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Effect of two enzyme systems on the removal of mucilage from coffee cherry beans (*Coffea arabica* L.)

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ABSTRACT

Objective: To evaluate and compare the percentage of mucilage removal from coffee (*Coffea arabica* L.) in pulping using two enzyme systems, Celuzyme and Macerex PM.

Design/methodology/approach: Seven treatments combinations were evaluated (type of enzyme and concentration level, plus a control) at 30 min intervals for 3 h. The experimental unit was 0.2 kg of pulped coffee.

Results: Results showed when using these enzyme systems (Macerex PM and Celuzyme) the percentage of mucilage removal increased and time was significantly reduced by 3 to 4 h compared to the natural fermentation time of 15 to 20 h.

Limitations on study/implications: Effect of two enzyme systems, Macerex PM and Celuzyme, at different concentrations (mg L^{-1}).

Findings/conclusions: The Macerex PM and Celuzyme enzyme systems showed 95% and 84.5% removed mucilage compared to 35% of the control.

Keywords: Degumming process, pectinase, cellulase, hemicellulase, fermentation time.

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INTRODUCTION

The process of transforming cherries coffee into parchment coffee consists of separating the seed from the pulp. This process can be carried out by two methods; the wet method begins with collecting and sorting coffee beans at optimum ripeness for pulping, mucilage removal and drying, whereas the dry method is based on drying coffee cherries in the sun for a prolonged time (Correa *et al.*, 2014).

Currently, Mexico ranks eleventh in terms of volume of coffee production after Brazil, Vietnam, Colombia, Indonesia, Honduras, Ethiopia, India, and Uganda, with a production volume of between 3 and 4 million sacks (60 kg per sack) per year (SIAP, 2019; ICO, 2019). Countries like Colombia and Ecuador have applied pectolytic enzyme systems and specific

Image by vandelino dias Junior at Pixabay

products to reduce the fermentation time in degumming coffee cherries, which they have achieved. These enzyme systems contain polygalacturonase and pectinesterase. Pectinase was developed especially for the degumming of coffee beans.

Users who use wet processing in Mexico have not paid much attention to automation and/or modernization. As it is well-known, water is the main element in the wet processing method since it lubricates, transports, washes and facilitates the processing operations. The traditional fully washed technique without recycling uses 4 to 5 m^3 of water per quintal of processed coffee cherry beans (1 quintal of coffee cherry beans=250 kg) whereas modern mechanical mucilage removal machines producing semi-washed coffee use only about 0.25 to 0.5 m^3 of water per quintal of processed coffee cherry beans (Enden & Calvert, 2010).

Thus, in the state of Veracruz, processing 1.2 million quintals of coffee cherry beans demands about 6 million cubic meters of water, which is used and returned to the water bodies with a high degree of contamination of organic origin (SIAP, 2019). The objectives of this research were: 1) To evaluate and compare the percentage of mucilage removal from coffee (*Coffea arabica* L.) in pulping using two enzyme systems, Celuzyme and Macerex PM and 2) Select the best enzyme system for removing mucilage from coffee beans.

MATERIALS AND METHODS

Analyzes were conducted in the coffee section of the food analysis laboratory at the Colegio de Postgraduados Campus Córdoba, located in Amatlán, Veracruz, Mexico at 650 masl, 18° 50' NL and 96° 51' WL.

Coffee cherries (31 kg) of the variety Colombia were harvested at optimum ripeness in the municipality of Ixhuatlán del Café, Veracruz, Mexico at 1180 masl, 19° 03' 01.95" N and 96° 54' 24.45" W, in the autumn of 2015. To preserve the quality of the coffee berries, prolonged exposure to high temperatures and humidity levels before starting the pulping and fermentation of the mucilage was avoided. The pulp was removed from 31 kg of coffee berries using a mechanical pulping machine (Mod. DV 255 CM, Penagos Brand, Santander, Colombia), obtaining 13.6 kg of pulp, 17 kg of coffee beans and a 0.7 kg reduction in juice and grain.

Enzyme systems

In this study, two enzyme complexes were assessed: Macerex PM (Enmex, México) and Celuzyme (Enmex, México). Macerex PM is a standardized enzyme system containing pectinase and cellulase obtained by controlled fermentation of *Aspergillus niger* and *Trichoderma reesei*. Macerex is a product designed to maximize the extraction of juice and solids in fruit maceration or liquefaction. On the other hand, Celuzyme is an enzyme system designed for degrading cellulose and other structural polysaccharides of plant cells, and this enzyme complex is produced by controlled fermentation of a strain of *Trichoderma longribrachiantum*. It contains cellulase, hemicellulase and beta-glucanase activities.

Treatments and experimental design

Two types of enzyme systems, Celuzyme and Macerex PM, at three different concentration levels (200, 300, and 400 mg L^{-1}) and a control were evaluated (seven

treatments). The control was allowed to demucilaginate until it reached 97% mucilage removal but without reaching an alcoholic phase. The experimental unit was 0.2 kg of pulped coffee, to which the enzyme was added and mixed in with a stainless-steel spatula. A completely randomized experimental design with repeated measures, as described below, was used (Equation 1):

$$y_{ijk} = \mu + \alpha_i + rep(\alpha)_{k(i)} + \tau_j + (\alpha * \tau)_{ij} + \varepsilon_{ijk} \quad (1)$$

where y_{ijk} is the response variable observed in the treatment i; time j in the replicate k; μ is the overall mean; α_i is the effect of the treatment i; $rep(\alpha)_{k(i)}$ is the random effect of the repetition k within treatment i; τ_j is the effect of the time j; $(\alpha * \tau)_{ij}$ is the effect of the interaction between the treatment i and time j; and ε_{ijk} is the experimental error independent and identically normal distributed with mean 0 and constant variance $\sigma^2\left(\varepsilon_{ijk} IIDN\left(0,\sigma^2\right)\right)$. For data analysis, the GLIMMIX procedure of SAS (SAS, version 9.3) was used. The autoregressive is of order 1. Mean comparisons were made using Fisher's least significant difference (LSD) test ($P \leq 0.05$).

Variables evaluated

Measurements of percentage of mucilage removed, pH and temperature were taken in 20 g samples at 30 min intervals for 3 h of fermentation in each of the treatments. A mercury thermometer (Mod. 360, LAUKA Brand, Import) was used to measure temperature and a potentiometer (Conductronic, Modelo pH120) for pH.

The technique of the four rinses was used for mucilage removal (López-Blanco, 2017). To determine the amount of mucilage removed water was added to the sample and kept stirring until the mucilage was removed, this water with coffee beans was weighed and by weight difference the percentage of mucilage in the coffee sample was estimated (Equation 2):

$$\%MS = \left(\frac{W_s - W_{bwm}}{W_s}\right) * 100 \quad (2)$$

where $\%MS$ is the percentage of mucilage present in the sample; W_s is the amount in grams of the coffee beans sample; and W_{bwm} is the amount in grams of coffee beans without mucilage.

Then the amount of mucilage that was removed in each of the six sampling times was evaluated using the following formula (Equation 3):

$$\%MR_t = \left(1 - \frac{SPR_t - SWM_t}{MS_t}\right) \quad (3)$$

where $\%MR_t$ is the percentage of mucilage removed at time t; SPR_t is the weight of the coffee beans sample with mucilage partially removed at time t; SWM_t is the total weight of

the coffee beans sample without mucilage at time *t*; and MS_t is the weight of mucilage of the sample at time *t* and this was estimated with the following equation (Equation 4):

$$MS_t = MR_0 * SWM_t \quad (4)$$

where MR_0 is the initial mucilage removed, estimated using the formula (Equation 5):

$$MR_0 = \frac{SPC - SWM_0}{SWM_0} \quad (5)$$

where *SPC* is the weight in the pulped coffee sample; and SWM_0 is the coffee sample without mucilage at time zero.

Water with the sample was weighed to calculate the water expenditure and this was estimated with the following equation (Equation 6):

$$W_w = W_{w+s} - W_s \quad (6)$$

where W_w is the water weight used in mucilage removal; W_{w+s} is the weight of the water with the sample; and W_s is the weight of the sample.

RESULTS AND DISCUSSION

Removal of coffee mucilage

Analysis of variance showed that there was a highly significant difference between treatments and the interaction between treatments and time in the percentage of mucilage removed. In Figure 1, Macerex PM enzyme at 400 mg L^{-1} can be seen to have obtained the highest mucilage removal percentage (65%) followed by Celuzyme enzyme at 400 mg L^{-1}

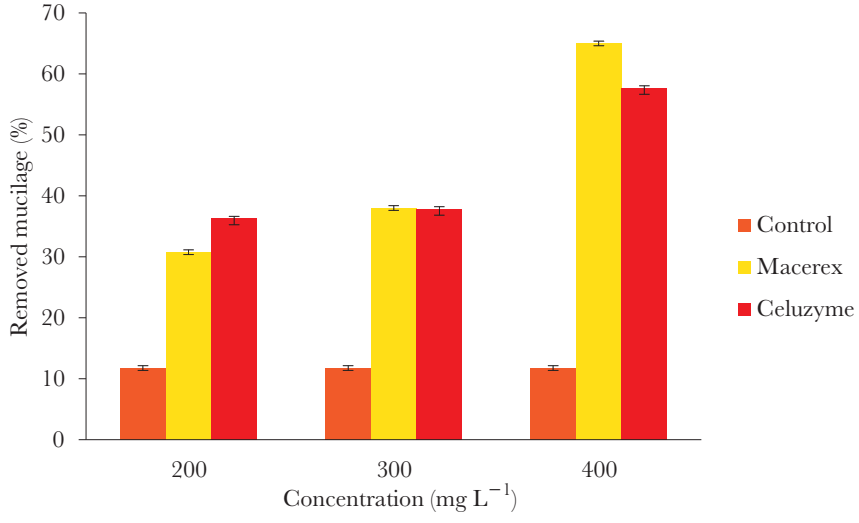

Figure 1. Average values of % of mucilage removed as a function of enzyme type and concentration (mg L^{-1}).

with 57.66% mucilage removed. Both treatments had the highest enzyme concentration compared to the rest of treatments and were statistically different from each other and with other treatments.

The use of enzymes (Macerex PM and Celuzyme) and the concentration added to the pulped coffee had a significant effect on the amount of mucilage removed relative to the control; for example, at concentrations of 200 and 300 mg L^{-1} of enzyme, average mucilage removal percentages were 22 and 26.2% higher than the control treatment (natural fermentation), respectively.

Coffee mucilage removal time

Analysis of variance results showed that there was a highly significant difference in mucilage removal time among treatments. In general, the time required to remove the largest amount of mucilage was lower in enzyme-added treatments compared to the control treatment (Figure 2). The time required to remove at least 90% of the mucilage adhering to the coffee beans under the traditional method (without enzyme) was greater than 10 h, while using 400 mg L^{-1} of the Macerex PM and Celuzyme enzymes resulted in removing 95 and 84.5% of the mucilage, respectively, in a 3 h period. Treatments with concentrations of 200 and 300 mg L^{-1} of enzymes (both enzymes) required more than 3 h to remove 80% of mucilage.

To study the mucilage removal response curve as a function of the enzyme type and concentration in the treatments, an orthogonal polynomial contrast was performed. Results indicate that the enzyme concentration has a quadratic effect on the average percentage of mucilage removed (P=0.0001). It can also be seen that for each added unit of enzyme in mg L^{-1}, the percentage of mucilage removed is higher in the Macerex than the Celuzyme enzyme (Figure 3).

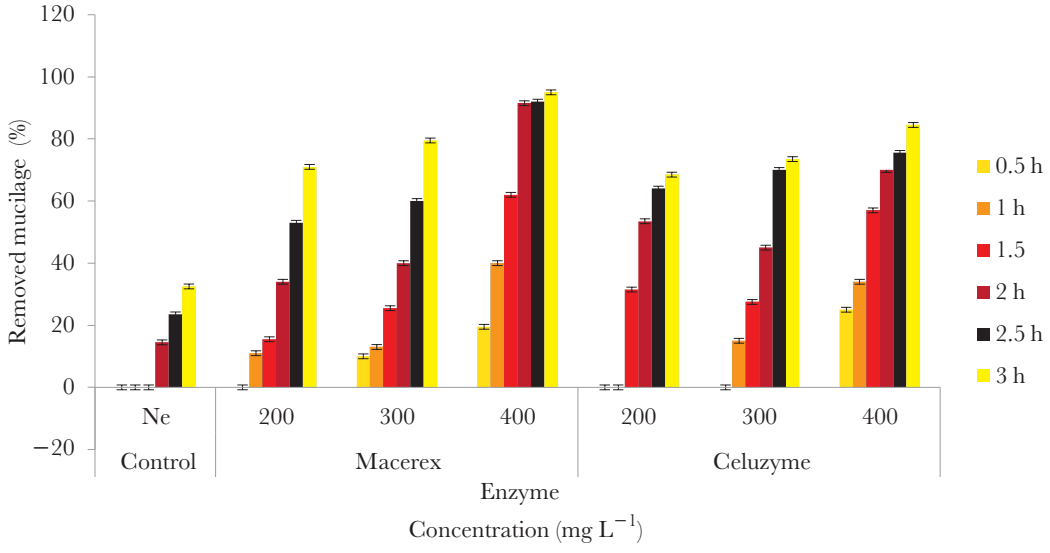

Figure 2. Average mucilage removal percentages based on enzyme type, concentration (mg L^{-1}) and time. Ne stands for no enzyme.

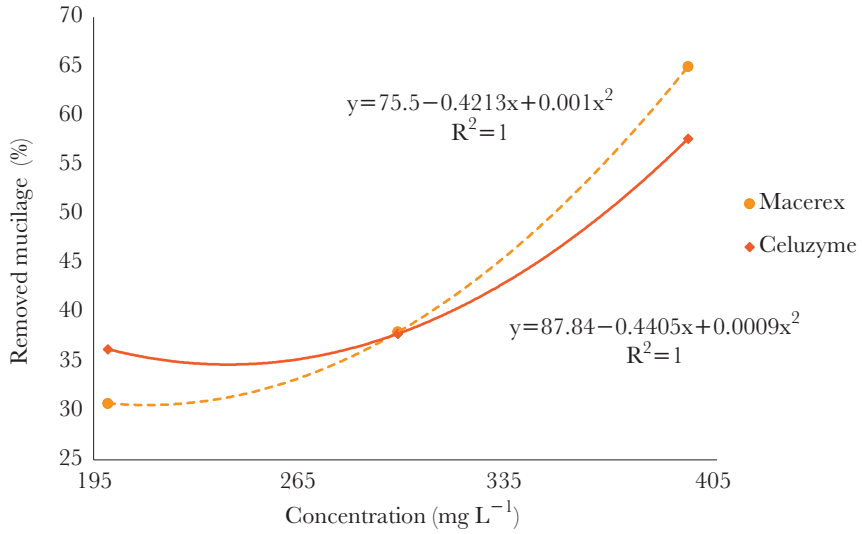

Figure 3. Mucilage removal response curve as a function of enzyme type and concentration (mg L^{-1}).

Enzymes have been used more frequently in industrial processes to accelerate juice extraction and substrate digestion processes. Macerex PM contains pectinase and cellulase, and Celuzyme contains cellulase, hemicellulase and beta-glucanase. Escalante-Minakata *et al.* (2013) showed that Macerex PM improved by 2.5 times the yield of juice extraction in banana while Glucozyme-400 had no a positive effect on the extraction of banana liquids at any stage of ripeness; thus, it seems that the effectiveness of the enzymes will depend on the chemical nature of the substrate.

In recent years the coffee agro-industry has been introducing the use of enzymes in wet processing (Peñuela-Martínez *et al.*, 2010); for example, the pectinase from *Bacillus subtilis* strain Btk27 (Oumer & Abate, 2017), and the pectinase produced by fermentation in solid state using coffee pulp with *Aspergillus niger* CFR 305 (Murthy & Naidu, 2011). It has been reported that the fresh mucilage has between 85 to 91% water and between 7.50 to 9.82% carbohydrates, the latter comprising 47.9% of reducing sugars, 29.8% of non-reducing sugars such as sucrose, 7.3% fiber and about 15.0% non-fibrous substances, such as pectic substances (Puerta-Quintero & Ríos-Arias, 2011).

The degradation speed of mucilage depends of variety coffee, for instance, coffee arabica to hydrolyze the mucilage requires more time than robusta (*Coffea canephora*) as well as depending on the inherent concentration of pectinolytic enzymes ambient temperature and pH. Murthy & Naidu (2011) reported that the duration for digestion in conventional coffee demucilage varies from 48 to 72 h depending on temperature and thickness of mucilage, while in treatments with the enzyme system in the wet fermentation process 50 and 76% pectin was degraded in about 1 and 2 h respectively depending upon the type of enzyme compared to 8% degraded pectin in about 1 h and continued up to 48 h with 100% pectin decomposition in natural fermentation for remove coffee fruit skin, mucilage and the parchment in robusta coffee.

Puerta-Quintero (2009) showed that by using pectin concentrates (enzymes), mucilage removal time (fermentation) was reduced from 20 h (traditional method) to 2 h depending

upon the type of enzyme and concentration; on the other hand, En-Sheng *et al.* (2014) found that when applying extracts of crude enzymes of *Aspergillus tubingensis* they eliminated the mucilage of cherries coffee in 3 h at 30 °C at pH 6.

Quite often the mucilage breakdown is not complete even after 72 h of fermentation. Haile & Kang (2019) mention that if the degumming time is extended, the sugars (present in coffee pulp) can degrade into acids while other enzymes that can cause deterioration to the grain are synthesized, producing a heterogeneous product and sometimes one of poor quality. Peñuela-Martínez *et al.* (2011) reported that the use of enzymes allows a greater control in the beneficiary reducing the risks of deterioration of quality due to prolonged or incomplete fermentations.

pH behavior in coffee mucilage removal

Analysis of variance results showed a highly significant difference between treatments, time, and the interaction between both treatment factors on pH (P=0.0001). A significant statistical difference was found in the average pH level between the enzyme-added treatments and the control. However, treatments with the same enzyme concentration level were not statistically different. In general, a decrease in pH over time was observed in all treatments and these average pH levels over time were higher in the control treatment compared to the treatments with enzymes added (Figure 4).

According to the literature, during coffee fermentation, the pH of the substrate decreases in the first 20 h due to the formation and dissociation of acids, mainly due to the effect of lactic acid (Puerta-Quintero, 2012). Considering the above, the results in this study demonstrate an accelerated decrease in pH in treatments with enzymatic fermentations, reaching below 4.5 in just 3 h, so an important effect of pH on the degradation of the mucilage in enzyme treatments. These results agree with those obtained by Puerta-Quintero & Ríos-Arias (2011).

Temperature during mucilage removal

The temperature of the cherry coffee beans varied between 22.6 and 25 °C during mucilage removal. In Figure 5 we can see that the treatments with the Celuzyme enzyme

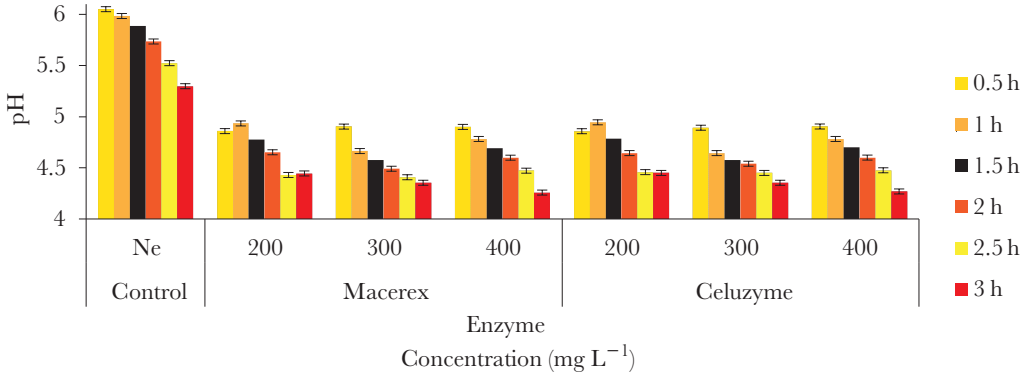

Figure 4. Average pH values of the wash water in coffee degumming based on enzyme type, concentration (mg L^{-1}) and time. Ne stands for no enzyme.

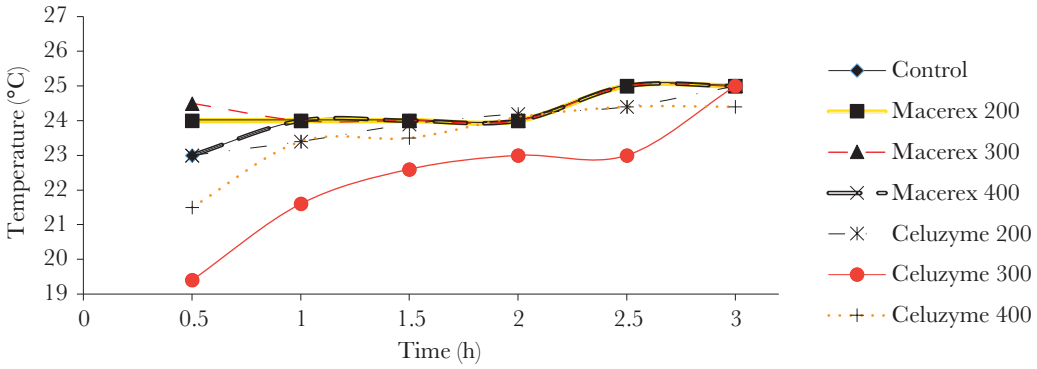

Figure 5. Average temperature during mucilage removal from coffee.

showed lower temperatures in the initial phase of the experiment, but as the experiment progressed the temperature was very similar in all treatments, while the Macerex PM enzyme showed higher temperatures throughout the process. This may be because the added enzymes and natural bacteria and enzymes from coffee tissues need a certain time to activate, but once stabilized in the medium they begin to act on the substrate causing the temperature to increase; that is, greater microbial and enzyme activity was observed over time.

Murthy & Naidu (2011) and En-Sheng *et al.* (2014) found that the use of commercial enzyme preparations in cherry coffee beans requires a certain temperature at which these enzymes accelerate the mucilage removal process through fermentation. On the other hand, Peñuela-Martínez *et al.* (2010) determined that the removal of mucilage using the TPL Rohapect® enzyme does not depend on the interaction of temperature and concentration. In this regard, the type of enzyme used to accelerate mucilage removal is very important since its activity influences factors affecting the formation of odors and flavors in the coffee.

Water consumption during mucilage removal

The traditional coffee beneficiary consists of a manual pulper and a fermentation tank where the process takes approximately 12 h and consumes 25 to 30 L of water per kg of cherry coffee. Using the technique of the four rinses to remove the mucilage from the grain 4.2 L of water are required per kg of dry parchment coffee obtained at the end of the process (Peñuela-Martínez *et al.*, 2010; Puerta-Quintero, 2012).

Innovations in the process of beneficiary allow to reduce water expenditure during washing, example of this we have to López-Blanco (2017) used a modified tank with discontinuous washing with a water expenditure of 5.3 L per kg of dry parchment coffee, reporting a water saving of 84% compared to conventional scrubbing channel washing which spent 33.8 L per kg of dry parchment coffee. On the other hand, modern mechanical mucilage removal machines that produce semi-washed coffee use only approximately 4.5 L of water per kg of dry parchment coffee (Enden & Calvert, 2010).

In the present study, the technique of the four rinses was used for mucilage removal. The total water consumption during mucilage removal of 31 kg of processed coffee cherry

beans was 28.9 L of water, the equivalent of 0.93 L per kg of coffee cherry beans or 4.2 L per kg of dry parchment coffee. This water expense represents a saving of 87.6% compared to conventional scrubbing channel washing (López-Blanco, 2017).

CONCLUSIONS

The use of pectic enzyme systems, such as Macerex PM and Celuzyme, significantly reduces the time for removing mucilage from coffee compared to the natural fermentation time. The Macerex PM enzyme showed a higher mucilage removal percentage. The use of commercial enzyme preparations is a viable technological alternative in the coffee agro-industry since it allows better pH, temperature and fermentation control in wet coffee processing; also, water consumption is significantly lower than in traditional coffee processing. These results have important implications in the wet method to help coffee industry in optimizing its process to be more sustainability without compromise the quality of coffee beans, and finally improve its revenues.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Phytophagous invertebrates that damage orchids in gardens of western Tabasco, Mexico

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ABSTRACT

Objective: To record six species of invertebrates that damage orchids in gardens in the western area of the state of Tabasco, Mexico.

Methodology: As part of the project "Fauna associated with plants cultivated in the south-southeast region of Mexico", non-systematic collections of invertebrates that were observed damaging orchids were made in three gardens in the western part of the state of Tabasco. The invertebrates were identified through descriptions and taxonomic keys.

Results: Four species of insects of the order Coleoptera and two species of mollusks of the class Gastropoda are recorded. The insects are: (1) *Diabrotica adelpha* Harold (Chrysomelidae), (2) *Stethobaris* sp. (Curculionidae), (3) *Cyclocephala guttata* Bates (Scarabaeidae), these three damaging flowers of *Brassavola nodosa* (L.) Lindley; and (4) *Stethobaroides nudiventris* Champion (Curculionidae) damaging flowers of *Catasetum integerrimum* Hook. The mollusks are (1) *Subulina octona* (Bruguière) (Subulinidae) scraping leaves of *Gongora leucochila* Lem., *Phalaenopsis* sp. and *C. integerrimum*, and (2) *Sarasinula plebeia* (P. Fischer) (Veronicellidae) damaging leaves of *C. integerrimum*.

Study limitations: It was not possible to specifically identify one of the insect species (*Stethobaris* sp.) and one of the damaged orchid species (*Phalaenopsis* sp).

Conclusions: This study contributes to the knowledge of invertebrates that damage orchids in Mexico, and is a basis for future studies to determine the impact and importance of these organisms in the conservation and cultivation of orchids in this area of Tabasco.

Keywords: Orchidaceae, Insecta, Mollusca, southeastern Mexico.

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INTRODUCTION

Orchids constitute a diverse group of plants popularly valued by the beauty of their flowers, although presently they face a series of problems that threaten their conservation, among which overexploitation, loss of habitat, and impacts from climate change stand out (Swarts and Dixon, 2009). In addition to these factors, which combined can lead to the loss of species, there are others that make difficult the conservation and production of these plants, among them bad cultural management and phytosanitary problems (Rivera and Corrales, 2007). The latter include the damage caused by insects and other invertebrates, which should be identified in order to implement control measures (Kawate and Sewake 2014). These organisms, in addition to affecting the plants' health, affect their aesthetic

value (Ramos *et al.*, 2008), and could alter the expectations of conservation in orchids that are relocated outside of their natural habitat (Light and MacConaill, 2011).

In general, the invertebrates that are found causing damages in orchids are insects from the orders Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera and Thysanoptera, as well as springtails (Collembola), mites (Acari), millipedes (Diplopoda), and slugs and snails (Gastropoda) (Rivera and Corrales, 2007; Light and MacConaill, 2011; González et al., 2012; Kawate and Sewake, 2014). In Mexico few studies have been published about invertebrates that damage orchids. For the state of Jalisco, the species reported as harmful are *Aphis spiraecola* Patch., *Macrosiphum luteum* (Buckton), *Toxoptera aurantii* (B. de F.), and *Diaspis* sp. (González *et al.*, 2012), and in Veracruz and Tamaulipas the beetle *Stethobaroides nudiventris* Champion has been recorded (Morales *et al.*, 2016; Rosas *et al.*, 2020). The objective of this study is to record six species of invertebrates that damage orchids in gardens in the western area of the state of Tabasco.

MATERIALS AND METHODS

Sites of registry

The registry was carried out in three sites: (1) in a garden of a rural household located in Ejido Chicozapote 1st Section of the municipality of Cárdenas (18° 13' 0.15" N, 93° 51' 3.44" W), (2) in a garden of an urban household located in the city Heroica Cárdenas of the same municipality (17° 59' 45.44" N, 93° 22' 54.61" W), and (3) in a garden of the enclosure of Colegio de Postgraduados, Campus Tabasco, belonging to the municipality of Huimanguillo, although located next to that city as part of that urban zone (17° 58' 36.45" N, 93° 23' 8.40" W). In sites 1 and 3 orchids were located on the trunk of trees (1.5 to 3 m height), while in site 2 they were found in pots (Figure 1). In addition to orchids, in the three sites there were other ornamental plant species. In Chicozapote 1st Section, the vegetation is made up mainly of mangroves and grasses with disperse trees and palm trees. In the city Heroica Cárdenas the vegetation consists mainly of various ornamental plants and fruit trees located in gardens and throughout the streets and avenues; in the surrounding area there are mainly cacao plantations (*Theobroma cacao* L.), pasturelands with disperse trees, and ruderal vegetation. The physiography of the zone corresponds to Tabasco plains. The climate is warm humid with abundant summer rains, isothermal variation of 26 to 28 °C, and rainfall variation from 2,000 to 2,500 mm (INEGI, 2017).

Collection and identification

The adult insects that were observed causing damage were caught manually and placed in glass containers with ethylic alcohol at 70%. The larvae of Coleoptera that were found damaging flowers were placed together with these in cages until the adults emerged. Each cage consisted of a plastic container of 300 ml capacity, inside of which there was a layer of moist soil 2 cm thick. The cap had small holes to allow the circulation of air inside. For the collection, preparation and preservation of mollusks the recommendations from Naranjo and Gómez (2011) were followed. The identification of insects was done by consulting studies by Jacoby (1880-1892), Bates (1886-1890), Champion (1906-1909), Davis (2009), Prena and O'Brien (2011), Derunkov and Konstantino (2013), and Prena (2017), and

Figure 1. Partial view of the three gardens with orchid plants. A) Chicozapote 1st Section. B) Enclosure of Colegio de Postgraduados, Campus Tabasco. C) City of Heroica Cárdenas.

of the mollusks by consulting studies by Auffenberg and Stange (1988), Caballero *et al.* (1991), Araújo and Bessa (1993), Herbert (2010) and Velázquez *et al.* (2014). The specimens were deposited in the collection of invertebrates associated to cultivated plants at Colegio de Postgraduados, Campus Tabasco. The species of orchids were identified *in situ* by consulting studies by Beutelspacher (2013) and Campos *et al.* (2019).

RESULTS AND DISCUSSION

The invertebrates that are registered next are four species of insects of the order Coleoptera and two species of mollusks of the class Gastropoda. For each species, the locality, date, individuals, development stage, and species of host orchid were recorded.

Information about their distribution and a comment about where their importance is reported in the literature are also presented.

***Diabrotica adelpha* Harold, 1875 (Insecta: Coleoptera: Chrysomelidae)**

Registry. Ejido Chicozapote 1st Section: December 29, 2021; one adult (♀) feeding and damaging flowers of a *Brassavola nodosa* (L.) Lindley plant, on the trunk of an *Annona muricata* L. tree (Figure 2A).

Distribution. North America: United States and Mexico (Chiapas, Coahuila, Colima, Durango, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Nayarit, Nuevo León, Oaxaca, Tabasco, Tamaulipas and Veracruz). Central America: Costa Rica, Guatemala, Honduras, Nicaragua and Panama. South America: Colombia (Jacoby, 1880-1892; Raigosa *et al*., 1978; Derunkov and Konstantino, 2013; Torres *et al*., 2021; GBIF, 2022).

Commentary. Polyphagous species. In Central America it is considered a pest of low importance in various agricultural crops (Saunders *et al*., 1998); however, it is capable of mechanically transmitting different viruses when the adults feed off foliage of the bean, *Phaseolus vulgaris* L. (Hallman, 1985). In Tabasco, adults were recorded feeding off flowers of *Caesalpinia pulcherrima* (L.) Swartz (Hernández and Sánchez, 2017), and in Tamaulipas the adults attacked the foliage of a crop of *Portulaca oleracea* L. (Torres *et al*., 2021).

***Stethobaris* sp. (Insecta: Coleoptera: Curculionidae)**

Registry. Ejido Chicozapote 1st Section: December 25, 2021, two adults damaging a flower button of a *B. nodosa* plant on the trunk of a *Pachira aquatica* Aubl. tree (Figure 2B). January 2, 2022, two adults damaging a flower of a *B. nodosa* plant on the trunk of

Figure 2. A) Adult of *Diabrotica adelpha* damaging a flower of *Brassavola nodosa* in Chicozapote 1st Section. B) Adult of *Stethobaris* sp. damaging a flower bud of *B. nodosa* in the same locality.

a *Tabebuia rosea* (Bertol.) DC tree. January 30, 2022, one adult damaging a flower of a *B. nodosa* plant on the trunk of a *P. aquatica* tree.

Distribution. The genus *Stethobaris* is distributed from Canada to Argentina and Uruguay, including the Caribbean islands (Prena, 2017).

Commentary. More than 100 species of the genus *Stethobaris* are known (Prena, 2017). Their hosts are mainly orchids (Prena and O'Brien, 2011; Prena, 2017). Approximately 50 species of *Stethobaris* have been found in 33 genera of orchids (Prena, 2017). In Costa Rica, *Stethobaris* sp. is registered in orchids of different genera, including *Brassavola* and *Catasetum* (Rivera and Corrales, 2007). Some species of *Stethobaris* cause much worry among producers and several species are disseminated outside of their natural environment through trade of ornamental plants (Prena, 2017). The damage caused by adults of the species found (*Stethobaris* sp.) consisted in small perforations on the flower organ.

***Stethobaroides nudiventris* Champion, 1907 (Insecta: Coleoptera: Curculionidae)**

Registry. Enclosure of Colegio de Postgraduados, Campus Tabasco: November 1, 2018, larvae damaging flowers of a *Catasetum integerrimum* Hook plant on the trunk of a *T. rosea* tree (Figure 3A); through larvae breeding, 34 adults were obtained (19 ♀, 15 ♂); 24 larvae obtained from a flower were preserved in ethylic alcohol at 70%. November 2, 2018; five adults (2 ♀, 3 ♂) collected in flowers of a *C. integerrimum* plant on the trunk of a *T. rosea* tree (Figures 3B and 3C).

Distribution. Mexico (Veracruz and Tamaulipas), Belize, Costa Rica, Panama and Colombia (Champion, 1906-1909; O'Brien and Wibmer, 1982; Rosas *et al.*, 2020; GBIF, 2022).

Commentary. In Mexico, Belize, Costa Rica and Panama it is associated to *Catasetum* sp. (Prena and O'Brien, 2011). In Veracruz and Tamaulipas it is found damaging flowers of *C. integerrimum* (Morales *et al.*, 2016; Rosas *et al.*, 2020). The larvae cause withering of the petals (Figure 3), and the flower dies within a period of three days, which is why it can become a potential pest (Morales *et al.*, 2016).

***Cyclocephala guttata* Bates, 1888 (Insecta: Coleoptera: Scarabaeidae)**

Registry. Ejido Chicozapote 1st Section: October 10, 2021, two adults (♀♂) damaging flowers from a *B. nodosa* plant on the trunk of a *T. rosea* tree (Figure 4A). January 30, 2022, one adult (♂) feeding from a flower of *B. nodosa* on the trunk of a *P. aquatica* tree (Figure 4B).

Distribution. Mexico (Chiapas, Hidalgo, Morelos, Oaxaca, Puebla, San Luis Potosí, Tabasco and Veracruz) and Central America: El Salvador, Guatemala, Honduras and Nicaragua (Bates, 1886-1890; Maes and Ratcliffe, 1996; Morón *et al.*, 1997; Sánchez, 1997; Pacheco *et al.*, 2008; Morón and Rojas, 2011; Maes, 2021; GBIF, 2022).

Commentary. Similar to other species of *Cyclocephala*, the habits and biology of this species are unknown (Morón *et al.*, 1997).

***Subulina octona* (Bruguière, 1792) (Mollusca: Gastropoda: Subulinidae)**

Registry. Heroica Cárdenas, Pueblo Nuevo neighborhood: November 17, 2021, and January 12, 2022, one individual scraping the foliage of a plant of *Gongora leucochila* Lem.

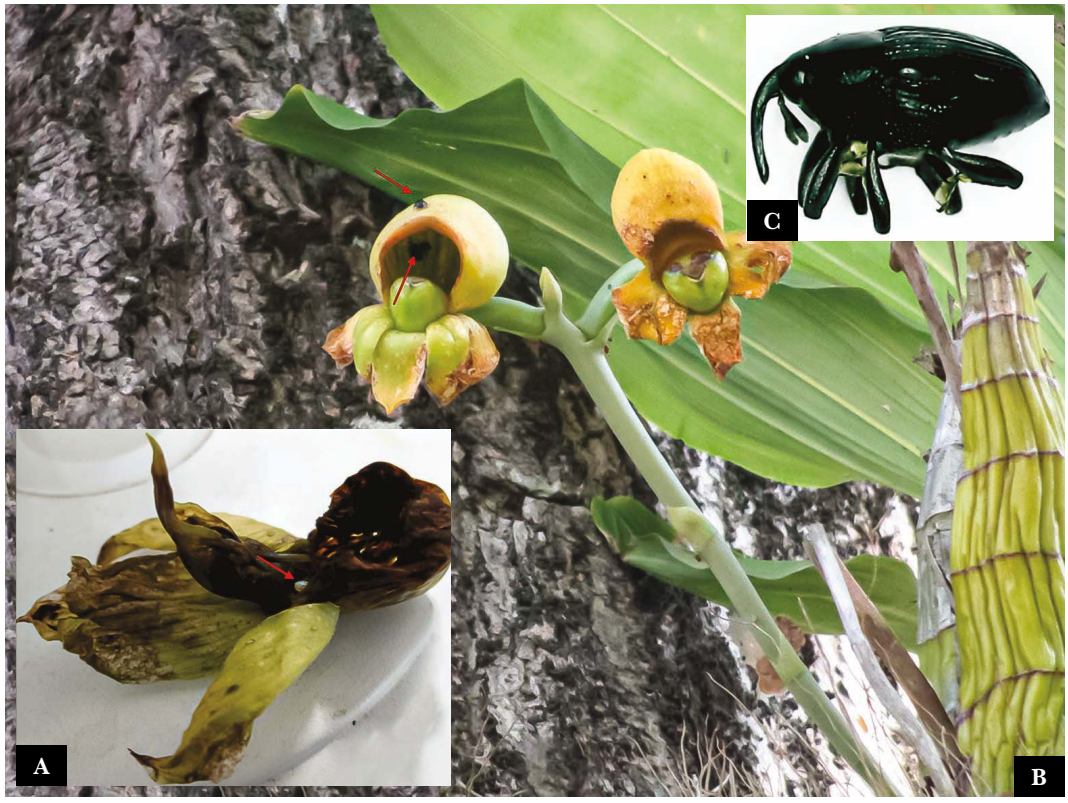

Figure 3. A) Flower of *Catasetum integerrimum* damaged by larvae of *Stethobaroides nudiventris* (the arrow points to a body part of the larvae). B) Flowers of *C. integerrimum* with damaged petals, and adults of *S. nudiventris* in a flower (signaled with the arrows). C) Female of *S. nudiventris* in lateral left view. Enclosure of Colegio de Postgraduados, Campus Tabasco.

(Figure 4C). November 17, 2021, one individual damaging the foliage of a *Phalaenopsis* sp. plant. January 27, 2022, two individuals scraping the foliage of a *C. integerrimum* plant.

Distribution. In different countries around the world, where it was introduced through vegetable and agricultural imports. Its geographical origin is unknown, although it is suggested that it is Neotropical (Auffenberg and Stange, 1988; Herbert, 2010; GBIF, 2022). In Mexico, it is found in the states of Chiapas, Campeche, Distrito Federal, Guerrero, Jalisco, Michoacán, Morelos, Nuevo León, Quintana Roo, San Luis Potosí, Sinaloa, Tabasco, Tamaulipas, Veracruz and Yucatán (GBIF, 2022).

Commentary. In Cuba it constitutes a phytosanitary risk for agriculture, since it causes damages in various crops, including orchids (Herrera *et al.*, 2013; Matamoros, 2014). In the north of Australia it is considered a garden pest (Solem, 1998). Since it is a species with quarantine importance, in Costa Rica it has caused substantial economic losses due to the interception of shipping of ornamental plants with presence of the species (Monje, 1996). It also has medical and veterinary importance, because it is an intermediary host of parasites that affect human beings and domestic animals (De Faria, 1980; Armiñana *et al.*, 2020).

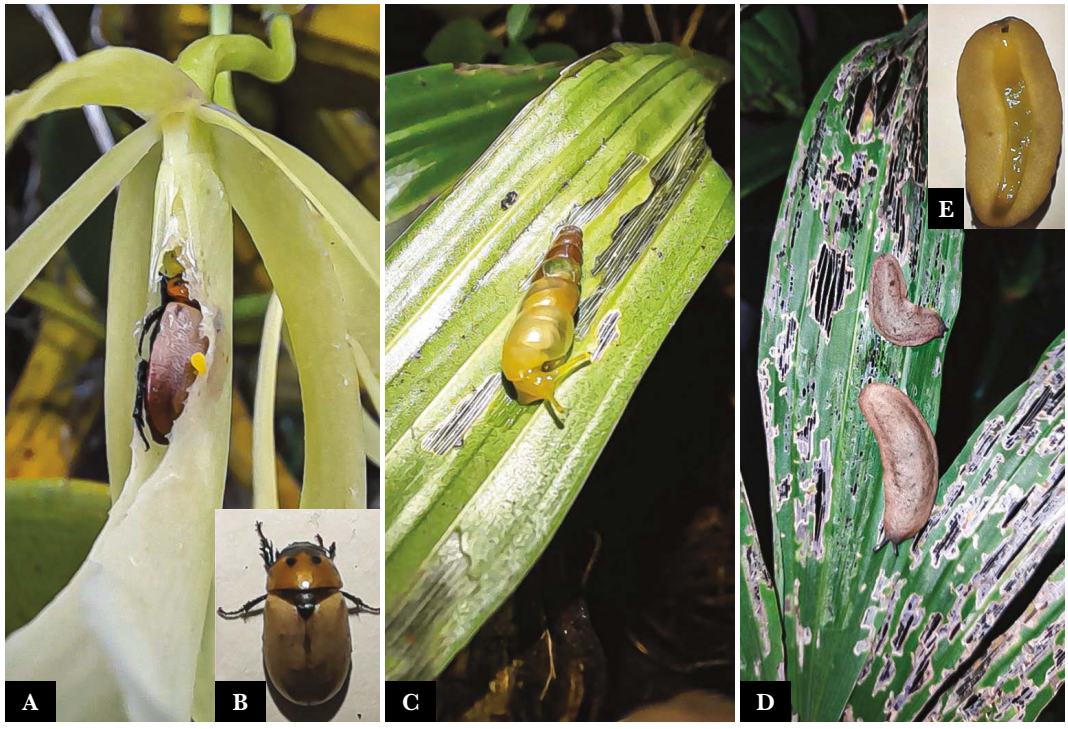

Figure 4. A) Adult of *Cyclocephala guttata* damaging a flower of *Brassavola nodosa* in Chicozapote 1st Section. B) Another adult of *C. guttata* in dorsal view. C) Adult of *Subulina octona* scraping the foliage of a *Gongora leucochila* plant in the city Heroica Cárdenas. D) Adults of *Sarasinula plebeia* scraping the foliage of a *Catasetum integerrimum* plant in that city. E) Adult of *S. plebeia* in ventral view.

***Sarasinula plebeia* (P. Fischer, 1868) (Mollusca: Gastropoda: Veronicellidae)**

Registry. Heroica Cárdenas, Pueblo Nuevo neighborhood: January 27, 2022; four adults and one juvenile damaging the foliage of a *C. integerrimum* plant (Figures 4D and 4E).

Distribution. It is considered non-native of Mexico (Naranjo *et al*., 2007). Presently it is distributed in the north, center and south of America, Caribbean islands, southeast of Asia, north and northeast of Australia, and islands of the Pacific (GBIF, 2022). In Mexico it is found in the states of Chiapas, Puebla, San Luis Potosí, Tamaulipas, Veracruz and Yucatán (Naranjo *et al*., 2007; Velázquez *et al*., 2014; GBIF, 2022). It lives in altered environments such as gardens. The horticultural industry, as part of global trade, is probably the most important dispersion vector of this and other species of gastropods (Cowie *et al*., 2008).

Commentary. In Central America it is one of the main pests of the bean crop (Sobrado and Andrews, 1985). It can cause losses of 100% (Rodríguez *et al*., 1987), has limited the harvests and motivated the abandonment of this crop (Sobrado *et al*., 1987; del Río *et al*., 1990). In Los Tuxtlas, Veracruz, it has caused bean producers to change the crop (Naranjo *et al*., 2007). In Veracruz and Puebla it has caused important reductions in the production of vanilla, *Vanilla planifolia* Jackson, a species of orchid (Velázquez *et al*., 2014). In Colombia it has caused the mortality of coffee plants, *Coffea arabica* L., in nursery and newly sown in the field (Constantino *et al*., 2010). In Hawaii, it causes severe damages to various ornamental plants, being one of the main pests of orchids from genus *Dendrobium*.

It has also been cause for quarantine rejections in export shipments (Kawate and Sewake, 2014). It has medical importance, since it is an intermediate host of nematodes of the genus *Angiostrongylus* which are pathogenic for human beings (Caballero *et al.*, 1991; Araya *et al.*, 2015).

CONCLUSIONS

This study contributes to the knowledge of invertebrates that damage orchids in Mexico. *Diabrotica adelpha*, *Stethobaris* sp., *Cyclocephala guttata* and *Subulina octona* are registered for the first time damaging orchids in the countries. Likewise, this study constitutes a basis for future studies that allow defining the impact and importance of these organisms in conservation and cultivation of orchids in this zone of Tabasco.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Long-term response planting method on wheat under conservation agriculture

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ABSTRACT

Objective: To compare different bed planting systems: narrow beds, wide beds, and flat soil, on the growth and yield of wheat under conservation system.

Design/methodology/approach: Treatments were established on a complete block design with three replicates. Also, wheat crop was grown during five seasons. Treatments were as follow: wide beds (100 cm), narrow beds (80 cm) flat soil. Response variables were dry weight of 50 stems, weight of 1000 grains, number of spikes (m^2), harvest index and yield. Also, the relationship between relative yield and cold units and degree-days were measured.

Results: The flat beds treatment reached the highest stem weight, while the narrow beds had the highest number of spikes m^2. Yield was the same between flat beds and narrow beds. No differences were found in the harvest index between the evaluated treatments. When the variables were compared in the years evaluated, the dry weight of 50 stems increased and the harvest index was reduced, negatively affecting yield. A negative association was identified between the reduction of chilling hours and crop yield.

Study limitations/implications: The use of wide planting beds reduced the yield of the wheat crop, and its use is not recommended.

Findings/conclusions: The performance was the same between the treatment of flat beds and narrow beds. The reduction in yield was mainly associated with the reduction in cold hours that occurred in each year of production.

Keywords: climate; environment; grain yield; plant density; topological arrangement.

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INTRODUCTION

The Mexicali valley is located in the state of Baja California in Northwest Mexico. It has an approximate area of 105,000 irrigated hectares, of which 98,000 hectares are crops of wheat (*Triticum aestivum* L.) cultivated during the months of November through May

Image by Levke Hannemann at Pixabay

(SIAP, 2014). Traditionally, most growers employ the flat planting system for wheat under conventional tilling practices. The system includes the subsoiling, disking and mould board plough. Besides, straw is usually burned after harvest, which substantially affects microbial activity in soils (Wuest *et al.*, 2005).

Wheat grain yield attained by the use of this technology has reached an average of 6.8 Mg ha^{-1} (Salinas-Zavala, Salvador, Lluch-Cota & Fogel, 2006). However, it can be enhanced by increasing the number of irrigations (Kakar & Iqbal, 2015) and employing different management practices such as bed planting system. Additionally, profitability of this crop can be increased by employing conservation tillage system (minimum tillage, crop rotation, straw incorporation) (Govaerts *et al.*, 2009).

In conservation agriculture system, the soil is ploughed as in conventional system during the first year. After that time, soil is not disturbed; crop rotation and all crop remains are incorporated over the surface. The planting method consists of raised beds (80-100 cm) with two or three rows on the top of the bed (Limon-Ortega, Sayre, Drijber, & Francis, 2002).

With conservation agriculture, growers can achieve fuel costs savings up to 30% (Lal, 2004), increase nitrogen concentration, organic matter content (Zuber *et al.*, 2015)and keep stable yield (Kassam *et al.*, 2009). Fahonga, Xuqinga & Sayre (2004) mention that bed planting system provides advantages in water saving (30%) when compared to flat soil planting. They also indicate that soil crusting problems are reduced because the seed bed surface improves physical structure. Besides, N use efficiency is increased up to 30%, microclimate favors crop stand, reduces lodging and presence of plant diseases. Weed management and fertilization timing practices are substantially improved (Govaerts, Sayre, & Deckers, 2005).

The use of bed planting in wheat crops has been recommended for more than a decade in the Yaqui valley (state of Sonora) as well as in high valleys of central Mexico (Govaerts *et al.*, 2005). Nevertheless, the use of this technology in Mexicali valley is under research due to different cropping systems (type of soil, irrigation water quality and environment) which are contrasting to the places above mentioned. In that sense, a study was conducted during five years in order to compare different bed planting systems (flat soil, wide beds (100 cm) and narrow beds (80 cm) on the growth and yield of wheat under conservation system.

MATERIALS AND METHODS

The research was carried out at the experimental platform for conservation agriculture located at the agricultural science institute (32° 24' 12.34" N; 115° 11' 47.37" W). The weather of this region is arid with periods of rain in winter (BW [h'] hs [x´] [e']; (Ruiz-Corral *et al.*, 2006), summer temperatures up to 50 °C and winter temperatures of −7 °C, the mean annual temperature is 22.3 °C and the mean annual precipitation is 58 mm.

The soil from the experimental site is classified as clayed (vertisol), bulk density of 1.16 g cm^3, electrical conductivity (EC) of 4.44 dS m^{-1}, pH 7.83, 33 ppm of phosphorus content, 395 ppm of potassium, 5236 ppm of calcium, 1255 ppm of magnesium and 672 ppm of sodium respectively. Irrigation water flowed from Colorado River, the pH was of

8.18, EC of 1.28 dS m^{-1} and 122.0, 61.4, 154.8, 177.1, 243.5, 421.8 mg L^{-1} of calcium, magnesium, sodium, bicarbonates, chlorides and sulfates approximately.

The experiment was established in a big area of land under conservation agriculture system. The tillage techniques were those recommended by Verhulst *et al.* (2015). The first year of the study (2011-2012), land was ploughed using conventional system (subsoiling, disking and leveling). Subsequently, all treatments were established on a complete block design with three replicates. Plots had a length of 140 m and 12 m wide. Treatments were: A) wide beds (furrows at 100 cm), wheat was planted in twin line (two rows 27 cm apart) (Figure 1) and seed density of 80 kg ha^{-1}; B) narrow beds (furrows at 80 cm) (two rows 27 cm apart) and the same seeding rate; C) flat soil with a seed density of 160 kg ha^{-1}.

Planting and harvest dates were as follow: A) December 6th 2011 and June 15th 2012 first growing season; B) December 15th 2012 and June 15th 2013 second growing season, C) November 15th 2013 and June 7th 2014 third growing season, D) November 22nd 2014 and June 4th 2015 fourth growing season, E) November 25 2015 and June 15th 2016 the fifth growing season. The variety planted was Rio Colorado during all growing seasons.

Planting on wide and narrow beds was realized with a multifunctional seed drill (manufactured by Industrias Vázquez S.A de C.V); flat soil planting system was realized with a special seed drill (Dobladense 290-17®). Fertilization rates were of 276 kg of nitrogen using urea [$CO(NH_2)_2$] and 78 kg of phosphorus using monoammonium phosphate (MAP-11-52-00). Total P and 25% of N were pre-plant applied, 33% of N applied in the first post-plant irrigation, 25% in the second post-plant irrigation while the last 17% in the third post-plant irrigation. Pest and weed management were realized following the guideline provided by Hernández-Vázquez *et al.* (2010).

Response variables were dry weight of 50 stems (season 2013-14; 2014-15 and 2015-16), number of spikes (m^2) (season 2014-15 and 2015-16), weight of 1000 grains (season 2013-14, 2014-15 and 2015-16), harvest index (season 2011-12, 2013-14 and 2015-16) and yield (from 2011 through 2016). It was also identified the ratio of annual yield relative to chill hours and degree-days from planting to February (year). The reason for considering February is because heading usually occurs during this time (Verhulst, Kienle, Sayre, Deckers, & Raes, 2014).

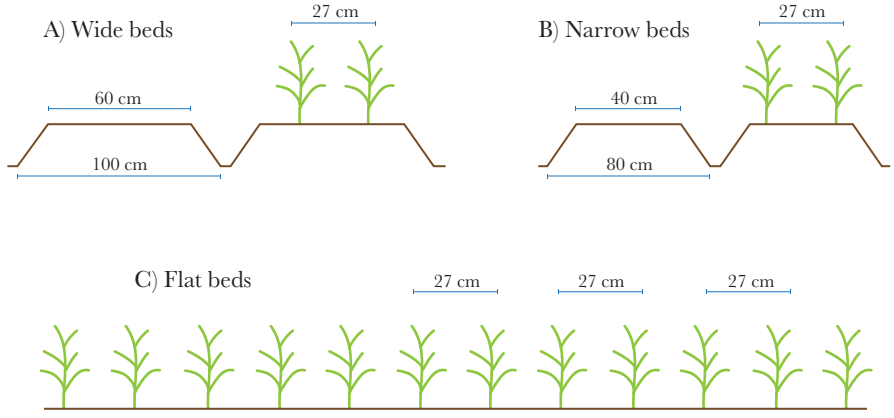

Figure 1. Different planting systems on wheat production.

To determine $DW_{50\ stems}$, plant material was taken at harvest, placed on a forced air-dry oven (65 °C) until constant dry weight. Harvest index (HI) was obtained by the ratio of dry weight of 50 stems relative to grain weight. Number of spikes were also counted (m^2). Yield was estimated by harvesting plants on 2 m^2 segment. Climatic conditions were monitored with a meteorological station located at 840 m from the experimental site during all years of the study. The daily chill hours and degree-days were obtained from a meteorological network station (SIMABC, http://www.simarbc.gob.mx/).

All data obtained was analyzed separately due to different variables measured. Analysis of variance was conducted between treatments, treating years as the fixed factor, ANOVA between years considering treatments as the fixed factor, mean differences between treatments or years, were separated using Tukey's least significance difference (LSD) at $P \leq 0.05$. Additionally, regression models were fitted between accumulated chill hours and degree-days with respect to grain yield.

RESULTS AND DISCUSSION

Table 1 shows the maximum and minimum temperatures during the time of the experiment. It also shows the average monthly maximum and minimum temperatures from 1961 through 2003. It is shown that temperatures obtained during the study were very similar to those recorded from 1962 to 2003. Maximum temperatures registered from January through may increase from 4.9 a 8.7 °C as compared to those registered in the period of 1961 to 2003.

Table 2 shows the monthly Degree-days (DD) and chill hours (CH) accumulation during the study. As the time passed during the period of the experiment, the months from February through March exhibited a reduction in the CH accumulation and increased the DD accumulation.

Table 1. Maximun and minimum temperatures registered on wheat crop under conservation agriculture. Mexicali, Baja California, México.

Month	Year cero (2011-12)		Year two (2012-13)		Year two (2013-14)		Year three (2014-15)		Year four (2015-16)		Average 2011-16		1961-2003†	
	Minimum	Máximum	Minimum	Máximum	Minimum	Máximum	Minimum	Máximum	Minimum	Máximum	Minimum	Máximum	Minimum	Máximum
November	7.4	24.7	11.8	31.8	9.4	25.3	9.4	27.0	-‡	-	9.5	26.9	9.3	26.0
December	2.6	19.2	4.5	27.5	5.1	22.0	7.1	20.8	-	-	4.8	22.1	6.4	21.4
January	4.6	23.7	3.3	18.8	6.5	24.3	6.4	22.6	5.1	20.8	5.1	26.3	6.4	21.4
February	6.2	24.0	3.7	21.3	8.1	25.7	9.4	27.5	8.3	26.3	7.1	29.7	7.7	23.9
March	7.1	26.6	9.9	28.8	11.3	30.5	11.7	29.2	9.1	27.8	9.8	33.8	9.7	26.4
April	11.5	30.7	11.4	31.0	11.5	31.2	11.7	30.4	12.2	29.4	11.6	36.5	12.0	29.8
May	14.8	36.2	16.1	36.6	15.5	35.2	13.3	31.9	13.7	32.9	14.6	41.3	15.5	33.9
June	19.0	39.5	19.7	39.6	19.6	39.6	19.6	39.9	21.4	39.0	19.8	47.2	19.1	38.5

†: Average from 1961-2001. Ruiz-Corral *et al.*, 2006. ‡: Not determined.

Table 2. Degree-days (DD) and chill hours (CH) accumulation registered on wheat crop under conservation agriculture. Mexicali, Baja California, México†.

Month/year	2011-12		2012-13		2013-14		2014-15		2015-16	
	CH	DD	CH	DD	CH	DD	CH	DD	CH	DD
November	139	254	0	113	70	292	76	337	267	0
Dicember	375	105	0	0	306	154	211	180	235	59
January	269	206	0	0	195	242	188	211	266	150
February	203	199	195	110	110	269	67	322	107	303
March	139	309	61	392	23	384	36	441	40	365
April	25	446	12	455	12	456	8	442	9	443
May	0	626	0	631	0	602	7	529	0	549
June	0	731	0	747	0	716	0	743	0	767
TOTAL	1150	2876	268	2448	716	3115	593	3205	924	2636

†: SIMARBC. 2016. http://www.simarbc.gob.mx/

According to analysis of variance, there were significant differences in variables of dry weight$_{50\ stems}$, spikes (m^2) and yield of crop by the effect of planting methods (Table 3). The greatest weight of 50 stems was registered on flat soil planting. The greatest number of spikes was recorded on narrow bed planting. Lastly, the lowest yield was attained on wide bed planting. However, there were no significant differences on weight of 1000 grains and HI.

Significant differences were found in variables of $DW_{50\ stems}$, $DW_{1000\ grains}$, HI and yield of crop during the years of the study (Table 4). The highest value of $DW_{50\ stems}$ was attained during the season 2015-2016. The yield and HI decreased as the time passed. In addition, $DW_{1000\ grains}$ decreased on the season 2014-15 as compared to seasons 2013-14 and 2015-16. Furthermore, the number of spikes (m^2) was not affected during the time of the experiment.

Table 4 shows the analysis of variance and mean comparison for the variables of $DW_{50\ stems}$, spikes m^2, $DW_{1000\ grains}$, HI and yield in five years of study. It was observed that as time passed, $DW_{50\ stems}$ significantly increased ($P>0.01$) to double the weight attained during the season 2013-14 as compared to season 2015-16. However, there were no significant differences on the number of spikes (m^2) during the years of study ($P>0.05$). The highest value of $DW_{1000\ grains}$ was attained on the seasons 2013-14 and 2015-16 as compared to season 2014-15. HI increased on seasons 2011-12 and 2013-14,

Table 3. Effect of planting method on dry weight of 50 stems, spikes (m^2), weight of 1000 grains, HI and yield of wheat crop (2011-12 through 2015-16).

Planting method	$DW_{50\ stems}$ (g)	Spikes m^2	$DW_{1000\ grains}$ (g)	HI	Yield (Ton ha^{-1})
Flat soil	193.54 a	270 b	77.61	0.393	6.45 a
Narrow beds	158.83 b	370 a	73.73	0.385	6.40 a
Wide beds	176.21 b	255 b	76.66	0.398	5.23 b
Significance	**	*	NS	NS	*

NS: non significant; *, **. Significant at $P<0.05$, $P<0.01$, respectively.

Table 4. Mean comparison of yield components on wheat in five growing seasons (2011 to 2016).

Year	$DW_{50\ stems}$ (g)	spikes m^2	$DW_{1000\ grains}$ (g)	HI	Yield (t ha^{-1})
2011-12	-	-	-	0.433 a	6.87 a
2012-13	-	-	-	-	6.98 a
2013-14	117.07 c	-	83.22 a	0.414 a	6.03 a
2014-15	160.93 b	298	63.12 b	-	5.33 ab
2015-16	250.58 a	309	81.66 a	0.327 b	4.94 b
Significance	**	NS	*	*	*

NS: non significant; *, **, ***. Significant at P<0.05, P<0.01, y P<0.001 respectively.

but it decreased on season 2015-16. The same tendency was observed when analyzing yield between years. Yield potential significantly decreased as time passed.

The regression model showed that yield increased as the number of cold units also increased (R^2=0.4355, P<0.007; Figure 2). The regression equation indicated that yield increased 1.35 ton ha^{-1} for every 100 units accumulated to February. A different behavior was observed with respect to degree-days accumulation and yield (Figure 3). The regression equation showed that yield decreased 0.94 ton ha^{-1} for every 100 degree-days accumulated to February (R^2=0.4487; P<0.006).

It is estimated that conservation agriculture system worldwide is growing at a rate of 5.3 million of hectares per year (Kassam, Friedrich, Shaxson & Pretty, 2009). Recently, number of studies show the benefits of this production system. This practice involves the evaluation of climatic and edaphic conditions for each region and the crop response to them (Verhulst, Kienle, Sayre, Deckers, Limon-Ortega, Tijerina-Chavez & Govaerts, 2011). It is observed that yield of wheat under raised bed planting system has not exceed the yield obtained under flat planting system in regions with arid climate and soil saline conditions (Saifuzzaman *et al.*, 2011). However, when there is an increase in yield, it has been associated to improvement in soil conditions (incorporation of crop remains) (Limón-Ortega *et al.*, 2011).

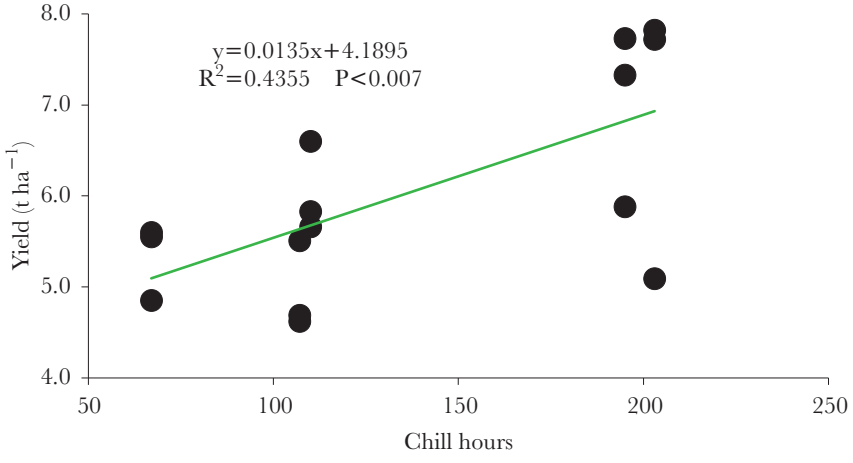

Figure 2. Relationship between chill hours accumulation and yield of wheat in five years.

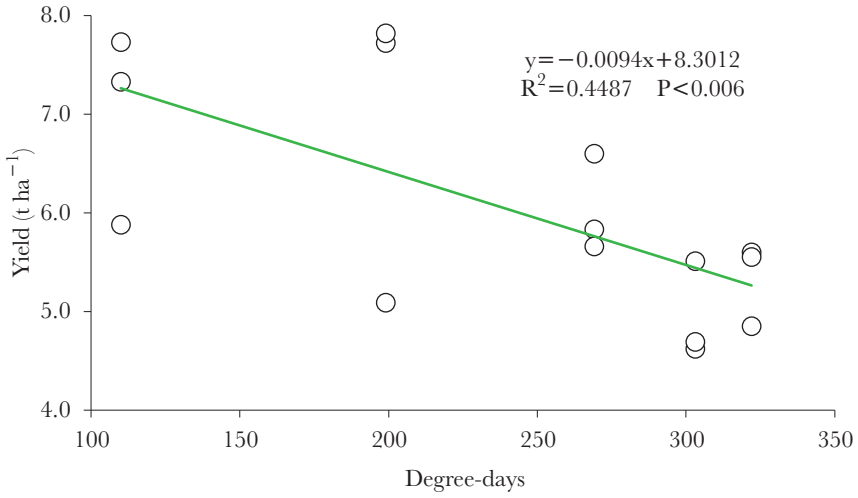

Figure 3. Relationship between degree-days accumulation and yield of wheat in five years.

This study evaluated three planting systems on the growth and yield of wheat under environmental conditions of Mexicali Baja California. The flat planting and the narrow bed planting system provided the same yield; while the yield under wide bed planting system was reduced. It is possible that decrease in yield on this system was due to spacing between them. Fischer *et al.* (2019) have also documented this response. Nevertheless, they stated that yield could fluctuate between years because of low temperatures at the time of planting and crop emergence. They also mentioned that wheat late plantings (past January) lead to better yields.

It was identified a significant relationship between yield drop and increase of temperatures on February ($P<0.006$). According to Verhulst *et al.* (2011), it is just the period for completing the stage of heading and beginning of stem elongation. This process could have modified the pattern of dry matter partitioning to stems, leading to a decrease in harvest index (Banerjee and Krishnan, 2015). The hypothesis is contrasting because of the increase in chill hours during the same month (February). Nuttall *et al.* (2018) indicated that heat waves in period before flowering affected the yield. On the other hand, Calderini *et al.* (1999) exhibited that lowering yields are associated to temperature drop between stages of growth.

CONCLUSIONS

The dry weight of the stems, the number of spikes and the yield of the wheat crop were affected by the sowing method. The lowest yield resulted from planting in wide beds. Likewise, the weight of the grain and the harvest index were not modified with the sowing method. On the other hand, the weight of the stems increased and the harvest index was reduced due to the effect of the years of study. An association between performance and hot or cold hours in February was identified. In general, the use of narrow beds can be a management practice in wheat cultivation because the yield is not affected.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Analysis of the origin and training of the labor dedicated to the post-harvest process of banana in Tecomán, Cerro de Ortega, Colima, Mexico

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ABSTRACT

Objective: To analyze and identify the place of origin, skills and working conditions of the laborers dedicated to the banana cutting and packaging process in the town of Cerro de Ortega, Tecomán, Colima.

Design/Methodology/Approach: A field checklist was designed and applied to 32% of the local crews and a survey directed to the members of the crews, surveying 21.3% of the total population.

Results: Information was obtained to carry out the analysis of origin and the current situation of laborers dedicated to the post-harvest process of banana in the study area.

Limitations of the study: An important limitation for the research was insecurity during the field surveys, which was present derived from the organized crime settled in the area.

Findings/Conclusions: The most specialized workers in the area who are dedicated to the activities of cutting and packing bananas are not native people from the municipality of Tecomán, nor from the state of Colima. They belong to the states of Chiapas and Michoacan.

Keywords: Training, agricultural labor.

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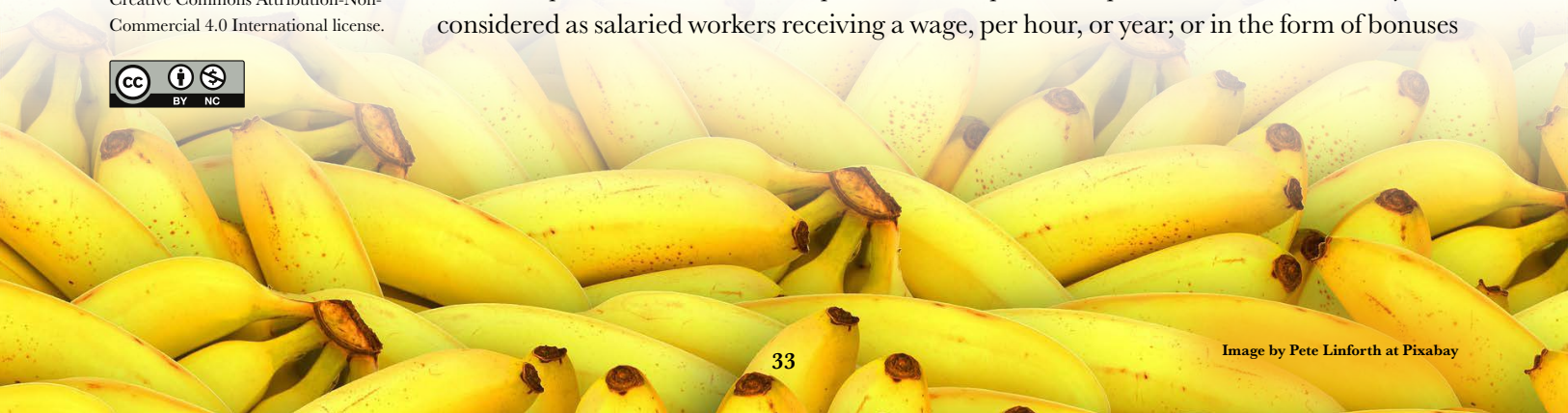
INTRODUCTION

In the State of Colima, the banana is a crop that requires a lot of labor. Although it does not require many personnel in the cultivation tasks that are carried out within the orchard, it does require many people during the harvesting process and post-harvest. A clear example is that regarding the production process, two contracted laborers and the owner of the orchard can easily take over the management of up to 10 hectares. While for the harvest and cutting of those same 10 hectares, between 8 to 12 people are required to carry out this process (Consejo Estatal de Productores de Plátano de Colima - CEPPC, 2016).

For Spencer (1993), labor is defined as all those services that laborers, semi-qualified workers, professionals and independent entrepreneurs provide. Therefore, they are considered as salaried workers receiving a wage, per hour, or year; or in the form of bonuses



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or commissions. The salary is then, the cost paid for the use of their services; which can also be a specific rate in agreement for a certain amount of work.

Gamboa *et al.* (2015) defines agricultural worker or laborer, as the person who receives a salary for their labor in an activity in the field within a production process. In most cases, the wage is obtained per day and in Mexico is called "jornal" (a labor-day payment).

In the case of laborers engaged in the cutting and packaging of bananas in the region, they can be categorized as a specific type of hand-work. They provide a unique service in the area, that any other group can not particularly provide and do so based on the definition of Spencer (1993). This labor force works for a salary, which is a specific remuneration on a countable amount of work, since they charge payment for a full truck, instead of per weight or per boxes.

Previously, in the state of Colima, people from states such as Tabasco and Chiapas were mobilized to go to work in the Colima fields. One main reason was that these people could be paid less for their services than native workers of the state; coupled with the scarcity of skilled labor in several of the post-harvest processes of agricultural products in the state of Colima. Day laborers of other states accepted, since that represented an income greater than what they received in their states of origin carrying out the same agricultural activities. Once they arrived to Colima, they were distributed in the different crops that demanded this labor and in the activities in which they had already had experience. In the case of banana, plantain it is a perennial crop in which harvest can be made every week due to their staggered plantings. This fact gave foreign laborers the baseline to have a constant source of employment.

CONAPO (2018) defines migration as the action by which a person stops residing in a specific geographic unit (either way a municipality or delegation, a federation state or a country) to establish their habitual residence in another.

Pimineta (2002) defines migration as any spatial movement that implies a change of residence and results in the continuous stay in the destination. In this sense, the necessary condition for migration to exist is that there must be a change in the geographical place of residence of one or a group of individuals, in order to settle permanently in another place different from their origin.

Despite the fact that this practice was carried out seasonally, agricultural workers from other states of the republic have been settling in the Colima territory. In the case of the workers of the banana post-harvest process, little by little they have got to live in Colima. Moreover, they have been developing and improving empirically the knowledge they have in these tasks; they have formed non-formal groups now called crews which have rustic work equipment to carry out the packaging of banana in a differentiated way in process. Even with difference in price between first quality (packing in one-time use cardboard boxes) and second or third quality (packing in reusable wooden boxes). They work in a mobile way; obviously these crews are not fully trained, which causes waste during the process of cutting and packing the fruit, thus land owners and producers income is reduced.

In the state of Colima during 2015, 179,820 tons of bananas were produced in an area of 5,567 hectares. These yields place the state as the fourth most important producer at the national level (SAGARPA, 2016). Within the Colima state the main banana areas are located

in the municipalities of Tecomán, Armería, and Manzanillo. In all municipalities, small-scale producers predominate. This productive activity is carried out by approximately 463 producers represented in five local associations and 8500 agricultural workers that are not organized in any union and do not have formal training to perform cutting nor packaging activities. There is then an increase in unnecessary fruit waste during this part of the post-harvest process, affecting the income of the banana producers in the state (CEPPC, 2016).

Tecoman is the largest municipality in agricultural production in the state, with a sown fields cover of 4140.04 hectares, reaching 3512.84 hectares of harvested area, a production of 136 904.00 tons and a total production value of $348 102 352.72 (SIAP, 2016). In the territory there are agricultural companies dedicated to produce and export bananas of the giant-dwarf variety. There are large areas planted with this crop and specialized facilities for the packaging process. Therefore, they attract a lot of the labor force in the region. However, they have a large labor rotation over a very short time, meaning that these companies do not retain workers as they should. As the packers are unemployed and for small- and medium-scale producers, those packers are the only alternative they have, a negotiation process begins, because the producers need means to pack their fruit on time. Thus, despite any other circumstance the producer has to pay more for their services, also facing the risk that the packers or crews do not completely know how to carry out the appropriate process, then causing losses in the quantity and quality of their product.

For this reason, a research was carried out that allowed to analyze the current situation of the workforce in the town of Cerro de Ortega, Municipality of Tecomán, state of Colima, in order to characterize the activities carried out within the process of cutting and packaging of banana and to identify skills and technical capacities that cutting and packing laborers should have in order to improve post-harvest handling so that plantain producers can offer quality fruit to the market.

MATERIALS AND METHODS

According to the State Council of Banana Producers of Colima SC. (CEPPC) the strongest banana region in the municipality of Tecomán in terms of production and specialization in cutting and packaging is in the town of Cerro de Ortega, which is why this area was defined as the study area.

In the region of Cerro ortega there are 25 gangs identified by producers and works n in the process of cutting and packaging this is to universe such , on average each comprises gangs of 12 people , in field is sampled at 32% of the total number of crews in the community, applying a checklist to evaluate the activities they carry out, was surveyed 21.3% of the total population that is dedicated to the process of cutting and packing plantains in the town of Cerro de Ortega , with the purpose of knowing the origin and origin as well as the labor capacities they have and analyze the current situation in which they are organized as a productive work unit.

This research was carried out in the municipality of Tecomán, Colima, which is located in the southeastern portion of the state between coordinates 103° 59' to 103° 73' of West longitude and 18° 41' 20" to 19° 06' North latitude. The approximate distance from the municipal seat to the state capital is 46 km, the municipal area of Tecomán is 789.7 square

kilometers. Due to extension, it is the second largest municipality in the state of Colima, since Tecomán encompasses the 14% of its territory (INAFED, 2017).

In this municipality, the main types of crops are perennial and to a lesser extent cyclical. There are 38,308 hectares in agricultural use (INEGI, 2016). In the state of Colima, Tecomán stands out for the production of banana, ranking as the main producer of this fruit at the state level (SIAP, 2016). To the south of Tecomán at 26 km it is located the town of Cerro de Ortega which has 3899 cultivable hectares. Among the main crops grown in the area, banana, coconut palm and lemon are predominant. The community of Cerro de Ortega has 7598 inhabitants (INEGI, 2017) dedicated mainly to plantain cultivation (D. Castillo *et al.*, 1998). Among the activities that plantain cultivation requires are the activities of cutting and packing the fruit, this is banana postharvest handling, which demands a lot of labor due to the large number of workers it requires. In the town of Cerro de Ortega, the inhabitants have specialized in these practices, besides those cultivation practices in the field.

RESULTS AND DISCUSSION

Among the activities that the banana cutting and packing process comprises, there are some that workers can carry out regardless of gender, age or experience. In the field, it was found that the minimum age of the surveyed laborers was 15 years old and the oldest was 60 years old. The person with the most work experience has 47 years, while the average in the sample is 10 years; the person with less time of work experience had 0.5 years on the job.

As part of the research objective, it was proposed to identify the place of origin of the labor force dedicated to the banana cutting and packing process, since it was hypothesized that the majority of the most trained labor force dedicated to these activities is not native to the state. That is, they are people who have been immersed in a process of migration and come from different states of the Mexican republic.

The data collected in the field have provided information that supports this hypothesis, 72% of the people who comprise the sample come from other states of the Mexican Republic and even other countries. They have acquired their knowledge in an empirical way and have been improving through time.

It is important to define the concept of labor skills or job competencies, because the search for new, better-paid work opportunities is a function of those. These skills should be an important factor to be considered by laborers before they intend a migratory movement. Hoffman (1999) defines labor skills and work behaviors of people, those which are needed to perform an effective work. The concept is applied on individual basis to a person that does a particular work.

Martens (1996) indicates that the competences are the real capacities of an individual to master the set of tasks on performance in a specific work offer.

The National Council for standardization and certification of work competences in Mexico (CONOCER, 2018) defines the job skills of people as individual-based knowledge, skills, and behaviors. This is what makes them competent to develop a given activity through their working life. It is worth mentioning that the competences are developed by

Table 1. Places of origin of the labor force.

Distribution of the States of origin of the workers		
Mexican state or country of origin	**Workers (number)**	**%**
Chiapas	34	46.58
Tabasco	7	9.59
Colima	20	27.40
Michoacán	9	12.33
Puebla	1	1.37
Guatemala	1	1.37
El Salvador	1	1.37
TOTAL	73	100

Source: own elaboration with data collected in the field.

any type of person who performs a job. Competences are not limited on whether a person is a professional or not.

For Levy-Leboyer (1997), cited by Díaz *et al.* (2002), competences are repertoires of behaviors that some people master better than others, which makes them effective in a given situation. These behaviors are observable in the daily reality of work and also in testing scenarios.

In the field, it was found that the necessary skills that workers dedicated to the banana cutting and packing process must develop were a response to the type of packaging where the fruit would be packed and transported. According to Tharanathan (2003), the main advantage of the packaging of the various types of products is product protection and preservation; food and raw materials are their highest-priority in terms of development. Those products require attention due to the contamination generated by microorganisms (bacteria, spores, fungi, etc.) during handling.

The main types of packaging used to storage bananas are wooden boxes covered by a plastic bag and newspaper wrap for second-quality fruits that are destined for Mexico's national market. But for the Premium export and national first-quality fruits cardboard single-use boxes, fruits covered with thin plastic bags or Kraft paper (in some cases even, they are closed at high-vacuum depending on the distance of the destination of the fruit).

In the case of wood packaging, it is transported in open wooden cabinets on the chassis of big trucks with an average capacity of 14 tons. Of that people surveyed, 22% declared that they have specialized in this type of packaging and transport, since it does not require too much work specialization. The market where this fruit is placed is not very demanding, workers take around 8 hours on average to fill these trucks, so for one day they only pack fruit to fill one truck.

78% of respondents stated that they are specialized in packing in cardboard boxes, indicating that a person needs to perform one series of more delicate and specialized activities, therefore, these crews get a higher salary than those that pack in wooden boxes. The market to which higher-quality fruit is directed is more demanding due to the first-

quality national and Premium export quality standards. A special means of transport in dry-ambient closed trailers with a Thermo King™ refrigeration system with an average capacity of 22 tons, the equivalent to 1200 boxes packed and stowed. The estimated time for one crew to fill one of these trailers is 8 to 10 hours depending on the field and technological conditions at the orchards, therefore it was found that they also can only fill one trailer per day.

Regarding the degree of training of the crew members, it was found that only 39% of them have received proper training, while the remaining 61% have obtained their knowledge by seeing how the activities are carried out by their crewmates. Of the people who reported that they had got a training course, it was found that 72% of them had access to these courses from the companies where they worked; that is, from the private capital sector. Finally, it was found that 12% have received training by close family members.

It is worth mentioning the absence of Governmental or producers' organizations; only 4% of the people who affirmed that they had got training, reported that it was obtained from an organization. The 90% of respondents stated that they were not asked any minimum experience to start working with the crew. General assumption is that in fact, workers learn by daily experience as working occurs.

Derived from the difference in the types of packaging used for fruit, the workers have had to develop knowledge and competences to fit in more than one working position within the crew. Of those interviewed 81.2% have stated that they know how to make more than one activity in the crew, while the remaining 18.8% have limited themselves to work in a single position and have not shown interest in training.

Training represents a better alternative for work because laborers can temp-replace other workers in other crews. This gives them flexibility to obtain a better income on certain occasions.

Table 2. Percentage of workers trained by activity.

Activity	Workers who know how to carry out the activity (%)	Workers who do not know how to carry out the activity (%)	Total (%)
Cutting	93.75	6.25	100
Carrying	90.62	9.38	100
Reception	93.75	6.25	100
Fruit bunch trimming	89.06	10.94	100
Selection	76.56	23.44	100
Rinse	85.93	14.07	100
Tray-delivering of fruit bunches	68.75	31.25	100
Labelling	78.12	21.88	100
Aspersion	85.93	14.07	100
Packing	76.56	23.44	100
Paper-wrap coverage and box packing	93.75	6.25	100
Stowing	85.93	14.07	100

Source: own elaboration with data collected in the field.

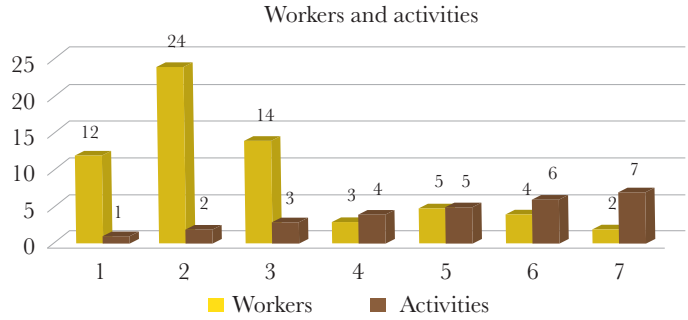

Figure 1. Number of workers and number of activities they know how to perform.
Source: Own elaboration with data collected in the field.

It can be seen in the previous graph that very few people know how to carry out more than 4 activities. That would give people more flexibility and the opportunity to work with more than one crew per week. An important fact that should be highlighted is that these more trained people arrived from the states of Chiapas and Michoacan. This is noticeable because it shows a direct relationship between the workers' level of training and the state from which they proceeded.

Within the whole packaging process there is a differentiation among payments for activities. This is due to two factors, the first one is the degree of specialization that is required to carry out the activities; as in the specific case of the people dedicated to cut and carry, and the others packing the fruit. The second factor that drives payment differentiation is the physical effort necessary to carry out the activity. As there are some activities that demand more effort than others, such as the trimming of the fruit bunches and the carrying.

Below is a table showing the average payment per activity:

Table 3. Average wages per activity according to the type of package.

Activity	Average salary	Minimum wage paid for activity in wood	Maximum wage paid per activity in cardboard
Cutting	$525.00	$400.00	$800.00
Carrying	$500.00	$500.00	$500.00
Reception	$400.00	$350.00	$500.00
Fruit bunch trimming	$400.00	$400.00	$400.00
Selection	$490.00	$450.00	$600.00
Rinse	$375.00	$350.00	$450.00
Tray-delivering of fruit bunches	$345.00	$300.00	$450.00
Labelling	$340.00	$300.00	$400.00
Aspersion	$300.00	$300.00	$300.00
Packing	$527.00	$400.00	$650.00
Paper-wrap coverage and box packing	$450.00	$400.00	$500.00
Stowing	$512.00	$350.00	$700.00

Source: own elaboration with data collected in the field.

It is worth mentioning that 95% of sampled workers stated that they work in up to four crews a week; that they exchange their working positions, diversifying their income. Whereas the remaining 5%, worked for a packaging company and had their positions established within their crew. These latter workers are guaranteed to work 6 days a week while the others can work 4 days a week on average. Because of that, they have had to find a way to insert themselves into other crews.

The crews in Cerro de Ortega are well-organized structures within their functions, the workers have specific positions and tasks. 95% of the crews interviewed have a representative or manager for the crew, who in general terms is their employer, he is the one who hires them and agrees with the producer.

The head of the crew carries out the coordination activities, recruitment, work position assignment and promotion of the work of the crew; advertising offers for them to producers and traders in the area. That person is responsible for transporting all workers from a meeting point towards the orchards where the work is needed. Usually, the head of the crew is the owner of the specialized equipment they use to pack in cardboard boxes, such as trays, iodine, roller belts, water tanks, tubs, spray backpacks, racks, tarps and equipment/workers transportation, which usually occurs in a three-ton truck. 64% of respondents stated that these tools are owned by the head of the crew. While 36% said they each had their tools. However, this statement was made primarily by personnel who perform their activities with just a curved knife (named chaveta) or a machete. Therefore, we can affirm that the most specialized equipment and material are properties of the head of the crew.

In the case of packaging in wooden boxes, only a tub, iodine, tarps, a water tank, umbrellas and a truck are needed. The truck is used to transport personnel and material, which are also the property of the head of the crew.

As it was mentioned above, the two types of packaging render not the same results. Thus, they are not made the same, mainly due to the equipment and materials used during these two processes neither are the same.

CONCLUSIONS

The main hypothesis of this work mentions that the place of origin of the members of the crews dedicated to the activities of cutting and packing bananas is not the town of Cerro de Ortega, municipality of Tecomán, state of Colima. We confirmed that hypothesis by finding that the workers dedicated to the activities of cutting and packing bananas are not predominantly native people of the municipality of Tecomán, neither even from Colima. It was then confirmed that the most qualified workers in this job are people who have arrived to Colima from states like Chiapas, Tabasco and Michoacán. The prior knowledge that these people have developed in their place of origin helps them to position themselves more easily in this job.

In a similar way, a direct relationship is seen between the number of activities they know how to perform and the wage that workers can achieve. Their level of training is the result of the time they have dedicated to the banana post-harvest activities. Since in this work they can begin at a very early age, and to start performing these activities they do not require prior experience, the visually learning process is easier for them, following the

trial-and-error sequence, mastering it through repetition. Thus, we concluded that it is a process of work evolution, and a type of non-formal interpersonal training.

The workers who come from other states are specialized in the handling of first quality fruit and Premium export quality, that is, they are dedicated only to packing the fruit in cardboard boxes. Since it is better paid than packaging in wooden boxes, the settlement of this labor force has been more accentuated in the municipality of Tecomán, because it is the main bananas producer in the state of Colima. The town of Cerro de Ortega is located in a privileged geographical area as it borders the municipality of Coahuyana, Michoacán. Both communities are among the most specialized in terms of production, post-harvest handling and commercialization of bananas in the central Pacific region of Mexico. This fact makes Tecomán and Cerro de Arteaga ideal places for the settlement of this migrant labor force from other states who have already had experience in this type of field work.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Reaction of inbred lines of habanero hot pepper (*Capsicum chinense* Jacq.) to inoculation of *Fusarium oxysporum*

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ABSTRACT

Objective: To evaluate the reaction of five inbred lines of habanero pepper (*Capsicum chinense* Jacq.) to the inoculation of *Fusarium oxisporum* and to identify genotypes with possible levels of resistance to this pathogen.

Design/methodology/approach: Samples of hot pepper plant stems with typical symptoms of vascular wilt disease were obtained. Then, due to microscopic morphological characteristics of mycelium and conidia in the samples, patogen was identified as *Fusarium oxisporum*. By immersion of roots, at a concentration of 1×10^6 conidia per mL, five inbred lines of habanero pepper were inoculated. In addition to the serrano type cultivar Criollo de Morelos 334 (CM-334). The percentage disease index and area under the disease progress curve (ABCPE) were estimated in a randomized complete block design, with three replicates.

Results: Significant differences ($p\leq0.05$) were observed for disease index and ABCPE in the genotypes evaluated; evidencing the difference in the genetic basis of the genotypes and the resistance or susceptibility to *Fusarium oxisporum*. The CM-334 cultivar, the Habanero 5 and Habanero 8 lines showed the lowest percentage of incidence of the disease (10, 20 and 30%, respectively) and ABCPE of 300, 560 and 880. In turn, lines Habanero 9 and Habanero 6 reached the highest ABCPE values with 2220 and 2190, respectively.

Study limitations/implications: The disease resistance response in plants is complex and contains multiple interactions between genes, proteins, and metabolites.

Findings/conclusions: The grouping of genotypes according to their reaction to *Fusarium* wilt disease facilitated the identification of resistant and susceptible lines, which can be considered in subsequent genetic improvement studies for hot pepper cultivation.

Keywords: genetic resistance, inoculation, inbred lines, plant breeding.

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INTRODUCTION

The genus *Capsicum* is made up of around 35 species, among which the most important for their domestication and cultivated area are *Capsicum annuum* L., *Capsicum frutescens* L.,

Image by jacqueline macou at Pixabay

Capsicum pubescens Ruíz & Pavón, *Capsicum baccatum* L. and *Capsicum chinense* Jacq. (Carrizo *et al.*, 2013). Mexico is the center of origin and diversity of *C. annuum* and the species *C. frutescens*, *C. pubescens* and *C. chinense* are also present. Aguilar-Rincón *et al.* (2010) reported for Mexico 56 types of chilli pepper among those cultivated, semi-cultivated and wild.

The habanero pepper (*Capsicum chinense* Jacq.) is a crop of importance in Mexico, its demand is increasing in national and international markets (Medina-Lara *et al.*, 2008). In 2019, 1135 ha of habanero pepper were planted, with an average yield of 18.4 Mg ha^{-1} and a total production volume of 20 829.61 Mg (SIAP, 2019). This crop is distinctive of the Yucatan Peninsula, Mexico; it was introduced by the Caribbean, adapted to the agroecological conditions and traditional management of Yucatecan farmers. In addition, it was integrated into the culture and gastronomy of the region, from where it was dispersed throughout the country and is currently planted at commercial scale in at least 17 states (Latournerie *et al.*, 2002).

This type of hot pepper is used as an important ingredient in the cuisine of countries such as Mexico, China, Thailand and South Korea (Nass *et al.*, 2015). It is known for its high pungency, ranging from 250 000 to 700 000 Scoville Heat Units (SHU), in addition to the unique aroma of the fruits. The size of the fruit normally varies from 2.9 to 6.0 cm long, 2.5 to 4.6 cm wide and from 7 to 12 g in weight. Habanero pepper is also an important source of phytochemical compounds for the human diet, such as vitamin C (Teodoro *et al.*, 2013), capsaicinoids (Jeeatid *et al.*, 2018), phenolic compounds (Campos *et al.*, 2013), carotenoids, flavonoids (Butcher *et al.*, 2012) and other secondary metabolites with antioxidant properties (Castro-Concha *et al.*, 2014).

Fusarium wilt is one of the most important diseases in chilli pepper cultivation; the causative agent *Fusarium oxysporum* causes significant yield losses (Mushtaq & Hashmi, 1997). The pathogen invades the vascular system causing wilt, chlorosis and leaf necrosis. The first symptoms are observed in the basal leaves as a unilateral yellowing, subsequently the leaves wither, dry but remain attached to the plant. The main roots and the base of the stem present vascular necrosis; when cutting transversely or longitudinally the diseased stems or the base of the petioles, necrotic tissue in the vessels of the xylem is observed (Villa *et al.*, 2014). The pathogen causes vascular wilt in a wide variety of economically important crops worldwide (Ortoneda *et al.*, 2004). It is a necrotrophic, soil-borne fungus with worldwide distribution in tropical to subtropical areas (Booth, 1971).

Chemical control is not effective in treating this disease, in addition to increasing production costs, it causes damage to the health of consumers and represents a negative environmental impact (Moran-Bañuelos *et al.*, 2010). In this regard, the use of resistant cultivars is a cheap, safe, non-polluting and reliable control method to treat diseases in agricultural production. Hence, one of the important options is to find varieties of cultivated plants capable of resisting the damage caused by these diseases (Cheema, 2018). A key aspect for the incorporation of resistance to a variety of commercial hot pepper is to use parents with genetic resistance.

Therefore, the objective of this study was to evaluate the reaction of five lines of habanero pepper (*Capsicum chinense* Jacq.) to the inoculation of *Fusarium oxisporum* and identify genotypes with possible levels of resistance to this pathogen.

MATERIALS AND METHODS

Genetic material

Five lines of habanero pepper (*Capsicum chinense* Jacq.) were evaluated, the seed was provided by the National Institute of Forestry, Agricultural and Livestock Research (in Mexico, INIFAP), obtained from the Genetic Improvement Program of chilli pepper cultivation. The serrano type cultivar Criollo de Morelos 334 (CM334) was also evaluated.

Isolation and identification of the pathogen

Samples of stems were obtained in chilli pepper plants (*Capsicum annuum* L.) with typical symptoms of vascular wilt disease, in which longitudinal cuts were made to the stem for the purpose of observing symptoms of internal brown necrosis in the conductive vessels. Then, sections of the stem of approximately 3 mm were cut, disinfected, and sown in a potato dextrose agar (PDA) culture medium in Petri dishes, incubated at 25 °C for eight days. After which hyphae tip transfer was performed to obtain monosporic cultures (Amini, 2009). The identification of *Fusarium oxysporum* (Fo) was made considering the symptomatology present in diseased plants and by the microscopic morphological characteristics of mycelium and conidia in culture medium (Gerlanch and Nirenberg, 1982, Leslie and Summerell, 2006).

Inoculum preparation and inoculation

The *Fusarium* cultures (10-day-old) grown in PDA medium were washed with sterile distilled water to obtain an inoculum suspension of the pathogen. For each isolate, the concentration of spores was measured by performing macro conidia counts using a Neubauer hematocimeter in a 40X magnification microscope. For inoculation, Habanero pepper seedlings with 68 d of germination were used, using the technique of immersion of roots in a suspension of 1×10^6 conidia per mL. After inoculation, the plants were placed in polyethylene vessels of 240 mL capacity with a mixture of soil and peat moss, kept in a greenhouse for 20 d at a temperature of 25±2 °C.

The response of plants to inoculation was performed using a severity scale proposed by Apodaca-Sánchez *et al.* (2004) and Clavijo-Castro (2014) to estimate the severity of the disease, where 0: corresponds to plants without visible symptoms of the disease, 1: necrotic points in hypocotyl, 2: withered leaves, darkening at the base of the hypocotyl or decrease in plant growth, 3: wilting, necrotic lesions and decrease in plant growth, 4: necrotic injury, defoliation and decrease in growth, 5: death of the plant. Using the scale values assigned to the plants, a percentage disease index was estimated for each of the genotypes using the following formula:

$$IE = \left[\left(\frac{\sum_{i=1}^{n} X_i}{n}\right)0.2\right]100$$

where: X_i=severity of the disease in the *i-th* seedling; n=number of seedlings evaluated; 0.2=correction factor for disease percentages.

The disease indices obtained were used to determine the progression of the disease and the response of these materials to inoculation of the pathogen, by calculating the area under the disease progress curve (ABCPE) according to the following equation:

$$ABCPE = \sum_{i=1}^{n-1} \left\{ \left(\frac{y_{i+1} + y_i}{2} \right) \left(t_{i+1} - t_i \right) \right\}$$

where "*t*" is the time of each reading, "*y*" is the percentage of plants affected in each reading and "*n*" is the number of readings (Shaner and Finney, 1977).

Statistical analysis

All pathogenicity tests were performed in a random complete block design, with three replicates. The experimental unit consisted of 5 pots per genotype. With the values obtained for disease index and ABCPE, an analysis of variance was performed using the GLM procedure (SAS Institute, 1999) and comparison of means with the Tukey test ($p \leq 0.05$). The analysis of variance for the disease index was performed with values obtained at 6, 12 and 18 d after inoculation.

RESULTS AND DISCUSSION

The results obtained in this study show significant differences ($p \leq 0.05$) for disease index and ABCPE in the genotypes evaluated. The above mentioned highlights the difference in the genetic basis of the evaluated lines and the resistance or susceptibility they presented to *Fusarium oxisporum*. One way to assess the damage caused by pathogens in chilli pepper plantations is through the quantification of the incidence, which measures the number of affected plants expressed as a percentage. It is also used to determine resistance to this pathogen.

In this study, the first symptoms of the disease (chlorosis) were observed 8 d after inoculation (DDI), with increased severity over time. The CM-334 cultivar, the Habanero 5 and Habanero 8 lines, were the genotypes that showed the lowest percentage of incidence of the disease (10, 20 and 30%, respectively) and ABCPE of 300, 560 and 880. On the other hand, lines Habanero 9 and Habanero 6 presented the highest ABCPE values, 2220 and 2190, respectively (Table 1). In this regard, the area under the disease progress curve is a quantitative summary of the inoculation of the pathogen; the lowest values correspond to the materials with the lowest incidence of disease, that is, with a higher level of resistance (Bautista *et al*., 2009).

The disease resistance response in plants is complex and contains multiple interactions between genes, proteins and metabolites. Horizontal and vertical resistance are a continuum of different mechanisms developed by the plant to interact with the external environment, including pathogens (Burbano-Figueroa, 2020). In this regard, plants and pathogens develop complex mechanisms of attack and defense. The defense system of plants is the ability to perceive pathogens and activate effective defense responses (Grube *et*

Table 1. Percentage of disease development over time and area under the disease progress curve (AUDPC) in chili pepper plants after artificial inoculation by root immersion of *Fusarium oxisporum*.

Genotypes	Disease index			AUDPC
	12 DAI†	16 DAI	20 DAI	
Habanero 10	75.0 a	85.0 a	97.0 a	2120 c
Habanero 9	75.0 a	75.0 b	80.0 b	2220 a
Habanero 6	70.0 a	75.0 b	80.0 b	2190 b
Habanero 8	30.0 b	30.0 c	30.0 c	880 d
Habanero 5	20.0 c	20.0 d	20.0 d	560 e
CM-334	10.0 d	10.0 e	10.0 e	300 f

†Days after inoculation.

al., 2000). Resistance in plants involves resistance proteins (R) that detect specific effector proteins (Avr) produced by the pathogen.

The use of chilli pepper varieties with genetic resistance represents a good alternative of sustainable production replacing frequent applications of pesticides. Therefore, the management of plant diseases is one of the main objectives in genetic improvement programs. However, host-pathogen interactions involving resistance are far from simple. Plants develop resistance mechanisms, pathogens develop strategies to overcome plant resistance; plants, in turn, develop new defensive measures that select for additional changes in the pathogen (Stahl and Bishop, 2000; Gururani *et al*., 2012).

Figure 1 shows the progress of vascular wilt disease along the development of plants inoculated with *Fusarium oxysporum*. During the evaluation time, it can be observed that genotypes CM-334, Habanero 5 and Habanero 8 presented the lowest incidence values of the disease in the former days of evaluation, delaying the onset of the disease probably as a defense mechanism of the plants to the attack of the pathogen.

This does not prevent plants from being infected but reduces the rate of increase of the disease in each of the points of infection. Therefore, this delays the spread of the disease

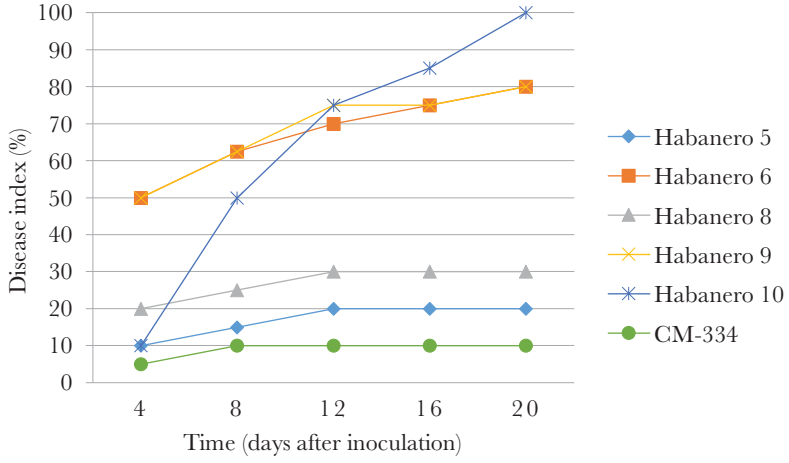

Figure 1. Response to *Fusarium oxysporum* inoculation in six chili pepper genotypes.

and the development of outbreak events in the field (Van der Plank, 1984). In this study, disease incidence values greater than 80% were observed; in contrast to other studies, the incidence and severity was variable, because the environmental conditions, variety and virulence of the pathogen were different.

The grouping of genotypes according to their reaction to *Fusarium* wilt disease facilitated the identification of resistant and susceptible lines, which can be considered in subsequent genetic improvement studies for hot pepper cultivation. Vallejo-Gutierrez *et al.* (2020) assessed variation in *Fusarium* wilt damage response in 16 genotypes (M1-M16) of Manzano pepper (*Capsicum pubescens* R. and P.), identifying the M8 genotype with resistance to *F. oxysporum* and *R. solani*. In this regard, Anaya-López *et al.* (2011) collected chilli pepper plants with symptoms of wilt and identified 26 collections resistant to *Fusarium* spp. Thus, they represent sources of resistance genes potentially useful in programs of genetic improvement, oriented to the control of the wilt on chilli pepper in the producing regions of central and northern Mexico.

CONCLUSIONS

The Habanero 5 and Habanero 8 lines were identified as the genotypes with the lowest incidence of this pathogen under greenhouse conditions. Therefore, they represent a potentially useful alternative in genetic improvement programs, aimed at controlling chilli pepper wilt.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Slaughter of pregnant cows in the municipal abbattoir of Pijijiapan, Chiapas, Mexico

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ABSTRACT

Objective: To quantify the percentage of breeding heifers slaughtered and the stage of gestation of the product in the municipal slaughterhouse of Pijijiapan, Chiapas.

Approximation: Many breeding heifers are sent to the slaughterhouse due to various factors, such as the few gestations diagnostic tests carried out in the field and erroneous diagnoses. Another cause is the dry season, in which the forage capacity is reduced, and the producer must dispose of animals in his productive unit, including breeding heifers, thus decreasing the cattle herd population. This situation can be considered an animal welfare problem.

Methodology: The total number of cattle slaughtered in four months was recorded, as well as the number of females, and it was recorded whether they were pregnant or not, and the gestation period of the calf.

Results: Cattle individuals (428) were slaughtered, of which 399 were females (93.22%) and 29 males (6.78%). Of the females, 211 (53%) were pregnant, 39.81% in the first third of gestation, 27.48% in the second and 32.22% in the third.

Limitations: It is necessary to increase the study period and not only in this municipality but also to implement similar research in other slaughterhouses in Chiapas to understand this problem better.

Keywords: breeding heifers, bovine slaughter, municipal slaughterhouses, cattle herd, animal welfare.

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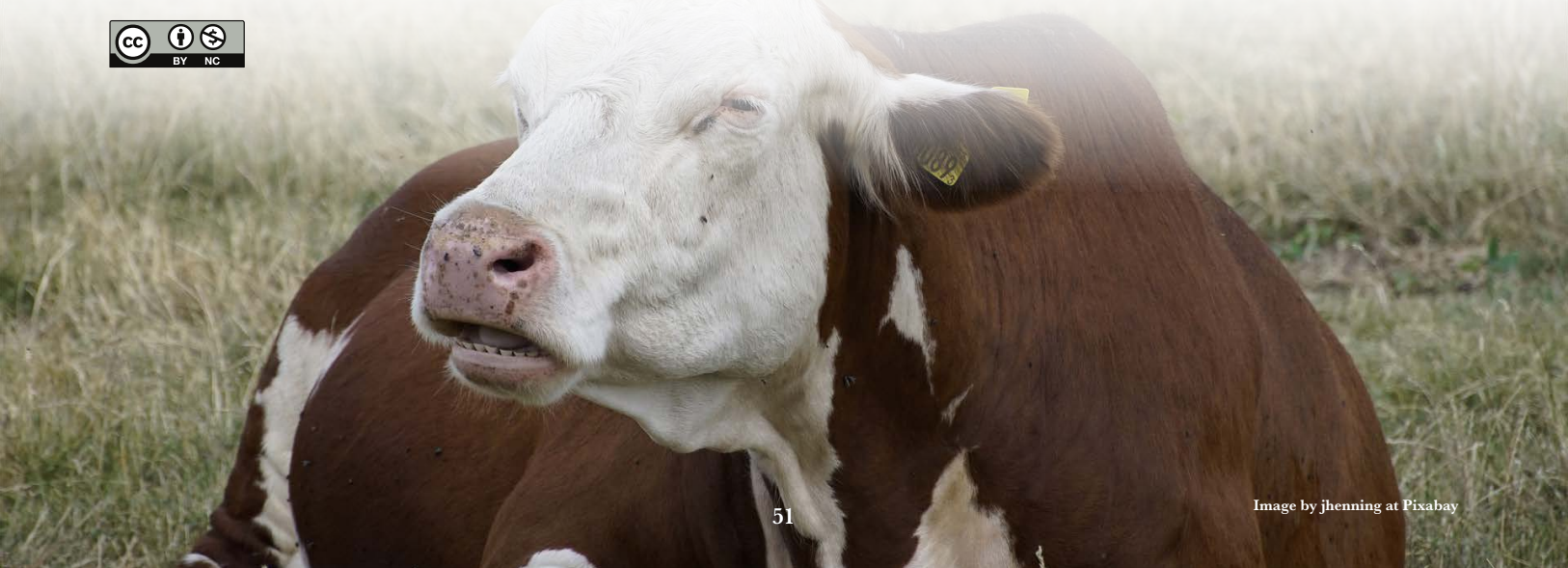
INTRODUCTION

Cows are sent to the slaughterhouse at the end of their production cycle. At the same time, female calves are admitted to the slaughterhouse after a fattening period or due to reproductive disorders (Zitterer and Paulsen, 2021). However, in some cases, breeding



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heifers are slaughtered (Saxmose *et al.*, 2019). Producers may need to be made aware that animals sent to the slaughterhouse are pregnant for different reasons, such as the lack of pregnancy tests, misdiagnosis, or little information on the part of farmers (Nielsen and Hawkes, 2019). The slaughter of animals in slaughterhouses has shown that not only conventional non-breeding cattle are slaughtered for meat but also pregnant and lactating productive females (Gregory and Grandín, 2007).

In Mexico, since gestation diagnosis is not common, many bovine breeding heifers are sent to the slaughterhouse in the early stages of gestation (Fernández-Figueroa *et al.*, 2015); this situation contributes to the reduction in the number of bovines. In addition, the marked dry season favors that a high number of animals per hectare is maintained in the rainy season. When the drought arrives, the forage capacity is reduced. Producers are then compelled to reduce the number of animals in their productive unit, including breeding heifers, which brings as a consequence that in the short term, the livestock herd must be repopulated to substitute those slaughtered bellies (FORBES, 2013; Moreno *et al.*, 2017).

In the case of the state of Chiapas, Orozco (1987) indicated that in the municipality of Tapachula, 53% of the slaughtered bovine females were pregnant. In the municipality of Tonalá, Pozos (1999) found out of 998 genital organs of bovine females in the slaughterhouse, 44% of the females were in a state of gestation. Due to this problem, it is necessary to generate information to contribute to the analysis of the impact of the slaughter of breeding heifers on bovine depopulation. On the other hand, the slaughter of breeding heifers has ethical implications from an animal welfare point of view, as well as about the quality of the meat, which would contain a higher content of steroid hormones (Zitterer and Paulsen, 2021).

MATERIAL AND METHODS

The study was carried out for four months (October-January) in the municipal slaughterhouse named "Sociedad Cooperativa de Bienes Tablajeros (SENASICA)" in the municipality of Pijijiapan, Chiapas, located in the Economic Region IX Istmo-Costa (Isthmus-Shore) (CEIGE, 2021), with a humid tropical climate with rains in summer (García, 2004).

The animals were slaughtered by disgorgement at the slaughterhouse, without previous stunning. The total number of slaughtered cattle and slaughtered females was counted daily. After slaughter, the reproductive organs of the females were separated from the carcass and deposited in nylon bags after identification. They were separated into empty and pregnant. The uterus was extended on a table. Later an incision was made in each uterine horn along its dorsal surface, from the uterus-tubal junction to the bifurcation of the uterus' body to expose the lumen. Similarly, the body of the uterus was sectioned following a straight line to the dorsal corner of the vulva to completely expose the cervical canal and the vagina (McEntee, 1990, Erales-Villamil *et al.*, 2008). Immediately, the products (fetuses) were extracted.

Keller's formula was used to classify the age of the product, according to the third of gestation in which they were, based on the fetuses' or embryos' development and measurements.

To do so, we measured from the occipital vertex to the gluteal region, as follows: 1st third (17 cm), 2nd third (18-60 cm), and 3rd third (60 cm) (Hafez, 1978; Sosa *et al.*, 1988). In this study, the breed of the slaughtered animals was not considered since it was primarily commercial Zebu cattle and crossed with European cattle.

RESULTS AND DISCUSSION

A total of 428 cattle individuals were slaughtered, of which 399 were females (93.22%) and 29 males (6.78%) (Figure 1). The data obtained are higher than those reported by Sosa *et al.* (1988), Franco *et al.* (1991), and Erales-Villamil *et al.* (2008) in studies conducted at the slaughterhouses in Chetumal (76.4%), Quintana Roo, and Mérida (37.3%) and Uman (66.3%), Yucatán, México. The differences reported by those authors with the data in this study can be attributed to the time of the period evaluated, the number of animals that arrived at the slaughterhouse, and the geographical area of the municipal facility with a more significant number of animals slaughtered daily.

Of the 399 females slaughtered, 211 were pregnant, corresponding to 53% of the total (Figure 2). Similar results were reported by Sosa *et al.* (1988) and Franco *et al.* (1991), with 55.9% and 52.1% of pregnant females at slaughter. Those latter authors indicated that there is a significant tendency to sacrifice pregnant females in the dry season; with an economic

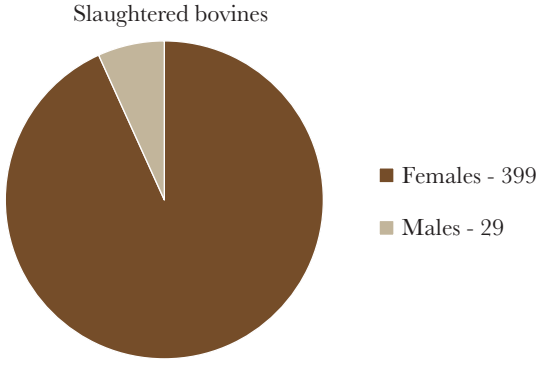

Figure 1. Female-male ratio of the slaughtered bovines in the municipal slaughterhouse of Pijijiapan, Chiapas, Mexico.

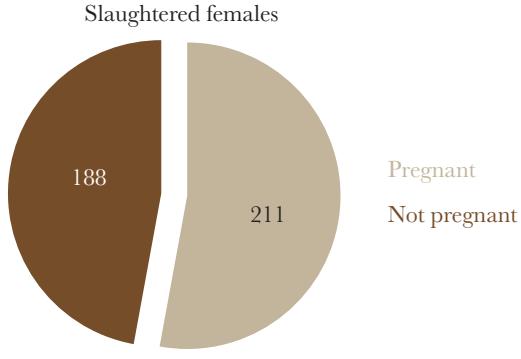

Figure 2. Pregnant-non-pregnant females ratio among the bovines slaughtered at the municipal slaughterhouse of Pijijiapan, Chiapas, Mexico.

impact on the concepts of calves at birth and dairy production (Fernández-Figueroa *et al.*, 2015).

On the other hand, Zitterer and Paulsen (2021) recorded a prevalence of 6.4% of pregnant females in a study conducted in Austria, where the slaughter of pregnant females is more restricted due to animal welfare. Since this study was carried out in the rainy season, the sale of breeding heifers can be attributed to weight gain caused by gestation with a higher economic remuneration for the producer (Sosa *et al.*, 1988). In addition, the animal may be susceptible to diseases, injuries, or other unforeseen conditions that make the producer decide to sacrifice the animal (Saxmose *et al.*, 2019).

Of all pregnant females, 85 (40.28%) were found in the 1st third of gestation, 58 (27.48%) in the second (2nd), and 68 (32.22%) in the last (3rd) third (Figure 3).

The data obtained differ from Sosa *et al.* (1988), who reported 45% of pregnant females in the 2nd third of gestation; but coincide with Franco *et al.* (1991), who referred that 30.8% of breeding heifers were slaughtered in the 1st third of gestation. Since the diagnosis of gestation is not a common practice, the producer was likely unaware that the cow was in the first third of gestation at the time of sale, which could explain why the slaughtered cows were mainly found at that stage.

Regarding the perspective of animal welfare, so far, there needs to be more information on the activation of signals that accompany brain processes for the manifestation of pain in slaughtered pregnant animals. Some studies have shown that bovine fetuses do not feel pain with electrical stunning. However, it is necessary to carry out other research using biomarkers for pain, the pain perception in pregnant female cattle when they are slaughtered with or without stunning (Fayemi and Muchenje, 2013).

CONCLUSIONS

The results showed that 53% of the slaughtered bovine females corresponded to breeding heifers, mainly in the first third of gestation, which contributes to the decrease in the repopulation and renewal of the cattle herd of the study region.

In addition, it is worth considering the lack of welfare in slaughtering cattle by killing many pregnant females and how they are slaughtered.

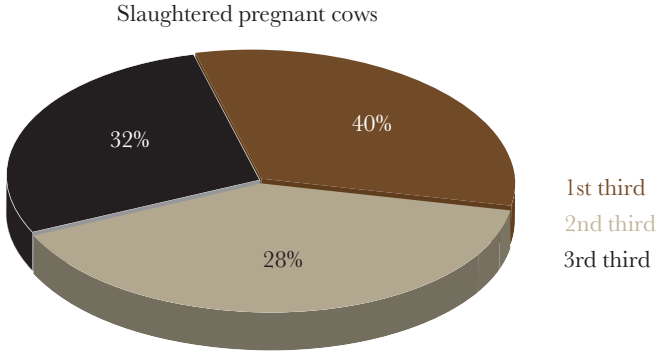

Figure 3. Distribution per third-of-gestation of the breeding heifers slaughtered in the municipal slaughterhouse of Pijijiapan, Chiapas, Mexico.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Characterization of agricultural practices in the community Mulato Viejo in Oaxaca

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ABSTRACT

Objective: To describe the standard of living in the "Mulato Viejo" community; culture and their agricultural practices facing the current governmental development policies.

Design/methodology/approach: The study was conducted during 2019, applying surveys and interviews to key informants with experience in community-elected positions.

Results: The results showed that families practice some traditional farming techniques based on family labor. There was also a lag in the incorporation of women (5.5%), and in the generational renewal of producers. For example, 84% are older than 40 years-old and out of those, only 69% completed elementary education. Thus, they do not know the rationality fundamentals of the agricultural practices they use. This limits the use of machinery to reduce the workload and the production costs, or to innovate.

Study limitations/implications: The main limitations were the sample size and the replicability of the study; a single community was analyzed. It is proposed then to expand the study to other communities with similar characteristics.

Findings/conclusions: Semi-traditional agriculture has preserved local landraces of maize. Yet, it is necessary for the community to incorporate dynamics that may diversify agricultural activities, crops; and allow establishing networks of cooperation and intra-community trade. So that agricultural activities may become an economic option profitable to further generations, who would have a greater capacity to acquire the bases for a sustainable agriculture. With the goal to improve yield but, with the challenge of keeping the traditional agricultural practices intact.

Keywords: Mulato Viejo, rural nucleus, solidarity networks.

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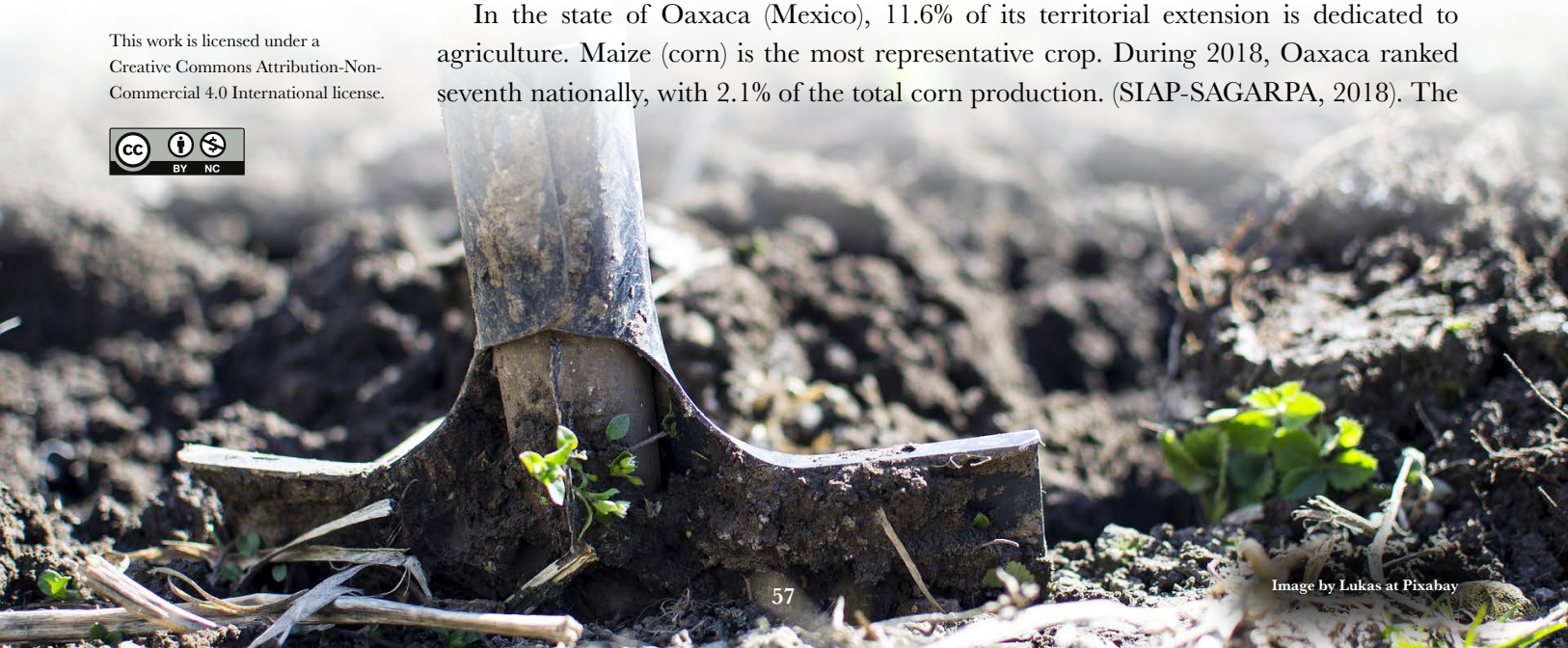
INTRODUCTION

In the state of Oaxaca (Mexico), 11.6% of its territorial extension is dedicated to agriculture. Maize (corn) is the most representative crop. During 2018, Oaxaca ranked seventh nationally, with 2.1% of the total corn production. (SIAP-SAGARPA, 2018). The



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most important regions for the cultivation of this cereal are the Isthmus, the Mixteca, the Central Valleys and the Coast (Government of Oaxaca, 2015). In addition to those regions there is the Papaloapan region, which is defined by the watersheds of the Tonto and Cosamaloapan rivers. White corn is the one mainly sown, which is used for self-consumption and transformation activities. In the municipality of Loma Bonita, a total of 5293 hectares were planted in 2019, out of which 1320 ha produced maize grain; 4062 ha, sugar cane; 1460 ha, pineapple, and 39 ha, Hevea (rubber tree) (ASERCA, 2019).

In 2017, the Cuenca region (named after the basin territory) produced 80 000 to 90 000 Mg of corn. Which represents about 10% of the state's production. However, within the basin itself there are large variations in maize planting yields. In the particular case of the Municipality of Loma Bonita, Oaxaca, the corn yield is 2.8 Mg ha^{-1}; this figure is below the national average for the grain, which is 3.84 Mg ha^{-1}. (Sánchez-Hernández *et al.*, 2019). Maize cultivation is important but not a priority for the economy in this municipality, as they base their economy on livestock, sugar cane, and pineapple plantations. However, due to cultural and food affinities, many of the producers maintain the planting of corn as one of their main agricultural activities.

In recent years, there has been a strong impulse on social programs that stimulate rural development, through a variety of social and economic supports, such as "Crédito a la palabra" (agreement-based credits), "Programa de producción para el bienestar" (Pro-Welfare production program), "Programa de precios de garantía" (Guarantee-prices program) for corn and beans. Other governmental programs are "Apoyo a construcción de vivienda" (Housing construction Support), the program "Jóvenes escribiendo el futuro" (Youth Writing the Future), "Becas para el bienestar" (Welfare through scholarships), etc. Additionally, there has been renewed interest in analyzing and get to know the traditional agricultural practices.

In this regard, Oaxaca has been a strong beneficiary of those initiatives. However, it is important to characterize the population of communities with small number of inhabitants, in order to use them as models for evaluating the impact of social support programs. All of this, to generate proposals that make the programs more appropriate, but at the same time try to influence the communities. Onto the goal of adapting some organizational and community participation proposals to the type of producers, regions and products; In order to generate analyses that correlate and link the productive, environmental, social, cultural and economic aspects involved.

The aim of the study is to describe as a case study, one of the rural communities comprised in the Municipality of Loma Bonita in the state of Oaxaca (Mexico); with particular interest on the cultural practices for planting maize.

MATERIALS AND METHODS

The study was carried out in the community of "Mulato Viejo", located in the Municipality of Loma Bonita, Oaxaca (17° 57' 40" N, 95° 54' 27" W). A non-probabilistic method was used, through a heuristic approach, called "snowball". The study team contacted Zenaida Zuñiga, inhabitant of the community, who introduced them to her relatives, whom are key informants with experience in community positions (community

representative, president of the ejido, etc.); in turn, they assisted us for the distribution and application of surveys to heads of household who are direct producers. The data were collected during October 2018 and until June 2019.

In order to meet the objective proposed in the research, a survey was designed to analyze and evaluate the characteristics of the cultural practices of maize planting (use of machinery, origin of the seed, fertilization, etc.); Likewise, information was collected on the socioeconomic characteristics of the community (schooling, land tenure, sex, secondary occupations, etc.); The surveys were conducted on the total number of maize producers, this is, 18 heads of household. Additionally, cob samples were collected from the September-October-2018 harvest and recorded using photographic evidence. The results were analyzed using descriptive statistics (means, mode, percentages).

RESULTS AND DISCUSSION

The community of Mulato Viejo belongs to the municipality of Loma Bonita in Oaxaca, is part of the 4 communities that make up the ejido of San Benito el Encinal. It is located at 2 km from San Benito, 11 km away of the municipal seat. The rural nucleus consists of two sectors. The first consists of the houses that are organized around the main street, in north-south orientation, along an approximate distance of 2 km. The second sector includes the plots, surrounding the houses, an approximate area of 200 ha.

The Organic Law for the municipalities of the state of Oaxaca, (Municipal Organic Law of the state of Oaxaca 2018) defines "Mulato Viejo" as a "rural nucleus", based on the number of inhabitants. The administrative organization includes a municipal agent, who is assisted by an alternate, a secretary and a treasurer. They are responsible for managing the necessary support for the community and serve as a liaison with the municipal seat; who validates their election by citizen vote every three years. Its legal organization includes a judge; a police commander, and 5 active police officers. The headquarters of this administration is located in the facilities of the so-called "Agency"; which includes the multipurpose hall and the prison. Although there is a remuneration for the positions, the time of dedication to the activities of communal representation is partial, so the attention to the people who require it happens under personal request.

In "Mulato Viejo", there is a commercial sector with: 3 grocery stores with basic products, 2 commercial mills, a beer store (franchise of Grupo Modelo). In terms of services, it has a church (Catholic), a medical office, which functions as a headquarters for National vaccination campaigns, although it does not have a staff doctor; the first attention is received in the clinic of San Benito el Encinal. The entire community is electrified by the Federal Electricity Commission (CFE); It has a drinking water system, although it is also common to use water from the well or the stream that passes through the community. In regard to the education sector, it has three schools, a preschool that has been operating since 1991 and a secondary (junior-high) school inaugurated in 2006. Both are dependent on the National Education Commission (CONAFE) as well as a primary school under the State Institute for Education of the Oaxaca People (IEEPO) (Figure 1).

In the visit to the community, it was observed that there is no drainage system, nor industry of any kind, the economic activity is of a primary type (agriculture and livestock,

Figure 1. Social infrastructure in Mulato Viejo, Oaxaca (Mexico). a) "Niños Héroes" community preschool; b) "Belisario Domínguez" primary school; c) "Octavio Paz" community junior (secondary) school; d) the Catholic Church; and e) Agency (Photography: own elaboration).

forest management, etc.), when there is surplus in production, transformation activities are carried out with maize corn. In the case of the tertiary sector, this consists of commercial activities and transport services that are provided outside the community.

In 2019, a total of 255 people lived in the community of "Mulato Viejo"; 64.7% are women and 35.3% are men. This population is distributed in about 55 families, of which 47.0% are engaged in agricultural activities. Mainly, to the planting of corn, pineapple, chili pepper, pumpkin, and sugarcane; as well as to the collection of rubber, and livestock. Meanwhile, the other 53% carry out activities other than those mentioned above, such as retail trade, transport, day laborers for wage, etc. (Figure 2).

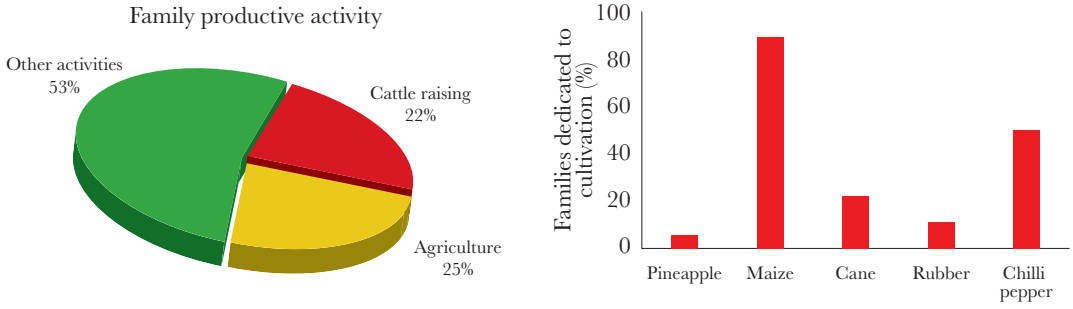

Figure 2. Primary economic activities in the community of Mulato Viejo, Oaxaca, Mexico. The percentages of families dedicated to this activity and the main crops obtained are shown (Source: own elaboration).

The combination of agricultural activities with activities that are not, is called multiactivity. Osorio-García *et al.* (2015) described this phenomenon and emphasized that it rarely implies the total abandonment of agricultural labor. This is the case of the producers in Mulato Viejo, who carry out transport activities or salaried work outside the community; However, they continue to carry out their agricultural work, in particular, the planting of corn, since they consider it essential for family food security. Additionally, the multiactivity in the community is directly related to age. Young people are the ones who look for other alternatives in addition to agricultural work; this trend changes as one advances within the age intervals.

The land tenure regime is the ejido. The distribution of land properties was made during the founding of the ejido San Benito el Encinal in 1940, and only included those who at that time lived and worked the land in the community. The successive inheritances or sales of land resulted in that, currently, not all those who cultivate the land are "ejidatarios" (ejido tenants). Among the interviewees, only 38.9% own land and are ejidatarios; among them, the proportion is 73.3% men and 26.6% women. The latter, although they are the owners, delegate the activities to the male heads of family. Likewise, 16.7% have their own land, inherited or purchased; and 44.4% do not have their own land, but rent the land. Therefore, for the purposes of this study, it was called as "Producer" who performs the tasks of planting, cultivation and harvesting of corn, regardless of whether or not he owns the land.

In "Mulato Viejo", the corn producers are mostly (95%) male, while women are engaged in household-related tasks. In contrast, the National Agrarian Registry (RAN), in 2018, indicated that 26.3% of the ejidatarios of the country, belong to the female gender who do develop activities related to agriculture. Then, it can be observed that in the community of "Mulato viejo" there is a lag in the feminization of agricultural work. In Mexico, generally, ejidatarias (female land tenants) access land and engage in agricultural activities since they inherit the land by death of the original owner, father or husband; therefore, they become the head of the family (Ramírez, 2011). However, the ejidatarias of "Mulato Viejo" are not in this case. In the first place, there are only 2 ejidatarias who dedicate their lands to the planting of corn, and in both cases, there is a man as head of the family; so, the planting work falls on the latter.

The 84% of producers are classified in two age intervals, 40 to 60 and 60 to 80 years old; only 11% are between 20 and 40 years old. Therefore, the population dedicated to the lands is aging, which will affect the trend that the community continues to dedicate to agriculture. This is also related to the level of schooling; mainly, 69% of producers have elementary schooling, and 22% secondary. Then, young people who do not have farmland that allows them to obtain economic remuneration for this activity, become demotivated and look for other economic activities to which to dedicate outside the community, which generates migration.

Cultural practices are defined as the techniques that are performed for the management of a crop; before, during and after harvest. In regard to corn, three types are managed. The community call them "criollo", "hybrid" and "unknown" variety. The creole corn is 39% of the planted material, it comes from free mixtures made during

the various planting cycles. Other 33% of producers indicate that they sow commercial hybrid varieties, which are acquired through social programs. Whereas the remaining 28% do not know the variety they sow. All producers alternately sow all three types depending on their availability (Table 1).

From the samples of cobs obtained in the community, using the method proposed by Aragón *et al.* (2006) and through the graphic comparison with the images proposed by those authors, three different races were tentatively identified: "criollo" (creole), which may correspond to cv. large Zapalote; "hybrid", which corresponds to cv. Vandeño and the unknown variety may be a Tepencintle. The values of the analyzed characteristics are shown in Table 1.

The average area destined for planting is 0.785 ha, the largest planting area is 3 ha, and the smallest is 0.118 ha. The cultural practices prior to planting are limited to primary tillage of the land (78%), the orography of the community prevents the use of heavy machinery leaving as an option manual work and the use of small-scale machinery such as motor growers and mini-tractors, brush cutter, etc., tough local producers do not know them. All of producers (100%) use agrochemical methods for fertilization and pest control and only 6% have incorporated the use of sustainable technologies, specifically, the use of compost. The planting method used by 83% of producers is manual, using the "coa" (a wooden tool similar to a tree planter, which they call "espeque"). However, it should be noted that the innovation that has been most appropriated by the producers of this community is the method of spraying fertilizers or pest control chemicals with a sprayer backpack (Figure 3).

Table 1. Evaluated characteristics of the maize varieties planted in the community of Mulato Viejo, Oaxaca (Mexico).

Sample 1 /Unknown variety/ Zapalote	**Sample 2/hybrid/Vandeño**	**Sample 3/Creole/Tepencintle**
		
Length/width ratio		
2.88	3.23	3.95
Number of rows		
7	5	5

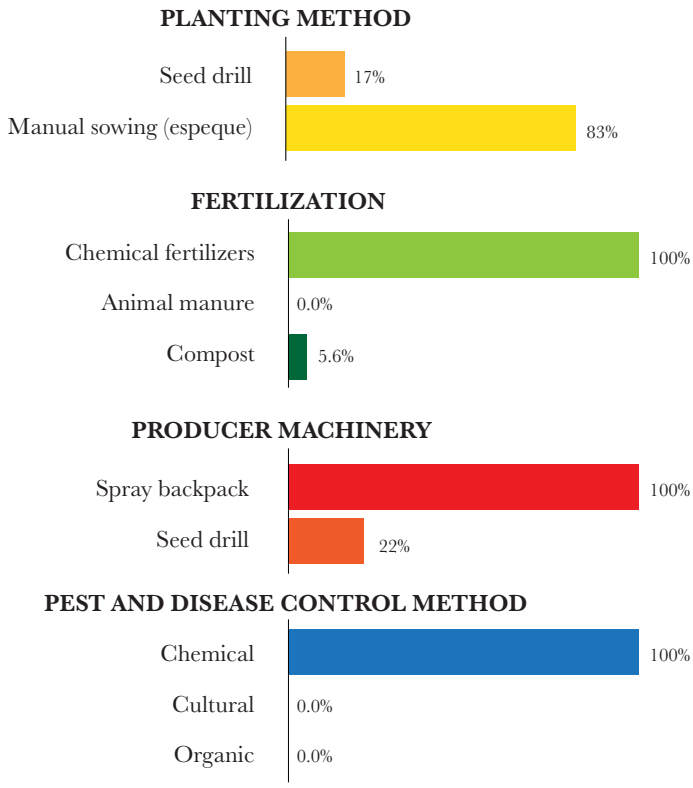

Figure 3. Agricultural practices in the community Mulato Viejo, Oaxaca (Mexico) (Source: own elaboration).

These results show that producers have a very low level of incorporation of new technologies. Damián-Huato *et al.* (2013) evaluating the degree of appropriation of technologies by producers in Puebla, did not find improved yields. However, they could observe that producers progressively assimilate technology that in the long run improves production. This process was also observed in "Mulato Viejo" where the results of technological appropriation by producers is similar to those of the rest of the state. In 2014, for example, the MasAgro (Crops for Mexico) project reported that 60-70% of producers in Oaxaca have adopted the use of spray backpacks, staggered fertilization, and use of pesticides (Rodríguez-Vázquez, 2014). This figure is close to the national average of 65%.

On the other hand, it is important to note that what at first glance seems to be lag in the modernization of the field, has indirectly facilitated the permanence of the traditional agriculture, which has preserved both the ancient cultural-related practices and local maize breeds. Although the producers interviewed do not record their production per year, they estimate that 89% is destined for family consumption and the remaining 11% is marketed in small quantities.

The production is sold as grain and other by-products such as "olotes" (cob cores, to feed livestock) and cob husks (as envelopes for the food called "tamale") or as processed products. Regarding this processing, 89% is for tortillas; followed by 56% for tamales, 22% is destined for the elaboration of atoles (beverages made of cooked corn), and smaller quantities for thickening (17%) and silage (11%) (Figure 4). Transformation activities are

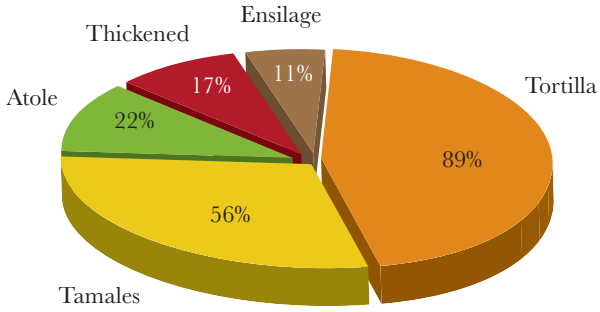

Figure 4. Products after the transformation of maize production (corn) in the community of Mulato Viejo, Oaxaca (México) (Source: own elaboration).

made by the women of the community who also commercialize the processed products. There are no clear records of the revenue generated by those sales.

In this study, the agricultural practices of the Mulato Viejo community were described. This rural nucleus is a case study environment where you can observe the conditions of small communities, where peasant agriculture is developed with traditional practices in small areas (0.76 ha on average). Primarily through family labor, with limited application of technologies and contemporary methods of production. The harvest is mainly destined for self-consumption (Sánchez-Olearte *et al.*, 2015).

In this rural nucleus, some of the problems nationally identified that contribute to the progressive abandonment of agricultural activities are present; agriculture is no more the main support of the family economy. Those problems include the fragmentation of the rural population of producers, and scarce opportunities for marketing their derived products. Additionally, in Mulato Viejo there is lag, both in the generational rotation of producers and in the incorporation of women into agricultural activities. All of the inhabitants dedicated to planting (100%) are men, although, the available data on land tenure in Oaxaca indicate that a large percentage of tenants are women. In this community, the property documents are registered with the name of the head of the family, the male who is also in charge of the work of planting, harvesting and direct marketing of the grain. The participation of women is limited to the processing of grain for self-consumption or to the sale of derived products.

It is important to involve young people in the knowledge of maize cultivation, so that in the future when they are in charge of agricultural practices, they shall have knowledge and skills that allow them to do it effectively. Additionally, it is considered that young people are more open to the incorporation of new technologies, which would contribute to improving yields and reducing the economy of self-consumption. A greater challenge is the incorporation of women, the support programs to the field have stimulated such incorporation. However, most of the time, due to the social customary division of activities, women are reluctant to join the field, since it would represent an increase in their daily workload for the family.

Finally, it is essential to make improvements in production, in order to obtain a surplus, and then decide what to do with the harvested corn or the processed product (whether sell it individually or as bulk at wholesale prices). To obtain greater profits and improve the

economic conditions of the inhabitants of the community of Mulato Viejo, it is important that all producers establish networks of solidarity collaboration. These networks would allow the reinforcement of local or regional systems where the subsistence of all individuals is guaranteed within a solidarity logic, which in turn generate products and services useful enough to be socially reproduced, even if these are not competitive in the market (Coraggio, 2007; Soria Sánchez *et al.*, 2015).

It is also important to identify the key roles and functions of social actors for management among stakeholders in order to obtain self-sufficient food production (Núñez-Ríos *et al.*, 2020). Or else, establishing a network of solidarity collaboration that would allow the best use of resources, the delimitation of functions and the identification of areas of opportunity or empty market niches. After identifying the networks and the functions of the actors, a multi-year strategic plan should be implemented based on them, to ensure implementation, monitoring and evaluation.

CONCLUSIONS

The agricultural practices of the community of Mulato Viejo (Oaxaca, Mexico) show the typical characteristics of the rural nuclei of the country. Due to this, they are models that allow us to observe variables and evaluate the effect of small-scale actions, with the aim of implementing them in larger communities or even nationally. These actions should include a multiannual strategy, the delimitation of the roles of the social actors already present (authorities, producers, etc.), identifying the empty market niches and encourage producers to create networks of solidarity collaboration.

All of the above must be monitored in order to make the necessary adjustments. Considering that the objective is to raise the standard of living of the associated producers, their families and the community, aiming to create sources of work, the rational extraction of natural resources, agricultural practices that allow the conservation and improvement of native maize races. As well as to produce, transform and market, both the direct products from harvest and others transformed within the community, promoting the generational renewal of producers and the incorporation of women into agricultural practices.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Participatory agroecological diagnosis in small and medium-sized producers in Michoacán and Morelos, Mexico

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ABSTRACT

Objective: To integrate an agroecological diagnosis to small and medium production units by identifying: (i) crop management practices, (ii) types of inputs and technologies used, and (iii) health status of soils and crops.

Design/Methodology/Approach: This implemented research was of the type known as "In-depth case study", where the minimum sample size is 6 to 10 cases. Descriptive, correlational, and explanatory aspects corresponding to the selected production units were considered; based on qualitative and quantitative information.

Results: For indicators of crop management and soil health, the predominant type of agriculture was transition to agroecology. Crop health indicators were the most agroecological.

Study limitations/Implications: The results obtained allowed to establish the current state of the production units; with which further comparisons of the condition of those production units in the future can be made.

Findings/Conclusions: The methodology used allowed to evaluate participatively, from an agroecological approach, the sustainability of soils and crops in production units of the municipalities Ario de Rosales, Michoacán and Tetela del Volcán, Morelos. Within the six productive units studied, it was found a productive unit with the appropriate characteristics to be considered as an "agroecological beacon".

Keywords: sustainability, agroecological management, agroecological beacon.

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INTRODUCTION

In recent decades, the industrial agriculture model has allowed a massive increase in agricultural production through: (i) plants selected according to their high productive potential; (ii) standardization of modes of production; and (iii) the use of synthetic inputs that minimize the effect of production-limiting factors and environmental heterogeneity (Duru *et al.*, 2015). Among them, the loss of biodiversity, including agrobiodiversity, negative impacts on the environment (pollution, climate change, depletion of fossil fuels

Image by Albrecht Fietz at Pixabay

and water resources), and ethical issues related to the lack of consideration of animal welfare in agro-productive units (Clark *et al.*, 2016). All these elements question the relevance of the agro-industrial production model for the future. In this context, a major challenge for farmers is to simultaneously contribute to the food and nutrition security of humanity, on the basis of limited resources, while reducing the negative impacts of agriculture on human health and the environment, maintaining decent living conditions (Biggs *et al.*, 2012). Many researchers believe that agroecology is a promising way to overcome all these challenges (Altieri *et al.*, 2017).

As a scientific discipline, agroecology is defined as "the application of ecological concepts and principles to the design and management of sustainable agroecosystems (Wezel *et al.*, 2009). This definition emphasizes the fact that natural processes, and in particular biodiversity and interactions between biotic and abiotic elements, can support the sustainability of agricultural systems. Thus enabling production at appropriate levels while reducing dependence on agricultural and agrochemical inputs; as well as negative impacts on human health and the environment, even in sub-optimal conditions (Bell & Bellon, 2018). In order to reorient the management and production systems administration towards agroecological practices, it is essential to implement diagnoses that allow evaluating management practices, the types of inputs and the technologies used (Wezel *et al.*, 2020).

Participatory agroecological diagnosis is a methodology that allows identifying and prioritizing the needs and problems of agricultural production units. Therefore, seeking sustainable management alternatives opposed to the agro-industrial production model (Barrios *et al.*, 2020). In addition, participatory methodologies are based on learning close to families in rural communities as key actors, through work dynamics that favor the exchange of ideas, experiences and knowledge oriented to the common good (FAO, 2011).

It is essential that small and medium-sized producers in rural communities recognize which management practices affect the quality and health of their crops and soil (Madsen *et al.*, 2020). Thus, the objective of this research was to implement an agroecological diagnosis to small and medium production units by identifying: (i) management practices for crops, (ii) types of inputs and technologies used, and (iii) health status of soils and crops. This research contributes to the process of observation, registration and decision-making for the agroecological management of small and medium-sized rural producers in Mexico.

MATERIALS AND METHODS

Description of production units

The study was carried out in 6 production units located in Ario de Rosales, Michoacán and Tetela del Volcán, Morelos. The municipality Ario de Rosales is located in the center of the state of Michoacán, within the coordinates 19° 12' N and 101° 40' W, at an altitude of 1910 m. Its area is 696.91 km^2. Climate is defined as temperate with rains in summer and as tropical in some parts. It has an annual rainfall of 761.6 mm and average temperatures of 21 °C (Figure 1).

The municipality Tetela del Volcán is located in the northeast of the state of Morelos, within the coordinates 18° 57' N and 98° 14' W, at an altitude of 2040 m; it has a total area of 98.5 km^2. Climate is wet and cold with dry winters, except in the north, whose climate is

Figure 1. Geographical location of the Municipality Ario de Rosales, Michoacán, Mexico.

typically mountainous. It is frequently cloudy, and characterized by stormy rainfall, usually accompanied by hail. The average annual rainfall is 2341.63 mm and average annual temperature is 23.6 °C (Figure 2).

Research description

Type of research

The research was of the "In-depth case study" type, where the minimum sample size is 6 to 10 cases (Hernández-Sampieri and Torres, 2018). Descriptive, correlational and explanatory aspects corresponding to the selected production units were considered; both qualitative and quantitative information was also used. Farmers were the direct source of information; however, valid documents providing related information were also considered.

Figure 2. Geographical location of the Municipality Tetela del Volcán, Morelos, México.

The methodology of this study was carried out through the following steps:

1. The research topic was defined: "Agroecological management of crops and the health of soils and crops".
2. Techniques for data collection were established: "Interview. Participatory agroecological survey and participant observation".
3. Data were collected in the field.
4. The data were organized and statistically analyzed.
5. Data were interpreted.
6. Conclusions and experiences obtained were recorded.

Data collection and analysis

A registration form was used, with which participatively with the farmer in his productive units, the indicators proposed by Padilla and Suchini (2013) were evaluated; grouped into three components, crop management, soil health, and crop health (Rutebuka *et al.*, 2019). All indicators were evaluated on a scale from 1 to 10, where values 1-4 will were assigned to conventional agriculture; 5-7 for agriculture in transition; and 8-10 for agro-ecological farming.

After organizing and generating the database, the statistical analysis was run in STATISTICA® (version 10.0; Statsoft, Tulsa, OK, USA). Measures of central tendency and analysis of absolute and relative frequencies were carried out. For depicting results, radial graphs and a scatter plots were generated to highlight the agro-ecological beacons found (Altieri and Nicholls, 2000).

RESULTS AND DISCUSSION

Based on the participatory diagnosis of crop management with farmers in their production units, the total relative weight of the characteristics of agriculture was determined based on each of the indicators observed and evaluated during the field trips. The results indicate that the predominant crop management in the production units is a management in agroecological transition. This management represented 49% to 68% of the production units, in 6 of the 10 indicators evaluated.

The second dominant management was agroecological, with a participation of 50% to 83% of the production units, in 4 of the 10 indicators evaluated. Conventional management had the participation of 50% of the farms in 2 out of 10 indicators evaluated, this management was found the least frequent in the production units.

In Figure 3, it is graphically observed that the management in transition of the crops was the one that predominated; the preparation of the land was highlighted, along with the management of insect pests, and the management of wild plants, with a contribution of 67% of the production units. The strength in terms of agroecological management was in the application of fertilization and inputs for the management of wild plants.

This type of graph, also known as a graph of nets or cobweb plot, was used in research as part of a quick and participatory agroecological method, where together with the farmer they were able to estimate the quality of soils and the health of crops in vineyard systems

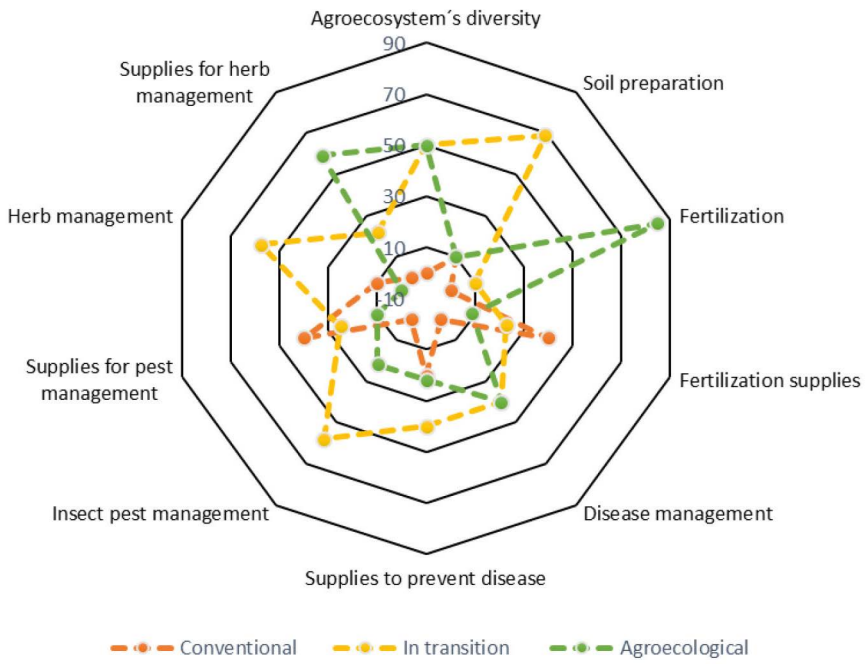

Figure 3. Radial representation of the type of crop management in the production units.

(Nicholls *et al.*, 2004). At the same time, it allows to show the areas that require greater attention in the management of crops and soils, to ensure a greater focus on ecological processes.

The weakness of the production units in terms of conventional management was in the use of inputs for fertilization and inputs for pest management. This situation indicates that farmers tend to use more synthetic fertilizers and moderate amounts of organic fertilizers. However, the application of fertilizers occurs under an awareness of agroecological management, where fertilization is applied to nourish the plant, and to replenish nutrients and improve life in the soil.

Conventional management of the use of inputs in pest control implies a strong dependence on commercial insecticides and their doses accustomed by farmers. In a study for the adoption of agroecological pest management (MAP) practices in watermelon cultivation, it was found that, in a sample of 96 farmers, a low level (20%) of adoption of MAP practices predominated (Brzozowski and Mazourek, 2018).

The results of the soil health indicators of the productive units show that the dominant type of agricultural system is that of transition towards agroecology (Figure 4). The indicators of soil depth, soil cover and biological activity were the most agroecological in the production units evaluated. The physical characteristics of the soil such as structure, infiltration and moisture retention, are at a transitional level. Likewise, the characteristics of light brown color without much odor, and with little visible organic matter, represented transitional conditions for the health of the soil.

Crop health indicators revealed that most production units have an agroecological profile based on crop health. However, one of the production units was found in a

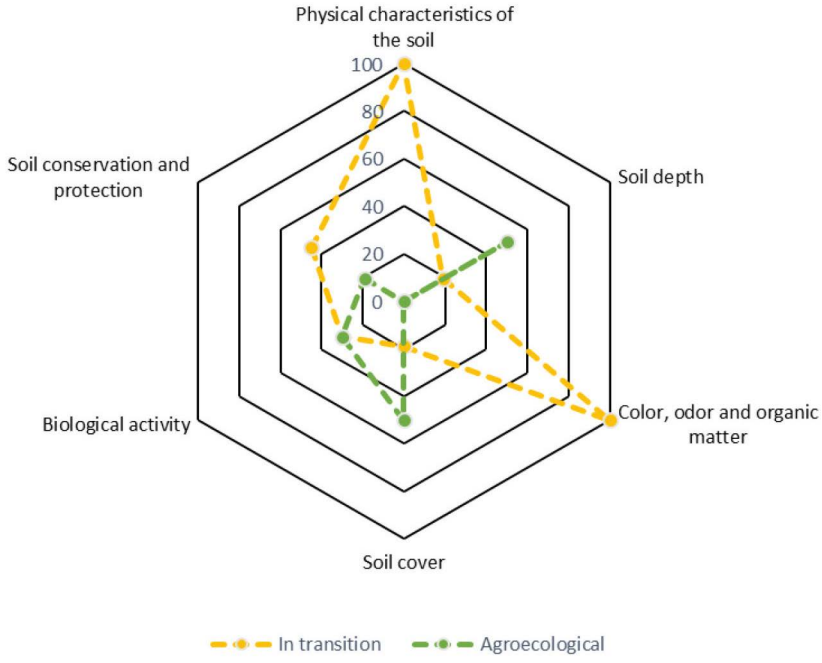

Figure 4. Radial representation of the health condition of the soil in the productive units.

conventional state. Figure 5 depicts that most crop health indicators were classified as agroecological.

The visualization of the average values of the productive units in a dispersion plot, allows to determine the status or condition of the units of production in regard to the threshold value (5) for crop management, soil health and crop health. It also allowed us to identify

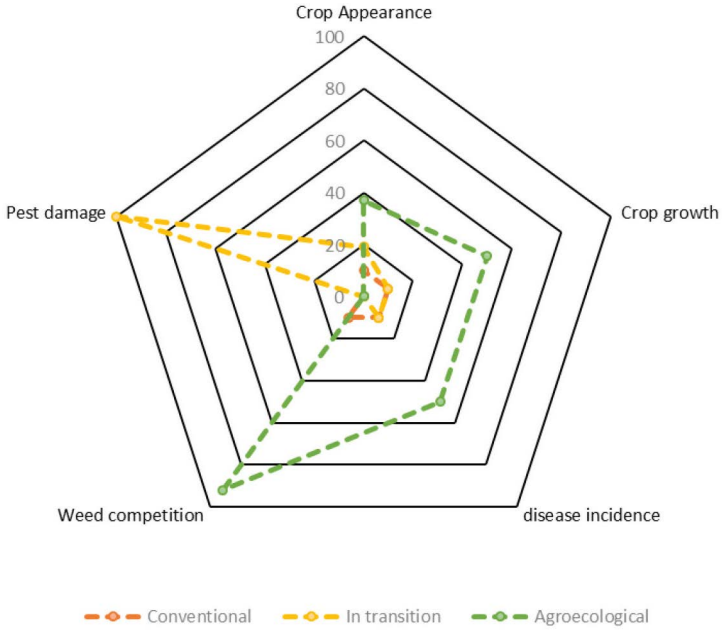

Figure 5. Radial representation of the state of health of the crops in the productive units.

the productive units that presented high averages. According to Altieri and Nicholls (2015), the productive units with higher values are considered as "agroecological beacons", in which ecological interactions and synergisms that explain the proper functioning of the system can be studied.

Ideally, farmers should not copy the techniques used by the farmer from the "agroecological lighthouses", but rather try to reproduce the processes and interactions promoted by the ecological infrastructure of such a production unit, which leads to the success of the system from the point of view of crop management, soil health and crop health. Within this context, efforts should be made to promote that farmers in other productive units tend to use those techniques that are within their reach and that optimize the same processes (Nicholls *et al.*, 2004).

Figure 6 shows that four of the production units are in the status of agroecological threshold in terms of crop management, which is closely linked to the type of agriculture in transition which is dominant in that production units. However, productive units 1 and 4 obtained high average values of 8 for crop management.

The health condition of the crops of the productive unit 4, as indicated by the farmer, was related to significant losses of the harvest due to the attack of pests in the reproductive stage, which was observed during the field tours with the farmer. This situation was further affected by poor management practices for wild plants, which generated greater stress on crops.

The comparison of the different cases allows to identify the healthiest systems. The outstanding systems are demonstrative beacons called "agroecological beacons" that allow generating information on specific agroecological practices that shall optimize the desired processes in the productive units that show values below the threshold (Altieri and Nicholls, 2015).

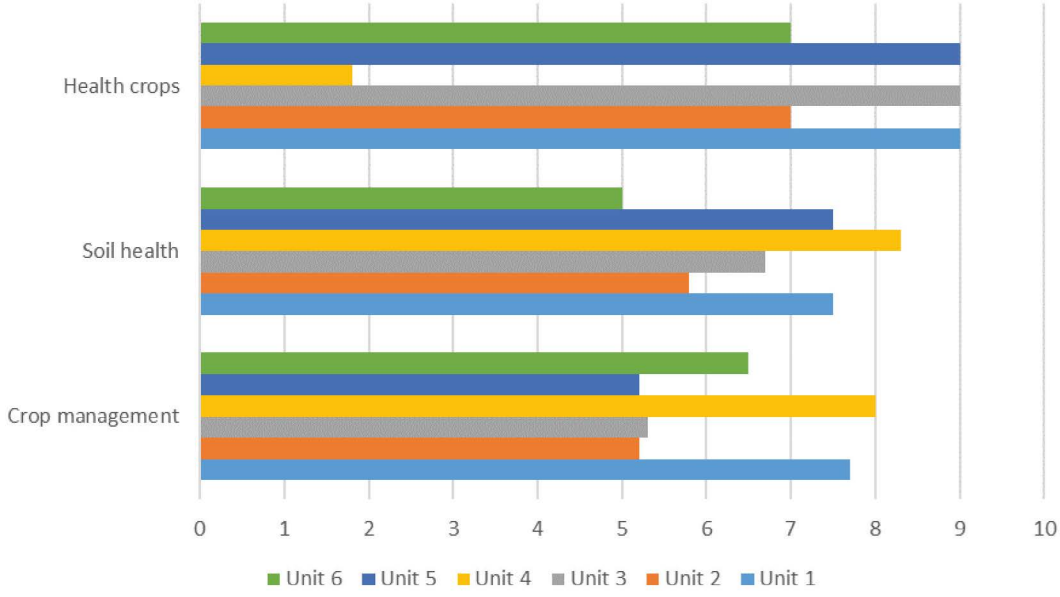

Figure 6. Comparison of the average indicators of crop management, soil health and crop health in the productive units.

Of the six productive units diagnosed in this research, productive unit 1 was the only case with average values of 7.5 to 9 for indicators of crop management, soil health, and crop health. Due to high values in the three components evaluated, this productive unit is considered as a potential "agroecological beacon".

In an agroecological evaluation study of the production units in Centella de Dagua, Colombia (Cerón *et al.*, 2014), those authors mentioned that "agroecological beacons" are examples for the management and conservation of resources; for the planning, the promotion of techniques that contribute to ecological integrity, and the promotion of sustainable human development in the field.

CONCLUSIONS

The methodology used allowed to evaluate participatively, from an agroecological approach, the sustainability of soils and crops in production units of the municipalities Ario de Rosales, Michoacán and Tetela del Volcán, Morelos.

The results obtained allow to establish the current state of the productive units, with which further comparisons can be made of the state of those productive units in the future.

For indicators of crop management and soil health, the predominant type of agriculture was determined as transitional towards agroecology. Crop health indicators were the most agroecological. Of the six productive units studied, it was found a productive unit with characteristics to be considered an "agroecological beacon".

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Genome mining for bioprospecting of biosynthetic genes clusters for bacterial metabolites potentially useful in agroecological production

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ABSTRACT

Objective. To describe the relevance and some tools of genome mining to explore genetic and molecular determinants encoded in bacterial genomes to address agronomic problems.

Design/Methodology/Approach. Literature review of the importance of bacteria as a reservoir of biosynthetic gene clusters (BGC), involved in the production of metabolites with biological activity as anti-pathogens; and of genome mining as a tool to reveal this potential.

Results. Bioinformatic tools are useful for the exploration of bacterial genomes and have the potential to contribute to the resolution of agronomy problems. For example, the use of bacteria, their genes and metabolites for the control of phytopathogens that attack crops of global importance. Likewise, the limitations of the genome mining and their coupling with other experimental approaches to achieve bioprospecting of BGC or their related metabolites are summarized.

Limitations of the study/implications: Although the use of genome mining to explore the potential of bacteria is a very powerful approach, it will always be necessary the experimental corroboration at the laboratory level, to confirm the hypotheses generated by bioinformatics tools.

Findings/conclusions: Genome mining allows to take advantage of the large number of bacterial genomes currently sequenced, that are available in public databases to understand the genetic bases of their biological activities. As well as for the heterologous expression of biosynthetic genes, or the identification and purification of new metabolites. The foregoing with the objective of contributing with more effective and environmentally friendly solutions that address agronomic problems.

Keywords: bacterial genomes, bioprospection, genome mining, biosynthetic gene clusters (BGC), phytopathogens.

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INTRODUCTION

It is well known that bacteria produce a wide variety of chemical compounds with different metabolic origins and different biological activities. A biosynthetic gene cluster (BGC) is a group of genes that, when expressed together, produce enzymes involved in

Image by Gerd Altmann at Pixabay

the biosynthesis of a bioactive metabolite, also called secondary (Medema *et al.*, 2015). BGCs are the toolbox available to bacteria to produce complex chemical structures that can be applied in different areas, such as solving some agronomic problems. There are many applications of bacteria, their metabolites or enzymes in the field of agronomy that have not yet been exploited in Mexico according to their potential. For example, the great losses caused each year by phytopathogens that attack crops of economic importance. This and other problems could be addressed through bioprospecting of bioactive microbial metabolites (BMMs).

The isolation and chemical identification of BMM until their application in a given product is a long run. However, current advances in accelerated genome sequencing, and the use of bioinformatics tools to "mine" information, accelerate and smooth processes, allowing the identification of which biosynthetic genes are potentially involved in some biological activity and what are the possible production routes of metabolites. Such proposals for possible genes subsequently have to be addressed from a biochemical point of view or using other complementary tools such as metabolomics or genetic engineering. This review recapitulates the main route for the systematic and exhaustive search of clusters of genes involved in the biosynthesis of bioactive compounds, emphasizing genome mining applied in the identification of possible BGCs and the bioactive metabolites they produce, for agronomic use in the control of phytopathogens.

Genomic and metabolic plasticity of bacteria

Bacterial genomes are very diverse. They contain a core genome, which includes genes that provide essential information and is conserved for most bacteria. They also contain a flexible part in which are the genes that give identity to each species. These genes provide additional characteristics that differentiate one bacterium from another both genetically and metabolically. This flexible part of the genome is where genomic plasticity occurs in bacteria, which is defined as the ability to incorporate genes or complete sets of genes (operons) into their genome (Bennett, 2004).

Among the mechanisms that contribute to genomic plasticity are point mutations that alter the expression of genes or the metabolic activity of encoded enzymes; mechanisms of genetic exchange such as recombination between genomes, or conjugation (plasmid exchange) between bacteria. As well as the horizontal transfer of genes promoted by bacteriophages, mobility of genome fragments by transposons, repairs and integration of DNA regions of different sizes (Dobrindt and Hacker, 2001; Sela *et al.*, 2018). Genomic plasticity is considered one of the keys to the evolution of bacteria (Sela *et al.*, 2018); the infinite metabolic possibilities that result from it make bacteria a source of numerous bioactive metabolites that can be of beneficial use with different applications.

Main types of biosynthetic gene clusters

There are different classes of biosynthetic gene clusters, classified according to the type of metabolite in whose biosynthesis participate. In addition to their origin, bacterial bioactive metabolites are grouped into ribosomal and non-ribosomal products. Here we address the main groups that belong to the non-ribosomal type; non-ribosomal peptide

synthetases and polyketide synthases which are two large sets of biosynthesis enzymes that have been studied with greater emphasis due to their frequent prevalence in bacterial genomes.

The BGC of non-ribosomal peptide synthetases (NRPS) have as their core, multi-modular enzymes that condense amino acids in a linear way through the formation of thioester-type peptide bonds (Figure 1, right), forming peptides different from those synthesized in the ribosome since, in addition to the 20 amino acids, they integrate more than 500 non-proteinogenic amino acids, heterocyclic elements and glycosylated residues, through biosynthetic pathways from other origins (Walsh and Fischbach, 2010).

Polyketide synthases (PKS) are an extensive group of enzymes classified into three types, whose activity is the modular condensation of monomers of acyl-CoA, malonyl and methyl malonyl, important intermediates in the biosynthesis of many organic compounds (Fischbach and Walsh, 2006).

NRPS genes such as PKS contain functional domains that once the enzyme is expressed become modules for substrate activation and for initiation, elongation, modification and termination of the product that can be a bioactive metabolite. Additionally, they may have transport proteins, transferases, and other auxiliary enzymes (Figure 1, left).

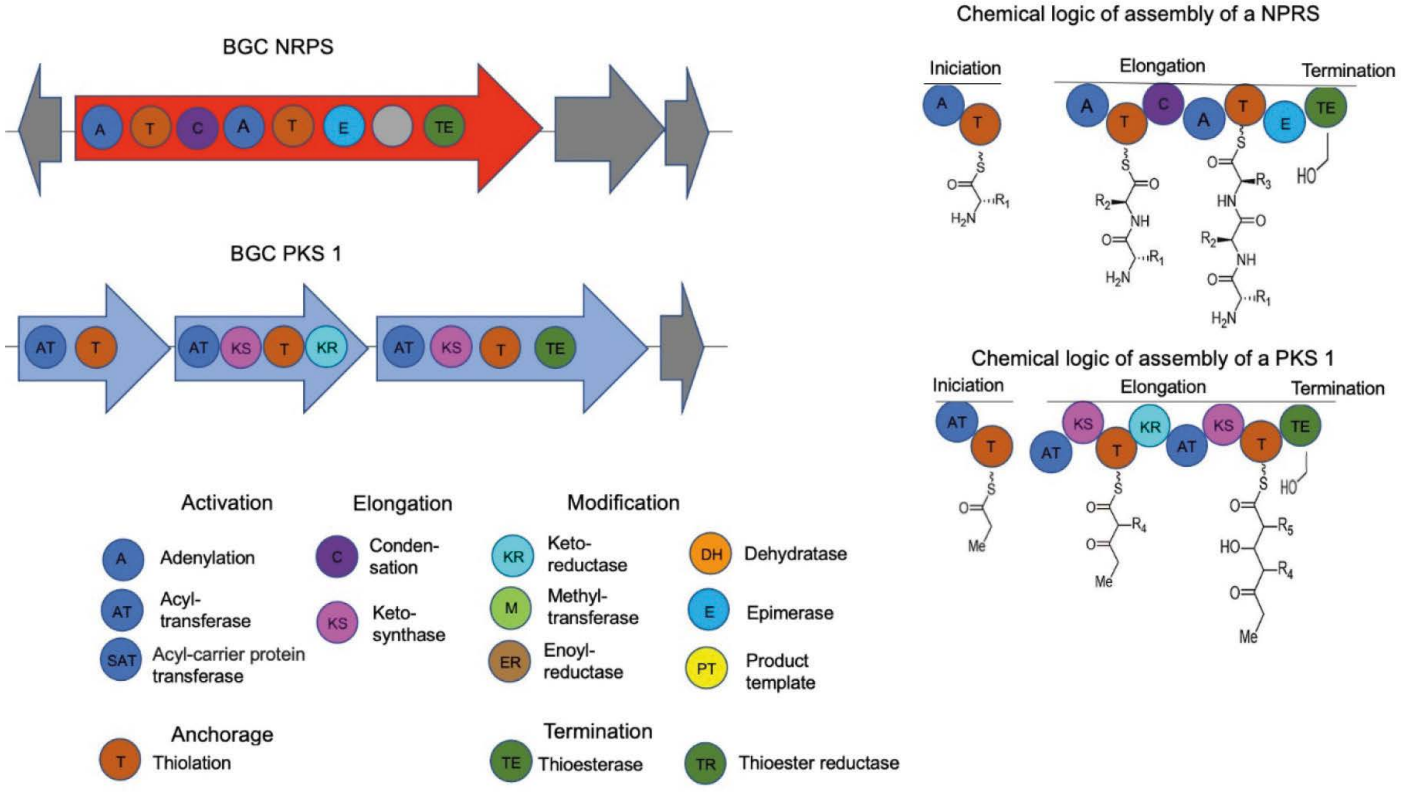


Figure 1. Example of bacterial biosynthetic clusters coding for an NRPS or a PKS 1 and the assembly of the metabolites they produce. Arrows represent the genes of a BGC that encode biosynthetic enzymes (red or blue), as well as genes that encode transport proteins, auxiliary enzymes, etc., (gray). On the right is the assembly logic (biosynthesis) of non-ribosomal peptides and polyketides. As well as the most common functional domains or modules (circles) of NRPS and PKS and their function (below).

Traditional strategy for the identification of BGC of bioactive metabolites in microorganisms

The traditional method for identifying biosynthetic gene clusters of secondary metabolites in bacteria (Figure 2, top) is based on the isolation of the metabolite guided by the bioactivity of supernatants from bacterial cultures. Also, on the use of chemical methods, such as mass spectrometry and nuclear magnetic resonance, to deduce the chemical structure of the bioactive metabolite.

Subsequently, the corresponding biosynthetic genes are inactivated by induced mutagenesis or randomized genetic deletion, followed by the detection and isolation of non-producing clones (Bachmann, Lanen, & Baltz, 2014; Read *et al.*, 2020). These mutant strains that do not produce the metabolite are analyzed to identify their genes affected; therefore, they are responsible for the biosynthesis of the metabolite. Despite the great advantage of directly isolating and characterizing the active product, this approach has as its main limitation that the expression of BGC is controlled by factors found in the natural habitat of bacteria. This means that, in laboratory conditions those genes may not be expressed, which generates the so-called cryptic genes (Clardy *et al.*, 2006; Lee *et al.*, 2020).

Genome mining

Genome mining is the set of steps and tools, totally dependent on bioinformatics, that allows to identify bacterial clusters from genome sequences, by comparing against

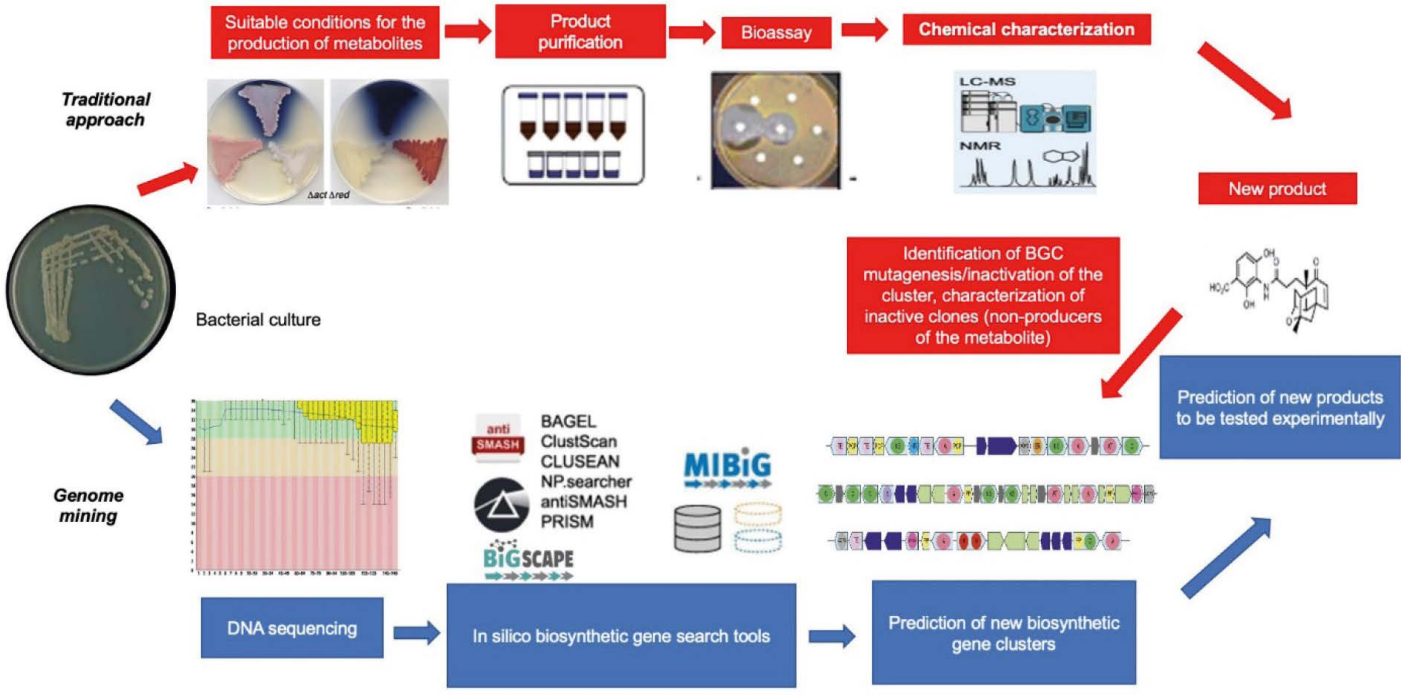


Figure 2. Comparison of the traditional strategy and the genome mining approach for the seeking of new bioactive metabolites and the BGCs that synthesize them.

previously described clusters. The existence of conserved regions between clusters of various bacteria greatly facilitates the genome mining; although bioactive metabolites can be highly diverse in their chemical structure, the biosynthetic machinery that includes assembly enzymes, belongs to the same families of highly conserved enzymes, especially PKS and NRPS (Lee *et al.*, 2020).

This new approach is radically different from the traditional approach and allows new BGCs to be identified more quickly (Figure 2, bottom). In recent years; as a result of new sequencing technologies and the easy and inexpensive access to genome sequence data, many bioinformatics tools have been developed based on identifying the highly conserved regions of the BGC, for the annotation or labeling of coding regions in the genomes (Ren *et al.*, 2020; Cibichakravarthy and Jose, 2021). The application of these technologies has revealed that the biosynthetic potential of bacteria has been underestimated. For example, following the traditional strategy, only four BCCs were discovered and characterized in the bacterium *Streptomyces coelicolor* in 30 years; but it is currently known that members of this genus possess approximately 30 clusters of biosynthetic genes, including many whose products have not yet been identified (Bentley *et al.*, 2002; Lee *et al.*, 2020).

Bioinformatics tools most commonly used in genome mining

Below are the general route, hotspots, and bioinformatics tools most commonly used for genome mining in the search for BGC.

1. **Evaluating the quality of genomes**. The completeness and quality of a sequenced genome is a fundamental part of mining genomes in search for BGC. It is known that a large number of genomes that are sequenced and reported in the databases are not complete. For example, 36% of the genomes of the genus *Streptomyces* show poor completeness (Studholme *et al.*, 2016). Likewise, the quality of the sequenced nucleotide bases is of great importance for the prediction of BGC since high-quality genomes improve the prediction of coding sequences (CDS), which in turn improves the prediction of the large genes that usually compose the core machinery of BGC.
2. **Identification of genes and their function (Annotation of genomes)**. For the identification of genes encoding proteins, rRNA and tRNA, etc., a wide variety of tools are used. One of the most used is RAST (Rapid Annotation using Subsystem) that identifies genes, assigns functions, predicts groups of genes associated with abstract functional roles that are represented in the genome (subsystems), uses this information to reconstruct the metabolic network and makes the result easily manipulated for the user. In addition, the annotated genome can be explored in an environment that supports comparative analysis with other different genomes (Aziz *et al.*, 2008).
3. **Use of BGC databases**. Currently the largest reservoir of BGC is in the Atlas of biosynthetic gene clusters (Integrated Microbial Genomes IMG-ABC, Joint Genome Institute JGI) (Hadjithomas *et al.*, 2015). This gene bank contained more than 960 000 groups of putative genes as part of some BGC in 2016. Only a small fraction of these BGCs make it to the final product description. Recently, the

Minimum Information for Biosynthetic Gene clusters (MIBiG) initiative has carried out a manual re-annotation of approximately 1300 BGCs representing an important highly curated reference dataset (Medema *et al.*, 2015). A significant number of programs have been developed to conduct searches for biosynthetic gene clusters within genomes including BAGEL (de Jong *et al.*, 2006); ClustScan (Starcevic *et al.*, 2008); CLUSEAN (Weber *et al.*, 2009); NP.searcher (Li *et al.*, 2009); PRISM (Skinninder *et al.*, 2017); and antiSMASH (Blin *et al.*, 2019). Most of these programs rely on searching for highly conserved BGC sequences to map their location. The Secondary Metabolite Bioinformatics Portal is http://www.secondarymetabolites.org a platform that updated and exhaustively groups the specialized software for the search for new BGCs. In this platform is the antiSMASH program that has been the most widely used in the prediction of gene clusters and which we described below.

4. **BGC prediction**. Since its initial launch in 2011, antiSMASH has become the most widely used tool for the search for clusters of secondary and specialized biosynthetic genes in fungi and bacteria; including those producing polyketides, non-ribosomal peptides, terpenes, aminoglycosides, aminocoumarins, indolocarbazoles, antibiotics, bacteriocins, nucleosides, beta-lactams, butyrolactones, siderophores, melanins and others (Medema *et al.*, 2011). In addition to using the characteristic gene of each cluster type using HMM (Hidden Markov Models) profiles for gene cluster identification, antiSMASH uses an algorithmic approach to extend the gene pool by 5, 10 or 20 kb on both sides; therefore, poorly spaced clusters can be merged into "superclusters" (Blin *et al.*, 2021). AntiSMASH also provides additional domain analysis options of the NRPS/PKS; as well as annotation, prediction of the central chemical structure of polyketides and non-ribosomal peptides, comparative analysis of gene groups (ClusterBlast) and also the analysis of families of enzymes involved in secondary metabolism (Blin *et al.*, 2021). There are other tools based on phylogenetic reconstructions such as EvoMining (Cruz-Morales *et al.*, 2016) and NaPDoS (Ziemet *et al.*, 2012); as well as in similarity networks such as Genome Neighborhood Networks (GNNs) (Zao *et al.*, 2014). In both cases there is the limitation that they require extensive prior knowledge of homologous enzymes.

5. **Integrating Genome Mining with Metabolomics and Targeted Search.** The integration of different strategies can be crucial for the discovery and study of bacterial secondary metabolites. Metabolomics is an analytical profiling technique used to measure and compare a large number of metabolites present in biological samples (Manchester and Anand, 2017). Once combining genomics and metabolomics, a new non-ribosomal lipopeptide Stendomycin (antifungal) was discovered from *Streptomyces hygroscopicus* (Kersten *et al.*, 2011). In another study, bioactive metabolites of *Streptomyces roseosporus* were identified by integrating correlation networks between metabolite fragmentation patterns by mass spectrometry and metabolomics, leading to the discovery of Stenothrycin, a metabolite with antimicrobial activity against Gram positive and negative bacteria (Liu *et al.*, 2014). Additionally, the search for metabolites directed by function, by tracking their biological activity, can make the

search for secondary metabolites more successful and give it a more applied meaning as we may see below.

Bioactive compounds of bacteria applied in agronomy: the case of biological control against phytopathogens

An example of BGCs useful to promote agroecological production are those that code for the biosynthesis of metabolites applicable to the control of phytopathogens. Over several years various microorganisms including different bacteria have proved to have the ability to suppress diseases in economically important crops (Table 1). This antagonistic action of bacteria against phytopathogens is given among other factors by a variety of secondary metabolites including iron-chelating siderophores, antibiotics, volatile biocides, as well as lytic enzymes and degradation enzymes (Compant *et al.*, 2005; Pal and Gardener, 2006; Colla *et al.*, 2018; Vurukonda, Giovanardi and Stefani, 2018; Köhl, Kolnaar and Ravensberg, 2019). A recent example of the use of genome mining is presented in the work of Siupka *et al.* (2020), who found multiple clusters of biosynthetic genes in a strain of *Streptomyces* isolated from chimney ash, which showed antagonistic activity against pathogenic strains of *Fusarium avenaceum*, *Aspergillus niger*, *Nigrospora oryzae* ssp. *roseF7*, and *Curvularia coatesieae* ssp. *junF9*. To give one more example, in our laboratory a confrontation test was carried out with phytopathogenic fungi to discriminate within a strain collection of more than 300 bacteria; starting only from antagonistic bacteria to those phytopathogens at the genome sequence and perform mining in search of the BGC that carry out the activity for which they were selected. Once the BGC has been identified, they can be heterologously expressed in controlled systems such as bioreactors, for the isolation or purification of metabolites and their eventual application (unpublished study data).

CONCLUSIONS

Genome mining is an important tool for the quick and easy prediction of biosynthetic gene clusters, based on the genome data of bacteria. This approach easily and quickly opens a door for the possible resolution of problems in the agricultural sector. However, bioprospecting of metabolites derived from these genes requires a subsequent and extensive laboratory analysis. The activation of biosynthetic clusters of secondary metabolites that are silenced; purification of the final metabolic products; and successful elucidation of their chemical structure should be done.

The information available in the databases is scarcely exploited in applications and developments directed towards agriculture. The education of human resources, trained in the management of these bioinformatics tools and their integration into task groups is essential; towards the bioprospecting of the microbial resources identifiable in Mexico, thus accelerated through genome mining. On the knowledge of this genetic richness, it may be possible to contribute to the resolution of some agricultural problems in the country, in order to offer the use of natural products and their mass production as alternatives, through technological developments based on biosynthetic gene clusters.

Table 1. Examples of some of the antibiotics produced by bacteria antagonistic to fungi; bacteria that produce them, as well as the target pathogen, and the diseases they cause are shown.

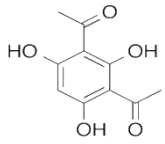
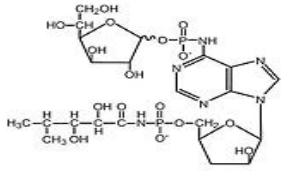
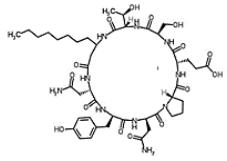
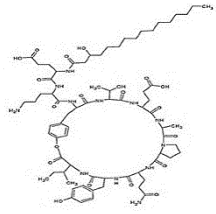
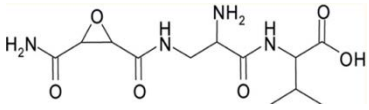
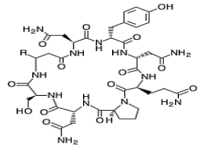
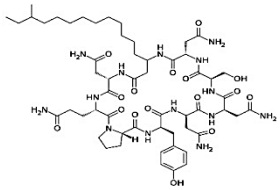
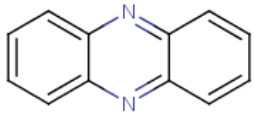
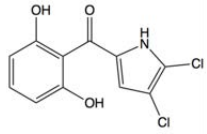
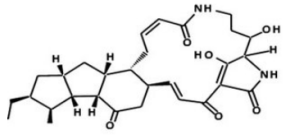
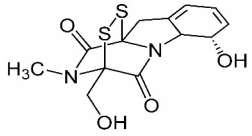
Antibiotic	Source / Target pathogen / Illness	Chemical structure	Ref.
2,4-diacetylphloroglucinol	*Pseudomonas fluorescens* F113 / *Pythium* spp. / Damping off		Shanahan *et al*., 1992
Agrocin 84	*Agrobacterium radiobacter* / *Agrobacterium tumefaciens* / Crown gall		Kerr, 1980
Bacilomycin D	*Bacillus subtilis* AU195 / *Aspergillus flavus* / Contamination of aflatoxin		Moyne *et al*., 2001
Fengycin	*Bacillus amyloliquefaciens* FZB42 / *Fusarium oxysporum* / Rot		Koumoutsi *et al*., 2004
Herbicolin	*Pantoea agglomerans* C9-1 / *Erwinia amylovora* / Fire blight		Wright *et al*., 2001
Iturin A	*B. subtilis* QST713 / *Botrytis cinérea* y *R. solani* / Damping off		Paulitz y Belanger 2001
Mycosubtilin	*B. subtilis* BBG100 *Pythium* / *Gaeumannomyces graminis* var. *Tritici* / Damping off		Leclere *et al*., 2005
Phenazin	*P. fluorescens* 2-79 and 30-84 / *Gaeumannomyces graminis* var. *tritici* / Foot or crown rot		Thomashow *et al*., 1990

Table. 1. Continues...

Antibiotic	Source / Target pathogen / Illness	Chemical structure	Ref.
Pyoluteorin	*P. fluorescens* Pf-5 / *Pythium ultimum* y *R. solani* / Damping off		Howell y Stipanovic 1980
Xanthobaccin A	*Lysobacter* sp. strain SB-K88 / *Aphanomyces cochlioides* / Damping off		Islam *et al.*, 2010
Gliotoxin	*Trichoderma virens* / *Rhizoctonia solani* / Root rot		Wilhite *et al.*, 2001

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Profitability and comparative advantage: Analysis of strawberry production in Michoacán, Mexico

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ABSTRACT

The production of berries is an issue that has become relevant over time due to the increase in the popularity of these fruits; its use extends every day to more markets, and the fact that its production requires specific geographical conditions and specific care restricts the amount of supply in the international market. Therefore, it is noteworthy that the state of Michoacán presents the appropriate characteristics for planting and harvesting berries. Because of this, the objective of this study is to determine the profitability and comparative advantage of Mexican strawberry production in Michoacán for the year 2021, through the Policy Analysis Matrix (PAM). A limitation to the study is the period of time selected in this case, which corresponds to the year 2021. Among the main conclusions, it stands out that with a value of 0.14 and a value of 0.37, it can be deduced that strawberry production is a competitive activity with an advantage for Michoacán producers.

Keywords: strawberry, berries, profitability, production in Michoacán.

INTRODUCTION

Strawberry is a fruit from a plant of the Rosaceae family. Most people know or can relate to the shape and color of strawberry due to its peculiar red tone, and because its sweet flavor has many uses in gastronomy. The attributes of this fruit in the nutritional aspect are also diverse and varied, which make it a natural selection in diets and in nutritional plans, and its use is one of the most widespread, reason why it is one of the fruits with extended consumption.

Strawberry is a fruit that requires large land extensions, as well as a careful harvest because of how delicate and sensitive it can be; for example, it requires a controlled temperature usually below 15 °C from the moment of cutting, storage, transport, and final sale; these details impact the product's general cost.

In particular for 2019, according to the Food and Agriculture Organization of the UN, there are three countries at the global level that show supremacy concerning strawberry exports, particularly Spain, Mexico and the United States, which exceed by at least double the amount to the Netherlands, which in the particular case of Mexico allows to see that strawberry production is intensive and large-scale.

Image by Jérémie Perron at Pixabay

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Therefore, Mexico has the capacity to produce and export large volumes of product if required.

Likewise, the countries that imported strawberry in 2019 were 134 which allow to see that strawberry has a generalized and much extended acceptance among diverse nations, where sometimes it is known as an exotic fruit and its price per kilogram can exceed the $150 MX pesos.

These data allow asserting that strawberry, despite being an expensive fruit in its production and transport, is a desired product and with an interesting volume of commercialization in different countries (FAO, 2019).

Regarding Mexico, there are 14 states in the country related to strawberry production which sow and harvest this fruit, in part because of the climate conditions of the country, as well as the geographical conditions that offer advantages for the production of this fruit, which is reflected in the total production volume of the country.

According to the agrifood and fishing information service of Mexico's government, by 2019, the state with highest production was Michoacán with 66 percent of the national production, where there are six municipalities that generate all of the strawberry in the state and they are the universe of the study. From the data presented, the objective of the study is to determine the profitability and comparative advantage of Mexican strawberry production in Michoacán for the year 2021, through the Policy Analysis Matrix (PAM). For this purpose, the study is divided into four sections, the first being the introduction which shows the production and demand of strawberry at the international, national and state level; the second section explains the methodological treatment used to fulfill the objective set out; in the third section, the main results derived from the methodological study are presented, divided into two parts: the private analysis that accounts for the profitability, and the social analysis that accounts for the comparative advantage in the meta market selected; and finally, the conclusions derived from the study are presented.

MATERIALS AND METHODS

The Policy Analysis Matrix for agricultural development (PAM) published and developed by Eric A. Monke and Scott R. Pearson in 1989 is made up by two countable identities: countable profitability or rather the difference between production costs and profits; and the divergence effects or policy effects, which measures the effects caused by the existence or absence of policies for producers.

The private profitability is made up of private prices and social prices. Therefore, PAM is composed of two accountability identities, the first defines the profitability and the second measures the effects of policy and the market distortions. PAM accounts for the results and separates them to determine the profitability that is obtained from the producer's point of view (private profitability) and the profitability that the country obtains through the efficient use of their resources (social profitability), so that the private profitability indicates the level of competitiveness of producers and the social profitability indicates the level of comparative advantages of the country in the production of a specific good.

$$Profits = \sum P_i X_i - \left[\sum P_j X_j + \sum P_k Z_k \right]$$

Where: P_i=Price of the product in the national market; X_i=Amount of tons produced per hectare; P_j=Price of marketable inputs in the national market; X_j=Amount of marketable inputs and indirectly marketable inputs applied per hectare; P_k=Price of the internal factors in the national market; Z_k=Amount of internal factors applied per hectare.

The first accounting identity is defined by $\sum P_i X_i$ (price of the product in the national market multiplied by the amount of tons produced per hectare); this identity is the amount of income received by the producer from the harvest of the product.

The second identity is identified by $\sum P_j X_j + \sum P_k Z_k$ (the sum of the prices of the marketable inputs in the national market, as well as the direct and indirect marketable inputs applied per hectare multiplied by the sum of prices of the internal factors in the national market by the amount of internal factors applied per hectare), which are the costs for the producer of producing the harvest.

Likewise, other ratios are derived from PAM where the following stand out:

Private Cost Ratio

$$PCR = \frac{C}{A - B}$$

Where: PCR=Private cost ratio; A=Gross income valued at private prices; B=Production cost of the marketable inputs valued at private prices; C=Cost of the internal factors valued at private prices.

This ratio shows the difference between the production value and the costs of marketable inputs; it shows how the system allows paying the domestic factors and continuing to be competitive.

PCR<1 then the producer is competitive.

PCR>1 then the producer has profits higher than the average.

PCR=1 then the producer generates the resources that he spends.

Domestic resource cost ratio

$$DRC = \frac{G}{E - F}$$

This ratio serves to measure indirectly the social benefits and as this ratio is smaller, the relationship between the social benefits that are being obtained will be larger; it is an indicator of efficiency.

DRC (+) 0-1 then there is comparative advantage in the production of the good.

DRC (+) >1 then there is no comparative advantage in the production of the good.

DRC (−) then there is a waste of resources that impact the efficiency that would allow aspects to be improved.

RESULTS AND DISCUSSION

As has been mentioned before in the previous sections, strawberry production in Michoacán represents 66% of the national production, which is why for this study it is considered the most important agricultural production from the region, taking data from eight regions that cultivate these fruits: Zamora, Pátzcuaro, Zitácuaro, Sahuayo, Uruapan, La Piedad, Morelia and Apatzingán. It should be highlighted that each region presented a considerably different level of infrastructure and investment in the harvest of these berries, although it largely represents an important income for families of these regions.

The first part of PAM requires obtaining the profits at private prices for the industry (private prices refer to the costs incurred and the profits obtained at the price that it costs for the companies or industries to produce their products).

When contrasting the income that is obtained per hectare harvested by crop versus the cost that implies sowing a hectare to determine the profitability that each crop offers in the presence of average scenarios of price and yield. Strawberry offers a slightly higher profitability at the cost volume that is required, as observed in Figure 1.

Because for these berries the sale prices depend on factors such as quality, amount harvested, season, among others, the different scenarios that can be used to identify which factors are more sensitive and which combination of factors can severely damage the utilities of the producers in case of the appearance of internal distortions are shown graphically. In order to measure these imperfections of the market, four sensitivity analyses were conducted per crop: 1. Minimum price expected by yield per hectare minimum expected; 2. Minimum price expected by yield per hectare maximum expected; 3. Minimum price expected by yield per hectare average expected; and 4. Maximum price expected by yield per hectare average expected.

Figure 2 shows four different scenarios; it is worth pointing out that the minimum prices were established based on historical data obtaining the price expected in each scenario, which is why the minimum price of $20 pesos per kilogram (kg) was established, and maximum price of $33 pesos per kg. Then the average yield per hectare harvested was obtained, which the producers can get based on historical data and experience gathered by some producers, establishing the amount of 35 tons per hectare in normal conditions

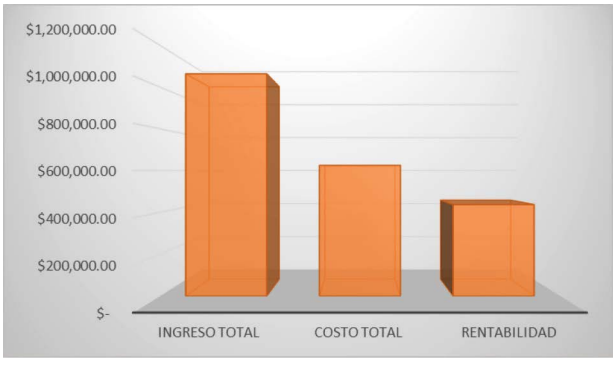

Figure 1. Income-cost ratio of strawberry.
Source: Prepared by the authors with data obtained from PAM.

Figure 2. Sensitivity analysis for strawberry.
Source: Prepared by the authors with data obtained from PAM.

for production, that is, not from a pest or loss from any natural disaster. For the case of a reduced harvest, the amount of 55 tons per hectare was established, in perfect conditions. Performing the operations in Figure 3, it is seen that the income obtained by producers responds strongly to the price that each kilogram is sold at, showing that it is necessary to maintain a price in balance through time so as to not destabilize the income of producers from Michoacán.

Considering the assumption where a producer obtains income different from the average, in a scenario in which the producer is forced to decrease his sale price at the time of harvest, the profitability would be impacted as a response to the one who maintained his production costs during the year, which is a habitual practice for strawberry producers from Michoacán who must finance its production for months with the hope of selling the fruit at the time of harvest and thus obtain a profit; to understand which would be the results expected from these assumptions, operations were conducted where the profitability was obtained in the presence of stressed scenarios.

Facing a stressed scenario, then, in which the income results obtained in the prior study were used but subtracting the production costs of the fruit, where a similar result is obtained in which the utility is strongly linked to the price and a decrease in it generates

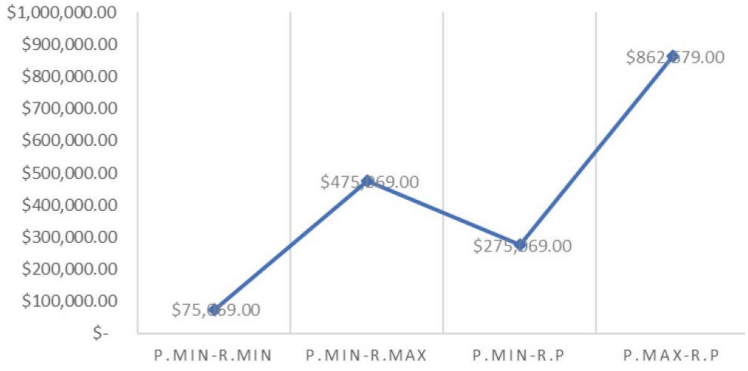

Figure 3. Sensitivity analysis in profitability for strawberry.
Source: Prepared by the authors with data obtained from PAM.

minimum profits for the producers. Although there are still profits, they would not be as expected because in the case of the producer this profit must be enough to cover a year of production to begin leveraging the following year and to be able to harvest again. With this in mind, we could say that there may be an incentive or a barrier to keep the producer from sowing in his fields again. Because of this, then, it is recommended to agree on a price before starting the harvest and that this price is respected through agreements and solid contracts; support could also be given to the producers, finding an adequate way of leveraging or financing their harvests, with this allowing to maintain a stable utility even when facing pessimist scenarios.

PAM analysis at private prices

Once the different analyses presented before were conducted per crop, the table of results is presented in terms of PAM, which as was specified in the methodological section, has a base structure in which the revenues are established and the costs in separate columns at private and social prices.

The first column shows the revenues obtained, if the result obtained in the line of social prices is subtracted from the line of private prices, we obtain an amount which in case of being negative (as is the case in our study in which the amount of −873,075 is obtained), it means that there are market distortions that impact the income that producers can obtain.

The second and third column group the production costs that strawberry producers face in Michoacán, using the same sequence of operations, subtracting from the production costs at private prices the production costs at social prices, where a negative result is obtained (−624.25 and −611) which implies the existence of policy effects. However, we can highlight that the differences obtained are not a considerable amount, so it can be established that the costs present effects in favor of producers in terms of level of protection for the inputs required.

The last column shows the profits or profitability obtained; we apply the sequence of operations and we observe that both at social prices and at private prices, the strawberry producers from Michoacán obtain profit. The fact that a negative result is obtained applying the sequence of operations means that the presence of market distortions have a negative effect on the level of profitability obtained from the producers. This then indicates that a greater volume of utilities could be generated without the existence of these distortions that can be taxes, duties or custom barriers.

Table 1. Policy analysis matrix (PAM) for strawberry.

PAM				
Strawberry				
Concept	Income	Production costs		Profit
		Commercial inputs	Internal factors	
Private prices	$1,062,360.00	$371,449.00	$223,482.00	$467,429.00
Social prices	$1,935,435.18	$372,073.25	$224,093.00	$1,339,268.92
Effect or divergence	−$873,075.18	−$624.25	−$611.00	−$871,839.92

Source: Prepared by the authors based on PAM results.

When there is an efficient use of products, there are prices in balance in revenues, costs and profits at the international level. If, on the contrary, they are in imbalance they could be a signal of distortions whether in public policies of the country or of the market, for which PAM allows comparing between social and private prices to obtain a result between them.

Table 3 shows the PAM indicators, and each of the indicators refers to information of great use for knowledge of strawberry production in Michoacán. If the indicator PCR is <1 it means that the producer is competitive in the market, which is fulfilled for the case of strawberry.

The higher that the RRP indicator is, it means a higher profitability level for the producers; in the case of strawberry it is not very high so there must be work done at the level of profitability obtained by producers.

The VAP and PVAP indicators show the same information, except one is reflected in the monetary amount and the other in the percentage; the information that these indicators show is the added value to the work factor, both of labor and of the producer.

The first contrast was conducted between the indicator of cost ratio of internal resources DRC and the private cost ratio PCR through quadrants. It must be mentioned that the first quadrant shows an efficient and profitable crop (with comparative advantage[2] and

Table 2. Ratios at private prices of PAM for strawberry.

Relationships at private prices		**Crop**
Indicators	**Formulas**	**Strawberry**
PCR	PCR=C/(A−B)	0.37
RRP	RRP=D/(B+C)	0.70
VAP	VAP=(A−B)	$690,911.00
PVAP	PVAP=(A−B)/A	0.65

Source: Prepared by the authors with data obtained from PAM.

Table 3. Contrast of RCP-PCR ratio for strawberry.

Contrasts of PAM ratios for strawberries			
Result obtained 0.14/0.32		**Ratio of internal resources DRC**	
		DRC <1 o > 0	**DRC <0 o >1**
		With advantage	**Without advantage**
Private cost ratio	PCR <1 o >0	I	II
	Competitive		
	PCR >1 o 0	III	IV
	Not competitive		

Source: Prepared by the authors based on results from PAM.

[2] Competitive: a competitive product is such that can be sold in an appropriate volume within a specific market, because the buyers consider that its price and quality are acceptable, taking into account the services of support, credit, shipment conditions, guaranteed reparations, and publicity (Smith, An inquiry into the nature and causes of the wealth of nations, 1776).

competitive); the second quadrant encompasses an inefficient although profitable crop (without comparative advantage but competitive); the third quadrant signals an efficient and non-profitable crop (with comparative advantage and non-competitive); and the fourth quadrant shows an inefficient and non-profitable crop (without comparative advantage and non-competitive).

The last analysis that is done is the Subsidy Ratio to Producers. This indicator shows whether there were subsidies from the government or if, on the contrary, the taxes established deplete the utility of the producer.

If it is positive, there are subsidies and sufficient backing from the sectors of the economy towards the producers. On the contrary, if it is negative, there are distortions of policy, duties and taxes that deplete the utility of the producers, the producers could increase their utility received in a market without the appearance of those distortions.

For the case of strawberry a value of 0.37 was obtained, which according to the previous parameters is located in the cell for PCR of <1 or >0 which is why strawberry production in Michoacán is a competitive activity in commerce, since strawberry producers in Michoacán obtain profits from their products and the final price at which they can sell their products without the need to affect the utility has a considerable margin of difference compared to the prices at which the product is found in international markets. For the DRC indicator, the same value of 0.37 is respected and according to the parameters, the value is located in the cell <1 or >0 which indicates that the strawberry production in Michoacán is an activity that has advantage compared to other production zones. In a more exhaustive analysis, this corresponds to the fact that the necessary inputs are purchased at adequate prices, the level of taxes is low, and it is an activity that is protected within Mexico. In addition to this, the climate conditions, as well as the cost of water and the price of the land, allow planting normally over average and of good quality, without requiring a costly chemical treatment.

CONCLUSIONS

An important aspect in this study is to identify whether there is a comparative advantage or not. For this purpose, the PAM methodology has an indicator that identifies the existence of comparative advantage within its analysis. For PAM, this is the indicator of cost of internal resources where, if the value obtained is higher than 0 and lower than 1, then there is comparative advantage in the production of that good. Once the calculations were carried out, the following results were obtained: strawberry 0.14, found in the range of 0 to 1, so there is comparative advantage, which is why it can be inferred that there is comparative advantage.

Likewise, a deeper analysis was conducted through a contrast between the indicators of cost of internal resources and of private cost, to ratify whether the value is consistent with a comparative advantage, giving as a result a value of 0.37 which indicates that strawberry production is a competitive activity and with advantage for producers from Michoacán.

It can be concluded that the PAM methodology also allows analyzing the comparative advantage to determine if it is a profitable product. Therefore, according to the PAM analysis, strawberry is one of the most harvested berries in Michoacán; it offers a

profitability of 41% corresponding to the investment, offering a profitability of $437,429 pesos per hectare sown and harvested, on average, according to the analysis.

Once the study was conducted, it can be concluded that strawberry production is a profitable activity. However, it should be pointed out that for the cultivation of this berry, the number of hectares that are required is considerable, because their production is not scalable in the vertical plane.

Within the problem observed, the lack of financial resources is identified, which is why it will be necessary to work in favor of a financial structure that allows generating resources, as well as for the required reinvestment in the new harvests, and maintaining a savings plan that allows reducing leveraging progressively as the years advance.

Investment in specialized machinery is recommended, for cutting and selecting the fruits, as well as packaging and labeling, which considerably influences the costs generated by exports. Therefore, purchasing the appropriate containers for storage and transport, cost that can be reduced by having a system of their own, which could also be acquired through groups or societies of farmers.

Likewise, it is recommended to establish anticipated sales, with firm contracts that stipulate the final prices, as well as using insurance for harvest and production, with the objective of ensuring a higher investment return and guaranteeing continuity in the market for the producer. Likewise, it is recommendable to study deeply the sanitary norms that countries of high demand have, and to work in fulfilling each of them; at some point in time it can be investment without utility, although in the long term it would be a differentiation of the competitors. It is recommended to work on a financial structure that does not affect the investment returns, but which protects the producer in case of reductions in their harvest.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Endoparasitic infections in captive wild mammals under human care in San Luis Potosí, Mexico

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ABSTRACT

Objective: To determine the prevalence, richness and parasitic load in wild mammals of Tangamanga I and II Parks in San Luis Potosí, SLP. To assess whether infections are related to the type of feeding and weather seasons.

Design/methodology/approach: Analysis of fresh feces collected for three consecutive days at two sampling stations. Passive flotation techniques with sodium nitrate (qualitative) and McMaster (quantitative) were used.

Results: The overall prevalence observed was 36% (n=242). The parasitic richness is formed by protozoa: *Eimeria* sp., *Isospora* sp. and *Cystoisospora* sp.; by the cestode *Moniezia expansa*, nematodes: *Toxocara* sp., *Toxascaris* sp., *Ancylostoma* sp./*Uncinaria* sp., *Strongyloides* sp., *Trichuris suis* and Strongylid eggs. The parasite load in the case of protozoa was in the range of 0-8505 oocysts per gram of faeces, and 0-1400 eggs per gram of faeces in the case of helminths. Statistical analyses showed that the prevalence of parasites does not depend on the climatic season, and only in Tangamanga II Park is the prevalence dependent on the type of feeding (herbivores).

Study limitations/implications: The conservation method used limits the stool test techniques that can be employed (stool culture or sporulation).

Findings/conclusions: Endoparasitic infections can be a potential risk to the health of animals. In particular to those of great genetic value such as species threatened with extinction. In addition, the potentially zoonotic parasites observed pose a threat to the health of caregivers.

Keywords: wildlife, captivity, parasites, zoonoses.

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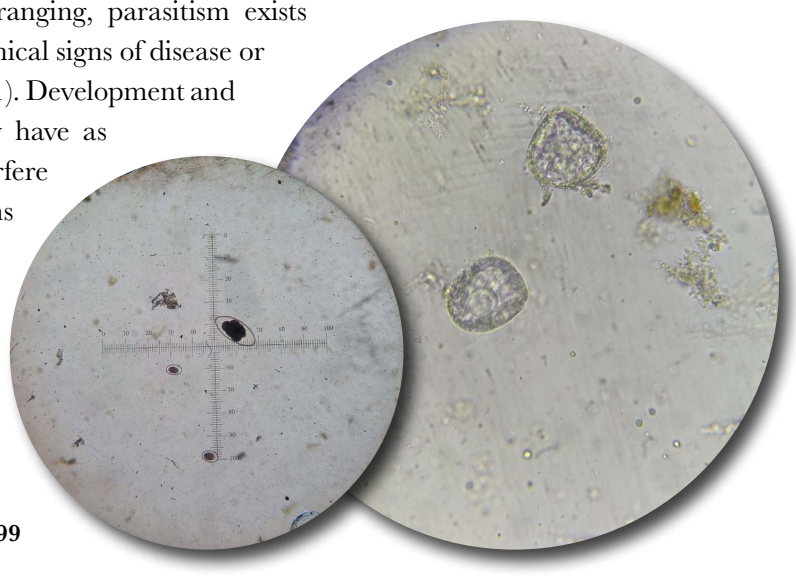
INTRODUCTION

Parasitism is the ecological relationship between one organism (the parasite) that benefits from causing harm, while another (the host) tolerates it. In wild animals of free ranging, parasitism exists naturally and often has no clinical signs of disease or parasitosis (Hossain *et al.*, 2021). Development and expansion of human activity have as intrinsic consequence to interfere with the balance of ecosystems and their populations, affecting them directly or indirectly. This leads to the creation of *ex situ* care centres for conservation or



management, such as the Management Units for the Conservation of Wildlife (UMA) (SEMARNAT, 2008) in Mexico; within them, wild mammals are kept in conditions of captivity (Sierra *et al.*, 2020). In that condition, they depend totally and exclusively on their keepers, therefore animal welfare must be a priority (OIE, 2021).

Animal-caregiver dependence implies that health conditions go beyond feeding; involving the management that the specimens receive, which include the cleaning and hygiene of their enclosures and food. This is of paramount importance when considering that intestinal parasites are one of the most important causes of disease and mortality in captive animals (Sierra *et al.*, 2020; OIE, 2021). In addition, there are potentially zoonotic parasites reported in wild mammals for example *Toxoplasma gondii* (Dărăbuş *et al.*, 2014). All of this is important in terms of public health, for animal keepers, as they work directly with animals and their excreta, and for the visitors of the zoo (Sierra *et al.*, 2020).

Therefore, this study was carried out with the aim of determining the prevalence, load and richness of intestinal parasites (protozoa and helminths) in the wild mammals of the UMA of the Tangamanga I and II Parks in San Luis Potosí, SLP. As well as to evaluate if parasites present are related to the type of feeding and the weather (seasons). Additionally, this is the first parasitological study carried out with the mammals of the Tangamanga I and II Parks; so, it is important to provide basic information necessary for decision-making by those responsible for the health and welfare of animals and workers.

MATERIALS AND METHODS

The study was developed in the UMA (DGVS-200-E-0055-SLP/98-SEMARNAT Registry) of the State Center for Culture and Recreation Tangamanga Park "Prof. Carlos Jonguitud Barrios"-CECURTI I and II. Tangamanga Park I (22° 07' 29.32" N and 101° 00' 01.74" W) is located in the west of the city of San Luis Potosí; while Tangamanga II Park (22° 10' 48.99" N and 100° 59' 05.78" W) in the north of the same city. The central area of the state of San Luis Potosí is characterized by a dry and semi-dry climate with rains in the months of June to September (average annual rainfall, 950mm). The average minimum temperature of the state is around 8.4 °C in January, while the average maximum temperature of the state is 32 °C in the month of May (INEGI, 2016).

The study was observational and included the analysis of stool samples from wild mammals in Parks I and II in two different seasons (dry: January-February; post rainy: October) during 2019. Fresh stool samples were collected, non-invasively, for three consecutive days in each season. The samples were collected directly from the floor (cement or wood) or soil (earth or grass) of the enclosure, collecting the portion that was not in direct contact with the substrate, to avoid possible contamination of the sample (Gallina, 2015). The collection of the samples was carried out in the morning before 10:00 AM.

In the field, the samples were collected, identified and stored in a thermal box (cooler) under refrigeration (5-10 °C) kept with frozen gels until transport to the laboratory, where they were stored in 5% formaldehyde solution until subsequent analysis. Parasites or sections of parasites that were observed in feces were collected and preserved in 70%

alcohol (Gallina, 2015) for subsequent analysis and taxonomic identification of genus using identification guides based on eggs and oocyst (Foreyt, 2001).

The sampling design varied according to the number of existing specimens and the type of enclosure in which the different species were found. The enclosures where there was only one specimen allowed the correct identification sample-specimen. In enclosures with two to five specimens, faecal samples were collected at random (simple randomized sampling), assuming that samples were collected from different individuals (100% of the population). In the case of enclosures with more than five specimens, as in the case of white-tailed deer in Tangamanga II Park, random faecal samples were collected (simple randomized sampling), assuming that samples were collected from different individuals among the 20% of the population (Daniel, 2017; Gallina, 2015).

In the case of llamas (*Lama glama*) which have the behaviour of defecating in pre-established places (dung deposits), samples were collected from deposits containing fresher faeces. Each day of collection the samples were taken from different deposits with the aim of increasing the probability of collecting the faeces of the three animals existing in each of the parks.

Passive flotation (Willis) techniques were used with sodium nitrate with 1.2 specific gravity and McMaster in the positive samples for gastrointestinal parasites. The McMaster technique allows the determination of the parasitic load, that is, the amount of eggs/oocysts per gram of faeces (Foreyt, 2001).

The information regarding the management of the specimens (feeding and cleaning) was obtained through interviews with the operational manager of the parks and also with the staff in charge of the daily care of the animals.

The statistical analysis method used was the χ^2 independence test considering a $p \leq 0.05$ (Daniel, 2017) used to evaluate, through a hypothesis test, whether the prevalence of parasites depends on the type of food of the animals studied, seasonality or both. In this study, parasite frequencies were obtained by type of mammalian feeding (carnivore, omnivore and herbivore) and by treatment of the environment where they live in relation to climatic variations (dry and post-rainy sampling season).

RESULTS AND DISCUSSION

All endoparasites found in the analyses have already been reported in wild mammals both in captivity (Dărăbuş *et al.*, 2014; Snak *et al.*, 2017; Sierra *et al.*, 2020; Hossain *et al.*, 2021) as of free-living (Mukul-Yerves *et al.*, 2014; Mino Botello *et al.*, 2016; Jones *et al.*, 2019).

The overall prevalence was 36% ($n=242$). The parasitic richness for the group of protozoa included oocysts of the genera *Eimeria* sp., *Isospora* sp. y *Cystoisospora* sp. For helminths, eggs were observed of the cestode *Moniezia expansa*, and nematodes of the genera *Ancylostoma* sp./ *Uncinaria* sp., *Nematodirus* sp., *Toxascaris* sp., *Toxocara* sp., of the Strongylid type, *Trichuris suis*, and larval eggs and larvae of the genus *Strongyloides* sp. The overall results are shown in Tables 1 and 2, and Figure 1.

A higher prevalence of protozoa in the dry season was found compared to the post rainy season (Figure 1). This can be explained by the fact that protozoan oocysts are

Table 1. Species sampled in Tangamanga I Park in the dry and post rainy seasons. Prevalence (%), richness and parasitic load (HPG=eggs per gram of faeces; OPG=oocysts per gram of faeces) observed.

Species	Total samples analyzed	Total positive samples	Prevalence (%)	Parasitic Richness	Parasitic Load
DRY SEASON					
Lama glama	9	5	56%	*Moniezia expansa*	50 - 1,050 EPG
				"Strongyles"	0 - 50 EPG
Odocoileus virginianus	9	6	67%	*Moniezia expansa*	0 - 50 EPG
				"Strongyles"	50 - 350 EPG
Potos flavus	3	0	-		
Procyon lotor	9	9	100%	*Eimeria* sp.	50 - 850 OPG
				Isospora sp.	0 - 50 EPG
Urocyon cinereoargenteus	6	0	-		
Panthera onca	6	2	33%	*Toxascaris* sp.	100 - 250 EPG
Lynx rufus	6	3	50%	*Toxascaris* sp.	150 - 200 EPG
				Toxocara sp.	0 - 50 EPG
Canis latrans	3	0	-		
Prevalence	51	25	49%		
Post RAINY SEASON					
Lama glama	12	5	42%	*Moniezia expansa*	100 - 150 EPG
				"Strongyles"	50 - 100 EPG
				Strongyloides sp.	0 - 50 EPG
				Nematodirus sp.	0 - 100 EPG
Odocoileus virginianus	6	4	67%	*Eimeria* sp.	50 - 100 OPG
				Moniezia expansa	0 - 50 EPG
				"Strongyles"	0 - 50 EPG
Potos flavus	3	0	-	-	
Procyon lotor	6	1	17%	*Strongyloides* sp.	0 - 50 EPG
Urocyon cinereoargenteus	12	6	50%	*Eimeria* sp.	0 - 8,050 OPG
				Cystoisospora sp.	0 - 550 OPG
Panthera onca	6	0	-	-	
Puma concolor	3	3	100%	*Ancylostoma* sp./ *Uncinaria* sp.	800 - 1,400 EPG
Lynx rufus	9	0	-	-	
Prevalence	57	19	33%		

forms resistant to certain unfavourable environmental conditions. In addition, we must consider the route faecal-oral transmission that occurs by the lack of hygiene and cleaning measures; as well as the types of floor and other objects existing in each enclosure where positive results were presented that may be acting as "shelters" for the oocysts (*e.g.* cracks or holes). Even the presence of parasitized and asymptomatic animals that maintain the reinfection cycle, or the combination of two or more of these factors (Sierra *et al.*, 2020; Hossain *et al.*, 2021).

Table 2. Species sampled in Tangamanga II Park in the dry and post rainy seasons. Prevalence (%), richness and parasite Load (HPG=eggs per gram of faeces; OPG=oocysts per gram of faeces) observed.

Species	Total samples analyzed	Total positive samples	Prevalence (%)	Parasitic Richness	Parasitic Load
DRY SEASON					
Pecari tajacu	3	1	33%	*Eimeria* sp.	0 - 50 OPG
Lama glama	10	3	30%	*Moniezia expansa*	50 - 300 EPG
Odocoileus virginianus	21	13	62%	"Strongyles"	50 - 1,300 EPG
				Eimeria sp.	50 - 350 OPG
Urocyon cinereoargenteus	6	0	-		
Lynx rufus	6	0	-		
Canis latrans	3	1	33%	*Eimeria* sp.	0 - 50 EPG
Bubalus bubalis	12	4	33%	*Eimeria* sp.	0 - 50 OPG
Prevalence	61	22	36%		
Post RAINY SEASON					
Pecari tajacu	3	3	100%	*Isospora* sp.	0 - 350 OPG
				Trichuris suis	0 - 50 EPG
				Oesophagostomum sp.	0 - 50 EPG
Lama glama	6	0	-	-	
Odocoileus virginianus	31	8	26%	*Eimeria* sp.	50 - 150 OPG
				Moniezia expansa	50 - 100 EPG
				"Strongyles"	50 - 1,150 EPG
Procyon lotor	3	3	100%	*Eimeria* sp.	200 - 600 OPG
				Strongyloides sp.	*
Urocyon cinereoargenteus	6	3	50%	*Ancylostoma* sp./ *Uncinaria* sp.	0 - 50 EPG
				Cystoisospora sp.	100 - 4,050 OPG
Lynx rufus	3	1	33%	*Strongyloides* sp.	*
Canis latrans	6	1	17%	*Ancylostoma* sp./ *Uncinaria* sp.	0 - 50 EPG
Bubalus bubalis	12	1	8%	"Strongyles"	0 - 50 EPG
Prevalence	70	20	29%		

Moniezia expansa was observed in both sampling seasons, since the route of infection is related to grazing, it may follow the fact that the lawn is constantly irrigated in the Park, which can favour the presence of the intermediate host, both in the dry season and post rainy season (Fassi-Fehri, 1987).

The presence of *M. expansa* in *Lama glama* and *Odocoileus virginianus* in Park I can be explained because both species share the same enclosure where they defecate and graze. This is consistent with the observation that in Park II the species do not share the enclosure and only the parasite was observed in *Lama glama*. It could also be explained by the movement of specimens from one park to another without proper preventive

measures; this is, without quarantine or deworming according to the information obtained in the interview.

The difference in parasitic loads could be explained by the differences in the conditions of the enclosures; therefore, in the possibilities of viability of intermediate hosts and reinfection. In Park I the enclosure has trees that provide many areas of shade and the grass receives frequent irrigation, always maintained green and abundant, which favours the establishment and proliferation of the oribatid mite and consequently the persistence of the reinfection cycle of the cestode. Whereas, in Park II there are not many shaded areas and there is practically no grass, prevailing a dirt floor that does not receive any type of irrigation; therefore, most of the feeding of the specimens is composed of hay fodder and grains, thus interrupting the biological cycle of the cestode.

Due to their direct biological cycle, infection with *Toxocara* sp. and *Toxascaris* sp. is via oral-faecal, so it is related to good hygiene and cleaning practices of the enclosures, although it may involve a paratenic host (Pariyar *et al.*, 2021). The positive results for *Toxocara* sp. and *Toxascaris* sp. observed in *Lynx rufus*, and *Toxascaris* sp. in *Panthera onca* in Park I could be explained by the presence of possible paratenic hosts, contamination or infection by street dogs or cats, contamination through fomites (Hossain *et al.*, 2021; Pariyar *et al.*, 2021) or by the exchange of animals between different enclosures without adequate hygiene or previous disinfection of the enclosure (OIE, 2021).

Parasitosis by *Toxocara* sp. and *Toxascaris* sp. are of relevant medical importance due to their complex extraintestinal larval migration that causes significant lesions in the organs of animals and also because they are potentially zoonotic, causing *larvae migrans* disease (Gakosso *et al.*, 2020). This becomes a public health problem and a potential risk to the health of the staff in charge of cleaning the enclosures of the animals (Sierra *et al.*, 2020).

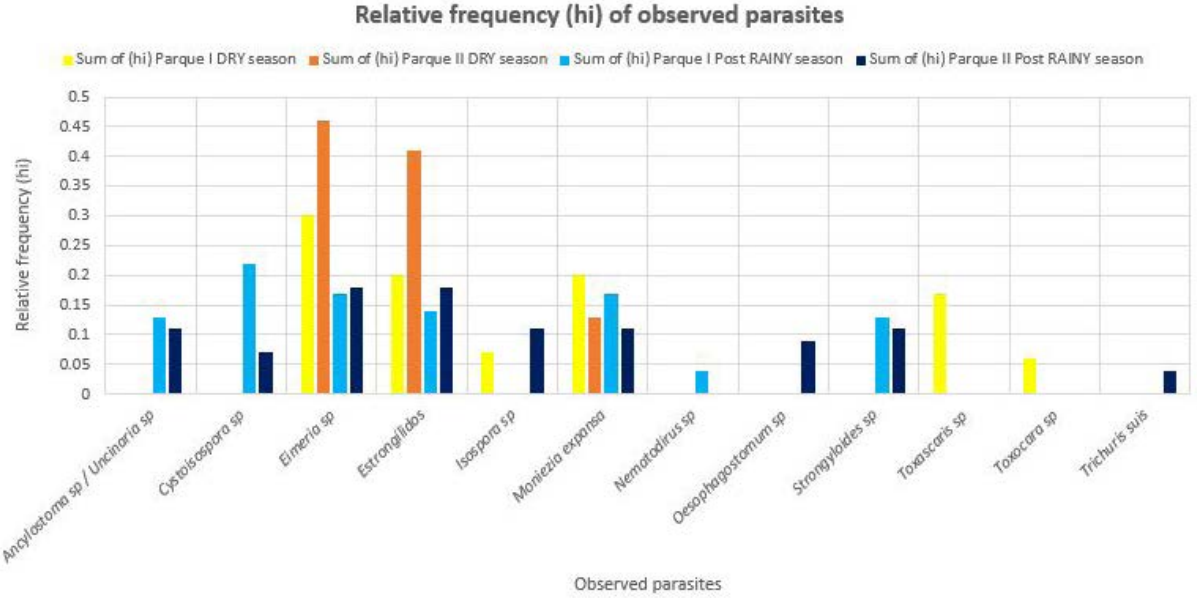


Figure 1. Graph of relative frequency (hi) of the parasites observed in the Tangamanga I and II Parks, in the sampling in dry and post rainy seasons.

The magnitude of the lesions in the organs and tissues of the animals is directly related to the magnitude of the parasitic load they present. However, when there are no preventive medicine protocols, the risk is that the animals do not present clinical signs and are in conditions of continuous reinfection, increasing the parasite load and consequently the number of larvae that shall migrate causing lesions (Klockiewicz *et al.*, 2019). Due to this, the existence of established biosecurity protocols is essential.

The Strongylids found in *Lama glama* and *Odocoileus virginianus* in Park I, both species with access to grazing which explains their infection and reinfection; also in the case of *Odocoileus virginianus* of Park II, despite the enclosure is mainly made of land, it is necessary to consider the movements of animals between the parks without the necessary preventive measures (OIE, 2021). Considering that the development and survival of L3 larvae depend on the temperature and humidity conditions of the environment, it was expected to find an increase in the prevalence and parasitic loads in post rainy season (Paixão *et al.* , 2018); nevertheless, the opposite was observed.

The positive results and parasitic loads in the dry season samples could be explained by the aforementioned lawn irrigation, and the presence of asymptomatic animals, which allow the infection-reinfection cycle to be established. All of this may be associated with inefficient management measures, as well as the absence of periodic stool test collection studies to detect asymptomatic animals, or deworming schedules with the use of specific drugs, and rotation of grazing sites in order to avoid the consumption of pastures contaminated with L3 larvae (Paixão *et al.*, 2018).

Strongyloides sp. larvae were found in some of the samples of *Procyon lotor* and *Lynx rufus* in Park II, and larval eggs in samples of *Procyon lotor* and *Lama glama* in Park I (Aranda *et al.*, 2013). Considering that one of the routes of infection is cutaneous, contaminated enclosures maintain the parasite cycle in those enclosures, in addition to its potentially zoonotic (Veraldi *et al.*, 2013).

Other results are reported as "*Ancylostoma* sp./*Uncinaria* sp." because it was impossible to distinguish both genera by the similarity of their eggs. They are phylogenetically related genera that cause similar clinical signs and lesions; both are present in Mexico. Therefore, similar treatment or prevention protocols can be used (Solorzano *et al.*, 2017). Both parasites are of direct life cycle and in general the route of infection is faecal-oral, although in *Ancylostoma* sp. they can also be lactogenic or cutaneous. Skin infection in humans is known as cutaneous larva migrans, so this genus is important for its zoonotic potential (Veraldi *et al.*, 2013). Therefore, enclosures with soil, grass or other vegetation, under favourable climatic conditions, allow and favour the development and permanence of the larvae of these parasites in the same way as enclosures with cement or wood floor that present porosities, cracks or holes that hinder their proper cleaning and disinfection. All of which may explain the results found in raccoon, grey fox, puma and coyote (Solorzano *et al.*, 2017; OIE, 2021).

The route of infection of *Trichuris suis* is oral through the intake of larvated eggs, which causes inefficient cleaning to contribute to the establishment and spread of the parasite (Hossain *et al.* , 2021). *Trichuris suis* is a typical parasite of pigs, but because of the similarities of their digestive systems, it is possible that they also parasitize collared peccaries (Jones *et al.*, 2019), which justifies the finding of the parasite in the faeces sample of the collared

peccary of Park II. In addition, the possibility of contamination through fomites must be considered (Pariyar *et al.*, 2021).

Regarding the genera of the parasites found, it is also observed that the relative frequency of parasites is different between both parks both in the dry season and in the *post* rainy season (Figure 1).

Prevalence results according to the sampling season were: 42% (n=112) in the dry season and 31% (n=127) in the post rainy season. Prevalence according to the park was: Park I, 49% (n=51) in the dry season and 33% (n=57) in the *post* rainy season (Table 1); in Park II, 36% (n=61) in the dry season and 29% (n=70) in the *post* rainy season (Table 2). Cases of multi parasitism, with the presence of two or more genera of parasites, were observed in both parks and in both sampling seasons.

Regarding weather season (dry and *post* rainy) it was observed that the prevalence of protozoa (coccidia) and helminths (cestodes and nematodes) did not depend on the season, both in Park I ($\chi^2_{(1,\ 0.05)}$=0.577; p=0.477) and in Park II ($\chi^2_{(1,\ 0.05)}$=0.278; p=0.598). Climate has an effect on parasites and their infectious stages (Paixão *et al.*, 2018), but in the parks evaluated this effect was not observed due to the management applied (frequent irrigation).

In regard to the type of feeding (carnivores, omnivores and herbivores) the highest prevalence of parasites was observed mainly in herbivores. Statistical analyses showed that, in both sampling stations, the prevalence of parasites in the specimens of Park I did not depend on the type of feeding (protozoa: $\chi^2_{(2,\ 0.05)}$=3,600; p=0.058 and helminths: $\chi^2_{(2,\ 0.05)}$=1.675; p=0.433). However, for the specimens of Park II the prevalence of parasites depended on the type of feeding (protozoa: $\chi^2_{(2,\ 0.05)}$=10.876; p=0.004 and helminths: $\chi^2_{(2,\ 0.05)}$=10.600; p=0.005).

CONCLUSIONS

Wild mammals in Tangamanga I and II Parks have endoparasitic infections caused by protozoa and helminths. However, although most animals do not show clinical signs of disease, there may be a potential risk to the health of the animals and the staff in charge of them, because potentially zoonotic parasites were found.

Weather seasons and the type of feeding of the animals of the study are related to the differences found in the prevalence, richness and parasitic load observed in the stool test results of the wild mammals of the Parks Tangamanga I and II.

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AGRO PRODUCTIVIDAD

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Evaluation of herbal choline in productive performance and blood metabolites of ewes

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ABSTRACT

Objective: To evaluate the effect of different levels of protected herbal choline (CHP) on productive performance, carcass characteristics, and serum metabolites in ewes.

Design/Methodology/Approach: Fifty-two Hampshire × Suffolk ewes (29.95±3.90 kg of initial body weight, IBW) were randomly assigned to one within four treatments: 0, 0.3, 0.6 and 0.9% choline kg^{-1} dry matter (DM). The experimental design was complete randomized, in order to detect linear or quadratic trends.

Results: No treatment effects were detected in the production variables, dorsal fat thickness and *Longissimus dorsi* muscle area, and AML with the addition of protected herbal choline in the diet ($p>0.05$). CHP linearly increased the concentration of cholesterol, glucose, albumins, globulins, total proteins ($p\leq0.05$) and phosphatidylcholine ($p\leq0.10$). The triglyceride concentration had a quadratic response ($p\leq0.05$) to the addition of CHP.

Study limitations/Implications: The level of choline supplementation in sheep depends on whether the source is herbal or synthetic.

Findings/Conclusions: The addition of CHP in ewe diets raised the concentration of phosphatidylcholine, modified the concentration of protein and lipid metabolites. However, no improvements in production were found.

Keywords: sheep, energy metabolites, herbal choline.

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INTRODUCTION

Choline is considered a metabolically essential B-complex vitamin in sheep (NRC, 2007). Choline metabolites in the body are important for the synthesis of proteins, phospholipids, acetylcholine, bone growth; also, as an essential factor in fat metabolism in the liver and methylation processes (NRC, 2007).

Image by Freddy at Pixabay

Dietary choline is extensively degraded in the rumen and a limited amount passes to the intestine (Baldi and Pinotti, 2006). Sources of protected choline are available on the market; however, in the feeding of lambs are not included regularly. The addition of protected choline in the diet of ruminants may reduce choline deficiencies and improve productive performance, health, and reproduction (Pinotti *et al.*, 2009; Jayaprakash *et al.*, 2016; Gutierrez *et al.*, 2019; Mendoza *et al.*, 2020).

Choline requirement in sheep has not been clearly established, but dietary supplementation may enhance productive performance (NRC, 2007). There is evidence that the addition of protected choline improves productive performance in lambs in completion, modifies hormones related to fat and protein metabolism, and stimulates the synthesis of glucose and cholesterol (Bryant *et al.*, 1999; Godínez-Cruz *et al.*, 2015; Li *et al.*, 2015; Rodríguez-Guerrero *et al.*, 2018).

Choline chloride is the synthetic source of choline commonly used in animal diets. However, under poor storage conditions, high hygroscopicity can accelerate the oxidation of food vitamins and premixes (Tavcar-Kalcher and Vengust, 2007). On the other hand, the use of levels higher than 3 g d^{-1} $animal^{-1}$ of protected choline chloride (CCP) would have adverse or no effects on the productive behavior of fat sheep (Bryant *et al.*, 1999; Li *et al.*, 2015), due to its low bioavailability (40-80%) and toxicity of secondary metabolic products (Sharma and Erdman, 1989; Jayaprakash *et al.*, 2016).

In addition, organic animal production is restricting the use of synthetic compounds such as choline chloride. This is the reason why researchers seek for alternative-natural dietary supplements in diets for ruminants.

Previous studies with fattening lambs (Godínez-Cruz *et al.*, 2015; Rodríguez-Guerrero *et al.*, 2018; Martínez-Aispuro *et al.*, 2019) have demonstrated the possibility of replacing CCP with protected herbal choline (CHP), because CHP contains phospholipids (mainly phosphatidylcholine) instead of choline chloride. The metabolic pathway of phosphatidylcholine in the body is different from free choline, as phosphatidylcholine requires less energy expenditure and does not require various metabolic processes to be available to cells. Free choline requires transporters to enter cells (some require ATP), then requires an ATP molecule for phosphocholine formation, followed by the conversion of phosphocholine to cytidine-di-phosphocholine, which determines the biosynthetic flow of choline to phosphatidylcholine (Fagone and Jackowski, 2013).

Supplementation with CHP in diets for ewes in completion could be a strategy to make metabolic and methylation processes more efficient, being an alternative source to the use of protected choline chloride. Therefore, the objective of this study was to evaluate the effect of different levels of CHP on productive performance, carcass characteristics and serum metabolites in ewes.

MATERIALS AND METHODS

Location of the study

The experiment was implemented following the standards of ethics, biosecurity and animal welfare of the Colegio de Postgraduados (CP), under the Official Mexican Standard NOM-062-ZOO-1999 at the CP Experimental Farm, located in Montecillo, State of

Mexico (19° 27' 38" N, 98° 54' 11" O, 2250 m). The climate is sub-humid temperate, with average annual temperature and rainfall of 15.8 °C and 663.7 mm, respectively.

Animals and treatments

The experiment used fifty-two Hampshire × Suffolk ewes (30.36±3.75 kg BW and four-month-old) housed in individual cages equipped with a feeder and drinker. The study factor was the dietary supplementation of Biocholine Powder® (Nuproxa Mexico, Querétaro, Mexico), a supplement containing choline of herbal origin with 16 g kg^{-1} of choline conjugates and is a polyherbal mixture based on *Achyrantes aspera*, *Trachyspermum ammi*, *Azadirachta indica*, *Citrullus colocynthis* and *Andrographis paniculata*. In each treatment, 13 ewes were randomly assigned, in a complete randomized design with four treatments. The treatments consisted of different dietary concentrations of Biocholine Powder® of 0, 3, 6 and 9 g kg^{-1} base MS in its incorporation of a basal diet (metabolizable energy 2.9 Mcal kg^{-1}, crude protein 189.0 g kg^{-1}, non-degradable protein in rumen 75.2, detergent acid fiber 150.5 g kg^{-1}, calcium 5.0 g kg^{-1} and phosphorus 4.6 g kg^{-1}) formulated in accordance with the recommendations of the NRC (2007). The composition of ingredients (g kg^{-1} MS) of the basal diet was as follows: corn (567.8), soybean paste (227.3), alfalfa hay (100.5), oat straw (50.0), cane molasses (40.0), calcium carbonate (2.4), sodium chloride (2.0) and a vitamin plus mineral premix (10.0).

The ewes were adapted to the basal diet for a period of 8 d the experimental phase lasted 60 days. The feed was offered at 08:00 and 16:00 h and the water *ad libitum*. The production variables were dry matter intake (DMI, kg d^{-1}), daily weight gain (DWG, g d^{-1}), feed conversion (FC) and final body weight (FBW). The average DWG was calculated with the initial body weight (IBW) and final body weight (FBW) of the experiment during the fasting period. The dorsal fat thickness and *Longissimus dorsi* muscle area were measured using a Sonovet 600 real-time ultrasound (Medison, Inc., Cypress, California, USA) with a 7.5 Mhz transducer between the 12^{th} and 13^{th} rib, on days 1 and 60 of the experiment.

At the end of the experiment (08:00 preprandial) 5 mL of blood were collected, by puncture of the jugular vein, in a tube without anticoagulant (BD Vacutainer®) for serum separation and placed in refrigeration (4 °C). The samples were centrifuged (Sigma 2-16 k, Germany) at 2500 x *g* for 20 min to obtain blood serum and stored in Eppendorf tubes at −20 °C until further analysis. In each sample, the concentration of total cholesterol (enzymatic oxidase-peroxidase method) was determined; triglycerides (enzyme method); high-density lipoproteins (HDL, enzyme method); phosphatidylcholine (enzymatic method); glucose (enzymatic method); total protein (Biuret method) and albumin (bromocresol-green method) using specific kits from the Spinreact trademark (Barcelona, Spain). By difference between total proteins and albumin, the blood concentration of globulins was obtained.

Statistical analysis

A complete randomized design was used, with four treatments and 13 replicates considering each ewe as an experimental unit. The Shapiro-Wilk and Levene tests were used to verify the normal distribution and homogeneity of the variance of the variables.

Data were analyzed using PROC GLM (SAS, 2010) and for the effect of choline intake orthogonal polynomials were used to detect linear or quadratic responses. Initial body weight was used as a covariate.

RESULTS AND DISCUSSION

Productive performance

The inclusion of protected herbal choline in the diet had no effect on the productive variables, dorsal fat thickness and *Longissimus dorsi* muscle area ($p>0.05$, Table 1) of the ewes.

Generally speaking, the dietary inclusion of choline in ruminants has beneficial effects. The addition of CCP in the diet of dairy cows improved productive performance during the postpartum period (Baldi and Pinotti, 2006); increased production and improved milk composition, and reproductive parameters (Jayaprakash *et al.*, 2016). In cattle and goats, feed conversion and weight gain were improved (Pinotti *et al.*, 2009; Habeeb *et al.*, 2017). However, when very high levels of choline chloride were used, no improvements in productive performance were observed (Pawar *et al.*, 2015; Budiarsana *et al.*, 2016).

In this study, the intake of 4.65-14.76 g d^{-1} of CHP had a null efect on the productive variables and the carcass characteristics. Which is consistent with the results observed in other studies (Godínez-Cruz *et al.*, 2015; Rodríguez-Guerrero *et al.*, 2018), where the supplementation of 4 g d^{-1} of CHP in the diet of finishing lambs did not modify the productive performance or the carcass characteristics. Is in contrast to some studies where the inclusion of CHP in ruminants may improve, the overall health of dairy cattle (Gutiérrez *et al.*, 2019; Mendoza *et al.*, 2020), and the productive performance of fattening lambs (3, 6 and 9 g kg^{-1} of DM; Martínez-Aispuro *et al.*, 2019).

In contrast, Li *et al.* (2015) found that production parameters and carcass characteristics responded quadratically to the inclusion of SPCs in lamb diets, observing the best response with 2.6 g d^{-1}. However, adverse effects were found with 7.8 g d^{-1} of CCP on daily weight gain. Bryant *et al.* (1999) observed increase in daily weight gain by suplement 2 g d^{-1} of CCP in the lamb diet but suplement 4 and 10 g d^{-1} of CCP, no additional benefit

Table 1. Productive variables of ewes fed with a diet supplemented with herbal choline.

Item	Herbal choline (g kg^{-1} DM)†				SEM	P-value	
	0	3	6	9		Linear	Cuadratic
Initial BW, kg	29.69	30.01	30.09	30.02	1.11	-	-
Final BW, kg	46.37	46.06	46.82	45.94	0.69	0.86	0.81
DWG, g d^{-1}	0.293	0.289	0.300	0.287	0.012	0.87	0.82
DMI, kg d^{-1}	1.54	1.55	1.55	1.64	0.06	0.57	0.52
Feed conversion	5.30	5.39	5.18	5.50	0.06	0.57	0.58
Backfat, mm	4.52	4.64	4.60	4.46	0.15	0.74	0.63
Chop area, mm^2	1107	1125	1108	1132	21	0.53	0.53

† Biocholine Powder® (Nuproxa Mexico); IBW: Initial body weight; FBW: final body weight; DWG: daily weight gain; DMI: dry matter intake; FC, feed conversion; SEM: standard error of the mean.

was observed. Thus, it is inferred that the use of choline chloride levels above 3 g d^{-1} has adverse or no effects on the productive performance of fattening lambs (Bryant *et al.*, 1999; Li *et al.*, 2015). However, Kawas *et al.* (2020) when supplementing 0, 0.1, 0.2, and 0.3% CCP (concentration of 25%) in fattening lambs, did not observe improvements in productive performance, although obtaining an increase in dorsal fat.

The difference in dietary supplementation tolerance between CCP and CHP is related to the presence of secondary compounds because choline chloride has an *in vitro* or *in situ* degradation between 40 to 80%, and the rest can be converted to trimethylamine (toxic compound) by intestinal bacteria (Sharma and Erdman, 1989; Jayaprakash *et al.*, 2016). Whereas CHP does not show this problem, since the phosphatidylcholine present in herbal choline is a source of esterified choline conjugated to a phosphate molecule, which is more active and bioavailable than choline chloride (Fagone and Jackowski, 2013).

In dorsal fat thickness and *Longissimus dorsi* muscle area, the addition of herbal choline had no effect, which coincides with other experiments by including levels of 2-10 g d^{-1} of protected choline (Bryant *et al.*, 1999; Godínez-Cruz *et al.*, 2015; Li *et al.*, 2015).

Another possible explanation for the lack of response to CHP supplementation in this research is that only females were used, whereas males and females were used in previous studies (Bryant *et al.*, 1999; Godínez-Cruz *et al.*, 2015; Li *et al.*, 2015; Rodríguez-Guerrero *et al.*, 2018). Studies in people reported a marked differentiation in methylation processes between males and females (McCarthy *et al.*, 2014); which would lead to think that the choline requirement is different between sexes.

Serum metabolites

The addition of CHP linearly increased the concentration of cholesterol, glucose, total proteins, albumins, globulins ($p<0.01$) and phosphatidylcholine ($p<0.08$) (Table 2). The concentration of triglycerides presented a quadratic response ($p<0.05$) to the addition of CHP. The lowest concentration of this metabolite was recorded with the use of 4.5 and 9.3 g d^{-1} of CHP. The addition of CHP did not affect the HDL concentration and the albumin/globulin ratio in blood serum ($p>0.10$).

In this study, CHP supplementation showed a linear increase in phosphatidylcholine in plasma, confirming that CHP supplementation was effective in incorporating choline into the lamb metabolism (Martínez-Aispuro *et al.*, 2019). Similar to what was reported by Habeeb *et al.* (2017) who found that choline supplementation in goats increased plasma concentrations of the metabolite.

Choline supplementation has been documented to show a linear trend to increase blood glucose in cows and goats (Zhou *et al.*, 2016; Habeeb *et al.*, 2017). In lambs, it is observed that the inclusion of herbal choline in the diet increases the concentration of glucose in blood serum (Rodríguez-Guerrero *et al.*, 2018; Martínez-Aispuro *et al.*, 2019). Choline could alter intracellular signaling of energy metabolism, as it occurs in cases of insulin resistance, where choline supplementation reduces glucose utilization for fatty acid and triglyceride synthesis and increased muscle glycogen (Taylor *et al.*, 2017).

Table 2. Blood metabolites in ewes fed a diet supplemented with herbal choline.

Item	Herbal choline (g kg^{-1} DM)†				SEM	P-value	
	0	3	6	9		Linear	Cuadratic
Cholesterol, mg dL^{-1}	96.10	103.55	118.76	114.83	3.59	0.001	0.01
PCho, mg dL^{-1}	106.14	108.40	116.11	115.10	8.24	0.08	0.23
Triglycerids, mg dL^{-1}	32.27	26.55	28.82	30.95	1.87	0.84	0.04
HDL, mg dL^{-1}	55.07	56.13	55.24	55.68	1.67	0.91	0.85
Glucose, mg dL^{-1}	75.09	80.82	83.61	86.08	2.85	0.008	0.57
Total protein, g dL^{-1}	5.75	5.81	7.58	7.91	0.39	0.001	0.73
Albumins, g dL^{-1}	2.78	2.81	3.17	3.50	0.12	0.001	0.27
Globulins, g dL^{-1}	2.98	3.00	4.40	4.42	0.36	0.001	0.98
Albumins/ Globulins	0.94	0.96	0.80	0.87	0.06	0.20	0.68

† Biocholine Powder® (Nuproxa Mexico); PCho: Phosphatidylcholine; HDL: high-density lipoproteins; SEM: standard error of the mean.

Similar to the results found in this study, the addition of 4 g d^{-1} of herbal choline in lamb diets increased serum cholesterol (Rodríguez-Guerrero *et al.*, 2018). In dairy cows, choline supplementation significantly increased cholesterol concentration (Soltan *et al.*, 2012). Nevertheless, the consumption of CCP (2-10 g d^{-1}) in lamb diets did not change serum cholesterol concentration (Bryant *et al.*, 1999; Li *et al.*, 2015). Choline is necessary for the transport and metabolism of lipid cholesterol (Zeisel and Costa, 2009), which could explain the changes in blood cholesterol concentrations.

A deficiency of choline in muscle cells leads to the accumulation of triglycerides in cattle (Bryant *et al.*, 1999). Similar to what was found in this study, choline supplementation in goats reduced triglyceride concentration (Habeeb *et al.*, 2017; Rodríguez-Guerrero *et al.*, 2018; Kawas *et al.*, 2020). However, in other studies the consumption of 2-10 g d^{-1} of choline did not modify the serum concentration of triglycerides in lambs (Bryant *et al.*, 1999; Li *et al.*, 2015).

Consistent with the results obtained in this study, choline supplementation in goats and fattening sheep increased globulin concentrations (Habeeb *et al.*, 2017; Martínez-Aispuro *et al.*, 2019). Whereas, in lambs supplemented with CHP (4 g d^{-1}), it did not modify serum albumin or total protein concentration (Rodríguez-Guerrero *et al.*, 2018).

Although in this research the concentrations of high-density lipoproteins were not modified; there is conflicting evidence that choline supplementation (2.6 g d^{-1} of CCP) to fattening lambs reduces (Li *et al.*, 2015) or does not affect (with CHP, Martínez-Aispuro *et al.*, 2019) the concentration of high-density lipoproteins.

CONCLUSIONS

Herbal choline is effective in raising the concentration of phosphatidylcholine in the body of ewes during fattening. It also modified the concentration of protein, lipid and energy metabolites. However, the consumption of 4.5-14.0 g d^{-1} of herbal choline does not favor the productive performance.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Pollution in the lower basin of Jamapa River by the application of antibiotics for veterinary use

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ABSTRACT

Objective: To analyze the level of use of antibiotics in livestock and aquaculture activities developed in the lower basin of Jamapa River, to know the possible impact of these compounds on water quality and the risk to public health.

Methodology: A survey of 60 farmers was conducted using a structured questionnaire with 41 open and closed questions during the year 2020. The use of key informants with experience in the livestock sector and related to the livestock sector in the study area was applied.

Results: Regarding the profile of the producers, it was found that 95% were men, and women only represented 5% (2 women). The 50% of producers have a basic level of schooling; 85% of respondents used antibiotics as a preventive treatment for disease control, 8.3% apply vitamins and 6.7% use dewormers. Among the most frequently used antibiotics is Oxytetracycline.

Study limitations/implications: to assess the toxic risk that exists when using antibiotics in the livestock sector, it is necessary to develop procedures and to plan new regulations for the use of antibiotics in this sector.

Findings/conclusions: a relationship was found with the use of oxytetracycline by the livestock sector. There is great concern about the impact that pharmaceuticals can have on water bodies and outside them. It is necessary to carry out risk assessments based on traditional fish responses, such as changes in growth or survival.

Keywords: veterinary drugs, antibiotics, diseases.

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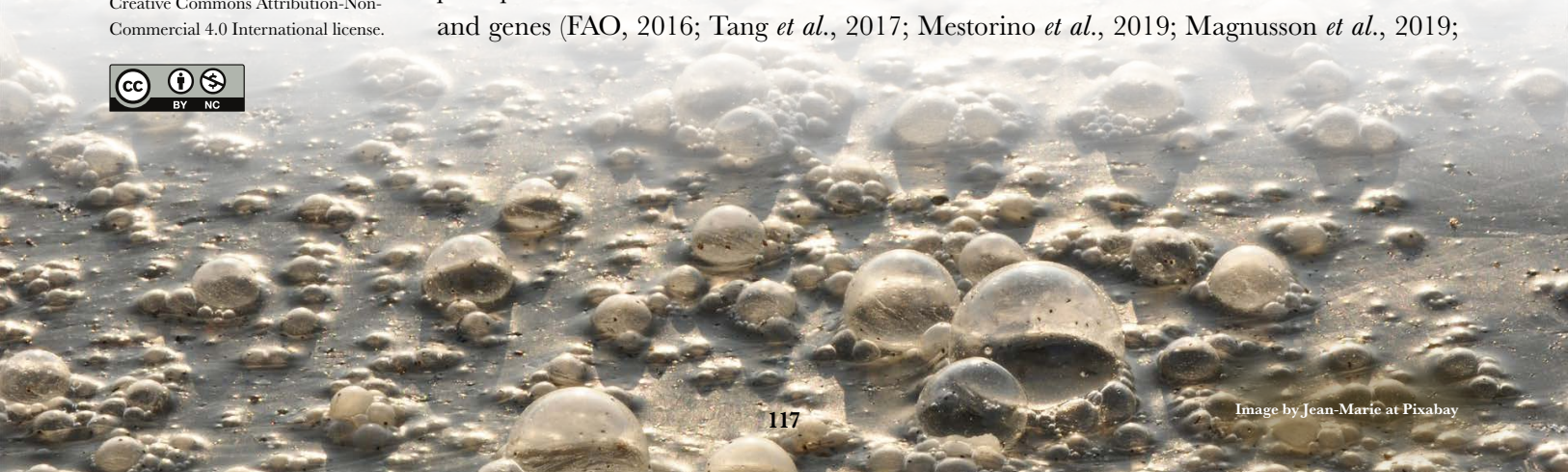
INTRODUCTION

Antibiotics are the group of pharmaceutical products of extensive use worldwide for their therapeutic purposes in human medicine, veterinary medicine and agriculture (Tang *et al.*, 2017). Extensive administration of antibiotics due to inadequate regulation and poor practices in their use is associated with the increase in antibiotic resistant bacteria and genes (FAO, 2016; Tang *et al.*, 2017; Mestorino *et al.*, 2019; Magnusson *et al.*, 2019;



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Fernández-Rodríguez *et al.*, 2020). The residuality of these compounds has been detected in livestock derived products such as meat (Aguilar-Gálvez *et al.*, 2018; Rivera-Alegría *et al.*, 2018; Vega-Sánchez *et al.*, 2020), kidney (Guerra and Elera, 2021), and raw milk (Cruz-Mendoza *et al.*, 2017; Jáuregui and Celis-Vielman, 2018). In Mexico, numerous problems related to the inappropriate use of antibiotics used in human and veterinary medicine have been reported (INSP-UNAM. 2010). However, research on the management of these compounds by users and their presence in surface water such as rivers is scarce in Mexico. Main routes of entry of antibiotics into the environment is through human excretion, elimination of unused products, and agricultural use (Gil *et al.*, 2012).

Agricultural and livestock activities such as cattle and pig farming (Cancho-Grande *et al.*, 2000; Echtermann *et al.*, 2019), poultry farming (de Assis *et al.*, 2016; Mestorino *et al.*, 2019), and aquaculture activities such as shrimp farming (Le Truong and Thanh, 2019; Thiang *et al.*, 2021). Together they constitute the main sources of antibiotics for veterinary use (Lara *et al.*, 2019). Antibiotics presence has been reported by various sectors using aquatic ecosystems, along with associated impacts on the organisms that inhabit those ecosystems.

A diversity of antibiotics and types of compounds for veterinary use are applied in the farm animal industry; they are administered primarily through feed and water. Advantages from those compounds include improving feeding and growth efficiency, reducing morbidity for therapeutic, and prophylactic purposes (Kozarova *et al.*, 2004; Grossi-Botelho *et al.*, 2015; Khatun *et al.*, 2018). The impact of antibiotic mixing on the diversity of microbial populations may be associated with their contribution in the ability to adapt and resist antibiotic compounds from wastewater in pig production (Reynoso-Varela *et al.*, 2020). Likewise, their presence is associated with the generation of DNA damage in living organisms (Zirena Vilca *et al.*, 2018).

The adverse effects caused by the use of these compounds have contributed to be included within the group of emerging pollutants, considered as non-persistent but whose solubility in water contributes to their ability to enter all stages of the hydrological cycle, and be detectable in aquatic ecosystems (Gil *et al.*, 2012; Snow *et al.*, 2018). The diversity of antibiotic compounds for veterinary use is incorporated into aquatic systems by the entry of effluents without prior treatment. They can cause effects on the biodiversity and health status of the organisms that inhabit these aquatic ecosystems (Barceló *et al.*, 2007; Jimenez, 2011; Salcedo, 2019).

In the state of Veracruz, most of the rivers that cross through the main population centers receive discharges of municipal wastewater, industrial discharges and various types of pollution from non-point sources (Torres, 2013). In addition, the 14 most important rivers in Veracruz register significant levels of pollution, which implies serious consequences for human health, limits productive activities and deteriorates the environment (CSVA, 2004). Nutrients and pollutants are naturally recycled in aquatic and terrestrial ecosystems. However, the anthropic influence has altered these cycles by increasing the amount of nutrients and pollutants that end up in water bodies, causing problems associated to eutrophication, disruption of natural ecosystems and damage to human health (Torres, 2013). This represents a risk for wild aquatic species such

as fish given their potential incorporation into their tissues and generation of adverse effects such as bacterial resistance, among others (Zaragoza *et al.*, 2020). Therefore, the importance of evaluating the use made by users of the different productive activities developed in this basin is highlighted, given the potential impact of antibiotics on public health and the environment. The objective of this research was to determine the level of use of antibiotics in livestock and aquaculture activities developed in the lower basin of Jamapa River, to know the potential impact of these compounds on water quality and the risk to public health.

MATERIALS AND METHODS

Study area

The basin of Jamapa River is part of the Jamapa-Cotaxtla and Medellín river system (Figure 1). It is located between 18° 45' and 19° 13' N, and 95° 56' and 97° 16' W; with an approximate area of 3,912 km^2, it covers 28 municipalities of the state of Veracruz. Within the limits of the basin, two main channels are comprised, the Jamapa and Cotaxtla rivers that originate in the Citlaltepetl or Pico de Orizaba (5700 m) and flow discharged into the Gulf of Mexico (Ortiz-Lozano, 2013).

Sampling techniques and data collection

Data were obtained by applying a structured survey as a sampling instrument (Piña-Guzmán *et al.*, 2019; Costa *et al.*, 2021; Ogwuche *et al.*, 2021). This was applied to cattle ranchers in the municipalities of Jamapa, Cotaxtla and Medellín in Veracruz, Mexico. The study population included antibiotic users located in the municipalities indicated above; which allowed to include a greater representativeness in relation to sex and age of the participants. It also included data on the production unit of the families, the surface of the farm, and the distance from the farm to the lower basin of the Jamapa River.

The questionnaire was the data collection tool on demographics, antibiotic use practices and antibiotic management regulations (Ogwuche *et al.*, 2021). The questionnaire was structured with 41 open or closed questions. A randomly selected representative sample of 60 livestock producers was interviewed individually and in groups. The questionnaire

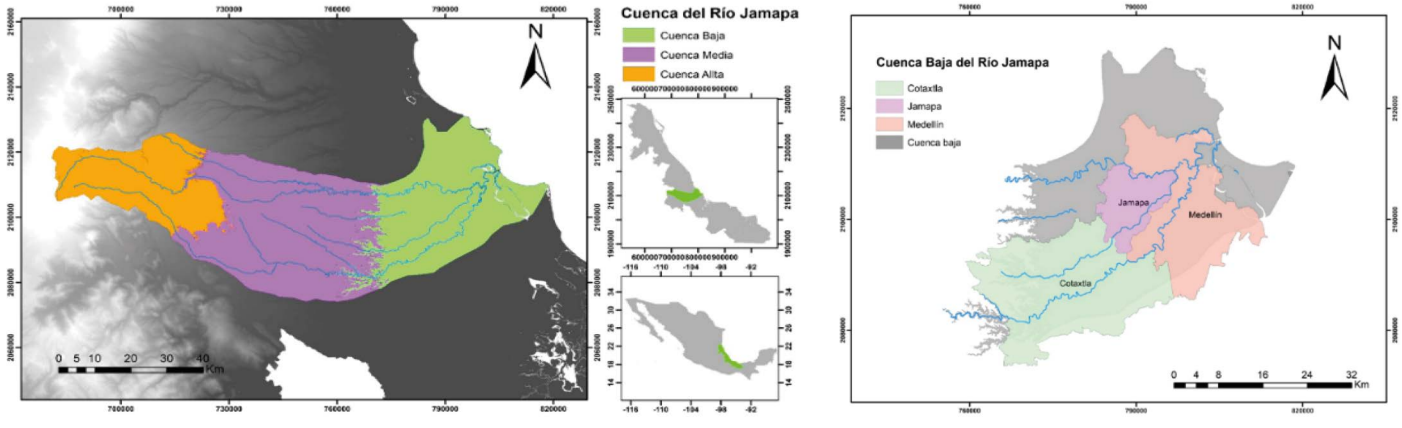

Figure 1. Location of municipalities in the lower basin of Jamapa River, Veracruz.

was previously evaluated as pilot within a group of producers from the study area to verify the quality of the questions and the proper functioning of the instrument designed. The use of key informants with experience in or related to the livestock sector in the study area was included.

Statistical analysis

Descriptive statistics were used with the statistical package TIBCO Statistica 14.0.0.15 (TIBCO Software Inc., Palo Alto, CA, USA) to group all the information collected on the demographic variables of the interviewees. Antibiotic use for the livestock herd, frequency of application of antibiotics and compounds with greater frequency of use; those were the most important data considered in the management of livestock herds.

RESULTS AND DISCUSSION

Socio-economic characteristics of producers and livestock activity

Figure 2 shows the average age of cattle farmers in the municipalities analyzed in this research (50.6±14.6). Orantes-Zebadúa *et al.* (2014) reported the same trend in livestock producers in Chiapas, with an average age of 50 years. Meanwhile, Vilaboa-Arroniz *et al.* (2009b) indicated in three age groups for cattle ranchers in the region of Papaloapan, Veracruz, with average ages of 53±13. 54±15 and 56±12 years old. Also, De los Santos-Lara *et al.* (2015) indicated the same age trend in cattle ranchers in the central region of Chiapas, they reported an average age of 52.4 years, out of which 53% indicated being older than 50 years old. Chalate-Molina *et al.* (2010) reported that dual-purpose livestock producers in the state of Morelos had an average age of 52±12 years.

The average age obtained in those previous studies indicates that there is little generational replacement in livestock activity in the study area; which agrees with Chalate-Molina *et al.* (2010) who indicated that livestock producer groups are characterized by an

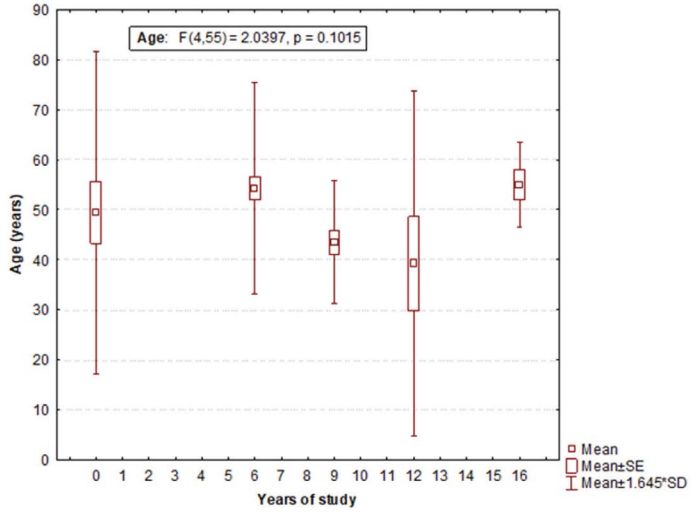

Figure 2. Distribution of age and schooling (years) of cattle ranchers in the municipalities of Medellín, Jamapa and Cotaxtla, in Veracruz, Mexico.

advanced age and low level of education. The latter statement also coincided with this research since it was identified that only 3 interviewees (5%) indicated to have higher level studies such as a bachelor's degree with a 12-year schooling. Chalate-Molina *et al.* (2010) mentioned that INEGI in 2005 recorded that the average schooling of farmers was 6±4 years; this value is lower than the national average of 8.1 years.

Similarly, Orantes-Zebadúa *et al.* (2014) indicated that in farmers there is a basic level of study with a schooling of 6 years (primary completed). On the other hand (Vilaboa-Arroniz *et al.*, 2009b) reported that traditional farmers indicated a schooling at the basic level with 6±6 years, although they also pointed out that 12.1% had a bachelor's level. In contrast, De los Santos-Lara *et al.* (2015) indicated that 90% of their interviewees had a bachelor's degree, with an average schooling of 17 years; 3% had postgraduate studies and 7% basic studies. Vilaboa-Arroniz *et al.* (2009b) identified differences in schooling; reporting that 31% of farmers in transition had a bachelor's degree, whereas 12% of traditional ranchers reported the same.

Regarding gender, 95% of the interviewees were men; indicating that cattle ranching is an activity dominated by the males in the study area, as females only represented 5% (2 women) among the participants (Table 1). In contrast, De los Santos-Lara *et al.* (2015) reported that women with higher education (professionals) had a greater participation in agricultural activities and these represent 10% of the study population analyzed. This may indicate a difference in women's participation in the livestock sector associated with the academic level and the region of the country, because other works do not mention the participation of women in this sector.

In the study area, it was identified that the interviewees had a work experience in livestock of 25.55±50 years. A low correlation was identified with an $r^2=0.5377$ between age and years of experience in the livestock sector as indicated in (Table 1). This coincided with the years of experience in the activity indicated for traditional farmers with 25±13 years, a maximum of 60 and a minimum of 1 year (Vilaboa-Arroniz *et al.*, 2009b). According to Chalate-Molina *et al.* (2010), the experience of the producer in the cattle ranching activity is the main strength that is based on the human resource in dual-purpose livestock (DP); they also highlighted the importance of other attributes such as the availability of family labor, the experience in production and the rusticity of the breeds used. In contrast, De los

Table 1. Characteristics of cattle ranchers in the analyzed municipalities of the central area of Veracruz.

Variable	Average	Minimum	Máximum
Age (years)	50.63	18	85
Escolarity (years)	6.45	0	16
Gender (%)	Male 95%		
	Female 5%		
Cattle experience (years)	25.55	2	60
Available area (ha)	20.5	2	72
Cattle heads number	37	7	150

Own elaboration.

Santos-Lara *et al.* (2015) indicated that Swiss cattle breeders in Chiapas are characterized by experience, education, economic and social potentials to innovate and improve the processes of breeding and selection of livestock.

In this research, 96.66% of interviewees operate extensive livestock farming and only two producers (3.33%) indicated to manage the intensive type. Extensive grazing is the main management system, 98% (Vilaboa and Díaz (2009). And 86.9% of the dual-purpose bovine production system is developed mainly under the extensive grazing management system in the tropical regions of Latin America (Vilaboa-Arroniz *et al.* (2009b). In agreement with the above, Herrera-Calvo and Majadas-Andray (2018) indicated that extensive livestock takes advantage of the resources of the territory, has a behavior of integration and respect with local ecological processes, since it must necessarily adapt to them to maintain the production process.

It should be noted that the information on the area available for livestock activity and the number of herd animals was not provided by the producers. But it should be appointed that this type of information would allow characterizing the scale of livestock production in the study area and the use of antibiotics for this farming activity.

The breeds with the highest predominance in the municipalities analyzed were Dual Purpose Creole with 40% (24 interviewees) and Swiss Cebu (17). In contrast, Vilaboa-Arroniz *et al.*, 2009b) indicated the racial pattern of the cross Swiss × Cebu (79.8%) as the most representative, and it has as fundamental objective the production of milk. Also, Orantes *et al.* (2010) indicated that, in livestock production systems through extensive grazing, Cebu × Swiss breeds have as their genetic source the breeds Cebu × Dutch and Cebu × Simmental, among the main ones. The predominance of Creole cattle in the study area indicates the adequacy of the farmers to obtain livestock according to their economic conditions and the production area.

Livestock producers interviewed in this research reported frequent use of 11 different types of antibiotics. They also indicated that Oxytetracycline with 43.33%, was the antibiotic most frequently used by cattle farmers in the three municipalities analyzed; followed by Penicillin with 15% and Forfenicol with 8.33%. The rest of the antibiotics indicated a less recurrent use such as 3 Sulfas and Tetracicin with 6.66%, Derriengue and Draxxin with 5.0% (Table 2). In this regard, Ogwuche *et al.* (2021) indicated in livestock the use of oxytetracycline (82.6%, 317/384), tylosin (44.5%, 171/384) and gentamicin (43.8%, 168/384), penicillin 39. 3% (151/384) and enrofloxacin 38. 5% (148/384). Herrera-Calvo and Majadas-Andray (2018) highlighted the use of tetracyclines, penicillin, sulfonamides and polymyxins in cattle in Spain; although they highlighted that a figure greater than 50% of antibiotics were destined for pig production.

In Figure 3, the livestock producers in the study area did not indicate to implement any type of clinical analysis for the application of these drugs. Likewise, Ogwuche *et al.* (2021) indicated that 32.0% of respondents did not undergo antimicrobial susceptibility testing (AST) prior to antibiotic treatment.

Recently, the need for adequate administration of antibiotics to preserve the efficacy of existing antibiotics against pathogens has been highlighted (Aslam *et al.*, 2018).

Table 2. Frequently used antibiotics in disease control (diarrhea) reported by cattle ranchers in the municipalities of Medellín, Jamapa and Cotaxtla; Veracruz, Mexico.

Antibiotics for Veterinary Use	Frequency	Percentage	Antibiotic used in diarrhea control	Frequency	Percentage
Penicillin	9	15.00	Penicillin	6	10.00
Oxytetracycline	26	43.33	Oxytetracycline	38	63.33
Florfenicol	5	8.33	Florfenicol	3	5.00
Derriengue	3	5.00	Ampicillin	1	1.66
3 sulfas	4	6.66	Derriengue	2	3.33
Tetracycline	4	6.66	3 sulfas	2	3.33
Flunixin	1	1.66	Tetracycline	2	3.33
Draxxin	3	5.00	11 Via	1	1.66
Shotapen L.A.	1	1.66	Shotapen L.A.	1	1.66
Fluvicin	2	3.33	Oxitetlacycline	3	5.00
7 via	2	3.33	Bactrex	1	1.66

Own elaboration.

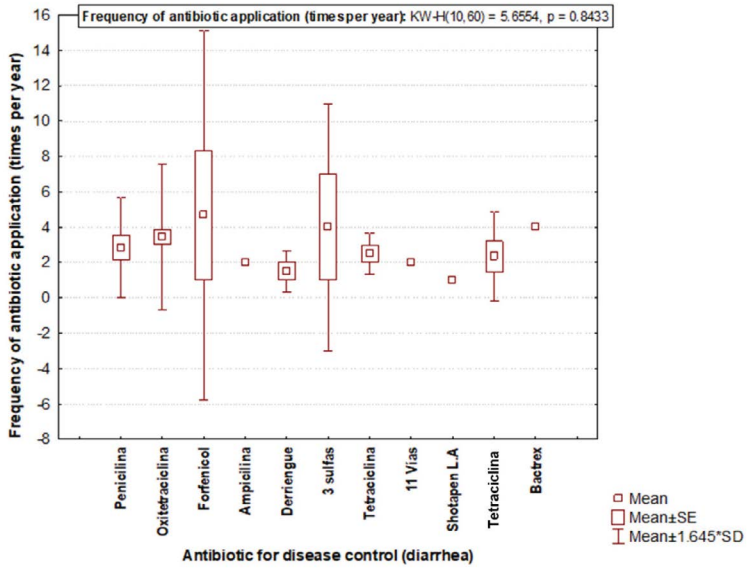

Figure 3. Frequency of antibiotic use reported in the control of diarrheal diseases used by cattle farmers in the study area.

Antibiotics are essential in the therapeutic treatment of bacterial diseases in livestock production, because they affect the health, production and well-being of livestock (Van *et al.*, 2020). Ogwuche *et al.* (2021) reported that 60% of respondents recommended the use of antibiotics for the treatment of non-bacterial pathogens, including viral, helminth and fungal pathogens. This may indicate an abuse of antibiotics for the treatment of pathologies not indicated by these compounds.

The different types of antibiotics indicated by farmers in this research involve a diversity of compounds applied on an extensive production scale. However, Herrera-Calvo and

Majadas-Andray (2018) indicated that regardless of the scale of extensive and industrial production there is an impact on these activities, and the use of antibiotics in extensive livestock farming that must be improved as observed in Figure 3.

The 100% of the interviewees indicated that the route of administration of these antibiotics is through injection. This implies that an elimination period is required for antibiotics and avoid their residuality, reported in milk and meat. Likewise, it was identified in this research that only 20% of the interviewees (12 farmers) indicated having knowledge about the use of antibiotics, they responded that "they kill bacteria". The rest of the interviewees (80%) indicated that they had no knowledge about antibiotics. The latter indicates the importance of the knowledge that producers need to possess about the action of antibiotics for a better decision making on their application to livestock. Regarding access to technical advice, 85% of the interviewees indicated to pay for the advice of a veterinary or zootechnician (MVZ); 15% indicated that they did not have this type of professional advice.

Despite, 100% of those interviewed in this research indicated that they acquire antibiotics supplied to livestock in veterinary clinics. In contrast, Vilaboa-Arróniz *et al*. (2009b) indicated that only 47% of producers receive technical advice from the MVZ related to the Local Livestock Association and 0.25% receive advice from Research Centers, or public and private universities. The latter indicates the importance of technical advice with MVZ professionals, particularly in the management of antibiotics for veterinary use.

Antibiotics are among the best-selling and consumed drugs in Mexico, according to IMSH data (2005) this represents an annual market of 960 million USD and ranks second in annual sales (14.3%) in private pharmacies in the country (Dreser *et al*., 2008). It should be noted that in Mexico there is no official published information on the volume and management of antibiotics in human and veterinary medicine. This type of information is not required of producers in official statistics such as the National Agricultural Census (INEGI, 2019), this instrument is focused on collecting information on available area, number of livestock heads (cattle, sheep, pigs, poultry), type of feed, among others. In addition, it collects information on the management of compounds such as hormones and anti-tick baths, but the use of antibiotics is not considered in livestock activity.

It was identified that 85.00% of the interviewees in this research used antibiotics as a preventive treatment for disease control, 7.5% applied deworming and vitamins (Table 3). In agreement with the above (Ogwuche *et al*., 2021) indicated a similar trend, 96.6% reported providing a therapeutic treatment of a disease was the most frequent reason for recommending the use of antibiotics, followed by the prevention and prophylaxis of diseases with 41.7% and for the promotion of growth 4.7%. It should be noted that the preventive treatment with antibiotics reported in the study area should be added to that

Table 3. Preventive treatment for disease control in the cattle herd.

Preventive treatment for disease control	Frequency	Percentage
Antibiotics	50	85. 00
Dewormers	5	7.5
Vitamins	5	7.5

Own elaboration.

implemented for disease control and cases of diarrhea in livestock. This increases three times the consumption of antibiotics given to cattle in this research.

The non-specific use of antibiotics without knowing the causative agent of the disease in animals in the study area is a common practice in agricultural activity worldwide. Likewise, Ogwuche *et al.* (2021) indicated that a high proportion of interviewees in their research corresponding to 81.3%, recommended the use of antibiotics to control non-bacterial pathogens, including viruses, protozoa and even fungi. Although they also reported that 98.7% of the interviewees indicated recommending others to the use of antibiotics for the control of bacterial pathogens.

The 100% of the interviewees indicated that they did not have any type of government support, this may imply that there is no support for the management of cattle and compounds of controlled use such as antibiotics in the study area. Chalate-Molina *et al.* (2010) indicated that there is little dissemination of federal, state and municipal support programs, highlighted the limited contact with people involved in the promotion of farming activity and how this situation makes it difficult to request government support. Cuevas-Reyes *et al.* (2012) reported with information from the Agricultural, Livestock and Forestry Census in Mexico, that the coverage of technical assistance is low, 3% of the national total of production units with agricultural activity have this service, and only 11.7% perceive the lack of technical assistance and training as a problem. In contrast, Vilaboa-Arróniz *et al.* (2009b) indicated that 50.1% of the farmers in the Papaloapan region do not receive technical assistance.

Environmental risk in the mode of application and management of antibiotics

It was previously indicated that oxytetracycline was the most frequent antibiotic for farmers in the study area, because they indicated its easy access to acquisition and affordable economic cost. This coincides with what was reported by León-Aguirre *et al.* (2017) who indicated the detection of oxytetracycline in pig wastewater from small and medium-sized farms in Yucatan. It also demonstrated the persistence and recurrent use of this antibiotic in the water used for livestock activities in Mexico. The antibiotics with the highest detection report in aquatic ecosystems correspond to diverse chemical groups that include tetracyclines (Dang *et al.*, 2007), and aminoglycosides (Shakil *et al.*, 2008).

Pollution by antibiotic residues can reach the consumer through the food chain. It has been identified that antibiotics can cause allergic reactions or alter the intestinal bacterial flora of humans. This contributes to potentiate the risk of generation and proliferation of strains of antibiotic resistant bacteria (ARB) and antibiotic-resistance genes (ARG) (Ventola, 2015; WHO, 2018). As well as, causing marketing problems by affecting product quality and market competitiveness (Guzmán-Carrillo *et al.*, 2012; Grossi-Botelho *et al.*, 2015). The data on the extent of the area used by farmers allow us to know the impact of this activity due to the use of chemical compounds. For example, drugs that include mainly antibiotics and pesticides, or insecticides and herbicides, among others.

In this research it was identified that 93.33% of farmers discard antibiotic residues when throwing bottles in the trash and that only 6.66% of bottles with antibiotic residues are burned.

In agreement with the above, Piña-Guzmán *et al.* (2019) noted that expired veterinary drugs are considered hazardous waste in Mexico, according to NOM-052-SEMARNAT-2005. However, those authors indicated the disorganization in the procedures that include the management and final disposal of antibiotics, since these are eliminated jointly with municipal solid waste or are discharged into the drainage. All of this can expose the general population and the environment to low doses of those antibiotics, which may involve an additional pathway for these compounds.

The main pathways of pharmaceuticals such as antibiotics to the environment are through excretion. A wide range of products are identified in surface, groundwater, and wastewater (Gil *et al.*, 2012). Also, Cheng *et al.* (2018) indicated that approximately 70% are discarded in their original formula through excreta and urine. Meanwhile, Kümmerer (2009) indicated that between 80 to 90% of antibiotics (ATB) are excreted as original compounds in the environment, which means that the compounds have not been metabolized in any animal body. The latter has special relevance since the proximity of water sources, such as the lower basin of the Jamapa River; water was identified at an average distance of 18046. 97±90291.0.6 m, with a minimum distance of 0.0 and a maximum distance of 500 000 m. In addition, the use of antibiotics was identified in the study area, 100% of the farmers indicated to perform anti-tick baths to their livestock herd. The intensive use of antibiotics has generated pollution of environmental matrices such as soil, water, sediments, plants, and their negative effects have been particularly highlighted in biota (Grossi-Botelho *et al.*, 2015). The above agrees with Zirena Vilca *et al.* (2018) who highlighted the need to monitor various environmental matrices to detect and quantify the presence of antibiotics in order to have a better understanding of their long-term effects on living organisms.

The potential ecological risk of antibiotics in the environment should be evaluated to develop management strategies for those substances, with the aim of contributing to the reduction of such compounds in aquatic systems (Grossi-Botelho *et al.*, 2015). Dreser *et al.* (2008) indicated that the sale of antibiotics in Mexico has a higher proportion compared to developed countries and in transition with large pharmaceutical markets. In the case of our country, as previously indicated, there is no official information on the use of veterinary antibiotics, which hinders the establishment of mechanisms to contribute to their rational use in this sector. Ogwuche *et al.* (2021) indicated that there are no guidelines in Nigeria restricting access to veterinary medicines. Grossi-Botelho *et al.* (2015) recommended that public health and regulatory institutions should commit to the prudent use of veterinary antibiotics (VA) due to the risk they pose to human health and the environment.

CONCLUSIONS

The proper management of antibiotics should be a central issue in the field of animal health in the livestock sector in Mexico. Cattle ranchers in the lower basin of Jamapa River use antibiotics in the treatment of diseases, such as diarrheal diseases, without a corroborated bacterial cause. The interviewees reported 11 different antibiotics in the municipalities evaluated in the lower basin of the Jamapa River; that is, the use of a mixture of compounds whose residues can reach the riverbed, given the proximity of the livestock production units to the water source.

A total of 11 antibiotic compounds were found in the three municipalities located in the lower basin of Jamapa River. It is possible that the residues of this mixture of antibiotics could reach the Jamapa riverbed given the proximity of the livestock production units.

The management of antibiotic residues and their final disposal should be addressed as part of good livestock production practices, because producers recurrently use Oxytetracycline for the treatment of diarrhea in livestock. This indicates frequent use of this compound with the risks associated with the presence of these compounds in the environment and public health.

The farmers interviewed have knowledge in the management of livestock production. However, a high percentage do not know the risks of antibiotics. Farmers and all those dedicated to the value chain in the production and fattening of cattle should receive information through government advice and research institutions, to provide them with knowledge about antibiotics and contribute to reduce inappropriate use, and to promote better management practices.

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AGRO PRODUCTIVIDAD

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Evaluation of three commercial feeds for the farming of Japanese quail (*Coturnix japonica* Temminck y Schlegel)

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ABSTRACT

Objective: To evaluate, for the farming of Japanese quail (*Coturnix japonica*) three brands of feed, with different nutritional content, enzyme complexes, particle size and presentation, in the variables of production, weight and length of organs, height of intestinal villi and cost per quail.

Design/Methodology/Approach: 300 young birds of both sexes were used; three commercial starter feeds of different brands (A, B and C) were evaluated. The response variables evaluated were feed consumed (AC), total feed consumed (ACT), live weight (PV), weight gain (GP), total weight gain (GPT), feed conversion (CA), total feed conversion (CAT), carcass yield, length, and weight of organs, as well as the size of the intestinal villi and the cost of the quail (CC). An analysis of variance was performed using the SAS® GLM procedure.

Results: From week three the birds were classified by sex, and the lowest AC and best CA ($p<0.05$) was obtained with treatment A in both sexes. Carcass yield was not affected by treatments ($p>0.05$); the longest and heaviest proventriculus and gizzard ($p<0.05$) were for treatment B. Regarding the villi, it was found that the enzymes favored ($p<0.05$) their size.

Limitations/Implications: The study showed that the size of the intestinal villi is influenced by the type of enzymes that are used, as well as by their concentrations or combinations.

Findings/Conclusion: It is concluded that feed presentation influences the consumption of feed. Enzyme complexes improve the size of the intestinal villi.

Keywords: enzymes, granulometry, intestinal villi, carcass yield.

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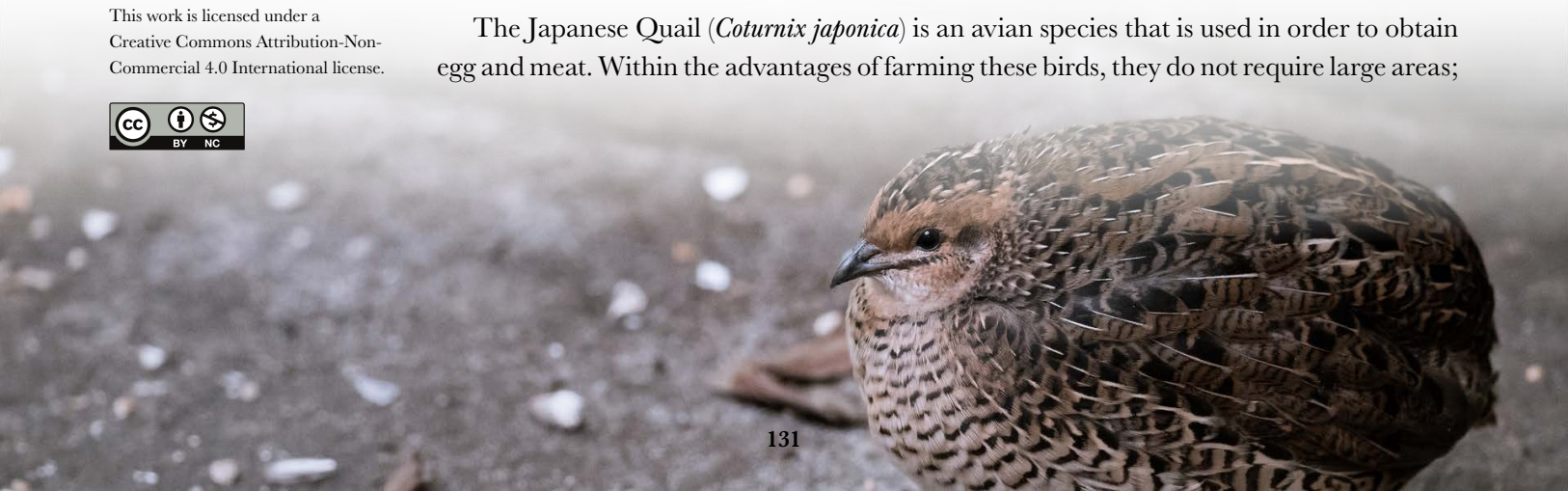
INTRODUCTION

The Japanese Quail (*Coturnix japonica*) is an avian species that is used in order to obtain egg and meat. Within the advantages of farming these birds, they do not require large areas;



are resistant to diseases; their growth is fast and have low feed consumption. Currently, in Mexico there are different brands of commercial feeds for quail farming, which are made of various ingredients, nutrient content, particle size, the use or not use of enzyme complexes and type of feed presentation. There are tables with recommendations to satisfy the nutritional requirements of protein (PC) and metabolizable energy (EM) in quails. Leeson and Summers (2005) suggested 28% CP for 2900 kcal kg^{-1} EM and Rostagno (2017) recommended 25.12 to 22.23% PW for 2900 kcal kg^{-1} EM. In the formulation of commercial feeds, in addition to considering the nutritional requirements, the thermal processing technique is important to obtain the pelletizing since the size and physical shape affects some organs, the digestibility of the diet, and consequently the yield of birds (Netto *et al.*, 2019).

Regarding the use of additives, exogenous enzymes such as xylanase, amylase and protease have been used in poultry farming for decades. In Mexico diets are formulated based on corn, sorghum and soy paste, ingredients with non-starchy polysaccharides (PNA), and enzymes that decrease the viscosity of the digest and increases the digestibility of nutrients, also improving the size of intestinal villi (Zhu *et al.*, 2014; Sugiharto, 2016; Alagawany *et al.*, 2018). Therefore, the objective of the study was to evaluate three brands of feed with different particle size, presentation, nutritional content, with or without enzymatic complexes, during quail farming, for production variables, carcass yield, weight and length of organs, height of intestinal villi and cost per quail.

MATERIALS AND METHODS

The experiment was conducted at a quail farm located in Texcoco, State of Mexico, Mexico. For the proper handling of birds, the Regulation for the use and care of animals for research of the Colegio de Postgraduados (2016) was complied; 300 young birds of both sexes were used, with an initial age of 1 d and a final of 42 d; The birds were randomly assigned to three treatments, with five replicates of 20 birds each. The birds were housed in spaces with electric breeders of 30×55 cm, linear feeders and automatic drinkers. When the birds were three weeks old the heat source was removed, in addition, they were grouped by sex to obtain the productive variables. Three commercial starter feeds of different brands (A, B and C) indicated in Table 1 were evaluated, the water and feed were offered *ad libitum* and determined the size of the feed particle.

Production variables

The response variables evaluated weekly were feed consumed (AC, g d^{-1} per bird), total consumed feed (ACT), live weight (PV, g), weight gain (GP, g), total weight gain (GPT, g), feed conversion (CA, kg of feed/GP), and total feed conversion (CAT).

Histological study

At 42 d of age, three males by replicate were randomly selected, weighed, and subsequently fasted for 12 h; then sacrificed by cutting the section of the jugular vein and carotid artery following the recommendations of the Official Mexican Standard NOM-033-ZOO-1995. The intestinal samples were collected after bird death was confirmed, a

Table 1. Nutritional content of quail feed in the initiation-growth stage in three different brands.

Label content	A	B	C
CP (%)	27.00	28.00	28.5
CF (%)	2.50	3.00	3.00
CFB (%)	4.00	3.00	5.00
Ashes (%)	7.50	8.00	10.00
Humidity (%)	12.00	12.00	12.00
NFE (%)	47.00	46.00	43.50
Dehydrated enzymes	-	Phytase 500 FTU/kg	Phytase 500 FTU/kg
		Xylanase 1200 U/kg	β Xylanase 1100 U/kg
		β glucanase 200U/kg	β glucanase 100U/kg
		Cellulase 200/kg	Cellulase 200/kg
		Manase 1800U/kg	Xylanase 75 U/kg
			Protease 1000U/kg
Presentation	mash	pellet	pellet
Cost ($ kg^{-1})	10.70	11.67	12.95
Particle size			
GMD	791	958	767
GSD	2.19	1.49	1.65

PC, crude protein; GC, crude fat; FC, crude fiber; ELN, nitrogen-free extract; PGD, geometric average of diameter; DEG, geometric standard deviation.

sample per replicate was taken of duodenum (descending) and jejunum (proximate). Each intestinal segment was collected closed with a size of 2.0 cm in length, and after cutting the samples the intestinal lumen was washed with distilled water and 10% buffered formalin to eliminate intestinal content. All samples were placed in individually identified bottles containing 10% buffered formalin for processing and cutting (Gava *et al*., 2015).

Later, the samples were then included in paraffin, cut into 4.0 μm thick sections (with an optical micrometer), rinsed in xylene, dehydrated at graduated alcohol concentrations, and stained with hematoxylin-eosin. Once the slides were obtained, 15 intestinal villi of each sample were measured. The measurements were made as follows: height of the villi (AVLL), from the crypt to the apex of the villi, micron scale was used in a light microscope with the 4X objective (Image J, Version 1.8.0).

Carcass yield, length, and weight of organs

Sequentially, with the males slaughtered from each replicate, variables were evaluated for carcass yield (RDC, %), viscera weight (PVISC), small intestine weight (PID), small intestine length (LID), large intestine weight (PIG), large intestine length (LIG), proventricular length (LPROV, mm), proventricular width (APROV, mm), proventricular weight (PPROV); gizzard length (LMOLL, mm), gizzard width (AMOLL, mm), gizzard plus digest (MOLLMD, g), gizzard without digest (MOLLSD, g), clean gizzard (MOLLLI, g), liver weight (PHIG, g) and length of the gastrointestinal tract (LTGI, cm). All measurements were done with a digital Vernier ruler (Steren®).

Feed cost per quail

The cost of quail (CC) was determined by multiplying the accumulated feed consumption by the cost of feed.

Statistical analysis

For the production variables, carcass yield, weight and size of organs, intestinal villi, feed cost per quail, a completely random experimental design (SAS® GLM procedure) was used. Mean differences per treatments were obtained with the Tukey test (SAS, 2011).

RESULTS AND DISCUSSION

When quails were housed in mixed sexes arrangement (Table 2), the birds that consumed the most feed ($p \leq 0.05$) were those of treatment B with a cumulative consumption of 297.03 g. The presentation of this feed was in pellet which increased the consumption capacity of the animals as reported by Massuquetto *et al.* (2019). However, the high consumption was not reflected in the PV; they are the birds that had the lowest weight, in addition this treatment had the largest particle size. The gizzard of young birds is not yet fully developed; therefore, it has a limited ability to grind coarse particles (Kheravii *et al.*, 2018). It is possible that the temperature of the pelletizing process affected the digestibility of the nutrients reflected in the low weight.

Table 2. Production variables initial (1 d) and per week (1 to 3 weeks) in mixed housing of Japanese quails.

Weeks	**1d**	**1**	**2**	**3**	
Treatment	**Weekly body weight (g)**				
A	9.56	37.64	79.49a	137.46a	
B	9.78	34.35	74.27b	128.51b	
C	9.43	33.7	73.71b	129.16b	
SE	0.06	1.17	0.68	1.00	
	Feed intake g/bird/d				**AFI (g)**
A		5.82b	12.14b	17.90b	252.32b
B		7.39a	13.70a	21.37a	297.43a
C		6.38b	12.48b	20.30a	272.78b
SE		0.19	0.24	0.49	5.79
	Weekly weight gain (g)				**TWG (g)**
A		24.85	37.64	58.00	127.93
B		24.51	34.35	54.24	118.73
C		24.26	33.70	55.45	119.73
SE		0.65	0.96	0.84	1.79
	Weekly feed conversion				**TFC**
A		1.19b	1.07c	0.92b	1.83c
B		1.50a	1.29a	1.16a	2.31a
C		1.33b	1.18b	1.09a	2.11b
SE		0.04	0.02	0.03	0.06

EEM, standard error of the mean. Means with different letters among columns are different ($p \leq 0.05$). ACA, accumulated feed consumed; GPT, total weight gain; CAT, total feed conversion.

The birds that were fed with the diets A and C had a lower intake ($p \leq 0.05$) (45.11 and 24.65 g respectively). In addition, those birds of treatment A that consumed less feed were the birds with higher PV and better CA ($p < 0.05$). GP was not affected ($p > 0.05$) although treatment A had 7% better gain (Table 2). This is related to the presentation of the A feed that was in flour, since when that is the case, the ingredients are ground and mixed homogeneously. Whereas, for the granulates, the feed undergoes a process that combines high temperature and humidity, which improves the physical quality of the granule, increases the consumption of feed but affects the digestibility of nutrients such as amino acids and energy (Loar *et al.*, 2014; dos Santos *et al.*, 2020).

Table 3 shows the results by sex from week 4 to week 6, it was observed that in males no differences were found among treatments. However, in females at the end of farming the PV was the highest ($p \leq 0.05$). The best PV was obtained with treatment B compared to C because at this age the gastrointestinal tract was already developed; the size of the feed 985 PGD favored the growth of the gizzard and consequently the digestibility of the

Table 3. Production variables from 4 to 6 weeks (Sem) in Japanese quails fed with different commercial feeds.

	Males				**Females**			
wk.	**3**	**4**	**5**	**6**	**3**	**4**	**5**	**6**
T	**Weekly body weight (g)**							
A	135.76a	179.03a	214.08	226.66	139.06	188.59	224.90ab	244.55ab
B	122.80b	169.49b	212.66	222.40	134.76	192.73	232.20a	249.05a
C	125.48b	166.42b	208.28	222.14	133.97	185.46	218.35b	236.72b
SE	1.3	1.24	0.10	0.10	1.29	1.35	1.42	1.51
	Feed intake g/bird/d							
wk.	**4**	**5**	**6**	**AFI (g)**	**4**	**5**	**6**	**AFI (g)**
A	23.73c	23.46b	19.27b	465.28c	24.36b	24.53b	25.12c	518.22c
B	28.81a	30.51a	25.78a	595.69a	30.66a	30.98a	31.65a	653.03a
C	26.42b	27.94a	23.66a	546.30b	26.84b	26.23b	27.49b	563.94b
SE	0.61	0.90	0.81	15.21	0.87	0.81	0.78	15.79
	Weekly weight gain (g)							
wk.	**4**	**5**	**6**	**TWG (g)**	**4**	**5**	**6**	**TWG (g)**
A	43.20ab	35.22b	12.54	90.96b	49.52b	36.31ab	19.54	105.39ab
B	46.53a	43.59a	10.13	100.26[a]	58.13a	39.54a	16.84	114.51a
C	41.57b	41.59a	11.45	94.61b	51.60b	31.93b	18.74	102.29b
SE	1.70	1.1	0.70	1.26	1.2	1.09	0.89	2.01
	Weekly feed conversion							
wk.	**4**	**5**	**6**	**TFC**	**4**	**5**	**6**	**TFC**
A	0.93c	0.66	0.59b	2.05c	0.90b	0.76b	0.72c	2.12c
B	1.19a	0.70	0.80a	2.66a	1.11a	0.93a	0.89a	2.62a
C	1.11b	0.67	0.74a	2.48b	1.00ab	0.84b	0.81b	2.38b
SE	0.03	0.08	0.02	0.05	0.03	0.02	0.01	0.07

EEM, standard error of the mean. Means with different letters among columns are different ($p \leq 0.05$). ACA, accumulated feed consumed; GPT, total weight gain; CAT, total feed conversion.

nutrients. Coarse particles are known to improve the bird yield, compared to fine ones (Lv *et al.*, 2015). In addition, the feed presentation in pellets increased the CA and the weight of the birds (Massuwuetto *et al.*, 2020). Treatment A, due to it was a flour had lower CA, but the nutrients were not compromised by high temperatures which in the end improved bird weight.

In ACA, both males and females consumption were higher ($p \leq 0.05$) with treatment B, followed by C; it is important to mention that the presentation of both feeds was in pellets, but they had different particle size. The one feed that had the lowest consumption was treatment A, which presentation was as flour; it is known that the physical form of the pellet increases feed consumption (Abdollahi and Ravindran, 2014).

In the GP variable in males and females it was recorded that the best gain was obtained by treatment B ($p \leq 0.05$) with 100.26 and 114.51 g respectively, which is related to high consumption and particle size of the feed. However, the best CA for both sexes was with treatment A ($p \leq 0.05$), followed by C and the worst was for B. The above is related to the presentation of the feed, since although treatment A had the lowest consumption because it was a flour, its nutrients were not affected by high temperatures. On the contrary, what happened with treatment B was that the consumption was so high that the CA was affected, which is related to the temperature of the pelletizing since it decreases the digestibility of nutrients (protein and energy) and it is known that birds eat to satisfy these requirements (Lesson and Summer, 2005; dos Santos *et al.*, 2020).

In this research, the weight and size of the intestines was not modified ($p > 0.05$) due to the effect of enzymes or particle size. While Hussein *et al.* (2020) found that the length of the duodenum, ileum, and cecum increased with enzyme supplementation, as well as the weight of the duodenum. This increase in size of the gastrointestinal tract occurs as an adaptive mechanism to an increased demand for exogenous enzymes (Brenes *et al.*, 1993).

Regarding the DRC, PVISC, PID, LID, PIG, LIG, LPROV, PHIG and LTGI, no differences were obtained among the treatments (Table 4). In the variables APROV and PPROV it was found that the widest and heaviest stomach was that fed on treatment B ($p < 0.05$) (Table 4). As the gizzard was significantly affected ($p \leq 0.05$) the LMOLL, AMOLL, MOLLMD, MOLLSD and MOLLLI, where the length, width and weight of the gizzard was greater in treatments B and C. Since these contained the largest particle size, which increased the crushing activity of the gizzard to grind the particles and consequently increased its development (Chewhin *et al.*, 2012; Jacobs and Parsons, 2013). Whereas treatment A had the smallest particle size, and this tends to reduce the mechanical stimulation in the gizzard because flour is retained for less time than pelletized coarse particles; less time causes a reduced size of the organs (Mateos *et al.*, 2012; Manyelo *et al.*, 2019).

In week 7 the highest intestinal villi (VI) in duodenum and jejunum were obtained with treatment B ($p \leq 0.05$). This is attributed to the fact that this treatment had a complex of enzymes which favored growth with greater height. This is related to a larger absorption area (Bogustawska-Tryk *et al.*, 2012). All of which coincides with Pérez *et al.* (2013) and Zhu *et al.* (2014) who found that the addition of enzyme complexes significantly increases the height of VIs during birds fattening. Treatment C also had a complex of enzymes,

Table 4. Carcass yield, length, and weight of internal organs at 42 d in male Japanese quails fed with different commercial feeds.

TRAT	A	B	C	SE
CPF (%)	80.60	79.44	78.80	0.38
VW (g)	14.50	15.55	13.80	0.51
SIW (g)	3.24	3.38	3.60	0.10
SIL (cm)	46.56	46.59	47.93	0.45
LIW (g)	2.17	2.51	2.45	0.10
LIG (cm)	13.16	14.36	14.50	0.43
PL (mm)	17.67	18.38	18.03	0.22
BP (mm)	9.18b	9.9a	9.24b	0.10
PW (g)	0.82b	1.02a	0.88b	0.02
GL (mm)	3.42b	4.17a	3.92a	0.29
WG (mm)	24.49b	26.41a	25.58ab	0.29
GPD (g)	4.50b	5.83a	5.0ab	0.17
GWD (g)	3.9b	4.7a	4.48a	0.10
CG (g)	3.42b	4.17a	3.92a	0.09
LW (g)	4.18	4.78	4.12	0.19
GITL (cm)	77.57	78.05	80.60	1.04

RDC, carcass yield; PVISC, viscera weight; PID, small intestine weight; LID, small intestine length; PIG, large intestine weight, LIG, large intestine length, LPROV, proventricular length; APROV, proventricular width; PPROV, proventricular weight; LMOLL, gizzard length; AMOLL, gizzard width; MOLLMD, gizzard plus digest; MOLLSD, gizzard without digest; MOLLLI, clean gizzard; PHIG, liver weight; LTGI, length of the gastrointestinal tract. EEM, standard error of the mean. Means with different letters in row are different ($p \leq 0.05$).

Table 5. Height of intestinal villi in six-week-old male Japanese quails.

Treatment	Duoenum (μm)	Jejunum (μm)
A	66.39b	32.49c
B	83.79a	76.70a
C	67.59b	61.88b
SE	2.64	1.66

EEM, standard error of the mean. Means with different letters in column are different ($p \leq 0.05$).

Table 6. Feed cost per quail for Japanese quail farming.

Treatment	Females USD$	Males USD$
A	0.4124b	0.384b
B	0.554a	0.521a
C	0.541a	0.530a
EEM	0.53	0.63

Feed cost for females and males (1 d to 6 weeks). EEM, standard error of the mean. Means with different letters in column are different ($p \leq 0.05$).

but it was different from that of B (Table 1), which could influence the villi size. Whereas treatment A was the one with the smallest VIs since this feed did not contain enzymes.

Treatment A had the lowest cost to produce a quail ($p \leq 0.05$) female and male (USD$0.41 and USD$0.38 respectively). This is related to the fact that the feed of this treatment was the least expensive, also to the fact that the accumulated consumption over the six weeks was the lowest ($p \leq 0.05$) (Table 3). Whereas, with treatments B and C the cost experienced an increase for females of USD$ 0.142 and USD$ 0.129 respectively, and in males increased USD$0.137 and USD$ 0.146 respectively. This cost increase occurred because these feeds are more expensive and greater consumption was recorded in both sexes ($p \leq 0.05$).

CONCLUSIONS

Pelletizing increased the feed consumption of quails, but nutrients are affected by the process which can reach minor feed conversions. The flour presentation is the best alternative for feeding during fattening for better feed conversion.

The particle size of the feed improves the size of the gizzard in quails. The enzymes used in these commercial feeds increase the size of the intestinal villi. Finally, the feed cost per quail depends on the feed conversion and the cost of balanced feeds.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Flowering of *Euphorbia pulcherrima* Willd. ex Klotzsch var Valenciana under blue and red LED light

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ABSTRACT

Objective: To determine the effect of blue light (BL) and red light (RL), applied five hours at the end of the day (AED) and that of temperature, on the flowering of *Euphorbia pulcherrima* var Valenciana.

Design/Methodology/Approach: Three groups of plants were established under greenhouse conditions, all received sunlight (SL), AED one was under BL (460 nm) and another under RL (660 nm) with a photosynthetically active photon flux of 440 μmol m^{-2} s^{-1} and 550 μmol m^{-2} s^{-1} respectively; from the beginning of the experiment until 144 d later. In a second flowering cycle, residual effects of the treatments were evaluated.

Results: In the first flowering cycle, the appearance of cyathia and bract pigmentation under BL occurred on average at 177.5 d after initiation of the treatments, and under RL at 178 d. Compared to the application of SL alone (138 d) the process was delayed, on average 39 and 40 d, under BL and RL, respectively. No residual effects of the treatments on flowering were recorded.

Limitations on study/implications: It is necessary to evaluate other levels of temperature below and above the ones reported in this study, and also to increase and decrease the photoperiod.

Findings/conclusions: The prolonged delay in flowering can be attributed not only to the quality and intensity of light, but also to the photoperiod and the daytime temperature above that documented for flower initiation in var Valenciana.

Keywords: Photoperiod, light quality, late flowering, daytime temperature.

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INTRODUCTION

Light modulates metabolic, morphological and development responses in plants, among which there is flowering [1]; the effect on flowering is manifested fundamentally in species considered photoperiodic or dependent on the daily light duration to flower [2]. There are two groups of plants in function of their response to the photoperiod, which flower during long days and short nights (long day plants-LDP), and those that flower during short days and long nights (short day plants-SDP) [3].

Image by Ulises Moreno at Pixabay

The plants detect the characteristics of light through different families of photoreceptors, through which they adjust their growth and development in various environmental conditions [4].

Light management is very important in horticulture, to manipulate the growth and development of plants. Managing light has been made easy through the use of light emitting diodes (LED) technology. Through their management, it is possible to control desired responses in plants [5], such as: increasing the yield in crops, extending the production season, improving the quality of the product, and controlling the flowering of species sensitive to the day duration through management of the photoperiod [6]. *E. pulcherrima* or poinsettia is considered a national plant genetic resource, important as germplasm for research, genetic improvement and commercial exploitation [7]. On the other hand, its commercial use is mainly ornamental, but there are records of its use in fields such as Chinese traditional medicine [8]. Likewise, there are studies of the potential use of some of their metabolites or the total extract of leaves and bracts, as agents of disease control in plants [9].

The objective of this study was to generate information that can contribute to already existent studies on poinsettia, and for this, the effect of the extension of the photoperiod in five h at the end of the day (AED) was evaluated under two qualities of LED light, blue (BL-460 nm) and red (RL-660 nm), on flowering and pigmentation of bracts of *E. pulcherrima* var. Valenciana (in this variety they happen simultaneously), since both light components can be manipulated and are of great impact in flowering of this plant, as a result of their condition of having long night photoperiods.

MATERIALS AND METHODS

The experiment was established in a greenhouse with glass cover that is located in the Universidad Autónoma Chapingo (Coordinates: 19° 29' 23" LN and 98° 53' 37" LW; 2250 masl). During the research period the average, minimum and maximum temperatures were: 17.85, 10.1 and 30.24 °C in the first year and 18.2, 10.5 and 30.6 °C in the second, respectively. The average relative humidity was 62.6% in the first year and 60.8% in the second. The data were recorded through two HOBO®, model MX2300 tem/RH, ONSET 1-800-LOGGERS.

The experiment was conducted with already rooted cuttings of *E. pulcherrima* var. Valenciana, from Tetela del Monte, Morelos. Previous to light treatments, the shoots were trimmed to leave plants with four internodes. The plants were placed in black polyethylene bags with capacity of four liters. A substrate formed by a mixture of soil with pine-oak litter and worm castings in 3:1 proportion was used. In the transplant a root development promotor (ROOTEX®) was applied, and then two applications in eight-day intervals.

Light sources and treatments

Light was supplied with monochromatic LED lamps for use in horticulture (LED Grow Light, E27). Blue light (BL; All Blue 460 nm; 36W) and deep red (RL; All Deep Red 660 nm; 36W) were used.

Blue light (BL) and Red light (RL) were applied from 18:00 h and until 23:00 h. The photosynthetically active photon flux that was supplied with BL was 440 μmol m^{-2} s^{-1} and with RL it was 550 μmol m^{-2} s^{-1}. The control treatment plants received only sunlight (SL= 109 μmol m^{-2} d^{-1} (daily integral light)).

The Photosynthetic Photon Flux Density (PPFD) was measured in the center of the light source and at a distance of 20 cm from the plant canopy, through a Quantum Meter (Model QMSW-SS, Apogee Instruments, Logan, UT). The treatments began on July 28 and ended in December 19, 2019 (144 days). The lamps were placed 20 cm above the plant canopy. To conserve this distance, the lamps were elevated as the plants grew.

Twenty-four (24) experimental units were established, eight for each treatment. Each unit was made up by three plants, with a separation between units of 50 cm. The experimental units that received the BL and RL treatments were isolated with polyethylene curtains of double color: white inside and black outside. The curtains stayed open during the day for all the plants to receive SL that falls on the greenhouse.

Evaluation of flowering times and bract pigmentation

The analysis and the comparison of the flowering times of poinsettia plants that developed under different lighting conditions were conducted through the so-called Survival Analysis (SA) [10]. The SA allows evaluating the occurrence and time when an event takes place [11], as is the case of flowering [12]. The event that was recorded was the appearance of cyathia and bract pigmentation, since it is an indication of the start of flowering in poinsettia plants.

Applying the SA allowed to: a) define the type of distribution and the functions of probability associated to the starting times of flowering and bract pigmentation; b) statistically compare the distribution of flowering times and bract pigmentation in plants exposed to the three lighting conditions; and c) estimate a regression model with the aim of evaluating the effect of the three lighting conditions in starting times of flowering and bract pigmentation. In addition, with the SA the statistical model that best represented the distribution of the times of occurrence was found and inferences to obtain the regression model were made based on it [13].

Distribution of starting times of flowering and bract pigmentation

By virtue of the presence of cyathia, the distribution and the probability function associated to flowering was recorded, obtained through the SAS LIFETEST procedure [14]. The comparison of the distributions of flowering times in plants exposed to the three lighting conditions was carried out through the Log-Rank test [11].

The effect of lighting conditions on the times of flowering and bract pigmentation was evaluated with a maximum likelihood parametric regression through the SAS LIFEREG procedure [14]. To compare the effect of the three lighting conditions, the condition of only SL was selected as reference variable. The coefficients of regression and their standard errors ($\beta_i \pm$SE) were improved based on a goodness of fit test, which allowed establishing the type of distribution that adjusted more to the starting times of flowering. The distribution

observed was compared with the theoretical Weibull, Exponential, Gamma, Log-logistic and Log-normal distributions [11].

According to Allison [11], the coefficients of regression (β_i) obtained were transformed based on the relationship $e^{\beta i}$. The result estimated the change in average time of flowering and bract pigmentation, under BL and RL conditions, in relation to the condition of only SL.

RESULTS AND DISCUSSION

The light environment of the greenhouse modified the times of flowering and bract pigmentation of poinsettia plants. The variety used, called sun variety, begins flowering at the beginning of October, under the experiment's conditions, and this process happened until the second week of December of the year 2019.

The plants that were subjected only to SL presented a distribution of times of flowering and bract pigmentation that was statistically different than the one presented by the plants subjected to BL ($\chi^2=47$; d.f.$=1$; $p<0.0001$) and RL ($\chi^2=47$; d.f.$=1$; $p<0.0001$). However, between BL and RL no significant differences were found in flowering times ($\chi^2=0.6$; d.f.$=1$; $p=0.438$). On average (± standard error), the flowering time of plants subjected only to SL (20±0.01) was six weeks shorter than the ones exposed to BL (25.87±0.193) and RL (26±0.209) (Figure 1A).

The estimated regression model (Table 1) indicates that the times of flowering and bract pigmentation presented statistically significant increments when the RL and complementary BL were applied. In both cases, the average increase of flowering time was estimated to be close to 30%.

In the year 2020 the same plants, which were no longer subjected to the addition of LED light, presented a distribution of flowering times significantly different to that presented in 2019 ($\chi^2=150.9$; d.f.$=1$; $p<0.0001$). In contrast to what happened in 2019, when the average flowering time (± s. e.) was 23.9 (±0.345) weeks, in 2020 it was only 13.07 (±0.163) weeks (Figure 1B).

The delay in flowering and bract pigmentation of the Valenciana var. under lighting treatments can be related not only with the photoperiod, but also with the quality and intensity of the light, as well as with the temperature. Poinsettia is a plant considered to be of long nights. Ecke III *et al.* [15] indicate that the flowering initiation requires a critical duration of the night longer than 11 h and 40 minutes and in particular Galindo-García *et al.* [7] report that this variety requires more than 11 h of darkness, for flowering initiation. Increasing the lighting period by five h (8 of darkness) was, possibly, one of the factors that contributed to the delay in flowering. The cyathia were visible and bract pigmentation started in the month of January, events that ought to happen the first week of the month of October, since most of the ecotypes of sun variety poinsettia, such as the Valenciana var., are called premature [7].

It should be pointed out that the number of long nights to which the plant is exposed is decisive. Kannangara and Hansson [16] subjected *E. pulcherrima* to conditions of short days for three weeks and then to continuous light, which prevented the production of flower primordia or bract pigmentation, while another group of plants, under four

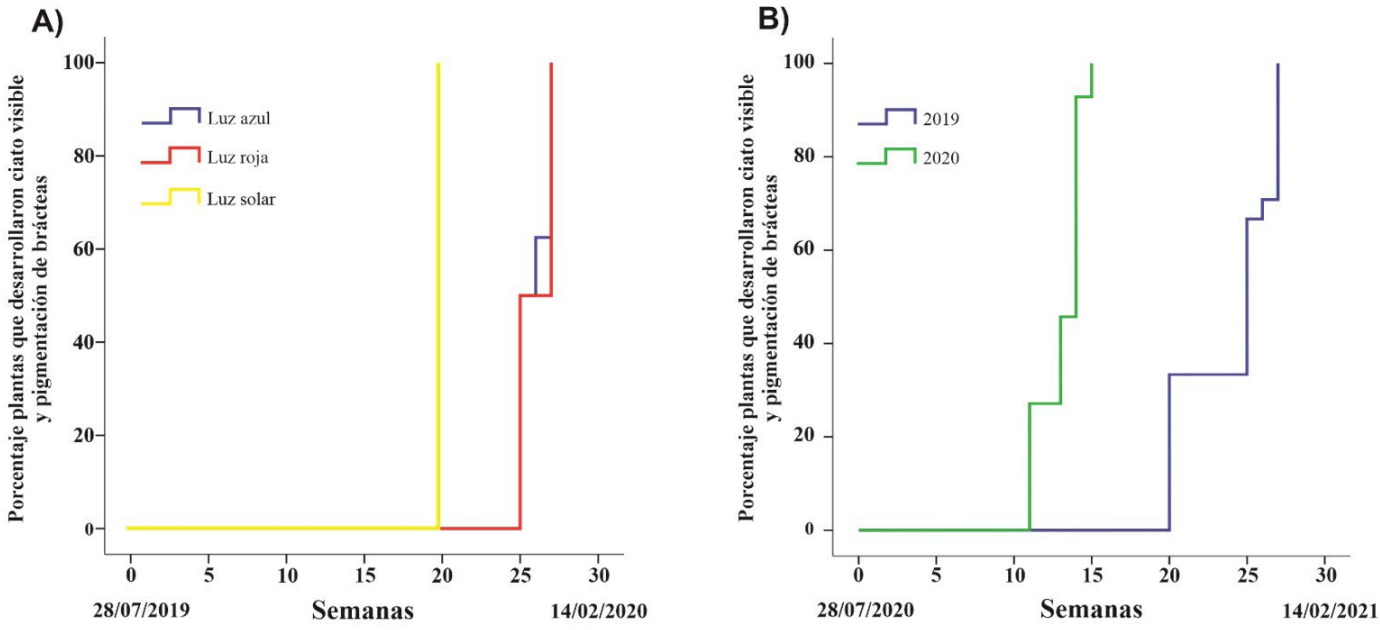

Figure 1. Distribution of the times of flowering and bract pigmentation of *E. pulcherrima* var. Valenciana. A) Distribution of the times of flowering and bract pigmentation under two lighting conditions: BL, blue light and RL, red light; without extension of the SL photoperiod, sunlight. B) Distribution of flowering times and two evaluation cycles. In 2019 plants that were subject to the extension of the photoperiod with BL and SL were considered, and in 2020 the same plants without extension of the photoperiod.

Table 1. Maximum likelihood regression model for the flowering times of *E. pulcherrima* var. Valenciana, under only SL, BL and RL. The condition of SL was considered the reference variable. The parameter ebi indicates the proportion of change in the flowering time in relation with the reference variable. It was considered that the flowering times have a Log-logistic distribution.

Parámetro	G. l.	$\beta_i \pm$e. e.	$e^{\beta i}$	χ^2	Pr>χ^2
Intercepto	1	2.996 ± 0.004	.	553691	<0.001
LA	1	0.2543 ± 0.0059	1.29	2382.44	<0.001
LR	1	0.2616 ± 0.0058	1.30	2503.54	<0.001
LS	0	0			
Escala	1	0.019 ± 0.002			

weeks of short days, did flower. This indicates that a specific amount of time is required in short days for flowering and could explain why in the Valenciana var., flowering under BL and RL happened three weeks after interrupting the treatments and increasing the hours of darkness.

Temperature is another factor that could be related to the extension of the vegetative period in the Valenciana var., by virtue of it being considered decisive for flowering [15]. For Schnelle *et al.* [17] the initiation stage of flowering seems to be very sensitive to high temperatures which can cause its delay ("delay from heat"). In this study the average daytime temperatures, in the last week of September, when flower induction should have happened [18], were up to four degrees higher than the ones recommended for this stage, 20-22 °C according to Ecke III *et al.* [15]. In this sense, Runkle and Heins [19] indicate

that above the optimal temperatures, flowering initiation can present "delay from heat"; on the other hand, Berghage and Heins [20] point to the delay from heat making synergy with the reduction in the number of hours of darkness.

In the second cycle of flowering and bract pigmentation, without extending the photoperiod, a delay in flowering was found, without significant differences between treatments. In the plants that had been under only SL the events happened in the third week of October (it could said to be one week of delay) and in the plants that had been under BL and RL (extended photoperiod) they happened in the first and second week of November (delay of three weeks). The delay in this second cycle could be attributed to temperatures above those recommended for flowering induction.

Finally, the third factor that possibly contributed to the delay of flowering is the quality of light (light spectrum). Craig and Runkle [3] describe that flowering of long-night plants can be inhibited with night lighting, without defining a spectrum capable of controlling, since the times of exposure and its quality have different effects. Runkle [21] points out that at a high intensity, 20 μmol m^{-2} s^{-1} or more, BL can inhibit flowering of short-day plant, although changes were also detected in function of the time of exposure, the moment of the day when it is applied, or both [22].

Flowering of *E. pulcherrima* can be affected by the RL AED, although the same as with BL, its effect is in function of its proportion, intensity and time of exposure [23]. Islam *et al.* [24], for example, report that the appearance of cyathia and bract pigmentation was not affected when subjecting two poinsettia varieties to 30 min of low radiation RL (5 mol m^{-2} s^{-1}) AED; however, Zhang and Runkle [23] report that four h AED of a high proportion of red: far red light (0.73:0.04), plus two h of far red (six h in total), delayed flowering of two cultivars of poinsettia.

Therefore, the delay in time of flowering and bract pigmentation of the Valenciana var., as pointed out by Kami *et al.* [25], could be the joint result of high daytime temperatures, the photoperiod, the quality and the light intensity.

CONCLUSIONS

The extension of the photoperiod by five h, the daytime temperature above 24 °C in the flowering initiation period, and the red or blue lighting do not favor flowering of *E. pulcherima* Valenciana var., for its sale as ornamental plant in the December season.

Daytime temperatures above 24 °C during flowering initiation, in addition to the expansion of the photoperiod, as well as light quality and intensity, were able to exercise synergy and delay for a long time (39 and 40 d, under blue and red light, respectively) the flowering and bract pigmentation of *E. pulcherrima* var. Valenciana.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Use of pulp and husk of coffee in animal feed

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ABSTRACT

Objective: To review the literature related to the use of husk and pulp of coffee in the feeding of ruminant and non-ruminant animals.

Approximation: The husk and pulp of coffee are by-products that remain after cleaning the bean. These are a potential source of pollution; however, they can also be used for animal feed, reducing costs for the producer.

Study limitations/implications: Coffee by-products have been used in animal feed globally, especially in those countries that are the highest producers of this bean. Therefore, it is important to know the results obtained there when those by-products are supplied as fed to animals in different conditions.

Conclusions: The results obtained vary when these coffee by-products are included in animal diets, depending on the species and the amount offered.

Keywords: coffee husk, coffee pulp, ruminants, non-ruminants, feeding.

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INTRODUCTION

In the last decade in the world, up to a third of the total production of cereals has been used for livestock feed (Makkar, 2016). Due to this, there is research focused on replace them with agricultural and industrial by-products, such as those of coffee.

Coffee is for long the most traded food product and one of the most widely consumed, as varied beverages in the world (Farah, 2009); it is the most important crop plant for trading and the second most valuable international product after petroleum (Esquivel and Jiménez, 2012; Skibba, 2016; Alves *et al.*, 2017).

Brazil is the largest global producer, contributing a third of world production; followed by Vietnam, Colombia, Indonesia, Ethiopia, Honduras, India, Mexico, Peru and Guatemala, among others. In 2020, world production of green coffee exceeded 9.9 million Mg (Megagrams, *i.e.* tons) (ICO, 2020). In Mexico, coffee cultivation is an activity with economic, social and ecological relevance (Hernández and Nava, 2018).

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There are two methods of processing cherry coffee to obtain the beans (Figure 1); the wet benefit and the dry or natural benefit. The wet benefit consists of pulping the harvested cherry coffee, followed by a fermentation and washing process to remove mucilage (exocarp and mesocarp), leaving together the coffee parchment and grain. Whereas, in the dry method once the fruits are dehydrated, the grain is separated from the husk (also called hull when dried), which is then composed of the pulp, mucilage and parchment (exocarp, mesocarp and endocarp remain together) (Berecha *et al.*, 2011).

Both methods generate great amounts of both solid and liquid waste (Kebede *et al.*, 2010). For every two Megagrams of fresh cherry coffee that are processed, one Megagram of coffee pulp and 0.5 Mg of husk is produced. Such by-products are mainly rich in carbohydrates, proteins, pectin and bioactive compounds, such as polyphenols.

The coffee husk has a high content of carbohydrates, as well as other organic compounds such as protein and lipids. In addition to bioactive compounds and phytochemicals, which allows its use in animal feed, compost production, used directly as fuel, biogas production and edible mushrooms production (Franca and Oliveira, 2009).

Of the total polysaccharides present in coffee husk, the most abundant are cellulose up to 35%, followed by hemicellulose (35%). It also has soluble carbohydrates such as fructose, glucose, galactose and arabinose; raffinose and sucrose (Hejna, 2021).

The protein content ranges from 6.6-11%, which are mainly made up of glutamic acid (7.7%) and aspartic acid (7.1%); as well as leucine (4.7%), glycine (4.2%), proline and valine (3.7%), alanine (3.5%), lysine (3.4%), serine (3.3%), threonine (3.1%) and phenylalanine (3%) (Hoseini *et al.*, 2021).

This by-product of coffee is also rich in minerals, especially potassium, calcium and magnesium. In addition, it contains polyphenols such as chlorogenic acid, which is considered a soluble polyphenol derived from the esterification of caffeic acid with quinic acid (Hoseini *et al.*, 2021), with antioxidant properties (Moraczewski *et al.*, 2018), which when ingested by animals can reduce the damage of reactive oxygen species [di oxygen

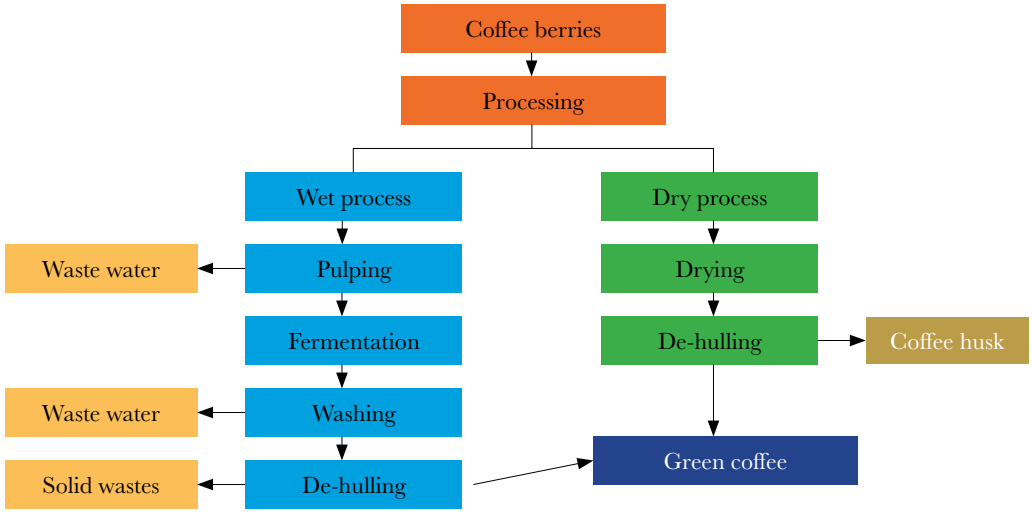

Figure 1. Coffee processing.

(O_2), superoxide anion $\left(O_2^-\right)$, hydroxyl (OH), peroxide (ROO), alkoxy (RO) and nitric oxide (NO)] (Chaves-Ulate and Esquivel-Rodríguez, 2019).

The pulp is the fraction of the mesocarp that is removed during the wet processing of ripe cherry coffee (Albes *et al.*, 2017) and is the most abundant by-product constituting approximately 40% of the fresh weight of the cherry coffee (Hejna, 2021). Pulp is mainly composed of carbohydrates such as glucose, fructose and pectin, has characteristics similar to husk (Pereira *et al.*, 2020). It has been used as a substrate for microorganisms in the synthesis of amino acids, for the production of bioethanol, as an ingredient in the diet of animals and as a source of antioxidants. It contains bioactive compounds, such as flavonoids and phenolic compounds that are of great interest not only for their high antioxidant power, but also for their anti-inflammatory, antimicrobial, antiallergic and anticancer activities (Murthy and Madhava, 2012).

Coffee pulp is characterized by a high level of moisture compared to husk, as well as a higher percentage of protein, tannins and caffeine. This coffee byproduct is a natural source of antioxidants. Of these compounds, caffeine and tannins are found in greater proportion. The antioxidant effect of caffeine is the result of the increase in the production of glutathione, in addition, it activates the enzymes glutathione reductase and superoxide dismutase (Dorsey and Jones, 2017). It is rich in potassium, followed by phosphorus, magnesium, calcium and sulfur (Martínez *et al.*, 2019).

Use of the husk and pulp of coffee in ruminant feeding

Use of coffee husk in bovines

Souza *et al.* (2006a) tested different levels of inclusion of coffee husk (0.0, 8.75, 17.5 and 26.25%) as substitute for maize in Holstein × Zebu heifer diets; they found that digestibility, consumption and weight gain were not affected when using up to 17.5% husk in the diet. However, in another study, Souza *et al.* (2010) noted that increased levels of husk in the diet would alter microbial protein synthesis by promoting N excretion.

The substitution of corn for coffee husk in the diet of Holstein × Zebu breeding heifers in lactation in a percentage of up to 10.5% did not affect either the production, quality of milk (Souza *et al.*, 2005), nor the balance of N (Souza *et al.*, 2006b). On the other hand, Oliveira *et al.* (2007a) observed that a level of up to 25% of coffee husk in diets with 60% fodder and 40% concentrate for lactating Holstein breeding heifers, did not affect milk production or microbial protein synthesis in those heifers that reached a production of 20 kg of milk per day (Oliveira *et al.*, 2007(b). Cyprian *et al.* (2006a), mentioned that it was possible to use up to 12% coffee husk to replace maize silage in lactating Holstein heifers whose diet consisted of 60% fodder and 40% concentrate. In another study Cipriano *et al.* (2006) noted that it was possible to replace up to 15% maize corn with coffee husk without affecting milk production in lactating Holstein heifers with a diet consisting of 60% maize silage and 40% concentrate.

Use of coffee pulp in livestock

The consumption and preference of Swiss-zebu bullocks for silage with different levels of coffee pulp (25, 50, 75 and 100%) in substitution for *Pennisetum purpureum* were evaluated

by Pinto *et al.* (2017), who reported that by increasing coffee pulp levels in silage, the percentage of crude protein increased, and the percentage of fiber decreased.

Pedraza *et al.* (2012) evaluated the effect of supplementing concentrates with three levels of coffee pulp (10, 15 and 20%) in diets for Holstein × Swiss-zebu dairy cows in grazing, on milk production and forage intake. Those authors did not observe a negative effect on those variables; thus, coffee pulp can be used as an alternative ingredient with the ability to reduce feed costs.

Use of coffee husk in sheep

Chemical treatment of coffee husk to improve digestibility was tested by García *et al.* (2000), who used 15.2% of coffee husk (hulls), untreated or treated with 4% urea, to replace maize in diets for lambs, without observing differences in DM intake, daily weight gain, feed conversion, weight of the carcass, nor in the cuts or composition of leg, shoulder or loin (García *et al.*, 2003b). However, they did find differences in the size of the reticulum-rumen of animals fed diets with coffee husks (García *et al.*, 2003a), associated with the husk fiber. However, by using calcium oxide (5%) to treat coffee husk, it is possible to include up to 16% of this as a substitute for maize silage in lamb diets, composed of 50% silage and 50% concentrate, without affecting DM intake, nutrient digestibility, weight gain, carcass yield or meat quality (Nunes *et al.*, 2020).

On the other hand, Pires *et al.* (2009) observed that by adding 15% coffee hulls to elephant grass silage in diets for lambs composed of 60% forage and 40% concentrate, lambs feeding behavior was modified increasing DM intake; while substituting up to 22.5% of coffee hulls for oat hay in diets for lambs composed of 30% forage and 70% concentrate, did not affect the digestibility of nutrients and became an alternative to reduce production costs (Rego *et al.*, 2019).

Souza *et al.* (2004) mentioned that the use of up to 25% of coffee husks in substitution of maize in the diet of adult sheep did not affect DM intake or nutrient digestibility and resulted in an alternative feed.

Use of coffee pulp in sheep

Nurfeta (2010) evaluated the effect of feeding castrated rams with diets containing two levels of coffee pulp 16.6% or 12.7% plus poultry manure (12.9%), without finding a difference in DM intake or weight gain; tough the digestibility of DM decreased in the diet with coffee pulp and poultry manure, while the N balance was also affected by that diet.

Salinas *et al.* (2014) evaluated the effect of including 0, 8 and 16% coffee pulp (ensiled for two months) in the diet of housed Blackbelly lambs, on carcass characteristics and meat antioxidant capacity; observed that the carcass yield improved compared to the control. In addition, rumen and intestine fat decreased in proportion to the increase in coffee pulp in the diet; however, the antioxidant capacity of meat was not affected. The inclusion of 0, 8 and 16% of ensiled coffee pulp did not affect feed intake, daily weight gain or feed conversion in the study carried out by Salinas *et al.* (2015); although the water consumption increased when the percentage of coffee pulp in the diet increased, the digestibility of FAD

was reduced in the treatments with 8 and 16 % of pulp; while the rumen concentration of acetic, propionic and butyric acids, as well as ammoniacal N, increased with the inclusion of 16% of coffee pulp.

Hernandez *et al.* (2018) evaluated the productivity and health of Pelibuey lambs housed for fattening, fed with different levels of inclusion of coffee pulp in the diet, 0, 7, 14, 21 and 28%; without finding differences in DM intake, weight gain or feed conversion, nor were they observed in cholesterol, glucose, protein, urea, or creatinine levels in the blood. In the hematological analysis, no changes were observed in the hematocrit or in the number of leukocytes.

The inclusion of 25% coffee pulp in the diets of multiparous Dorset × Suffolk ewes 14 d before the application of the progestogen until 25 d after the service, had no effect on the duration of estrus; although it may affect the percentage of gestation at 30 d, also increasing the antioxidant capacity of blood plasma at the time of applying the progestogen (Salinas *et al.*, 2016).

In another study, Gutiérrez *et al.* (2019) reported that when using 0.5 and 10% of coffee pulp in the diet of Dorset × Suffolk primiparous ewes 16 d before mating, it did not affect the duration of estrus, nor the gestation percentage, nor the weight of the offspring. In addition, the antioxidant capacity of sheep blood plasma was increased by including coffee pulp in the diet, while plasma lipid oxidation decreased by including 10% coffee pulp.

Use of coffee husks in goats

The use of 15% coffee hulls as an additive in elephant grass silage in diets for Saanen dairy goats, composed of 60% silage and 40% concentrate, did not affect DM intake, digestibility or milk production (Oliveira *et al.*, 2010).

Use of husk and pulp of coffee in the feeding of non-ruminants

Fishes

Aquaculture plays a very important role in providing high-quality protein to the population. In addition, it is characterized as a sector with rapid growth in food production (Yue and Shen, 2021). Nutrition is an important factor, since meeting the nutritional requirements of the fish ensures production and reduces the incidence of diseases (Yu *et al.*, 2021), but when formulating a diet, the availability and cost of feed must be considered as well as ingredients or alternative ingredients (Celada and Fuertes, 2016).

Coffee pulp has been used up to 30% in the diet of tilapia (*Tilapia aurea*) for 5 months, without presenting differences in weight gain compared to diets without coffee pulp (Brahan, 1979). Christensen (1981) used 10 and 30% coffee pulp in the diet of common carp (*Cyprinus carpio* L.) and catfish (*Clarias mossambicus* Peters). In both species, the daily weight gain was reduced from 1.65 g to 0.34 g when using 30%, extending the growth time, although production costs decreased.

In another study carried out by Fagbenro and Arowosoge (1991) to evaluate the feeding of catfish (*Clarias isheriensis*) in ponds for 150 d, with the use of coffee pulp at 10, 20 and 30% inclusion in the diet in substitution for yellow maize corn, they found that by increasing the inclusion level of coffee pulp, daily weight gain was reduced, although when using up to

20% of coffee pulp, no differences were found in the final weight compared to the control diet, thus it is possible to reduce the production costs.

When using different levels of inclusion of dehydrated coffee pulp (10, 20 and 30%) in diets of red tilapia fingerlings (*Oreochromis aureus* × *Oreochromis niloticus*), no differences in weight gain were reported between the control diet and the diet with 10% coffee pulp; however, the economic feasibility was better in the diets with 20 and 30% coffee pulp compared to the control diet (Castillo *et al.*, 2002).

Moreau *et al.* (2003) evaluated the feeding of fresh and ensiled coffee pulp protein by Nile tilapia fingerlings (*Oreochromis niloticus*), by testing a control diet with 100% protein and diets with 80% protein, plus a contribution of 20% protein from fresh and ensiled coffee pulp. When feeding the fingerlings for 28 d, no toxicity was observed, although growth was negatively affected when using fresh coffee pulp as well as silage.

Ulloa and Verreth (2003) reported that by including 13, 26 and 39% dehydrated coffee pulp in diets for tilapia (*Oreochromis aureus*) housed in ponds, no difference was found in the final weight when using 13% coffee pulp in comparison to control treatment.

Bautista *et al.* (2005) fed fingerlings of the hybrid cachamay (*Colossoma* × *Piaractus*) for 84 d with diets composed of ensiled coffee pulp with 5% molasses and without molasses, with three levels of pulp inclusion: 10, 15 and 18% and a control treatment without pulp. The best weight gain was found in the individuals that were fed with a level of 18% coffee pulp silage without molasses, although the highest survival rate of the fish was associated with the treatments with added molasses.

Poultry

The production of chicken meat in 2019 was more than 118 million Mg in the world. Among the countries with the highest production, the United States stands out in first place with more than 20 million Mg, followed by China with 15.1 million Mg and Brazil with 13.5 million Mg. Mexico is positioned in the ninth place with a production of 3 476 622 Mg (FAO, 2019).

The large production of chicken meat demands a large amount of inputs and raw materials, so it is essential to search for alternative inputs that avoid direct competition with food for humans and that reduce the pressure on the environment (Govoni *et al.*, 2021). The use of by-products can partially replace traditional ingredients such as maize and soy flour, reducing costs and avoiding competition for food (Pires Filho *et al.*, 2021). Coffee by-products have a considerable amount of carbohydrates, although they are also high in fiber (dos Santos *et al.*, 2021); however, it has been used in the diet of chickens (Funes *et al.* 1986; Donkoh *et al.*, 1988; Molina *et al.*, 1990; Zelaya *et al.*, 1994; Acosta *et al.*, 1997).

Funes *et al.* (1986) tested the inclusion of 0, 10, 20 and 30% of dehydrated coffee pulp in diets of Indian River hybrid male chickens, in the starter and fattening stage. They evaluated feed intake, daily weight gain, and feed conversion. In the initiation stage, feed intake and weight gain decreased in relation to the increase in coffee pulp in the diet, also feed conversion was negatively affected. While in the fattening stage, consumption, weight gain and feed conversion were only affected with levels equal to or greater than 20% of coffee pulp.

The use of coffee pulp in chicken diets was also evaluated by Donkoh *et al.* (1988), who added 0, 2.5, 5, 7.5 and 10% of coffee pulp in diets for chickens, fed for 8 weeks; demonstrating that by increasing the levels of coffee pulp, weight gain tends to decrease; therefore, an acceptable level of this by-product in the diet is 2.5%.

Molina *et al.* (1990) reported that the use of coffee pulp subjected to solid fermentation, inoculated with *Aspergillus niger*, in addition to adding urea (2.5%) and dicalcium phosphate (2%), improved the composition of the pulp by reducing the levels of polyphenols, caffeine and fiber. In addition, they tested different levels (0, 5, 10 and 15%) of fermented and unfermented coffee pulp in broiler diets, where feed intake was increased by using fermented and unfermented pulp, while gain of weight and feed conversion were not affected when using up to 15% of fermented coffee pulp. In contrast, a level greater than 10% of unfermented pulp negatively affected these variables.

Zelaya *et al.* (1994) evaluated the effect of the use of 0, 6, 9 and 12% of dehydrated coffee pulp in Single Comb White Leghorn hybrid chicks in the initiation and development stage. In the first stage, feed intake was not affected, while weight gain decreased by increasing the levels of pulp in the diet, in the same way feed conversion was negatively affected, during this first stage there was a high mortality of individuals especially in the treatments with 9 and 12% of coffee pulp. Whereas in the development stage, the feed intake and daily weight gain of the pullets were not affected, although the feed conversion increased when using 12% of coffee pulp in the diet.

Acosta *et al.* (1997) evaluated the digestibility of DM, N and metabolizable energy of corn-based rations with different levels of ensiled coffee pulp with 5% molasses and without molasses (0, 5, 10, 15, 20, 25, 30 and 35%) in Hy Line roosters of 32 weeks; finding that by increasing the ensiled coffee pulp with or without molasses to more than 5%, digestibility of DM, N and metabolizable energy decreased.

Pigs

The use of coffee pulp in pigs was evaluated by Parra *et al.* (2008), who measured the digestibility of diets with levels of 25% fresh coffee pulp with different particle sizes, in growing male pigs weighing 45.7±4.12 kg and 15 finishing pigs weighing 77.5±6.28 kg. Those authors observed that the particle size did not influence the digestibility of the DM, although the fresh pulp presented greater digestibility compared to the dry pulp; they also found that the pigs in the growth stage presented a better digestibility coefficient compared to the growing-finishing pigs.

Carvalo *et al.* (2011) also evaluated the digestibility of a diet with a level of 25% ensiled and not-ensiled coffee pulp, in a group of 15 castrated male pigs with an initial average weight of 43.06±4.12 kg, without finding differences in digestibility. of the coffee pulp when subjected to the silage process. Those authors also evaluated the productive performance of 30 castrated male pigs and 30 females with an average weight of 35.52±3.21 kg, in the growth phase, and 55 pigs in the finishing phase with an average weight of 61.7±3.56 kg. The evaluated diets contained 0, 2, 8, 12 and 16% of ensiled coffee pulp, in both phases feed intake, weight gain, feed conversion and carcass yield were not affected.

CONCLUSIONS

Due to the characteristics of the digestive system of ruminants and their greater capacity to digest fiber, these species may have a greater use of these by-products compared to non-ruminant animals, although the results of providing them are variable.

Both in the case of ruminant and non-ruminant animals, if the husk or pulp is subjected to a chemical treatment or the silage process, its digestibility and use may increase.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Cañón del Río Blanco National Park: A Forgotten Protected Natural Area

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ABSTRACT

Objective: To submit a report about the current situation of the Cañón del Río Blanco National Park and to gather all the existing information about this Protected Natural Area (PNA) in a single document.

Design/Methodology/Approach: A bibliographic research and field work were carried out to determine the current state of the PNA.

Results: The decree, the geographical location, the history of its protection, the biological, sociocultural and economic characteristics, the environmental services, the CONANP work program, the environmental problems, and the category issues are described.

Study Limitations/Implications: There were no limitations.

Findings/Conclusions: The area still protects an important biodiversity and fulfills vital environmental services; however, assigning an adequate category to the park and appointing a director is an urgent matter.

Keywords: Protection, Center of Veracruz, CONANP, Conservation.

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INTRODUCTION

Mexico is a megadiverse country and this affirmation is supported by current specialized literature. Mexico is part of an exclusive group of nations where almost 70% of the worldwide animal and plant diversity can be found (CONABIO, 2021).

However, Mexico has faced —like most Latin American countries— a serious biodiversity loss over the years. One of the main tools used by the Mexican government to counteract this loss is the creation of a Protected Natural Area (PNA) network. Nevertheless, this strategy has not always been successful. Therefore, the objective of this study is to set out the current situation of the Cañón del Río Blanco National Park and to gather all the available data about this PNA in a single document.

Decree

General Lázaro Cárdenas del Río —then president of Mexico— issued a decree on March 22, 1938, establishing the Cañón del Río Blanco National Park as a Protected

Natural Area (PNA) (Departamento Forestal de Caza y Pesca, 1938). This National Park (NP) included 55,690 ha (González and Sánchez, 1961); however, no traversing was carried out. Therefore, the Comisión Nacional de Áreas Naturales Protegidas (CONANP) plotted a traverse survey, which included 48,799.77 ha (CONANP, 2018; CONANP, 2017).

Geographical Location

The Cañón del Río Blanco NP is located in the center of Veracruz, in the Altas Montañas region, which is one of 10 socioeconomic regions in which Veracruz is divided (INAFED, 2010). Twelve municipalities are fully or partially located within the NP. To the West, it borders with the State of Puebla. The municipalities included in its territory are: Acultzingo, Aquila, Camerino Z. Mendoza, Huiloapan de Cuauhtémoc, Ixhuatlancillo, Ixtaczoquitlán, Maltrata, Nogales, Orizaba, Rafael Delgado, Río Blanco, and Soledad Atzompa (Google, 2020; INEGI, 2019) (Figure 1). Orizaba —one of the most important cities of Veracruz— is located within the National Park.

Management Program

There is no management program that guides or determines the actions that must be carried out in the National Park.

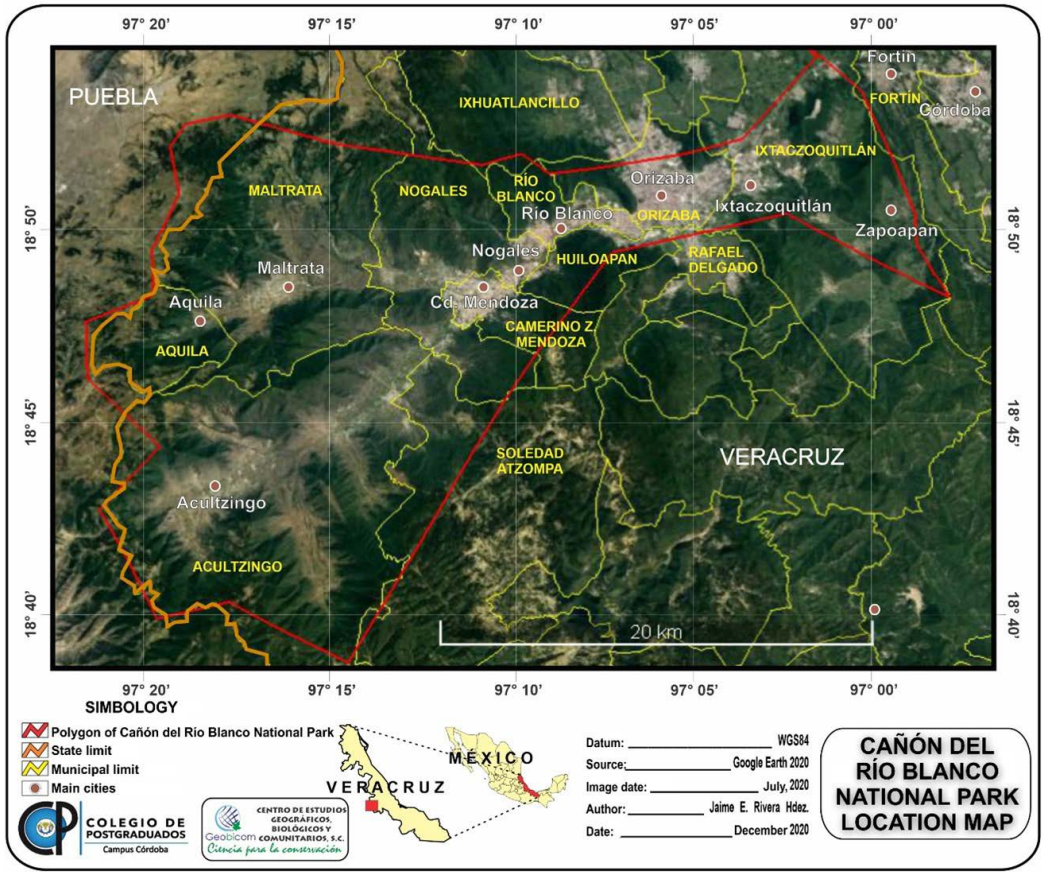

Figure 1. Map of the Cañón del Río Blanco National Park.

Brief history of the protective measures taken in this region

The main objective of the creation of this NP was to protect the flora and fauna of the region, as well as the river itself. In fact, other protection decrees had already been implemented in this region: a) Zona Protectora Forestal Cuenca Superior del Río Blanco, issued on November 30, 1933, which included 16,700 ha; b) Reserva Forestal Cuenca Hidrográfica del Río Carbonera, issued on November 26, 1936, which included 22,050 ha; and c) Zona Protectora Forestal Cuenca Hidrológica del Río Carbonera, issued on November 26, 1936, which included 12,000 ha.

All previous and current decrees share the same aim: the protection of natural resources and environmental services that humans can enjoy.

Biological Characteristics

Flora and Vegetation

The biological diversity of this PNA has been documented since the last decade (2010-2020). Regarding flora and vegetation, Rivera-Hernández (2015) reported five ecosystems protected by this PNA: 1) xerophitic scrub, 2) oak forest, 3) cloud forest, 4) gallery forest, and 5) tropical rainforest (Figure 2).

All these ecosystems are of unparalleled importance. The xerophitic scrub (1) of this NP is one of the three semiarid areas in Veracruz and is the only one officially protected through a PNA decree. Recent botanical studies (Rivera-Hernández *et al.*, 2019), have

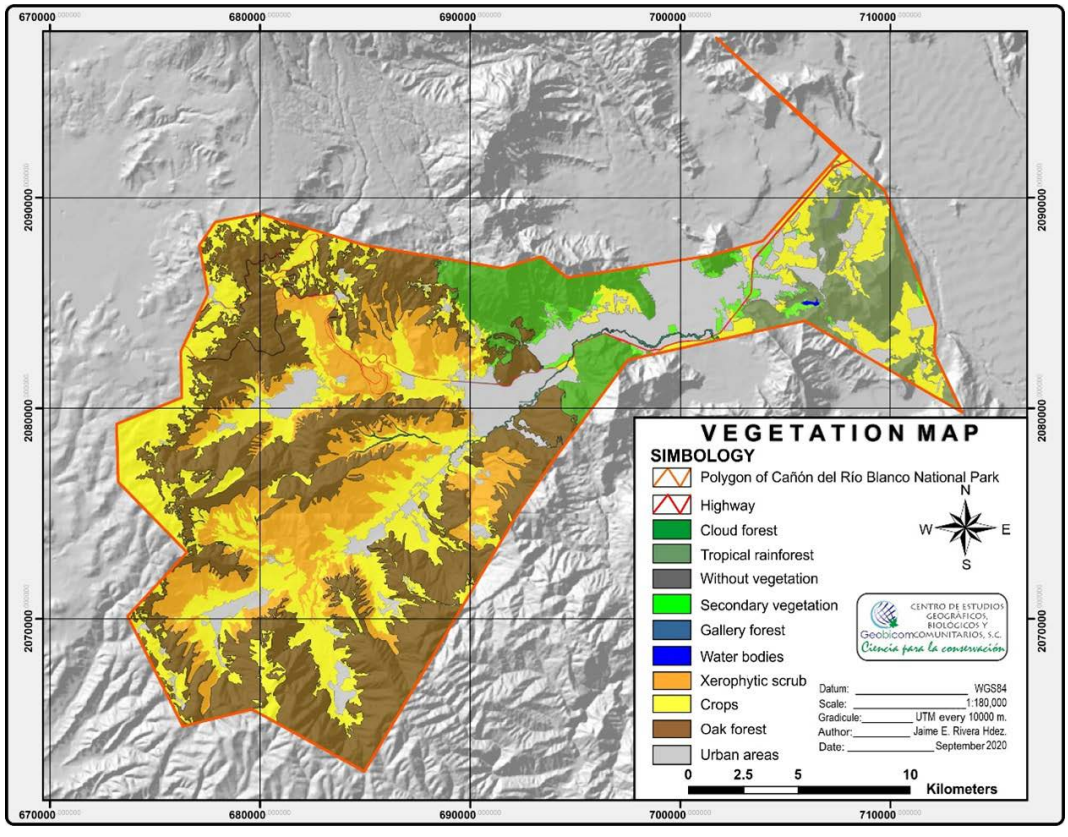

Figure 2. Vegetation and soil use map of the Cañón del Río Blanco National Park.

found that there are about 13 plant species in this semiarid area that were previously considered endemic to the Valle de Tehuacán-Cuicatlán. The oak forest (2) can also be found in the NP either as dry forest (adjacent to the xerophitic scrub and the gallery forest) or as a very humid forest (in a spot called Puerto del Aire, in the highest part of this PNA). Pines are spread throughout this oak forest, but they are not enough to be considered as a proper pine forest. Most of these pines have been felled, although many areas have been reforested with pines (mainly *Pinus patula* Schltdl. & Cham.) and other species (Figure 3). The cloud forest (3) is one of the most threatened ecosystems in Mexico. It has an important number of endemic species. This forest is also considered as one of the most

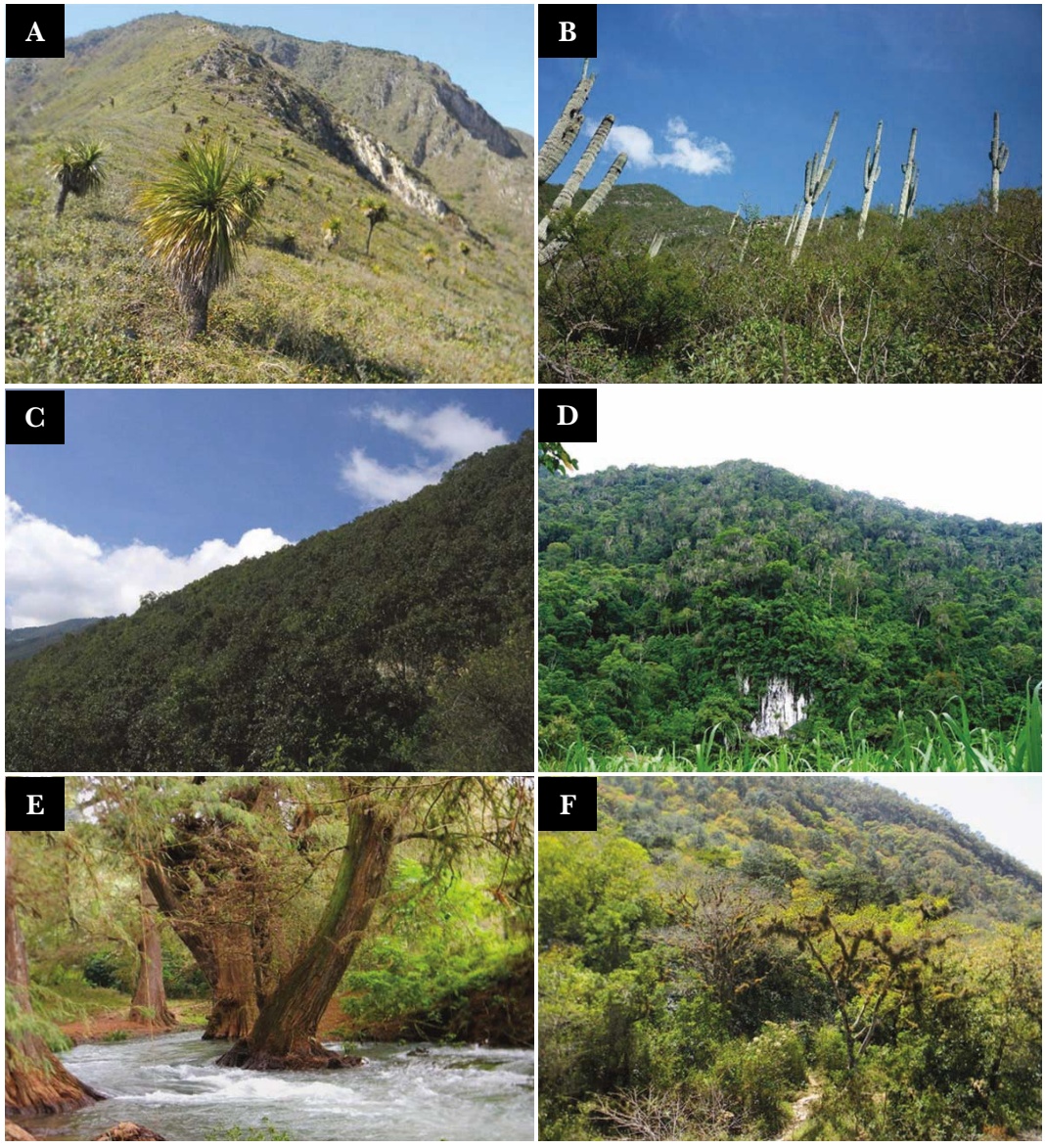

Figure 3. Types of vegetation that prevail in the Cañón del Río Blanco NP. A) Xerophitic scrub where *Quercus sebifera* and *Nolina parviflora* prevail; B) Xerophitic scrub where *Cephalocereus macrocephala* prevails; C) *Quercus* forest, D) Tropical rainforest, E) Gallery forest, and F) Cloud forest. Photographs by Jaime E. Rivera Hernández.

diverse forests of the country, because it houses a great number of flora species. The gallery forest (4) is the only forest in Veracruz were ahuehuete (*Taxodium mucronatum* Ten.), the national tree, predominates. Consequently, this forest has a high cultural importance. The tropical rainforest (5) of this NP is one of the last bastions of the karst tropical rainforests that widely prevailed in the past and that used to spread from this region up to Uxpanapa. Meanwhile, Rivera-Hernández (2015) reported a 1,688 plant species diversity in this PNA, more species have been reported in specific studies about the cloud forest (Vargas-Rueda, Rivera-Hernández, Álvarez-Aquino, Salas-Morales, Alcántara-Salinas and Pérez-Sato, 2020) and about the semiarid area of this PNA (Rivera-Hernández, Flores-Hernández, Vargas-Rueda, Alcántara-Salinas, Cházaro-Basáñez and García-Albarado, 2019), so far over 1,700 plant species have been reported.

Wildlife

Most of the studies focus on birds, recording about 417 bird species in this NP; this represents approximately 81% of the total birds reported in the Altas Montañas of Veracruz region (Alcántara-Salinas *et al.*, 2020). Regarding amphibia and reptiles, Canfield-Limón (2009) recorded 147 species (100 reptiles and 47 amphibia).

Mammals have been the subject of less studies. Only one study (Cid-Mora, 2015) has been recently carried out: 17 medium and large mammals were recorded in the cloud forest. The river otter was recorded for the first time in the high part of the Río Blanco in this PNA by Cid-Mora, Rivera-Hernández, Alcántara-Salinas, Sánchez-Páez, and Aranda-Sánchez (2018). The characteristics of this area are so peculiar and unique that a considerable number of species stand out as a result of their micro-endemism (Table 1). Regarding only vegetable species, Rivera-Hernández (2015) recorded 297 endemic Mexican species, out of which 20 are endemic to Veracruz. Approximately 20 bird species in the NP are endemic to Mexico (Figure 4).

Table 1. Species that stand out in the Cañón del Río Blanco NP.

Species	Importance	Species	Importance
Mammillaria haageana subsp. *acultzingensis* (Linzen, Rogoz. & F. Wolf) D.R. Hunt	Endemic to Cañón del Río Blanco NP	*Dioscorea orizabensis* Uline	Endemic to Córdoba-Orizaba region
Ipomoea eximia House	Endemic to Córdoba-Orizaba region	*Hylorchilus sumichrasti*	Endemic to centre zone of Veracruz and adjacent zones of Puebla and Oaxaca
Sedum lucidum R.T. Clausen	Endemic to Cañón del Río Blanco NP	*Dendrortyx barbatus*	Endemic to the mountains along Golf of Mexico, from North Oaxaca to South Tamaulipas
Ribes orizabae Rose	Endemic to Córdoba-Orizaba region	*Pseudoeurycea granitum*	Endemic to centre zone of Veracruz
Peperomia cordovana C. DC.	Endemic to Córdoba-Orizaba region	*Mesaspis antauges*	Endemic to centre zone of Veracruz
Ceratozamia decumbens Vovides, Avendaño, Pérez-Farr. & J. González-Astorga	Endemic to Córdoba-Orizaba region	*Aquiloeurycea cafetalera*	Endemic to centre zone of Veracruz

Source: Rivera-Hernández (2015), Vargas-Rueda *et al.* (2020), Rivera-Hernández *et al.* (2019), Alcántara-Salinas *et al.* (2020), Roberto Mora-Gallardo (personal communication), Cid-Mora *et al.* (2018).

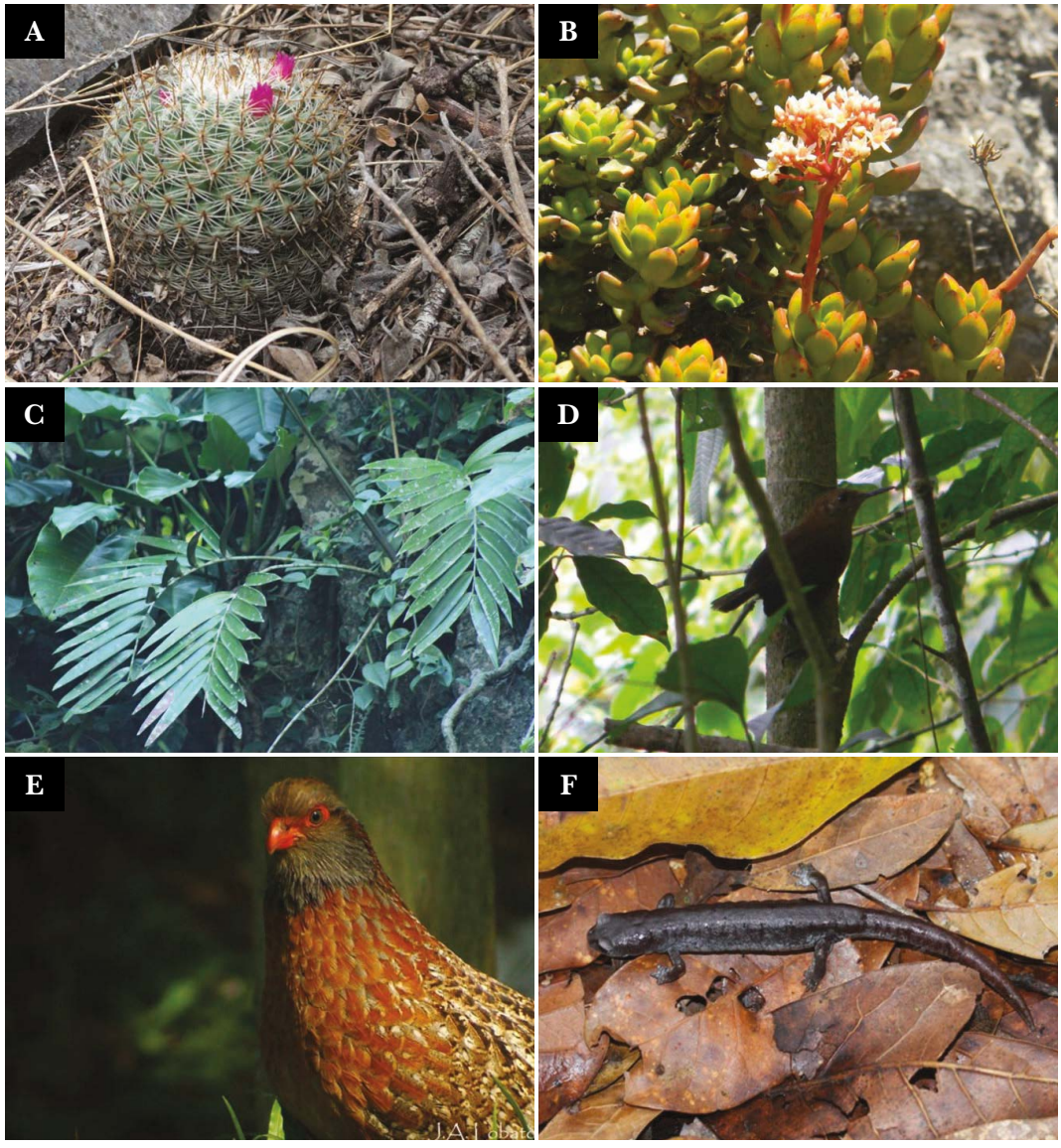

Figure 4. Some species that stand out in the Cañón del Río Blanco NP. A) *Mammillaria haageana* subsp. *acultzingensis*, B) *Sedum lucidum*, C) *Ceratozamia decumbens*, D) *Hylorchilus sumichrasti*, E) *Dendrortyx barbatus*, and F) *Aquiloeurycea cafetalera*. Photographs: A, B, C, and D) by Jaime E. Rivera-Hernández, E) by José Alberto Lobato García, and F) by Sean Michael Rovito (from Naturalista.mx).

Socio-cultural characteristics

The Cañón del Río Blanco NP was one of the first sites included in a PNA decree in Mexico. The PNA includes cities of different size and Orizaba is the most important city in the area. In 2015, Orizaba was added to the Pueblos Mágicos (Magical Towns) Program of the Secretaría de Turismo (SECTUR, 2021). This city is part of an industrial corridor that links several conurbations, including: Río Blanco, Nogales, and Ciudad Mendoza. Additionally, this area includes rural communities, located in municipalities, such as: Acultzingo, Aquila, Camerino Z. Mendoza, Huiloapan de Cuauhtémoc, Ixtaczoquitlán, Nogales, and Soledad Atzompa.

Some nahua communities also live in these municipalities, including: Acultzingo, Soledad Atzompa, and Camerino Z. Mendoza (INPI and INALI, 2020). The Río Blanco city is also located in this area. This city is historically important because it witnessed one of the first worker's uprising that led to the Mexican Revolution. The worker's uprising was carried out in a yarn and fabric factory. Unfortunately, there were many deaths; this episode is known as the Río Blanco rebellion (Sánchez-Hernández, 2010).

Economy Characteristics

Orizaba and its metropolitan area are one of the most important industrial areas in Veracruz. One of the main breweries in Mexico (Cuauhtémoc Moctezuma) is located in this area. In addition, several industries and companies are located throughout the metropolitan area of Orizaba, including several fabric and manufacturing industries of historical importance. Eleven of the localities that make up the conurbation are municipal seats; therefore, this area is the conurbation with the highest number of municipalities of Veracruz (Jiménez-Trejo, 2007).

Environmental Services

The NP provides the following environmental or ecosystem services: capture and filtering of water and control of the hydrological cycles; oxygen generation and assimilation of several pollutants —particularly environmental pollutants produced by the regional industries—; maintenance of the gas quality in the air —which helps to regulate weather—; generation and conservation of fertile soils; shelter for wild animals; and biodiversity conservation. In addition, the NP provides food, raw materials, genetic resources, and medicinal and ornamental plants. It also provides natural spaces with outstanding landscapes for recreation, scientific research, and education.

Work Program

Despite the decrees and preservation aims of the PNA, the area remained abandoned for a long time, until 2004, when the Comisión Nacional de Áreas Naturales Protegidas (CONANP) took charge of the NP. In 2008, it appointed an official manager (rather than an official director) to manage the NP, as well as two other areas: the Pico de Orizaba National Park and the Cofre de Perote National Park. The Cañón del Río Blanco National Park was never managed by an exclusive director or team. In 2018, the park lost its manager and, to this day, the NP remains under the management of the Dirección Regional Planicie Costera y Golfo de México. Finally, in 2022 CONANP has designed an administrator (not director) exclusively to Cañón del Río Blanco National Park, who is in charge of the different social support programs in this PNA.

As many other PNAs, the CONANP management is based on the allocation of projects through subsidy programs; these subsidies are granted specifically by the Programa de Conservación para el Desarrollo Sostenible (PROCODES), which mainly supports the following projects: biodiversity technical studies, communal ecotourism, communal birdwatch, and clean-up of water bodies. A key role of the administration of this NP was to provide technical opinions for more than 74 Environmental Impact Statement

applications, most of which were related to infrastructure construction sites (Raúl Álvarez, personal communication).

Environmental Problems

This region has been inhabited since colonial times. Villa de Córdoba and Villa de Orizaba were founded in 1618 and 1774, respectively. Over time, this area has become an important industrial zone and, therefore, the region has suffered a great environmental degradation, mainly in terms of loss of habitat, air pollution, and riverbed pollution. Based on Rivera-Hernández (2015), the following problems were identified:

a) Agricultural Areas

The main problem in the highlands —located in Acultzingo, Maltrata, and Aquila, in the westernmost side of the PNA— is the change in land use from forest to agricultural. The agricultural area of Puebla ends where the Cañón del Río Blanco starts; in this area, the rough conditions of the soil do not allow the establishment of more crops. However, crops have also been established in the flat areas of the high part of some of the hills of these municipalities. The same situation can be found in the lowlands of Acultzingo and Maltrata, where the agricultural areas spread to all the valleys and reach the skirts of the hills.

Meanwhile, growing coffee is also a widely-spread practice in the region, mainly in the tropical rainforest of Ixtaczoquitlán and, to a lesser degree, in the cloud forests of Orizaba and Huiloapan. In these areas, the natural ecosystems have been replaced by coffee crops and, only in small areas of very wild lands, these natural ecosystems remain as a memory of the vast tropical rainforests that once prevailed in the region. Coffee crops have practically replaced the shrub layer, both of the tropical rainforest and the cloud forest, extinguishing an outstanding number of plant species. Nevertheless, several studies (Manson *et al*., 2008; López, 2004) prove that coffee is one of the less harmful crops to biodiversity and that it even works as biodiversity reservoirs (Macip and Casas, 2008).

Finally, sugarcane (*Saccharum officinarum* L.) is the most spread crop in the lowest lands of the National Park, followed by chayote (*Sechium edule* (Jacq.) Sw.). Unlike coffee plantations, these two crops completely modify the ecosystems, reducing the quality of the soil; additionally, they do not promote biodiversity. Fortunately, these crops are only established in the lowest and flattest parts of the NP and, therefore, they do not impact the hillsides. However, maize and coffee are also established on the hillsides. Consequently, the tropical rainforest is one of the most fragmented and threatened ecosystems of the National Park (Figure 5).

b) Illegal Human Settlements

These settlements are considered one of the main problems faced by the National Park. To date, several ecosystems adjacent to the urban sprawl have been impacted by illegal settlers, mainly in the Río Blanco, Huiloapan, Nogales, and Ciudad Mendoza areas, as well as in the Acultzingo and Maltrata municipalities. They remove the primary vegetation to increase the urban sprawl (Figure 6A).

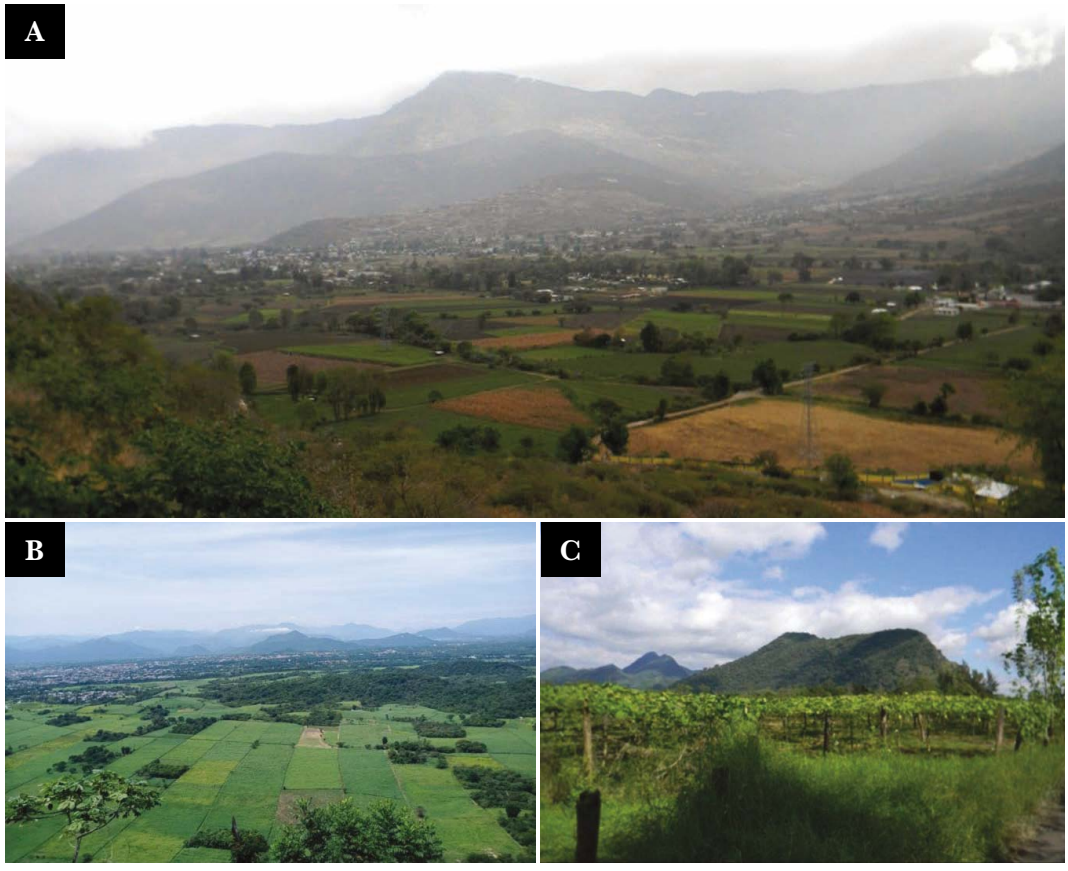

Figure 5. Crops grown in the Cañón del Río Blanco National Park. A) Crops in the lowlands of Acultzingo, B) Sugarcane plantations in lowlands of NP, and C) Chayote in the Tuxpanguillo area. Photographs: A) and C) by Jaime E. Rivera Hdez. and B) by Raúl Pablo García Sánchez.

c) Air Pollution

The problem is mainly focused in the metropolitan industrial area of Ixtaczoquitlán-Ciudad Mendoza, where a high number of factories —such as the Kimberly-Clack paper mill, the Sabritas fried potato manufacturer, the Proquina pharma, the Cuauhtémoc-Moctezuma brewery, and the yarn and fabric manufacturer Coats, among others— are established. These industries produce diverse air and water pollutants, as well as high volume of solid waste (Figure 6B).

d) Quarries or rock extraction banks

At least four opencast mines are destroying the biodiversity habitat in the Orizaba-Ixtaczoquitlán area. The largest is located in Ixtaczoquitlán, in the Buenavista hill, and it belongs to the Holcim-Apasco multinational. This is the only multinational that has an environmental restoration program, working together with the Instituto de Ecología, A.C. of Xalapa, Veracruz. The other three mines are located as follows: the first one is located in the Escamela hill, Orizaba; the second, in the San Cristóbal hill, in Huiloapan; and the third, also in the San Cristóbal hill, but it belongs to the Rafael Delgado municipality. The last one is located outside the traversing of the NP, but adjacent to it. These mines pose a

real problem to the biodiversity conservation and to the existence of the NP as a whole, because, day by day, they destroy the local hills, reducing the fragile habitat of the cloud forest ecosystem (Figure 6C).

e) Water Bodies Pollution

Most of water bodies of the NP are polluted: domestic wastewater and industrial liquid waste alike are poured into them. The crops established in the whole region are another important source of pollution: both weed-killers and chemical fertilizers are dragged by rainfalls and end up in the rivers. In addition, solid waste (garbage) pollutes the river when it flows by human settlements. The Río Blanco originates in Acultzingo and it is polluted throughout its course. Once the river reaches the lowest parts of the NP, it is polluted to such a degree that the pollution can be perceived by the color and odor. This river flows into the Laguna Alvarado and, then, into the Gulf of Mexico; its pollution impacts the Parque Nacional Sistema Arrecifal Veracruzano. This situation has a highly negative impact on the oceans (Figure 6D and 6E).

f) Charcoal Production

This is a low-scale activity that, nevertheless, is constantly carried out in the holm-oak wood near Ciudad Mendoza (Figure 6F). This situation has had a negative impact on these ecosystems of the PNA, because the number of oaks is constantly being reduced.

g) Goat Grazing

Billy goat grazing is a common practice in Maltrata and Acultzingo and it has a direct impact on the local xerophitic scrub (Figure 7A).

h) Poaching

Despite the existing legislation, poaching is a common practice in the whole NP and adjacent areas. Different ejidos and groups have expressed their worries and disagreements about external people (sport hunters) who hunt in their territories without asking for their permit. Additionally, some landowners also engage in poaching and members of the local communities allow hunters to enter the area (Figure 7B).

i) Forest Fires

This type of accidents takes place during the dry season, mainly in the dry oaks zone of Ciudad Mendoza (Figure 7C).

j) Introduction of exotic species

This is a common problem througout Mexico, mainly in the areas adjacent to human settlements. Many inappropriate reforestation and restoration activities are carried out. Non-native species are used for this purpose, including: jacaranda (*Jacaranda mimosifolia* D. Don), African tulip tree (*Spathodea campanulata* P. Beauv.), Chinese privet (*Ligustrum lucidum* W.T. Aiton), and southern silky oak (*Grevillea robusta* A. Cunn. ex R. Br.). In addition, Maltrata has commercial *Cupressus* spp. and *Pinus* spp. plantations. Finally,

Figure 6. Canón del Río Blanco NP problems. A) Illegal human settlements, B) Air pollution, C) Quarries or rock extraction banks, D) Wastewater discharge in the Río Blanco, E) Water pollution of the Río Blanco as a result of solid waste, and F) Charcoal production. Photographs: A, B, C, D, and E) by Jaime E. Rivera Hdez and F) by Oscar Cid Mora.

domestic cats pose a threat to local fauna, including several birds, reptiles, and wild mammals (Figure 7D).

Problem of the Category

The National Park category was the first category used in Mexico and, therefore, it is also one of the most limiting categories regarding the use and exploitation of natural resources in the PNA. Originally, its only aim was biodiversity conservation and, consequently, the National Park category does not allow any sustainable exploitation, just research activities.

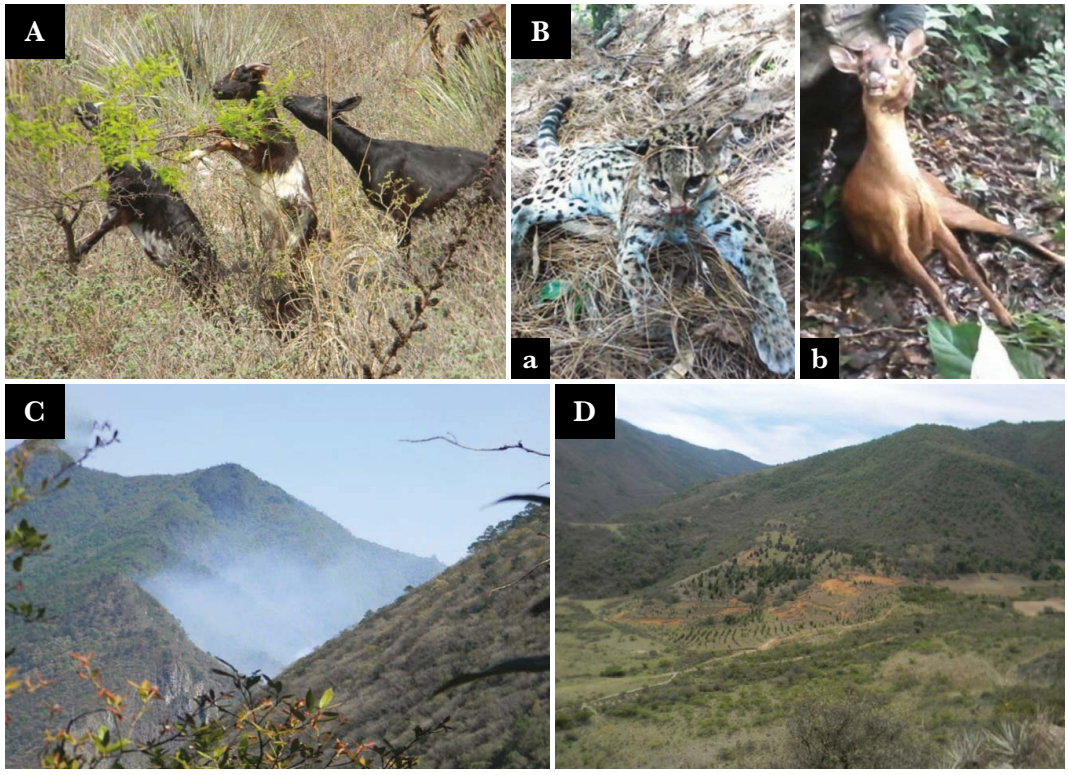

Figure 7. Cañón del Río Blanco NP Problems (cont.). A) Goat grazing, B) Poaching (a) margay (*Leopardus wiedii*) and (b) Central American red brocket (*Mazama temama*), C) Forest fire in the NP, D) *Cupressus* spp. and *Pinus* spp. commercial plantations. Photographs A, C, and D) by Jaime E. Rivera Hdez. and B) by Oscar Cid Mora.

In this context, it is obvious that placing the Cañón del Río Blanco under this category was a mistake from the beginning, because it included several cities. Currently, dealing with all the violations against the natural resources of this PNA is a very difficult task, because even agriculture should be prohibited in this area. Additionally, the authorities cannot authorize sustainable exploitation projects in this NP, because they would violate the protection decree.

Therefore, CONANP is currently analyzing the modernization of the decree of this NP. The proposal includes a change of category to an Área de Protección de Recursos Naturales, taking into account that the main reason behind its establishment was to secure water supply and environmental services in the Córdoba-Orizaba area. This objective should have been achieved through the restoration of the forests; already in 1930, the region was highly deforested. In addition, the main urban areas of the NP would be excluded from this decree.

The proposed Área de Protección de Recursos Naturales category would establish the respect for the forestry calling and promote the restoration of the biodiversity of the forest and the expansion of the forest limits. It would open and promote models for the sustainable exploitation of the natural resources and would support sustainable agricultural production processes that are appropriate for the PNA.

The legislation also allows the establishment of a restoration sub-area within the zoning, in those areas where the natural resources have been seriously altered or modified, establishing especial programs for their ecological restoration. Likewise, areas that include representative ecosystems of the PNA can be established, as a result of their conservation interest, excluding them from any type of exploitation.

CONCLUSIONS

The area still shelters an important biodiversity and also performs vital environmental services —such as oxygen supply, carbon capture, and biodiversity conservation, among others. In addition, humans can still enjoy its outstanding landscapes (Figure 8).

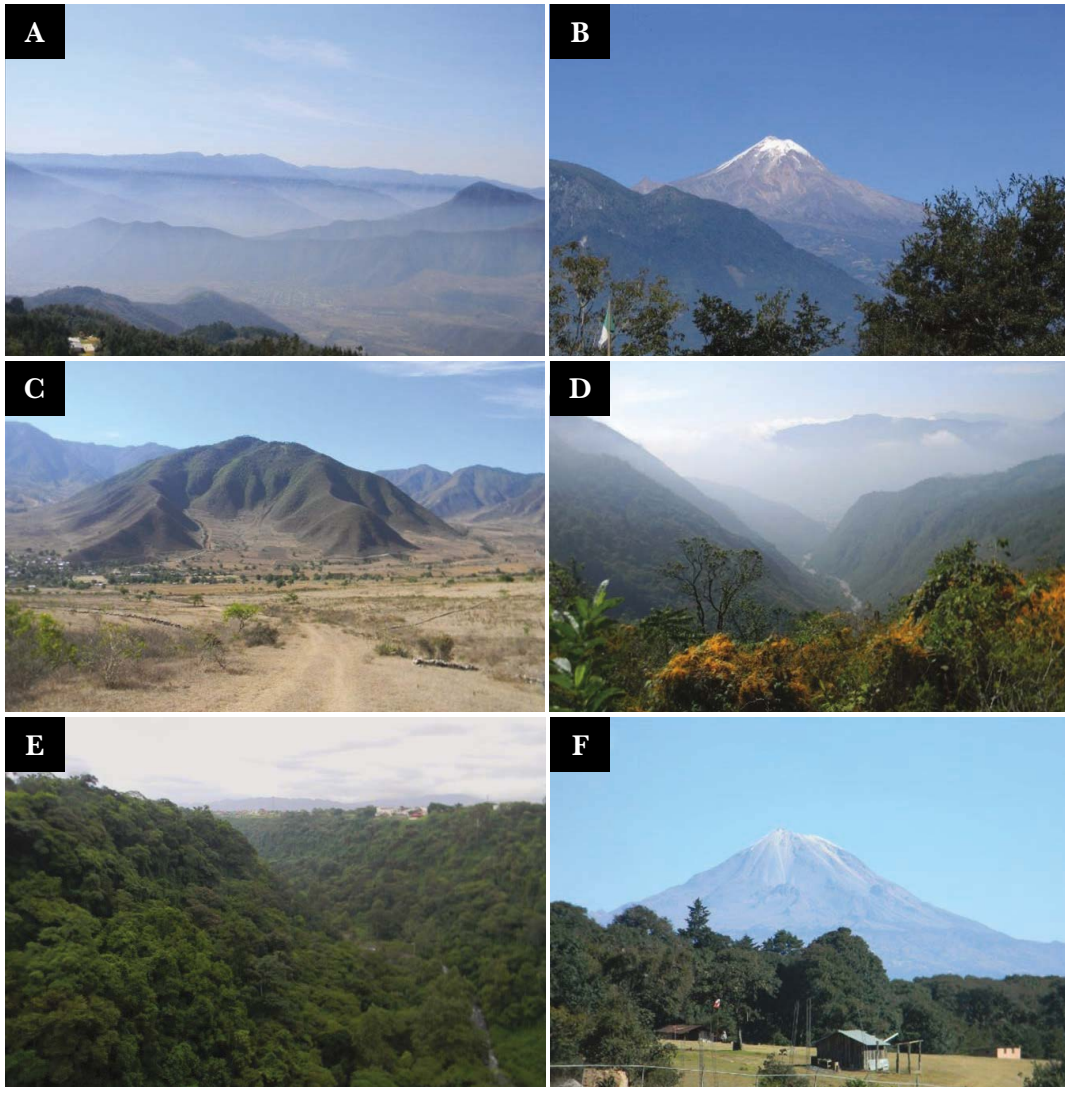

Figure 8. Some landscapes of the Cañón del Río Blanco NP. A) View of the mountains from the heights of the municipality of Nogales, B) View of the Citlaltépetl Volcano or Pico de Orizaba from the Escamela hill in the municipality of Orizaba, C) Hill in the municipality of Acultzingo, D) Carbonera Canyon, E) Metlac Ravine, in the municipalities of Fortín and Ixtaczoquitlán, and F) View of the Citlaltépetl volcano from the municipality of Nogales. Photographs by Jaime E. Rivera Hernández.

A deep analysis of the current situation of this NP must be carried out, in order to provide the NP with the appropriate category, taking into consideration the reshaping of the polygon. This measure would allow it to function as a protected natural area enabling the implementation of the appropriate regulation. In addition, the support aimed to the development of projects for the conservation and sustainable exploitation of the natural resources by the rural communities in the PNA should be arranged. Finally, CONANP must promptly appoint a new administration or person in charge of this National Park, who can reliably face the problems discussed in this study and can materialize the modernization of the decree, allowing this PNA to comply with the objectives for which it was created.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Characterization of mamey [*Pouteria sapota* (Jacq.) Moore & Stearn] in the Totonacapan Poblano of Puebla, Mexico

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ABSTRACT

Objective: The objective of this study was to morphologically characterize mamey in 10 municipalities of the Sierra Nororiental of Puebla (Totonacapan Poblano), Mexico.

Design/methodology/approach: In the study, 206 accessions obtained from simple random sampling using qualitative and quantitative morphological descriptors were evaluated, using an analysis of variance, principal components and hierarchical cluster analysis with the Gower distance and the Ward grouping method.

Results: Quantitative descriptors had high coefficients of variability (12.66% to 43.40%). The principal components analysis indicated that components 1 (41.66%), 2 (19.97%) and 3 (16.62%) accounted for 78.25% of the variance and the cluster analysis identified eight groups; the tree and leaf variables turned out to be the most important to group the samples.

Limitations on study/implications: For greater accuracy on the morphological diversity of mamey, it is necessary to include more descriptors of the fruit and the flower.

Findings/conclusions: The descriptors that contributed the most to the grouping were those related to the shape, height and size of the tree, and the weight, size and shape of the leaf; in relation to the fruit, the descriptor that contributed the most was the shape. These results could be useful in programs of conservation, improvement, and use of the mamey.

Keywords: morphological diversity, conglomerates, Sierra Norte of Puebla, Sapotaceae.

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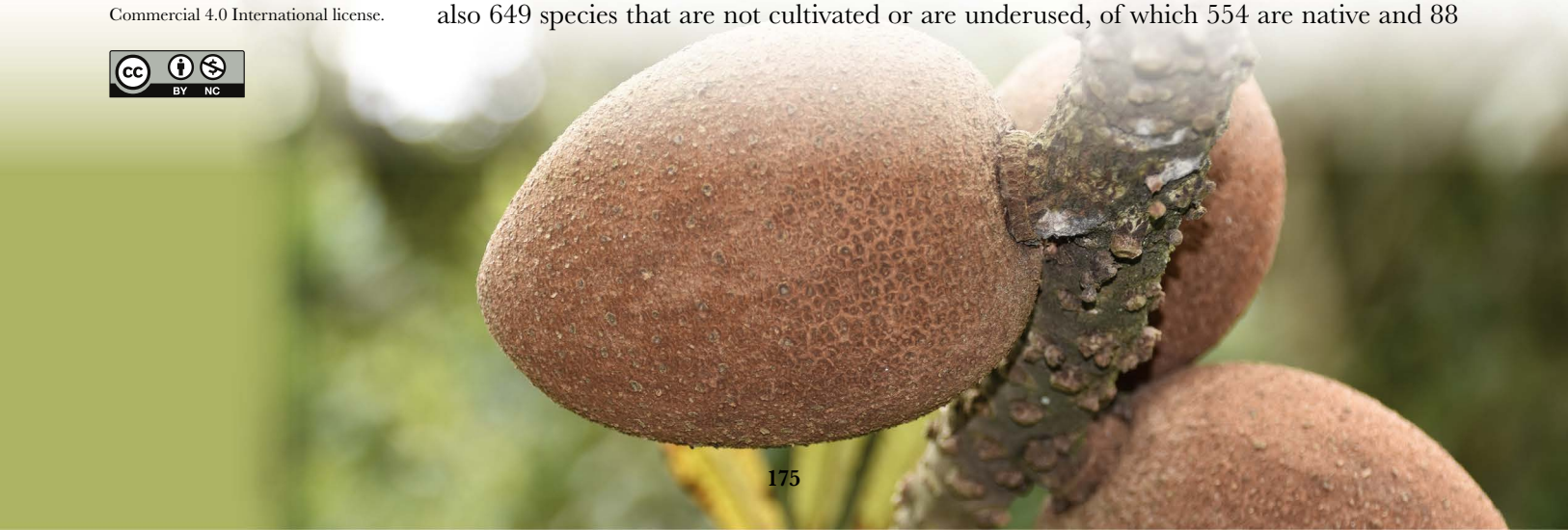
INTRODUCTION

Segura *et al.* (2018) mention that 762 species of edible fruits are cultivated in Mexico, which are distributed into 87 families and 288 genera; in addition, they mention that there are 113 species of fruit trees cultivated, of which 53 are native and 60 introduced; there are also 649 species that are not cultivated or are underused, of which 554 are native and 88



introduced. One of these underused species is mamey [*Pouteria sapota* (Jacq.) Moore and Stearn] from the Sapotaceae family and native to the country, which together with other species from the families Myrtaceae, Cactaceae, Annonaceae and Fabaceae were already consumed by the native peoples of Mesoamerica (González & Del Amo, 2012). Presently, *P. sapota* is valuable for its fruit because it presents several nutritional properties such as fiber, fat, iron, calcium, proteins, sodium, vitamins and healthy carbohydrates for human beings (Velázquez *et al.*, 2015).

Mamey is a species native to tropical and subtropical zones of Central America and southeastern Mexico, which is considered to be its possible center of domestication (Martínez-Castillo *et al.*, 2019). This species is distributed from the south of Sinaloa to Chiapas and in the Gulf of Mexico, from Tamaulipas to Tabasco, as well as in the Yucatán Peninsula and central states such as Morelos, Guanajuato, Estado de México, San Luis Potosí, Hidalgo, Querétaro and Puebla; some of the states mentioned have zones with tropical and subtropical climates (Núñez-Colín *et al.*, 2017). Mexico is the principal mamey producer in the world, with a surface sown of 1,731.71 hectares and a production of 21,800.42 tons, and more than 87.87% of national production is concentrated in the states of Yucatán with 62.28%, Guerrero 12.23%, Veracruz 6.09%, Michoacán 3.87% and Puebla 3.40% (SIAP, 2021).

In Mexico there are studies about the morphological characterization of mamey (Bayuelo-Jimenez & Ochoa, 2006; Gaona-García *et al.*, 2008), focused on maturation of fruits in ecotypes (Domínguez *et al.*, 2010), characterization of post-harvest management (Villarreal-Fuentes *et al.*, 2015), characteristics of mamey quality (Espinosa-Zaragoza *et al.*, 2016), and tolerance to forced warm air in the fruits (Ariza *et al.*, 2018). On the other hand, Villegas-Monter *et al.* (2016) analyzed aspects about the diversity and uses of mamey, while Carpio *et al.* (2015) studied the phenology of mamey. However, there are few studies regarding variations of growth habits, tree shape, and leaf shape (Calderón *et al.*, 2011). The morphological characterization of plant genetic resources is a procedure used to measure and know the genetic variability of a population, to differentiate plants, and to conserve genetic resources (Hernández, 2013). This study had the objective of morphologically characterizing mamey (*P. sapota*), using morphological variables of trees, leaves and fruits in 10 municipalities of the Totonacapan Poblano region, with the purposes of improvement and conservation.

MATERIALS AND METHODS

Study area and sampling

A total of 206 accessions were collected in 10 municipalities of the Totonacapan Poblano region, located in the Sierra Nororiental of Puebla (Figure 1). The climate in these municipalities is warm with rainfall nearly all year long, with altitudinal zones that range from 90 m to 1020 m of altitude with annual rainfall of 800 mm to 2000 mm, zones of up to 4000 mm and a mean annual temperature between 18 °C and 26 °C (Barrera-Rodríguez *et al.*, 2009).

The field phase was carried out from November to December 2020 and February to March 2021. The sampling was simple random, five leaves were included per tree, labeled

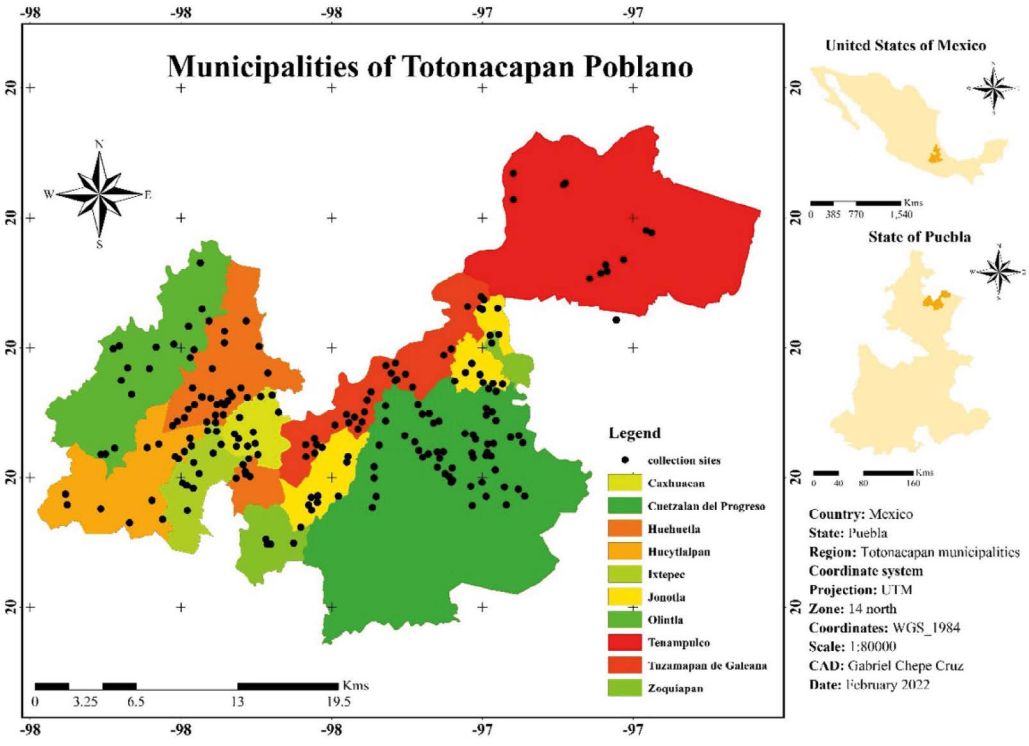


Figure 1. Geographical location of the 206 accessions of *P. sapota* in 10 municipalities of the Totonacapan Poblano region.
Source: Prepared by the authors with vectorial data from INEGI (2020).

and stored in a cooler with average temperature of 4 °C to later store them at −20 °C. For the morphological characterization, quantitative and qualitative descriptors adapted to mamey were obtained as presented by Azurdia *et al.* (1997) to characterize mamey fruits and trees (Table 1).

The length, width and weight of the leaf were measured from each sample using a Mitutoyo brand Vernier. The weight was determined with an analytical scale Velab VE-204. The qualitative descriptors of the tree, leaf and fruit were determined visually.

Statistical analysis

The Principal Components Analysis (PCA) and the conglomerate analysis were conducted with the software RStudio version 4.1.1 (RStudio Team, 2021). For the Pearson's coefficients and descriptive statistics, the functions used were summary and corr; the principal components analysis was done with the prcomp function, of the factoextra package (Kassambara & Mundt, 2020). For the Conglomerate analysis (Cluster) the daisy and hclust functions from the cluster package were used (Maechler *et al.*, 2021). To determine the number of conglomerates, the function used was kgs, of the maptree package (White & Gramacy, 2012).

The PCA was obtained with standardized data through the function scale of R, and then the traits that contributed most to differentiate mamey materials were identified. To determine the number of principal components (PC), the Cliff (1988) criterion and the

Table 1. Morphological descriptors used to characterize mamey.

Descriptor	Code	Unit of measure or state of the descriptor
Tree		
Height	ATL	Meter (m)
Height to the first branch	APR	Meter (m)
Diameter at chest height	DAP	Centimeter (cm)
Sample status	EM	1) Inserted, 2) Native, 3) Wild
Type of vegetation or production system	TV/SP	1) Acahual, 2) Kuojtakiloyan, 3) Pepper, 4) Paddock, 5) High evergreen forest, 6) Cedar agroforestry system, 7) Shade coffee system, 8) Citrus system, 9) Milpa system, 10) Backyard
Tree habit	HA	1) Columnar, 2) Pyramidal, 3) Rectangle, 4) Round, 5) Irregular
Canopy shape	FC	1) Pyramidal, 2) Round, 3) Irregular
Leaf		
Leaf blade length	LL	Centimeter (cm)
Leaf blade width	AL	Centimeter (cm)
Leaf Length/width ratio	RL/A	Dimensionless
Leaf blade area	AF	Square centimeter (cm^2)
Leaf blade weight	P	Gram (g)
Leaf shape	FH	1) Cuneate, 2) Linear, 3) Oblanceolate, 4) Obovate, 5) Ovate
Fruit		
Fruit shape	FF	1) Ellipsoid, 2) Spheroid, 3) Ovoid
Fruit tip shape	FA	1) Curved, 2) Straight

Kaiser (1960) criterion were used. In the cluster analysis, a dendogram based on both quantitative and qualitative descriptors, was constructed; pondering was based on Gower (1971) distances. Grouping was based on Ward's (1963) hierarchical method. The number of conglomerates was estimated using the penalization function by Kelley-Gardner-Sutcliffe through the KGS statistics (Kelley *et al*., 1996).

RESULTS AND DISCUSSION

Descriptive statistics

The descriptive statistics of the eight quantitative descriptors measured in the 206 accessions of mamey in ten municipalities of the Sierra Nororiental of Puebla are shown in Table 2. In this study the traits had high coefficients of variability (CV) (12.66% to 43.40%); according to Hidalgo (2003), the traits that present CV lower than 20% indicate low variability, although this is not a decision criterion to identify the use of traits in the analysis.

The frequencies of scoring of each qualitative descriptor of *P. sapota* are described next: in the state of sampling, 54.9% are native and 44.2% are introduced. In type of vegetation or production system, it was found that 28.2% corresponded to acahual, 23.8% to a shade coffee sustem, 18% to backyard, 9.7% to the milpa system, 7.3% to the system called Kuoujtakiloyan, 5.3% to the citrus system, 3.4% to pastureland, 2.4% to the pepper system, and lastly the tall sub-evergreen forest and cedar agroforestry system only presented

Table 2. Quantitative descriptors and statistical parameters in mamey samples.

Code	Average*	S	R	Minimum	Maximum	CV (%)
ATL	12.65	4.32	25.21	5.22	30.43	34.13
APR	3.15	1.37	9.43	0.56	9.99	43.40
DAP	44.16	16.38	90.35	1.16	91.52	37.10
LL	34.28	6.85	34.94	18.96	53.89	19.99
AL	9.97	2.35	20.94	4.50	25.44	23.53
RL/A	3.48	0.44	3.64	1.28	4.92	12.66
AF	180.94	69.67	396.81	49.86	446.67	38.50
P	5.84	1.93	13.92	1.37	15.30	33.03

S=standard deviation; R=range of variation; CV=coefficient of variation. ATL=tree height, APR=height of the first branch, DAP=diameter at chest height, LL=laminar length, AL=laminar width, RL/A=length:width ratio of the leaf, AF=leaf area, P=weight, *n=206.

1%. Regarding the tree's growth habit, the results were: pyramidal 46.6%, round 41.7%, without shape 8.7%, rectangular 2.4% and columnar 0.5%. In the descriptor crown shape, it was found that 47.1% was pyramidal, 44.7% semi-circular and 8.3% without shape. For leaf shape, it was found that 71.4% were oblanceolate, 19.4% obovate, 5.3% cuneate, 3.4% linear and 0.5% oval. In fruit shape, the results were: ellipsoidal 54.4%, spheroid 24.3% and ovoid 21.4%, with straight apex in 99% and 1 % curve.

Correlation between the descriptors

Pearson's correlation coefficients for the eight quantitative descriptors are shown in Table 3. Most of the correlations (15) between the descriptors were significant ($p<0.05$) and 13 were not. The highest correlations were found in the morphological descriptors of the leaf, the strongest positive correlations were found between AF and AL (0.91), between AF and LL (0.88), between P and LL (0.76), and P and AF (0.74). These correlations mean that the leaf width and length are directly related with the leaf area and weight. On the other hand, RL/A were negatively correlated with AL (−0.41); this signals that as the leaf

Table 3. Matrix of Pearson's correlation coefficients of the quantitative descriptors of 206 mamey samples.

	ATL	APR	DAP	LL	AL	RL/A	AF	P
ATL	1.00							
APR	0.33***	1.00						
DAP	0.45***	0.16*	1.00					
LL	0.16*	0.10	0.14*	1.00				
AL	0.08	0.08	0.15*	0.63***	1.00			
RL/A	0.07	0.02	−0.01	0.36***	−0.41***	1.00		
AF	0.12	0.10	0.16*	0.88***	0.91***	−0.07	1.00	
P	−0.02	0.02	0.09	0.76***	0.58***	0.06	0.74***	1.00

*, **, *** indicate statistical significance in the p value of 0.05, 0.01 and 0.001, respectively. ATL=tree height, APR=height at first branch, DAP=diameter at chest height, LL=leaf length, AL=leaf width, RL/A=leaf length:width ratio, AF=leaf area, P=weight.

width decreases, the leaf length/weight ratio will increase. The minimal correlations were present in the following descriptors: ATL and LL (0.16), APR and DAP (0.16) and in turn DAP and LL (0.14), DAP and AL (0.15) and lastly DAP and AF (0.16). This shows that there is a minimal relation between the descriptors of height of the first branch with leaf width and length.

Principal components analysis

The principal components analysis indicated that PC1, PC2 and PC3 explain 41.66%, 19.97% and 16.62% of the variability, and together they explain 78.30% (Table 4). PC1 explained 41.66% of the variance, including the descriptors AF, LL, AL, P, which are leaf variables. PC2 contribute 19.97% of the variance, where the descriptors that contributed most were: ATL, DAP and APR. Finally, PC3 explained 16.62% of the variance, where descriptors RL/A and LL stood out.

In general, the descriptors evaluated contributed to a large extent to the variance (Figure 2), which agrees with results from Bayuelo-Jimenez & Ochoa, (2006), Gaona-García, Tejacal-Alia *et al.*, (2008) and Rodríguez-Gaytán *et al.*, (2021) who used some similar desciptors; this suggests that these descriptors are useful to carry out the characterization of mamey. The PCA presented high levels of variability, which helped to identify the groups that were generated through the cluster analysis.

Hierarchical conglomerate analysis

Eight groups were identified at a Gower distance of 1.3 (Figure 3). The characteristics of each group are summarized next. Conglomerate one grouped 21 individuals, most from the municipality of Tuzamapan de Galeana, with trees of low height (16.62 m to 6.03 m), with

Table 4. Results from the PCA of the quantitative descriptors used to characterize the mamey accessions.

Components	1	2	3
Eigen value	3.33	1.6	1.33
Variance percentage	41.66	19.97	16.62
Cumulative variance percentage	41.66	61.63	78.25
Descriptors	Eigen values		
ATL	0.208	−0.817	−0.080
APR	0.167	−0.597	−0.096
DAP	0.260	−0.665	−0.179
LL	0.903	0.009	0.387
AL	0.862	0.172	−0.414
RL/A	−0.013	−0.214	0.962
AF	0.976	0.112	−0.052
P	0.827	0.210	0.180

ATL=tree height, APR=height to the first branch, DAP=diameter at chest height, LL=leaf blade length, AL=leaf blade width, RL/A=leaf blade length to width ratio, AF=leaf blade area, P=leaf blade weight.

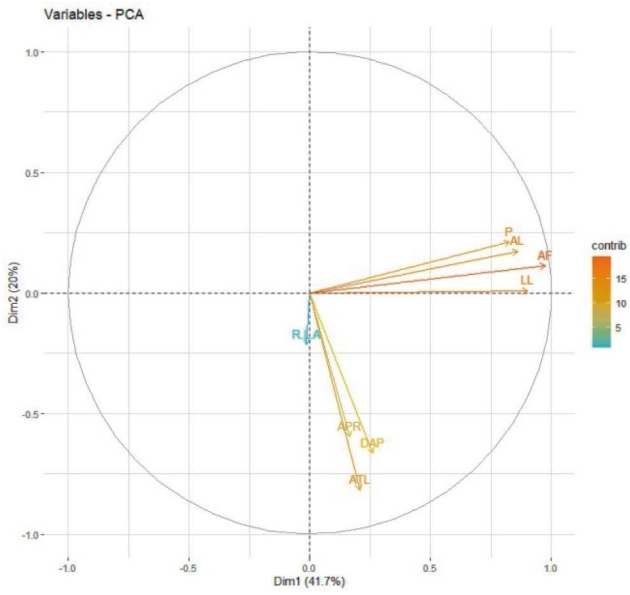


Figure 2. Contributions of the mamey descriptors according to what is determined in PC1 and PC2. ATL=tree height, APR=height at first branch, DAP=diameter at chest height, LL=leaf length, AL=leaf width, RL/A=leaf length:width ratio, AF=leaf area, P=weight.
Source: Prepared by the authors with Rstudio v.4.1.1.

large leaves of average leaf area of 189.16 cm^2, with a tree growth habit and crown without shape and ellipsoidal fruit; these results can be due to the management by producers since the municipality is known for producing mamey, among other fruits. Cluster two showed 32 accessions from the municipalities of Jonotla, Ixtepec, Cuetzalan del Progreso, Olintla and Huehuetla, with the lowest height at first branch with an average of 2.70 m and a high length/width ratio of the leaves, which can result from management practices; most of the accessions were found in a shade coffee system. Group three had 23 samples from the municipalities of Hueytlalpan, Huehuetla, Caxhuacan, Cuetzalan del Progreso and Tenampulco, the trees presented a high height with average of 15.53 m; this group had the highest DAP (56.65 cm), with round tree growth habit and semi-circular crown, the trees were found in the acahual vegetation which implies low human intervention, generating the conditions for greater development. Conglomerate four presented 32 individuals from the municipalities of Tuzamapan de Galeana, Cuetzalan del Progreso and Olintla, and in the descriptors related to the tree presented averages of: ATL (12.92 m), APR (3.33 m), DAP (53.18 m) and in those related to the leaves: LL (33.68 cm), AL (9.68 cm), R L/A (3.49), AF (174.97 cm^2), P (5.69 g); they are present in acahual vegetation or shade coffee system, with fruit of spheroid shape and two accessions presented a round apex, this group is similar to a group reported by Gaona-García, Alia-Tejacal *et al.* (2008) in the southwest region of the state of Morelos.

Conglomerate 5 had 19 accessions from the municipalities Caxhuacan, Cuetzalan del Progreso and Huehuetla; this conglomerate presented the highest data in ATL (16.02 m), APR (3.67 m), LL (40.68 cm), AL (11.39 cm), AF (238.99 cm^2) and P (7.34 g), the accessions were found in the shade coffee system with pyramidal tree growth habit

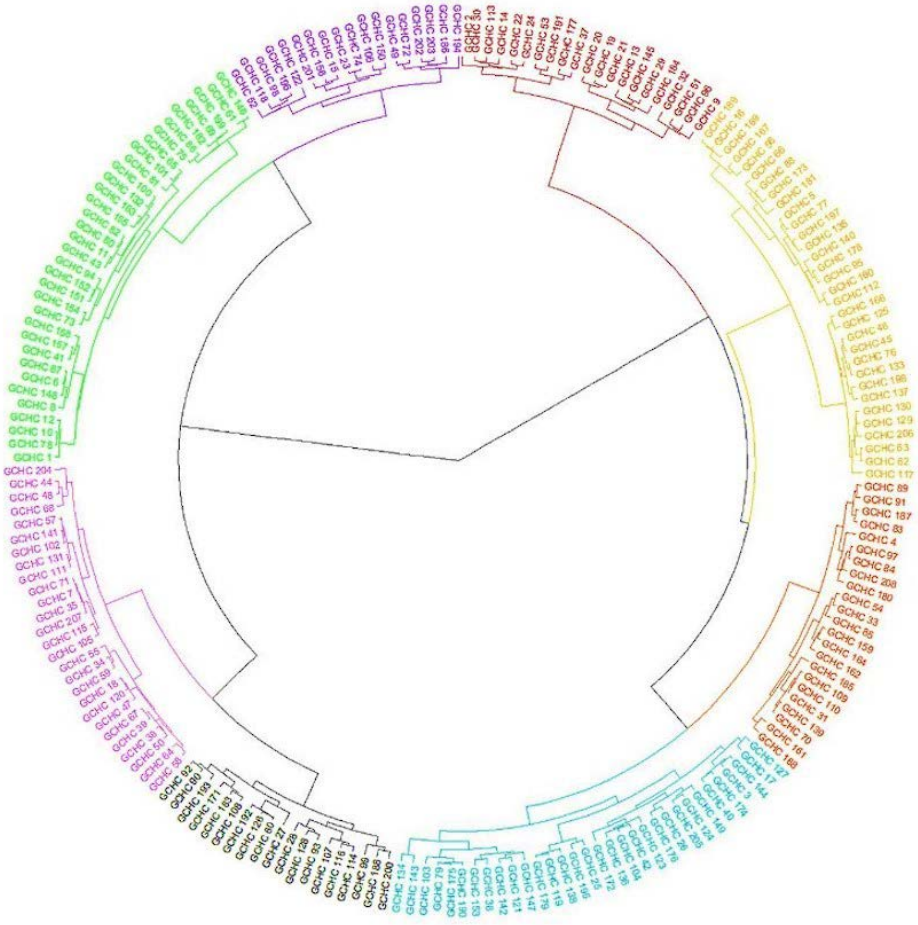


Figure 3. Dendogram constructed through Ward's accumulative hierarchical algorithm and Gower distances of 15 morphological variables in *P. sapota*, eight conglomerates are observed.
Source: Prepared by the authors with Rstudio v.4.1.1.

and shape of the crown, with obovate shaped leaves and spheroid fruits; these growth conditions of the tree can be influenced by the management of the system where they belong and favor the conditions such as nutritional state, relative opening of the crown, initial height of the seedling, and size of the seed (Ricker *et al*., 2000)secondary forest, and open pasture in Los Tuxtlas (Veracruz, Mexico. Conglomerate six had 27 samples from the municipalities of Huehuetla, Tuzamapan de Galeana, Jonotla and Cuetzalan del Progreso; in this group the lowest data in LL (30.33 cm), RL/A (3.23), AF (156.58 cm^2) and P (4.9 g) were found, with native accessions in acahual vegetation with pyramidal tree growth habit and crown shape, with leaves of oblanceolate shape and ellipsoidal fruit. Conglomerate seven presented 34 individuals from the municipalities Huehuetla, Ixtepec, Cuetzalan del Progreso and Tenampulco; this group presented the lowest data of DAP (31.28 cm), and in addition they are of low growth habit with ATL of 10.93 m with an APR of 3.05 m; they are accessions introduced to acahual vegetation with pyramidal growth habit and crown shape, oblanceolate leaves, and ellipsoid fruits. Conglomerate eight presented 18 individuals from the municipalities of Huehuetla and Cuetzalan del Progreso; this

conglomerate had the lowest data in descriptors ATL (10.12 m) and AL (9.44 cm), with samples introduced in backyard productive systems of pyramidal growth tree habit and crown shape, oblanceolate leaves, and spheroid fruits; this last trait is similar to what was reported by Bayuelo-Jimenez & Ochoa (2006) in some genetic materials of *P. sapota* from the center-west of Michoacán, and because of their nature of being introduced they can be scarcely adapted to the conditions of these two municipalities with a low height.

CONCLUSIONS

In the Sierra Norte of Puebla, in the Totonacapan Poblano region, a high morphological variability was detected in *P. sapota* where eight conglomerates of mamey were differentiated. The descriptors that contributed most to the grouping were those related to the shape, height and size of the tree, as well as the weight, size and shape of the leaf; regarding the fruit, the descriptor that contributed most was the shape. These results could be useful in programs for conservation, improvement and exploitation of mamey.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Implementation of stubble in agricultural production: A review

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ABSTRACT

Objective: To elucidate the benefits that agricultural residues or stubble have in agricultural activities, through a bibliographic and objective review that could help to make the actors of agricultural sector aware of the benefits and their correct implementation in production systems.

Design/methodology/approach: A detailed review of scientific articles from the main academic databases and repositories was performed. We took into account aspects such as the use of stubble as plant cover; effects and changes in physical and chemical structure of soil; crop yield and use for livestock feeding.

Results: The use of stubble has beneficial effects on agricultural activities. In agriculture, they have positive effects for the proliferation of beneficial organisms and assimilation of nutrients, which are easily absorbed by plants. Consequently, crop yield is maximized both in quantity and quality terms. In livestock sector, the stubble implement provides nutrients such as proteins, ashes and vitamins, which together with conventional diets, reduce production costs and improve meat and milk production.

Limitations on study/implications: The repertoire of scientific articles related to stubble is very broad, making it difficult to assimilate the information. In Mexico, particularly, more research is needed to inform farmers of the possible uses of stubble.

Findings/conclusions: The use of stubble in agricultural activities generates benefits at ecological and economic levels, which ensure the sustainability and resilience of agroecosystems. Nutritional content of stubble is a function of the species and/or varieties of agricultural species. The lack of knowledge in the management of stubble leads to these being incinerated, releasing particles and toxic and polluting substances for the environment, further favoring the conditions for greenhouse effect.

Keywords: stubble, vegetation cover, yield, sustainability, resilience.

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INTRODUCTION

Stubbles are by-products derived from agricultural activities, which remain on the ground after harvesting and are mainly used as plant covers and for livestock feed [1]. Stubble is mainly linked to the cultivation of corn. The Consultation Agrifood Information System [2], registered 17.5 million tons of corn stubble (15.5 million tons), wheat, triticale, sorghum and oats.

Image by S. Hermann / F. Richter at Pixabay

Cereals are grass-like plants that form the basis of food in many regions of the world. Most important cereals include maize, rye, wheat, rice, sorghum and oats. Maize is the most cultivated and important cereal in Mexico. At the time of corn harvest, the cob is the usable element of the plant, the remaining elements such as stems and leaves, which are considered foliar residues [3].

Agriculture is an economic activity carried out in a large part of the Mexican territory, with international importance. When fields are homogeneous with flat surfaces, agricultural practices such as planting and harvesting are easier to carry out. In rocky or steeply sloping surfaces, besides making these agricultural tasks more difficult, the rain cause erosion, and therefore the soils gradually decrease their productive capacity due to the loss of nutrients.

Soil erosion is one of the many problems facing agriculture and is a serious threat to food sustainability [4]. Cultivating on eroded or degraded soil will result in a poor harvest in terms of quantity and quality. Soil erosion due to hydric factors is the most important type of erosion and is the one that causes the greatest losses, especially in soils that do not have vegetal covers and those that are found on slopes [3].

Residue cover can reduce evaporation of water from the soil surface, thus conserving moisture and increasing the number of days a crop can survive in the absence of rains. Most farmers are unaware of the usefulness that stubble can have, so they resort to burning it, causing pollution to the environment and affecting the nutritional balance of the soil. When stubble is burned, volatile compounds such as carbon dioxide, carbon monoxide, nitrogen oxide, sulfur oxide, methane and aerodynamic particles are produced [5].

Cattle feeding is another secondary activity for farmers that can be economically draining. Stubble also contains nutrients that can be incorporated into animal feed. However, it is important to consider that they are not a substitute for balanced feed, but rather a complement that can help reduce costs. For animal feeding, stubble must be processed: cut, chopped and ensiled, which may require certain machinery or equipment that can be expensive [6].

From an ecological and socioeconomic point of view, the use of stubble becomes essential in sustainable agricultural systems. However, its use is limited by the lack of knowledge and technical advice to farmers. The objective of this research was to elucidate the benefits that agricultural residues or stubble have in agricultural activities. With that aim, we performed a deep bibliographic and objective review that may help to make stakeholders of agricultural sector aware of the benefits and their correct implementation in production systems.

MATERIALS AND METHODS

For this review the following sources were surveyed: JSTOR, ProQuest, Wiley Library, Taylor & Francis, Redalyc, World Wide Web, BioOne, PubMed, Natural Journal, Dialnet, ACS Publications, Springer, Annual Reviews, Scielo and MDPI. The topics of interest were related to agricultural implementation, plant cover, animal production, crop yield and pollution. Information relevant to the searches carried out in the sources consulted is shown in Table 1. It is important to mention that the use of stubble dates back several centuries. Furthermore, the searches also yield stubble information related to disciplines such as sociology, where its use in agricultural sector is not scientifically based.

Table 1. Academic repositories and stubble search features.

Database	Number of publications	Interval of years	Features
JSTOR	18,406	1699-2022	Focused on agricultural sciences. Scientific articles (68%) and book chapters (25%) predominated.
ProQuest	11,056	1805-2020	Dominate scientific articles related to agriculture.
Wiley Library	10,800	1818-2020	Nealy 90% of the sources were scientific articles; 30% of which linked to agricultural production.
Taylor & Francis	5,038	1901-2020	About 50% of scientific articles are related to environment, 15% to geography and 14% to earth sciences.
Redalyc	1,619	2001-2021	Articles mostly in Spanish (95%), mainly Mexico (32%), Colombia (25%) and Argentina (10%). Of them, 30% were related to agrosciences, 9% to biology and 6% to veterinary medicine.
World Wide Web	1,356	? - 2022	Researches performed in the USA acomplisgshed 580 articles. Of them, 30% refer to soil science and management.
BioOne	1,131	1965-2022	Scientific articles related to agriculture and conservation.
PubMed	415	1943-2022	Scientific articles related to agricultural research.
Nature Journal	395	1873-2022	Scientific research focused on agricultural systems.
Dialnet	327	1964-2021	Approximately 77% are scientific articles; 11% is represented by thesis.
ACS Publications	214	1894-2022	Scientific articles related to agriculture and food chemistry.
Springer	187	2005-2022	Scientific articles stand out in the area of agronomy, sustainable development and ecology.
Annual Reviews	100	1934-2019	About 26% were scientific articles related to entomology.
MDPI	79	1966-2022	Of the total, 90% were scientific articles focused on agricultural production.
Scielo	52	2000-2020	Articles are associated with agricultural and biological sciences.

RESULTS AND DISCUSSION

Agricultural implementation and vegetation cover

Agriculture is an economic activity carried out in a large part of the Mexican territory. On flat terrain, activities related to the field and the implementation of agricultural machinery are usually facilitated, but not so on irregular, rocky terrain and with steep slopes. Water erosion in soils induces leaching of nutrients and loss of essential particles (sand, silt, clay) causing low fertility and low productivity of soils with agricultural use [4].

The value of the use of stubble can have significant contributions to producer's economy [7]. In Morocco agricultural systems, cereal stubble can represent 25% of the total production value, taking into account that the irrigation and/or rain conditions are adequate.

Cong *et al.* [8] showed that thickness of ideal mulch or plant cover is up to 20 cm, thus ensuring the retention of a greater amount of water and an increase in temperature, which are factors that regulate and improve seed germination and optimal seedlings growth.

Huang *et al.* [9] evaluated the soils fertility of Loess Plateau, China, where the incorporation of stubble increased nitrogen levels and consequently grain yield of cereals. This translates into increased water and nutrient absorption capacity, which will later provide vigor and productivity to plants, as well as tolerance to abiotic factors, such as frost or drought. Stubble cover is an ideal habitat for arthropods, which feed on weed seeds and release organic matter.

Addition of soybean residues [*Glycine max* (L.) Merr.] and nitrogenous fertilizers in a wheat rotation (*Triticum aestivum* L.) increased NH_4^+ (ammonium) concentrations by 153%, and relative abundance of saprophytic fungi that contribute to the mineralization of plant compounds, which is important since they form the so-called humus. Humus is a mixture of organic compounds that has positive effects on soils by maintaining physical-chemical structure and providing availability of nutrients; Additionally, the amount of ammonium and beneficial organisms increase significantly with the application of nitrogenous fertilizers [10].

In subtropical regions, there is incomplete information on the long-term effects of tillage or soil removal, the use of stubble, and the application of nitrogen fertilizers on the soil. Hernández-Flores *et al.* [11] examined soil effects with conventional tillage, zero tillage, stubble management, stubble burning, and nitrogen fertilization to analyze the impact on microbial flora growth, glomalin, and nitrogen mineralization. Results showed that the aforementioned variables significantly increased with stubble retention and external nitrogen inputs; the increases in glomalin (glycoproteins) had statistically significant increases, this is due to the fact that this compound is a natural sequestrate of carbon and nitrogen, which are essential elements in the synthesis of biomolecules.

Stubble can be used to minimize the problems caused by water erosion, since they work as soil protectors when used as plant covers or mulches [3]. Hernández-Flores *et al.* [11] studied Australian tropical regions over the last 50 years to assess the effects of incorporating stubble into soil and zero tillage. They showed that microbial activity of soil improved due to mineralization of nitrogen, which makes soil nutrients available and can be used by plants [12].

Animal production

Agroecosystems usually use products derived from crops for certain activities related to agriculture. Cereal crops bring about not only grans but also organic residues that can be used as feed for livestock. These residues are important sources of fiber, proteins and minerals [3]. The amounts and percentages of nutrients depend on the crop in question and the parts used as stubble, whether stems, leaves and roots, as well as genetic interactions of livestock with the environment and stubble management [13].

In sorghum, research has been carried out on genetic breeding to release varieties with a dual purpose, grain and stubble production. Thomas *et al.* [14] evaluated dual-purpose sorghum genotypes in Nicaragua and analyzed agronomic variables, highlighting a high

protein content in fresh leaves with high heritability indices (≥0.5). The use of stubble has benefits related to chewing, rumination, food particles and liquids in the digestive system [15]. In maize, it is recommended to chop stubble to a size close to 2 cm, the most suitable in practical terms and beneficial for sheep diet [16].

Arellano-Vicente *et al.* [17] analyzed the characteristics of maize stubble compared to different types of weeds in the diet of cattle in La Frailesca, Chiapas, Mexico. Dry matter availability is higher in maize stalks compared to other plant components (leaves, bracts, grain, panicle, and cob). At the end of stubble use, statistical differences were found in the percentage of crude protein and ashes compared to the first five and half days of stubble use. The elements richness of stubble is essential to improve the quality of animal diets.

In Western Australia, the dietary effects of barley (*Hordeum vulgare* L.), lupin (*Lupinus* spp.), canola (*Brassica napus* L.) and wheat stubble on sheep nutrition were evaluated. It was quantified that sheep obtained a weight gain of 89 g day^{-1}, taking as reference the metabolizing energy of wheat (5.9 MJ metabolizable energy kg^{-1} dry matter); barley residues were higher by 5%; 19% higher in lupine and 5% lower compared to canola [13].

Livestock production and rice cultivation are important activities in Thailand. In goat diets it is important to implement cereal stubble. Vorlaphim *et al.* [18] elaborated a diet based on rice stubble plus the incorporation of urea and *Pleurotus ostreatus* to increase the nutritional value. The combination of these three elements had significant effects on goat body weight gain, which was 82.3% higher than the diet that only included rice stubble. The total weight gains with the mixed diet (rice stubble, urea and *P. ostreatus*) was 85% higher compared to the exclusive feeding of rice stubble. However, corn stubble can also contain toxins if not managed properly. López *et al.* [19] reported aflatoxins and mycotoxins, such as deoxynivalenol (0.7 mg kg^{-1}) and T-2 toxin (4.1 mg kg^{-1}) in grain and zearalenone (3.0 mg kg^{-1}) in stems and leaves, by means of two-dimensional thin-layer chromatography. This means a health risk to both animals and humans when consuming contaminated meat.

Crop yield

Rice cultivation is one of the main foods included in the diets of the Asian continent, with China, Bangladesh, Indonesia and India producing more than 500 million tons of rice a year [20]. Consequently, the amount of waste generated by the crop can be a problem that could be difficult to manage in these countries.

In India, technologies have been implemented for the management of rice stubble, where by means of machinery these can be used for vegetable covers or for silage. When rice stubble is managed efficiently, the cost of inputs per acre (0.404 hectares) decreases and crop yield increases significantly, since stubble contributes with organic matter and nutrients to the soil. These contributions function as a substitute in the use of chemical fertilizers, which tend to have a higher cost. However, not all producers have access to agricultural machinery, so it is recommended that producers are organized in cooperatives to reduce the costs related to the acquisition of machinery to handle stubble [21].

China is one of the main producers of rice and wheat worldwide, since it constitutes the staple food in its culture. When stubble is left on the ground, it can cause problems

for seed germination, therefore, it should be incorporated after sowing or when the plants have a considerable height. Xu *et al.* [22] determined that the length of the straw (30 mm, finely chopped), the quantity or height of the incorporated stubble significantly influence the yield of the rice crop. Keil *et al.* [23] developed a machine that has the ability to sow wheat on a thick layer of stubble, thus facilitating the timely sowing of cereals, showing a significant saving of 120 dollars per hectare in Punjab, Pakistan. A promising new approach is the *Happy Seeder*, which combines the functions of stubble mulching and seed sowing in one machine. Stubble is cut and collected in front of the seeding tines, flattening into bare soil, and deposited behind the drill as mulch allowing wheat to be sown on rice stubble [24].

Wheat and soybean stubbles were applied at different levels (0.5%, 1% and 2%) to cultivate cucumber, with both types of stubble significantly increasing soil porosity and thus improving aeration and drainage. The treatment with wheat stubble at 2% decreased the electrical conductivity (EC), the opposite was the case for treatments with soybean stubble. By increasing the EC, plants have an easier time absorbing and assimilating nutrients from the soil. In general, wheat and soybean stubble increased yield cucumber crop in quantity and quality [25].

Stubbles also have the ability to incorporate nitrogen into soil, which is one of the main elements for synthesizing proteins. Rice yield is achieved with the contribution of nitrogen from stubble and reduce the cost of chemical fertilizers [26]. The combination of stubble with chemical fertilizers such as urea significantly increases wheat yields and minerals such as nitrogen and organic carbon [27].

A correct stubble decomposition increases soil microbial flora. To achieve such a result, a carbon:nitrogen ratio of 30:35 and a moisture content of 60-65% are required. For stubble decomposition the following genders stand out: *Chaetomium*, *Myrothecium*, *Trichoderma*, *Fusarium*, *Aspergillus*, *Penicillium*, *Trichonympha* and *Clostridium* [28].

Phosphorus (P) is present in stubble and can be released into soil as soluble P or assimilated by microorganisms that carry out the mineralization process. Noack *et al.* [29] analyzed stubble of various cereals in South Australia finding 1-5 kg ha^{-1}. Using nuclear magnetic resonance spectroscopy, it was determined that 50% of P was in the form of soluble orthophosphate, which is easily assimilated by plants and organisms; the remaining 50% phosphorus belonged to phospholipids, nucleic acids and pyrophosphates.

Contamination

Stubble burning in India generates around 150 million tons of CO_2, 9 million tons of CO, 250 thousand tons of sulfur oxide and one million tons of particulate matter (PM2.5 y PM10) [30]. Smog can cause health damage, such as skin and eye irritation, cancer, as well as neurological, coronary and respiratory diseases [5]. When stubble is burned, the amount and efficiency of nitrogenous fertilizers (urea), diammonium diphosphate (DAP, 18-46-00) and monoammonium phosphate (MAP, 11-52-00) are reduced [31].

Additionally, stubble can also be used to generate charcoal and as compost elements, it can even be added to cement mixtures and brick production [5]. In Mexico, studies were also carried out on ethanol production based on corn stubble, despite the fact that

the addition to gasoline is effective, the production costs are still high and they are not profitable. In the near future, we expect to develop technology to reduce production costs. It is important that countries have food and energy sovereignty to ensure development, security and socioeconomic prosperity [32].

CONCLUSIONS

Stubble use in agricultural activities generates benefits at ecological and economic levels, which ensure the sustainability and resilience of agroecosystems. The nutritional content of stubble depends on the species and/or varieties of agricultural species. The lack of knowledge in the management of stubble leads to these being incinerated, releasing toxic particles and polluting substances for the environment, further promoting the conditions for greenhouse effect.

Edaphic erosion problems can be mitigated with the use of plant covers or mulches, since these improve humidity conditions, soil physical properties, and provide assimilable nutrients for plants. Moreover, they function as a habitat for arthropods, which reduce the incidence of weeds because they feed on their seeds.

The results of this review show that stubble, together with the addition of other organic compounds and external sources of fertilizers, significantly increase crop yields. Incorporation of stubble to cattle feed has beneficial effects on body weight gain and influences cost reduction. Although not addressed in this article, stubble has also applications in diverse industries including construction, architecture, manufacture and bioenergy.

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AGRO PRODUCTIVIDAD

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Yield and quality of Guinea grass cv. Mombasa according to plant spacing and season of the year

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ABSTRACT

Objective: The purpose was to evaluate the yield, the chemical composition and the *in vitro* digestibility of Guinea grass (*Megathyrsus maximus*, Jacq.) cv. Mombasa according to plant spacing and season of the year.

Design/methodology/approach: The Mombasa grass was stablished at spacing between plants of: 25×25, 50×25, 70×25 and 100×25 cm between rows and plants, respectively, and with broadcasting (traditional planting); cutting was conducted in cool, dry and rainy seasons. A factorial split-plot design in randomized blocks was used, with four replications.

Results: The dry matter yield (DMY) of Mombasa grass was equal ($p>0.05$) in cool and rainy seasons and lower ($p<0.05$) in dry season (27.2, 27.5 and 12.7 kg ha^{-1} d^{-1}, respectively). The crude protein (CP) increased with greater plant spacing ($p<0.05$). The fodder of the dry season showed a higher ($p<0.05$) CP content than cool or rainy seasons (10.5, 9.7 and 8.7%). The content of all fiber fractions, except lignin, did not differ between plant spacing ($p>0.05$).

Study limitations/implications: The season of the year affects the quality of tropical grasses. On the other hand, there is no information about the effect of plant spacing on the quality of Mombasa grass.

Findings/conclusions: The highest DMY was in the cool season at 75×25 cm and 100×25 cm. The fodder harvested in the dry season showed lower fiber content in cell walls and higher *in vitro* digestibility ($p\leq0.05$), but lower DMY. To ensure proper yield and quality, it is recommended to establish the Guinea grass cv. Mombasa at greater plant spacing, such as 75×25 cm or 100×25 cm.

Keywords: digestibility, *Megathyrsus maximus*, morphology, tropical grasses, seed yield.

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INTRODUCTION

Grasses of the genus *Megathyrsus* are extremely important fodder species for bovine livestock production in the Mexican humid tropics (Hernández *et al.*, 2020). The Guinea grass (*Megathyrsus maximus*) cv. Mombasa (Poaceae) presents yields of 650 kg ha^{-1} of dry matter in the dry season and 1,696 kg ha^{-1} in the rainy season (Ramírez *et al.*, 2010). The fodder presents 11.6% of crude protein (CP), 68.6% of soluble neutral detergent fiber (NDF) and 41.1% of soluble acid detergent fiber (ADF) (Molina *et al.*, 2015), and degradability of the dry matter of 47% (Almaraz-Buendía *et al.*, 2019). However, factors such as the

season of the year and the population density of plants affect the yield and nutritional quality of the grasses (Freitas *et al.*, 2012; Pereira *et al.*, 2012). In a study it was observed that the Guinea grass presented better chemical composition in the early rainfall season, followed by the winter season, and the worst chemical composition in the dry and late rainfall season (Juárez *et al.*, 2009); for their part, Muñoz-González *et al.* (2016) reported that the chemical composition was better in the dry season. Although the density of plants has an effect on the biomass production of Guinea grass (Freitas *et al.*, 2012; Pereira *et al.*, 2012), there is no evidence of its effect on the quality of the fodder (Freitas *et al.*, 2012). Manipulating the plant density by varying the distance between rows can be an option to improve the yield and quality of the Guinea grass.

In Mexico, information about the effect of plant spacing and season of the year on the yield and nutritional quality of Guinea grass is non-existent. Therefore, this study evaluated the effect of plant spacing and season of cutting on the biomass yield and nutritional quality of Guinea grass cv. Mombasa harvested at 30 d of regrowth.

MATERIALS AND METHODS

Study area

The study was carried out under rainfed conditions in lands of La Posta Zootecnica at Universidad del Papaloapan Campus Loma Bonita, Oaxaca, Mexico (18° 06' 35" LN and 95° 52' 47" LW at 25 masl). The climate is warm, sub-humid with summer rains (Aw). The mean annual precipitation is 1,845 mm and the average temperature is 24 °C (INEGI, 2016). Soil in the plot is crumbly sandy texture with pH of 3.68 (strongly acidic) and 1.03% of organic matter. The climate data found in each period of evaluation are presented in Figure 1.

Grassland establishment

The fodder samples were taken from a Guinea cv. Mombasa grassland established in August 2015. Seed of the Guinea grass [*Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs] cv. Mombasa was used to establish the grassland. Fertilization was done only at the

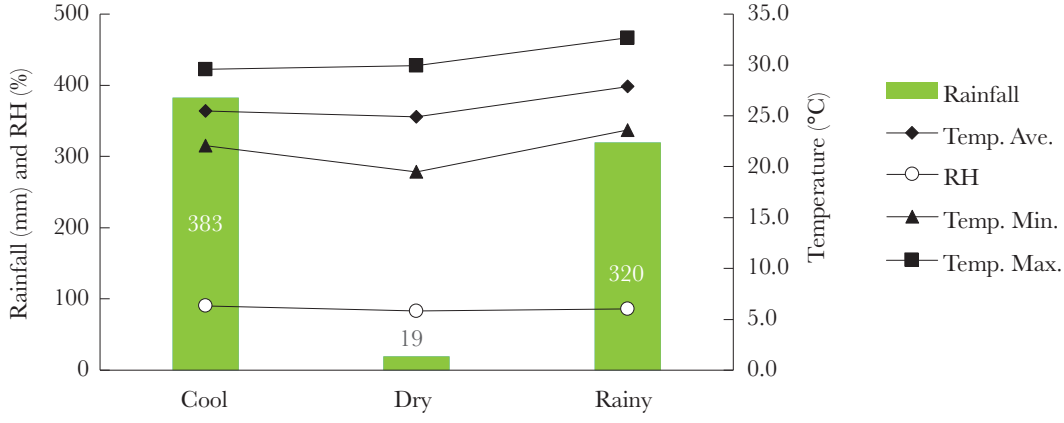

Figure 1. Climate data obtained from the meteorological station of the Military Air Force No. 8, located in Loma Bonita, Oaxaca. Periods: Oct-Nov/2015, Feb-Mar/2016, Jun-Jul/2016.

beginning with the N:P:K 50-50-50 formula. Sowing was done manually: by broadcasting (traditional) on flat terrain, at 5.5 kg ha^{-1} of seed, and in rows at spacing of 25, 50, 75 and 100 cm between rows, seeking 25 cm of distance between plants.

Experimental plots

The grassland was divided into 20 experimental plots to apply the treatments. Each treatment had four experimental units of 12 m^2 each. Two uniformity cuts were made in the grassland: September 1st and October 9th.

Treatments and experimental design

The treatments consisted of four distances between plants: 25×25 cm, 50×25 cm, 75×25 cm and 100×25 cm of separation between rows and plants, respectively, and broadcasting (traditional sowing). To measure the effect of the season of the year three fodder cuts were made at different dates: 17/11/2015, cool season; 21/03/2016, dry season; 21/07/2016, rainy season. All the cuts were made at 30 d of regrowth and at 5 cm above ground. A factorial experimental design was adjusted into divided plots, in completely randomized blocks; plant spacing constituted the main plot and season of the year the sub-plot, with four replications per treatment.

Variables evaluated

Yield variables. To determine the dry matter content (DM, %) and to calculate the DM yield (DMY) in each plot and in each season of the year, all of the grass present was cut at 1 m^2 and the weight in green was recorded. Then, the samples were taken to a drying furnace at 65 °C for 48 h and the dry weight was recorded. The humid content and DM were obtained from the difference between green weight and dry weight. The rate of DMY was expressed in kilograms per hectare per day (kg ha^{-1} d^{-1}). Then, all the samples were ground to 1 mm in a Wiley® mill for their later chemical analysis and digestibility study.

Chemical composition variables. The following were estimated: total dry matter (105 °C/12 h; AOAC, 934.01); organic matter and ash (600 °C/6 h; AOAC, 942.05); crude protein content (CP, % of N × 6.25; AOAC, 978.04). Soluble neutral detergent fiber (NDF) and soluble acid detergent fiber (ADF), and acid detergent lignin (ADL) were calculated sequentially in the equipment Ankom200® using filter bags F57® with pore size of 25 μm. To determine the ADL, after the ADF test, the bags were immersed in H_2SO_4 at 72% for three hours.

Digestibility variables. The *in vitro* digestibility of DM (IVDMD) and of NDF (IVNDFD) were determined by incubating for 48 h the duplicated samples of each sample in Ankom F57® filter bags in the DaysiII® incubator. The methodology proposed by the company Ankom® was used for these tests. The ruminal culture that was used was a mixture made with the inoculum taken directly from the rumen of three recently slaughtered cows in the municipal meat processor. It is worth pointing out that the diet of these animals was based 100% on grazing of tropical grasses.

Statistical analysis

The data from each variable were analyzed independently, considering plant spacing, season of the year, and their interaction as fixed effects, while the block was randomized. Means comparison was done with Tukey's test at significance level $p \leq 0.05$. The analyses were conducted with the SAS software (SAS Institute, 2004).

RESULTS AND DISCUSSION

The interaction of plant spacing × season of the year for DMY was observed. All the variables studied differed as a result of the season of the year, while the DMY and the CP and ADL contents differed in response to plant spacing.

Dry matter yield (DMY)

In this study, the DM content of Guinea grass cv. Mombasa was 24% and it was lower in plant spacing of 100×25 cm. The DM content was higher in the dry season ($p<0.05$), intermediate in the rainy season, and lower in the cool season (Table 1).

The DMY was 22.5 kg ha^{-1} d^{-1} on average and it was 27.2, 12.7 and 27.5 kg ha^{-1} d^{-1} in the cool, dry and rainy seasons, respectively. The DMY showed an interaction between plant spacing × season of the year ($p=0.0167$); in the cool season the highest DMY was seen with distances of 100×25 cm and 75×25 cm and in the dry season with 100×25 cm (Figure 2).

Table 1. Chemical composition and digestibility of Guinea grass cv. Mombasa established at five distances between plants and harvested in three seasons of the year, in Loma Bonita, Oaxaca, Mexico.

Item	Plant spacing (cm)					SEM	p-value
	Broadcast	25×25	50×25	75×25	100×25		
DM	24.9^{a}	25.0^{a}	24.6^{a}	23.9^{ab}	22.8^{b}	0.440	0.0036
CP	9.1^{b}	9.1^{b}	9.3^{b}	9.8^{ab}	10.4^{a}	0.205	<.0001
NDF	66.9	66.6	66.1	67.1	67.4	0.360	0.1483
ADF	34.5	34.8	34.6	35.3	35.7	0.435	0.2821
ADL	2.97^{ab}	2.60^{b}	3.36^{a}	3.46^{a}	3.29^{a}	0.153	0.0014
IVDMD	70.4	69.7	69.0	69.5	68.9	0.588	0.4116
IVNDFD	55.6	54.6	53.3	54.8	53.9	0.797	0.3347
	Season of the year						
	Cool		Dry		Rainy		
DM	20.9^{c}		27.1^{a}		24.8^{b}	0.341	<.0001
CP	9.4^{b}		10.5^{a}		8.7^{c}	0.159	<.0001
NDF	68.5^{a}		63.5^{b}		68.5^{a}	0.279	<.0001
ADF	36.3^{a}		31.3^{b}		37.3^{a}	0.337	<.0001
ADL	3.38^{b}		2.16^{c}		3.87^{a}	0.119	<.0001
IVDMD	67.9^{b}		74.3^{a}		66.3^{c}	0.456	<.0001
IVNDFD	53.2^{b}		59.4^{a}		50.8^{c}	0.618	<.0001

Values with different letter in the same row are statistically different (Tukey, $p \leq 0.05$); SEM=standard error of the mean.

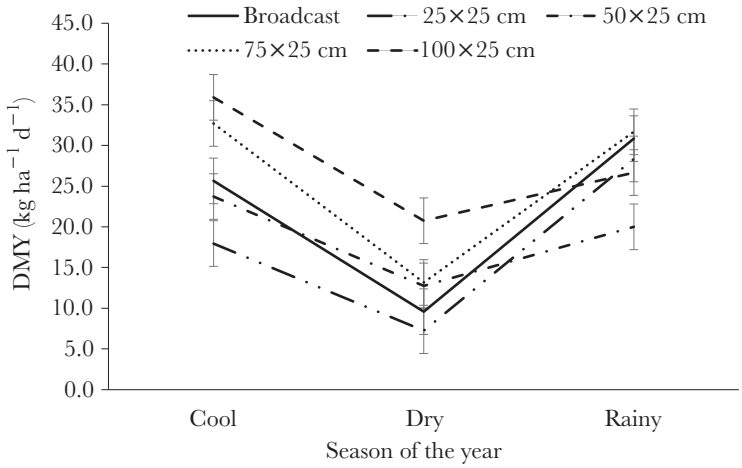

Figure 2. Dry matter yield (DMY) of Guinea grass cv. Mombasa established at five distances between plants and harvested in three seasons of the year, in Loma Bonita, Oaxaca, Mexico.

The lower DMY obtained in the dry season was attributed to the lack of rainfall recorded in this period (Figure 1). Water availability and temperature influence the development and nutritional quality of tropical fodder plants, in addition to modifying their morphological, physiological and biological structure (Verdecia *et al.*, 2012). Figure 2 shows for the rainy season a lower variation between plant spacing for DMY, which is explained by the high temperatures and relative humidity, and by the amount and better distribution of rainfall that happened in this period. In addition, it shows that the broadcasting treatment (higher plant density m^2) responds better in the rainy season (period of greater precipitation and higher temperatures, Figure 1), and the inverse is observed with the distance of 100×25 cm (lower plant density). This confirms the relationship between the number of plants and the amount of water available.

The highest DMY was obtained with the distances of 100×25 cm, 75×25 cm and by broadcasting (27.8, 25.8 and 22.0 kg ha^{-1} d^{-1}, respectively). In their study, Basile *et al.*, (2019) evaluated distances between plants of 20×20, 40×40, 60×60 and 80×80 cm, and they observed that the highest DMY (6.4 t ha^{-1}) was found at spacing of 60×60 cm. The highest biomass yield is a common condition in tropical grasslands with low plant density m^2 (Hare *et al.*, 2014; Basile *et al.*, 2019); however, in a study it was concluded that plant density showed low effect on the DMY (Freitas *et al.*, 2012).

The DMY, as a result of the effect of season of the year observed in this study, is expected, since they are patterns of growth that are characteristic of tropical grasses (Ramírez *et al.*, 2009). Regarding this, Ramírez *et al.* (2009) reported in their study an increase of 79.6% in the fodder yield of the Mombasa grass harvested in the rainy season compared to the dry season. Finally, it is important to highlight that the DMY obtained in this study was low (Figure 2), which is explained primarily by the lack of fertilization of the plots and the acid pH (3.68) of the soil. In the same study area, Sánchez-Hernández *et al.* (2019) reported 81.8% of the increase in DMY of Mombasa grass, harvested at 29 d of regrowth, going from a fertilization N:P:K formula of 00-00-00 to 140-20-00. For their part, Ramírez *et al.* (2009) reported a growth rate of 21.4 and 118.8 kg ha^{-1} d^{-1} of DM at similar days of

regrowth in the dry and rainy seasons, while Hernández *et al.* (2020) reported values of 680±240 and 2,270±240 kg ha^{-1}, respectively. Fodder accumulation as a result of the season of the year confirms the seasonality in biomass production in tropical grasses.

Chemical composition

Crude protein (CP). The CP content varied due to plant spacing and was higher with distances of 75×20 and 100×20 cm. The CP was the highest in the dry season ($p<0.05$), intermediate in the cool, and lowest in the rainy season (Table 1).

The highest content of CP observed at 75×25 and 100×25 cm is explained by the strong activity of nitrogen fixation by the plant, which is more intense at the beginning of the vegetative stage and is normally accompanied with a low content of DM and a higher proportion of leaf (Ramírez *et al.*, 2009).

Typically, when increasing the biomass and the DM, the CP tends to decrease from the effect of dilution of the plant's nitrogen (Patiño *et al.*, 2018), fact that results from the effect of maturity (Van Soest, 1994). However, the lowest content ($p<0.05$) of CP observed in the cool and rainy seasons, in comparison to the dry season (Table 1), is explained by the higher accumulation of biomass that occurs in these two seasons promoted by higher rainfall, fact that causes greater dilution of the nitrogen (Muñoz-González *et al.*, 2016). In this regard, Muñoz-González *et al.* (2016) evaluated three fodder grasses in three seasons of the year in the southeast of Mexico, and they observed that the CP was higher in the dry season (13.9%), intermediate in the cool season (10.8%) and lower in the rainy season (9.3%), behavior similar to the one obtained in this study. For their part, Verdecia *et al.* (2012) reported a negative correlation between the availability of water and the CP content in the fodder.

Normally, tropical grasses present low content of CP. In our study, the Mombasa grass harvested at 30 d of regrowth presented values between 9.1 and 10.4%; however, ranges were reported of 8.1% (Santos *et al.*, 2014) to 12.4% (Fernandes *et al.*, 2014) at similar days of regrowth. Finally, it is important to highlight that in this study the Guinea grass cv. Mombasa presented levels of CP over 7%, value required as minimum for there to be an adequate digestion in a bovine (Van Soest, 1994).

Cell wall components. In general, the cell wall components varied in function of the season of the year. The NDF, ADF and ADL were lower ($p<0.05$) in the dry season and higher in the cool and rainy seasons. The ADL showed an effect from spacing between plants and season at a distance of 25×25 cm and the lowest value was seen (Table 1).

The fact of observing lower content of fiber components in the Mombasa grass during the dry season can be explained by the lower biomass production, consequence of the lack of rainfall. On the contrary, in the cool and rainy seasons, where there was higher precipitation, a better development of the plant was promoted and as consequence an increase in the fiber content, behavior that is characteristic of tropical grasses (Muñoz-González *et al.*, 2016).

Edaphoclimatic factors exert a strong effect on the yield and the nutritional quality of the grasses (Verdecia *et al.*, 2012). In this study, in addition to the lack of fertilization, another limiting factor was rainfall, particularly in the dry season (Figure 1). There is a

strong correlation between the rain factor and the quality of the grasses. Another study showed that the cell wall components were positively correlated to the rain factor, while the digestibility presented a negative correlation (Verdecia *et al.*, 2012).

Other authors report fiber values different from those seen in this study, which can be due to the different conditions in which the experiments were carried out, mainly the days of regrowth when they were harvested (Santos *et al.*, 2014; Patiño *et al.*, 2018; Hernández *et al.*, 2020). In these studies the effect that the days of regrowth have on the cell wall components can be appreciated.

It is possible for the effect of season of the year on the behavior of the fiber fractions (NDF, ADF, ADL) to be explained by the intensity of development of the plants in each season. It is documented that the physiological response of the plant in the accumulation of cell walls is strongly influenced by environmental factors such as temperature, soil humidity and luminosity. These factors influence biomass accumulation, leaf:stem ratio, and maturity of plants (Verdecia *et al.*, 2012). It is possible that differences in the cell wall contents found in this study, as a result of season of the year, are because of the influence of these factors, such as temperature which was higher in the rainy season, intermediate in the cool season, and lower in the dry season (Figure 1). It is known that high temperatures promote an increase in lignin accumulation, and it seems that the enzymes that synthesize lignin present greater activity at higher temperatures (Van Soest, 1994). The variation in the cell wall components is consequence of the intensity and combination of factors present in each study (Van Soest, 1994).

In vitro digestibility

The IVDMD and IVNDFD only changed from the effect of the season ($p<.0001$). The values of IVDMD and IVNDFD were higher in the dry season, intermediate in the cool season, and low in the rainy season (Table 1).

The higher values of IVDMD and IVNDFD observed in the dry season are attributed to lower contents of ADF and ADL that the Guinea grass cv. Mombasa presented in that season (Table 1). This relationship between the IVDMD and the IVNDFD with the fractions of the cell wall (lignin and cellulose, mainly) and the CP are to be expected; the strong relationship present between these fractions and the digestibility is documented (Van Soest, 1994; Fernandes *et al.*, 2014). It is also documented that there is a strong relationship between the digestibility of the cell wall and the digestibility of organic matter (Moore and Mott, 1973). At the same time, the digestibility of the cell wall depends on its degree of lignification. Therefore, lignin is considered to be the main inhibiting component of the quality of tropical grasses (Moore and Mott, 1973).

Finally, it is of interest to point out that the low fiber content and the higher CP content and digestibility of Mombasa grass can be attributed to the fodder being harvested in an early stage of maturity (30 days of regrowth).

CONCLUSIONS

In this study, the dry matter yield of the Guinea grass cv. Mombasa is higher at greater distance between plants, and is higher in the cool and rainy seasons, when there

is more rainfall. The crude protein content is better in the dry season. Plant spacing does not affect the fractions of the cell wall or the digestibility of the fodder, although it does the lignin content. The fodder harvested in the dry season presents better quality, since it contains fewer cell walls and is more digestible. Due to its higher yield and crude protein content, the quality of the Guinea grass fodder improves at greater plant spacing (75×25 and 100×25 cm).

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