METHODS

Figure 1 shows how a multiple case study was carried out to characterize the goat production units (GPUs) of the central mountain area of Veracruz (Villareal-Larrinaga y Landeta-Rodríguez, 2010). An interview card was developed, taking into account the following criteria: 1) socio-economic aspect of the producer; 2) grasses and feeding type; 3) reproduction and improvement; 4) health; and 5) milk production (Mendoza and Ortega, 2009). Six goat production units located in Pacho Viejo, Coatepec, Perote, Tatatila, and Xico were analyzed. Table 1 shows the agroecological conditions of the sites where the goat production units are located.

RESULTS AND DISCUSSION

Characterization of the Donelo GPU

The owner of this GPU is 49 years old, has a bachelor degree, and his family has three members. Activities such as cattle-raising and agriculture are carried out in this GPU, as

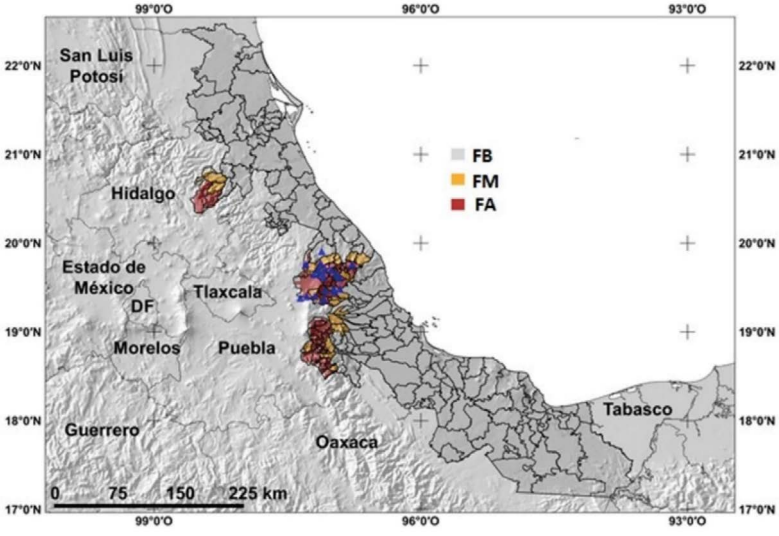

Figure 1. Predominance of goat production systems in Veracruz; LF: low favorability, MF: medium favorability, and HF: high favorability (Ramírez-Rivera *et al.*, 2017).

Table 1. Location of GPU and agroecological conditions.

GPU	Rainfall Mm	Altitude Masl	Dominant vegetation	Climate type	[4]AAT °C
Donelo	1,500	1,208	MMF[1]	C(M) y C(FM)	18
Las Lajas	1,500	1,440	MMF[1]	C(M) y C(FM)	18
La Chiviada	1,750	2,011	MMF[2]	C(FM)	19
Rincón del Rio Frio	1,346	1,867	MMF[3]	C(fa)	20
Hermanos- Enríquez	493.6	2,400	FF, PF & XS[3]	BS1kw	12

GPU: Goat Production Unit; MMF: Mountain Mesophyll Forest; Mm: milimeters; Masl: Meters above sea level; [1]García-Franco *et al.* (2008); [2]García-de la Cruz *et al.* (2013); FF, PF, XS; Fir forest, Pine forest and Xerophytic scrub; [3]Márquez y Márquez (2009). [4]AAT: Average Annual Temperature (INAFED, 2005).

well as production and sale of artisanal cheeses. Fifty percent of its income comes from goat production and the sale of cheeses. The GPU has 1 ha and it can be classified as a small property. A temporal worker is hired per day's wage. The inventory is made up of 127 goats (80 breeding females, 3 bucks, 20 goatling, 4 weaned goatling, 20 replacement males and females) of the Alpine and Saanen breeds, acquired in Celaya, Guanajuato. This GPU is focused on milk and artisanal cheese production. Goats are fed with the following grasses: King grass (*Saccharum sinense* L.), CT-115 (*Cenchrus purpureus*), maralfalfa (*Pennisetum* sp.), and Giant Star grass (*Cynodon plectostachyus* Schum, Pilger). Their diet is supplemented with white mulberry (*Morus alba* Linn.) and orange bagasse (*Citrus sinensis* L.), acquired in local juice bars (Figure 2a). The grasses are grown in the GPU and fertilized with goat faeces. The commercial product "La Posta" (15.0% protein, 3% fat, 9% fiber) is used as supplement. Controlled mating is carried out, using three males. Kids are born from November to May and they are weaned after a two-month lactation period. Deworming is carried out every four months and 5% Closantil is applied to eliminate parasites of the *Strongyloides* spp. genus. The goats are vaccinated against brucellosis (*Brucella melitensis*) and clostridial disease (*C. perfringens*, *C. chavoei*, *C. sordelli*, *C. haemolyticum*). Each goat produces approximately 2 L of milk; forty goats are hand milked, obtaining 80 L day^{-1}. Lactation lasts from 8 to 10 months. The GPU uses a stall-feeding arrangement.

Characterization of the Las Lajas GPU

The owner of this GPU is 50 years old, has a bachelor degree, and his family has two members. This GPU is focused on milk production, breeding stock, and agriculture. This producer makes and sells different types of artisanal cheeses, which account for 20% of his income. The GPU has 55 ha and mechanical methods are used to prepare the land. The producer employs two temporal workers and two permanent workers. The goat inventory is made up of 46 goats (20 breeding females, 1 buck, and 25 kids) of the Alpine breed (Figure 2b), acquired in Querétaro and Veracruz. The goats' diet includes Kikuyo grass (*Pennisetum clandestinum*, var. Whittet), Giant Star grass (*Cynodon plectostachyus* Schum, Pilger), and King grass (*Saccharum sinense* L.). Goat and cow manure is used to fertilize a 5.5 ha area. Fodder seasonality is addressed using ensilage, supplemented with a Purina commercial product (20% raw protein). Controlled mating is carried out. Births take place

from September to October and goatling are weaned 2.5 months later. Albendazole (10%) is used to control internal parasites, while ivermectin is used to eliminate external parasites. This producer mentioned that his flock is free from brucellosis. Milk production per goat varies from 1 to 1.5 L; twenty goats are hand milked per day and lactation lasts seven months. Milk is used to produce artisanal cheeses. This GPU uses a partial stall-feeding arrangement.

Characterization of the La Chiviada GPU

The owner of this GPU is 62 years old, has a bachelor degree, and his family has three members. Cattle-raising activities account for 50% of his income. He produces and sells artisanal cheeses. The GPU has 19 ha and animal and manual traction are used to prepare the land with the help of a temporal worker, hired per day's wage. Goat inventory is made up of 62 goats (45 breeding females, 2 bucks, 5 weaned goatling, 10 replacement males and females) of the Alpine and Saanen breeds (Figure 2c) acquired from other local GPUs. The type of grasses grown to feed the goats are Kikuyo and Orchard (*Dactylis glomerata*). The grasses are fertilized using goat manure. The commercial product La Posta (15% raw protein) is used as supplement all year round. Controlled mating is carried out, using two males from the Saanen and Alpine breeds. Births take place from September to October and weaning is carried out after 2.5 months. Parasites from the *Haemonchus* sp. genus are controlled with the Famacha© method and the goats are vaccinated against brucellosis. Milk production per goat reaches 0.75 L. Forty-eight goats are hand milked, obtaining a total of 36 L day^{-1}. Lactation lasts eight months. This GPU uses a partial stall-feeding arrangement.

Characterization of the Rincón del Río Frío GPU

The owner of this GPU is 54 years old, has a bachelor degree, and his family has five members. This GPU carries out cattle-raising activities and produces artisanal cheeses. Both activities account for 100% of his income. The GPU has 35 ha and animal and manual traction are used to prepare the land. The producer employs one temporal worker and one permanent worker. Goat inventory is made up of 143 goats (60 breeding females, 3 bucks, 20 goatling, 20 weaned goatling, and 40 replacement males and females). The goats are crossbred Alpine-Saanen (Figure 2d) and they were acquired in the Tatatila region. Goats are feed Orchard grass (*Dactylis glomerata* L.), Taiwan grass (*Pennisetum purpureum* Schum), and forage oat (*Avena sativa*) grown in the GPU. Grass is cut by hand and 5 ha are fertilized using organic manure. Fodder seasonality is minimized using peach (*Prunnus persica*), apple (*Pyrus malus* L.), pear (*Pyrus communis*), avocado (*Persea americana*), acorn (*Quercus ilex*), and plum (*Prunus domestica* L.). During grazing, the goats eat different wild plants, such as silverleaf nightshade (*Solanum eleagnifolium*.), flameberry (*Urera caracasana*), bracken fern (*Pteridium aquilinum* L.), tree tobacco (*Nicotiana glauca* Graham), field bindweed (*Convolvulus arvensis*), and Alder (*Alnus acuminata* Kunth). Controlled mating is carried out and births take place from February to March. Weaning is carried out when kids are 2.5 months old. Deworming is carried out every four months. The producer stated that his goats are free from brucellosis. Vaccination is carried out twice a year to prevent blackleg (*Clostridium*

chauvoei) and pneumonia, which can affect goats from May to December, as a result of the sudden temperature changes. Milk production per goat reaches 1.5 L and a total of 40 goats are milked per day, obtaining approximately 60 L day^{-1}. Lactation lasts 10 months. This GPU uses an extensive system.

Characterization of the "Enríquez" GPU

This GPU is located in the high plateau of the state of Veracruz. The producers are 29 and 54 years old. Their education level ranges from primary to high school. They carry out cattle-raising and agricultural activities, which account for 50% of their total income. The average surface of the GPU is 6±2 ha and they are used to grow alfalfa (*Medicago sativa*) and corn (*Zea mays*). Mechanical and manual techniques are used to prepare the land and the labor is carried out by the family itself. The inventory is made up of 68 heads (40 breeding females, 3 bucks, 3 goatling, 1 weaned goatling, 20 males, and 1 replacement female), acquired in the state of San Luis Potosí (Figure 2e and f). Goats are fed alfalfa, corn waste, and annual wall-rocket (*Diplotaxis muralis*). Overall, 3.5 ha are fertilized with goat urea and faeces. Providing dried fodder minimizes the fodder seasonality problem, supplemented with the Bayer™ Magnaphoscal commercial product. The producers carry out controlled mating procedures with three males (two Saanen and one Alpine). Females give birth from November to January and from March to May. Goats are dewormed every four months and they are vaccinated against brucellosis, pasteurellosis (*Pasteurella multocida*), clostridial diseases (*C. perfringens*, *C. chavoei*, *C. sordelli*, *C. haemolyticum*), and coccidiosis (*Eimeria arloingi*, *Eimeria faurei*, *Eimeria gilruthi*, *Eimeria caprovina*, *Eimeria ninakohyakimovae*). The average milk production fluctuates between 1.5 to 3 L per goat and the producers milk 23 to 30 goats by hand, in order to produce 34.5 to 90 L day^{-1}. Lactation lasts from 6 to 7 months. This GPU uses a partial stall-feeding system.

Regarding the family composition and age of the producers, Dorantes *et al.* (2012) reported that goat producers in the southern Estado de México have an age range of 51.9±11.1 years and that their families have 6.7±2.5 members. However, there was a noticeable difference in the education level: producers from the municipality of Perote have the greatest education gap. These results match the findings of Hernández *et al.* (2013) who determined that producers from Tehuaxtla, Puebla have a maximum education level of high school. Land ownership is similar to the findings reported by Hernández *et al.* (2011) for producers from the state of Coahuila, 20% of which were ejidatarios and 60% owned small properties. Mendoza and Ortega (2009) and Hernández *et al.* (2013) reported similar goat inventories in the states of Oaxaca (53-470) and Puebla (30-110). Family members' participation in goat production has also been documented in the GPUs of Comarca Lagunera, Coahuila, and the area of the Mixteca Sierra located in the state of Puebla (Hernández *et al.*, 2011, 2013; Escareño-Sánchez *et al.*, 2011). Another contrasting aspect is the type of feeding, which can be the result of several factors, including the geographical location of the GPU. For example, the type of feeding used in the Perote GPU matches the type of feeding used by GPUs located in arid areas (Coahuila, San Luis Potosí, Nuevo León, etc.), which are limited to alfalfa, oats, maguey, and corn waste (Baraza *et al.*, 2008). The mountain mesophyll forest (MMF) vegetation may explain the greater diversity of food

Figure 2. Goat Production Unit. a) Donelo; b) Las Lajas; c) Chiviada; d) Rincón del Rio Frio; e) Hermanos Henríquez; f) Familia del Ángel.

available for goats in the Las Lajas (Coatepec), Rincón del Río Frío (Tatatila), and Chiviada (Xico) UPCs; goats can eat various fruits in this region, including toxic plants that may cause miscarriages and death (Moreno *et al.*, 2010). The reproduction and improvement results match the findings of Escareño-Sánchez *et al.* (2011), who reported births from November to February. Bucks are selected based on phenotype, race, and record; meanwhile, in the state of Oaxaca, goat producers choose goats based on visual characteristics (Mendoza and Ortega, 2009). The low number of brucellosis cases in the GPUs included in this study matches the findings of Martínez-Herrera *et al.* (2010), who reported a 0.5% occurrence

of brucellosis in the municipalities of Coatepec, Perote, Tatatila, and Xico. The producers who participated in this research were helped by technicians from the Sistema Especie Caprino from the state of Veracruz (SIPECAV A.C.); they are organized in GGAVATs (Cattle-Raiser Groups for Technology Validation and Transfer). The system used by the GPUs included in this research prevails in the states of Oaxaca, Aguascalientes, and Puebla (Mendoza and Ortega, 2009). In some cases, the milk production results (2-3 L/day) are higher than the results reported by Escareño-Sánchez *et al.* (2011) for Comarca Lagunera: a daily milk production of 1.5 L per goat and 56.9 L per flock. Goat producers who participated in this research are considered to be innovative producers, given their openness to technological changes, group activities, entrepreneurial attitude, etc. (Issaly *et al.*, 2007). For his part, only the producer from the "Donelo" GPU is considered to be a "producer-spreader", because he shares information about goat production, cheese making, and health and handling practices courses, among other activities.

CONCLUSIONS

Goat production in Veracruz is carried out in mountain mesophyll forests and xerophytic scrub, located in mountains and high plateau regions, respectively. This situation has determined the establishment of goat systems with partial or full stall-feeding arrangements. Family members participate in every aspect of this activity, including crop growing, animal handling, milk production, and artisanal cheese making. Goat inventory is below 150 heads and the average milk production ranges from 16 to 90 L per GPU.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Assessment of postoperative recovery and post-penopexy return of teaser bulls to natural mounting

Abarca-Acero, Mario A.[1*]; Arieta-Román, Ronnie de J.[1]; Lara-Rodríguez, Daniel Alejandro[1]; Fernández-Figueroa, José A.[1]

[1] Universidad Veracruzana, Facultad de Ingeniería en Sistemas de Producción Agropecuaria, Carretera Costa del Golfo km 220, Colonia Agrícola y Ganadera, Acayucan, Veracruz, México, C. P. 96100.
* Correspondence: mvzabarca87@gmail.com

ABSTRACT

Objective: To assess the perineal penopexy surgical technique in the preparation of teaser bulls for the bovine industry.

Design: To assess the technique, three 11-month-old Swiss-Zebu bovines, with an average weight of 280 kg, were subject to a surgical procedure. The assessed parameters were time of procedure, postoperative recovery (infectious processes), and return to normal mounting.

Results: This study shows a total average time —from the beginning of the procedure until the animal can stand— of 23.7 min, plus nine days of postoperative recovery, without infectious processes.

Implications: During the postoperative process, the ring might be rejected, fibrous tissue might form as a reaction to the ring's material, and the penis might be overexposed because of the ring's diameter.

Limitations: There might be limitations resulting from the surgeon's experience regarding the location of the sigmoid flexure.

Results: Using this technique contributes to the improvement of the teaser bull preparation protocols for bovine artificial insemination programs.

Key words: Animal reproduction, Estrus detection, Teaser bulls.

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INTRODUCTION

A series of physiological, zootechnical, and nutritional processes must be taken into consideration to improve the reproductive indicators of bovine cattle in their zootechnical function as teaser bulls. An inadequate management will result in reduced reproductive parameters. One of the most relevant variables is the length of estrus, which is represented in the production unit by its most important feature: the postpartum interval. Ideally, a bovine female in reproductive age is expected to give birth to one calf per year. Estrus detection is

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one of the main problems in production units where artificial insemination programs are in place. Low estrus detection levels could impact gestation up to 90 days after the cow has given birth, as well as the service rate, both of which extend the parturition-to-conception and parturition intervals. In this context, the preparation of teaser bulls may contribute to increase estrus detection in female cows by up to 80% (Hidalgo *et al.*, 2018). Reproductive biotechnologies (*e.g.*, artificial insemination (AI)) help to improve zootechnical efficiency. AI maximizes the potential of a bull with outstanding genetic characteristics by adding a genetic value: the bull's distinctive features are expanded and assessed in the number of offspring (Rosete-Fernández *et al.*, 2019).

Reproduction is one of the most important aspects when establishing the yield rate of bovine cattle productions, both in the dairy and meat industries. The traditional methods of reproduction, feeding, and management could improve the biological and economic effectiveness of bovine cattle, through the adequate application of controlled breeding techniques (Blandón-Mairena and Blandón-Palacio, 2016).

One of the most essential stages in the artificial insemination process is observing the heat. In this regard, surgically prepared males (teaser bulls) are an important tool to increase reproductive parameters within an improvement program (Silva and Pimentel, 2017). Using a teaser bull (a sterile animal) hinders the transmission of venereal diseases, while at the same time the libido remains high (Morgan and Dawson, 2008). The techniques to prepare teaser bulls can be classified into two categories: mating and emission of semen hindering (Franco-da Silva et al., 2002). Therefore, the objective of this work was to assess the perineal penopexy surgical technique to prepare teaser bulls for the bovine industry.

MATERIALS AND METHODS

The field work was conducted in three production units located in the State of Chiapas, Mexico. The Frailesca region is mainly characterized by a large agricultural activity. It is in the Sierra Madre, in the central region of Chiapas (16° 14' N, 93° 16' 09" W). It consists of 23 municipalities distributed in an area of 12,629 km^2 (16.7% of the state territory), which makes it one of the largest areas of the state. The regional seat is Tuxtla Gutiérrez. The surgical technique was performed on three bovine males of Swiss×Zebu genotype, with an average age of 15 months, and 280 kg of live weight. The animals were clinically healthy, in proper body condition, and sexually active.

Surgical technique

Surgical instruments: Needle holder, scalpel handle and blades, Kelly hemostatic clamps, Backhaus clamps, straight and curved scissors, hypodermic needles.

Surgical material and medications: Nylon surgical gloves. The following medications were used: 2% xylazine hydrochloride (tranquilizer), benzalkonium chloride and iodopovidone (disinfectant), 2% lidocaine (local anesthetic), Negasunt powder (healer, anti-insect repellent), 10% enrofloxacin (antibiotic), and flunixin meglumine (analgesic).

Patient preparation: The animals followed a 24 h fasting period for food and 12 h for water. They were tranquilized with 2% xylazine hydrochloride, using an intramuscular

dose of 0.05 mg kg^{-1} of live weight (the equivalent for the product's brand presentation is 0.25 mL per 100 kg of live weight).

Animal positioning: The animals were placed in the right lateral decubitus position with the limbs held separately to better expose the perineum. Before administering the anesthetic, the operation area of the body was prepared: a round area, two to three times larger than the surgical site, was washed with soap and water. Subsequently, the area was shaved with a razor blade and antisepsis was performed with benzalkonium chloride and iodopovidone (Figure 1).

Local anesthesia: To ensure the numbness of the surgical site, 2% lidocaine was used to block the area of the perineum where the incision would be made. The injection was administered subcutaneously with a 3.5-cm long 18-gauge needle.

Asepsis of the perineum: Once infiltration anesthesia had taken effect in the perineum, the disposable surgical drapes were put into place using Backhaus clamps.

First stage: To begin the surgical procedure, we protected the open area where the incision would be made with surgical drapes. Once the animal was in the lateral decubitus position, we rapidly located the sigmoid flexure of the penis with the help of an assistant.

Second stage: The penile flexure was located and held in a fixed position by an assistant, who maintained it in the distal third of the perineum, above its midline, approximately 10 cm above the scrotal neck (Figure 2).

Third stage: We made two small incisions (2 cm) on each side of the penis or median raphe.

Fourth stage: We inserted a trocar through the two lateral incisions and mounted the flexure on it (Figure 3).

Fifth stage: We removed the trocar and placed the previously disinfected nasal ring, which remained fixed once its corresponding screw was closed.

Sixth stage: Only part of the nasal ring remained exposed. The lateral wounds healed by second intention (Figure 4).

Figure 1. Preparation of the perineum with the animal in the decubitus position.

Figure 2. Identification of the sigmoid flexure. a) Palpation of the flexure, where the nasal ring will be inserted; b) placement of surgical drape.

Figure 3. a) Incision on the distal part of the sigmoid flexure; b) subsequently, blunt scissors inserted through the incision served as guide for the nasal ring.

Figure 4. Nasal ring placed on the sigmoid flexure for anatomical fixation.

Seventh stage: We cleaned the surgical area with benzalkonium chloride and applied an antibiotic spray (oxytetracycline). The males that underwent surgery were kept in a clean place. Although the technique is minimally invasive, supervising cicatrization is crucial. We administered IM antibiotics for five days.

RESULTS AND DISCUSSION

Results indicated that perineal penopexy has a rapid recovery rate due to its low invasiveness. There was no significant tissue damage, and the average recovery time was eight days. According to Vizcarra *et al.* (2011), other surgical techniques used to prepare teaser bulls —such as the surgical removal of the penile dorsal apical ligament— have shown a longer recovery time.

Recovery time

Table 1 shows an average total surgery time of 23.7 min, including preoperative and intraoperative care, as well as patient recovery.

Macroscopic changes after the procedure

Table 2 shows the cardinal signs of inflammation (redness, heat, swelling, and pain).

Table 3 shows macroscopic changes on the incision site, eight days after the procedure.

Postoperative assessment at 90 days of animals that underwent surgery showed no physiological changes concerning loss of libido, and total exposure of the penis was observed. As for complications resulting from the technique, one animal presented exudative secretion that did not compromise its physiological state (Table 3). The investment required for this surgical technique is very low and all three bulls fulfilled their zootechnical function as teaser bulls (Table 4).

Table 1. Assessed indicators and average clinical results.

Animal number	Preoperative time (min)	Transoperative time (min)	Recovery time (min)
1	25	40	20
2	20	30	20
3	30	15	10
Average time	25	28.3	16.6

Table 2. Physiological indicators of inflammation.

Animal number	Blushing	Pain	Warmth	Tumor
1	Pale pink	Moderate	Moderate	Absence
2	Pale pink	Moderate	Moderate	Absence
3	Pale pink	Moderate	Moderate	Absence

Table 3. Macroscopic changes on the incision site.

Animal number	Wound healing	Macoscopic changes
1	Tissue with the presence of exudate	Presence
2	Healthy tissue	Presence
3	Healthy tissue	Presence

Table 4. Total costs of the penopexy technique in Mexican pesos ($20.00 equivalent to one USD).

Drug/Supplies	Supplies costs ($Mex)	Quantity	Total costs of Tx/8 days ($Mex)
Oxytetracycline-topical antiseptic	8.6	8 atomizations	68.8
Enrofloxacin 10%	4.2	25 mL	110
Xylazine hydrochloride	3.3	2 mL	6.6
Syringes	10 each	4 pieces	40
Surgical gauze	2.2 each	10 pieces	22
18×3.2 cm gauge needles	1	8 pieces	8
Scalpel blades	8	1 piece	8
Metamizole sodium	0.7	60 mL	42
Povidone-iodine	1.7	10 mL	17
Benzalkonium chloride	0.02	200 mL	4
Copper ring	170	1	170
Total			496.4

CONCLUSIONS

The results of this study uphold our conclusion that the perineal penopexy surgical technique is highly efficient in the preparation of teaser bulls. It is minimally invasive, the surgery time is short, and the investment required to prepare males decreases. Moreover, it presents a favorable recovery rate: animals are ready to fulfill their corresponding zootechnical function within a 15-day period.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Trends and research on Covid-19 and farmers using VOSviewer

Ramírez-Vásquez, J. Daniel[1]; Figueroa-Rodríguez, Katia A.[1*]; Velasco-Velasco, Joel[1]; Aguilar-Rivera, Noe[2]

[1] Colegio de Postgraduados Campus Córdoba. Carretera Córdoba-Veracruz. Congregación Manuel León, Municipio de Amatlán de los Reyes km 348, Veracruz, México. C.P. 94946.
[2] Universidad Veracruzana Facultad de Ciencias Biológicas y Agropecuarias, km 1 carretera Peñuela-Amatlán, 94945, Córdoba, Veracruz, México.
* Correspondence: fkatia@colpos.mx

ABSTRACT

Objective: The aim of this study was to use bibliometric analysis to provide an overview of the empirical and theoretical research that has been carried out regarding COVID-19 focusing on scientific publications on the topic of farmers.

Design/methods/approach: The global literature on COVID-19 and agricultural producers (farmers) published between 2019 and 2022 (August 8), was obtained from the SCOPUS database, comprising a total of 665 documents. VOSviewer was used to perform a bibliometric analysis of these papers.

Results: The two countries that published the most research related to the terms studied were the United States and India. Research conducted in these countries was found in the most cited studies. The studies focused on five major topics: agriculture, epidemiology, psychology, economic impact, as well as rural areas and risk determination. The evolution of the topics over time showed that the research originally began with health-oriented studies, and that once the protocols for the return to normal were generated, studies were carried out to visibilize the producers and their challenges during the pandemic in addition to the support strategies that were generated and the impact that the pandemic had on them, as well as on the local, regional, national, and global economy.

Limitations/implications: The documents analyzed are exclusive to the SCOPUS database, so literature was excluded from other sources such as Google Scholar or Web of Science, which could contain important information on the subject in relation to other disciplines.

Findings/conclusions: This type of study makes it possible to better understand the current state of the art regarding the effect of COVID-19 on the agri-food sector, thus allowing researchers to visualize the relevance of, and guide, their research on the topic.

Keywords: bibliometric analysis; resilience; farming; SARS-CoV-2; pandemic; farm workers.

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INTRODUCTION

In January 2020, the WHO defined SARS-CoV 2 as a new disease caused by the coronavirus or COVID-19 (Radulova *et al.*, 2020). This disease spread rapidly among the population, causing symptoms similar to those of atypical pneumonia (Shirani and Toghyani, 2020). It became a global pandemic that led to the greatest economic, social, and health crisis of the modern era. It is estimated that at least 10% of the world population contracted the disease (Mora-Alvarado, 2022), causing the governments of each country as well as international organizations to urgently implement various strategies to mitigate the effects and adapt to this new reality and global emergency (Davila *et al.*, 2021).

The COVID-19 pandemic resulted in movement restrictions and changes in consumption habits (González-Alejo *et al.*, 2020), which socioeconomically affected the

population and various sectors around the world (Lopez-Ridaura *et al.*, 2021). The agri-food sector was no exception (Lopez-Ridaura *et al.*, 2021). Agribusiness, the supply of inputs, and sales of agricultural products were interrupted (Amjath-Babu *et al.*, 2020). The restrictions on ports and borders, the curfews, as well as social distancing reduced productivity and therefore the competitiveness of the agri-food sector (Rahimi *et al.*, 2022). Agricultural producers were one of the most vulnerable actors because in some cases they did not stop their activity, putting themselves at risk. At the same time, those who limited social contact in their capacity as food suppliers caused a negative effect (Triana *et al.*, 2021).

Due to the importance of this global phenomenon, unprecedented scientific research related to COVID-19 was conducted in various countries (Lou *et al.*, 2020). The literature on COVID-19 is relatively recent (since 2019), but abundant, with over 350,000 publications in the Scopus database. Nearly 10% are literature reviews. This type of publication allows scientists to learn about general aspects of the disease (Lai *et al.*, 2020) and its impact on the global population (Nicola *et al.*, 2020).

Bibliometric analysis is a method that determines scientific activity by quantitatively analyzing scientific publications (Soytas, 2021). One of the first bibliometric analyses regarding COVID-19 was carried out by Al-Zaman (2020), who concluded that the most numerous articles were those related to medicine. Zyoud *et al.* (2022) found that studies related to clinical research were the most frequent. Other very recent bibliometric analyses regarding COVID-19 focused on topics such as: business and management (Verma and Gustafsson, 2020); supply chains (Fabeil *et al.*, 2023); agrifood chains (Das and Roy, 2022); nutrition (Zyoud *et al.*, 2022); tourism (Viana-Lora and Nel-lo-Andreu, 2022); agricultural production and food security (Okolie and Ogundeji, 2022); social sciences (Singh and Verma, 2022); education (Zhang *et al.*, 2022); and nanotechnology (Lunardi *et al.*, 2022). Despite the availability of previous reviews, there are, to our knowledge, no publications that address the topic of farmers. Hence, the objective of this study was to use bibliometric analysis to provide an overview of the empirical and theoretical research that has been conducted on COVID-19 with an emphasis on scientific publications that address the topic of farmers.

MATERIALS AND METHODS

The data used in this paper was obtained from the Scopus database (www.scopus.com), one of the largest indexed databases in the world (Hamidah *et al.*, 2020). The words entered in the Scopus search engine on August 8, 2022 were 'COVID-19' and 'farmers'[1]. Of the 710 documents found, only articles, reviews, conference papers, and book chapters were considered in the study, because they have the greatest scientific impact. In the end, 665 documents were retained.

In order to improve the consistency of the results, the database was homogenized (Niñerola *et al.*, 2019); for example, the word COVID-19 was used for all words related

[1] The word 'farmers' was used in order to represent agricultural producers.

to the disease such as: 'coronavirus', 'coronavirus disease', 'coronavirus infection', 'sars-cov-2-19', 'betacoronavirus', and 'covid-19 pandemic'; plural words were replaced by the singular, for example 'coping strategies' by 'coping strategy', 'fruits' by 'fruit', and 'animals' by 'animal'.

Content analysis

VOSviewer version 1.6.9 (Centre for Science and Technology Studies, 2018) was used for metadata analysis. This software builds and visualizes bibliometric networks (van Eck and Waltman, 2010). An analysis of the co-occurrence of keywords and academic terms in the titles and abstracts of the publications was carried out, using the co-occurrence method, showing only related elements, and using the association strength (AS) normalization method, a resolution of 1.00, a visualization scale of 100%, TLS weight, a label variation size of 50%, and a kernel width of 30% (van Eck and Waltman, 2010). For the analysis, only those that coincided at least 5 times in the count were considered. Of the 3996 results, only 205 were below this threshold. Generic words such as 'COVID-19', 'human', 'pandemics', 'farmer', 'survey', and 'article' were removed from the software base.

RESULTS AND DISCUSSION

A total of 665 documents were analyzed, of which 78% were articles, 13% were conference papers, 7% review articles, and 3% book chapters. The aim was to use the bibliometric analysis to visualize the empirical and theoretical research that has been conducted regarding COVID-19 with an emphasis on those that have addressed the topic of farmers.

Performance analysis

Table 1 presents the 10 main scientific journals and the countries with the highest number of publications related to COVID-19 and farmers. A total of 367 documents were found. The source with the highest number of publications was IOP Conference Series: Earth and Environmental Science, which has an SJR-2021 indicator of 0.20. It should be noted that these are conference papers, which makes sense due to the time required to publish in scientific journals, providing the scientific community with information on the subject in a shorter amount of time. It is also consistent with the fact that these conference papers have been cited 22,362 times. The topics addressed by the main sources, which, except for the case described above, are scientific journals, focus on aspects related to health sciences, sustainability, and the agri-food sector, in addition to multidisciplinary topics. The journals with topics related to health sciences were: *Journal of Agromedicine, Frontiers and Public Health*, and *International Journal of Environmental Research and Public Health*; those with topics related to sustainability were *Sustainability, Frontiers in Sustainable Food Systems*, and *Food Security*; those with topics related to the agri-food sector were *Agricultural Systems* and *Indian Journal of Animal Sciences*; finally, the multidisciplinary scientific journal *PLOS ONE*.

192 countries were listed, of which 108 had at least one publication, with the United States, India, and China being the most productive (Figure 1). The United States was the country with the highest number of documents, with 19% of the total publications; this is because it is a leading country in science and innovation. Regarding affiliation, the documents came from 676 different affiliations, none of which was outstanding in terms of the number of publications.

Table 2 shows the ten most cited documents. Of the total number of documents, 401 were cited at least once, accumulating a total of 4395 citations. Only three documents had

Table 1. Performance analysis: number of publications by source and country.

Rank	Source	NPs	Country	NPs
1	IOP Conference Series: Earth and Environmental Science	36	USA	125
2	Sustainability	25	India	118
3	Agricultural Systems	29	China	75
4	Journal of Agromedicine	12	Indonesia	74
5	Frontiers in Sustainable Food Systems	10	United Kingdom	47
6	Food Security	10	Australia	27
7	Indian Journal of Animal Sciences	10	France	23
8	PLOS ONE	8	Germany	22
9	Frontiers in Public Health	8	Netherlands	22
10	International Journal of Environmental Research and Public Health	7	Nigeria	22

NPs: number of publications.Source: Own elaboration with SCOPUS data (August 8, 2022).

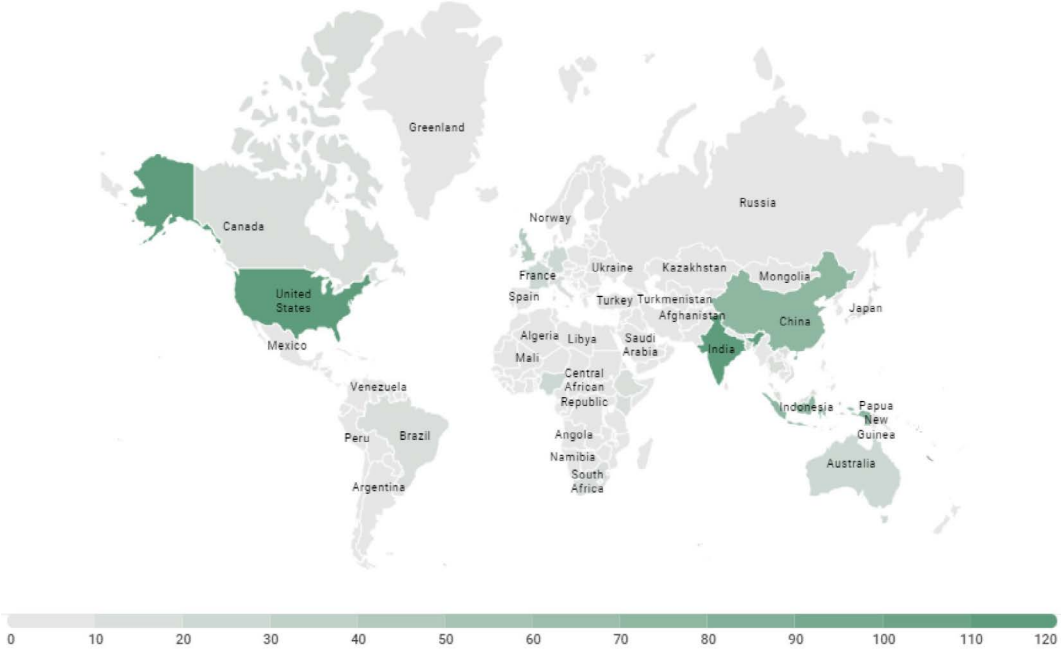

Figure 1. Worldwide distribution of publications related to research on COVID-19 and farmers. Source: Own elaboration with SCOPUS data (August 8, 2022).

more than 100 citations. Among the most cited documents, seven correspond to research articles and three to literature reviews. The articles deal with various topics; some do not involve farmers but rather the general population, for example, Di Renzo *et al.* (2020) studied the impact of the pandemic on eating habits; Kansiime *et al.* (2021), evaluated the effects of the pandemic on household income and food security; Li *et al.* (2020) determined the behavior of consumers purchasing grocery products; and Abedin *et al.* (2021) identified trends in the public's willingness to be vaccinated. The articles that focused on the topic of farmers were as follows: Harris *et al.* (2020) evaluated the impact of the pandemic on the livelihoods and dietary effects of vegetable producers; Ceballos *et al.* (2020) determined the impact of the pandemic on farmer income; and finally one article focused on the agri-food sector: Mahajan and Tomar (2021) analyzed the disruption of food supply chains due to the economic shutdown caused by COVID-19.

Table 2. Most cited documents on COVID-19 and farmers (August 8, 2022).

#	Title	Document type	Authors (year)	Source	Citations
1	Eating habits and lifestyle changes during COVID-19 lockdown: An Italian survey	Article	Di Renzo *et al.* (2020)	Journal of Translational Medicine	815
2	Impact of COVID-19 on the food supply chain	Literature review	Aday and Aday (2020)	Food Quality and Safety	203
3	COVID-19 implications on household income and food security in Kenya and Uganda: Findings from a rapid assessment	Article	Kansiime *et al.* (2021)	World Development	138
4	Challenges to the poultry industry: current perspectives and strategic future after the COVID-19 outbreak	Literature review	Hafez and Attia (2020)	Frontiers in Veterinary Science	80
5	Food system disruption: initial livelihood and dietary effects of COVID-19 on vegetable producers in India	Article	Harris *et al.* (2020)	Food Security	77
6	Impacts of a national lockdown on smallholder farmers' income and food security: Empirical evidence from two states in India	Article	Ceballos *et al.* (2020)	World Development	71
7	Changing grocery shopping behaviours among Chinese consumers at the outset of the COVID-19 outbreak	Article	Li *et al.* (2020)	Tijdschrift voor Economische en Sociale Geografie	70
8	COVID-19 and supply chain disruption: evidence from food markets in India	Article	Mahajan and Tomar (2021)	American Journal of Agricultural Economics	65
9	Willingness to vaccinate against COVID-19 among Bangladeshi adults: Understanding the strategies to optimize vaccination coverage	Article	Abedin *et al.* (2021)	PLOS ONE	64
10	Preliminary report of an outbreak of SARS-CoV-2 in mink and mink farmers associated with community spread, Denmark, June to November 2020	Literature review	Larsen *et al.* (2021)	Eurosurveillance	56

Source: Own elaboration with SCOPUS data (August 8, 2022).

The literature reviews also focused on various topics; the most cited review addressed a more general topic. In other words, it focused on the impact of COVID on agrifood chains (Aday and Aday, 2020); another review addressed the challenges of the poultry industry in the face of the pandemic (Hafez and Attia, 2020); and the topic of the third was mink-farm-owning producers in Denmark and how COVID-19 spread between farms (Larsen *et al.*, 2021).

Scientific mapping

The VOSviewer results established 3996 keywords, of which those with co-occurrences greater than five were retained, forming a total of 5 clusters with 205 links. Figure 2 shows these clusters as follows: the first (red) refers to agriculture; the second (green) to epidemiology; the third (blue) brings together topics related to psychology (occupational and mental health, poverty, and migration); the fourth (yellow) deals with economic impact; and the fifth (purple) covers topics related to rural areas and risk determination.

Figure 3 shows that the publications on research related to COVID-19 and farmers are recent. They began in 2019, a few months after the appearance of the disease, which is an indicator that the scientific community quickly started publishing information about the disease and assessing the impact that COVID-19 had on the population and the world economy.

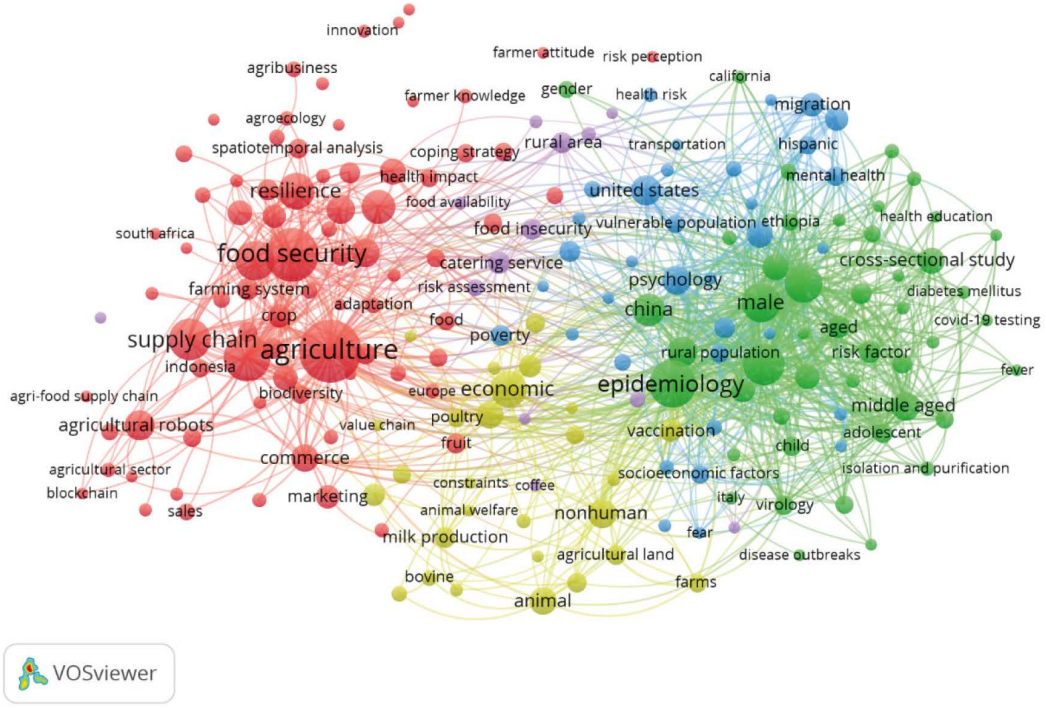

Figure 2. Network visualization of COVID-19 and farmers (665 documents) using the VOSviewer program. The following terms were removed: 'COVID-19', 'human', 'article', 'farmer', 'pandemic', and 'survey'. Source: Own elaboration with SCOPUS® data (August 8, 2022).

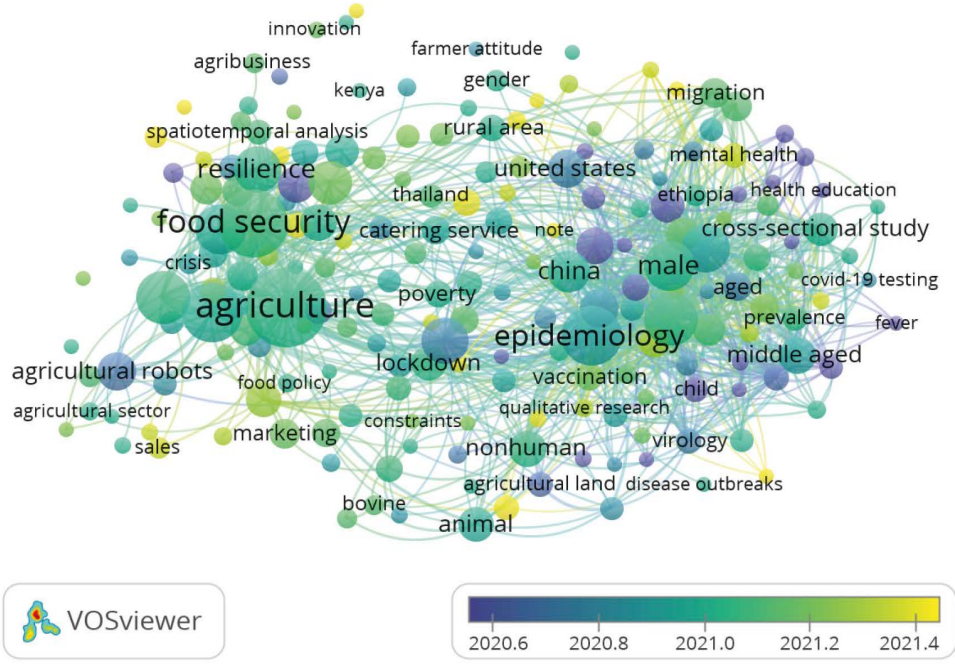

Figure 3. Overlay visualization of COVID-19 and farmers (325 documents), using the VOSviewer program. The following terms were removed: 'COVID-19', 'human', 'article', 'farmer', 'pandemic', and 'survey'. Source: Own elaboration with SCOPUS® data (August 8, 2022).

The published research on COVID-19 and farmers has focused mainly on five aspects: the first being issues related to agriculture, the second to epidemiology, the third to economic impact, the fourth to livestock, and the fifth to the rural population and food insecurity.

Agriculture, food security, and resilience

The publications analyzed in this cluster focus mainly on the effects of the pandemic on food security, supply chains, and the role of resilience in agricultural systems as well as for farmers.

Food security refers to physical and economic access to sufficient, safe, and nutritious food (Rozaki, 2020). The application of strict measures during the pandemic caused significant repercussions on agriculture and therefore on food security (Kansiime *et al.*, 2021). This crisis exposed the fragility of the farmers' livelihoods, threatening their food security and well-being (Kumar *et al.*, 2020). As agriculture is one of the main components of agri-food supply chains, the analysis of the negative effects of COVID-19 on these chains became relevant (Kumar and Kumar Singh, 2021).

With regard to resilience, Ahmed *et al.* (2020) defines this term as the capacity of a system to absorb disturbances and maintain the same structure and functions. Studies on resilience focused mainly on agricultural systems (Dixon *et al.*, 2021; Middendorf *et al.*, 2021), agricultural markets (Varshney *et al.*, 2020), and the creation of adequate public policies in order to mitigate the effects caused by the COVID-19 crisis (Kumar and Kumar Singh, 2021).

Epidemiology

Since COVID-19 is an infectious disease, one of the biggest challenges was understanding how transmission occurred between humans and whether animals were also transmitters or at what points in agricultural and market activities humans could become infected. The most cited study in this cluster was that of Larsen *et al.* (2021), who implemented an epidemiological surveillance system around mink farms in order to determine infections in the community. They found that the minks were infected and developed a COVID-19 strain that could in turn be retransmitted to humans. Other studies related to the topic have been carried out in countries such as China (Li *et al.*, 2020), the United States (Quandt *et al.*, 2021), and India (Ceballos *et al.*, 2020).

Psychology, mental health, and vulnerable populations

The publications that belonged to this cluster analyzed the impact that COVID-19 had on different aspects of human life; for example, on mental health (Paul *et al.*, 2021), access to health services (Quandt *et al.*, 2021), resilience (Dixon *et al.*, 2021), and consumption habits (Paul *et al.*, 2021).

Later studies focused on agricultural workers who continued to work when carrying out essential activities, so the researchers reported them as invisible and unprotected workers who needed to be visibilized and studied (Dudley, 2020). Once the impact of COVID-19 on the sector was established, researchers began to propose support models for farmers and inhabitants of rural areas (Ramos *et al.*, 2020).

In a third phase, and as vaccines were developed, a series of studies focused on understanding the reluctance of farmers and inhabitants of rural areas to be vaccinated, in order to propose strategies that would allow for the optimization of vaccination coverage (Abedin *et al.*, 2021).

Economic impact

The documents in this cluster focused on analyzing the impact that COVID-19 had on the economy at the global, national, and individual levels with an emphasis on the agri-food sector. At the global level, Mohapatra *et al.* (2020) described the impact on the agricultural sector and on the economy in general. At the national level, for example, Pan *et al.* (2020) determined the impact on China's agricultural economy; Varshney *et al.* (2020) evaluated the impact on the price of agricultural products and on market chains in India; and Dokić *et al.* (2020) conducted a study on the impact on Croatia's trade balance. At the individual level, Jaacks *et al.* (2021) evaluated whether there was an effect on farmer income, and Quandt *et al.* (2021) determined whether there were negative effects on the incomes of Latino families in urban and rural areas, just to mention some of the studies.

Rural areas and risk determination

The smallest cluster grouped few concepts, the most relevant of which was rural areas. This included aspects related to rural inhabitants such as agricultural workers, agribusiness, and agrifood chains. These studies highlighted the fact that the population in rural regions was particularly vulnerable to infection by COVID-19 due to misinformation or the need

to work, while adults whose children had migrated to cities were more likely to suffer from isolation (Meredith *et al.*, 2020). In some cases related to agrotourism, the negative impact of the pandemic was reported, along with strategies recommended for owners in order to create alternative sources of income (Zanetti *et al.*, 2022).

Risk was a topic related to rural areas. The studies addressed aspects such as resilience and risk factors for transmission of COVID-19. For example, in one study conducted in a market in China (Li *et al.*, 2021), the authors observed that most of the positive samples were found in the aisles of the market, with multiple microspheres in various routes, as well as on bathroom handles and other frequently touched surfaces. They also found positive samples on products for sale (aquaculture, soy-based products), so transmission occurred through droplets or fomites (passive vectors such as viruses, fungi, or parasites). This type of study allowed governments to establish disinfection policies and maximum capacity in public spaces, and also make general recommendations for the population to stay at home and disinfect the products they purchase in the market.

CONCLUSIONS

Bibliometric studies are a powerful tool that make it possible to systematize large amounts of research to determine trends and establish the relevance of different lines of research. In this study, we conducted a bibliometric analysis of the terms 'COVID-19' and 'farmers', using data from the titles, abstracts, and keywords of articles published in major journals and other peer-reviewed documents, available in the SCOPUS database from 2019 to 2022 (August 8). The two countries that published the most research related to the terms studied were the United States and India. Research conducted in these countries was found in the most cited studies.

The studies focused on five major topics: agriculture, epidemiology, psychology, economic impact, as well as rural areas and risk determination. The evolution of the topics over time showed that the research originally began with health-oriented studies, and that once the protocols for the return to normal were generated, studies were carried out to visibilize the producers and their challenges during the pandemic in addition to the support strategies that were generated and the impact that the pandemic had on them, as well as on the local, regional, national, and global economy. This type of study makes it possible to better understand the current state of the art regarding the effect of COVID-19 on the agri-food sector.

The limitation of the study is the fact that the documents analyzed are exclusive to the SCOPUS database, so literature from other sources such as Google Scholar or Web of Science, which could contain important information on the subject, was excluded. Therefore, it is suggested that further studies analyzing these databases be conducted and that other, more multidisciplinary approaches be taken.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Paula Poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) indoor variety

García-Pérez, Faustino[1]; Canul-Ku, Jaime[1*]; Barrios-Gómez, Edwin J.[1]; Portas-Fernández, Blanca[2]; Rangel-Estrada, Sandra E.[1]

[1] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Zacatepec de Hidalgo, Morelos, México, C.P. 62780.
[2] Profesional independiente
* Correspondence: canul.jaime@inifap.gob.mx

ABSTRACT

Objective: To evaluate the behavior of the Paula poinsettia variety (*Euphorbia pulcherrima* Willd. ex Klotzsch) and to describe its characteristics, based on the guidelines established by the International Union for the Protection of New Varieties of Plants (UPOV).

Design/Methodology/Approach: The genetic improvement included hybridization and selection techniques. To promote compaction and greater branching, it was grafted on a commercial variety. The cuttings obtained gave rise to the Paula variety (*Euphorbia pulcherrima* Willd. ex Klotzsch), which was described in 2020, based on the Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability of the International Union for the Protection of New Varieties of Plants. A completely randomized experimental design with 10 replications was used. Vegetative and bract characteristics were recorded and an analysis of variance was applied, as well as a Tukey's mean comparison test ($P \leq 0.05$).

Results: The Paula variety is medium-sized and has a wide breadth and medium branching. It is pale green in the middle third of the stem. It has oval-shaped, long, and broad leaves of the same medium-intensity color, and a wedge-shaped base. It has a long petiole with a pale green upper side. This variety also has many elliptical red bracts of medium length and narrow width. Compared to other INIFAP varieties, the vegetative and bract characteristics of Paula presented statistical differences.

Study Limitations/Implications: The commercial production of poinsettia is carried out through asexual propagation. The mother plant is kept in a vegetative state, through comprehensive and intensive management, preventing its flowering and obtaining a continuum of propagation material.

Findings/Conclusions: Paula has an intermediate cycle, with quality and competitive size, branching, and color of the bract (red). It can satisfy the taste and preference of consumers in the domestic and even foreign markets.

Keywords: Poinsettia, genotype, graft, clone, bract.

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INTRODUCTION

In Mexico, 16,268,524 finished poinsettia plants —from 30 varieties developed abroad— were produced in 2020. Ninety percent of the plants has red bracts and the remaining percentage is made up of yellow, pink, white, striped, and marbled tones. In Mexico City, Jalisco, Michoacán, the State of Mexico,

Morelos, Oaxaca, and Puebla, 240.6 hectares are used to grow poinsettias. Morelos was the main producer with 6,549,818 plants: approximately 40.3% of the national production (SADER, 2020).

E. pulcherrima is a tropical Euphorbiaceae native to Mexico (Canul *et al.*, 2013). However, the 30+ commercial varieties that are grown in the country have been developed abroad (SADER, 2020). This situation has the following consequences: 1) technological dependency; 2) right payment for the use of the variety, included in the cost of the cutting; 3) capital flight; 4) economic losses, resulting from pigmentation outside the commercial period; and 5) restrictions to the importation of vegetative material of new varieties (García-Pérez *et al.*, 2020).

In order to lessen the aforementioned consequences, the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), started in 2010 the Programa de Mejoramiento Genético de Nochebuena, at its Campo Experimental Zacatepec, located in Morelos, (Canul *et al.*, 2017a). To establish a broad genetic base —an essential requirement in any genetic improvement program—, germplasm (seeds and twigs) was collected in 10 Mexican states (Canul *et al.*, 2013). Their description was based on the morphological characteristics of their stem, leaf, bract, and plant (Canul *et al.*, 2014), applying the graphic handbook for variety description of poinsettia (Mejía *et al.*, 2006) and the Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability of plant organisms (UPOV, 2008). Subsequently, the scientific bases for hybridization were established (Canul-Ku *et al.*, 2015), which allowed the crossing of parents with contrasting characteristics. The outstanding genotypes were evaluated in the C. E. Zacatepec (Canul-Ku *et al.*, 2017a) and in Tetela del Monte, Cuernavaca, Morelos (Canul-Ku *et al.*, 2017b). Grafting techniques were defined to support the genetic improvement of poinsettia (García-Pérez *et al.*, 2017), in order to promote compaction and a greater plant branching (García-Pérez *et al.*, 2019, 2020).

After a 10-year research about the *E. pulcherrima* genetic improvement, one of the results obtained is the varietal description of the material called Paula, as well as its evaluation and comparison with other varieties. Given the quality and competitiveness of its size, branching, and color of the bract (red), its cultivation and commercialization at national and international level are a viable option, which benefits different links in the poinsettia production chain.

MATERIALS AND METHODS

Table 1 shows the process used to obtain the indoor variety of the Paula poinsettia. Manual crossing between the OAX10 and MORPR13 parents was performed in 2013. The F_1 progeny were evaluated in 2014 and the best plants were selected based on the poinsettia ideotype established by Canul-Ku *et al.* (2017a). From 2014 to 2017, in the C. E. Zacatepec (18° 39' 16" N, 99° 11' 54" W, altitude 910 m.a.s.l.), the number of plants was increased by vegetative propagation, during four selection cycles, applying the poinsettia production technological package (García *et al.*, 2017).

Table 1. Process used to obtain the Paula poinsettia indoor variety.

Year	Generation	Process
2013	OAX10 X MORPR13 ↓	Hybridization
2014	F_1 seed ↓	Evaluation and selection
2014-2017	F_1 clonal ↓	Increase of plants and selection
2018	F_1 clonal /commercial variety ↓	Graft
2019	Graft F_1 clonal ↓	Evaluation and selection
2020	Paula	Varietal description

In 2018, the clonal hybrid progeny was grafted onto a commercial variety (pattern) using the wedge technique, in Tetela del Monte, Cuernavaca, Morelos (García-Pérez *et al.*, 2017). In 2019, the obtained cuttings were rooted and cultivated in Tehuixtla, Jojutla de Juárez, Morelos (18° 36' 21" N, 99° 11' 55" W, 870 m.a.s.l.), until they reached the final stage. Finally, in 2020, the varietal description of *Euphorbia pulcherrima* Willd. ex Klotzsch was made applying the Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability (UPOV, 2008).

In 2020, ten Paula poinsettia plants (*i.e.*, ten repetitions) were established in 15.24 cm wide×11 cm high pots (1.61 L); control consisted of an equal number of pots with the Alhely, Beatriz, and Vicky varieties from INIFAP, which were likewise planted in Tehuixtla. A completely randomized experimental design was used. The plants were pruned once and, according to the UPOV guidelines (2008), growth regulators were not applied —a common practice in the commercial management of the crop. Vegetative and bract characteristics were recorded: plant height, stem diameter (mm), number of branches, number of internodes, leaf length (cm), leaf width (cm), leaf petiole length (cm), bract length (cm), bract width (cm), bract petiole length (cm), bract canopy width, and cyatium diameter (cm). An analysis of variance and a Tukey's mean comparison test ($P \leq 0.05$) were performed using the SAS 8.1 statistical software (SAS, 2000).

RESULTS AND DISCUSSION

Characteristics

The Paula poinsettia is medium-sized and has a wide breadth and medium branching. It is pale green in the middle third of the stem. It has oval-shaped, long, and broad leaves of the same medium-intensity color, and a wedge-shaped base. It has a long petiole with a pale green upper side. This variety also has many elliptical red bracts of medium length and narrow width (in both cases including the petiole). The color of the upper side of the bract is 45 B and of the underside is 46 C, based on the Royal Horticultural Society color chart (RHS, 2007). Paula reaches the bract pigmentation phenological stage 8 to 9 weeks after autumn begins; consequently, it is classified as an intermediate cycle variety (UPOV, 2008) (Figure 1).

Figure 1. Canopy of the Paula poinsettia indoor variety.

Response

Highly significant statistical differences were found in plant height, stem diameter, number of branches, leaf petiole length, bract length, bract width, and bract canopy width. Statistically significant differences were obtained regarding the number of internodes, leaf length, bract petiole length, and cyathium diameter. No significant differences were found in leaf width.

In terms of plant height, Paula and Beatriz belong to the same statistical group. However, the former was 5.3 cm smaller than the latter, a relevant aspect in ornamental species grown in containers (Table 2). The compact size of this poinsettia its appropriate for its commercial presentation and the transportation of the finished plant to the point of sale.

Table 2. Means comparison of vegetative and bract characteristics between the poinsettia Paula variety and control INIFAP varieties.

Caracter/descriptor	Paula	Alhely	Beatriz	Vicky	DMSH[1]	CV (%)[2]
Plant height (cm)	38.5 b[3]	59.9 a	43.8 b	57.4 a	0.80	9.10
Stem diameter (mm)	10.9 b	14.5 a	10.7 b	13.3 a	0.70	8.93
Number of branches	6.4 b	6.3 b	6.7 b	12.2 a	0.63	25.34
Number of internodes	17.8 ab	18.2 a	14.8 b	17.4 ab	0.23	15.17
Leaf length (cm)	13.0 a	12.1 ab	12.1 ab	10.9 b	0.27	10.70
Leaf width (cm)	8.6 a	7.9 a	7.7 a	7.7 a	0.10	12.92
Leaf petiole length (cm)	4.7 a	4.2 a	3.8 ab	3.2 b	0.36	19.14
Bract length (cm)	11.5 a	9.5 b	10.2 ab	11.9 a	0.32	13.75
Bract width (cm)	5.6 b	5.6 b	4.3 c	7.2 a	0.64	14.57
Bract petiole length (cm)	1.9 b	2.0 ab	2.5 a	2.4 ab	0.24	21.86
Bract canopy width (cm)	28.9 a	22.0 b	29.2 a	27.0 a	0.48	11.85
Cyathium diameter (cm)	4.5 ab	3.4 ab	5.6 a	2.7 b	0.25	47.83

[1]DMSH=Honest Least Significant Difference, [2]CV=Coefficient of variation. Values with different letters within rows indicate significant differences (Tukey, 0.05).

Paula had the longest leaves (13.0 cm) and leaf petioles (4.7 cm), although it is statistically similar to Alhely and Beatriz. Regarding bract length and bract canopy, it was also similar to Beatriz and Vicky. However, Paula recorded the lowest averages in bract width and bract petiole length. Alhely had the largest stem diameter (14.5 mm), as well as the higher number of internodes (18.2).

The commercial value of a poinsettia plant lies in its aesthetics: compact size, greater branching, and bract color (mainly red). Figure 2 shows the Paula variety ready for commercialization; we foresee a good acceptance among producers, propagators, and consumers.

CONCLUSIONS

Paula is a poinsettia indoor variety with an intermediate cycle. It is medium-sized and has a wide breadth and medium branching. It has many red, elliptical bracts, of medium length and narrow width. Its quality and competitiveness can satisfy the taste and preference of consumers in the domestic and even foreign markets. It can also benefit different links in its production chain.

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Figure 2. Front view of the Paula poinsettia indoor variety.

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