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pág. 3

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Contenido

Año 16 • Volumen 16 • Número 4 • abril, 2023

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Silicon and titanium affect the percentage of juice and color attributes in tomato fruits of plants exposed to salt stress

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ABSTRACT

Objective: To evaluate the leaf application of silicon (Si) and titanium (Ti) in three doses (0, 75, and 150 mg L^{-1}), independently, on tomato plants cv. Río Supremo, subjected to saline stress (0, 50, and 100 mM NaCl), on the percentage of juice and color attributes of the fruit.

Design/methodology/approach: Two independent essays were carried out under a completely randomized experimental design in a 3^2 factorial arrangement, where the first study factor was the NaCl concentration in the nutrient solution and the second factor was the leaf application of Si or Ti. The percentage of juice and color attributes in fruits were determined. An analysis of variance and the comparison of means by Tukey ($p \leq 0.05$) with the SAS software were performed.

Results: Salinity was found to reduce the percentage of juice, the color index, and the ratio of "a/b" indexes. Regarding the interactive effects, NaCl with both Ti and Si increases the "b" index. Leaf applications of Si increased the "b" index and reduced the percentage of juice, the color index, and the ratio of "a/b" indexes. Also, Ti improved the color index and the "b" index.

Limitations of the study/implications: The results were obtained in the Río Supremo tomato variety under greenhouse conditions. Other varieties should be tested too.

Findings/conclusions: Si and Ti applied to the leaves have positive effects on the color of the fruits of tomato plants under saline stress.

Keywords: beneficial elements, inorganic biostimulants, saline stress, quality of *Solanum lycopersicum* fruits.

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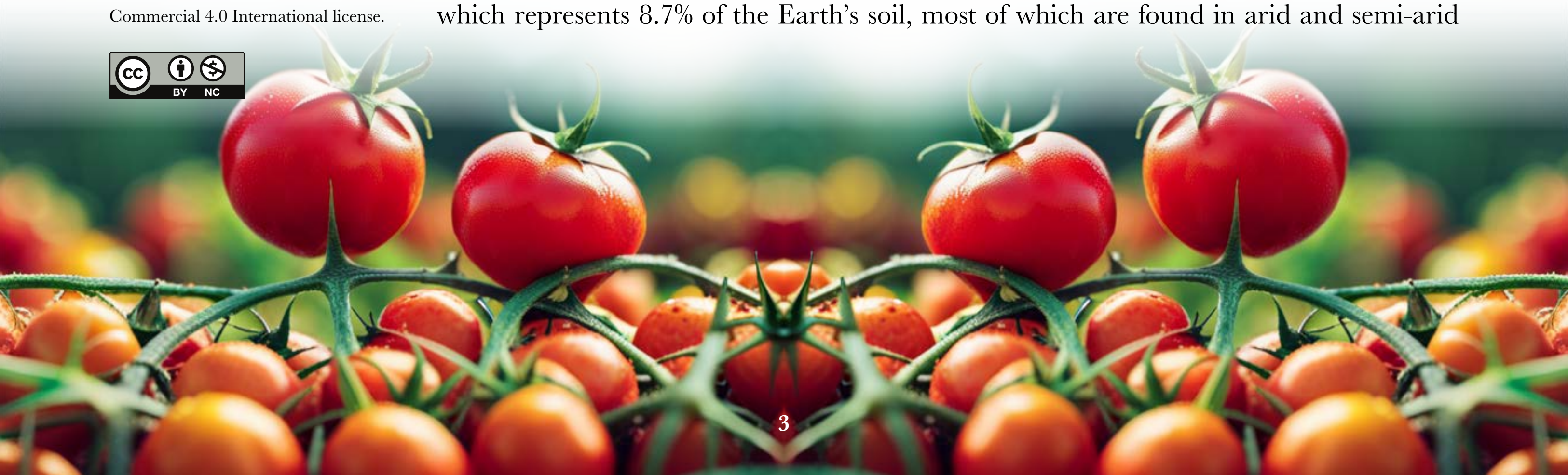
INTRODUCTION

In 2018, the global area of saline soils was 397 million ha; of which, 45 million ha were irrigated soils affected by salinity, which represented a third of those used for food production [1]. In 2022, 833 million ha of soils with saline problems were estimated, which represents 8.7% of the Earth's soil, most of which are found in arid and semi-arid



zones. Similarly, between 20 and 50% of irrigated soils located on all continents are highly saline [2]. In the Americas, the total area devoted to crops is approximately 3.9 million, 65.1% of which has salinity problems [3]. In Mexico, this problem has increased due to the high rates of evaporation in shallow groundwater areas, the use of brackish irrigation water, saline intrusion into the groundwater table in coastal areas (50 in total), the over exploitation of 157 aquifers, and the effects of climate change [4, 5].

Excess salts in the soil are a stress factor that affects metabolic processes in the plant, since the osmotic pressure generated in the rhizosphere induces less water absorption, causing ionic and nutritional imbalances. The excessive accumulation of sodium and chloride in the cytosol increases oxidative stress, interferes with the absorption of potassium and calcium, in addition to unbalancing the absorption and translocation of nitrate [1], all of which negatively affects crop growth and development, in addition to limiting production and reducing yield [6].

Tomato has a medium tolerance to salinity. Because it is a glycophyte species, it supports electrical conductivities no greater than 2.5 dS m^{-1}. Salinity affects seed germination, reduces lateral and vertical elongation of the root, induces a decrease in bioactive compounds and antioxidant capacity, reduces chlorophyll content, photosynthetic capacity, and decreases the size and number of fruits. However, salinity may improve organoleptic attributes by increasing organic acids and sugars [7-10].

Both Si and Ti are non-essential elements with the ability to promote growth and development of plants, thus they are classified as biostimulants within the group of inorganic compounds. Biostimulants are materials that, in small quantities, stimulate nutritional absorption, increase tolerance to abiotic stress, and improve the quality of crops. Biostimulation is an integral process in crop systems that has gained momentum in recent years, by optimizing production through physiological adjudgments of plants [11, 12].

Si is one of the most abundant elements in the Earth's crust (second only after oxygen) with 27% of its composition. Various investigations have shown its beneficial role in alleviating the negative effects of abiotic stress factors. In addition, Si may constitute between 0.1 and 10% of the plant biomass in dry basis [13, 14]. In tomato plants, it induced tolerance to *Orobanche ramosa* infections [15], improved commercial yield and fruit quality [16], as well as postharvest quality [17]. In avocado (*Persea americana*) trees, K_2SiO_3 applications improved fruit quality and yield [18]. Also, foliar applications of K_2SiO_3 in loquat (*Eriobotrya japonica* Lind.) trees increased fruit set, as well as number, weight, and quality of the fruits [19].

Ti ranks ninth in the Earth's crust with 0.56%. In strawberry (*Fragaria* × *ananassa*) cvs. Selva and Elkat, Ti increased antioxidant capacity, while in the cv. Daewang it improved yield and fruit quality [20, 21]. In peach (*Prunus persica*) cv. Sevilla II and nectarine (*Prunus persica* var. *nucipersica*) cv. Silver King, Ti increased fruit growth and firmness at harvest [22]. In okra (*Abelmoschus esculentus*), it positively influenced germination and chlorophyll content [23]. Furthermore, Ti improved fruit quality characteristics such as the relationship between total soluble solids and titratable acidity (TSS/TA) in tomato cv. Río Supremo [24]. The objective of this study was to evaluate the independent leaf application of Si and

Ti in three doses (0, 75, and 150 mg L^{-1}), to tomato plants cv. Río Supremo, subjected to saline stress (0, 50, and 100 mM NaCl), on fruit quality variables.

MATERIALS AND METHODS

Experimental conditions and plant material

The experiments were carried out in a tunnel-type greenhouse with an anti-aphid mesh and a plastic cover with a roof window. Using a Data Logger (Onset Hobo), the following climatic variables were measured: the mean daytime and nighttime temperatures (31.7 and 15.1 °C, respectively), relative humidity during the day and night (30 and 86.9%), as well as the duration of the photoperiod; it was an average of 11.3 h with a mean light intensity of 137 μmol m^{-2} s^{-1}. Seeds of hybrid tomato cv. Río Supremo of determined size were sown in trays of 200 cavities with peatmoss as substrate. Seedlings, 32 days after sowing (das), were transplanted into 400-gauge black polyethylene bags (30×30×30 cm) containing the local volcanic gravel tezontle as a substrate (particle size ≤ 6 mm).

Design of treatments and experimental design

Two essays were established with a completely randomized experimental design in a 3^2 factorial arrangement. The first study factor was the NaCl concentration in the nutrient solution with three levels (0, 50, and 100 mM NaCl). The nutrient solution (NS) used was the Steiner's universal [25]. The leaf application of Si or Ti was supplied from SiO_2 and TiO_2 (Sigma Aldrich®, St. Louis, MO, USA) respectively, at three levels each: 0, 75, and 150 mg L^{-1}. Si or Ti were considered as the second study factor. With the combination of factors and levels, nine treatments were obtained in each trial, and each treatment had three replicates. The experimental unit consisted of a 61 das plant transplanted in a black polyethylene bag.

An experiment with a spaghetti drip irrigation system was established; for this, nine 200 L tanks were placed, each container had a ½ HP pump, a PVC branch with a return to the tank regulated with a valve and an outlet line with a ring filter, which was connected to a 16 mm black agricultural hose with three drippers per container. Each dripper had a crosshead for four tubes and each tube with a stake that went directly to the experimental unit.

The leaf sprays with Si and Ti for each experiment began 62 das in the morning (6:00 h). For greater adherence of the sprayed solution to the leaf blade, the Tween™ 20 surfactant (Hycel; Gualajara, Mexico) was added at a concentration of 0.5 g L^{-1}. Eight leaf applications were carried out at intervals of 10 days between one and the next applications.

Variables evaluated

The quality variables were evaluated in fruits of the second bunch in the state of maturity known as "red" according to UPOV [26]. Whole fruit weight was determined using an analytical scale (ADAM, model CQT1501, Kingston, UK). Subsequently, the tomato juice was obtained with an extractor (Hamilton Beach, model: 67606-MX, China) and the percentage of the total fruit weight that corresponded to the juice was estimated.

The color indexes, “a” and “b”, were obtained using a colorimeter (Hunter Lab, model D25-PC2, Reston, VA, USA). The measurement was determined at two opposite points of the equatorial zone of each fruit. The ratio of the “a/b” indexes was calculated with the data obtained from the colorimeter as proposed by Domene and Segura [27].

Statistical analysis

With the results obtained, analysis of variance and Tukey’s means comparison tests ($p \leq 0.05$) were performed, using the SAS software [28].

RESULTS AND DISCUSSION

NaCl and Si in juice percentage and color parameters of tomato fruits

Juice percentage. Crops affected by salinity show osmotic stress at the root level; that is, high salt concentrations in the soil solution reduce the water potential in the rhizosphere of the plant, causing less water absorption and water deficit in its organs [29]. In this study, NaCl significantly affected the percentage of fruit juice, which decreased by 24.2 and 20.9% with the addition of 50 and 100 mM NaCl, respectively, compared to the control (Table 1).

The absorbed Si tends to be deposited in leaf cells; this deposition has been observed in the cell wall of guard cells, causing slight deformations and structural changes that affect the opening of the stomata, reducing conductance and transpiration flow [30, 31]. It is possible that the doses used in this research affected the moisture content of the plant, as well as that of the fruits, as it was observed that the 75 and 150 mg Si L^{-1} doses decreased the percentage of juice in fruits; however, only the 12.7% decrease with the 75 mg Si L^{-1} dose was significant compared to the control (Table 1). These results are not consistent with those of other studies. For example, in orange (*Citrus sinensis*) cv. Olinda Valencia treated with K_2SiO_3 in doses of 2 and 4%, the fruits showed higher juice content at harvest time, compared to control [32]; and in 40-year-old pomegranate (*Punica granatum*) trees cv. Manfalouty, where the juice percentage was higher when leaf doses were applied with 0.5 and 1% of K_2SiO_3, compared to the control [33].

The interactive effect of the study factors was significant; the doses with Si decreased the juice percentage even in combination with NaCl compared to the treatment without NaCl and without Si (Table 1).

Color Index (CI). The color of fruits is associated with organoleptic characteristics such as aroma, flavor, and texture. These sensory properties are indicators of freshness and quality, which determine if the consumer’s preferences [34]. CI values between 20 and 40 indicate that the fruit is in a coloration from “orange” to “dark red” [27]. In this research, the results obtained exceeded this interval (both treatments and the control). Nevertheless, 50 and 100 mM NaCl were significantly lower, by 50.9 and 41.8% when compared to the control (Table 1). The application of 75 and 150 mg Si L^{-1} decreased the CI of the fruits by 13 and 16%, respectively, compared to the control. However, only the high dose of Si was significant. In the interactive effect, there were no significant differences among treatments tested (Table 1).

Table 1. Main effects and interaction of NaCl and Si on the juice percentage and color parameters of tomato (*Solanum lycopersicum*) cv. Río Supremo.

NaCl (mM)	Juice (%)	Color Index	"a" Index	"b" Index	"a/b" Index Ratio
0	57.4±5.9 a	70.5±6.22 a	35.6±3.6 a	17.7±0.9 a	2.0±0.27 a
50	43.5±4.4 b	34.6±6.70 b	19.6±3.9 c	17.6±2.4 a	1.2±0.28 b
100	45.4±4.8 b	41.0±1.85 b	25.0±1.1 b	17.6±1.6 a	1.4±0.13 b
Si (mg L^{-1})	**Juice (%)**	**Color Index**	**"a" Index**	**"b" Index**	**"a/b" Index Ratio**
0	52.1±7.7 a	53.9±8.2 a	29.8±3.5 a	16.2±1.6 b	1.8±0.21 a
75	45.5±6.0 b	46.9±7.6 ab	25.4±3.8 a	17.5±1.2 ab	1.4±0.23 b
150	48.7±2.5 ab	45.3±12.0 b	25.1±5.8 a	19.1±2.1 a	1.4±0.36 b
NaCl (mM) - Si (mg L^{-1})	**Juice (%)**	**Color Index**	**"a" Index**	**"b" Index**	**"a/b" Index Ratio**
0-0	70.7±3.2 a	75.7±3.4 a	39.0±1.8 a	16.9±0.5 ab	2.31±0.18 a
0-75	54.3±4.1 b	62.8±8.4 a	31.7±5.0 abc	18.8±1.1 ab	1.75±0.34 abc
0-150	47.0±1.9 bc	73.0±4.5 a	36.3±2.7 ab	17.3±0.7 ab	2.12±0.23 ab
50-0	45.3±3.2 bc	44.0±1.0 b	24.3±0.7 cde	13.7±0.9 b	1.78±0.09 abc
50-75	37.1±5.5 c	35.0±1.4 bc	20.0±1.4 de	17.0±0.5 ab	1.18±0.09 d
50-150	48.1±2.6 bc	24.9±9.7 c	14.6±5.9 e	22.0±2.9 a	0.80±0.31 d
100-0	40.1±4.6 bc	42.0±0.5 bc	26.0±0.6 bcd	18.0±2.1 ab	1.48±0.12 bcd
100-75	45.1±5.5 bc	43.0±1.3 bc	24.5±1.2 cde	16.8±1.6 ab	1.48±0.11 bcd
100-150	51.0±2.9 bc	38.0±2.3 bc	24.5±1.5 cde	18.0±1.4 ab	1.40±0.16 bcd

Means±SD with different letters in each column and study factor indicate significant statistical differences (Tukey, $p \leq 0.05$).

"a" Index. In this study, the treatments with NaCl (50 and 100 mM) significantly reduced the "a" index of the fruits (44.9 and 29.8% with respect to the control). That is, the fruits of plants without saline treatment showed the highest value of index "a" (35.6), and this value was 34.8% higher than 26.4 (referred to the "bright red" color), so they enter the classification of "dark red" [35]. With the saline treatments, the values obtained in index "a" were 20 (50 mM NaCl) and 25 (100 mM NaCl), which were within the rage of "bright red" classification. The independent effect of Si and the interactive effects of the study factors (NaCl×Si) were not significant for this variable (Table 1).

"b" Index. For the "b" index, values below 20.7 (classified as "dark red") are considered ripe fruits with acceptable quality [35]. In this study, the treatment without Si (control) showed the lowest value of the "b" index (16.2), which is less than 20.7 by 21.7%. The doses of Si applied to the leaves progressively increased the variable with means of 17.5 (75 mg Si L^{-1}) and 19.1 (150 mg Si L^{-1}) which meant an increase of 8 and 17.9%, where only the high dose of Si (150 mg L^{-1}) had a significant difference with the control. Although these values are still below the "dark red" classification, it can be seen that Si improved this variable. The independent effect of NaCl and the interactive effects of the study factors (NaCl×Si) were not significant for the variable (Table 1).

Ratio of the "a/b" indexes. The ratio between the "a" and "b" indexes was significantly affected by salinity, since the 50 and 100 mM NaCl doses decreased this ratio by 40 and 30%, respectively, compared to the control. Likewise, the application of Si in concentrations of 75 and 150 mg L^{-1} significantly decreased this variable by 22.2%. Regarding the interactive effects, in the treatment with 50 mM NaCl and 150 mg Si L^{-1}, the lowest and most significant value was observed (55.1% compared to the 50 mM NaCl and 0 mg Si L^{-1} treatment, and 65.4% when compared with the 0 mM NaCl and 0 mg Si L^{-1} treatment), as shown in Table 1.

The maximum organoleptic quality of a tomato fruit is in its maturity state with a "bright red" coloration, which is the result of the synthesis of carotenoids, mainly lycopene. Within the intervals proposed by Cantwell *et al.* [35], the values in "a" and "b" indexes for the stage of "bright red" coloration of the fruit are 26.4 and 23.1, respectively.

NaCl and Ti in juice percentage and color attributes of tomato fruits

Juice percentage. When a plant absorbs salts in excessive amounts, it transports them through the transpiration flow to the leaves, where it stores them. The overaccumulation of ions such as Na^+ and Cl^- induces cell toxicity causing ionic stress. High concentrations of cytoplasmic Na^+ affect the absorption of K^+, an important ion involved in stomatal opening and closure; when the activity of the guard cells in the stomata is affected, the flow of masses is reduced, decreasing the percentage of humidity in the various organs of the plant, such as the fruits [29, 36]. This effect was reflected in this study only with the 50 mM NaCl dose, which caused a 31.9% decrease in the percentage of fruit juice compared to the control (Table 2).

The doses of 75 and 150 mg Ti L^{-1} non-significantly increased the juice percentage by 4.6 and 6.3%, respectively, compared to the control (Table 2).

The interaction of the study factors did not show significant differences, except for the 100 mM NaCl with 75 mg Ti L^{-1} treatment, which increased the percentage of juice by 15.3% with respect to the treatment without NaCl and without Ti (Table 2).

Color Index (CI). The perception of color by the human eye depends on the passage of light through the cornea and the processing of information by the brain. The CI is a parameter that mathematically describes the colors by means of the CIE-L*a*b* color space and serves to evaluate the color of the fruits, determining the consumer's acceptance. In tomato, chlorophyll degradation and lycopene synthesis in chloroplasts dictate the color change from green to red [37, 38]. In this experiment, the CI was decreased by 33.9 and 41.7% with the 50 and 100 mM NaCl doses, compared to the control (Table 2). This is consistent with the results obtained with the lycopene variable (data not shown), since the lycopene concentration decreased under saline stress.

The 150 mg Ti L^{-1} dose increased the CI by 20.5% when compared to the control (Table 2). Regarding the interactions of the study factors, the treatments consisting of 50 mM NaCl with 75 or 150 mg Ti L^{-1} decreased the color index by 33.2 and 20.2%, respectively, compared to the treatment without NaCl and without Ti (Table 2).

"a" Index. The color change in the ripening process (chlorophyll degradation and carotene synthesis) can be measured with the "a" (red) and "b" (yellow) indexes, with values of 26.4 and 23.1, respectively, for the classification called "bright red" [35, 39]. In the fruits of plants treated with the 50 and 100 mM NaCl doses, the "a" index was reduced

Table 2. Main effects and interaction of NaCl and TiO_2 on the juice percentage and color parameters of tomato (*Solanum lycopersicum*) cv. Río Supremo.

NaCl (mM)	Juice (%)	Color Index	"a" Index	"b" Index	"a/b" Index Ratio
0	57.4±5.9 a	70.5±6.2 a	35.6±3.6 a	17.7±0.9 b	2.06±0.2 a
50	39.1±9.2 b	46.6±8.6 b	25.4±4.4 b	20.8±1.9 a	1.30±0.2 b
100	65.5±7.9 a	41.1±2.2 b	26.0±1.3 b	19.1±2.2 ab	1.42±0.1 b
Ti (mg L^{-1})	**Juice (%)**	**Color Index**	**"a" Index**	**"b" Index**	**"a/b" Index Ratio**
0	52.1±3.8 a	48.7±11.2 b	27.6±5.3 b	18.2±2.2 b	1.6±0.3 a
75	54.5±8.4 a	51.2±7.2 ab	27.8±3.3 ab	18.8±1.4 ab	1.5±0.2 a
150	55.4±6.8 a	58.4±7.4 a	32.1±2.9 b	20.6±1.7 a	1.6±0.2 a
NaCl (mM) - Ti (mg L^{-1})	**Juice (%)**	**Color Index**	**"a" Index**	**"b" Index**	**"a/b" Index Ratio**
0-0	70.7±3.2 ab	75.9±3.4 a	39.0±1.8 a	16.9±0.5 b	2.3±0.1 a
0-75	54.3±4.1 abc	62.8±8.4 ab	31.7±5.0 abc	18.8±1.1 b	1.7±0.3 ab
0-150	47.0±1.9 abc	73.0±4.5 a	36.3±2.7 ab	17.3±0.7 b	2.1±0.2 a
50-0	45.3±3.2 abc	28.6±7.9 b	17.5±4.7 d	21.3±3.2 ab	0.9±0.3 b
50-75	28.2±5.4 c	50.7±5.3 b	25.7±4.4 bcd	21.3±1.1 ab	1.2±0.1 b
50-150	43.7±10.5 abc	60.6±1.7 ab	33.1±1.7 abc	19.8±0.6 ab	1.6±0.2 ab
100-0	40.1±4.6 bc	41.7±0.7 ab	25.2±0.7 cd	16.5±2.2 b	1.5±0.1 ab
100-75	81.5±10.3 a	40.0±1.1 ab	26.0±0.8 bcd	16.2±0.6 b	1.6±0.1 ab
100-150	75.4±9.3 ab	41.5±3.8 ab	26.9±2.0 bcd	24.6±1.0 a	1.1±0.1 b

Means±SD with different letters in each column and study factor indicate significant statistical differences (Tukey, $p \leq 0.05$).

by 27.8% on average, compared to the control (Table 2). That is, the control treatment obtained a high value (36), which places it well above the "dark red" classification (value of 27.5; Cantwell *et al.* [35]); while the values obtained with both saline doses (25.5, on average) place them within the "bright red" classification (Table 2).

The applied doses of Ti did not show significant effects on the variable (Table 2).

The 50 mM NaCl with 150 mg Ti L^{-1} treatment obtained a value of 33, this meant an 89.1% increase in the "a" index with respect to the 50 mM NaCl treatment without Ti, which obtained a value of 17.5. This indicates that the treatment with the medium dose of salinity and with the high dose of Ti, produced fruits classified as "dark red" in comparison with the treatment without NaCl and without Ti located in the color "pink orange".

"b" Index. Regarding the "b" index, the effect of NaCl was only significant with the 50 mM dose, which obtained a value of 21, and represented a 17.5% increase in the variable compared to the control (Table 2).

The application of 150 mg Ti L^{-1} significantly increased the "b" index by 13.2% compared to the control (Table 2).

The increases described above suggest that both salinity and Ti application caused the fruits to be classified as "bright red".

The 100 mM NaCl and 150 mg Ti L^{-1} treatment increased the "b" index by 49.1 and 45.6% compared to the 100 mM NaCl treatment without Ti and the treatment without NaCl and without Ti, respectively (Table 2).

Ratio of the "a/b" indexes. The reduction in the values of the ratio of the "a" and "b" indexes indicates a greater accumulation of green pigments; in ripe fruits with reddish coloration, the values tend to increase [40]. In fruits treated with 50 and 100 mM NaCl, the values of the ratio were 1.3 and 1.4 respectively; that is, the "a/b" index ratio was reduced by 36.9 and 31.1%, when compared with the control (2.1) (Table 2). In other words, the NaCl-treated fruits were not a "dull red" color, but "bright red".

The independent effect of Ti and the interactive effects of both study factors were not significant (Table 2).

CONCLUSIONS

The fruits of tomato plants cv. Río Supremo treated with 50 and 100 mM NaCl are differentially affected in the juice percentage and color parameters. On the one hand, in the experiment with NaCl×Si, salinity reduces the juice percentage, the color index and the "a/b" index ratio of the fruits. Although, the "b" index is increased. In the study with NaCl×Ti, salinity decreased the juice percentage and color index variables, and it improved the indexes and "a/b" index ratio.

The foliar applications of Si increased the "b" index. Although, they reduce the juice percentage, the color index, and the "a/b" index ratio. In the interactive effects of the study factors, decreases were only found in the juice percentage and the "a/b" index ratio. It is concluded that the application of NaCl and Si improve some quality variables in tomato fruits cv. Río Supremo.

On the other hand, the leaf-applied Ti improves the color index and the "b" index of the fruits. These effects may be due to the fact that the TiO_2 contained in both plants and fruits modifies color characteristics in the latter, since it is considered a photocatalyst that is activated by ultraviolet light.

In the interactive effects of the study factors NaCl and Ti, the positively affected variables were juice percentage, color index, and the a and b indexes. Ti can improve color indicators that optimize the quality of tomato fruits. However, the action mechanisms of Ti at the molecular level are still unknown.

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Colegio de Postgraduados

Evaluation of the commercially important clam fishery in the Alvarado Lagoon System, Veracruz, México

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ABSTRACT
Objective: to evaluate the historical trends of clam fishery in Alvarado, Veracruz, Mexico, to determine the current state of its populations and to promote the incorporation of aquaculture activities, to conserve the resource and reduce the negative impact of its fishery.
Design/methodology/approach: data were obtained through the CONAPESCA on the arrival notices of clam fishery production from the offices in Alvarado, Veracruz. After analyzing this information, graphs on trends, composition, and proportion by species were made about the capture between the years 1998 to 2021 of the three most important species in the region (*Rangia cuneata*, *Rangia flexuosa* and *Polymesoda caroliniana*).
Results: the three clam species hit peak production in the years 2006 (*P. carolineana* with 700 t), 2012 (*P. carolineana* and *R. cuneata* with 650 t), and 2015 (*P. carolineana* with 800 t), respectively. Nevertheless, the trend in the last years shows an extraction decrease. Representing more than 50% of the composition, *P. carolineana* predominates the captures, even though this species enjoys special protection under NOM-059-SEMARNAT-2010.
Limitations of the research/implications: there were no limitations.
Findings/conclusions: the increase of clam fishery is not recommended due to the diminishing trend and the signs of overexploitation observed in the three species, maintaining less than 50% of peak production.

Keywords: *Rangia cuneata*, *Rangia flexuosa*, *Polymesoda caroliniana*, trends, artisanal fishery

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INTRODUCTION

In Mexico, the production of clams through fishery and aquaculture was 12,333 t in 2020, with a total value of 281.2 million MXN. By volume, it occupies the 18th place in the national fishery production and the 20th in its value. Since 2018, the production volume has decreased by around nine thousand tons annually (Table 1). Of the total national clam production, 8% of its volume was extracted from the shores of Veracruz through artisanal river fishery (CONAPESCA, 2020). Nonetheless, the Mexican government does not distinguish between clam species in these reports.

Table 1. Comparing the national clam production between the years 2016 to 2020.

Year	2016	2017	2018	2019	2020
Volume (live weight in t)	19,937	15,481	30,211	21,079	12,333
Value (thousands of MXN)	513,804	580,863	762,044	388,764	281,215

Even though clams are considered a high-value commodity and have high demand in the national and international markets, their fishery has had further development in the coastline states of the country. As a result, its production comes almost exclusively from the fishery (Figure 1), even though natural populations are getting closer, or in some cases, have exceeded their maximum sustainable production (Helm *et al.*, 2004), which highlights the need to turn to aquaculture as an option to propel the seed-production laboratories for producers.

In Veracruz, most species that comprehend this resource are exploited in the basin of the Papaloapan River, which includes the Alvarado Lagoon System, with 37 bodies of water between lakes and rivers. This lagoon system runs parallel to the coastline in an east-west direction through approximately 17 km, with a maximum width of 4.5 km and an area of 64 km^2 (Cruz-Escalona *et al.*, 2007). The species are exploited through artisanal methods (Baqueiro-Cárdenas, 2004), with the state of Veracruz occupying first place in clam production in the Gulf of México. During the period between the years 1993 to 2004, it comprised 94% of the total capture in Mexico with 15,505 tons (CONAPESCA, 2004).

The target fishery species in the coastal lagoons of the Gulf of Mexico is Rangia cuneata, at the same time, *Rangia flexuosa* and *Polymesoda carolineana* are incidental, the first one, given its size, and the second one for its low density. *R. cuneata* holds higher value and demand owing to its food appreciation, making it the most exploited clam species inhabiting the basin of the Papaloapan River (Rogers and García-Cubas, 1981). As for *P. carolineana* it is the second most captured clam species for human consumption in the Gulf of Mexico (CONAPESCA, 2014), even though it holds a place in the special protection category (NOM-059-SEMARNAT, 2010). The official statistics do not differentiate between species, not considering whether they are fresh, salt, or estuarine water species, just as it happens with other mollusks' fishery. In Veracruz, river clams constitute a significant fishery, sustaining and giving employment and income to several fishermen and their families. The fishery of *R. cuneata* is the only source of livelihood for around 400 to

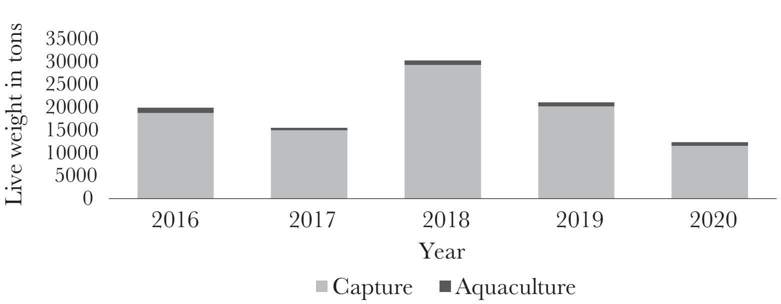

Figure 1. National clam production through fishery and aquaculture between the years 2016 to 2020.

500 families. On this account, this investigation aims to evaluate the historical tendencies of clam fishery in the Alvarado Lagoon System, Veracruz.

MATERIALS AND METHODS

Through the Sub-delegation of the National Commission of Aquaculture and Fisheries (CONAPESCA) in Veracruz, data was obtained on the arrival notices of the clam fishery production from the fishery office in Alvarado, Ver. (the most extensive extraction area in the Gulf of Mexico). The information was analyzed, and trend composition and proportion graphs were made on the captures between the years 1998 to 2021 for the three most important species in the region: *Rangia cuneata*, *Rangia flexuosa* and *Polymesoda caroliniana*. The Alvarado Lagoon System duels in the northeast central region of the Gulf of Mexico, and is integrated by several coastal lagoons: Camaronera, Buen País, Alvarado, Tlalixcoyan, and Popuyeca, amongst others (Figure 2).

RESULTS AND DISCUSSION

The capture trend from 1998 to 2021 (Figure 3) shows that the extraction of the three species has had essential variations. It can be observed that the three clam species experimented with production peaks in the years 2006 (*P. carolineana* with 700 tons), 2012 (*P. carolineana* and *R. cuneata* with 650 tons), and 2015 (*P. carolineana* with 800 tons); nevertheless, as reported by the trend followed during the last years, its extraction has diminished.

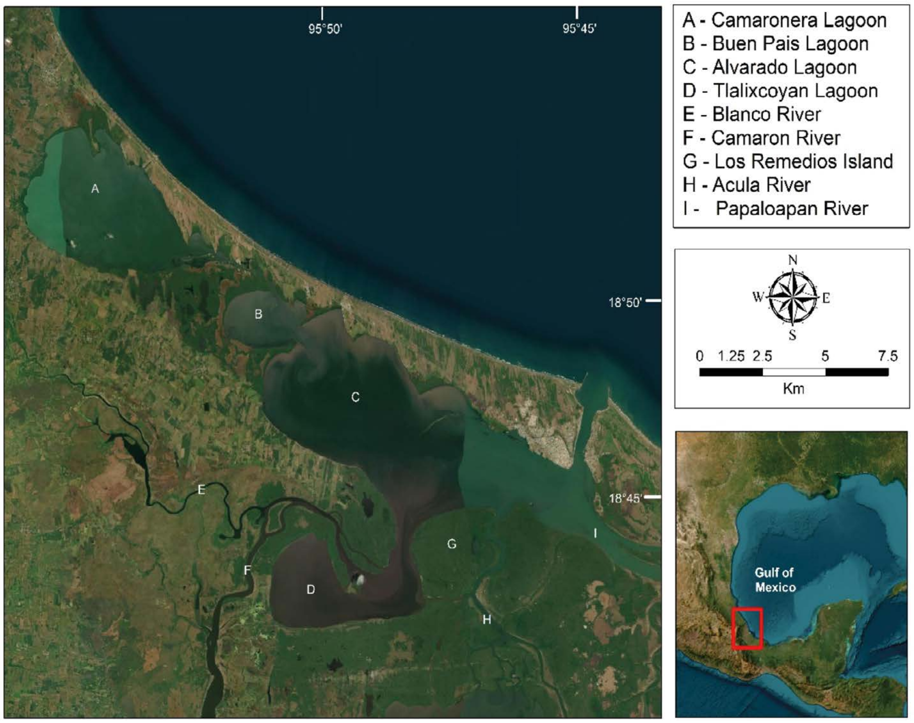

Figure 2. The Alvarado Lagoon System.

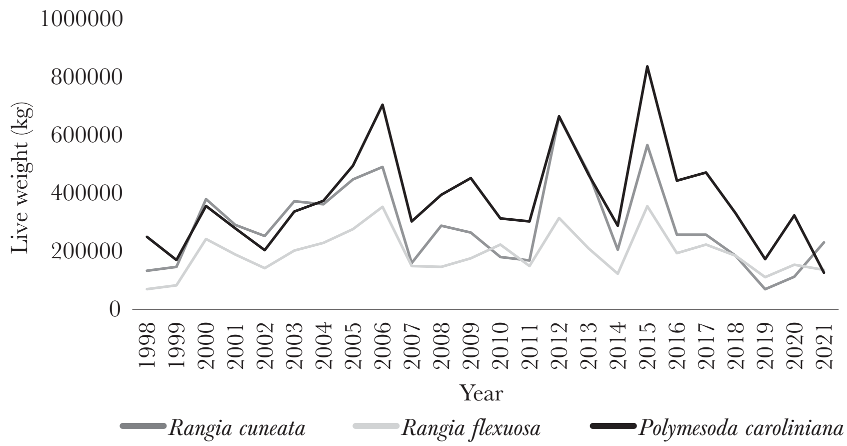

Figure 3. The trend of clam capture in the Alvarado Lagoon System, Ver., according to official production data (arrival notices) of *Rangia cuneata*, *Rangia flexuosa* and *Polymesoda caroliniana* species.

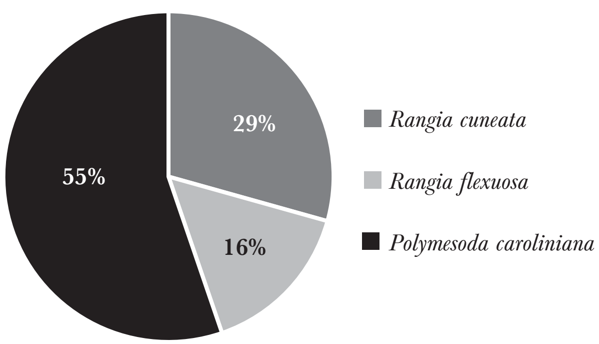

Figure 4. Composition by species of the captures in the Alvarado Lagoon System from 1998 to 2021.

Under the obtained data, the composition of the captures in the latest years in Alvarado, Ver., has been dominated in 50% by *Polymesoda caroliniana,* even though this species is placed under special protection by the NOM-059-SEMARNAT-2010 (DOF, 2010). The second place is *Rangia cuneata* (Figure 4) considered the target species for coastal lagoon fishery in the Gulf of Mexico (Rogers and García-Cubas, 1981; Baqueiro-Cárdenas, 2004).

The proportion of annual captures by species is shown in Figure 5, where *P. caroliniana* was found in higher proportion in the captures during the years 1998, 2007, 2009, 2015, 2017, and 2020, covering 50% of the captures in Alvarado, Ver. Although in 2021 its fishery diminished by more than half of what was reported in the year 2020; this capture represents 15% of the fishery of *P. carolineana* compared it with 2015, where 800 t were obtained. The extraction of *Rangia cuneata* has stayed constant throughout time, with its most significant captures occurring in 2005, 2006, 2012, and 2015. As for *Rangia flexuosa*, the proportion it's been captured has less than the other two species. Its extraction percentage has remained constant across the period between 1998 to 2021, with its most considerable percentage occurring in 2015. In their 2002 study, Froese and Kesner-Reyes suggested an approach for diagnosing the fishery's state based on historic statistics, demonstrating that in Alvarado, Ver., the clam fishery is overexploited. For

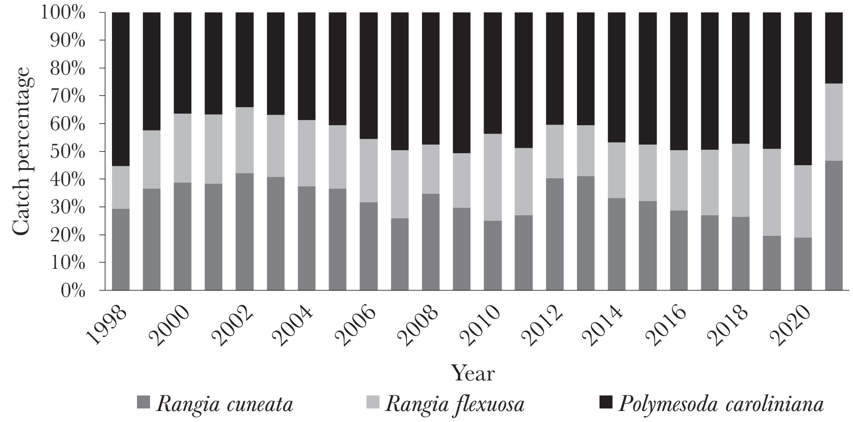

Figure 5. The proportion by species of the annual capture in the Alvarado Lagoon System, Veracruz, by arrival notices.

example, in the years following the highest registered capture, only 50% to 10% of the maximum value has been reached.

CONCLUSIONS

Limited information exists on clam beds and their biology, making imperative the need for the actualization of their distribution, biomass estimation, and density of the many species that conform to them, with the intent of knowing their availability to establish management measures for extraction. The increase of fishery is not recommended due to the declining trend observed through the last years, with the three species showing overexploitation signs, reaching only 50% of production peaks. Better overseeing the arrival data reports in fishery offices is needed; an option is elaborating a catalog for the accurate identification between clam species to make fishery statistics more precise as a response to the current inaccurate arrival data reports caused by species misidentification. An alternative for conserving and recovering the clam resource is promoting aquaculture investigation and reducing the negative impact of fishery where the population has been affected.

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Colegio de Postgraduados

Characterization of sheep production systems and their relation with gastrointestinal parasites in four municipalities of Campeche, Mexico

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ABSTRACT

Objective: The increase in the number of sheep in herds is accompanied many times by bad practices, which lead to nutritional deficiencies, rises in parasitism and loss of homeostasis. Therefore, gastrointestinal parasites from sheep were characterized from four municipalities of the state of Campeche.

Design/methodology/approach: The number of sheep studied was 243, evaluating the body weight, body condition, coloring of the eye mucosae, age, eggs per grams of feces, and family of parasites. The data were analyzed through an ANOVA and Tukey's means test ($P<0.05$), as well as Spearman's correlation analysis using the statistical package Statistica 7.

Results: Five parasite families were found (*Trichostrongyloidae*, *Strongyloidae*, *Trichuridae*, *Eimeriidae* and *Anoplocephalidae*). In the production systems of the municipality of Calakmul the five families of parasites were found, with a parasite load of 3,571 hpg. The family *Trichostrongyloidae* presented the highest frequency in the municipalities of Champotón and Calakmul with 52 and 75%, respectively. The animals with best body condition, FAMACHA© and lowest parasite load were observed in Hecelchakán.

Limitations on study/implications: It is suggested to carry out a study of anthelmintic resistance, to establish a management of prevention and control of gastrointestinal parasites.

Findings/conclusions: The frequency of gastrointestinal parasites is influenced by the management and the municipality of origin within the sheep production systems in the state of Campeche.

Keywords: sheep farming, technologies, gastrointestinal parasites, livestock systems

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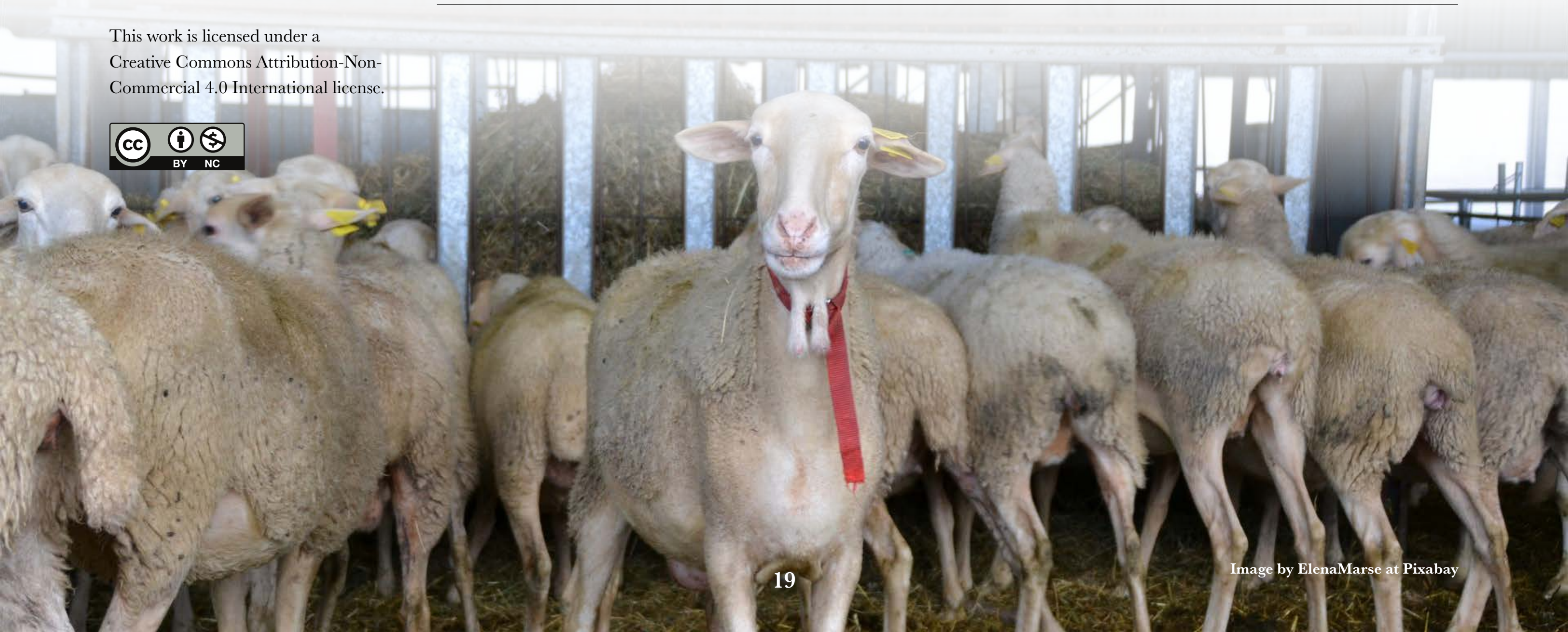

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INTRODUCTION

In the Mexican tropics, sheep farmers have focused on breeding sheep for meat production (González-Garduño *et al.*, 2013), opting mainly for the short-hair breeds, because of their greater rusticity and adaptability to environmental conditions in these zones (Pérez-Hernández *et al.*, 2011), which are characterized mainly by high temperatures and environmental moisture, and high solar radiation. In these systems, it is common for the productive performance in the herds to be affected by nutritional deficiencies, parasitism, and loss of homeostasis (Whitley *et al.*, 2014). To understand and respond to this quandary, characterizations have been made in sheep production agroecosystems in zones of the Mexican tropics, which reflect the deficient management of nutritional supplementation and sanitary management (Nuncio-Ochoa *et al.*, 2001; Dzib-Can *et al.*, 2006; Candelaria-Martínez *et al.*, 2015; Góngora-Pérez *et al.*, 2010; Pérez-Hernández *et al.*, 2011; Pérez-Bautista *et al.*, 2021), conditions that make herds susceptible to presenting gastrointestinal parasitosis (Herrera *et al.*, 2010; Fthenakis *et al.*, 2015). In Yucatán and Tabasco, Mexico, studies have been conducted on the prevalence and loads of gastrointestinal parasites in sheep (González-Garduño *et al.*, 2011; López-Ruvalcaba *et al.*, 2013), and high prevalence of larvae of *Haemonchus contortus* (68,9%), followed by *Trichostrongylus colubriformis* (30.9%) and *Cooperia curticei* (0.6%) (Zaragoza-Vera *et al.*, 2019) were reported until 2019. Likewise, studies have been conducted for the detection of anthelminthic resistance (Torres-Acosta *et al.*, 2012) through the analysis of immunological parameters (Alvarado-Alvarado *et al.*, 2017) and through the level of social hierarchy on the parasite load (Flota-Bañuelos *et al.*, 2019). Within the strategies of prevention or control, the following have been implemented: use of FAMACHA© (Soto-Barrientos *et al.*, 2018), copper oxide needles (Galindo-Barbosa *et al.*, 2011), selective removal of parasites (Medina-Pérez *et al.*, 2015), use of plants with presence of secondary metabolites (Herrera-Manzanilla *et al.*, 2017; Sepúlveda-Vázquez *et al.*, 2018), or residues of *Coffea arabica* (Ortiz-Campos *et al.*, 2016).

In the state of Campeche, there are reports of the types of sheep production systems and the morphostructural variability of black belly studs (Dzib-Can *et al.*, 2011). However, information about gastrointestinal parasites is scarce, with records about identification and presence of anthelminthic resistance to benzimidazole in gastrointestinal nematodes of cattle (Encalada-Mena *et al.*, 2014), with prevalence of 62.31 and 80.15% in calves younger than four months and from four to seven months old, respectively (Encalada-Mena *et al.*, 2009), and about the frequency of sheep resistant to anthelmintic in nine ranches of Campeche (Sepúlveda-Vázquez *et al.*, 2021), leaving aside the management aspects that impact this problem. Given the importance and relevance of this activity in the state (FIRA, 2010) and the increase in sheep production of 136,000 to 193,501 heads from 2009 to 2020 (SIAP, 2021), the objective set out for this study is to characterize gastrointestinal parasites in sheep production systems in four municipalities of the state of Campeche.

MATERIALS ANDY METHODS

The study was conducted during the months of September 2019 to March 2020, in 19 sheep production systems with cooperating producers in the state of Campeche. The municipalities of study were Champotón, located at 18° 34' and 19° 41' LN and 89° 54' and 91° 11' LW, altitude between 8 and 20 masl, mean annual temperature of 26.5 °C and mean annual precipitation of 1500 mm (INEGI, 2009a); Hecelchakán, located at 19° 54' and 20° 17' LN and 89° 51' and 90° 30' LW, altitude between 0 and 100 masl, mean annual temperature of 27 °C, mean annual precipitation between 800 and 1100 mm (INEGI, 2009b); Campeche, located at 17° 48' and 18° 31' LN and 90° 14' and 91° 20' LW, altitude between 100 and 200 masl, mean annual temperature of 26.5 °C, mean annual precipitation between 1200 and 2000 mm (INEGI, 2009c); and Calakmul located at 17° 49' and 19° 10' LN and 89° 25' and 90° 17' LW, altitude between 100 and 300 m, mean annual temperature of 26 °C, mean annual precipitation of 1500 mm (INEGI, 2009d).

Description of the management in production systems

A questionnaire was applied through direct interviews with those responsible for the sheep production systems, which contained the following information: general description (age and schooling of the person responsible, land tenure, workforce used, importance of sheep production, technical assistance, time devoted and purpose of sheep farming), type of production system, registry of production, number of females, number of studs, offspring, surface devoted to grazing, number of pens, sanitary management (disease control, removal of parasites/product, disease prevention), nutrition (ensilage, use of fodder plants, use of multi-nutritional blocks, supplement), and use of grasslands (grazing/grass/plant) (Dzib-Can *et al.*, 2006; Dzib-Can *et al.*, 2017 and Halimani *et al.*, 2021). After applying the questionnaire, a visit was made to the facilities to verify the conditions of herd management (Pérez-Bautista *et al.*, 2021).

The size of the sample was calculated through the formula of finite populations (Sierra, 1995), with a level of confidence of 95%, error of 5%, and total population size of 650 animals between all the production systems, obtaining a sample size of 243 Pelibuey sheep and their crosses, which are characteristic and representative of the municipalities of Campeche.

Weight and body condition

The animals were managed by the ranch owners (with prior consent) and aligned with the regulations for the use and care of research animals (ColPos, 2019). To measure the weight (BW), all the sheep were fasted for 12 h prior to weighing on a digital Torrey® PCp 500 scale. The body condition (BC) was measured with a comparative morphometric scale established by Russel *et al.* (1969), where 1 is very thin, 2 thin, 3 normal, 4 fat, 5 very fat, based on palpation and observation of different areas of the sheep to determine the level of fat coverage.

Coloring of the ocular mucosae

The degree of anemia was evaluated in function of the coloring of ocular mucosae using the FAMACHA© method, where 1 is intense red coloring, 2 light red, 3 pink, 4 light pink, and 5 very pale (Kaplan *et al.*, 2004).

Parasite load

The number of eggs per gram of feces (hpg) was evaluated through a coproparasitoscopic analysis, collecting 10 g of fresh feces taken directly from the rectum of each sheep selected, which were deposited into previously marked bags. Then, the feces were homogenized and processed individually to quantify the eggs for each gram of feces using the McMaster technique (Sandoval *et al.*, 2011). The eggs were identified at the level of family, genus or species in function of the morphometric characteristics (Figueroa-Castillo *et al.*, 2015).

Analysis of information

The data from the questionnaires were captured in worksheets of the Microsoft Office Excel 2007 software, then codified, classified, and a descriptive analysis of the variables was conducted (Santesmases, 2005). To compare the body weight, body condition, coloring of ocular mucosae (FAMACHA©), and parasite load per municipality, a one-way analysis of variance was carried out to determine statistical differences through Tukey's test ($P<0.05$). Spearman's correlation analysis ($P<0.05$) was conducted with the dependent variables using the statistical package Statistica 7.

RESULTS AND DISCUSSION

Description of the management in production systems

In 95% of the sheep production systems analyzed, the person responsible belonged to the masculine gender and only in 5% the person responsible was of the feminine gender. In this sense, Vázquez-García (2013) mentions that a gender approach is required to help understand the allotment of tasks between women and men, because women are fundamental pieces in this productive process in the rural sphere, by performing feeding activities of animals in the pen, grazing, and sanitary management of the animals (Estevez-Moreno *et al.*, 2019). The sheep under care of women showed lower total parasite load ($F=4.942032$, $P=0.027570$) in contrast with the sheep with only men responsible, with averages of 80.77 and 2,058.86 hpg, respectively. In this sense, Hulela (2010) mentions that women have more experience in the management of diseases and control of parasites in sheep and goats thanks to the association of empirical knowledge; this is the case of women shepherd in Chiapas, Mexico, who have improved the quality of the wool and have decreased the neonatal mortality in their herds (Vázquez-García *et al.*, 2013).

Of the total of survey respondents, 36.8% studied primary school, 31.6% studied secondary school, and 31.6% undergraduate university studies; the producers from Hecelchakán and Calakmul have more schooling with 14 years on average, compared to the producers from Campeche and Champotón, who have 9 years on average ($F=14.690$, $P=0.0001$), with values higher than those reported by Estevez-Moreno *et al.* (2019) in sheep producers from Zinacantepec, Mexico, with incomplete primary school (5.16 years of schooling) and in Yucatán, where 61% of the sheep producers have complete primary school (Góngora-Pérez *et al.*, 2010). Concerning age, the producers were in the range of 26 to 65 years old, with those from the municipality of Champotón being older ($F=79.695$, $P=0.0001$) with 56 years on average (Table 1), and age ranges are similar to those registered in sheep production systems from Campeche and Yucatán (Pérez-Bautista *et al.*, 2021; Candelaria *et al.*, 2015).

For 36.8% of the producers, sheep farming is a very relevant activity, because it generates important economic resources, and 63.2% indicate that it is moderately relevant to date, because they are in the phase of starting the activity, the sales are scarce because the objective of the business is to increase the size of the herd (Table 1). The producers

Table 1. Characteristics of the sheep production systems in Campeche, Mexico.

Variables	Municipality			
	Hecelchakán	**Champotón**	**Campeche**	**Calakmul**
Age of the person responsible (years)	47 ± 0.89^{b}	56 ± 11.95^{a}	42 ± 0.42^{b}	30 ± 8.42^{c}
Schooling of the person responsible (years)	14 ± 3.12^{a}	9 ± 4.89^{b}	9^{b}	13 ± 4.43^{a}
Time devoted to sheep production (months)	7^{c}	67 ± 50.26^{a}	33 ± 48.55^{bc}	36 ± 50.11^{b}
% Gender of the person responsible	100 masculines	100 masculines	95 masculines 5 feminine	100 masculines
Land tenure	Private property	*Ejido* property	Private and/or *ejido* property	Private and/or *ejido* property
Workforce used	Hired with payment	Family without payment and hired	Family without payment and/or hired	Family without payment and/or hired
Importance of sheep production	Medium	High	Medium	Medium
Purpose of sheep production	Consumption and sale	Sale	Sale, consumption, and sale	Sale
Number of females (>1 year)	10.5*	70*	42.8*	50*
Number of studs (>1 year)	3*	2*	1.3*	4*
Offspring (birth to 1 year)	25*	48*	10.7*	26*
Type of production system	Confined and grazing	Confined and grazing	Confined and grazing	Grazing
Surface devoted to grazing (ha)	7*	8*	3*	8*
Number of pens	4*	5*	2.1*	3*
Supplement	Yes	No	Yes	No
Disease control	Yes	Yes	No	Yes
Registry of production	Yes	No	No	Occasional
Removal of gastrointestinal parasites / product	4 months / Ivermectin	2 months/ Ivermectin, fenbendazole	3 months / Ivermectin	3 months / Ivermectin
Disease prevention	Yes	No	No	No
Ensilage	Yes	No	No	No
Use of fodder plants	Yes	No	No	No
Use of multi-nutritional blocks	Yes	No	No	Yes
Grazing/grass/plant	Yes / *B. decumbens*, *A. gayanus*, *M oleifera*, *B. alicastrum*	Yes / native grasses	Yes / native grasses	Yes / native grasses
Technical assistance	No	Yes	No	Occasional

[a,b,c] Different letters in the same line indicate significant differences $P\leq0.05$. The values indicate average ± standard deviation. *It refers to the average.

from Champotón had greater experience in the management of sheep (F=11.33414, P=0.000001) with more than 6 years in the activity. Both schooling and experience are relevant factors for success in the productivity of the systems (Herrera-Haro *et al.*, 2019), because in some cases, the null or minimum experience and the low schooling in those responsible lead to failure (Martínez-González *et al.*, 2011).

Of the production systems, 50% are developed in lands with private property tenure and 50% are *ejido*, differing from what is reported by Candelaria *et al.* (2015) in the eastern part of the state of Yucatán, where 89% of the sheep production systems are developed in private properties and 11% in *ejido* lands (Table 1). Access to lands is important to develop the activity and influences the possibility of expanding for pasturelands or fodder crops (Herrera-Haro *et al.*, 2019), although being in *ejido* presents shortages in rights that make agriculture and livestock production more difficult (Morett-Sánchez and Cosío-Ruiz, 2017), in contrast with private property where there are rights for control and of land transference (FAO, 2003).

Of the producers, 95% uses family workforce without payment and hired with payment, while 5% uses only hired workforce (Table 1). In this sense, Candelaria *et al.* (2015) reported a similar finding in sheep breeding systems in eastern Yucatán for which they mention that 100% use family workforce and 82% use hired or occasional labor.

From the production systems evaluated, only the municipality of Hecelchakán conducts supplementation, production records, disease prevention, removal of parasites from animals every 4 months, and they use fodder plants (*M. oleifera* and *B. alicastrum*) as well as improved grasses and multi-nutritional blocks (Table 1). The results are similar to those reported by Dzib-Can *et al.* (2006) and Dzib-Can *et al.* (2017) for the state of Campeche; however, in their studies they had not reported the use of forest shepherding or silo elaboration. In Yucatán, the sheep graze on secondary vegetation or introduced pastures, and only 30.4% of the producers supplement with concentrated commercial feed. In some cases, they use *B. alicastrum* and agroindustrial wastes (Góngora-Pérez *et al.*, 2010).

All of the producers (100%) mentioned that the main problem are the gastrointestinal parasites, and to combat them they eliminate parasites with ivermectin at a frequency of 2 to 4 months, the same as in sheep production systems of the state of Yucatán, where gastrointestinal parasitosis is the disease with highest frequency followed by respiratory diseases, and in 98% of the systems they use chemical anti-parasite drugs, with monthly, bi-monthly, tri-monthly and even annual applications (Candelaria-Martínez *et al.*, 2015); this disease can cause mortality rates of 41.18% (Muñoz-Osorio *et al.*, 2015).

Prevalence of gastrointestinal parasites in sheep

From the 243 sheep studied, 65.8% were positive to gastrointestinal parasites, showing a greater prevalence of the family Trichostrongylidae in the herds from the municipalities of Champotón and Calakmul, with 52 and 75%, respectively, followed by the family Eimeriidae, with 40% for Calakmul and less than 15% for the rest of the municipalities. The family Strongylidae had a prevalence of 23% in Calakmul, while the family Trichuridae showed the lowest prevalence (5%) recorded for Calakmul and Campeche. Likewise, the family Anoplocephalidae was found only for Calakmul with 30% (Figure 1). Similar results were reported in Pelibuey sheep from San Pedro Lagunillas, Nayarit, where the family Trichostrongylidade predominated, followed by the families Eimeriidae and Anoplocephalida (Salgado-Moreno *et al.*, 2017), and in the central region of the state of Tabasco, the family Trichostrongylidae dominated with the genera *Haemonchus* spp., *Trichostongylus* spp. and *Oesophagostomun* spp. (Herrera-Manzanilla *et al.*, 2017). However, in sheep from Bocayá, Colombia, the prevalence was higher with 89.4%, where the main families were Eimeriidae with 63%, followed by Trichostrongylidae (47.7%), Dyctiocaulidae (38.1%) and Strongylidae (21.5%) (Díaz-Anaya *et al.*, 2017).

The weights of sheep ranged from 26 to 34 kg on average, finding higher weights (P=3.1341, P=0.27127) in the sheep from Hecelchakán and lower in Champotón. The herds showed animals with ages from 12 to 30 months on average, with younger sheep in Calakmul and older in Campeche. The body condition of the sheep from the four municipalities did not present significant differences (Table 2).

The oldest sheep were found in herds from the municipality of Campeche with an average of 30.11 months (F=26.6507, P=0.00001) (Table 2), in these herds a lower total count of parasites was observed, while the youngest sheep were found in the municipality of Calakmul with 12.83 months, which presented a higher number of hpg (r=−0.254926, P=0.05); in this sense, it has been suggested that young animals are more susceptible to infestation from gastrointestinal parasites, compared to older animals (González-Garduño *et al.*, 2018).

In the herds from the municipality of Hecelchakán there was coloring of the mucosae (FAMACHA®) with 2.5 (between light red and pink) (F=8.758, P=0.000016) (Table 2); meanwhile, in the herds of the rest of the municipalities studied, the coloring of the mucosae is closer to the range 3 (pink) or limit (light pink), considered as intermediate value where the animal can be considered both anemic and non-anemic (Zárate *et al.*, 2017).

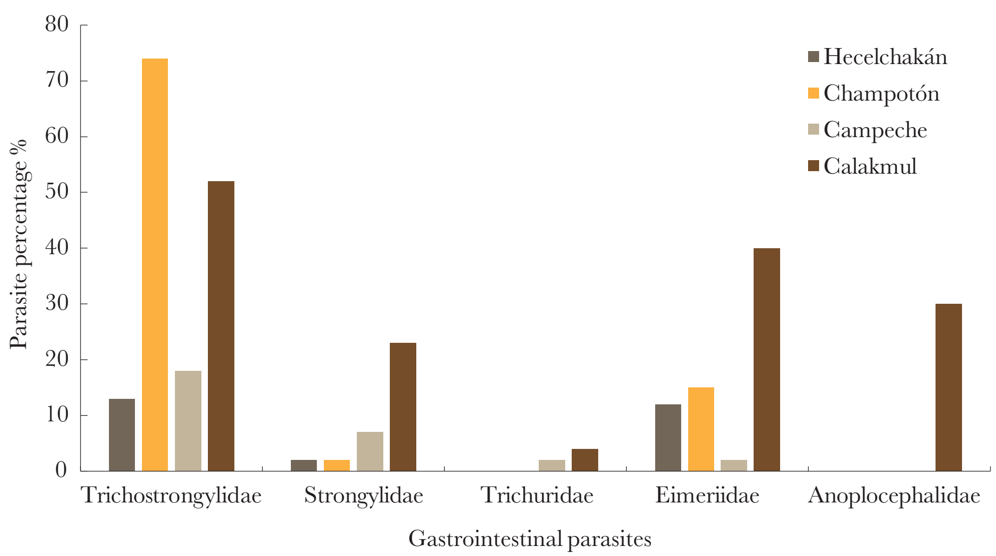

Figure 1. Prevalence of gastrointestinal parasites in municipalities of Campeche

Table 2. Composition of sheep and gastrointestinal parasites in sheep production systems of Campeche, Mexico.

Variables	Municipality			
	Hecelchakán	**Champotón**	**Campeche**	**Calakmul**
Weight (kg)	33.77±14.66[a]	26.11±9.56[b]	33.03±12.01[ab]	28.24±12.24[ab]
Age (month)	22.22±9.23[b]	17.88±5.94[b]	30.11±12.81[a]	12.83±7.93[c]
Body condition	2.46±1.67[a]	2.28±1.09[a]	2.30±1.11[a]	2.02±0.84[a]
FAMACHA	2.55±0.80[a]	3.09±0.92[b]	2.96±0.92[ab]	2.77±0.79[ab]
Trichostrongyloidae (hpg)	727.77±1066.92[a]	560.37±874.38[a]	207.69±313.59[a]	559.83±1248.59[a]
Strongyloidae (hpg)	1.85±9.62[b]	0.94±6.86[b]	11.53±32.58[b]	182.78±326.32[a]
Trichuridae (hpg)	0	0	0	4.09±21
Eimeriidae (hpg)	512.96±679.16[ab]	97.17±217.59[b]	15.38±78.44[b]	2373.77±5967.03[a]
Anoplocephalidae (hpg)	0	0	0	450.81±1208.01[a]
Total of parasites (hpg)	1242.59±1684.52[ab]	658.49±135.41[b]	234.61±62.98[b]	3571.311±1919.88[a]

[a,b,c] Different letters in the same line indicate significant differences P≤0.05. The values indicate average ± standard deviation.

The sheep with best coloring of the ocular mucosae presented better body condition (r=0.323408, P=0.05); this situation is because the sheep from the production units of the municipality of Hecelchakán are supplemented with commercial concentrate feed and fodder from shrub and tree species, which allow varying the diet, covering their requirements (Provenza, 1996), and in addition they promote the capacity to resist high parasite loads (Flota-Bañuelos *et al.*, 2019).

For their part, the sheep from production systems in the municipality of Calakmul presented the highest amount of parasites (F=8.40576, P=0.000025) with a value of 3571.31 eggs, and they had higher presence of Strongylidae (F=12.29291, P=0.00001), Eimeriidae (F=6.727639, P=0.000025) and Anoplocephalida (F=6.268243, P=0000413), with 182.78; 2373.77 and 450.81 hpg, respectively (Table 2). The families and frequency of parasites agreed with the reports in sheep from the municipalities of Teapa, Centro and Huimanguillo, Tabasco, where the families that were mainly found were Trichostrongylidae (*H. contortus*, with 1448, 1191 and 800 hpg) (González-Garduño *et al.*, 2011) and *H. contortus*, *Cooperia* spp., *Ostertagia* spp., *Chabertia* spp., and *Moniezia* spp. (Rivero-Pérez *et al.*, 2019). Likewise, in sheep production systems of Colombia, under three systems (confinement, semi-confinement and grazing), the highest frequency of Trichostrongylidae was found with *H. contortus*, *Teladotargia circumcincta* and *Trichostrongylus* spp., with 76, 61.3 and 25.5% (Zapata-Salas *et al.*, 2016), and in temperate zones of Mexico, *Cooperia* spp. and *Trichostrongylus colubriformis* (Mondragón-Ancelmo *et al.*, 2019).

From the four municipalities evaluated, the sheep from the production systems of the municipality of Calakmul presented all the families of parasites and the highest total amount of parasites, influenced mainly by the climate characteristics, with a warm humid climate (AW2), compared to Hecelchakán, Campeche and Champotón (García, 2004), and humidity being one of the main factors that favor the proliferation of gastrointestinal parasites (Quiroz *et al.*, 2011). Other factors that favor the frequency of parasites are the extensive grazing system in native grasses during 24 h, increasing the exposure to parasite re-infestations; in addition, the sheep do not have access to supplementation, which reduces the sheep's capacity to respond against gastrointestinal parasites (Aguilar-Caballero *et al.*, 2002). Likewise, the low recruitment of technical assistance with trained staff, for the prevention and control of diseases promotes bad sanitary practices for sheep management.

CONCLUSIONS

The parasite loads present in sheep from the different production systems evaluated in the state of Campeche depend on the producer's schooling and experience in the activity, which impact directly on the use and management of the herd, removal of parasites, and control of diseases; these characteristics are reflected in the systems of Champotón, with sheep with parasite loads lower than 700 hpg, and in sheep from production systems that belong to the municipality of Calakmul and Hecelchakán with loads higher than 1200 and 3500 hpg, as well as the presence of the five and three families of parasites, respectively (Trichostrongylidae, Strongylidae, Eimeriidae, Trichuridae and Anoplocephalidae). It is proposed to conduct a study of anthelmintic resistance in these systems, to later establish management of prevention and control of gastrointestinal parasites.

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Colegio de Postgraduados

Festulolium and annual ryegrass pastures associated with white clover for small-scale dairy systems in high valleys of Mexico

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ABSTRACT
Background: The implementation of polyphytic pastures composed of grasses and legumes is an important component of agricultural systems in temperate zones, since grazing pastures which can reduce feed costs— are a viable option for small-scale dairy systems (SSDS).
Objective: To evaluate the continuous grazing of dairy cows in Festulolium pastures associated with annual and perennial ryegrass and with clover in two farmrs.
Methodology: Two experiments were carried out. The first experiment was established in the municipality of Almoloya of Juárez using eight cows that were divided into two groups of four; the cows grazed on two pastures with Festulolium associated with annual ryegrass and they were fed with 3.6 kg DM/cow/day of commercial concentrate, for 16 weeks. The second experiment was carried out in the Northwest of State of Mexico; six multiparous cows grazed on two pastures, under a cross over design arrangement; one pasture features Festulolium cv Spring Green and the other, annual ryegrass. Milk and body condition ere measured every 3 and 12 d, respectively. Variables from both experiments were analyzed using a split-plot experimental design.
Results: Neither experiment recorded significant differences for the net accumulation of forage, the height of the pastures, and their chemical composition (P>0.05). No significant differences between treatments (P>0.05) were recorded regarding the yields and chemical composition of the milk.
Study Limitations/Implications: The study of mixed pastures can be an alternative for feeding grazing cows, helping to reduce costs in SSDS.
Findings/Conclusions: Festulolium/annual ryegrass pastures with white clover are a viable forage alternative for small-scale dairy systems.

Keywords: pastures; milk; grazing; grasses; white clover.

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INTRODUCTION

Small-scale dairy systems (SSDS) contribute more than 35% of production in Mexico (Hemme *et al.*, 2009); they have persisted over time and generate a constant income. They are characterised by herds of 3 to 35 cows plus replacements, by farming activities carried out in small farms (Fadul-Pacheco *et al.*, 2013), and by family labour-based production. They have proven to be an option to overcome rural poverty (Espinoza-Ortega *et al.*, 2007).

The economic scale of sustainability is the most vulnerable aspect of these systems, given the high production costs. Feed is the major component of these costs (Fadul-Pacheco *et al.*, 2013; Prospero-Bernal *et al.*, 2017), as a result of the use of large amounts of commercial concentrates, straws, and stubble (Martínez-García *et al.*, 2015), which represent up to 70% of a farm's expenses (Espinoza-Ortega *et al.*, 2007).

Grazing grass and legume pastures has proven to be a viable option for SSDS. This practice obtains better results than conventional grassland management (mowing and grazing), significantly reducing feeding costs (Pincay-Figueroa *et al.*, 2016; Prospero-Bernal *et al.*, 2017). However, future scenarios about the uncertain availability of irrigation water, changes in rainfall patterns, and an increasing frequency of extreme temperatures —factors which have a direct influence on pasture production— require the development of feeding strategies that enable the optimization of resources within the production unit, seeking the species and varieties of pasture that are best adapted to the agroecological and management conditions of the SSDS (Plata-Reyes *et al.*, 2018).

Perennial ryegrass (*Lolium perenne*) —which is usually associated with white clover (*Trifolium repens*)— is the grass of choice for temperate pastures, but it does not tolerate temperatures above 25 °C or water deficit (Parsons and Chapman, 2000). In this sense, an alternative to the low persistence of perennial ryegrass-based grasslands may be short-duration grasslands based on fast-growing, highly nutritional quality grasses whose lifespan is a long as that of perennial ryegrass grasslands. Grasses that meet these characteristics include annual ryegrass (*Lolium multiflorum*) and hybrids of ryegrass species and species of the genus *Festuca* known as festulolium.

Annual ryegrass is a native species of Europe and North Africa, but it is widely distributed throughout the world. It is a biennial plant used to establish short-term grasslands, because its establishment is faster than other grasses (Humphreys *et al.*, 2003) and it can be grazed within 70 d of sowing. It can achieve high forage yields of excellent nutritional quality and is widely adapted to the different temperate and semi-arid climate regions of Mexico (Hernández-Ortega *et al.*, 2011).

Fescue species are more persistent than ryegrass: they have a more developed root system that tolerates low nutrient levels and cold or drought stress. These characteristics improve performance in pastures associated with *Lolium* (Thomas *et al.*, 2003).

Festulolium is an inter-specific hybrid of perennial or annual ryegrass and *Festuca* species. It was developed to have the hardiness and ability to grow in hostile environments of fescue (higher drought tolerance) and the high nutritional value and rapid germination and establishment ability of ryegrass (Touno *et al.*, 2011; Barnes *et al.*, 2013). As a result of its high yield, Festulolium cultivars obtained from the cross between *L. multiflorum* and

Festuca arundinacea have been adapted to Nordic conditions; however, these hybrids have not been evaluated in Mexico.

One of the festulolium varieties available in Mexico is festulolium cv. Barfest, a *loliaceum*-type festulolium (×*Festulolium loliaceum* (Huds.) P. Fourn.) resulting from crossing perennial ryegrass with meadow fescue (*Festuca pratensis* Hudson) (Orloff *et al.*, 2016).

Therefore, the objective of this study was to compare, on the one hand, the continuous grazing of dairy cows on a short-duration fescue pasture associated with annual ryegrass versus a perennial ryegrass pasture. On the other hand, an annual ryegrass pasture was compared with a fescue pasture. All these pastures are associated with white clover and all are located in two small-scale dairy systems farms.

MATERIALS AND METHODS

The work was carried out in two communities in the Toluca Valley, within a rural participatory research framework, specifically through the participatory research approach for livestock technology development. This approach is characterized by on-farm experimentation with the participation of farmers aimed to identify, plan, develop, and establish new management practices that favour the improvement of their production processes and that are disseminated in their communities (Conroy, 2005). Each community was considered as a case study.

Bromatological analyses were performed at the Instituto de Ciencias Agropecuarias y Rurales (ICAR) of the Universidad Autónoma del Estado de México (UAEMex), following the standardized procedures described by Celis- Alvarez *et al.* (2016).

Experiment 1

Location

The farm is located in the ejido San Cristóbal, Almoloya de Juárez, in the State of Mexico, Mexico. Its production unit is located at 19° 24’ N and 99° 51’ W, at an altitude of 2650 m.a.s.l. The climate is temperate sub-humid with a rainy season from May to October and a dry season from November to April. The average annual rainfall ranges from 800 to 1,000 mm and the average temperature is 13 °C (Albarrán *et al.*, 2012).

Experimental specifications

Eight cows were divided into two groups of fourand were randomly assigned to the treatments. Cows were grouped in pairs (blocks), according to the number of calvings, days open, and performance prior to the experiment. The cows in each pair were randomly assigned to each experimental treatment. The experiment lasted 16 weeks.

Establishment of grasslands

Two 1.0-ha pastures were used. The first one was established in April 2011 with Festulolium cv. Barfest (*Lolium multiflorum* × *Festuca pratense*), associated with annual ryegrass cv. Maximus (*Lolium multiflorum*) and white clover cv. Ladino (*Trifolium repens*). The seeding rate was 22.5 kg/ha. 15 kg/ha, and 3 kg/ha for Festulolium, annual ryegrass, and white clover, respectively. The second grassland was established 2 years earlier with perennial ryegrass cv. Bargala, annual ryegrass cv. Maximus, and white clover cv. Ladino, at of 22.5 kg/ha, 15 kg/ha and 3 kg/ha rates, respectively. At the time of the experiment, the cycle of annual ryegrass had ended and this type of grass was non-existent; therefore, the area was considered a perennial ryegrass pasture associated with white clover. For both pastures, the fertilization rate at sowing was 80-80-60 kg/ha. A maintenance application was carried out every 28 d with 50 kg urea/ha.

Treatments

The following treatments were evaluated: FL-AR=grazing of fescue and annual ryegrass with white clover; and PR=grazing of perennial ryegrass and white clover. The cows of both treatments were fed 3.6 kg DM/cow/day of commercial concentrate (18% CP).

Animal variables

The cows were milked by hand twice a day (5:00 am and 4:00 pm). The stocking rate was four cows (larger livestock units) per ha. Milk yield (RL) was recorded in kg/cow/day once a week with a 20-kg Torino® clock scale, manufactured in Mexico. The protein and fat composition of milk samples collected every 15 days was determined using an Ekomilk® Ultra 40s ultrasound milk analyzer (BULTEH 2000 Ltd., Bulgaria).

Live weight (LW) and body condition (BC) were measured every 15 d. LW was determined using a 1,000-kg electronic scale while BC was estimated on a scale of 1 to 5 (Yabuta *et al.*, 1997).

Grassland variables

Net herbage accumulation (NHA) was estimated following the procedure established by Hoogendoorn *et al.* (2016), using six 0.70×3.0×0.70 m exclusion cages and cutting 0.25 m×2.0 m quadrats inside and outside, at the start and end of each measurement (15 d). The pasture was nominally divided in two.

The grassland was sampled by simulated grazing to determine the chemical composition of the forage (dry matter (DM), organic matter (OM) and crude protein (CP) content), following the standardized procedures described by Celis-Alvarez *et al.* (2016).

Statistical analysis

Grassland and production response variables were analysed with a split-plot experimental design (Kaps and Lamberson, 2004), according to the following model:

$$Y_{ijk}=\mu+B_i+M_j+E_{ij}+p_k+Mp_{jk}+e_{ijk}$$

Where μ=Overall mean; B=Block effect (pair) of cows per lactation stage $i=1,2$; M=Effect of treatments (major plot) $j=1,2$; E=Residual term for major plots; p=Effect of experimental period (minor plot) $k=1,...16$ (for milk yield); Mp=Effect of interaction between pasture type and experimental period; e=Residual term for minor plots. When significant differences were detected, Tukey's test was applied ($P<0.05$).

Experiment 2

Location

The farm is located in the northwest of the State of Mexico, at an altitude of 2440 m.a.s.l., with a temperate sub-humid climate, an average temperature of 14 °C, and an average annual rainfall of 800 mm (Plata-Pérez *et al.*, 2020).

Experimental specifications

Six multiparous cows crossbred with Brown Swiss, weighing between 415 and 480 kg, were used under a statistical cross over arrangement. The cows were in the third stage of lactation, grazed nine hours per day, and were subjected to three experimental periods of twelve days each (nine days for adaptation and three days for sampling and data recording). Cows were fed 2.0 kg DM of concentrate per cow/day (16% CP), divided into two 1-kg rations per day. The concentrate consisted of a mixture of ground corn and soybean paste (80% corn, 20% soybean).

Establishment of grasslands

Two 0.75-ha pastures were used: the first was established with Festulolium cv. Spring Green (*Lolium perenne/L.multiflorum* × *Festuca pratense*) and the second with annual ryegrass cv. Westerwold. The grasses were sown in each meadow on 25 November 2016, with a density of 30 kg/ha (ryegrass) and 3 kg/ha (white clover cv. Ladino) and a fertilization dose was 58N-30P-00K. Once the grasses were established, a maintenance fertilization was carried out every 28 days with 100 kg of urea.

Treatments

The treatments evaluated (Tx) were: FL=Grazing of Festulolium cv. Spring Green + white clover; and AR=Grazing of annual ryegrass cv. Westerwold + white clover. The cows from both treatments were provided with free access to water.

Animal variables

The milk yield (MY) was measured on the last 3 days of each experimental period, during the morning and afternoon milkings. One aliquot per day was prepared with the milk from the morning and afternoon milkings, taking the proportion of the yield of each milking. These aliquots were used to determine the protein and fat content with the Ekomilk® Ultra 40s ultrasonic milk analyzer (BULTEH 2000 Ltd., Bulgaria). Live weight (BW) and body condition (BW) were measured every 12 d.

Grassland variables

The NHA (Hoogendoorn *et al.*, 2016) was estimated using six 0.50×0.50×0.80 m exclusion cages in each pasture, cutting 0.40×0.40 m quadrat cuts on the inside and outside. at the beginning and end of each measurement (12 d). To determine the NHA, the pasture was divided into two parts. Pasture heights were recorded every 12 days, using the rising-plate technique described by Hodgson (1994).

Samples were taken from simulated grazing and cut pasture to determine DM, OM, CP, neutral detergent fiber (NDF), and acid detergent fiber (ADF) content, following the standardized procedures described by Celis-Alvarez *et al.* (2016). *In vitro* digestibility of organic matter (IVDOM) was determined by incubation with rumen fluid (López-González *et al.*, 2020). Metabolizable energy (ME) was estimated applying the equation ME=0.0157 (DOMD), where DOMD is organic matter in g/kg DM (AFRC, 1993).

Statistical analysis

Grassland variables were analysed with a split-plot experimental design (Kaps and Lamberson, 2004) and animal variables with a Double Crossover design, based on the following mathematical model:

$$Y_{ijkh} = \mu + S_i + C_j(i) + + + Tl + e_{ijkl}\,,$$

Where: μ=Overall mean; S_i=Fixed effect due to sequence; $C_j(i)$=Random effect due to cow within sequence; $P\ h(i)$=Random effect due to period within sequence; Tl=Fixed effect due to treatment; e_{ijkl}=Experimental error.

When significant differences were detected, Tukey's test was applied ($P<0.05$).

RESULTS AND DISCUSSION

Fodder production variables

Table 1 shows the net herbage accumulation and pasture height in experiments 1 and 2. No significant differences were recorded for the variables evaluated in either experiment ($P>0.05$). Forage availability in the evaluated pastures is low in both experiments. The NRC (1987) indicates that a forage availability of 2250 kg/ha must be ensured in order to guarantee an adequate intake in grazing cows. The lower forage availability in experiment 2 —which was carried out during the dry season— is attributed to the lower ANF (Muñoz-González *et al.*, 2013; Álvarez *et al.*, 2016).

The average NHA in experiment 1 (989 kg DM/ha) is higher than results reported byLópez-González *et al.* (2020) for perennial ryegrass (820 kg DM/ha); however, just like the treatments in experiment 2 (807 kg DM/ha), they are lower than the 1747 kg DM/ha in perennial ryegrass and fescue pastures likewise reported by López-González *et al.* (2017).

The compressed height of the FL treatment (17.3 cm) was greater than BA (12.3 cm) in experiment 2. Pasture height is an indicator of forage availability, Mayne *et al.* (2000) mention that, in continuous grazing, the meadow should be 5.0-8.0 cm tall to maximize forage consumption (Plata-Reyes *et al.*, 2018). The height of the pastures evaluated in experiment 2 is greater than the heights reported by Plata-Reyes *et al.* (2018) for festulolium (4.7 cm) and perennial ryegrass (5.5 cm), by López-González *et al.* (2020) for perennial ryegrass (7.1 cm), and by López-González *et al.* (2017) for perennial ryegrass cv Bargala (5.5 cm), perennial ryegrass cv. Payday (6.1 cm), and Festulolium (5.8 cm).

Table 1. Net herbage and height of the grasslands used in experimental 1 y 2.

Variable	Experiment 1		Mean	SEM_{MP}	SEM_{SP}
	FL-AR	PR			
NHA (kg DM/ha)	1319.0	659.0	989.0	388^{NS}	173^{NS}
NHA (kg DM/ha)	87.9	43.9	65.9	25.9^{NS}	11.5^{NS}
NHA (kg DM/cow/d)	21.9	10.9	16.4	-	-
	Experiment 2				
	FL	**AR**			
Height (cm)	17.3	12.3	14.8	3.5^{NS}	5.1^{NS}
NHA (kg DM/ha)	839.0	776.0	807.5	44.9^{NS}	188.2^{NS}
NHA (kg DM/d)	69.9	64.5	67.2	3.7^{NS}	15.68^{NS}
NHA (kg DM/cow/d)	23.3	22.4	22.4	-	-

FL-AR: grazing of fescue and annual ryegrass with white clover; PR: grazing of perennial ryegrass and white clover; FL: Festulolium associated with white clover; AR: annual ryegrass associated with white clover, NHA: net herbage accumulation; SEM_{MP}: Standard Error of the Mean of the main plot, SEM_{SP}: Standard Error of the Mean of the split plot; NS: Not Significant (P>0.05).

Table 2 shows the chemical composition of the grasslands evaluated in both experiments. No significant differences (P>0.05) were recorded for the variables evaluated in both experiments. As a result of low rainfall, the average DM values for FL and BP in experiment 1 are higher than those reported by Plata-Reyes *et al.* (2018) for Festulolium (212 g/kg) and perennial ryegrass (185 g/kg) associated with white clover.

CP, NDF and FDA content are important parameters of forage quality that determine intake and digestibility of forage. High protein content increases milk yield and milk protein, while NDF and FDA are related to digestibility (Yang *et al.*, 2017).

Table 2. Chemical composition of grazed grassland, cut grassland, and concentrates.

Variable	Experiment 1		Mean	SEM_{MP}	SEM_{SP}
	FL-AR	PR			
DM (g/kg)	246.0	251.3	248.5	26.3^{NS}	87.4^{NS}
Ash (g/kg MS)	114.0	113.2	113.6	5.0^{NS}	1.65^{NS}
OM (g/kg MS)	882.5	847.5	865.0	6.65^{NS}	30.2^{NS}
CP (g/kg MS)	170.8	182.2	176.5	24.3^{NS}	6.02^{NS}
	Experiment 2				
	FL	**AR**			
CP (g/kg MS)	101.1	106.3	103.7	3.7^{NS}	18.7^{NS}
NDF (g/kg MS)	615.7	626.1	620.9	7.3^{NS}	24.4^{NS}
ADF (g/kg MS)	320.2	331.7	326.0	8.1^{NS}	11.8^{NS}
DIVOM (g/kg MS)	722.1	723.6	722.8	1.0^{NS}	43.7^{NS}
ME (MJ/kg MS)	9.6	10.4	10.0	0.2^{NS}	0.3^{NS}

FL-AR: grazing of fescue and annual ryegrass with white clover; PR: grazing of perennial ryegrass and white clover; FL: Festulolium associated with white clover; AR: annual ryegrass associated with white clover; DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; DIVOM: in vitro dry matter digestibility; ME: metabolizable energy; SEM_{MP}: Standard Error of the Mean of the main plot; SEM_{SP}: Standard Error of the Mean of the split plot; NS: Not Significant (P>0.05).

The CP values of experiment 1 are slightly higher than those reported by Opitz *et al.* (2006), Dierking *et al.* (2008), and Touno *et al.* (2011), who evaluated different festulolium cultivars and, at different stages, obtained a CP content of 138 to 152 g/kh DM. Nevertheless, they fall within the values previously reported for these systems, which range from 176 g/kg DM (López-González *et al.* 2017), to 209 g/kg DM (Heredia-Nava *et al.* 2007). The CP content of the treatments in experiment 2 is below that reported by López-González *et al.* (2017) and Heredia-Nava *et al.* (2007). The average height of the pastures evaluated by López-González *et al.* (2017) did not exceed 6.0 cm, whilethe height of the treatments (14.8 cm) in experiment 2 indicates that the pastures had higher amounts of structural carbohydrates and, therefore, lower CP content. According to Arriaga-Jordan *et al.* (1999), CP contents for pasture ranging from 160 g/kg DM to 280 g/kg DM are sufficient to cover the requirements of cows with moderate milk production.

The NDF (620.9 g/kg DM) and FDA (326.0 g/kg DM) contents of the treatments in experiment 2 are higher than those reported by López-González *et al.* (2017) in festulolium and perennial ryegrass (515 g/kg DM for NDF and 265 g/kg DM for FDA) and by Plata-Reyes *et al.* (2018) in festulolium (485 g/kg DM for NDF and 234 g/kg DM for FDA) and perennial ryegrass (524 g/kg DM for NDF and 219 g/kg DM for FDA).

The DIVMO of the treatments in experiment 2 (722.8 g/kg DM) and ME (10.0 MJ/kg DM) are similar to those reported byLópez-González *et al.* (2017): a DIVMO of 721.5 g/kg DM and ME of 11.2 MJ/kg DM in festulolium and perennial ryegrass pastures.

Table 3 presents the results for milk yield and chemical composition, PV, and CC for experiments 1 and 2, showing that there were no significant statistical differences between treatments (P>0.05) for both experiments.

In experiment 1, the FL-BA treatment presented an RL of 17.1 kg/cow/day and a BP of 18.9 kg/milk/day, while the average milk fat and protein content was 31.9 g/kg and 30.0 g/kg, respectively. Cow weighed 544 kg (FL-BA treatment, with a CC of 1.6) and 524 kg (BP treatment, with a CC of 1.8).

The RL in experiment 2 was 10.1 kg/cow/day for the FL treatment and 10.6 kg/cow/day for the BA treatment, while the average milk fat and protein content was 39.1 g/kg and 30.8 g/kg, respectively. On average, cows in the FL treatment weighed 450 kg with a CC of 2.5, while cows in the BA treatment weighted 448 kg with a CC of 2.5.

López-González *et al.* (2017) and Plata-Reyes *et al.* (2018) obtained lower yields in grasslands of Festulolium cv. Spring Green associated with white clover cv. Ladino under similar production systems than the yields of experiment 1 (15.8 kg/cow/d and 15.4 kg/cow/d, respectively). However, their yield was higher than the LR of experiment 2. The same authors reported cows that weighted 519 kg and 495 kg, respectively.

Another factor that directly influences the LR is the physiological state of the cow and the third stage of lactation (when the cow is in production), which has an impact on the chemical composition of the milk.

A quality ryegrass pasture can cover the energy requirements for intensively grazed cows with milk yields of up to 30 kg/cow/d (Arriaga-Jordan *et al.*, 1999). Work in small-scale systems has obtained values of 19.0 kg/cow/d (Heredia-Nava *et al.*, 2007) and 14.6 kg/cow/d

Table 3. Productive response of cows in small-scale dairy systems.

Experiment 1				
Variable	**Experiment FL-AR**	**PR**	**Media**	**EEM**
MY (kg/cow/day)	17.10	18.90	18.0	1.26^{NS}
Fat (g/kg)	32.40	31.40	31.9	0.04^{NS}
Protein (g/kg)	29.10	30.90	30.0	0.05^{NS}
PV (kg)	544.20	524.50	534.3	32.50^{NS}
CC (1-5)	1.60	1.80	1.7	0.04^{NS}
Experiment 2				
	FL	**AR**		
MY (kg/cow/day)	10.1	10.6	10.3	0.8^{NS}
Fat (g/kg)	40.0	38.3	39.1	0.14^{NS}
Protein (g/kg)	30.8	30.8	30.8	0.3^{NS}
LW (kg)	450.8	448.7	449.8	3.1^{NS}
BC (1-5)	2.5	2.5	2.5	0.1^{NS}

FL-AR: grazing of fescue and annual ryegrass with white clover; PR: grazing of perennial ryegrass and white clover FL: Festulolium associated with white clover, AR: annual ryegrass associated with white clover, MY: Milk yield, LW: Live weight, BC: Body condition, SEM_{MP}: Standard Error of the Mean of the main plot, SEM_{SP}: Standard Error of the Mean of the split plot; NS: Not Significant (P>0.05).

(Plata-Reyes *et al.*, 2018), so the results of experiment 1 for the BP treatment are within those reported by these authors.

The average values of milk chemical composition (fat and protein), fall within the parameters acceptable by the Mexican standard -NMX-F-700-2004 COFOCALEC (fat ≥32g/kg and protein ≥31g/kg), which regulates Mexican standards. The fat and protein content of experiment 1 and the protein of experiment 2 result lower than those reported by López-González *et al.* (2017) (34.3 g/kg for fat and 31.76 g/kg for protein) and Plata-Reyes *et al.* (2018) (34.2 g/kg fat and 31.8 g/kg protein), however, the milk fat content of experiment 2 is above those reported by these authors.

According to Arriaga-Jordán *et al.* (2010), ryegrass can be used as a strategy to enrich the feed of dairy cows while decreasing the feed cost and thus increasing the level of sustainability (Juárez-Morales *et al.*, 2017).

CONCLUSIONS

The FL-AR treatment (Festulolium cv. Barfest and annual ryegrass) associated with white clover, from experiment 1, produced more forage compared to the rest of the treatments evaluated in both experiments.

As there were no differences in yield, milk chemical composition and nutritional quality of the grasslands evaluated in both experiments, it is concluded that the integration of the grasslands evaluated in these production systems represents a good alternative as a feed base for dairy cows.

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Compliance with ethical standards. This research was approved by the Institutional Committee for the Care of Laboratory, Teaching, Research, Service and Production Animals, following the procedures approved by the Universidad Autónoma del Estado De México.

Data availability. Data are available from the correspondent upon reasonable request.

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Colegio de Postgraduados

Corn-neem soil cover and neem (*Azadirachta indica* A. Juss.) extract application for the *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) management in corn (*Zea mays* L.)

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ABSTRACT

Objective: To evaluate the soil cover with corn-neem biomass for the *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) management in the INIAP H601 corn hybrid, at Pechiche, Los Ríos, Ecuador.

Design/Methodology/Approach: Two treatments were evaluated: 1) a treatment using corn-neem soil cover at 2.0 t ha^{-1} before sowing, plus two foliar applications, 10 and 20 days after sowing; 2) a control treatment without soil cover, in a completely randomized design. The variables evaluated were the percentage of corn plants with 1^{st} to 3^{rd} degree damage and 4^{th} to 5^{th} degree damage (30 days after sowing), Leaf Area Index (LAI), and agricultural yield.

Results: Under field conditions, both treatments had no significative differences in the 1^{st} to 3^{rd} degrees damages (69.0% impact). However, there were significative differences in the 4^{th} to 5^{th} degree damages (31.0%) in the control and the soil cover (15.0%). Additionally, LAI and dry grain yield were higher. There was a linear but inverse relationship regarding LAI and yield in plants with 4^{th} to 5^{th} degree damage.

Limitations/Implications: The availability of neem tree biomass could be a limitation, if this technology is applied to larger land areas.

Findings/Conclusions: Corn-neem soil cover plus a foliar application decreased the *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) impact and increased LAI and agricultural yield.

Keywords: pests, bioinsecticide, yield.

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INTRODUCTION

Corn (*Zea mays* L.) is native to Central America, particularly Mexico. Corn has a wide-spread and diversified tradition as human food; however, its growth, development,

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and final grain yield can be impacted by several causes, such as management practices, environmental factors, and pests (Ángel, 2015). *Spodoptera frugiperda* is one of these pests.

Armyworm (*Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)) is an invasive pest that causes losses in several crops. It is endemic to the tropical regions of the western hemisphere (Njuguna *et al.*, 2021). Armyworm is one of the main corn pests. Although it causes more damages during the initial growth and development of the crop, it can attack crops at any point of the growing cycle (Reséndiz *et al.*, 2006).

In order to attack and mitigate the effects of this pest, different control methods have been applied with different levels of success throughout the years. They have included chemical, traditional, mechanical, and biological methods (Ángel *et al.*, 2015).

From a control point of view, the greatest success is achieved applying chemical methods. However, they have adverse side effects because they pollute water, soil, and air. Additionally, this situation has side effects on human health and reduces the populations of beneficial insects which can (to a certain degree) control the pests. These beneficial insects include ladybugs, lacewings, and some parasitoid wasps (Hernández *et al.*, 2018), mainly from the Ichneumonidae super-family (Rodríguez, 2018).

One of the disadvantages of the indiscriminate use of chemical products to control pest insects is the resistance that they develop to this type of products; *Spodoptera frugiperda* larvae survive despite the chemical applications (Rodríguez, 2018). In contrast, an alternative pest control method is the use of biological products. This practice lacks the problems caused by chemical products (Badii y Abreu, 2006) and does not impact the staff that handles and applies the product in the field (Rodríguez, 2018).

Therefore, the objective of this research was to evaluate the soil cover using corn-neem biomass for the *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) management in the INIAP H601 corn hybrid, at Pechiche, Los Ríos, Ecuador.

MATERIALS AND METHODS

The experiment was carried out at Los Ríos, Pechiche, Ecuador. The soil of the area is clay loam (Soil Survey Staff, 2003). The variety of corn grown for the experiment was INIAP H601. Two treatments were evaluated: 1) a treatment using corn-neem soil cover at 2.0 t ha^{-1} before sowing, plus two foliar applications, 8 and 15 days after sowing; 2) a control treatment without soil cover.

Six-hundred certified seeds of the INIAP H601 corn cultivar were selected. The seeds were imbibed in distilled water for 24 hours before sowing.

Under field conditions, 200 m^2 were selected. They were divided into two plots of 100 m^2 each. After soil preparation and before the sowing took place, a 2.0 t ha^{-1} mixture of 90.0% corn (*Zea mays* L.) waste and 10.0% of fresh neem (*Azadirachta indica* A. Juss.) leaves was spread over the soil previous solarization for 15 days. Both corn and neem leaves had been previously cut and homogeneously mixed. In that same plot, neem leaf extract was applied to corn leaves, 10 and 20 days after germination. The applications were carried out following the method described by Osuma (2005): 800g of neem leaves per 8 liters of water. The completely randomized design was developed under field conditions.

Neither corn waste, nor neem leaf extract were applied in the 100 m^2 control plot. Corn was sown in November, 2020.

In order to determine the seriousness of the *S. frugiperda* attack, the field sampling was carried out 30 days after the germination of the plants, since the crops receive the greatest impact within the first 40 days. The x-shape sampling method was used and five points were randomly selected, avoiding the border effect at all times. From each point, 10 linear plants were evaluated, obtaining a 50-plant population per sampling point. The damage caused to the whorl and the last visible ligule of the leaf of each plant was observed. The five-degree scale proposed by Fernández and Expósito (2000) was used. These authors consider the fourth and fifth degrees as the degrees of greatest damage.

Both areas were divided into five 20-m^2 plots, in order to make it easier to weight the replicates in the final grain yield evaluation. Harvest was carried out 120 days after the sowing and dry grain yield was determined in t ha^{-1}. The spray watering method was used to meet the water requirements of the crops during the main stages of the vegetative cycle of the plants.

The following variables were evaluated:

1) Percentage of corn plants with 1^{st} to 3^{rd} degree damage caused by *Spodoptera frugiperda*. Evaluated according to the methodology proposed by Fernández and Expósito (2000), 30 days after sowing.
2) Percentage of corn plants with 4^{th} to 5^{th} degree damage caused by *S. frugiperda*. Evaluated according to the methodology proposed by Fernández and Expósito (2000), 30 days after sowing.
3) Leaf Area Index. The LAI was determined in the ripening stage, following the recommendations of Montgomery (1911), using 0.75 as correction coefficient for corn crops.
4) Corn agricultural yield. One-hundred fifty days after sowing, dried corn was de-kernelled and weighted.

The 1^{st} to 3^{rd} and the 4^{th} to 5^{th} degree damage percentage variables of the two treatments were analyzed using a test for comparing two proportions. Meanwhile, the LAI and the dry corn grain agricultural yield variables were analyzed using the Student's t-test (5.0% probability). Taking into account this data, the variables were adjusted to a normal distribution with a modified Shapiro-Wilk test (Rahman and Govindarajulu, 1997).

Two simple linear regression analysis were carried out. The first regressor variable was the amount of plants with 1^{st} to 3^{rd} degree damage, while the second variable included the amount of plants with 4^{th} to 5^{th} degree damage. Both LAI and dry corn grain yield were the dependent variables of the analysis. The aim of this analysis was to determine the existence of a linear relationship between both regressor variables, regarding each dependent variable by itself. The following statistic values of the simple linear regression variables were determined: adjusted coefficient of determination (R^2Aj.), estimated value (Est.), standard error of the estimation (EE), significance (p), and Mallow's Cp.

The data was processed using the Infostat 2019 statistical package software (Di Rienzo *et al.*, 2019).

RESULTS AND DISCUSSION

There were no significative differences between the treatments regarding the INIAP H601 corn cultivar with 1st to 3rd degree damage caused by *S. frugiperda* (Figure 1a). The treatments were: 1) corn-neem soil cover plus two neem extract foliar applications and 2) control without soil cover. The percentage of damaged plants was 69.0% for both treatments.

Meanwhile, there were significative differences (Figure 2b) between both treatments regarding the percentage of plants with 4th to 5th degree damage. In the treatment with soil cover plus two foliar applications only 15.0% of the plants were damaged, while the percentage of damaged plants was double in the control treatment, reaching 31.0%.

Neem leaf extracts do not only control pests; they also disturb their feeding habits, inhibit their growth, and impact their mating and oviposition, without phytotoxic effects and causing damage to the environment, inducing resistance in the pest, or generating any kind of waste on the plants or the harvest (Subbalakshmi *et al.*, 2012). However, Gutiérrez *et al.* (2010) did not record the expected results from the use of 20.8% neem oil, perhaps as a consequence of the phytotoxic effect of such concentration; nevertheless, the defoliation degree by *Spodoptera frugiperda* diminished.

Both treatments showed significative differences regarding the Leaf Area Index (Figure 2a). The highest LAI (5.2) was obtained with the corn-neem cover treatment plus two applications, significatively exceeding the value of the control treatment (3.8).

The LAI recorded a significative decrease in the control treatment which provides clear evidence of the defoliation caused by the attack of the pest. In the said treatment, the pest impacted 31.0% of the leaves, causing 4th and 5th degree damage (the most severe damages).

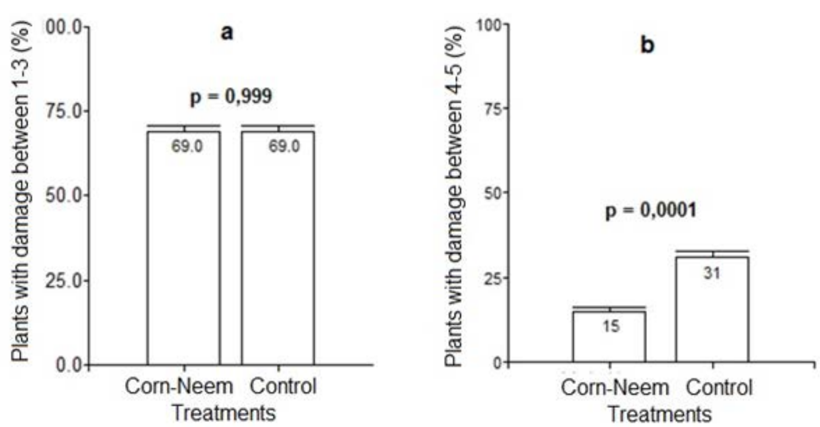

Figure 1. Percentage of plants of INIAP H601 corn cultivar with 1st to 3rd (Figure 1a) and 4th to 5th (Figure 1b) degree damage caused by *Spodoptera frugiperda*. Comparison of the corn-neem cover, plus two neem extract foliar application and control or treatment without soil cover. Different letters indicate significative differences ($p \leq 0.05$) in the proportion analysis.

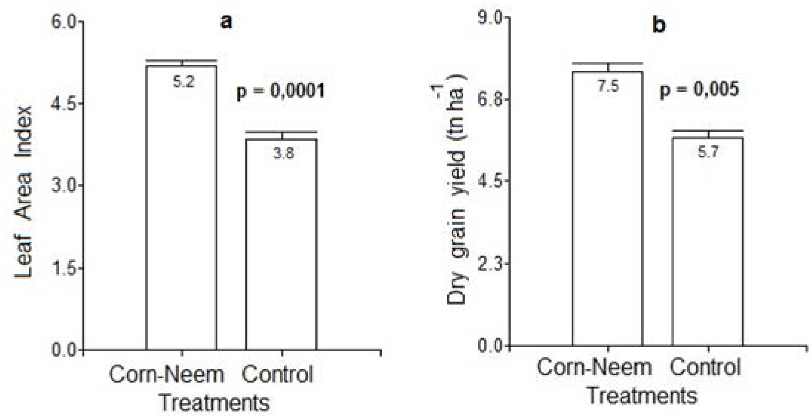

Figure 2. Leaf Area Index (Figure 1a) and dry grain yield (Figure 1b). Comparison of the effect of the corn-neem cover, plus two applications of neem leaves, with the treatment without cover (control treatment). Different letters mean significative differences ($p \leq 0.05$), using the Student's t-test.

The management of any agricultural plantation —particularly of corn, a plant with a C4 photosynthetic path— has the aim of improving solar energy use, through photosynthesis, a function that results in the production of dry matter (Castellanos *et al.*, 2017). This use is based on the development of an appropriate area of the leaves, whose surface can be determined through its Leaf Area Index (LAI).

The LAI provides an estimate of the relation between biomass production and the final crop yield. This relation can be impacted by management, climate, and pest factors (Intagri, 2017).

Rodríguez *et al.* (2007) consider that leaf area and the Leaf Area Index play a key role as regulators of the amount of solar radiation that reaches the plant's basal area. Regarding photosynthetic activity, it is the first responsible for solar radiation interception, consequently favoring a greater leaf area development. Therefore, as a consequence of its greater photosynthetic activity, plants cover a greater soil surface, favoring an increase in the plant's total biomass. This reduces the depopulation that hinders the development of weeds and plant coverage, which regulates soil temperature (thermoregulation).

The LAI obtained was higher when the soil was covered with corn-neem waste (plus two foliar applications) than with the control treatment. The former treatment could have resulted in a significative increase in dry grain yield (Figure 2b).

The simple linear regression (Table 1) in which the regressor variable was the number of plants with 1^{st} to 3^{rd} degree damage did not record significative differences ($p>0.05$) for either the LAI or the yield. Therefore, there is no simple linear relation between the regressor (independent variable) and the leaf area index or the yield an aspect that is corroborated by the value of the determination coefficient (0). This research does not intend to determine which of these variables best fits the various types of non-linear regressions.

Opposite results were found when the amount of plants with 4^{th} to 5^{th} degree damage was used as regressor variable: the value of p (<0.05) was significative for both the LAI and the yield. This proves the existence of a linear, inverse relation, as a result of the negative sign of the estimate value. In other words, as the pest attack intensifies and reaches the

Table 1. Statistic values of the simple linear regression for the number of plants with 1st to 3rd degree damage and the number of plants with 4th to 5th degree as regressor variables and LAI (IAF) and dry grain yield (Rend) as dependent variables. Significance level: $p<0.05$.

	Number of plants between damages 1-3					Number of plants between damages 4-5				
	R^2Adj	Est	SE	p	Mallow	R^2Adj	Est	SE	p	Mallow
LAI	0,00	−0,62	1,66	0,72	1,14	0,80	−7,87	2,26	0,01	13,1
Yield	0,00	0,37	1,24	0,77	1,09	0,94	−6,63	0,89	0,01	56,55

4th and 5th degrees in the damage scale caused to corn plants, the linear form of the Leaf Area Index and the crop yield diminish. The relation between yield and the LAI is more linear and inverse as a result of a greater adjusted coefficient of determination (from 0.80 to 0.94), a lower standard estimation error (from 2.26 to 0.89), and a greater Mallow's Cp coefficient (from 13.1 to 56.55). Therefore, yield fits better a linear regression model than the Leaf Area Index, when the regressor variable is the amount of plants with 4th and 5th degree damage.

To a great extent, this inverse linear relation is linked with the following phenomena: the pest worm mainly feeds on the plant's growth area (apical meristem), damaging the tissues in charge of growth and development —*i.e.*, the growth of the corncob (Cruz, 2009) and the development of new leaves.

CONCLUSIONS

The soil cover treatment with a 9:1 ratio of corn waste and neem plus two applications of neem extracts, 10 and 20 days after germination, resulted in a 15.0% decrease of the percentage of plants that suffer 4th and 5th degree damages as a consequence of the attacks of *Spodoptera frugiperda*. Likewise, it increases the Leaf Area Index (>5 values); meanwhile, the agricultural yield (grains) exceeds 7 t ha^{-1}, perhaps as a result of the control it has over the pest and the beneficial effects of harvest waste cover on the soil, which could be a potentially sustainable alternative for maize production. However, if pests cause 4th and 5th degree damages, there is a significative linear and inverse reduction in LAI and agricultural yield.

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Socioeconomic diagnosis of a group of meliponiculturists in the locality of San Antonio Cayal, Campeche, Mexico

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ABSTRACT
Objective: To identify the economic, technical, and social characteristics of a group of meliponiculturists established in the locality of San Antonio Cayal, Campeche, Mexico.
Design/methodology/approach: An interview card was applied to ten members of a group of meliponiculturists, and each card consisted of 20 open and closed questions distributed in the following sections: general data, technical aspects, characteristics of the meliponary, production, market, and perspectives of meliponiculture. An observation guide was also applied.
Results: It was found that in the locality of San Antonio Cayal, meliponiculture is a recent activity (four years); the activity started with a total of ten people, with ages between 47 and 64, using modernized boxes and the honey extraction technique using syringes. However, currently the activity is only practiced by two people.
Limitations on the study/implications: Meliponiculture is a scarcely practiced activity in the locality, and therefore, there are few records of this activity.
Findings/conclusions: The study allowed us to understand the limitations of the group of meliponiculturists that caused the dispersion of the group, and allowed finding different areas of opportunity (management, production) to strengthen the activity.

Keywords: Meliponiculture, *Melipona beecheii*, honey.

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INTRODUCTION

Native stingless bees (NSLBs) (Apidae: Meliponini) are characterized by the absence of a functional sting (Ayala, 1999; Michener, 2007), the reduction of veining of the anterior wings, and by having simple nails and presenting a line of thick hairs as a comb on the distal margin of the posterior tibia (Márquez-Luna, 1994; Arnold *et al.*, 2018a). NSLBs are distributed in the tropical and subtropical regions of Australia, Asia, Africa and America (Michener, 2007). In America there are approximately 400 species, distributed from Argentina to northern Mexico (Yáñez-Ordóñez *et al.*, 2008; Michener, 2007; Arnold *et al.*, 2018a). Mexico has 16 genera and 46 species of bees belonging to the Meliponini tribe, found in Puebla, San Luis Potosí and Veracruz (Salazar *et al.*, 2017; Ayala *et al.*, 2013), Guerrero (González-

Acereto, 2012; Padilla *et al.*, 2014; Patlán *et al.*, 2014), Oaxaca (Arnold *et al.*, 2018ab), Michoacán (Reyes *et al.*, 2017), Chiapas (Guzmán *et al.*, 2011), and Tabasco (Murillo, 1984; Cano *et al.*, 2013; Arnold and Burguete, 2015; Chan *et al.*, 2019). According to Contreras-Cortés *et al.* (2020), the genera that present the most species are: Plebeia (11), Melipona (7), Trigona (5) and Trigonisca (5) (Ayala, 1999; Ayala *et al.*, 2013; Quezada-Euán 2018).

In the Yucatan Peninsula (YP), there are records about NSLBs representing an important resource for the Maya people and that they took advantage of it since Pre-Hispanic times, with the trade of honey and wax. However, with the introduction of European bees, meliponiculture was gradually displaced by apiculture (Villanueva-Gutiérrez and Collí-Ucan, 1996). Based on archaeological information and the current geographic distribution, it is likely that meliponiculture had its origins in the YP (Kent, 1984; Narez, 1988; Crane, 1992; González-Acereto, 2012; Ayala *et al.*, 2013; Quezada-Euán, 2013).

Mayan culture developed meliponiculture at a level compared to the management of western honeybees in medieval times in Europe, with the result of a great impact in the economy (Cortopassi *et al.*, 2006; Quezada-Euán *et al.*, 2013). The NSLBs were an important part of the Mayan worldview and mythology, which considered meliponiculturists as guardians and keepers of the *Melipona beecheii* bee, known in the Mayan language as "Xunan-kab", because it is believed that they were given directly to them by the major god "Yun 'ku" or "Yumbil dios" (Quezada-Euán, 2011).

NSLBs fulfill an important role as pollinators of wild and cultivated plants, and they are also of cultural importance in different ethnic groups; in addition, the honey produced is attributed to have medicinal value for various respiratory, dermatological, and gastrointestinal conditions (Vit *et al.*, 2004; González-Venegas *et al.*, 2018). However, the effect of disturbances caused in tropical ecosystems such as demographic growth, livestock production, and the increase of extensive agriculture as a result of the high demand for foods, shows an important reduction of their natural habitats (Guzmán *et al.*, 2011; Mérida and Arnold, 2016; Ayala and Ortega, 2018; Vásquez *et al.*, 2021). NSLBs have faced numerous pests and predators such as the "nenem" fly (*Pseudohypocera kertezi*), the "xulab" ant (*Eciton burchelli*), the "sanjol" (*Eira barbara*), and the "muuch" (*Chaunus marinus* and *Cranopsis valliceps*), among others (Pat-Fernández *et al.*, 2018a; Camberos-Sánchez, 2019). Despite the ecological and economic importance of the *M. beecheii* bee, economic aspects have still not been evaluated in a general way (Martínez-Puc *et al.*, 2022), and this is why the objective of this study was to identify the main economic, technical and social characteristics of the group of meliponiculturists in the locality of San Antonio Cayal, Campeche, Mexico.

MATERIALS AND METHODS

Location of the study area

The locality of San Antonio Cayal is in the municipality of Campeche (−90. 175000°, 19.743056° at 50 masl), in Campeche, Mexico. According to the Koppen classification modified by García (1988), the climate of the locality is classified as Aw1(i')gw' warm sub-humid with abundant to very abundant summer rains, with an annual precipitation that ranges from 1,200 to 2,000 mm. The type of vegetation in the community is medium sub-deciduous forest, and in addition it has three different types of soil: Gleysol, Vertisol and Regosol. This locality has a population of 502 inhabitants, 257 men and 245 women (INEGI, 2020).

Data collection methods

Between February and June 2022, a total of ten interviews were carried out with meliponiculturists of the locality, who were identified through the referenced method with the aim of understanding the main socioeconomic, cultural and productive characteristics and the perspectives on meliponiculture of the group. Each interview respondent received a questionnaire that consisted of 20 open and closed questions, distributed in the following sections: general data (age, sex, marital status), technical aspects (harvest season, harvest technique, diseases, pests and treatments), use of workforce (hours devoted to meliponiculture), construction costs of the meliponary (investment in infrastructure), production and marketing (amount of honey obtained, sale price), and perspectives of meliponiculture (training, government backing, problems). The information gathered was analyzed through descriptive statistics.

RESULTS AND DISCUSSION

The interviews were carried out with the group of meliponiculturists from the locality of San Antonio Cayal (SAC), Campeche, Mexico, which does not have a properly established name; this group is made up of six women and four men, with an age that ranges between 47 and 64 years, they practice stingless beekeeping since four years ago, they devote two hours to it approximately every 15 days, since the members of the group have other types of occupations, such as paid work, domestic work, and farming. The group began with four hives, and currently they have 12.

Structure and investment in infrastructure and equipment for the meliponary

The traditional meliponary was built in the patio of the ejido commissioner's office, for which a right of usufruct contract was elaborated to be able to carry out the construction; the main components of the meliponary are the support structure and the roof. The first part is constituted by components commonly known as "horcón" and "balo". The second part is formed by crossbeams or "pachna" lateral beams, and the "hunquiche" vertical grafting rests on them, and on top of these the "hill" is placed which supports the "huano" palms (*Sabal mexicana*), and lastly, the "holná-che" trestle is placed on the upper part, which is also where the lateral grafting rests (Figure 1, Table 1).

The meliponary is named in Maya as "Najil kaab"; this structure provides the hives with shade and protection from the rain and it is oriented east to west (Pat-Fernández *et al.*, 2018ab; Quezada-Euán, 2018; Harvey-Lemelin, 2019). Camberos-Sánchez (2019) mentions that in the Mayan community of Felipe Carrillo Puerto, Quintana Roo, the construction of the meliponary is carried out in two to four weeks with an approximate cost of US$475.00, like what was invested for the construction of the meliponary in SAC.

Figure 1. Side view of the meliponary.

Table 1. Measures and material used for the elaboration of the meliponary.

Roof		
Description	**Quantity**	**Measured**
Hill (length)	10	5.70 m
Hill (width)	10	3.70 m
Huano	500	2.00 m
Easel	1	4.00 m
Support structure		
Descripción	**Quantity**	**Measured**
Horcon	8	2.00 m
Side beams (length)	2	6.00 m
Side beams (width)	2	4.00 m
Balo	1	4.00 m

Technical characteristics of meliponiculture management

The members of the group carry out honey harvesting between March and June, because this season is when the hive is the strongest and there is a greater floristic resource. Likewise, in this harvest period, approximately 300 to 500 mL of honey are obtained per hive. However, not all the hives are harvested each year. The harvest is done through the modernized process by which they use a syringe and a knife to perforate the superior part of the jars to extract the honey, which is deposited into recycled soda plastic containers with a capacity of 500 or 1000 mL (Figure 2). On the other hand, the practice of dividing hives is carried out between April and May.

Abandonment of the activity

The honey harvested from the *M. beecheii* bee (Figure 3) is used for auto-consumption and in traditional medicine to treat colds, coughs, fleshiness in the eyes, or to heal

Figure 2. Syringe extraction of honey from *Melipona beecheii*.

Figure 3. *Melipona beecheii* in the Yucatan Peninsula.

wounds; because of this, meliponiculturists do not trade the honey harvested, they do not know the economic value that it can reach and they do not have training to diversify the production which could help to improve their economy. Another limitation is the loss of interest; during the development of the study, four members of the group abandoned the activity, because there was no economic income from it; two from lack of time since they preferred to work in agricultural activities such as growing corn (*Zea mays*) and fruit tree management (*Mangifera indica*), which has a higher economic profitability; two other members lost interest in the activity, and it should be mentioned that, by the end of the study, eight meliponiculturists abandoned the activity and they are currently devoted to apiculture.

In the YP, the detriment of this activity is very evident; for example, in the Mayan zone of Quintana Roo, between the years 1981 and 2004, the decrease in hives was 93% (Villanueva *et al.*, 2005a); by 2011, a loss of 6.6% was reported (Villanueva *et al.*, 2013). In this study, the abandonment of meliponiculture was primarily due to the inexistent economic income, the lack of interest for meliponiculture, and the change in activity for apiculture where honey productivity is higher compared to meliponiculture (Villanueva-Gutiérrez *et al.*, 2005; Pat-Fernández *et al.*, 2018).

Although meliponiculture is an activity that is decreasing, some authors state that this activity allows improving the quality and economic income of families. However, it is important to give an added value to the honey to improve the quality of life of meliponiculturists (Montenegro *et al*., 2014).

In Maní, Yucatán, four groups of meliponiculturists are reported which receive an income between US$50 and US$100 monthly from the sale of honey, pollen, propolis, jobones, or modernized boxes. The sale of hives fluctuates between US$150 and US$100. It should be highlighted that the success of this group of meliponiculturists is also because of the economic support and technological support from various institutions (Parra-Argüello *et al*., 2018). Likewise, in the locality of "Ich ek", municipality of Hopelchén, Campeche, there is the group "Koolel Kab", which is devoted to meliponiculture since 1995; they maintain the activity as a result of the sale of packaged honey and the added value from trading it in cosmetics (facial cream and soaps), as well as in eye droppers (for ophthalmic treatment), which they offer in their locality, fairs, by order or internet purchases, and in addition they have an advertising strategy through brochures, exhibitions, and promotion via internet (Pumares-Chab, 2019; Martínez and Vázquez, 2019).

In the last 20 years, different initiatives have been presented to promote the knowledge, management and rescue of meliponiculture in the YP. The School of Veterinary Medicine at Universidad of Yucatán has offered courses-workshops to meliponiculturists from the YP, addressing topics such as the transference of bee nests to modernized hives, reproduction, division of the hives, and honey production; the courses-workshops are offered in Yucatán, Campeche and Quintana Roo, with the main result of an increase in 8% of meliponiculturists (González-Acereto *et al*., 2006).

Likewise, manuals have been made about breeding and management of the "Xunancab" bee in the YP (González-Acereto and Araujo Freitas, 2005; Villanueva *et al*., 2005a; Pat-Fernández *et al*., 2018a), and there was even a box designed, called González-Acereto con Bisagras (T.I.B.G.A), for *M. beecheii*, *Scaptotrigona pectoralis*, and *Nanotrigona perilampoides* bees, which can be used for breeding.

The efforts mentioned for the promotion and rescue of meliponiculture have been found through various studies, to understand the sites where meliponiculture is practiced and to learn about the traditional knowledge and management of NSLBs in Campeche (Moo-Huchin *et al*., 2015; Negrín and Sotelo, 2016; Pat-Fernández *et al*., 2018ab, Vásquez and García, 2019 and Uchin-Mas, 2021), Yucatán (Quezada-Euán *et al*., 2001; González-Acereto *et al*., 2006; Catzin-Ventura *et al*., 2008; Pinkus-Rendón, 2013; Moo-Huchin *et al*., 2015; Parra-Argüello *et al*., 2018), and Quintana Roo (Villanueva-Gutiérrez *et al*., 2005; Villanueva-Gutiérrez *et al*., 2013; Moo-Huchin *et al*., 2015).

The analysis of the economic, technical and social characteristics of meliponiculture allows understanding the producers' needs and problems, and to identify opportunities for the development of projects that favor management of the bees, to increase honey production, and to provide an added value to the byproducts obtained from the hive. This would increase the economic income of the families that practice this activity. Meliponiculture in Campeche has been studied more in the northern part of the state

(Tenabo, Calkiní and Hecelchakán); in Hopelchén, it is still necessary to conduct studies to understand more deeply the sites where meliponiculture continues to be practiced.

CONCLUSIONS

The group of meliponiculturists studied in SAC presented a low interest for meliponiculture, which is why 80% of the members of the group abandoned the activity and are currently devoted to apiculture. Although the members of the group know the procedure to harvest honey with syringes and to divide hives, they do not know the procedure to trade the honey and therefore they consider meliponiculture to be of low profitability.

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Reproductive characterization of hair ewe in the American tropics: a review part 1

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ABSTRACT

Objective: To carry out a reproductive characterization of the breeds of hair ewe in the tropical region of the Americas.

Design/Methodology/Approach: A search of scientific information about the reproductive variables of breeds of hair ewe was carried out and the results were analyzed in the Web of Science, Redalyc, Dialnet, PubMed, SciELO, and Latindex databases.

Results: We described and discussed the highly-variable onset of puberty in ewe lambs. Similar results were found in adult ewes regarding the duration and occurrence of the estrus, estrus cycle length, seasonal anestrus, ovulation rate, fertility, gestation, and prolificacy.

Study Limitations/Implications: Information about the reproductive variables of breeds of hair ewe is poorly known or non-existent.

Findings/Conclusions: The onset of puberty in 15-43 kg ewe lambs ranges from 175 to 335 d of age. The estrus cycle of hair ewe in Mexico lasts 17 d in autumn-winter and 21 d in spring-summer. In Brazil, this cycle lasts 17 d in spring-summer and 18 d in autumn-winter. In the United States of America and Venezuela, the estrus cycle length was similar in both periods of the year. The occurrence of the estrus reached 97.1% in autumn-winter and 76.5% in spring-summer. The duration of the estrus ranges from 16 to 52 hours. Seasonal anestrus occurs from May to July. The ovulation rate ranges from 1 to 3 oocytes. The percentage of fertility fluctuates between 80 and 100% in temperate months and reaches 37% in warm ones. Gestation lasts from 144 to 152 d. Prolificacy ranges from 1.0 to 2.2 offspring per ewe. The lambing interval ranges from 244 to 294 d.

Keywords: Puberty, estrous, estrous cycle, ovulation rate.

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INTRODUCTION

The success of a sheep production unit depends mainly on nutritional, reproductive, and health management, as well as on welfare, biosecurity, and other factors (Rojas-Rodríguez *et al.*, 2001). Reproduction is a fundamentally important factor, since it determines the ability to increase the size of the herd in each period. Therefore, an efficient reproductive management will allow the production unit to be profitable (Plakkot *et al.*, 2020). Because of their reproductive and adaptation characteristics, hair sheep are very important in the tropical regions of the Americas, the Caribbean, and certain warm and humid environments in the United States; some hair breeds and breeds crossbred with wool sheep have shown their potential for lamb production under marginal conditions (Wildeus, 1997). With the increase in sheep production, other hair breeds have become present in recent years, including Katahdin, Dorper, Santa Inés, and Saint Croix. Although they have a greater productive capacity, especially regarding weight gain, their reproductive characteristics are still largely unknown. Therefore, the objective of this work was to review the scientific data bases, in order to carry out a reproductive characterization of the main breeds of hair sheep in the American tropic.

Puberty in ewe lambs

Puberty is defined as the beginning of reproductive activity and is evidenced by the first ovulation and the presence of >0.5 ng mL^{-1} plasma progesterone (López-Sebastián *et al.*, 1985). In ewe lambs with a body weight ranging from 15 to 43 kg, the first estrous has been observed between 8 and 13 months of age (Camacho-Ronquillo *et al.*, 2008). Likewise, it has been established that ewe lambs begin puberty when they reach 60 to 75% of the live weight of the adult ewe (Simplicio and Santos, 2005). This reproductive process is strongly influenced on the one hand, by age and live weight (Table 1), and, on the other hand, by birth season and type of lambing (Table 2), as well as food availability and health (Valasi *et al.*, 2012).

Table 1 shows that Saint Croix, Pelibuey, and Blackbelly ewes began puberty at an earlier age (218 d average) and with a live weight of 29.4 kg. However, West African, Katahdin, Dorper, Morada Nova, Santa Inés, and Brazilian Somali ewes start their reproductive process later (292 d average) and with a live weight of 28 kg.

On the other hand, the ewe lambs born in September-October began puberty earlier than those born in July-August. For their part, the ewe lambs born in January-March and April-June took longer to begin their reproductive activity (Table 2).

Table 2 shows that the type of lambing (single or multiple) has a significant effect on the onset of puberty. In Mexico, multiple-birth ewe lambs began puberty 24 d earlier than ewe lambs that had single birth; meanwhile in Brazil, single-birth ewe lambs began puberty 16.8 d earlier than their multiple-birth counterparts. It is important to note that, in both

Table 1. Age (a) and live weight (LW) in ewe lambs of different hair breeds at the onset of puberty.

Breed	Place	Age (d)	PV (kg)	Reference
DR	Yucatán, Mexico	291.2±7.2	43.1±0.93	Zavala *et al.* (2008)
KN	Yucatán, Mexico	272.6±1.6	36.5±0.2	Zavala *et al.* (2008)
BY	Yucatán, Mexico	250.1±1.8	24.7±0.4	Zavala *et al.* (2008)
PY	Yucatán, Mexico	231.8±1.5	27.5±0.2	Zavala *et al.* (2008)
SC	Virginia, USA	175±17.7	36.0±0.7	Wildeus, (1997)
SI	Piauí, Brazil	294.6±13.8	28.0±0.7	Girão and Medeiros, (1988)
SB	Caerá, Brazil	335.4±12.8	21.8±0.6	Silva *et al.* (1987)
MN	Caerá, Brazil	292.1±11.7	23.8±0.6	Silva *et al.* (1987)
WA	Zulia, Venenezuela	268. 1±35.5	15.3±1.0	Rodríguez-Urbina *et al.* (2001)

DR=Dorper; KN=Katahdin; BY=Blackbelly; PY=Pelibuey; SC=Saint Croix; SI=Santa Inés; SB=Brazilian Somali; MN=Morada Nova; WA=West African.

Table 2. Effect of season and type of lambing on the onset of puberty in hair ewe lambs.

Time of birth	Place	Age (d)	Live weight (kg)	Reference
Jul-Aug	Yucatán, Mexico	262.5±0.6	31.0±0.6	Zavala *et al.* (2008)
Sep-Oct		233.8±1.2	30.6±0.1	
Jan- Mar	Maracay, Venezuela	420.1±126.3	30.1±2.0	Rondón *et al.* (2002)
Apr-Jun		439.3±149.4	30.4±2.2	
Type of lambing				
Single	Yucatán, Mexico	266.6±1.0	34.1±0.1	Zavala *et al.* (2008)
Multiple		242.5±0.7	28.6±0.1	
Single	Noreste de Brazil	290.3±9.9	26.2±0.5	Dias *et al.* (1988)
Multiple		313.1±9.8	24.2±0.5	
Single	Piauí, Brazil	291.8±17.7	28.5±1.0	Girão and Medeiros, (1988)
Multiple		302.5±26.0	23.3±1.5	

countries, single-birth ewe lambs were 4.2 kg heavier than multiple-birth ewe lambs. The low body weight of the latter could be associated with a lower intake of liquid feed during rearing. Finally, the differences in the onset of puberty between breeds are associated with their genetic structure, since the genetic factor influences the ability to adapt to the environment (Zavala *et al.*, 2008).

Estrus in ewes

Estrus is the period in which the ewe is receptive to the ram. When the ewe manifests the estrus behavior, it presents changes in its behavior and emphasize the constant urination, inappetence, nervousness, and changes in the coloration of the vulva (pink to red) (Rojas-Rodríguez *et al.*, 2001). Generally, the estrus lasts 25 to 28 hours; however, some ewes have a 30 to 48 h estrus (Table 3, Macías-Cruz *et al.*, 2015). It is important to mention that, when the estrus takes place, ewes do not mount each other, as it happens with cows and does; the only way to know which female is in estrus is with the help of a marker ram (Muñoz-García *et al.*, 2021).

Estrus cycle

The estrus cycle is the period that elapses from one estrus to another or the successive repetition of behavioral events that begins and ends with estrus behavior in adult ewes. Table 3 shows the estrus cycle length (Rojas-Rodríguez *et al.*, 2001).

The estrous cycle length ranges from 14 to 24 days (17 d average), depending on the time of year in which it takes place. However, in the case of Pelibuey ewes in Veracruz, Mexico, their estrus cycle was longer during the spring. In general, the occurrence of the estrous in the reproductive season is higher (95.4%, autumn-winter) than in the seasonal anestrus period (29%, spring-summer). The duration of the estrus ranges from 16 to 52 h, depending on the production system (intensive or extensive), as can be seen in Table 3. Both the estrus cycle length and the occurrence of the estrus will be strongly influenced by

Table 3. Influence of the season of the year on the cycle length, occurrence, and duration of the estrus in hair ewes.

Breed	Place	Estrus cycle (d) and % incidence of oestrus				Duration of oestrus (h)	Reference
		P	V	O	I		
PY	Baja California, Mexico	24.2 95.2	17.8 95.2	17.3 100	20.1 100	24 to 36	Macías-Cruz *et al.* (2015)
PY	Veracruz, Mexico	22.3 84.6	22.3 82.3	17.8 92.0	17.8 96.0	16 to 52 in grazing	Cruz-Lazo *et al.* (1994)
PY	Habana, Cuba	19 -	17 -	17 -	19 -	- -	Herrera *et al.* (2010)
KN	Baja California, Mexico	- -	- -	- 95.8	- 95.8	- -	Macías-Cruz *et al.* (2017)
KN	Durango, Mexico	- 72.2	- 29.7	- -	- -	- -	González-Godínez *et al.* (2014)
DR	Yucatán, Mexico	17.4±0.3 82.1	17.3±0.3 82.1	17.4±0.3 97.4	17.4±0.3 97.4	17.3±1.8 to 36.6±1.7	Aké-López *et al.* (2017); Aké-López *et al.* (2019)
SI	Ceará, Brazil	14 a 19* -	14 a 19* -	18.4±0.4 ** 90.2	18.4±0.4 ** 90.2	29.1±1.0	Simplício *et al.* (1981)
MN	Caerá, Brazil	18.5±0.3	18.5±0.3	18.0±0.3	18.0±0.3	24 to 36	Sousa *et al.* (2015)
SB	Caerá, Brazil	18.5±0.3	18.5±0.3	18.0±0.3	18.0±0.3	30.2±0.80	Simplício *et al.* (1981)
WA	Maracaibo, Venezuela	- 17	16.8±0.9 84	16.8±0.9 87	17.2±0.7 89	26.7±2.4	González-Stagnaro, (1993)
SC	Idaho, USA.	19 51	- -	19 74	- 25	-	Pope *et al.* (1989)

PY=Pelibuey; KN=Katahdin; DR=Dorper; SI=Santa Inés; MN=Morada Nova; SB=Brazilian Somali; WA=West African; SC=Saint Croix; *Rainy season (January to June) in Brazil; **Dry season (July to December) in Brazil; SP (P)=spring, SU (V)=summer, AU (O)=autumn, WI (I)=winter.

the rainy season and forage availability, since this food source will be used to feed the ewes and improve their body condition.

Seasonal anestrus

It is defined as the period of ovarian inactivity and it is characterized by the absence of sexual activity (Rosa and Bryant, 2003). The ewes of certain breeds have well-defined seasonal reproductive cycles, as in the case of wool ewes from temperate and cold climates (far from the equator); these ewes have a period of ovarian inactivity in spring-summer, heavily impacted by the photoperiod (Ortavant *et al.*, 1988). However, some hair breeds developed in equatorial regions have periods of reduced estrous activity during spring and early summer; such is the case of the Pelibuey ewes in Mexico, which have a reproductive inactivity of 36.1±8.1 d (Valencia *et al.*, 2006). However, Arroyo *et al.* (2007) reported a 67.5 d absence of sexual activity (spring-summer). The behaviour of the Saint Croix ewe had a similar pattern: reproductive inactivity in May, June, and July (Wildeus, 1997). Likewise, Balaro *et al.* (2014) reported that 85.7% of the Santa Inés ewes had a short seasonal anestrous in the spring (September to December), while González-Stagnaro (1993) determined that the West African ewe reduced its reproductive activity in the December-March season to 7.5%. At this time, the limited availability of forage has a reduced nutritional quality, which fails to meet the nutritional requirements, as reported for the Cuban Pelibuey sheep by Herrera *et al.* (2008).

Ovulation rate

The ovulation rate is defined as the number of oocytes released per estrus cycle (Nagdy *et al.*, 2018). The evaluation of the ovulation rate is determined by counting the corpora lutea (CL) present on the ovarian surface. CL can be counted in two ways: 1) with an ultrasound machine; and 2) by direct counting via laparoscopy. The choice of method will depend on the equipment available, and the staff trained to carry out the count. The ovulation rate has great variability between and within breed (Table 4). Generally, ewes ovulate between 24 and 27 h after the beginning of the estrus.

Table 4 shows that the Pelibuey ewe from San Luis Potosí, Mexico, had the highest ovulation rate, followed by the Morada Nova and Santa Inés ewes (both from Brazil), while the Dorper from Mexico had the lowest ovulation rate. It is important to highlight that the ovulation rate is strongly influenced by feeding, body condition, and age of the ewe.

Fertility

Fertility is defined as the number of ewes that get pregnant after services (natural mating or artificial insemination) multiplied by 100. Fertility can be affected by body condition, age, and health status. Table 5 shows that an acceptable fertility percentage ranges between 85 and 100% (Abecia-Martínez and Forcada-Miranda, 2010) and is strongly influenced by the time of year. Therefore, in the reproductive season (autumn-winter, 90.8%) ewes have a higher fertility than in the seasonal anestrus period (spring-summer, 84.8%).

Gestation

The gestation length in ewes is very similar between breeds; the gestation is 150 d average from the last service (natural mating or artificial insemination) to the lambing (Table 5).

Table 4. Ovulation rate in different breeds of hair ewe.

Breed	Place	Ovulation rate	Reference
PY	San Luis Potosí, Mexico.	3.00±0.18	Muñoz-García *et al.* (2021)
BY	California, USA.	2.04	Bradford and Quirke, (1986)
SI	Brasilia, Brazil.	1.2±0.1 - 2.2±0.1	Silva *et al.* (2010)
MN	Sobral, Brazil.	1.4 - 2.3	Silva *et al.* (1987)
SB	Sobral, Brazil.	1.0 - 2.0	Silva *et al.* (1987)
DR	Yucatán, Mexico.	1.2±0.0 - 1.4±0.1	Aké-López, (2019)
WA	Aragua, Venezuela.	1.6±0.2	Contreras-Solís, (2008)
SC	Utah, USA.	1.93-1.97	Wildeus, (1997)

PY=Pelibuey; BY=Blackbelly; SI=Santa Inés; MN=Morada Nova; SB=Brazilian Somali; DR=Dorper; WA=West African; SC=Saint Croix.

Table 5. Fertility percentages by time of year and gestation length in hair ewes.

Breed	Place	Fertily (%)				Length of gestation (d)	Reference
		P	V	O	I		
PY	Baja California, Mexico.	81	81	100	100	150.0	Macías-Cruz *et al.* (2009) Macías-Cruz *et al.* (2017)
PY	Habana, Cuba.	65	92	92	65	150	Herrera *et al.* (2010)
DR	Baja California, Mexico.	93	93	-	-	146.7	Macías-Cruz *et al.* (2009)
KN	Baja California, Mexico.	92	92	-	-	148.5	Macías-Cruz *et al.* (2009)
BY	Yucatán, Mexico.	80.6±2.7	79.2±2.7	-	80.6±2.7	-	Segura *et al.* (1996)
DR	Yucatán, Mexico.	84.4	84.4	85.3	85.3	-	Aké-López *et al.* (2019)
SI	Sao Carlos, Brazil.	100.0	-	100.0	100.0	148.3-150.1	Machado and Simplício, (1998)
SB	Ceará, Brazil	-	-	90	90	144-152	Sousa *et al.* (2015)
MN	Ceará, Brazil	-	-	92	92	144-152	Sousa *et al.* (2015)
WA	Maracaibo, Venezuela.	80.5	94.3	94.3	80.5	150.9±2.8	González-Stagnaro, (1993)
SC	Arkansas, USA.	37.7±7.6	71.2±7.3	88.6±7.4	86.7±8.3	-	Brown and Jackson, (1995)

PY=Pelibuey; DR=Dorper; KN=Katahdin; BY=Blackbelly; DR=Dorper; SI=Santa Inés; SB=Brazilian Somali; MN=Morada Nova; WA=West African; SC=Santa Cruz; SP (P)=spring, SU (V)=summer, AU (O)=autumn, WI (I)=winter.

Table 5 shows that, on the one hand, fertility is variable during the year: it is highest in autumn-winter and lowest in spring-summer. On the other hand, the gestation length was similar in all breeds and ranges from 144 to 152 d.

Prolificacy

It is defined as the total number of offspring born divided by the total ewes that were born multiplied by 100 (Abecia-Martínez and Forcada-Miranda, 2010). Table 6 shows the prolificacy in different breeds of hair ewes.

Table 6 shows that both Mexican and Cuban Pelibuey ewes have great variability in prolificacy, with a range from 1.2 to 2.1 offspring per ewe. Similarly, the prolificacy of Santa Inés ewes ranged from 1.1 to 1.8 offspring per ewe. The Pelibuey, Santa Inés, and Blackbelly breeds had the most offspring per ewe, followed by Dorper and Katahdin. West African and Brazilian Somali ewes had the fewest offspring per ewes.

Lambing interval

The lambing interval (LI) is defined as the time that elapses between one birth and the next one. In the ewes, the LI can be affected by external and internal factors. The season in which the births occur (dry and rainy) stands out among the former, while the age and weight of the ewe at birth, type of lambing (single- or multiple-birth), and number of births stand out among the latter (nulliparous or multiparous) (Neto Rego *et al.*, 2012). Table 7 shows the average values of the LI of the hair breeds.

On the one hand, the previous table shows that the breeds that had the lowest LI were Dorper from Brazil, followed by Blackbelly from the Caribbean, Mexico, and Trinidad and Tobago. On the other hand, the ewes that had a higher LI were Pelibuey from Cuba,

Table 6. Prolificacy in different breeds of hair ewes.

Breed	Place	Prolificity	Reference
PY	Baja California, Mexico	2.1	Macías-Cruz *et al.* (2009)
PY	San Luis Potosí, Mexico	2.1±0.1	Muñoz-García *et al.* (2021)
PY	Yucatán, Mexico	1.2	Segura *et al.* (1996)
PY	Yucatán, Mexico	1.46±0.1 a 1.7±0.0	Cansino-Arroyo *et al.* (2009)
PY	Habana, Cuba	1.2 - 1.7	Herrera *et al.* (2010)
SI	Brasilia, Brazil	1.11 - 1.87	Silva *et al.* (2010)
SI	Minas Gerais, Brazil	1.7±0.3 - 2.2±0.2	Saunders *et al.* (2012)
MN	Caerá, Brazil	1.51	Sousa *et al.* (2015)
SB	Caerá, Brazil	1.04	Sousa *et al.* (2015)
DR	Yucatán, Mexico	1.2±0.0 to 1.4±0.1	Aké-López *et al.* (2019)
BY	California, USA	1.71	Bradford and Quirke, (1986)
BY	Trinidad and Tobago	1.92	Rastogi, (2001)
KN	Durango, Mexico	1.3±0.3	González-Godínez *et al.* (2014)
SC	Arkansas, USA	1.4±0.0 to 1.7±0.0	Brown and Jackson, (1995)
WA	Maracaibo, Venezuela	1.1 - 1.2	González-Stagnaro, (1993)

PY=Pelibuey; SI=Santa Inés; MN=Morada Nova; SB=Brazilian Somali; DR=Dorper; BY=Blackbelly; KN=Katahdin; SC=Saint Croix; WA=West African.

Table 7. Lambing interval in different hair breeds.

Breed	Place	LI (d)	Reference
PY	Chiapas, Mexico	268 .0±66.2	González-Garduño *et al.* (2010)
PY	Habana, Cuba	294	Herrera *et al.* (2010)
SI	Piauí, Brazil	281.1±64.7	Neto Rego *et al.* (2012)
MN	Distrito Federal, Brazil	284.8±70.6	Quesada *et al.* (2002)
SB	Piauí, Brazil	281.3±9.8	Braga Magalhães *et al.* (2010)
DR	Piauí, Brazil	244	Rosanova *et al.* (2005)
BY	Tabasco, Mexico	256.2±79.1	Hinojosa-Cuellar *et al.* (2011)
BY	Caribbean Islands	257	de Almeida, (2018)
BY	Trinidad and Tobago	262.3±3.1	Knights *et al.* (2012)
SC	Utah, USA	262	Evans, (1987)
WA	Lara, Venezuela	268.8±72.5	Dickson *et al.* (2004)

PY=Pelibuey; SI=Santa Inés; MN=Morada Nova; SB=Brazilian Somali; DR=Dorper; BY=Blackbelly; SC=Saint Croix; WA=West African.

and Santa Inés, Somali, and Morada Nova from Brazil. The LI will mainly depend on the feeding that the ewe receives (Herrera *et al.*, 2010).

CONCLUSIONS

The onset of puberty in 15-43 kg hair ewe lambs ranges from 175 to 335 d. Ewe lambs born in September-October began puberty earlier than those born in April-June.

According to the type of lambing, single-birth ewe lambs in Brazil began puberty earlier than multiple-birth ewes. Meanwhile, single-birth ewe lambs born in Mexico began puberty earlier than multiple-birth ewe lambs. In average, the estrus cycle in Mexico lasts for 17 d and 21 d for the autumn-winter and spring-summer periods, respectively. In the case of Brazil, in spring-summer, the estrus cycle length is 17 d and 18 d in autumn-winter. In the United States of America and Venezuela, the estrus cycle length was similar in both seasons of the year. The occurrence of the estrus in Mexican ewes was, on average, 97.1% in autumn-winter and 76.5% in spring-summer. The estrus lasts from 16 to 52 h. Seasonal anestrus occurs from May to July and is closely related to rainfall and forage availability. The ovulation rate ranges from 1 to 3 oocytes. Fertility is higher in the temperate months (80-100%) than in the warm ones (37%). Gestation lasts from 144 to 152 d. The prolificacy ranges from 1.0 to 2.2 offspring per ewe. The lambing interval ranges from 244 to 294 d.

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Punica granatum: A plant with a high potential for the synthesis of silver nanoparticles

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ABSTRACT

Objective: To use pomegranate (*Punica granatum* L.) extracts as a reducing agent for a simple and eco-friendly biosynthesis of silver nanoparticles and to test the latter's antimicrobial potential against different bacteria.

Methodology: The leaves and peel of ripe fruits of pomegranate were used to prepare aqueous and methanolic extracts to synthesize silver nanoparticles (AgNP). Then we evaluated these nanoparticles with techniques that allowed us to define their size, shape, and dispersion quality in aqueous media. To characterize the AgNPs we resorted to UV-Vis spectroscopy and dynamic light scattering (DLS). We determined their antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Staphylococcus aureus*.

Results: UV-Vis spectra were observed with absorption peaks at 425 nm for the nanoparticles synthesized with both aqueous and methanolic extracts. Regarding size, distribution, and dispersion in solution, the zeta potential shows that the surface charge in all cases is negative, with an average value ranging from −28.0 mV to −52.0 mV. Smaller nanoparticles were obtained from the aqueous extracts of the peel and leaves, with values between 1.42 - 2.96 nm, while the methanolic extracts yielded nanoparticles of 10 - 19.04 nm. Inhibition of microorganisms proved to be best against *Escherichia coli*, with a minimum inhibitory concentration (MIC) of 0.83 μg/mL up to 6.68 μg/mL.

Conclusions: This study suggests that pomegranate extracts synthesize small-sized, well-dispersed silver nanoparticles with an almost spherical morphology, and good antimicrobial activity against gram-negative bacteria such as *Escherichia coli*.

Keywords: *Punica granatum*, Nanoparticles, Green synthesis, Antimicrobial properties.

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INTRODUCTION

Pomegranate (*Punica granatum* L.) originated in Central Asia. Its introduction in America dates to approximately 1500 - 1600. For centuries it has been cultivated for its fruit in warm regions of the world. It is a deciduous shrub or tree that grows in temperate and cold regions. Its fruit is edible, round-shaped, and usually pink-to-reddish-colored. It

Image by Albrecht Fietz at Pixabay

is famous for its beneficial properties for health, and is therefore a functional food, mainly because it is rich in polyphenols. In addition, its peels, leaves, and flowers are used to prepare different meals [1–8].

The ecological synthesis of silver nanoparticles has gained popularity because of its inexpensive and environmentally friendly approach. The green synthesis of nanoparticles uses different reducing agents, among which fruit extracts stand out for being innovative, ecological, profitable, and well preserved when synthesized [7, 9, 10]. Nanotechnology is a constantly evolving discipline with practical applications in various fields, which makes it appealing for industries such as food, cosmetics, construction, textiles, electronics, energy, and the health sector [6, 11, 12]. Metal-based nanoparticles ranging from 1 to 100 nm show good physicochemical properties, especially silver nanoparticles, which have chemical stability, good conductivity, and antimicrobial, antiviral, antifungal, and cytotoxic activity [6, 10]. They are particularly suitable as antimicrobials since bacteria do not have an adaptation strategy that can achieve universal resistance to all metals. Thus, if an antimicrobial metal fails, other metal-based antibiotics can intervene. This allows for research opportunities revolving around the properties of metallic nanomaterials to produce effective varieties of antimicrobial agents [13–16].

Knowledge about nanomaterials from biocompatible agents such as plants is relevant for broader applications. For this reason, our objective was to develop silver nanoparticles from pomegranate extracts to test their antimicrobial potential against gram-positive and gram-negative microorganisms.

MATERIALS AND METHODS

Vegetal material

We evaluated ripe fruits and leaves of pomegranate (*Punica granatum* L.) using the fruits' epicarp. First, we cut down the epicarp and let it dry in the shade at room temperature (25 ± 2 °C) until a constant weight was obtained. It was then reduced to fine powder using a mechanical mill and sieved through a no. 44 mesh. The same process was conducted with the leaves. The powders were stored at room temperature, and protected from light and humidity for subsequent experimental use.

Extract preparation

Aqueous extract

We prepared aqueous extracts by infusing the leaves and the epicarp. For this purpose, we used the fine powder previously obtained and distilled water brought to a boil (1:10 ratio). We removed the infusion from the heat and kept it covered for 10 min. Afterwards, we used Whatman paper grade 40 for filtering. We kept the filtrates protected from light for subsequent use. Each aqueous extract destined for evaluation was prepared at the time of each test.

Methanolic extract

We used 100 g of each materials' fine powder. Following Patra, Dhal, and Thatoi (2011), we macerated the vegetal powder in methanol for five days to obtain the polar compounds

[17]. Then, using a rotary evaporator (BUCHI R-124), methanol was evaporated and the dry extract retrieved.

Green synthesis of silver nanoparticles (AgNP)

We conducted a green synthesis of silver nanoparticles according to the procedures described by Rodríguez-Luis *et al.* (2016) [18]. We used an aqueous $AgNO_3$ solution (1.7×10^{-4} g/mL) to synthesize the AgNPs from the pomegranate extracts, adding 10 mL of each extract separately to the $AgNO_3$ solution via magnetic stirring. Once the extracts were added, we adjusted the pH value of the solution to 10 using an NH_4OH solution (Figure 1).

Physical characterization of the AgNPs

To characterize the AgNPs, we used spectroscopy and dynamic light scattering (DLS) according to the procedures described by Martínez-Castañón *et al.* (2008) and Espinosa-Cristobal *et al.* (2013) [13, 18]. We obtained UV-Vis absorption spectra with an S2000 UV-Vis spectrometer (OceanOptics, Inc.). The position of the plasmonic absorption peak is a surface phenomenon characteristic of the metals' material, morphology, and size at the nanometer scale. Silver nanoparticles absorb energy from the electromagnetic spectrum in the UV-Visible range between 400 and 450 nm. All data were obtained thrice for each AgNP. The hydrodynamic radius and the zeta potential were determined by dynamic light scattering using a Nanosizer DLS.

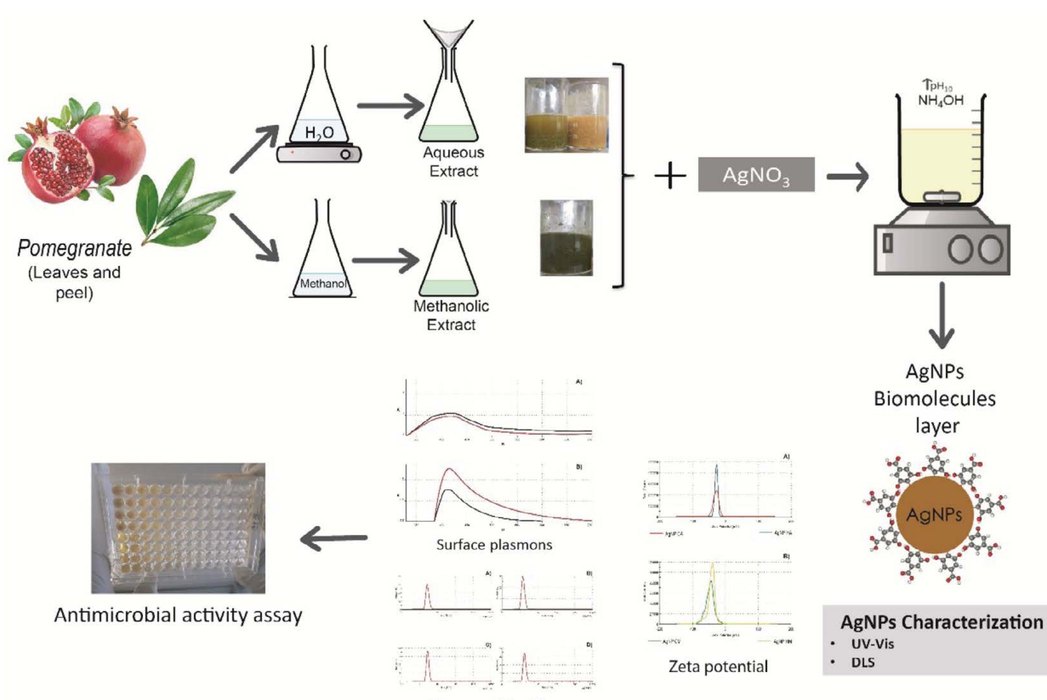

Figure 1. Graphic summary of the AgNPs synthesis process and their physical characterization.

Evaluation of the *in vitro* antimicrobial effect of AgNPs

Selected microorganisms

We resorted to American Type Culture Collection (ATCC) strains, which guarantee the quality of the chosen method. We selected microorganisms that can cause infections in different body parts such as lungs, intestinal tract, skin, or oral cavity. Both gram-positive and gram-negative microorganisms were considered. Among the gram-negative microorganisms, we chose *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Enterococcus faecalis (*ATCC 29212); among the gram-positive, *Staphylococcus aureus* (ATCC 29213) and *Streptococcus mutans* (ATCC 25175).

Standard Microdilution Method (MIC)

The antimicrobial activity of AgNPs was tested using the standard microdilution method that determines the minimum inhibitory concentration (MIC) leading to the inhibition of bacterial growth. For the assays, we followed the procedure described by Dealba-Montero *et al.* (2017) [20]. We used disposable microtiter plates with 96 wells and serial half dilutions of Mueller Hinton (MH) broth with microorganisms at a 105 CFU/mL concentration. We had two controls: the nanoparticles with no broth or bacterial suspension (SB) were added in the first line of wells, while only MH broth was added in the twelfth line. Lines two to eleven contained MH broth, AgNPs from each extract, and SB —all in serial dilution. The results were read after 24 h of incubation at 37 °C as the MIC of the tested substance to inhibit the growth of the bacterial strain. We extracted a culture from the well where no growth inhibition was observed to conduct a disk diffusion test with MH agar in plates previously labeled with the corresponding concentration. The incubation lasted 24 h at 37±2 °C. We took the results from the plates where the antimicrobial thoroughly eliminated bacterial growth. We tested for methanol to rule out any effect of said substance.

RESULTS AND DISCUSSION

Physical Characterization of AgNPs

We observed UV-Vis spectra with absorption peaks at 425 nm for the nanoparticles synthesized with the aqueous extract of epicarp (AgNP-CA) and for those synthesized with the aqueous extract of leaves (AgNP-HA) (Figure 1.) As for the nanoparticles synthesized with the methanolic extract of both epicarp (AgNP-CM) and leaves (AgNP-HM), absorption peaks appeared at 420 nm in the UV-Vis spectra.

In the absorption spectrum, the position of the absorption peak is associated with the size of the particle. Absorptions lower than 435 nm indicate the presence of small particles of around 3 nm. These results confirm the morphology of nanoparticles, since they comply with the absorption associated with small spherical nanoparticles (between 420 and 450 nm as described by [13]).

Regarding size distribution and dispersion in solution, we obtained the zeta potential for the synthesized AgNPs. As we can see, the surface charge in all cases is negative, with an average value of −28.0 mV for AgNP-CA, −28.7 mV for AgNP-HA, −42.3 mV for AgNP-CM, and -51.3 mV for AgNP-HM (Table 1).

In every stance, the surface charge values represent a state of stability that prevents the nanoparticles from coalescing and from subsequent aggregation. As for the size, the smaller AgNPs came from the methanol extracts and presented values of 1.42 for AgNP-CM and 1.72±0.01 nm for AgNP-HM. In all cases, values may represent the obtention of more homogeneous nanoparticles (Table 1).

Plant extracts have been shown to be rich in polyphenols and other compounds that contain hydroxyl groups, which can reduce silver ions to silver [20, 21]. The surface charge values of the AgNPs indicate that the particles are dispersed or have good dispersion. This characteristic is more notable in particles developed from the methanolic extracts because related molecules of said extracts coat the AgNPs and thus reduce the free energy on the particles' surface, preventing aggregation. This phenomenon is explained in the work of Yang *et al.* (2016) [21], which mentions that the free energy on the surface of nanoparticles—especially those that are being formed—is higher, which renders them unstable and allow them to agglomerate with each other to form larger particles, thus lowering the surface energy. This process is spontaneous; however, in the nanoparticles developed from pomegranate extracts by green synthesis, the phytochemicals adhered to their surface, which helped reduce their free energy and avoid agglomeration.

Evaluation of the *in vitro* antimicrobial effect of AgNPs

To assess the antimicrobial activity of the AgNPs developed in this study, we tested minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for the silver nanoparticles against *E. coli*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*. We present the results in Table 2, which shows the AgNPs from both aqueous and methanolic extracts.

Regarding the inhibition of microbial growth by nanoparticles, AgNP-CA and AgNP-HA showed better inhibition against *E. coli*, both with a MIC of 3.38 μg mL^{-1}. For their part, AgNP-CM presented a MIC of 6.68 μg mL^{-1}, while AgNP-HM had the lowest MIC with 0.83 μg mL^{-1}.

By observing the AgNP sizes, we can state that the smallest nanoparticles correspond to the lowest inhibition values. The MIC of all AgNPs is lower when tested against *E. coli*

Table 1. Particle size values for AgNPs obtained from aqueous and methanolic extracts of pomegranate epicarp and leaves.

	Size (nm)	Surface charge (mV)
AgNP-CA	2.89 ±0.01	−28.7
AgNP-CM	1.42 ±0.00	−42.3
AgNP-HA	2.96 ±0.01	−28.0
AgNP-HM	1.72 ±0.01	−51.3

AgNP-CA represents nanoparticles from the aqueous extract of the pomegranate epicarp, AgNP-CM stands for nanoparticles from the methanolic extract of the epicarp, AgNP-HA are nanoparticles from the aqueous extract of the pomegranate leaves, and AgNP-HM come from the macerated methanolic extract of the leaves. The values are the mean of three repetitions ± their standard deviation (SD); the value of SD in the surface charge is zero.

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for AgNPs from pomegranate aqueous and methanolic extracts.

	CMI (μg mL^{-1})				**CMB (μg mL^{-1})**			
	E. coli	***P. aeruginosa***	***S. aureus***	***E. faecalis***	***E. coli***	***P. aeruginosa***	***S. aureus***	***E. faecalis***
AgNP-CA	3.38	6.68	3.38	26.75	6.68	13.37	6.67	26.75
AgNP-CM	6.68	13.37	26.75	53.50	6.68	26.75	53.50	53.50
AgNP-HA	3.38	13.37	6.67	13.37	3.38	26.75	13.37	26.75
AgNP-HM	0.83	1.67	1.67	3.38	0.83	1.67	3.38	3.38

AgNP-CA represents nanoparticles from the aqueous extract of the pomegranate epicarp, AgNP-CM from the methanolic extract of the epicarp, AgNP-HA from the aqueous extract of the leaves, and AgNP-HM from the macerated methanolic extract of the leaves. The values are the mean of three repetitions with a standard deviation equaling zero.

and *P. aeruginosa* (gram-negative microorganisms) than when tested against other bacteria. These results may be due to the cell wall structure of each strain, since gram-negative strains can have thinner cell walls than gram-positive ones. Moreover, the nanoparticles interact with the charge of cell walls and affect permeability [13, 22].

The size of nanoparticles affects their antimicrobial effect. Particles of small dimensions within the nanometric scale were found in all AgNPs obtained from the pomegranate extracts. We know that small AgNPs and the phytochemicals adhered to their surface may have a better interaction with bacteria, which is reflected in their high inhibition activity on some bacterial membranes [13, 16, 20].

CONCLUSIONS

The nanoparticles synthesized from *P. granatum* extracts were of small size, showed good dispersion, and an almost spherical morphology, with a good antimicrobial activity against gram-negative bacteria such as *E. coli*. Therefore, pomegranate could be a source of reducing agents for synthesizing silver nanoparticles. Exploring new natural extracts that allow the development of nanomaterials is essential.

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Colegio de Postgraduados

Morphometric, weight, viability, and germination analysis of castor bean seeds (*Ricinus communis*) under two temperature and relative humidity conditions

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ABSTRACT

Objective: To analyze the morphometric, weight, viability, and germination evaluation under two temperature and relative humidity conditions, in sixteen local varieties of castor bean (*Ricinus communis*) from several states of Mexico (E1-E16), as well as two commercial varieties (k75B and k93B).

Design/Methodology/Approach: The following morphometric characteristics were analyzed using a vision system: area, elongation index (EI), and Feret's diameter (FD). Viability and germination tolerance (germination percentage (GP)), germination speed (GS), and emergence speed index (ESI)) were evaluated under two conditions of relative humidity and temperature (T1 - RH 80%/T 20 °C; T2 - RH 30%/T 40 °C), using a completely randomized block experiment design, with four replicates of 75 seeds.

Results: There are morphometric differences (EI, area, and FD) between and within the study varieties. There are significant differences between T1 and T2 regarding the following variables: days of radicle emergence (T1: 44.71 and T2:11.6), germination percentage (T1: 48.37 and T2: 56%), ESI (T1: 34.07±12.72 and T2: 77.02±23.78), and GS (T1: 9.93 and T2: 24.60). The results obtained show a positive correlation between the morphometric properties and the germination percentage in T1; however, there was no correlation in T2.

Study Limitations/Implications: There were no limitations to carry out this study.

Findings/Conclusions: The E4 and E15 local varieties obtained a >93% productive potential in T1, while the E3 and E16 local varieties obtained a >78% productive potential in T2. Meanwhile, the k75B commercial variety had a better performance in T1 (89.75%) than in T2 (78.67%).

Keywords: germination, local varieties, *Ricinus communis*, viability.

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INTRODUCTION

Castor bean (*Ricinus communis* L.) is an allogamous plant (Euphorbiaceae, 2n=20) native to tropical Africa. It spread out from Africa to the Middle East as a wild plant, giving birth to a wide range of different weights, sizes, shapes, and colors (Goytia-Jiménez

Image by Alejandro Sánchez

et al., 2011; Peña-Uribe *et al.*, 2021). In Mexico, castor bean is grown in the states of Chiapas, Chihuahua, Coahuila, Colima, Guanajuato, Guerrero, Jalisco, Estado de México, Michoacán, Morelos, Nayarit, Oaxaca, Sinaloa, Sonora, Tabasco, Tamaulipas, San Luis Potosi, Zacatecas, Tlaxcala, Veracruz, and Yucatán (Acosta-Navarrete *et al.*, 2017; Barrios-Gómez *et al.*, 2018; Solis Bonilla *et al.*, 2016; Llaven Valencia *et al.*, 2019; Canul *et al.*, 2021).

From the diversity point of view —and in order to evaluate the variability between and within genotypes or species—, the morphometric characterization of plants (seeds and fruits) is based on the qualitative or quantitative values (Barrios-Gómez *et al.*, 2018; Canul-Ku *et al.*, 2022; Pecina-Quintero *et al.*, 2013), the knowledge about sizes and shapes, and the useful data available. This information can be used to design innovative management and storage techniques in natural ecosystems and farms (Huang *et al.*, 2022; Peña-Uribe *et al.*, 2021). These measurement techniques include vision systems that have been implemented (Modarres Najafabadi and Farahani, 2012) to find out the morphometric dimensions of the objects, in order to generate databases (Acosta *et al.*, 2013). The analysis of images is a less expensive non-invasive technique than hand techniques (Pasato-Guanga and Fuentes-Pérez, 2021).

Mature seeds have a certain latency degree, which can cause a slow, erratic, and low germination, probably as a result of the hard shell that covers the seeds and limits water permeability. Therefore, the plants have developed several adaptative strategies to counteract the adverse effects, enabling their survival (Ricardo Abril-Saltos *et al.*, 2017). These strategies include the control of physical and chemical changes, such as the loss of the cell membrane integrity, the reduction of enzymatic activity, and lipid peroxidation (Ergin *et al.*, 2022). Consequently, the loss of vigor and the germinative potential are fundamental parameters for the certification and commercialization of castor bean seeds (Escobar-Álvarez *et al.*, 2021).

Castor bean seed can develop under extreme environmental conditions: 18-33.9 °C temperatures, 383-1083 mm annual precipitations, and a 21-69% relative humidity (Hernández-Ríos *et al.*, 2019; Llaven Valencia *et al.*, 2019; Solis Bonilla *et al.*, 2016). The optimal temperatures for the development of this plant range from 20 to 25 °C (Machado *et al.*, 2012). These measurements were recorded using fiber optic sensors, in order to determine the development and behavior of castor bean seeds under the various agro-climatic conditions to which they are exposed (Huerta-Mascotte *et al.*, 2016).

Both in Mexico and in the rest of the world, castor bean (*Ricinus communis*) is a high interest culture, as a result of its oil content —mainly ricinoleic acid, which is a raw material used in the production of high value-added products. Therefore, efforts are aimed to increase the agronomic yield of this crop, under erratic weather conditions (Vasco-Leal *et al.*, 2022). Consequently, the objective of this study was to evaluate the following vigor parameters: the emergence speed, the germination speed, and the germination percentage of 16 local varieties and 2 commercial varieties of castor bean. The vision system proposed takes into account their vigor and the morphometric characteristics, as well as their relationship with the weight of 100 seeds as dimensional reference.

MATERIALS AND METHODS

Castor bean seeds came from a national collection, which was initially made up of 120 wild accessions, stored at 4 °C (Bonner, n.d.) for two years before they were evaluated. Out of this collection, 16 indehiscent varieties (E1-E16) were chosen, because they had obtained a high agronomic yield in Guanajuato. These seeds were grown in the INIFAP-Bajío experimental unit (20° 34' 47" N and 100° 49' 14" W, at 1,767 m.a.s.l.). We used the technological package developed by Hernández Martínez *et al.* (2013). The materials were compared with 2 commercial varieties native to India (k75B and k93B). The offspring of the first generation of the original plants was obtained.

Weight and Morphometry

Seed weight was determined using an Oahus analytical scale (0.0001g accuracy), randomly taking one hundred seeds from each variety. To morphometry, an area (a), minor axis (ma), and major axis (MA) were established and, subsequently, the elongation index (EI) and the Feret's diameter (FD) were determined, using the equations proposed by Quintanilla Carvajal *et al.* (2015) and the image processing algorithm developed by Ojeda-Magaña *et al.* (2010). This process is based on three stages: i) binarization, ii) segmentation, and iii) extraction of morphometry. We used the Matlab R2011 version to process the digital images, with a Dino-lite 311-ST microscope, which was placed 29 cm from the base where the seeds were placed. As dimensional reference, the algorithm validation was confirmed by hand, with a ± 0.24 cm^2 average error.

In order to evaluate germination viability and tolerance (germination percentage, germination speed, and emergence speed index), indirect tests were carried out in the lab to measure the physiological attributes of the seeds (Martínez-Solís *et al.*, 2010).

Germination percentage (GP)

The test was carried out using polystyrene trays, with peat-moss as a substrate. The seeds were sown at a 2-cm depth. The percentage was determined through a standard germination test, following the International Rules for Seed Testing (International Seed Testing Association, 2016), using a modified Bitoronette Mark II-845 plant growth chamber. The seeds were subjected to two treatments, controlling relative humidity (RH) and temperature (T) throughout the stages of the germination process. The first treatment had 80% RH and a 20 °C T, while the second treatment had a 30% RH and a 40 °C T. Germination tests were carried out for every experiment, using a completely randomized block design, with four repetitions of 75 seeds each.

Germination speed (GS)

GS was determined with the equation proposed by González-Zertuche and Orozco-Segovia (1996). The number of germinated seeds was quantified on a daily basis, during the germination period:

$$GS = \sum \frac{ni}{t} \qquad \text{Equation 1}$$

Where *GS* is the germination speed; *t* is the germination time from the sowing to the germination of the last seed; and *ni* is the number of seeds germinated in a given day.

Emergence speed index (ESI)

The number of plants that emerged was counted every day, considering the day in which they were sown in the trays as the first day. ESI was calculated using the following equation (2) proposed by González-Zertuche and Orozco-Segovia (1996):

$$ESI = \frac{\sum(niti)}{N} \quad \text{Equation 2}$$

Where *ESI* is the emergence speed index; *ni* is the number of seeds that germinated that day; *ti* is the number of days since the beginning of the experiment until day *i*; and *N* is the total number of germinated seeds.

Experimental design and result analysis

The results were statistically processed through an Analysis of Variance (ANOVA), comparing the means through the Tukey-Kramer HSD method (α=0.01 and 0.5), using the JMP software ver 5.0.1 (JUM-Statical Discovery Software, SAS).

RESULTS AND DISCUSSION

The average weight of the sixteen local varieties evaluated was 49.31 g per 100 seeds (0.49 g per seed). Meanwhile, the heaviest varieties were E-15, E-16, and E-4, which recorded 91.65±14.78, 86.95±27.87, and 86.83±23.44 g, respectively. In contrast, both commercial varieties (k75B and k93B) had an average weight of 31.45 g per 100 seeds (0.31 g per seed). This last result is the closest to the findings of Goytia *et al.* (2011) and Acebedo *et al.* (2018), who recorded average values of 48.72 and 32 g, respectively. The different results reported by the authors corroborate the diversity that likely results from the various agricultural and weather conditions. A wide variation range in the weight and shape of the seeds is considered a significant element in the plants' reproductive strategies and adaptations.

Meanwhile, 4.14±0.64 to 4.41±0.44 cm^2 ranges were reported in the area. They belong to the E1, E3, E4, E15, and E16 varieties. For their part, the E7 and E8 varieties reported a 1.36 to 1.38 cm^2 range, respectively, which matches the findings of Goytia-Jiménez *et al.* (2011).

Table 1 shows the results of the morphometric values of sixteen local castor bean varieties (E1-E16), plus two commercial varieties (k75B and k93B). Those values were obtained through a statistical comparison of their average values and standard deviations. The weight of the seed is directly proportional to its dimensions (area and FD). Likewise, Salihu *et al.* (2014) reported that seed weight is related to the total number of seeds produced by the plant.

Table 1. Weight and morphometric characteristics of castor bean seeds.

Variety	Weight in 100 seeds (g)	Area (cm^2)	EI (Φ)	FD
E-1	79.98±0.86	4.30±0.42	0.78±0.07^{cde}	2.76±0.23^{ab}
E-2	48.02±16.03	2.61±0.19	0.77±0.06^{gh}	1.66±0.13^{d}
E-3	78.81±18	4.14±0.64	0.76±0.06^{de}	2.65±0.44^{b}
E-4	86.83±23.44	4.41±0.44	0.68±0.03^{cd}	2.76±0.46^{ab}
E-5	43.09±5.32	2.69±0.31	0.74±0.07^{fg}	1.78±0.42^{cd}
E-6	50.78±13.28	2.89±0.32	0.73±0.07^{ef}	1.84±0.25^{c}
E-7	31.08±18.24	1.36±0.29	0.75±0.16^{de}	0.94±0.18^{ij}
E-8	21.13±17.75	1.38±0.17	0.61±0.06^{k}	0.89±0.14^{j}
E-9	40.94±12.88	2.30±0.31	0.69±0.07^{g}	1.50±0.29^{e}
E-10	33.42±13.99	1.99±0.11	0.66±0.04^{ghi}	1.13±0.08^{h}
E-11	36.65±16.93	1.99±0.18	0.76±0.02^{cde}	1.27±0.11^{fg}
E-12	27.74±18.21	1.69±0.30	0.78±0.07^{cd}	1.08±0.21^{h}
E-13	31.76±15.85	1.80±0.15	0.61±0.04^{jk}	1.15±0.11^{gh}
E-14	38.65±25.94	1.99±0.14	0.79±0.02^{c}	1.26±0.09^{fg}
E-15	91.65±14.78	4.49±0.41	0.76±0.03^{cde}	2.86±0.26^{a}
E-16	86.95±27.87	4.16±0.61	0.75±0.12^{cde}	2.66±0.54^{b}
Average	**51.71**	**2.76**	**0.72**	**1.76**
Standard deviation	**24.32**	**1.16**	**0.05**	**0.73**
k75B	32.7±20.14	1.50±0.27	0.64±0.03^{hij}	1.36±0.07^{f}
k93B	30.2±19.05	1.46±0.12	0.89±0.05 ^{a}	1.37±0.09^{ef}
Average	**31.45**	**1.48**	**0.76**	**1.365**
Standard deviation	**1.76**	**0.02**	**0.17**	**0.007**

Average±Standard Deviation. Means compared using Tukey-Kramer HSD. The same letter indicates that there is no significant difference (95%).
EI: elongation index. FD: Feret's diameter.

The evaluated variables have contrasting morphometries and colors (silver grey, ochre, dark, and matt black). Even within the same variety, there are colors or intensity combinations that hinder their classification.

No differences regarding the shape of the seed (SS) were recorded between the castor bean populations evaluated, all of which were elliptical.

For its part, the Feret's Index (FI) shows the correlation of the accessions with a bigger area and a greater FI (1, 3, 4, 15, and 16), which they are elements of the seed shape. Consequently, as the area increases, its parameters record a proportional increase and vice versa, because they are dependent elements, according to the formula proposed by Quintanilla Carvajal *et al.* (2015).

Table 2 shows the parameters of the germination percentage (%), ESI, and GS, when the seeds were subjected to two different temperature and relative humidity conditions.

Table 2. Germination under different Relative Humidity (RH) and Temperature (T) treatments.

Variety	**T1 RH 80%, T 20 °C**			**T2 RH 30%, T 40 °C**		
	GP	**ESI**	**GS**	**GP**	**ESI**	**GS**
E-1	92.00 ab	11.04 ab	E-1	92.00 ab	11.04 ab	E-1
E-2	64.25 d	6.43 d	E-2	64.25 d	6.43 d	E-2
E-3	88.00 abc	10.56 bc	E-3	88.00 abc	10.56 bc	E-3
E-4	93.75 ab	10.31 bc	E-4	93.75 ab	10.31 bc	E-4
E-5	86.50 abc	6.92 d	E-5	86.50 abc	6.92 d	E-5
E-6	73.50 abc	5.88 e	E-6	73.50 abc	5.88 e	E-6
E-7	83.75 abc	9.21 c	E-7	83.75 abc	9.21 c	E-7
E-8	78.75 bc	6.30 d	E-8	78.75 bc	6.30 d	E-8
E-9	80.00 c	6.40 d	E-9	80.00 c	6.40 d	E-9
E-10	81.25 c	6.50 d	E-10	81.25 c	6.50 d	E-10
E-11	77.75 abc	13.22 a	E-11	77.75 abc	13.22 a	E-11
E-12	82.50 abc	7.43 c	E-12	82.50 abc	7.43 c	E-12
E-13	86.00 abc	7.74 c	E-13	86.00 abc	7.74 c	E-13
E-14	78.50 c	7.07 c	E-14	78.50 c	7.07 c	E-14
E-15	95.75 a	10.53 bc	E-15	95.75 a	10.53 bc	E-15
E-16	93.25 a	11.19 ab	E-16	93.25 a	11.19 ab	E-16
Average	**83.46**	**8.54**	**Average**	**83.46**	**8.54**	**Average**
DS	**8.27**	**2.29**	**DS**	**8.27**	**2.29**	**DS**
k75B	89.75 ab	9.87 c	k75B	89.75 ab	9.87 c	k75B
k93B	87.00 abc	9.28 c	k93B	87.00 abc	9.28 c	k93B
Average	**88.37**	**9.57**	**Average**	**88.37**	**9.57**	**Average**
DS	**1.94**	**0.41**	**DS**	**1.94**	**0.41**	**DS**

GP: germination percentage (%); ESI: emergence speed index; GS: germination speed (number of seeds/day). Means compared with Tukey-Kramer HSD. The same letters indicate that there is no significant difference (95%).

Tenorio-Galindo *et al.* (2008) highlight that bigger seeds have a greater possibility of survival and that they also increase the germination percentage and speed. However, both parameters depend on the chemical composition of the seed, the permeability of the husk, and the difference between the water potential and the thickness of the storage tissues. In this research, bigger seeds recorded greater GP and GS under T1, which provided greater relative humidity to the seeds than T2. There are few studies about castor bean seeds, as a result of their great phenotypic diversity.

According to Escobar-Álvarez *et al.* (2021), high relative humidity accelerates metabolism and, consequently, triggers germination. In their turn, these results are related with the findings of this research, since the emergence speed index (ESI) was higher in bigger seeds; therefore, a greater ESI entails a greater germination speed. These findings contrast with the results of Acevedo-Lara *et al.*, (2018), who determined that the ESI value is the result of the lower water volume required for the germination of smaller seeds (while bigger seeds require more water).

Table 3 shows that T1 had a positive correlation ($r>0.67^{**}$) regarding the GPT1, GST1, and ESIT1 variables. There was a positive correlation ($r>0.88^{**}$) regarding T2 between the A, FI, and W variables, and a negative correlation ($r>-0.50^{*}$) between EI and A.

On the one hand, seeds (E1-E16) under T1 (RH 80%, T 20 °C) recorded an 83.46%±8.27 germination percentage, an 8.54±2.29 ESI, and a 7.39±3.31 GS. On the other hand, when they were exposed to T2 (RH 30%, T 40 °C), the germination percentage was 52.34%±19.14, the ESI was 8.32±3.14, and the GS was 11.64±7.52.

Meanwhile, the germination percentage and ESI of commercial varieties (k75B and k93B) recorded similar values than other varieties (E1-E16), although their GS values were different (3.81±1.32). Under T2, the germination percentage (71.33±10.37), the ESI (13.10), and the ES (24.48±11.36) were higher than the same parameters for the E1-E16 varieties. These results prove that a greater index results in greater germination speed —an attribute that is related to morphometric dimensions—, since small seeds require less water, most of which is assimilated by the contact surface (Acevedo-Lara *et al.* 2018). Meanwhile, ESI was 77.02±23.78, while GS was 24.60.

Table 3 shows the results of the correlation analysis. There are positive correlations between the evaluated parameters (EI, area, germination percentage, FI, ESI, and GS), as well as a high correlation between area and germination percentage (0.678, $p<0.05$), area and FI (0.997, $p<0.05$), ESI and germination percentage (0.946, $p<0.05$), GS and ESI (0.892, $p<0.05$), GS and germination percentage (0.782, $p<0.05$), and ESI and area (0.851, $p<0.05$).

Table 3. Correlation between the evaluated parameters.

Variety	T1 RH 80%, T 20 °C	T1 RH 30%, T 40 °C	Variety	T1 RH 80%, T 20 °C	T1 RH 30%, T 40 °C	Variety	T1 RH 80%, T 20 °C
GPT1	GP	ESI		GP	ESI		GP
E-1	92.00 ab	11.04 ab	E-1	92.00 ab	11.04 ab	E-1	92.00 ab
E-2	64.25 d	6.43 d	E-2	64.25 d	6.43 d	E-2	64.25 d
E-3	88.00 abc	10.56 bc	E-3	88.00 abc	10.56 bc	E-3	88.00 abc
E-4	93.75 ab	10.31 bc	E-4	93.75 ab	10.31 bc	E-4	93.75 ab
E-5	86.50 abc	6.92 d	E-5	86.50 abc	6.92 d	E-5	86.50 abc
E-6	73.50 abc	5.88 e	E-6	73.50 abc	5.88 e	E-6	73.50 abc
E-7	83.75 abc	9.21 c	E-7	83.75 abc	9.21 cW	E-7FI	83.75 abc
E-8	78.75 bc	6.30 d	E-8	78.75 bc	6.30 d	E-8	78.75 bc
E-9	80.00 c	6.40 d	E-9	80.00 c	6.40 d	E-9	80.00 c
E-10	81.25 c	6.50 d	E-10	81.25 c	6.50 d	E-10	81.25 c
E-11	77.75 abc	13.22 a	E-11	77.75 abc	13.22 a	E-11	77.75 abc
E-12	82.50 abc	7.43 c	E-12	82.50 abc	7.43 c	E-12	82.50 abc
E-13	86.00 abc	7.74 c	E-13	86.00 abc	7.74 c	E-13	86.00 abc
E-14	78.50 c	7.07 c	E-14	78.50 c	7.07 c	E-14	78.50 c

FI=Feret's index; SI=sphericity index; GPT1=germination percentage (%) for T1; GPT2=germination percentage (%) for T2; W=weight of 100 seeds (g); ESIT1=emergence speed index for T1; ESIT2=emergence speed index for T2; GST2=germination speed for T2; A=area (cm^2).

This information contributes to the phenotypic characterization of the seeds. This process would enable the identification of desirable characteristics for seed improvement. Based on their handling, the resulting seeds can be useful for producers, as well as for the design of rolling, pressing, and milling equipment (Tönshoff *et al.*, 2002).

CONCLUSIONS

There were weight, size, and color variations between both the castor bean seeds varieties themselves and the commercial seeds. The proposed algorithm helped to determine the area, elongation index, and Feret's diameter dimensions, applying the image analysis with the adjustments required by the color diversity.

Although catalase can be considered a seed deterioration indicator, some seeds that have been stored for more than two years have managed to maintain an adequate germination percentage.

The relative humidity and temperature conditions may determine seed vigor, as a consequence of their potential for a quick and uniform emergence, producing a high number of normal seedlings (although they might not be optimal for the species). Seed vigor enables the identification of the desirable characteristics for seed improvement.

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Agriculture 4.0: Is Mexico Ready?

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ABSTRACT

Objective: To analyze the possibilities and actions required to foster the introduction of technologies consistent with the term "agriculture 4.0" in Mexico.

Design/Methodology/Approach: To identify providers of technology in Mexico. To present the cost-benefit equation regarding the adoption of said technology as applied to the cultivation of maize in different regions. To design and construct an adoption propensity index that will serve as a basis to propose focused and adequate actions to remove technology access barriers.

Results: Mexico has a young and wide offer of technology, both tangible and intangible, where digital platforms of agricultural management, mobile apps, and remote monitoring predominate. The cost-benefit relationship offers a large margin to adopt new technologies. However, there are adoption barriers (related to education or infrastructure, for instance) that represent a challenge to different regions of the country: the northern, northeastern, and western states of Mexico are more likely to adopt new technologies.

Study limitations/Implications: Further experimental and field analyses are required to delve deeper into potential additional barriers (culture-related, for example).

Findings/Conclusions: The cost-benefit analysis offers a large margin for adoption. However, the propensity to adopt is associated to restricting factors such as the producers' educational level, the production unit's size and level of mechanization, the access to and use of Information and Communication Technologies, and the telecoms infrastructure, whose geographic disparity is significant. The public sector's intervention is desirable to reduce the gap between the supply and demand of technologies, as well as the access barriers to the latter.

Key words: Precision agriculture, Digital gap, Technology adoption, Intangible technologies.

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INTRODUCTION

In 1968, the Club of Rome (Meadows *et al.*, 1972) challenged the limits that our natural environment imposes on the growth of human activity.[1] The results sounded the alarm due to the bleak conclusions drawn by the participants when illustrating the excess processes that can be reached, and to the scenario of collapse they laid out for humanity in the 21st century were the collective course of action not rectified. At the same time, the current technological advances combine diverse spheres of knowledge and have come to be called the Fourth Industrial Revolution (Schwab, 2017), the basic technologies of

[1] The dynamic modeling of five subsystems is considered, *i.e.* population, agricultural production, depletion of nonrenewable resources, industrial production, and pollution.

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the current revolution are, among others: the Internet of Things (IoT), cloud computing, big data management, 5G, artificial intelligence, 3D printing, robotics, and virtual and augmented reality.

At the center of this crossroads lies an essential element of the limits to growth: the supply of natural resources to provide food for the population. The consumption, growth, and depletion of said resources is largely related to the available amount of arable land. According to FAO, the amount of arable land per capita in the world in 1961 was 0.45 ha. This figure dropped to 0.21 available ha in 2016 and maintains an estimated annual reduction rate of 0.08 ha per capita. Based on FAO data, yield improvements will be the main source of production increases (78% of improvements between 1961-1999 and 70% towards 2030).

Unlike the previous periods, the subsequent expansion stage of agricultural productivity will face unprecedented challenges such as: i) a historically low availability of agricultural lands; ii) a changing climate that manifests in greater episodes of drought, floods, and extreme heat waves; and iii) restrictions in the use of energy and fertilizers due to the greater conscience regarding the impact of agricultural activities on the environment, among other limiting factors.

Two periods of significant advances are identified in the group of technologies related to the fourth industrial revolution: 1990 to 2014, a period associated to precision agriculture, and 2015 to date, linked to smart farming and agriculture 4.0. The new technologies will bring a greater availability of foods worldwide; however, foreseeing the impact of said technologies in a country like Mexico is still difficult due to the great inequalities that characterize its primary sector.

To help understand the technological advances, this article sets out to describe how the new technologies are made up, what their supply and demand potential is in the Mexican context, as well as to identify the potential barriers that are keeping us from turning these technologies into an opportunity to reduce —instead of widening— the development gap in rural Mexico.

MATERIALS AND METHODS

The adoption of new technologies for agricultural development is usually heterogenous among and within countries. A publication search in the “ScienceDirect” platform was done using the key terms “Precision Agriculture”, “Smart Farming”, and “Digital Agriculture” / “Agriculture 4.0”, in order to examine the performance reports, obstacles, and results of applying this technology. The presence of the terms in article key words, abstracts, or titles was emphasized.

To identify the degree of adoption and the provider profile in Mexico, we turned to the new technologies adoption database recently prepared by Fideicomisos Instituidos en Relación con la Agricultura (FIRA).[2] The nature of technological innovation was

[2] FIRA tends to Mexico’s rural, agricultural, forest, and fishing sectors by granting loans, guarantees, and technical assistance through a vast network of associate businesses and around 100 offices throughout the country. https://www.fira.gob.mx

emphasized based on a distinction commonly made in agricultural economics (Sunding & Zilberman, 2001) regarding the type of technologies for agricultural production, which in turn can be divided into tangible or embodied[3] and intangible or disembodied[4] technologies. The viability of the adoption of new technologies in the country was subsequently assessed based on the assumption that the total income after adopting said technologies should be higher than the total income before such adoption. The variable income per hectare I_{ha} was thus defined and expressed as a function of the yield per hectare Y_{ha} and the cost per hectare C_{ha}, according to the following equation:

$$I_{ha} = Y_{ha} - C_{ha} \quad (1)$$

The necessary condition to adopt the technology is:

$$I_{ha}^{t} \geq I_{ha}^{t0} \quad (2)$$

Where superscript t describes the technology that allows us to define income I_{ha}^{t}. We used $\alpha(t)$ to designate a change factor of production per hectare due to the adoption of technology t, and $\beta(t)$ to designate a change factor of cost per hectare due to the adoption of technology t. Both $\alpha(t0)=0$ and $\beta(t0)=0$, where $t0$ is the original technology. The variable is defined as follows:

$$Y_{ha}^{t} = N_{ha}^{t} * P_{crop}$$

and

$$C_{ha} = Cind_{ha}^{to} + Cdep_{ha}^{t}$$

where

$$N_{ha}^{t} = (1+\alpha(t)) * N_{ha}^{t0} \text{ and } Cdep_{ha}^{t} = Cdep_{ha}^{t0}(1+\beta(t))$$

Where N_{ha}^{t0} is the number of tons per hectare yielded by the crop when using the initial technology $t0$; P_{crop} is the price of the crop; $Cind_{ha}^{t0}$ is the cost per hectare of producing the crop, where the cost is independent from the used technology, while $Cdep_{ha}^{t}$ is the corresponding cost, where the cost is dependent on technology t. Substituting equation (1) in equation (2), the following condition ensues:

[3] These technologies are incorporated into physical devices such as agricultural machinery, sensors for animals or plants, drones and robots, among others.

[4] These technologies comprise, for instance, remote consultancy platforms, software to manage and monitor crops, and digital platforms boosted by data mining.

$$\left(1+\alpha(t)\right)*N_{ha}^{t0}*P_{crop}-\left(Cind_{ha}^{t0}+Cdep_{ha}^{t0}\left(1+\beta(t)\right)\right)\geq N_{ha}^{t0}*P_{crop} - \left(Cind_{ha}^{t0}+Cdep_{ha}^{t0}\right) \quad (3)$$

$$\alpha(t)\geq\frac{Cdep_{ha}^{t0}}{N_{ha}^{t0}*P_{crop}}\beta(t)$$

Inequality (3) represents the change factor in production necessary for the producer's income to be greater with technology t than with respect to the existing technology of each crop t0. Finally, to analyze the natural access barriers to new technologies we created a development index (UNDP, 2022) considering four development variables that restrict the adoption of agriculture 4.0 technologies: production unit size, 3G and 4G coverage, educational lag and level of Information and Communication Technologies (ICT) use, and degree of agricultural labor mechanization. All of this was based on information from four surveys.[5]

RESULTS AND DISCUSSION

The term agriculture 4.0 was first used as one of many extensions of the "fourth industrial revolution" (Schwab, 2017). Figure 1 shows two development stages of the new technologies as applied to agriculture. The first one (1998-2014) refers to precision agriculture, which according to the National Research Council (1998) is an "IT management strategy to obtain data from multiple sources to make them relevant in decisions related to crop production". Precision agriculture considers three basic components: data capture at an appropriate frequency and scale, their interpretation and analysis, and the implementation of a managerial response in the appropriate time and size. The information sources generally come from tools developed for other spheres of scientific activity, such as satellites, multispectral cameras, or sensors. The increase in the number of bibliographic references largely linked to digital and smart farming from the period 2014-2017 is noteworthy. From this period onwards, articles refer to big data technologies (Wolfert *et al.*, 2017), cloud computing (Pivoto *et al.*, 2018), digital innovation (Ayre *et al.*, 2019), the Internet of Things (Doshi *et al.*, 2019), and artificial intelligence (Spanaki *et al.*, 2021), none of which were available during the preceding period. All of these technologies led to a natural evolution of precision agriculture.

International service providers

Birner *et al.* (2021) identified four types of agricultural technology providers.

[5] Censo Agrícola, Ganadero y Forestal (2007) for production units and land areas; Instituto Federal de Telecomunicaciones and Secretaría de Agricultura y Desarrollo Rural (IFT-SADER, 2020) for 3G and 4G coverage; Encuesta Nacional Agropecuaria (ENA, 2019) for variables related to the use of tractors, educational level, and the use of ICT in production units.

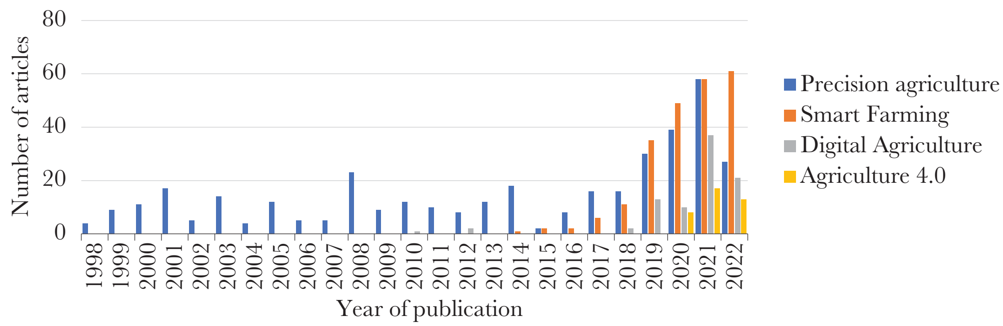


Figure 1. Bibliographical references relevant to agricultural technology innovation

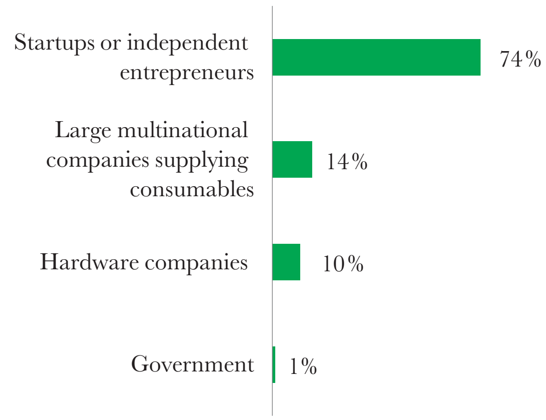
Table 1. Types of technology providers.

Type of provider	Value offer	Examples
Large multinational companies supplying consumables	They provide services alongside their products and have a great capacity to invest in research and development (R&D), and to distribute their services through their commercial networks.	Bayer, Syngenta, Monsanto, DuPont, Yara.
Large software and hardware multinational companies	Large software companies with the capacity to gather and process very vast databases.	IBM, Microsoft, SAP, TENCENT, Alibaba, Google.
Hardware companies (not necessarily agricultural)	They have economies of scales and give added value to their main lines of business (*e.g.* machinery).	Bosch, John Deere, Airbus, XAG, Massey Ferguson, Fendt, Kubota.
Startups or independent entrepreneurs (not necessarily specialized in the agricultural sector)	Agility and innovation, as well as specialization in added value services for producers.	DigiFarm, Hello Tractor, Xarvio app, RML AgTech, Cropin.

Based on the survey results, we identified 86 technology providers in Mexico, most of them (74%) startups or independent entrepreneurs who offer both tangible and intengible technology. In contrast, we could not find any software provider with a large multinational company profile. Meanwhile, the large hardware companies are scarce in the country; they represent 10% of the total. In the latter category, we found companies such as John Deere, Massey Ferguson, and XAG. In short, these providers offer technologies related to agriculture 4.0 in Mexico: 49 of them offer technologies classified as tangible (57%) and 37, as intangible (43%) (see Figures 2 and 3).

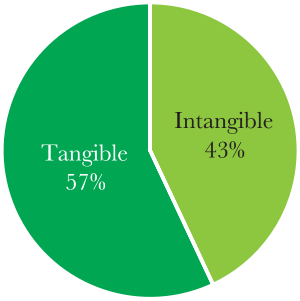
During the last 14 years, the adoption of these technologies has increased, with a tendency to adopt more intangible technologies than tangible ones. Digital platforms of agricultural management were more present in 2018, mobile apps in 2019, and drones and agrobots in 2020.

In the end, we found an adoption process in place for a wide range of technologies in different production chains in Mexico, comprising basic grains, vegetables, and perennial crops. Maize stands out as the main crop to have adopted the different types of technology, both tangible and intangible, which is consistent with the socioeconomic importance this crop has in Mexico.



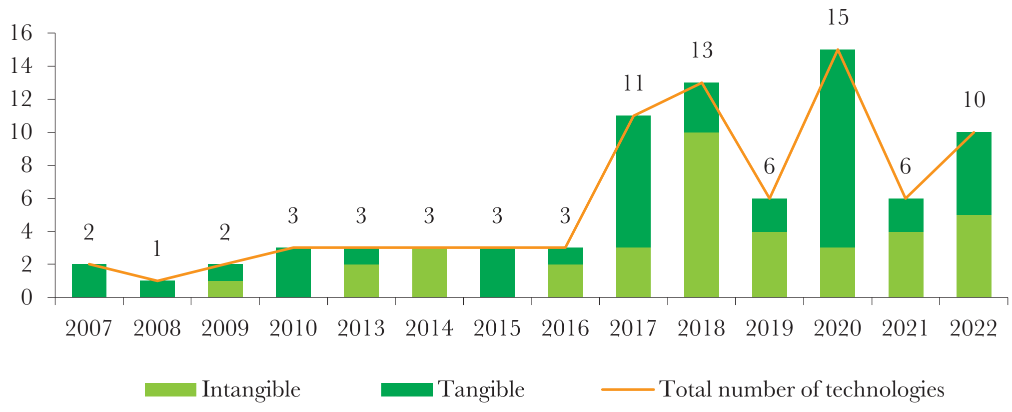
Source: Own elaboration based on information gathered by FIRA.

Figure 2. Technology providers in Mexico.



Source: Own elaboration.

Figure 3. Percentage of tangible and intangible technologies in Mexico



Source: Own elaboration based on information gathered by FIRA.

Figure 4. Historical series of technology adoption in Mexico, 2007-2022

Table 2. Characterization of technology types by agricultural chain and year of adoption.

Type of technology	Technology	Main agricultural chains where it is applied	Year of first adoption
Tangible	Drones	maize, wheat, cane, barley, vine, agave, cotton, tomato, citrics	2002
	Agrobots	Various crops	2016
	High-end tractors	maize, rice, banana, lemon, watermelon, melon, milk	2000
	Agricultural devices	maize, sorghum, bean, fava bean, sunflower, among other seeds	2005
	Monitoring system	maize, green chili, garlic, tomato, and milk	1995
Intangible	Apps	maize, barley, honey, vegetables, fruits, dairy products, meat, seafood, and pine tree	2013
	Digital platforms of agricultural management	maize, wheat, palm oil, apple, honey, lemon, vegetables, and coffee	2002
	Research & Development	Various crops	1982

Source: Own elaboration based on the information gathered by FIRA.

Potential demand of services in Mexico

The adoption of new technologies must meet the condition of generating a higher income for the producer, once the acquisition costs have been considered. This condition can be met under different parameters according to a series of production-related conditions, such as crop type, production region, currently available technology, product prices, as well as the component impacted by the new technology. Thus, considering production costs and yields databases[6] by geographical zone, we analyze different scenarios that will allow us to assess the productivity increase thresholds that are necessary to generate demand for new technologies.

The starting point to analyze this sensibility was the condition presented in equations (2) and (3). We chose three states with different advancement levels in maize productivity: Sinaloa (high productivity), Morelos (intermediate productivity), and Tlaxcala (low productivity). We assumed the adoption of a technology impacting fertilization costs.

Table 3. Analysis of the adoption of new technologies.

State	N_{ha}^{t0}	P_{maize} (\$)	$Cdep_{ha}^{t0}$ (\$)	$\frac{Cdep_{ha}^{t0}}{N_{ha}^{t0} * P_{crop}}$
Sinaloa	12	6,075	17,034	0.234
Morelos	7.5	6,500	9,722	0.199
Tlaxcala	4.85	6,300	3,974	0.130

6 Agrocostos FIRA www.fira.gob.m/agrocostos.

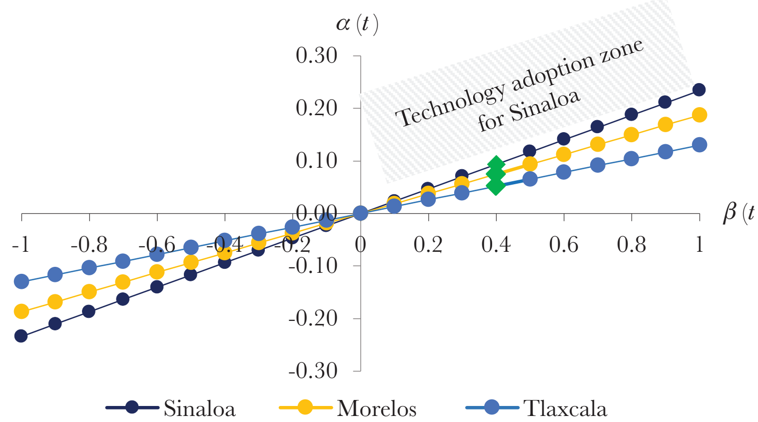


Figure 5. Change factor of maize productivity in Sinaloa, Morelos, and Tlaxcala

The slopes of the straight lines calculated define the new technologies adoption zone depending on the effect on production cost β_t. This zone is broad and lies above the straight lines according to:

$$\text{Sinaloa } \alpha(t) \geq 0.234*\beta(t), \text{ Morelos } \alpha(t) \geq 0.199*\beta(t), \text{ Tlaxcala } \alpha(t) \geq 0.130*\beta(t)$$

In turn, the line delimiting the adoption threshold depends on the region's productivity N_{ha}^{t0}, the price of the product P_{maize}, and the cost associated to the adopted technology $Cdep_{ha}^{t0}$. In this regard, Figures 6 and 7 analyze the sensibility of the increase in productivity necessary to adopt the new technologies (some scenarios of impact on cost $\beta(t)$ are additionally considered).

Figures 6 and 7 suggest three assumptions regarding the necessary conditions for the adoption of new technologies: i) the increase in productivity required to adopt technologies decreases as the price of maize rises; ii) the increase in productivity required for the adoption of technologies decreases when the degree of agricultural production development is higher; and iii) in a low performance setting, a relatively higher impact on productivity is required for the adoption of the new technology, regardless of its cost.

Figure 6. Isoquants for price of maize/return on productivity

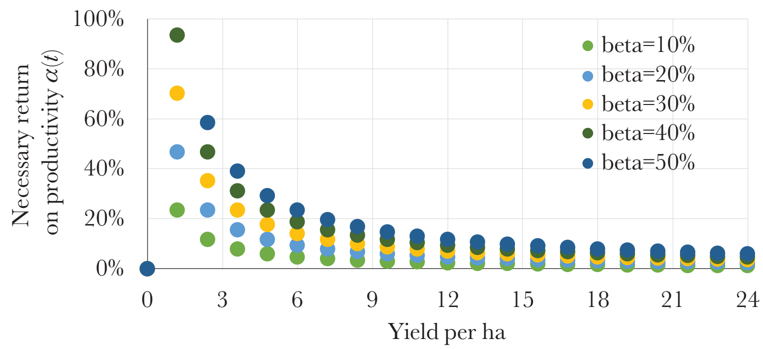


Figure 7. Isoquants for yield of maize/return on productivity.

Access barriers: infrastructure, technology, and capacities

As we already illustrated, the potential demand of digital technologies depends on different factors, such as geographical characteristics, level of mechanization, yield rate of the agricultural production units. All of these factors assume that attracting said technologies to the production setting is feasible. However, this is only possible if the minimal operation and adoption conditions required for the new technologies to perform are in place. As we have already mentioned, new technologies use cloud computing, the Internet of Things, and other digital devices that require basic access elements. Three relevant dimensions are analyzed below.

I) Telecoms infrastructure: access to 3G and 4G services.
II) Educational level, and use and adoption of ICT.
III) Mechanization of agricultural labor.

Infrastructure

The 3G and 4G mobile coverage is an enabling element for both tangible and intangible technologies, for the latter require data to be collected in the field and then sent to data centers to be processed. Although many service providers have set up capacities to save information and then exchange it when connectivity services are accessed (GSMA, 2019), this issue continues to be a natural barrier to the adoption of the technologies described. According to the Instituto Federal de Telecomunicaciones (IFT) and the Secretaria de Agricultura y Desarrollo Rural (SADER) 2020, 81% of agricultural production units in Mexico report having access to 3G technologies, while 75% of them report having access to 4G technologies. The percentage of production units (PU) with access to this service in the states of Oaxaca, Guerrero, Campeche, and Quintana Roo is the lowest of the country. On the contrary, as shown in Figure 8, production units in the northeastern and western states of the country report a higher access to this service. Figure 12 shows that the states whose production units have larger agricultural work areas usually have a higher level of 3G and 4G connectivity and coverage.

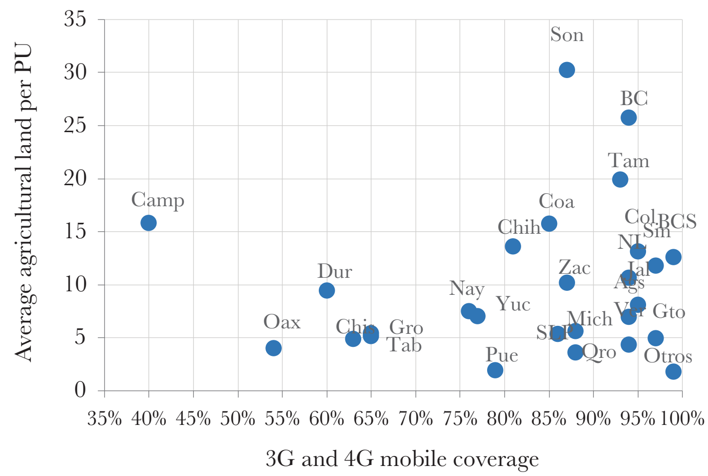


Figure 8. Average agricultural land according to their coverage level.

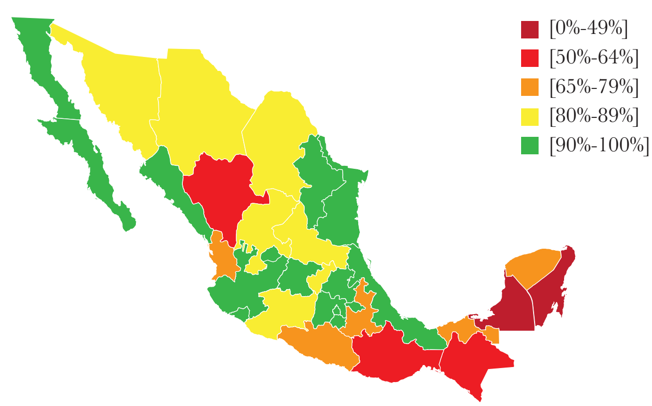


Figure 12. States according to their mobile coverage level.

Educational level, and use and adoption of ICT

The producer's or production unit owner's educational level is another aspect that restricts the demand for technologies, particularly those requiring digital interaction, such as remote consultancy or digital assessment apps. There are two types of abilities: basic education abilities, that allow the interpretation of figures and texts associated to crop development; and abilities that allow the use and adoption of communication and digital technologies. According to the Encuesta Nacional Agropecuaria (ENA, 2019), the average schooling level of agricultural producers is 5.9 years: 57.1% of producers have primary education, 16.8% have secondary education, and 14.8% do not have any studies. The states of Baja California (1.7%), Durango (2.1%), Mexico City (2.8%), and Aguascalientes (3.1%) have a lower percentage of uneducated producers, as can be seen on Figure 13.

Figures 9 and 10 show that states where production units have larger work areas are related with both a lower educational lag and a higher use of ICT. When

Figure 13. Educational level in Mexico (% of producers with no studies).

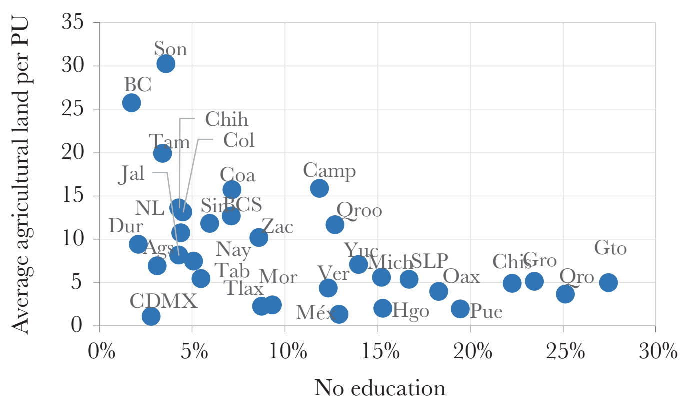


Figure 9. Average agricultural land according to the owner's educational level.

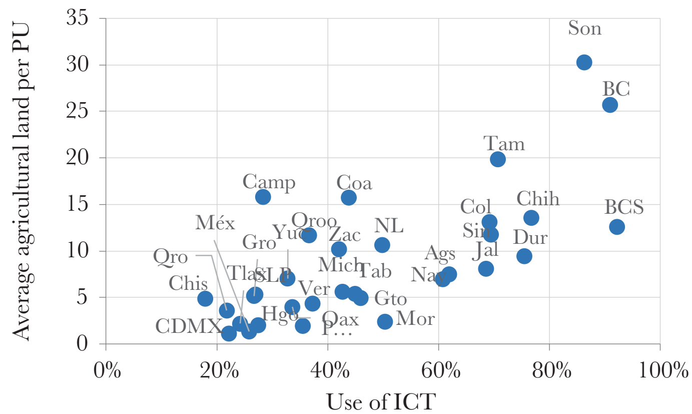


Figure 10. Average agricultural land according to use of ICT.

analyzing the producer's income in relation to the use of ICT in Mexico, we obtain a Pearson correlation coefficient of -0.7, which means that the larger the population[7] with incomes below the poverty line (PL), the lower the use of ICT.

[7] Living in municipalities with agricultural production units.

Mechanization

One of the main variables related to the potential demand of tangible technology is the level of mechanization of the production units. In this regard, the tractor is one of the more representative agricultural devices due to its versatility based on the large amount of implements it can use. According to ENA 2019 data, the higher percentage of production units that own a tractor are located in the northern, western, and northeastern states. In contrast, less than 40% of production units in most southern and southeastern states have tractors. This makes them less likely candidates to adopt tangible or embodied technologies, as Figure 14 shows.

The index of technology adoption potential is presented next in order to add in one dimension the basic conditions for the adoption of agriculture 4.0 technologies for each state of the country.

Figure 15 shows the geographic contrast between the northern, northeastern, and western production units, and those of the southern and southeastern regions with regard to their new technologies adoption potential. This evidence suggests the need of public policies to keep the new technologies from becoming a factor in further dividing the regions in terms of productivity.

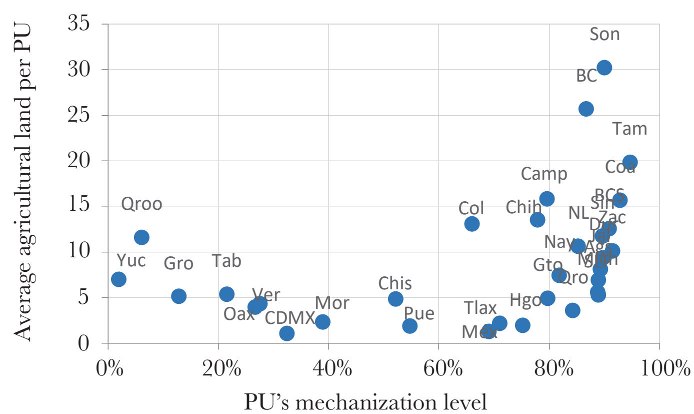


Figure 11. Average agricultural land according to PU's level of mechanization.

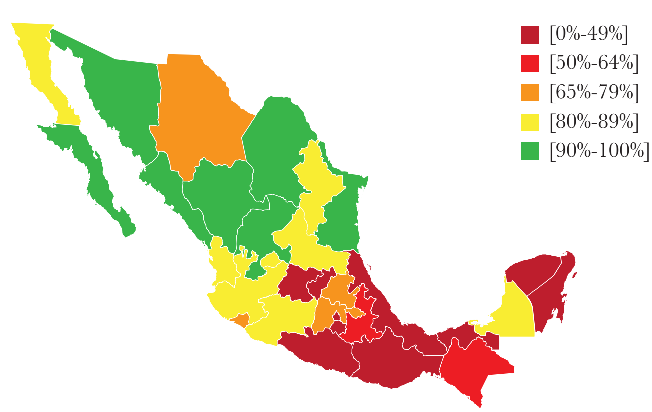


Figure 14. Mechanization level in Mexico.

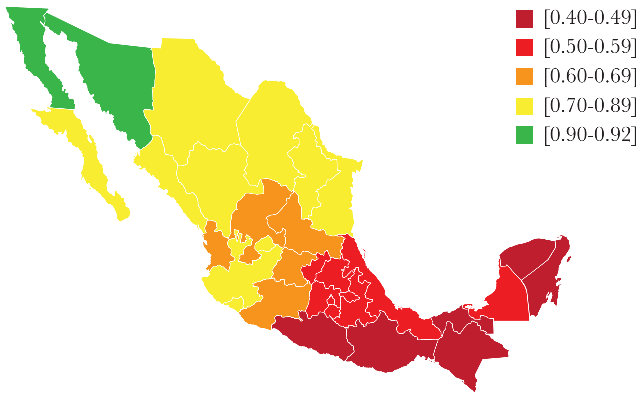


Figure 15. Index of technology adoption potential. (arithmetic mean)

Potential public policy actions to promote the adoption of new agriculture 4.0 technologies in Mexico:

Following the ideas that we have hitherto set out, we can offer a general outline of public policy actions that would, on the one hand, expand the offer of technology services and, on the other, foster their demand. In all cases, this development would take place within a regulatory framework that would encourage a more suitable adoption environment in the long run.

Fostering the conditions for supply

Course of action no. 1: Reducing the barriers between technology providers and the agricultural producers that currently operate in Mexico and could potentially receive the benefits of technological services related to the concept of agriculture 4.0.

- Promoting public-private alliances where public entities concentrate and order relevant information on rural production units according to their characteristics and make this information available to the private sector in exchange for benefits in the supply conditions of their services among the abovementioned production units.
- Including technology services of specialized companies in promotion programs that are currently on place, in order to enhance the value offered to producers and to tangibly expand the knowledge and benefits of new technologies among producers.
- Reducing the access barriers to the offer of technology services by concentrating the providers in a common marketplace.

Course of action no. 2: Encouraging the acquaintance with the offer of new technologies

- Generating public access information reports on service providers and periodically updating them so that they can serve as a radar for new technologies in the country.

- Validating the benefits of the new technologies either by fostering pilot tests within the private sector or through controlled tests in public facilities.
- Disseminating the benefits resulting from the use of new technologies in each agroecological region by encouraging the transmission of knowledge among producers or through public-access specialized dissemination seminars.
- Conducting pilot tests on the usage of new technologies subsidizing the adoption cost for the producer in order to tear down the cultural barriers to adoption with regard to the traditional production practices.

Course of action no. 3: Encouraging innovation and creation of infrastructure in Mexico

- Organizing seminars or awards revolving around the innovation or acceleration of new technologies in order to foster the research and development of said technologies in Mexico, with special emphasis on solving the idiosyncratic conditions of the country's productive regions.
- Facilitating the investment in infrastructure in Mexico by offering to share the risk and financing capital for development with the offerors.
- Creating public databases that enable the development of value-added services by providers whose size or condition is limiting, thereby allowing an even ground among providers of technology services.

Fostering the conditions for demand

Course of action no. 4: Investing in abilities and infrastructure to establish the necessary conditions for the adoption and development of new technologies in areas where said conditions are insufficient.

- Setting an agenda of digital inclusion and adoption of new technologies in the agricultural sector through the extension channels currently available in our country. Using the infrastructure and capacities of the public and private research and development centers of the country to consolidate them as technology transfer agents in each region: FIRA's Centros de Desarrollo Tecnológico (CDT); Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP); Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), etc.
- Investing in basic connectivity infrastructure.

Course of action no. 5: Reducing the adoption threshold for new technologies

- Pairing the adoption of new technologies with other traditional measures to increase productivity, thus securing the benefits of adopting digital technologies.
- Encouraging the adoption of new technologies by having an additional impact on the reduction of production costs where new technologies are involved, or else by reducing the adoption cost.

Conditions of governance and regulation of new technologies

Course of action no. 6: Consolidating a climate of certainty for investors and users of new technologies by creating and implementing an adequate regulation that establishes the rights and responsibilities of adopters, particularly regarding the information that results from the constant monitoring of the production processes with the new technologies.

Course of action no. 7: Working on the creation of a governing research and development center that convenes the public, private, and academic sectors for the introduction of new technologies in order to facilitate the adoption of agriculture 4.0 in Mexico, particularly in the less developed regions of the country.

CONCLUSIONS

This research has offered a general view of the current situation of agriculture 4.0-related technologies in Mexico. Agriculture 4.0 is a recent concept in academia and is recognized as a new paradigm with regard to previous technological changes in agriculture.

The offer of providers corresponds to the nature of Mexican agriculture, inasmuch as it focuses on maize, wheat, and bean as the main crops for the adoption of technology. We also identified that the demand for these technologies is heterogenous among the country's regions; it depends on the type of crop, the regions' characteristics, the prices of the products, and the natural barriers to access. Some ideal characteristics for the adoption of technologies are observed among the northern, northeastern, and western states: their production units are larger, they have a greater level of 3G and 4G connectivity and service coverage, their use of ICT and level of mechanization is higher, and the educational lag among their producers is lower.

For this reason, creating the necessary conditions for producers to adopt and continue developing digital agriculture is essential. According to our results, it is possible to present a general outline of public policy actions that, on the one hand, will expand the offer of technology services and, on the other, will foster their demand within a regulatory framework, so that all this may contribute to reduce the technology adoption gap between the different regions of the country.

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Colegio de Postgraduados

Glucosinolates: Structure, classification, biosynthesis and functions in higher plants

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ABSTRACT
Objective: To analyze concepts, structure, classification, biosynthesis and functions of glucosinolates (GSLs) in higher plants.
Design/methodology/approach: A search was performed into recent high-impact literature related to glucosinolates (GSLs).
Results: GSLs are secondary metabolites rich in N and S. They are divided into aliphatic, aromatic, and indole GSLs depending on the amino acid from which they arise. The products of their hydrolysis, mediated by thioglucoside glucohydrolase, thioglucosidase or myrosinase enzymes (EC 3.2.1.147), play a role in increasing tolerance to biotic and abiotic stress factors. Furthermore, given their composition, they can serve as a nutrient reservoir under nutrient deficiency conditions.
Limitations on study/implications: GSLs are synthesized only in species of the Capparidaceae, Brassicaceae, Resedaceae, and Moringaceae families.
Findings/conclusions: GSLs are sulfur compounds that can serve as defense mechanisms against biotic and abiotic stress factors and as sources of nutrients in plants, and molecules with important nutraceutical properties in food and human health.

Keywords: secondary metabolites, sulfur, defense compounds, Brassicaceae.

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INTRODUCTION

Glucosinolates (GSLs) are sulfur-rich secondary metabolites derived from amino acids (Kamal *et al.*, 2022). They are especially synthesized by species of the Brassicacea family, which includes cultivated plants of global importance for human consumption, such as cauliflower (*Brassica oleracea* var. *botrytis*), cabbage (*Brassica oleracea* L. var. *capitata*), broccoli (*Brassica oleracea* var. *italica*), bok choi (*Brassica rapa* ssp. *chinensis*), radish (*Raphanus sativus*), and Brussels sprouts (*Brassica oleracea* var. *gemmifera*). In plants, GSLs contribute to mitigating

Image by Helga Ulbing at Pixabay

damage from biotic (pests and diseases) and abiotic (salinity, extreme temperatures, and radiation) stress factors, and in turn, when consumed as food, they promote better health conditions in humans (Nguyen *et al.*, 2020).

Glucosinolates are hydrolyzed by the myrosinase enzyme after the plant perceives a stress signal. After hydrolysis, the resulting compounds comprise isothiocyanates, thiocyanates, epithionitriles, and nitriles (Hanschen and Schreiner, 2017). In species of the Brassicaceae family, the nutrients C, N, and S are used for the synthesis of GSLs (Koroleva *et al.*, 2010; Jeschke *et al.*, 2019), which fulfill important defense functions against biotic and abiotic factors (Feng *et al.*, 2022). At least 130 different structures of GSLs have been identified in species of this plant family (Essoh *et al.*, 2020).

The term glucosinolates refers to the glucosyl ("gluco") moiety, the presence of a sulfate (ate) group, and the property of being a precursor to a mustard oil (sinol). GSLs have been defined as natural substances found in different plants, and participate as part of a defense mechanism against herbivorous insects. Plants of the Brassicaceae family, such as cabbage (Chhajed *et al.*, 2020), mustard (*Brassica nigra*; Blažević *et al.*, 2020), broccoli, Brussels sprouts, cauliflower, kohlrabi (*Brassica* × *napobrassica*), and radish (Marcinkowska and Jeleń, 2020), show these metabolites in the highest concentration. The amount of GSLs varies from one species to another and directly influences the type of plant tissue (Nguyen *et al.*, 2020).

GSLs are responsible for the spiciness of species such as mustard or horseradish. In some cases, they may offer protection against some types of cancer. In particular, the raw consumption of species from the Brassicaceae family offers high bioavailability of isothiocyanates (produced by the myrosinase activity on GSLs). Among the isothiocyanates are benzyl isothiocyanate, phenethyl isothiocyanate, and sulforaphane [1-isothiocyanato-4-(methyl-sulfinyl) butane], which have proven to target proteins related to cell proliferation and homeostasis. The interaction of isothiocyanates with proteins involved in DNA repair inhibits the cell cycle and induces programmed cell death, actions that reduce tumor growth (Soundararajan and Kim, 2018).

GSLs are transported by the phloem and can help the plant defend itself against organisms that feed on phloem products and also acquire the ability to coordinate the synthesis and use of protective resources between different organs (Koroleva *et al.*, 2010). The GSLs that fulfill the defense function of plants are thioglycosides that are derived from their hydrolysis.

STRUCTURE AND CLASSIFICATION OF GLUCOSINOLATES

The structure of glucosinolates consists of a sulfonated aldoxime domain linked to a -D-thioglucose group together with a side chain (aglycone) derived from one or several amino acids (Figure 1; Blažević *et al.*, 2020; Sugiyama *et al.*, 2021).

GSLs contain an oxidized sulfur atom of 3'-phosphoadenosine 5'-phosphosulfate (PAPS), a reduced glutathione atom and, in the case of methionine-derived aliphatic GSLs, a third sulfur atom (Mitreiter and Gigolashvili, 2021).

GSLs are classified as aliphatic, aromatic, and indole GSLs (Kamal *et al.*, 2022), depending on the type of amino acid from which they come (Figure 2).

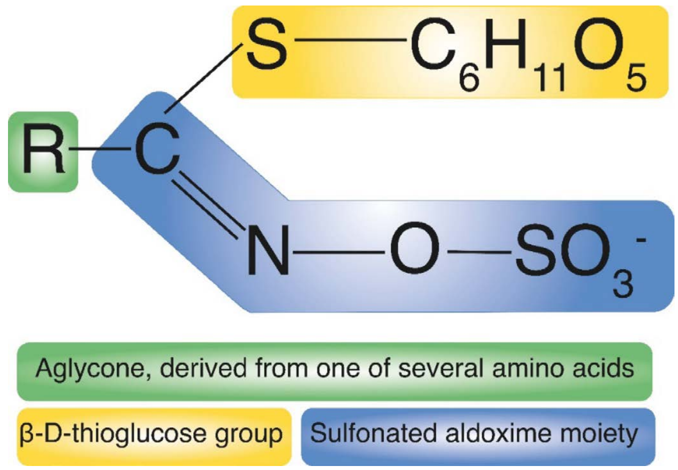

Figure 1. Basic structure of glucosinolates.

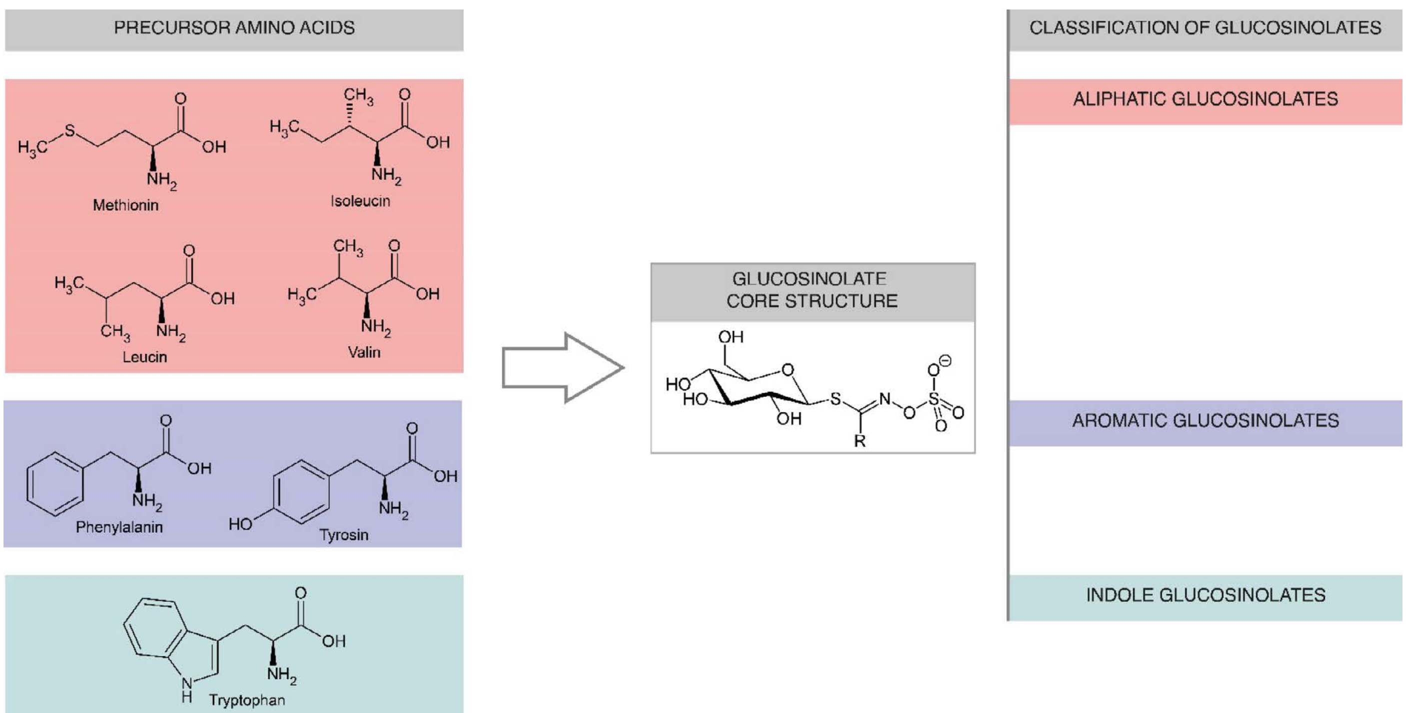

Figure 2. Classification of glucosinolates (GSLs) according to the type of precursor amino acid. Aliphatic GSLs are derived from methionine, isoleucine, leucine, or valine. Aromatic GSLs are derived from phenylalanine or tyrosine. Indole GSLs are derived from tryptophan.

GSLs are biologically inactive (Urbancsok *et al.*, 2018) and are degraded by the thioglucoside glucohydrolase, thioglucosidase, or myrosinase enzyme (EC 3.2.1.147), which hydrolyzes the glucose residue (Mitreiter and Gigolashvili, 2021). after cell damage, thus obtaining degradation products such as: glucose, sulfate and, depending on the chemical structure (Aghajanzadeh *et al.*, 2014), isothiocyanates, thiocyanates, epithionitriles, and nitriles (Bones and Rossiter, 2006) that function as defense substances

(Sugiyama *et al.*, 2021). The general reaction catalyzed by thioglucoside glucohydrolase is shown in Figure 3.

The final products of the degradation of GSLs depend on factors such as pH, availability of ferrous ions, and proteins that interact with the thioglucoside glucohydrolase enzyme (Martínez-Ballesta *et al.*, 2013).

The storage of GSLs and thioglucoside glucohydrolase enzymes is spatially distinct. Therefore, they only interact after the plant has faced some kind of stress. Specialized cell types can act as different storage locations: S-cells for GSLs and myrosin cells for classical myrosinases (Mitreiter and Gigolashvili, 2021). S-cells contain up to 40% of the total sulfur of *Arabidopsis thaliana* flower stem tissue (Koroleva *et al.*, 2010).

BIOSYNTHESIS OF GLUCOSINOLATES

The biosynthesis of glucosinolates occurs mainly in the leaves, from where they are transported to other organs of the plant. Their biosynthesis in different organs is more active in young growth stages and less so in mature stages (Feng *et al.*, 2022).

The biosynthesis of GSLs consists of three stages (Figure 4): I) chain elongation in which a methylene group is inserted into the side chain of aliphatic amino acids; II) the metabolic reconfiguration of the rest of the amino acids to produce the central structure; and III) the modification of the core structure to produce GSLs with various aglycone structures (Nguyen *et al.*, 2020).

The first stage begins with a deamination of the amino acids by branched-chain amino acid aminotransferase (BCAT) that transforms them into 2-oxoacids, which condense with acetyl-coenzyme A by the action of the methylthioalkylmalate synthases enzyme (MAMs) and thus forms a 2-malate derivative. This last compound is isomerized to a 3-malate derivative by isopropylmalate isomerase (IPMI). This is followed by decarboxylation by the isopropylmalate dehydrogenase enzyme (IPMDH) and produces an elongated 2-oxoacid intermediate that can undergo transamination to provide extended amino acids for the next stage or re-enter the transformation cycle for further elongation (Nguyen *et al.*, 2020; Figure 4).

In the second stage (Figure 4) there is an oxidation of the amino acid into aldoximes. This oxidation is catalyzed by three enzyme systems [cytochrome-P450 (CYP79) dependent monooxygenase, flavin-containing monooxygenase, and peroxidase]. The participation of each enzymatic system depends on the nature of the amino acid precursors. Cytochrome monooxygenases CYP83 activate the aldoxime resulting from oxidation of the amino acid to the corresponding thiohydroxymate. The activated aldoxime is conjugated with reduced

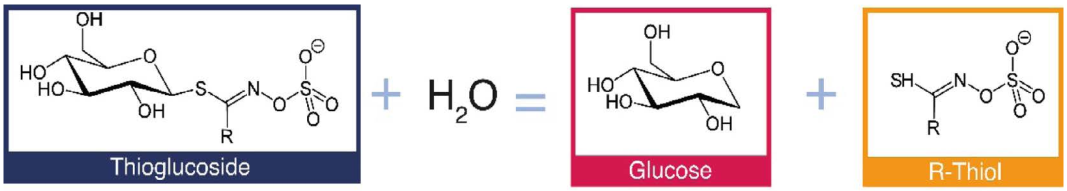

Figure 3. Representation of the reaction catalyzed by thioglucoside glucohydrolase (EC 3.2.1.147). R-thiol varies depending on the chemical structure, and can be isothiocyanates, thiocyanates, epithionitriles, and nitriles.

glutathione (GSH), which donates S to produce the intermediate thiohydroxymate. The S-alkyl-thiohidoximate intermediate formed is cleaved by the activity of a C-S lyase enzyme: SUR1 to form thiohydroxymates. These thiohydroxymates are transformed by the UDP-glucose:thiohydroxymic acid S-glucosyltransferases (S-GT) and desulfoglucosinolate sulfotransferases enzymes to produce the core structure of GSLs with the corresponding side chains (Nguyen *et al*., 2020).

In the third stage, chemical transformations of the GSLs side chains occur through enzyme-catalyzed oxidations, eliminations, alkylations, and esterifications (Figure 4). These modifications contribute to the structural diversity of GSLs (Nguyen *et al*., 2020).

FUNCTIONS OF GSLs IN PLANTS

GSLs are widely synthesized in species of the Capparidaceae, Brassicaceae, Resedaceae, and Moringaceae families (Lockwood, 1988), although most studies have been done on species of the Brassicaceae family.

When the species of the Brassicaceae family suffer an attack, the GSLs are hydrolyzed by thioglucoside glucohydrolases or myrosinases enzymes into different defense products, including isothiocyanates, which are the most characterized. Isothiocyanates are toxic to insect pests and disease-causing pathogenic microorganisms. However, when synthesized excessively, these compounds can be harmful to the plant, as they can cause stomatal closure, alter microtubules in the cytoskeleton, deplete reduced glutathione (GSH), inhibit root growth or induce cell death (Ting *et al*., 2020).

GSLs act as excellent defense mechanisms against generalist herbivores, but are less effective against specialist herbivores (Schweizeir *et al*., 2013). In addition, these sulfur compounds can also be toxic to microbial pathogens both in the soil and in the aerial part of the plant (Mitreiter and Gigolashvili, 2021).

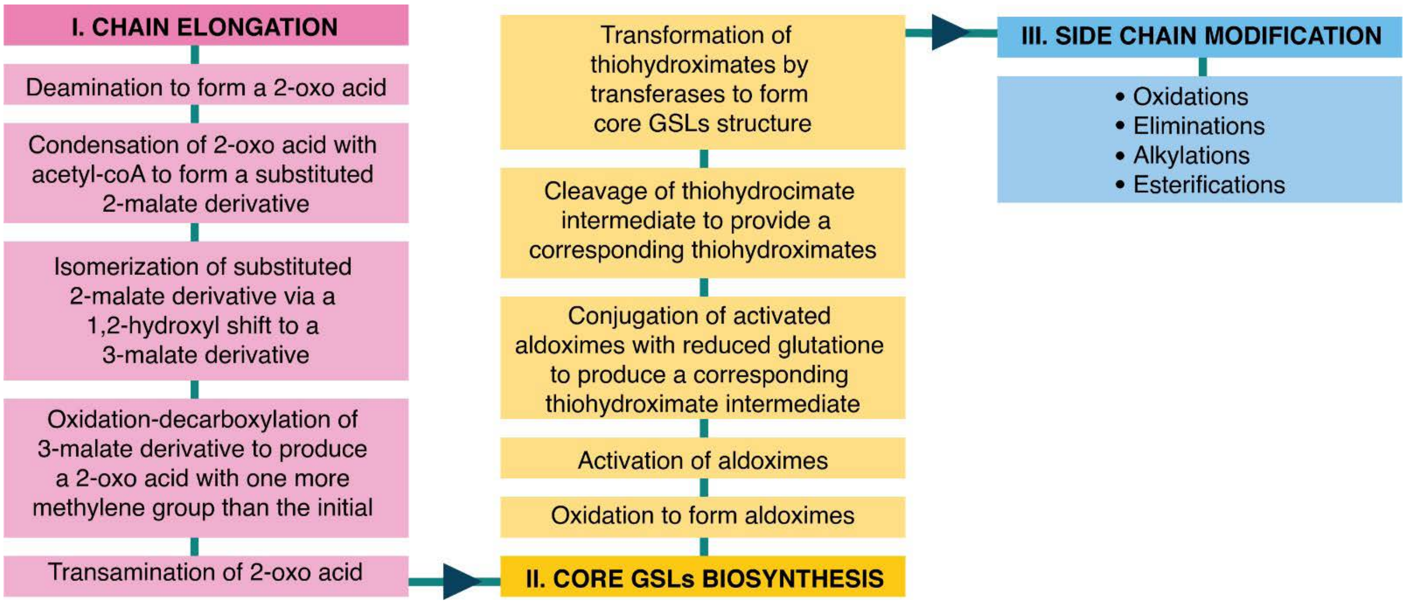

Figure 4. Biosynthesis stages of glucosinolates (GSLs) (Nguyen *et al*., 2020).

To produce crops with a greater amount of desirable compounds, several strategies can be followed. The first is to select species, genotypes, or cultivars that contain a genetically determined higher level of phytochemicals (Bouargalne *et al.*, 2022; Zhan *et al.*, 2022). The second is to manipulate the growth factors and environmental conditions for plant cultivation (Trejo-Téllez *et al.*, 2019; Šamec *et al.*, 2021). A third alternative is the use of genetic engineering, metabolic engineering, and genome editing (Miao *et al.*, 2021).

In adverse environmental conditions such as drought, salinity, extreme temperatures, and excessive exposure to UV radiation, plants activate defense mechanisms that include the accumulation of specialized metabolites or phytochemicals (Šamec *et al.*, 2021). These natural plant defense mechanisms can be stimulated during the cultivation of certain species, which triggers greater production of desirable compounds.

Eustressors are biological, physical, or chemical stressors that activate signaling pathways that lead to increased content of bioactive compounds. Salinity is considered a chemical stress factor that affects the physical quality and chemical composition of various plant products (Rouphael *et al.*, 2018).

By increasing the level of salinity in crops of species of the Brassicaceae family, a concomitant rise in the content of bioactive compounds can be observed, at the expense of their growth and yield (Santander *et al.*, 2022).

Salinity differentially affects the metabolism of GSLs in plants, which depends on environmental conditions such as temperature and radiation, nutritional management, type of GSL synthesized, and the genotype of the plant (Rios *et al.*, 2020).

In *Brassica oleracea* L. var. *italica* exposed to 40 and 80 mM NaCl for two weeks, an increase in the content of GSLs was observed, the same as in *Brassica rapa* L. exposed to 20, 40, and 60 mM NaCl for five days (Steinbrenner *et al.*, 2012).

In species of the Brassicaceae family, GSLs can represent up to 30% of the total sulfur concentrations (Falk *et al.*, 2007; Sugiyama *et al.*, 2021). This means that GSLs can be nutrient reservoirs, which under nutrient deficiency can be hydrolyzed by myrosinase enzymes, so that sulfur is reallocated to primary metabolites such as cysteine (Sugiyama *et al.*, 2021). Thus, under stress conditions, these secondary metabolites can be degraded for the formation of other molecules.

Given the importance of different species of the Brassicaceae family in human nutrition, it is important to highlight that GSLs can contribute to improving health as these compounds have shown protective properties against the incidence of cancer and cardiovascular diseases (Traka, 2016).

CONCLUSIONS

GSLs are secondary metabolites rich in N and S; they are mainly synthesized by plant species of the Brassicaceae family. By the type of amino acid from which GSLs come, they are divided into aliphatic, aromatic, and indole GSLs. The products of their hydrolysis mediated by myrosinase enzymes play a role in increasing tolerance to biotic and abiotic stress factors. In addition, given their composition, they can serve as a nutrient reservoir under deficiency conditions. Finally, GSLs have nutritional functions and can contribute to improving human health.

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Colegio de Postgraduados

Effect of coumaphos on *Rhipicephalus microplus* and entomopathogenic nematodes in cattle production units

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ABSTRACT

Objective: to determine the effect of coumaphos on *Rhipicephalus microplus* and entomopathogenic nematodes in bovine production units.

Design/methodology/approach: Two experiments were carried out: First) tick samples were collected and evaluated, using the Adult Immersion Test (10 ticks per humid chamber), the treatments were applied: 1: Control; 2: Coumaphos 0.1%; 3: Coumaphos 0.2%; 4: Coumaphos 0.4%. An ANOVA was carried out with a completely randomized design (mortality dependent variable and treatment independent variable with four levels) and a multiple comparison of means (Tukey's test). Second experiment) 10 samples of livestock soil where coumaphos is used, which correspond to the Mariano Matamoros ejido, Tamaulipas, and 10 non-livestock soil from the Las Huastecas Experimental Field, were analyzed to determine the presence of entomopathogenic nematodes (EPN). a cross-frequency table between the sampled soils and the Chi-square test.

Results: Experiment 1: In counting the tick mortality percentages on the second day after application in group 1: control was 37.5%; 2: 72.5%; 3: 80%; 4: 92.5%, on day 7 and day 8 there was no difference with 90% and 100% mortality. Experiment 2: The soils positive to EPN were 35% Non-livestock and 25% Livestock.

Limitations on study/implications: it is important to make producers aware of the correct use of chemical products.

Findings/conclusions: *R. microplus* was susceptible to coumaphos in cattle production units. A greater presence of EPN was found in non-livestock soils, which may be related to the use of chemical products to control parasites.

Keywords: Tick, ixodicide, resistance, nematodes, soils.

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INTRODUCTION

Ticks are arthropods belonging to the Arachnida class, which in their adult phase have four pairs of legs as a characteristic and their body is divided into two regions, cephalothorax (union of head and chest) and abdomen, these parasites are considered one of the most important sanitary factors that limit cattle farming in the tropics, worldwide they affect 80% of the cattle ranching in the herds. It should be noted that they are hematophagous ectoparasites that feed on the blood of their host during their life cycle (Polanco *et al.*, 2016).

Ticks mainly inhabit warm and humid weather, and cause losses in livestock production systems if not adequately controlled, ticks *Rhipicephalus microplus* is widely distributed worldwide and has a high negative economic impact for cattle industries (Hurtado and Giraldo-Ríos, 2018). In the southern region of Tamaulipas, herds are made up of native cattle and specialized breeds for meat production: Beefmaster, Simmental, Suiz-Bú, among others which are used for their ability to adapt to the tropical weather (Garay *et al.*, 2020). The main economic losses caused by ticks are due to decreased growth, skin damage, decline in meat and milk production, zoonotic disease transmission, increased costs of disease control and treatment, as well as reproductive problems in cattle (Vargas-Cuy *et al.*, 2019).

The use of dewormers to control ticks affects the fauna present in the soil, such as entomopathogenic nematodes (EPN) and other organisms (Ruiz-Negrete *et al.*, 2018); which constitute a group of importance in the biological control of insects (García *et al.*, 2008). EPN are transparent organisms with dorsoventral movement, unsegmented with bilateral symmetry, in their anatomy they have excretory, nervous, digestive, reproductive and muscular systems, but they don't have the respiratory and circulatory systems, these are mostly found on the ground and to continue with the life cycle it is necessary that they infest an insect (Rosales *et al.*, 2008).

EPN are lethal parasitic organisms and obligate only of insects, have a ubiquitous distribution and are used as biological control agents, founding a mutualistic relationship with bacteria of the Enterobacteriaceae family, which require this host to obtain protection and be able to transport from one insect to another (Stock and Goodrich, 2008). The two most important and studied families are Steinernematidae and Heterorhabditidae, these are associated with bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively (Vashisth *et al.*, 2013). Different strains of EPN have been used as biological control agents for various pests, mainly in the agricultural sector for the protection of various crops (Parada *et al.*, 2019), and in pests of livestock production animals (Alves *et al.*, 2012), although it is necessary to deepen its study in the livestock sector, as well as the effect that the chemical products used for animals have on the populations of these nematodes (Negrete *et al.*, 2018).

It is important to increase the effectiveness of tick control, always considering reducing economic costs, and taking care of animal health (Barrón-Bravo *et al.*, 2020a), using strategies to reduce environmental impact on non-target populations such as EPN (Barrón-Bravo *et al.*, 2020b). Considering the above, the objective of this study was to determine the effect of coumaphos on *Rhipicephalus microplus* and entomopathogenic nematodes in bovine production units.

MATERIALS AND METHODS

Two experiments were carried out during the months of August to December 2021, in the first experiment, tick samples were collected and evaluated from a cattle production unit (CPU) and was evaluated with coumaphos treatment at different concentrations. In the second experiment, samples of Livestock Soil (LS) were analyzed, corroborating the use of coumaphos for the control of parasites in the same CPU, Non-Livestock soil (NLS) samples were also collected, in which coumaphos is not used, collected at INIFAP, Las Huastecas Experimental Field located in Villa Cuauhtémoc, Altamira, Tamaulipas, Mexico, to determine the presence of EPN.

Location of the study area

The study was conducted at two locations: The first place was the private CPU that is located in the community Mariano Matamoros, Altamira, Tamaulipas (22° 39' 18.6" N and 98° 09' 47.5" W). The CPU aims to produce beef cattle in the cow-calf system, in extensive grazing, the herd has 12 breeding cows. The second place was the National Institute of Forestry, Agriculture and Livestock Research (INIFAP), Las Huastecas Experimental Field (22° 33' 57.2" N and 98° 09' 52.3" W), where is the Animal Health Laboratory located, in which the samples were worked, both locations are from the same municipality. Altamira has a warm sub-humid weather with rains in summer of medium humidity, an altitude between 0 and 600 m. Its temperature range is from 22 to 26 °C, its annual precipitation is from 900 to 1100 mm (Vargas *et al.*, 2007; INEGI, 2009).

First experiment: Susceptibility of *Rhipicephalus microplus* to coumaphos

The Adult Immersion Test was used for this experiment (Drummond *et al.*,1973), four treatments were evaluated: 1: Distilled water (control); 2: coumaphos diluted 0.1%; 3: coumaphos diluted 0.2% y 4: coumaphos diluted 0.4%, performing four repetitions per treatment, with 10 ticks each one of them.

Adult ticks were collected directly from the body of the cows, carefully not to leave the mouthparts on the skin of the cattle. To do this, with the help of dissecting forceps, gently grasped the body of the ticks, he turned on his back and then quickly pulled in a direction perpendicular to the skin. 10 ticks were grouped and placed in petri dishes with wet filter paper, a total of 160 ticks were tested, they were transported in an expanded polystyrene cooler at a temperature of 25±2 °C to the Animal Health Laboratory. After 24 h the treatments were applied, it started with the control group, first with the lowest concentration and ending with the highest concentration. 50 mL of distilled water were added for the control group and the same volume for the ixodicide of each treatment, in the beakers they were kept immersed for 30 s.

After the immersion time, filter paper was placed to extract the ticks from the solutions and they were returned to the petri dish with wet filter paper, finally, they were labeled for identification. Petri dishes were incubated at a temperature of 25±2 °C and 80 to 90% relative humidity. Tick mortality was assessed daily, considering the day after application (DAA) from 1 to 6 to register the result in a database (Drummond *et al.*, 1973; Jonsson *et al.*, 2007).

Second experiment: Determination of the presence of entomopathogenic nematodes in livestock soils and non-livestock soils

Soil samples were taken using the five-gold technique. Samples of LS in the CPU from the Mariano Matamoros community and samples of NLS from Las Huastecas Experimental Field, INIFAP. The samples were transported in an expanded polystyrene cooler to the laboratory at 25±2 °C (López-Llano and Soto-Giraldo, 2016). For the isolation of entomopathogenic nematodes, the trap insect methodology was used, described by Bedding (1984) and Kaya and Stock (1997), the one kg soil samples were sieved, moistened and placed in a plastic container with a capacity of 1,000 mL, and were added 10 larvae of Tenebrio molitor in samples collected from the area LS and NLS, they were left for seven days in the dark, at a temperature of 25±2 °C. After this time, the dead larvae or pupae were transferred to a petri dish with wet filter paper. It was observed daily for 7 days under the microscope, once identifying the infection by EPN on the dead larva, transferred to a White Trap moistening with distilled water for later identification (Nguyen and Smart,1996).

Analysis

Tick mortality data (dependent variable) were analyzed with the PROC GLM (SAS, 2002), under a completely randomized design, with four replications. The independent variables were the treatments and DAA. The comparison of means was performed using the Tukey test (α=0.05). Regarding the results of entomopathogenic nematodes, they were organized in a database in the Microsoft Excel Program, Subsequently, the general prevalence was determined. A cross-frequency table was made between the sampled soils and the Chi-square test was performed, in the statistical program Statgraphics, edition 18 (2017).

RESULTS AND DISCUSSION

The effect of coumaphos on *R. microplus* and entomopathogenic nematodes in cattle production units was determined, the results are shown in the first and second experiment below:

First experiment: Susceptibility of Rhipicephalus microplus to coumaphos

It was observed that from the day 1 DAA, mortality was greater than a 60 % (Table 1). No differences were observed between the different concentrations of coumaphos that were used ($P>0.05$). 100% mortality was observed in the 4th DDA at 0.20% of coumaphos. With the lowest concentration of coumaphos (0.05%), a 100% mortality was obtained on day 6th DAA (Table 1).

Growing tick populations are a serious problem worldwide, as well as the resistance to the products used for its control, the results show how these methodologies strengthen control programs and can be used anywhere this problem occurs, Bravo *et al.* (2008) conducted an experiment on *R. microplus* ticks collected from four dairy farms, its susceptibility did not present significant differences between the groups treated with the five concentrations evaluated in each farm; the increase in coumaphos concentration was

Table 1. Susceptibility of *Rhipicephalus microplus* to different concentrations of coumaphos.

Coumaphos Treatment (%)	Days after application					
	1	2	3	4	5	6
	Mortality (%)					
Control	22.5 b	37.5 b	37.5 b	40.0 b	42.0 b	45.0 b
0.1	60.0 a	72.5 ab	80.0 a	90.0 a	95.0 a	100.0 a
0.2	62.5 a	80.0 ab	95.0 a	95.0 a	100.0 a	100.0 a
0.4	67.5 a	92.5 a	97.5 a	100.0 a	100.0 a	100.0 a
P-Value	0.0224	0.0212	0.0013	0.0024	0.0003	0.0002

Different literals between lines indicate significant difference (Tukey; $\alpha=0.05$).

not directly proportional to the efficacy. *R. microplus* adults were resistant; however, 100% mortality was observed in larvae, which indicates high susceptibility of the larvae to this organophosphate, Similar results to the present study where a 45% mortality of the control group was obtained and in the concentrations that contained coumaphos 100% being susceptible.

Reyes-Domínguez *et al.* (2013) evaluated the resistance of *R. microplus* to coumaphos from San Juan Evangelista, Veracruz, Mexico, they worked in 16 CPU dedicated to the dual-purpose system (meat and milk). In each CPU, 50 female *R. microplus* ticks were collected to determine resistance to coumaphos, deltamethrin and amitraz, using the adult immersion test. In the CPUs sampled, populations of ticks with multiple resistance to organophosphates, synthetic pyrethroids and amitraz were identified, however, 2 of 16 did not present resistance to organophosphates. There are differences with the present study, since in the studied CPU a 100% mortality was obtained with the organophosphate coumaphos, which may be due to the different management in the dual-purpose production system in which regularly the control of ectoparasites is more frequent.

Rodríguez-Pacheco *et al.* (2017) evaluated the *in vitro* resistance of the *R. microplus* to organophosphates, synthetic pyrethroids and amitraz. Adult ticks were collected in Boyacá, Colombia, and they were subjected to the adult immersion test to evaluate the efficacy of the ixodicides, the effect on oviposition and the percentage of larval hatching. Their results demonstrated mortality rates of 43% in the control, 56% synthetic pyrethroids, 60% amitraz and 96% organophosphates, at 21 days DAA. The oviposition inhibition was 13.4% for the control, 44.6% for amitraz, 45.5% for synthetic pyrethroids and 96 % for organophosphates. The hatching percentage of eggs was 88% for the control, 16% for amitraz, 14% for synthetic pyrethroids and 4% for organophosphates, there was a significant difference in the organophosphate group. Their results agree with those of the present study, showing high mortality when using organophosphates, which may be since dewormers are rotated to make efficient use.

Coronado and Mujica (1997) studied the adult females of *R. microplus*, which they collected from cattle on farms in eight states of Venezuela, using the adult immersion test (Drummond test). The acaricides used were: coumaphos, chlorfenvinphos, amitraz, iminothiazole, cypermethrin and flumethrin, respecting the doses recommended by the manufacturers. The results obtained show variable levels of resistance according to the area of origin of the ticks. The highest levels of resistance were observed in organophosphates and in cypermethrin. Their results differ from those of the present study, where susceptibility on the part of the parasite is observed, most likely due to the indiscriminate use mentioned by the authors.

Rentería and Sevilla (2015) conducted an experiment in five cattle ranches in the state of Nuevo León (Terrabel, Bisonte, El Pájaro, Valle Escondido and 14 de Mayo), collected tick samples from cattle *R. microplus*, because the producers showed concern about diseases and economic losses caused by these ectoparasites, and to diagnose susceptibility to the products used in its control. Organochlorines (Lindane), organophosphates (Coumaphos, diazinon, chlorfenvinphos) and pyrethroids (Flumethrin, deltamethrin and cypermethrin) were used. Their results showed a greater susceptibility to Deltamethrin, followed by chlorfenvinphos, diazinon, flumethrin, cypermethrin, lindane and lower mortality for coumaphos, which is the most common product in tick control. Its results are similar to those obtained in the present study due to the common use of coumaphos in the CPU of both regions, so integral management methods for tick control should be suggested.

Second experiment: Determination of the presence of entomopathogenic nematodes in livestock soils and non-livestock soils

The presence of EPN in the LS was 25% positive and 25% negative, in contrast to the results for NLS with 35% positive and 15% negative of the total of 20 (100%) samples (Table 2), the EPN found were identified as belonging to the Steinernematidae family.

The issue of EPN has various applications, they are beneficial organisms for the agricultural and environmental sector, so their study is of great importance in order to make the most of their potential worldwide, Montoussé *et al.* (2008) investigated the presence of EPN in chestnut orchards *Castanea sativa* Mill (Fagaceae) from Galicia, España, since they are of interest to start the biological control program of the chestnut plague *Curculio elephas* Gyllenhal (Coleóptera: Curculionidae) and butterflies of the genus *Cydia* Hubner (Lepidoptera: Pyralidae), these pests cause severe damage. They collected samples in 30 characteristic points of the provinces Pontevedra and Ourense, analyzing the EPN using the insect trap technique with larvae of *Galleria mellonella* L. (Pyralidae). They were identified by morphological and morphometric analysis and by molecular techniques, the nematodes of the genus *Steinernema* and *Heterorhabditis*, in four of the sampled locations.

Table 2. Presence of entomopathogenic nematodes (EPN) in relation to the type of soil in Altamira Tamaulipas, Mexico.

EPN presence (%)	Soil type (%)		Total (%)
	Not livestock	Livestock	
EPN negative	3 (15.0)	5 (25.0)	8 (40.0)
EPN positive	7 (35.0) [a]	5 (25.0) [b]	12 (60.0)
Total	10 (50.0)	10 (50.0)	20 (100.0)

Different literal by column indicates significant differences (Chi-square test); $\alpha=0.05$.

The results were positive, since there is a presence of EPN in four of the 30 places analyzed, with an abundance of 13.3%. The presence of EPN in the soil varies due to numerous factors, not only physical such as texture, humidity, temperature, aeration or soil pH, but also by biological factors such as the abundance of host insects in the environment. Their results show lower percentages than those of the present study in terms of the presence of EPN in LS and NLS, which may be due to the different climatic conditions and the chemical products used in the management of these places.

Parada *et al.* (2006) They located zones that correspond to the area of greatest production of potato *Solanum tuberosum* L. (Solanaceae) in 24 municipalities of Colombia, where they collected insects whose movements were very slow, or corpses of yellow to dark cream coloration, of a soft consistency, but not rotten, in addition to soil samples to carry out the insect trap technique, with a total of 288 places, each sampling point comprising five subsamples, which were taken with a shovel at a depth of 25 and 35 cm, collecting 300 g of soil. Infected insects in the isolation of nematodes that were arranged in wet petri dishes and White traps. Their results show 56 % of positive soils to EPN, in addition to 100% of the nematodes that were associated with the collected insects, 55% of the population corresponded to species of the *Steinernematidae* family and the remaining 45% were located within the order Rhabditida, mainly Rhabditonematidae and Rhabditidae families. In the present study, the same insect trap technique was carried out, obtaining results below the percentage of EPN presence of this study, which is surely due to the richness in organic matter of the soils, as well as the favorable conditions for the EPN of the sampled points.

Molina-Acevedo *et al.* (2006) conducted an experiment in the State of Minas Gerais, Brasil, with the objective of determining the percentage of survival in storage of six species of EPN, Steinernemátidos (*Steinernema carpocapsae* Weiser, *Steinernema glaseri* (Steiner) Wouts, Mracek, Gerdin & Bedding and *Sedum arenarium* Brotero (Rhabditida: Steinernematidae)) and three Heterorhabdítidos (*Heterorhabditis bacteriophora* Poinar, *H. bacteriophora* HP88 and *Heterorhabditis baujardi* LPP7 Weiser (Rhabditida: Heterorhabditidae)), at five different temperatures (8, 12, 16, 20 and 24 °C), with two concentrations (1000 and 10000 Infective Juveniles/mL) in a period of 15 days and three months. Most of the Steinernematids gradually increased in a wide temperature range from 8 to 20 °C, in both concentrations and in the shortest time, registering survival between 87 and 95%. On the contrary, high temperatures 20 and 24 °C, together with the lowest concentration and time, favored the high survival of Heterorhabditids, being between 78 and 92% respectively. Thus, in this experiment it was possible to determine specific conditions for each EPN, which represents a high survival. Their results are similar in the EPN management conditions of the present study, although the objectives differ, the differences observed based on genera may be mainly due to the different EPN species of each place and their adaptation.

Mercedes-Lucero *et al.* (2006) conducted a study in Colombia, with the objective of evaluating the use of entomopathogenic microorganisms (*Beauveria bassiana* (Balsamo) Vuillemin (Cordycipitaceae), *Metarhizium anisopliae* (Mechnikov) Sorokin (Clavicipitaceae) and *Steinernema* sp. in pest insect control chiza (*Astaena* sp.), as well as the agroecological sustainability of wheat and potato production systems on smallholding farms in the municipalities of Ospina and Yacuanquer in the Department of Nariño. The experiments were carried out under greenhouse and field conditions on producer farms, located at an altitude between 2,650 and 2,850 m, with an average temperature of 12 °C, relative humidity between 75% and 80%, average rainfall of 800 to 1000 mm.

The application of entomopathogens was carried out using decomposed wheat chaff in a ratio of 1:10. With the application of entomopathogenic fungi, they achieved the percentage mortality of the pest with 28.75% (in Ospina) and 14.67% (in Yacuanquer), for chlorpyrifos there was a percentage mortality of 28.89% (Ospina) and 18.82%. (Yacuanker). With the Steinernema nematode, a mortality of 17.27 % (Ospina) and 12.74 % (Yacuanquer) was observed. The application of a mixture of entomopathogenic microorganisms and decomposed wheat chaff to the crops contributed to reduce environmental contamination and to improve the characteristics of the soils. Their results, although they differ from the present study because only the presence of entomopathogenic nematodes was evaluated, show the practical application that can be developed in control programs based on entomopathogens and the need to study their potential.

Méndez-Barceló *et al.* (2011) carried out a study to identify EPN in Colombia, in the towns of Buenaventura, Valle del Cauca, (Cisneros, Córdova and Llano Bajo), polyculture areas of chontaduro (*Bactris gasipaes* H. B. K.), papachina (*Coloasia esculenta*, Schott), banana (*Musa* sp.), árbol del pan (*Arthocarpus* sp.), yuca (*Manihot sculenta*, Krantz), borojó (Borojoa patiñoi, Cuatr.) and fruit and forest species. The weather of these areas is humid tropical forest with an average annual temperature of 28 °C and rainfall of 7000 mm per year. For the isolation, the trap insect technique was used. The EPN that were found were inoculated again into *G. mellonella* larvae in order to evaluate their pathogenicity and obtain samples for identification at the genus level. They detected EPN in 36.84% of the samples, identifying only the genus Steinernema in the three locations, mainly where the decomposing peach tree is found, which has a significant incidence of these organisms in traditional cropping systems in the rural area of Buenaventura. The isolated nematodes caused 100% mortality in *G. mellonella* larvae at 48 h. Their results are similar to the present study, because they found the same EPN family, and a very similar percentage to those of NLS, this may be due to the similarity in the conditions of the sampled places.

In the study of Barron *et al.* (2020b) simples from LS and NLS were analyzed, collected from the municipalities of Irapuato and León, Guanajuato, México, from which were isolated EPN from Steinernematidae and Heterorhabditidae family. A 37% of positive samples were obtained at EPN, which most were LS with 24.3% and in smaller quantity NLS with 13.5%. These results differ from those of the present study where a greater presence was observed in NLS, which is probably related to the use of chemical products related to parasite control, which is more frequent due to the favorable conditions of humidity and temperature for the development of parasites in this area, especially tick, LS LS are rich in organic matter and present better conditions for EPN, but this is affected by the indiscriminate use of chemical products related to pest control.

CONCLUSIONS

Rhipicephalus microplus was susceptible to coumaphos in the bovine production unit in southern Tamaulipas, the methodologies used can strengthen control programs, continuous monitoring of ixodicides is very important to detect resistance, emphasizing management training Integrated tick adapted to cattle production systems. The effect of coumaphos on the populations of entomopathogenic nematodes, was manifested whit a greater presence was found in non-livestock soils, which may be related to the recurrent use of chemical products to control parasites in livestock soils from cattle production units and the growing populations of ticks in the area, which forces the repetitive and indiscriminate use of ixodicides, affecting populations of beneficial organisms which are not their objective.

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Colegio de Postgraduados

Morphological characterization of Maax pepper (*Capsicum annuum* var. *Glabriusculum*) and Pico Paloma pepper (*Capsicum frutescens*)

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ABSTRACT

Objective: To morphologically characterize two wild species of *Capsicum annuum* Var. *Glabriusculum* (Maax pepper) and *Capsicum frutescens* (Pico Paloma pepper).

Methodology: Two seed compounds were evaluated, one from Maax pepper and another from Pico Paloma pepper, collected in Campeche, Mexico. Both ecotypes were evaluated *ex situ* in the 2016 and 2017 cycles, under greenhouse conditions. The evaluation was carried out jointly with eight other ecotypes in a randomized block design with three repetitions. The morphological characterization was based on the Capsicum descriptor of the IPGRI. The ecotypes were characterized and compared based on descriptive statistics.

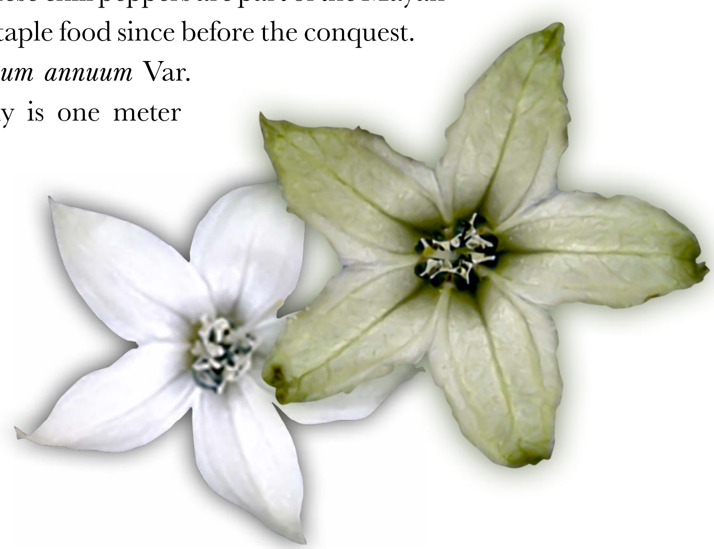
Results: The main differential characteristics were: shape, length, and width of the cotyledonous leaf; shape and margin of the leaf; number of flowers per axil; color and spot of corolla; number of fruit's locules, and seed surface.

Conclusions: The morphological characterization allowed to establish differences between the two genotypes; contributing to the knowledge of ex situ growth characteristics.

Keywords: Morphological characterization, wild chili peppers, *ex situ*.

INTRODUCTION

Mexico has a great diversity of plants, and it is one of the main centers of domestication worldwide. The genus *Capsicum* stands out as one of the first domesticated plants in the Americas. Specifically, more than 100 morphotypes of cultivated and wild chili peppers are known to be widely distributed in the national territory. This variation includes two types or species of pepper: Maax (*Capsicum annuum* Var. *Glabriusculum*) and Pico Paloma (*Capsicum frutescens*), with different morphotypes depending on the region where they grow. In the Yucatan peninsula, these chili peppers are part of the Mayan gastronomic culture and have been a staple food since before the conquest. The Mayans knew the fruit of *Capsicum annuum* Var. *Glabriusculum* —a plant that generally is one meter or less tall— as Maax Iik (Carnevali *et al.*, 2010). In this region, the Maax pepper grows in "milpa" or "solar" fields and jungles, where it behaves as a perennial plant. This chili pepper can be found mainly from July to December, during the rainy season. Immature



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fruits can be pale green to dark green, while ripe fruits are reddish orange to dark red. For its part, the Pico Paloma pepper is a wild chili pepper species found in the Mexican southeast, specifically in the states of Tabasco, Chiapas, and Campeche, and it has various morphotypes and populations (Pérez-Castañeda *et al.*, 2008). The wild chili peppers of *Capsicum frutescens* can be found all year round growing on the edges of roads, pastures, family vegetable gardens, banana plantations, and cocoa plantations (Castañón-Nájera *et al.*, 2008). They are used for local consumption and sale, as well as to garnish typical dishes of the local gastronomy (Pérez-Castañeda, Castañón-Nájera & Mayek-Pérez, 2008). As a result of the existing variation of the genus *Capsicum*, the morphological characterization is important, given the agroecological diversity of the chili pepper ecotypes, which represents a valuable collection of genes. Salinas *et al.* (2010), Moreno-Pérez (2011), Ramírez-Meraz *et al.* (2015), and Toledo-Aguilar *et al.* (2016) have carried out many characterization studies, while Alonso *et al.* (2008), Pérez-Castañón *et al.* (2008), Castañón-Nájera *et al.* (2008), Pérez Castañeda *et al.* (2015), Galvez *et al.* (2018), and Gutiérrez-Buron *et al.* (2020) have researched morphological diversity. However, a more detailed morphological description is required. Consequently, the objective of the research was the morphological description of the Maax pepper and the Pico Paloma pepper in *ex situ* conditions in the state of Campeche.

MATERIALS AND METHODS

The Maax pepper and Pico Paloma pepper ecotypes were collected in 2016. The former was collected in Escárcega, while the latter were collected in Candelaria, both municipalities located in southern Campeche. Ripe red fruits were collected from different plants, forming a compound of ripe fruits for each ecotype. The fruits were washed with sterilized water and kept at low temperature before seed extraction. The seed was extracted through artisanal techniques, using water and macerating the pulp. The extracted seeds were dried on metal trays in a ventilated and shaded place for 8 to 10 days. Subsequently, the seeds were stored at 0 °C. The number of seeds was enough for the two cycles of morphological characterization of the ecotypes. Sowing was carried out under greenhouse conditions in May 2016 and 2017, in 200-cavity polystyrene trays. Canadian Peat Moos® was used as substrate. The seeds were covered with black plastic bags to encourage a rapid and uniform germination. After eight days, the trays were uncovered and placed on terraces where the seedlings grew until they were taken to the field. The plants were transplanted 45 days after sowing. The study included eight different types of chili pepper. Planting was carried out under protected agriculture conditions, with 0.25 m between plants and 1.5-m wide furrows. Two furrows were used per ecotype. The agronomic management of the plants followed the technical recommendations for the region (Soria *et al.*, 2002). The qualitative and quantitative morphological characterization was carried out using the varietal description guide of the International Plant Genetic Resources Institute (IPGRI, 1995). The variables or stages considered for the morphological description of the plants were: seedling, plant, flower, fruit, and seed. The analysis of the results of the morphological description was based on descriptive statistics, estimating the average and the coefficient of variation. This coefficient was used as a measure to explain the contribution of morphological variables to the morphological diversity of the evaluated pepper ecotypes. The contribution of the qualitative morphological variables to the diversity of the morphological expression was determined by the different expressions of a specific characteristic of the Maax pepper and Pico Paloma pepper plants.

RESULTS AND DISCUSSION

Seedling

The Maax pepper had a staggered seedling emergence of five to eight days, while the Pico Paloma pepper had a more uniform seedling emergence. The cotyledonous leaf had a lanceolate shape, while Pico Paloma pepper had a deltoid shape and was greener (Table 1 and Figure 1). The cotyledonous leaf of the Pico Paloma pepper was longer and wider; however, the length and width of the cotyledonous leaf of the Maax pepper had a higher coefficient of variation (12.67% and 17.17%) than the Pico Paloma pepper (7.95% and 7.59%). This phenomenon is explained by the morphological variation of the ecotypes of wild chili peppers that can be found in the state of Campeche and the Yucatán peninsula (Gutiérrez-Burrón *et al.*, 2020). The value of the coefficients of variation determined that, for both types of pepper, the cotyledonous leaf can help to identify and differentiate the Maax and Pico Paloma peppers.

Table 1. Qualitative and quantitative *ex situ* characteristics of the seedling stage of the Maax (*Capsicum annuum* var. *Glabriusculum*) and Pico Paloma (*Capsicum frutescens*) pepper ecotypes. CV-Coefficient of variation.

Characteristics	Maax chili		Pico paloma chili	
Qualitative				
Hypocotyl colour	Green		Green	
Hypocotyl pubescence	Sparse		Sparse	
Cotyledonous leaf colour	green		green	
Cotyledonous leaf shape	Lanceolate		Ovate	
Quantitative	**Average**	**CV**	**Average**	**CV**
Cotyledonous leaf length (mm)	15.31	12.67	21.25	7.95
Cotyledonous leaf width (mm)	6.52	17.17	7.61	9.59

Figure 1. Leaf shape of the Maax (*Capsicum annuum* var. *Glabriusculum*) and Pico Paloma (*Capsicum frutescens*) pepper ecotypes.

Plant

The results found for the plant morphology indicate that the main qualitative differences between the Maax pepper and the Pico Paloma pepper were the presence of anthocyanins in the stem, leaf density, leaf color, leaf shape, and leaf blade margin (Table 2).

The Pico Paloma pepper plant had a more vigorous expression than the Maax pepper, judging by the size of the plant and the density of the branches and leaves, with deltoid-shaped leaves and undulate leaf margin (Table 2). The Maax pepper had lanceolate leaves and slightly undulated leaf margins. These characteristics provided the best explanation of the phenotypic variation, which matches the findings of De la Cruz-Lázaro *et al.* (2017), Gálvez-Muñoz *et al.* (2018), and Gutiérrez-Buron *et al.* (2020). Additionally, the presence of anthocyanins in the stem node of the Maax pepper was another characteristic that set it apart from the other ecotypes evaluated.

Quantitative differences could be observed in the morphology of the plants. The Maax pepper had an average plant height of 1.86 m. This result falls within the plant height range (0.77-2.35 m) established by Estrada *et al.* (2010). These findings were different from those reported by Domínguez-Orta and Herrera-Martínez (2019) under natural conditions (≤1 m). These differences can be attributed to the conditions of the place of growth with greater or lesser humidity and soil fertility. According to De la Cruz-Lázaro *et al.* (2017),

Table 2. Qualitative and quantitative *ex situ* characteristics of the plant of the Maax (*Capsicum annuum* var. *Glabriusculum*) and Pico Paloma (*Capsicum frutescens*) pepper ecotypes.

Qualitative characteristics	Maax chili		Pico paloma chilli	
Steam colour	Green		Green	
Nodal anthocyanin	Light purple		Green	
Stem shape	Angled		Angled	
Steam pubescence	Sparse		Sparse	
Plant grouth habit	Erect		Erect	
Branching habit	Dense		Dense	
Tillering	Dense		Dense	
Leaf density	Intermediate		Dense	
Leaf colour	Dark green		Green	
Leaf shape	Lanceolate		Ovate	
Lamina margin	Undulate		Undulate	
Leaf pubescence	Sparse		Sparse	
Quantitative characteristics	**Maax chili**		**Pico Paloma chili**	
	Average	**CV (%)**	**Average**	**CV (%)**
Plant heigth (cm)	173.82	8.47	1.85	5.54
Steam heigth (cm)	69.33	17.45	50.67	14.0
Plant width (cm)	119.00	5.86	144.67	31.11
Mature leaf lenght (cm)	12.40	12.90	14.43	4.72
Mature leaft lenght (cm)	4.83	1.84	4.53	9.95
Pedicel lenght (cm)	2.39	6.25	8.23	10.33

CV=Coefficient of variation.

Figure 2. *Ex situ* characteristics of plants of the Maax (*Capsicum annuum* var. *Glabriusculum*) and Pico Paloma (*Capsicum frutescens*) pepper ecotypes.

plant height is a major morphological characteristic in characterization and diversification studies, because its variation depends on environmental conditions.

Flower

Regarding its qualitative characteristics, the Maax pepper had two flowers per axil, white corolla, pale blue anther, white filament, and intermediate calyx margin. These characteristics coincide with the findings of Alonso *et al.* (2008) who reported two flowers per axil (Table 3). Meanwhile, the Pico Paloma pepper had yellowish flowers, greenish corolla spot, and two flowers per axil. These characteristics should be considered for the morphological description and differentiation of pepper morphotypes (Table 3). These results match the findings of the diversity studies of Alonso *et al.* (2008).

Fruit

The Maax pepper had an intermediate fruit set, a red fruit, and an intermediate persistence of the fruit on the stem, which made it qualitatively different from the Pico Paloma pepper. For its part, the Pico Paloma pepper had a low fruit set, intense red fruit during the ripe state, and persistence of the pedicel with the stem (Table 5). These results match the findings of Gutiérrez-Burrón *et al.* (2020), who considered that color, pigmentation, and fruit size are differential morphological characteristics of Maax pepper.

The Maax pepper was smaller and lighter than the Pico Paloma pepper. These results can be compared with different in situ conditions. Salinas-Hernández *et al.* (2010) recorded a fruit weight and fruit diameter of 0.21 g and 0.51 cm and 0.31 g and 0.57 cm for

Table 3. Morphological ex situ characteristics of the flower of the Maax (*Capsicum annuum* var. *Glabriusculum*) and Pico Paloma (*Capsicum frutescens*) pepper ecotypes.

Qualitative characteristics	Maax chili		Pico Paloma chili	
Number of flowers per axil	From one to two		Two	
Flower position	Erect		Erect	
Corolla colour	White		Light yellow	
Corolla spot colour	White		Green	
Corolla lenght (cm)	<1.5		<1.5	
Corolla shape	Rotate		Rotate	
Anther colour	Light blue		Blue	
Filament colour	White		Purple	
Stigma exsertion	Excert		Excert	
Calyx pigmentation	Absent		Absent	
Calyx margin	Intermediate		Dentate	
Quantitative characteristics	**Average**	**CV**	**Average**	**CV**
Anther lenght (cm)	1.94	4.37	1.98	12.54
Filament lenght (cm)	1.05	1.02	1.29	3.43

CV=Coefficient of variation.

Figure 3. *Ex situ* characteristics of the flower of the Maax (*Capsicum annuum* var. *Glabriusculum*) and Pico Paloma (*Capsicum frutescens*) pepper ecotypes.

Maax pepper and for Pico Paloma pepper, respectively. For their part, Alonso *et al.* (2008) indicates a fruit weight of 0.41 g for Pico Paloma pepper and a variable weight for Maax pepper.

The Pico Paloma pepper had longer fruits (1.72 cm) and two locules, while the Maax pepper had 1.15-cm long fruits and three locules. These characteristics were considered to explain the morphological variation, following the conclusions of Gálvez-Muñoz *et al.* (2018).

Table 4. Qualitative and quantitative *ex situ* characteristics of the fruit of the Maax (*Capsicum annuum* var. *Glabriusculum*) and Pico Paloma (*Capsicum frutescens*) pepper ecotypes.

Qualitative characteristics	Maax chili		Pico paloma chili	
Fruit set	Intermediate		Intermediate	
Fruit colour at mature stage	Red		Dark red	
Fruit shape	Triangular		Triangular	
Fruit shape at pedicel attachment	Obtuse		Obtuse	
Fruit shape at blossom end	Pointed		Pointed	
Fruit blossom end appendage	Absent		Absent	
Fruit cross-sectional corrugation	Slightly corrugated		Slightly corrugated	
Fruit surface	Semiwrinkled		Semiwrinkled	
Pedicel with fruit	Slight		Slight	
Pedicel with stem	Intermedite		Persistent	
Placenta length	>1/2 fruit length		>1/2 fruit length	
Quantitative characteristics	**Average**	**CV (%)**	**Average**	**CV (%)**
Fruit Weight (g)	0.26	22.12	0.58	23.40
Fruit lenght (cm)	1.15	2.58	1.72	11.15
Fruit width (cm)	0.54	42.27	0.58	9.86
Fruit pedicel lenght (cm)	2.83	12.64	2.65	22.30
Fruit wall thicknees (mm)	0.49	8.22	0.51	16.80
Number of locules	3.00	0	2.0	0

CV=Coefficient of variation.

Seed

The surface of the Maax pepper seed was considered rough due to the bumps it has on its surface, while the Pico Paloma pepper seed was considered smooth. Judging by its length and diameter, the Pico Paloma pepper had slightly larger seeds than the Maax pepper (Table 5).

Table 5. Morphological *ex situ* characteristics of the seed of the Maax (*Capsicum annuum* var. *Glabriusculum*) and Pico Paloma (*Capsicum frutescens*) pepper ecotypes.

Qualitative characteristics	Ecotype			
	Maax chili		Pico paloma chili	
Seed colour	Straw (deep yellow)		Straw (deep yellow)	
Seed surface	Rough		Smooth	
Seed size	small		small	
Quantitative characteristics	**Average**	**CV (%)**	**Average**	**CV (%)**
Seed lenght (mm)	3.08	7.79	3.62	8.01
Seed dianeter (mm)	2.48	36.69	2.97	6.39
1000-seed weight [g] (g)	2.0	4.03	3.43	6.06
Number of seeds per fruit	11.4	35.35	11.0	35.63

CV=Coefficient of variation.

The weight of 1,000 seeds was another differential characteristic between the two morphotypes of pepper. The weight of 1000 seeds for Pico Paloma (3.43 g) is similar to the 3.57 g found by Alonso *et al.* (2008).

CONCLUSIONS

The morphological characterization of the Maax and Pico Paloma pepper ecotypes allowed us to establish the *ex situ* differential morphological characteristics of both ecotypes. In all the growth stages of the plant (seedling, plant, flower, fruit, and seed), we were able to identify characteristics that help to explain the morphological variation. The results contribute to the knowledge about the Maax and Pico Paloma, chili peppers of great importance for the state of Campeche.

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Colegio de Postgraduados

History of lethal yellowing with emphasis on the susceptibility of royal palms (*Roystonea* spp.)

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ABSTRACT

Objective: To explore the available literature relating to lethal yellowing (LY) in order to assess the susceptibility of royal palms (*Roystonea* spp.) to this disease.

Design/methodology/approach: Bibliographic material in English and Spanish was consulted in physical and digital libraries in search of unequivocal and plausible LY reports in *Roystonea* palms. Information gathered was then reviewed and discussed.

Results: We found evidence of LY susceptibility of royal palms dating back to the beginning of the 20th century. In addition to Mexico, possible LY outbreaks in *Roystonea* palms might have occurred in Cuba, Haiti, the Dominican Republic, and Antigua and Barbuda.

Limitations on study/implications: Reports of LY predating molecular diagnostic tools, particularly in *Roystonea* palms, cannot be assumed as unequivocal evidence of susceptibility to this disease.

Findings/conclusions: Royal palms have shown evidence of susceptibility to the LY pathogen throughout the Caribbean Basin. In light of this, their potential role as long-term phytoplasma reservoirs should be examined in order to better comprehend this disease's pathosystem.

Keywords: Lethal yellowing, Texas Phoenix palm decline, lethal bronzing, royal palms, susceptibility.

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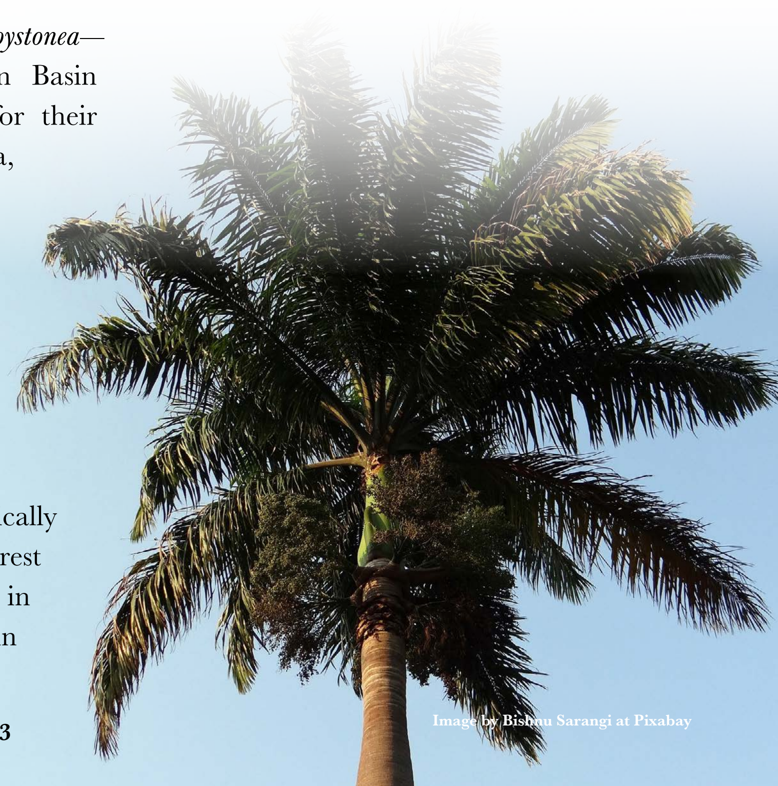
INTRODUCTION

Royal palms —genus *Roystonea*— are native to the Caribbean Basin and appreciated worldwide for their ornamental beauty (Zona, 1996). In Mexico, two species —*Roystonea dunlapiana* P.H. Allen and *Roystonea regia* (Kunth) O.F. Cook— are naturally distributed in the Yucatan Peninsula and in Tabasco, Chiapas and Veracruz (Orellana *et al.*, 2018). Both are economically important non-timber forest products for rural communities in the Yucatan Peninsula, used in



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construction and honey production (Noguera-Savelli and Cetzal-Ix, 2021). In addition, they are also frequently used as street trees in many cities in southeastern Mexico. However, they are also susceptible to diseases caused by several types of pathogens, including phytoplasmas (Bajwa *et al*., 2020).

Between 2018 and 2022, we noticed a few declining *Roystonea* sp. in the cities of Villahermosa, Tabasco, Coatzacoalcos, Veracruz, and Merida, Yucatan, Mexico, with leaf yellowing and inflorescence necrosis symptoms, indicative of phytoplasma infection (Figure 1) (Palma-Cancino and Ortiz-García, personal observations). This prompted us to explore bibliographic material with the intention of assessing the susceptibility of *Roystonea* palms to a phytoplasma disease currently endemic to the Caribbean Basin: lethal yellowing (LY). However, to provide the context necessary for this topic to be addressed, we will also present a summary of LY and its history and provide up-to-date information on its distribution, as well as an updated list of susceptible palm taxa.

MATERIALS AND METHODS

Bibliographic material in English and Spanish was consulted in physical and digital libraries in search of unequivocal and plausible LY reports in *Roystonea* palms. Digital libraries and databases were accessed and examined through the use of search engines, primarily Google Search, Google Scholar, and Scopus. Keywords used for searching materials in English included "lethal yellowing", "royal palms", "*Roystonea*" and "phytoplasma", among others. Likewise, "amarillamiento letal", "palma real" and "fitoplasma" were used for searching sources in Spanish. Information gathered was then reviewed and discussed and the notion of relative susceptibility to LY —as implemented by McCoy *et al*. (1983)— was applied to estimate how susceptible royal palms are to LY.

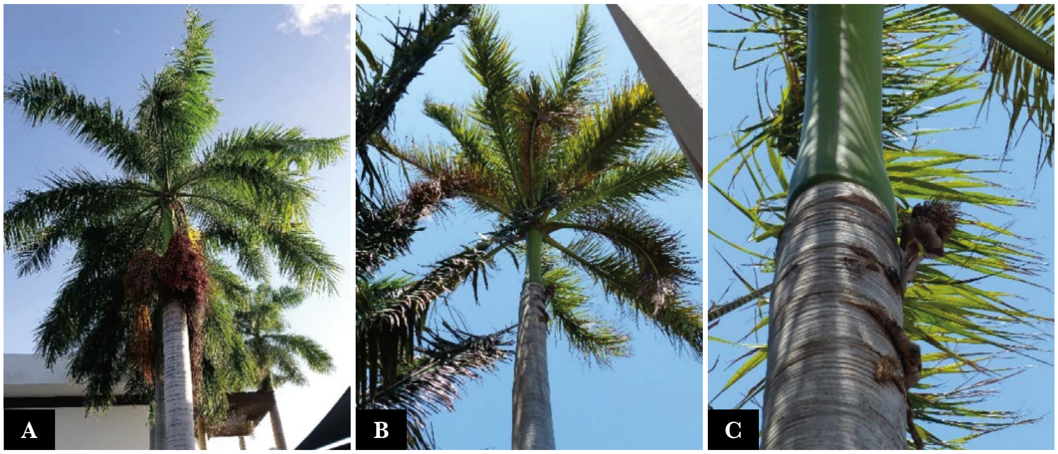

Figure 1. *Roystonea* palms in the city of Merida, Yucatan, Mexico. A. A flowering, visibly healthy *R. regia*. B. *Roystonea* sp. with leaf decay of mature leaves and foliar discoloration in the upper crown. C. Detail of the same palm showing necrosis and atrophy in an inflorescence, a symptom indicative of phytoplasma infection.

RESULTS AND DISCUSSION

LY: discovery and general characteristics

LY, a devastating disease of coconut (*Cocos nucifera* L.) and at least 44 other palm species (Table 1), has apparently been active in the Caribbean Basin for almost two centuries (Figure 2). Its first account may have been given by the 2nd Marquess of Sligo, who, in 1834, reported the destruction of all the coconuts in the leeward side of Grand Cayman, in the Cayman Islands, by a strange disease (Fawcett, 1889). By May 1888, the disease was still active at Grand Cayman according to the British botanist William Fawcett, who, upon inspecting its symptoms —which included premature fruit drop, blackening of new inflorescences, and leaf yellowing advancing upwards through the crown (Figure 3)— suspected that the disease was "due to the presence of a bacterium" (Fawcett, 1889). However, his statement had to wait until 1972 for confirmation, when phytoplasmas —which are considered a special type of parasitic bacteria— were finally determined to be the cause of LY (Beakbane *et al.*, 1972; Plavšić-Banjac *et al.*, 1972).

Despite some instances of success, the vast majority of phytoplasmas cannot as yet be cultured axenically (Contaldo and Bertaccini, 2021). For that reason, phytoplasma strains associated with LY are often referred to as members of group 16SrIV, subgroup A (Lee *et al.*, 1998), instead of having a conventional scientific name, although '*Candidatus* Phytoplasma palmae' has also been proposed as a name for the taxon (Bertaccini *et al.*, 2022). So far, the cixiid planthopper *Haplaxius crudus* Van Duzee is the only proven vector of this phytoplasma subgroup (Dzido *et al.*, 2020), nevertheless, additional putative vectors of group 16SrIV phytoplasmas have also been discovered (Brown *et al.*, 2006; Ramos-Hernández *et al.*, 2020; Fernández-Barrera *et al.*, 2022).

While LY diagnoses today consist of the specific detection of subgroup 16SrIV-A phytoplasma DNA in symptomatic palms, usually by nested PCR or real-time PCR

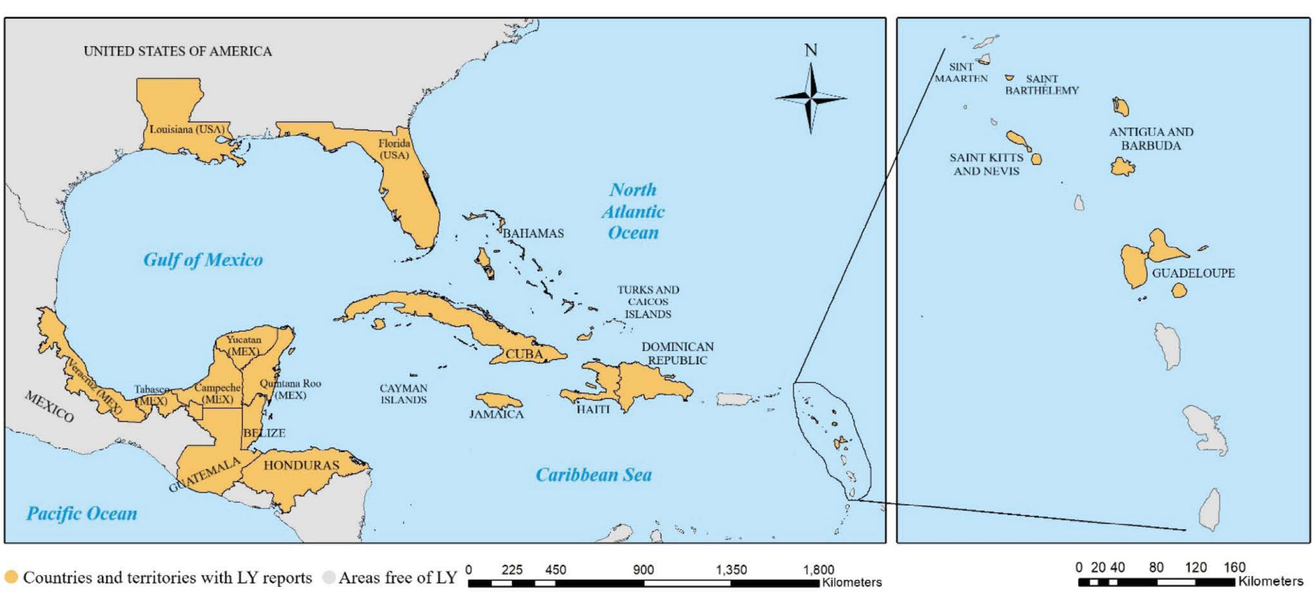

Figure 2. Current distribution of LY (associated with subgroup 16SrIV-A phytoplasmas) in the Caribbean Basin.

Table 1. Host range of subgroup 16SrIV-A phytoplasmas, associated with LY.

Taxa[1]	Known to be susceptible in	Phytoplasma ID[2]		Reference
		Confirmed	Unconfirmed	
Acrocomia aculeata (Jacq.) Lodd ex. Mart.	Mexico (Yucatan)	✓		Narvaez *et al.* (2016)
Adonidia merrillii (Becc.) Becc.	Mexico (Tabasco, Yucatan), USA (Florida)	✓		Lara *et al.* (2017)
Aiphanes lindeniana (H. Wendl.) H. Wendl.	USA (Florida)		✓	Harrison *et al.* (1999)
Allagoptera arenaria (Gomes) Kuntze	USA (Florida)		✓	Harrison *et al.* (1999)
Arenga engleri Becc.	USA (Florida)		✓	Harrison *et al.* (1999)
Bismarckia nobilis Hildebrandt & H. Wendl.	Antigua and Barbuda		✓	Myrie *et al.* (2014)
Borassus flabellifer L.	USA (Florida)		✓	Harrison *et al.* (1999)
Caryota mitis Lour.	USA (Florida		✓	Harrison *et al.* (1999)
Caryota rumphiana Mart.	USA (Florida)		✓	Harrison *et al.* (1999)
Chelyocarpus chuco (Mart.) H.E. Moore	USA (Florida)		✓	Harrison *et al.* (1999)
Cocos nucifera L.	Caribbean Basin (widespread)	✓		Ntushelo *et al.* (2013)
Coccothrinax readii H.J. Quero	Mexico (Yucatan)	✓		Narvaez *et al.* (2006)
Corypha utan Lam.	USA (Florida)		✓	Harrison *et al.* (1999)
Cryosophila warscewiczii (H. Wendl.) Bartlett	USA (Florida)		✓	Harrison *et al.* (1999)
Cyphophoenix nucele H.E. Moore	USA (Florida)		✓	Harrison *et al.* (1999)
Dictyosperma album (Bory) H.L. Wendl. & Drude ex Scheff.	USA (Florida)		✓	Harrison *et al.* (1999)
Dypsis cabadae (H.E. Moore) Beentje & J. Dransf.	USA (Florida)		✓	Harrison *et al.* (1999)
Dypsis decaryi (Jum.) Beentje & J. Dransf.	USA (Florida)		✓	Harrison *et al.* (1999)
Gaussia attenuata (O.F. Cook) Becc.	USA (Florida)		✓	Harrison *et al.* (1999)
Gaussia maya (O.F. Cook) H.J. Quero	Mexico (Yucatan)	✓		Narváez *et al.* (2018)
Howea belmoreana Becc.	USA (Florida)		✓	Harrison *et al.* (1999)
Howea forsteriana (F. Muell. & H. Wendl.) Becc.	USA (Florida)		✓	Harrison *et al.* (1999)
Hyophorbe verschaffeltii H. Wendl.	USA (Florida)		✓	Harrison *et al.* (1999)
Latania lontaroides (Gaertn.) H.E. Moore	USA (Florida)		✓	Harrison *et al.* (1999)

Table 1. Continues...

Taxa[1]	Known to be susceptible in	Phytoplasma ID[2]		Reference
		Confirmed	Unconfirmed	
Livistona chinensis (Jacq.) R. Br. ex Mart.	Antigua and Barbuda, USA (Florida)		✓	Myrie *et al*. (2014)
Livistona rotundifolia (Lam.) Mart.	USA (Florida)		✓	Harrison *et al*. (1999)
Nannorrhops ritchiana (Griff.) Aitch.	USA (Florida)		✓	Harrison *et al*. (1999)
Phoenix canariensis hort. ex Chabaud	USA (Florida)	✓		Harrison *et al*. (2008)
Phoenix dactylifera L.	Antigua and Barbuda, USA (Florida)	✓		Harrison *et al*. (2008)
Phoenix reclinata Jacq.	USA (Florida)		✓	Harrison *et al*. (1999)
Phoenix rupicola T. Anderson	USA (Florida)		✓	Harrison *et al*. (1999)
Phoenix sylvestris (L.) Roxb.	USA (Florida, Louisiana)	✓		Ferguson and Singh (2018)
Pritchardia maideniana Becc.	USA (Florida)		✓	Harrison *et al*. (1999)
Pritchardia pacifica Seem. & H. Wendl.	Antigua and Barbuda, Mexico (Yucatan), USA (Florida)	✓		Dzido *et al*. (2020)
Pritchardia remota Becc.	USA (Florida)		✓	Harrison *et al*. (1999)
Pritchardia thurstonii F. Muell. & Drude	USA (Florida)		✓	Harrison *et al*. (1999)
Ravenea hildebrandtii C.D. Bouché	USA (Florida)		✓	Harrison *et al*. (1999)
Roystonea regia (Kunth) O.F. Cook	Antigua and Barbuda, Mexico (Yucatan)	✓		Narvaez *et al*. (2016)
Sabal mexicana Mart.	Mexico (Yucatan)	✓		Vázquez-Euán *et al*. (2011)
Sabal palmetto (Walter) Lodd. ex Schult. & Schult. f.	USA (Florida)	✓		Mou *et al*. (2022)
Syagrus romanzoffiana (Cham.) Glassman	Antigua and Barbuda	✓		Myrie *et al*. (2014)
Syagrus schizophylla (Mart.) Glassman	USA (Florida)		✓	Harrison *et al*. (1999)
Thrinax radiata Lodd. ex Schult. & Schult. f.	Mexico (Yucatan)	✓		Vázquez-Euán *et al*. (2011)
Trachycarpus fortunei (Hook.) H. Wendl.	USA (Florida, Louisiana)	✓		Ferguson and Singh (2018)
Veitchia arecina Becc.	USA (Florida)		✓	Harrison *et al*. (1999)

[1]Botanical names included in this list are in accordance with their current usage in systematic botany and thus may differ from their original citing source.
[2]Phytoplasma ID was considered confirmed if the presence of subgroup 16SrIV-A phytoplasmas in that palm species was verified by DNA sequencing.

Figure 3. Symptoms induced by subgroup 16SrIV-A phytoplasmas (associated with LY) in *Cocos nucifera* and two other palms. A. Premature fruit drop in *C. nucifera*. B. Blackening of new inflorescences in the same species. C. Progressive (uppwards) leaf yellowing in *C. nucifera*. D. Leaf yellowing in *Thrinax radiata*. E. First stage of foliar decay in *Adonidia merrillii*.

protocols, it is important to keep in mind that, prior to the late 1980s, phytoplasmas were differentiated only by some of their biological properties such as host range, geographic distribution, and the symptoms induced in affected plants, thus, LY reports before that period, including those cited in this article, should be regarded as plausible, but not unequivocal evidence of subgroup 16SrIV-A's involvement. Also, it should be noted that LY was not always referred to as such. Previous names applied to this disease in the first half of the 20th century included "fever", "bud rot", and "West End bud rot" (Smith, 1905; Johnston, 1912; Ashby, 1915). Other authors used the terms "bronze leaf wilt" (Martyn, 1945) and "unknown disease" (Leach, 1946), to avoid confusion with the "common" bud rot caused by *Phytophtora palmivora* (Butler) Butler.

Early LY outbreaks in the Caribbean Basin: "unknown disease" period

Following the apparent emergence of LY in the Cayman Islands, the disease was next spotted in Cuba. Translating from Cuban naturalist Carlos de la Torre y Huerta: "The coconut plague, according to the people who studied it for the first time in Cuba, dates back to a time very close to the famous hurricane of 1870" (de la Torre, 1906). By the 1900s, LY became widespread in Cuba (Smith, 1905; Horne, 1908), and the devastation caused by it was such that banana groves had to replace many coconut plantations, especially around Baracoa (de la Torre, 1906).

About the same time as in Cuba, LY started to be noticed in Jamaica as well. In 1891, large-scale coconut deaths caused by an unknown disease —now considered to have been LY— were reported at Montego Bay, on the northwest shore of the island (Fawcett, 1891), however, prior to 1872, a disease of similar characteristics had already wiped out the coconut population along forty miles of the southwest shore, in Saint Elizabeth Parish (Martyn, 1945). By the beginning of the 20th century, LY was restricted to the western end of Jamaica, along the shoreline between Savanna-la-Mar and Montego Bay, and, though it was not greatly feared by local farmers at that time, it was nonetheless considered a potentially dangerous condition (Johnston, 1912).

Curiously, LY's first account in Haiti may have been given by the famous poet Oswald Durand, in a footnote to his poem *La mort de nos cocotiers*, included in the first edition of *Rires et pleurs* (Durand, 1896). According to him, around 1880, all the coconuts in and in the vicinities of Cap-Haïtien were affected by a disease that destroyed them in a short time, leaving only bare trunks. No further spread of LY was reported in Haiti until 1943, when the disease was observed in Gonaïves, killing nearly 8,000 coconuts (Leach, 1946).

Other countries within the Caribbean Basin that were affected by LY during the first half of the 20^{th} century were The Bahamas, in the 1920s (Leach, 1946), and the Dominican Republic, probably as early as 1925 (Ciferri and Ciccarone, 1949). Eventually, the disease reached United States territory in 1937 via Key West, Florida (Martinez and Roberts, 1967), however, it wasn't until 1955 that a new outbreak in Key West led to the constant monitoring of LY in that country (Corbett, 1959).

LY outbreaks in North America's mainland: "expanded host range" period

LY was documented for the first time on the Florida mainland in 1971, in Miami and Coral Gables (Seymour *et al.*, 1972). Until then, it was considered to be primarily a disease of *C. nucifera*, however, shortly after the emergence of said outbreak, multiple diseased individuals from three additional species, *Adonidia merrillii* (Becc.) Becc., *Pritchardia pacifica* Seem. & H. Wendl., and *Pritchardia thurstonii* F. Muell. & Drude, were detected in the southwest area of Miami (Parthasarathy and Fisher, 1973). By 1974, a total of 12 palm species were known to be susceptible to LY in Florida (Thomas, 1974). This number grew to 25 by 1978 (Thomas, 1979). Finally, three decades after arriving in Miami-Dade County, LY was known to affect 36 palm species in the whole of Florida (Howard and Wilson, 2001). This aggressive expansion in host range by the LY phytoplasma was viewed as a consequence of the higher diversity and abundance of palms, both native and exotic, present in the urban landscapes of South Florida (as well as in the living collections of botanical gardens located within the area) compared to that of other locations in the Caribbean that were also affected by LY at that time (the 1970s and early 1980s) (McCoy *et al.*, 1983). Naturally, this meant that new palm hosts of subgroup 16SrIV-A phytoplasmas had to be found in other regions with a different species composition than that of Florida.

Thus, Mexico would end up becoming a testing ground of sorts for this assumption to be proven, as the arrival of LY to Mexican shores during the same period was considered imminent.

History took its course and LY eventually reached Mexico via the Yucatan Peninsula: the disease was first hinted on the island of Cozumel, state of Quintana Roo, in 1977 (Romney and Harries, 1978), and was later confirmed during an outbreak in Cancun that started in 1981 (McCoy *et al.*, 1982). Subsequently, in 1985, LY was reported at El Cuyo, in the neighboring state of Yucatan (Villanueva *et al.*, 1987). Then, in 1990, the disease finally reached the state of Campeche, thus affecting the entire Mexican portion of the Yucatan Peninsula (Robert *et al.*, 1991).

Regarding the existence of additional hosts of subgroup 16SrIV-A phytoplasmas, they were studied in Yucatan since the 1990s. After PCR-based techniques became the standard for phytoplasma detection and identification, several studies demonstrated the existence of a total of six new palm hosts of subgroup 16SrIV-A phytoplasmas, all of them native to the Yucatan Peninsula (see Table 1). Surprisingly, one of such hosts was *R. regia* (Narvaez *et al.*, 2016), a species that, according to observations made in Florida, was not considered to be susceptible to LY (McCoy *et al.*, 1983). This apparent contradiction will be discussed in the following segments, meanwhile, let us to conclude this summary of LY in Mexico by mentioning its advance to the states of Tabasco, in 1993 (Escamilla *et al.*, 1995), and Veracruz, in 1999 (Sánchez Anguiano, 2002).

From the 1990s to today, LY continued spreading to the following countries and territories: Belize (Escamilla *et al.*, 1994), Honduras (Ashburner *et al.*, 1996), Guatemala (Mejía *et al.*, 2004), Saint Kitts and Nevis (Myrie *et al.*, 2006), Turks and Caicos Islands (Brown *et al.*, 2007), Antigua and Barbuda (Myrie *et al.*, 2014), Saint Barthélemy (Jeger *et al.*, 2017), Sint Maarten (Myrie *et al.*, 2019), and Guadeloupe (Pilet *et al.*, 2023). The disease is apparently spreading more rapidly in the Lesser Antilles than in Central America, threatening to eventually reach South America (Yankey *et al.*, 2018).

Evidence of susceptibility of royal palms to LY and other LY-like diseases

As previously mentioned, unequivocal evidence of susceptibility of royal palms to subgroup 16SrIV-A phytoplasmas was first presented in Yucatan, Mexico, during the last decade (Narvaez *et al.*, 2016). However, accounts from early literature concerning LY provide interesting glimpses that suggest an even longer history of interaction between *Roystonea* palms and this particular phytoplasma subgroup.

Cuba, home to five *Roystonea* species, although one of them now extinct (Moya López, 2020), previously had two instances of mortality of royal palms that were possibly associated with LY. Horne (1908), who studied LY in the 1900s, encountered several bare trunks of royal palms which might have died from the disease in a badly affected coconut grove near Naguaraje. He also observed a young royal palm around the same location, which "showed as nearly a typical case of bud rot [syn. LY] as one could imagine possible". A few years later, Johnston (1912) noted about 15 to 20 dead or diseased royal palms near Baracoa, over the course of three years. Though not without hesitations, he suspected the cause could be LY. Moreover, the symptoms he observed in the affected palms were very similar to the ones later described by Narvaez *et al.* (2016) in the Yucatan Peninsula.

In the island of Hispaniola, where *Roystonea borinquena* O.F. Cook is extremely abundant (Zona, 1996), two other accounts of LY possibly affecting royal palms were also provided. Upon a visit to Gonaïves, Haiti, in late 1945, Leach (1946) informed the following: "associated with the outbreak of the disease on coconuts [LY]… there has been an equally sudden and serious mortality of date palms... and even a few royal palms... have been killed in the same area". In addition, he noticed a "small amount of dieback" on the inflorescences of diseased royal palms after cutting open some immature spathes, a symptom that, given the circumstances described by the author, strongly suggests LY. Likewise, in Santo Domingo, Dominican Republic, a similar event was witnessed a year later. Translating from Ciferri and Ciccarone (1949): "A disease with similar symptoms to those of bronze leaf wilt [syn. LY] attacks royal palms in the same area".

No further evidence of susceptibility of royal palms to LY was provided until the disease reached Antigua and Barbuda and group 16SrIV phytoplasmas were detected in a *R. regia* palm by means of real-time PCR (Myrie *et al.*, 2014). Similarly, in Tabasco, Mexico, group 16SrIV phytoplasmas were detected in a *R. regia* in 2015 (Ramos-Hernández *et al.*, unpublished data). Eventually, the work of Narvaez *et al.* (2016) confirmed that *R. regia* was a host of subgroup 16SrIV-A phytoplasmas. However, group 16SrIV encloses at least five other closely related phytoplasma subgroups (Palma-Cancino, 2020), including subgroup 16SrIV-D, which is associated with Texas Phoenix palm decline (TPPD, syn. lethal bronzing), another serious LY-like disease which also affects palm species of both economic and ornamental importance (Bahder *et al.*, 2019; Ferguson *et al.*, 2020; Palma-Cancino *et al.*, 2020). In fact, subgroup 16SrIV-D phytoplasmas are considered by some to represent an entirely different species from '*Ca*. P. palmae', namely '*Candidatus* Phytoplasma aculeata' (Soto *et al.*, 2021). In view of the above and considering the widespread occurrence of TPPD in the Caribbean Basin (Ntushelo *et al.*, 2013), this article will also review evidence of susceptibility of royal palms to subgroup 16SrIV-D phytoplasmas.

Earlier in the past decade, an outbreak of a LY-like disease affecting several palms —including one *Roystonea* sp. —in Guaynabo, Puerto Rico, was confirmed to be associated with subgroup 16SrIV-D phytoplasmas (Rodrigues *et al.*, 2010; Ntushelo *et al.*, 2013). Additionally, group 16SrIV phytoplasmas were detected in a *R. borinquena* during a subsequent survey conducted in Puerto Rico in the mid-2010s (Simbaña Carrera, 2019). While this last example cannot be precisely ascribed to any particular phytoplasma subgroup within group 16SrIV, it is worth mentioning that, so far, only subgroup 16SrIV-D is known to occur in Puerto Rico (Ntushelo *et al.*, 2013; Agosto, 2021).

To conclude this section, additional diseases of royal palms associated with phytoplasmas not enclosed in group 16SrIV will be mentioned briefly as LY-like diseases —as a whole— negatively impact palms not only in the Caribbean Basin but all over the world (Hemmati *et al.*, 2020). So far, the only known examples are the association of group 16SrII phytoplasmas with *R. borinquena* in Puerto Rico (Simbaña Carrera, 2019) and of subgroup 16SrII-D phytoplasmas with *R. regia* in Oman (Hemmati *et al.*, 2021). Lastly, in Malaysia, a new 16SrI phytoplasma subgroup was reported as infecting *R. regia* (Naderali *et al.*, 2015) but the figures included in that report actually depict diseased palms of a different genus.

On the relative susceptibility of royal palms to LY and TPPD

Based on what was mentioned in the previous section, is it possible to estimate how susceptible royal palms are to both the LY and TPPD pathogens? The short answer is: only subjectively.

Field resistance to LY —typically expressed as disease incidence or mortality rate— has only been sufficiently tested for the economically important coconut palm. The concept of relative susceptibility is applied to several other species instead, with varying degrees of success (McCoy *et al.*, 1983; Howard and Wilson, 2001). Likewise, relative susceptibility to the TPPD pathogen has been preliminary estimated for a few species (Palma-Cancino, 2021). However, *Roystonea* species have not been included in any of these ratings, mainly due to a lack of data. Therefore, a final discussion of all the evidence previously mentioned will follow in an attempt to assess the relative susceptibility of royal palms to LY and TPPD.

According to Narvaez *et al.* (2016), in Yucatan, subgroup 16SrIV-A phytoplasmas were encountered very rarely in *R. regia* palms. To put that in perspective, *R. regia* is one of the palms most frequently used as street trees in the city of Merida, Yucatan, where both LY and TPPD are currently active. In some areas of the city, this palm represents nearly 12% of all observable Arecaceae, only *C. nucifera* and *A. merrillii* are more common (Palma-Cancino, personal observations in 2020 and 2021). Thus, it appears that, despite being fairly abundant in urban landscapes of the region, *R. regia* is one of the least susceptible species to subgroup 16SrIV-A phytoplasmas in Yucatan, Mexico. Interestingly, the comparatively lower abundance of *R. regia* in the LY-affected urban areas of Florida (Fitzpatrick, 2005) could explain why the disease was never observed in *R. regia* palms in that region, given a relative susceptibility rating of low to minimal.

Similarly, if we consider the evidence presented in the previous segment for both Cuba and Hispaniola as genuine LY outbreaks in *Roystonea* palms, it would appear that the relative susceptibility rating of the *Roystonea* spp. from both islands sits somewhere between low to minimal, given the comparatively low numbers of diseased individuals —as opposed to those of *C. nucifera*— encountered by the authors (Horne, 1908; Johnston, 1912; Leach, 1946). Nevertheless, it is also possible that these mortality events were not actually associated with LY. In that case, the *Roystonea* spp. from Cuba and Hispaniola could be considered immune to LY. This assumption was previously put forth by Harries *et al.* (2001) as part of a means to explain why LY —even to this day— has not become epidemic in the Dominican Republic. Supposedly, the abundant *Roystonea* spp. populations from that country contribute to a kind of "buffer effect" that protects the more susceptible—but somewhat isolated— *C. nucifera* populations from LY. We are of the opinion that the first scenario —that is, that the royal palms in Cuba and Hispaniola have a low to minimal relative susceptibility to LY— is the most likely.

In comparison, royal palms have only shown evidence of susceptibility to subgroup 16SrIV-D phytoplasmas in Puerto Rico. At the moment, it is difficult to say whether this is because TPPD is not as widely distributed in the Greater Antilles —where most *Roystonea* spp. are native and therefore more abundant (Zona, 1996)— as LY, or to some other factor. However, considering that royal palms have not been found to be susceptible to subgroup 16SrIV-D phytoplasmas in regions like Florida and Yucatan, where TPPD has been active for several years (Harrison *et al.*, 2008; Vázquez-Euán *et al.*, 2011), we are of the opinion that royal palms, in general, are some of the least susceptible palms that are known to be affected by this phytoplasma subgroup.

As a final point, we wish to draw attention to a quotation on this issue by Horne (1908): "If royal palms are attacked [by LY] it is so rarely that probably there is no practical importance to be attached to the matter". However, as our knowledge of the pathosystems of group 16SrIV phytoplasmas continues to increase, we can now say that this is not the case. Even if royal palms have a low to minimal susceptibility to the LY and TPPD pathogens, they are still key elements of the pathosystems of both diseases for a number of reasons: 1) royal palms are amply distributed throughout the Caribbean Basin (Zona, 1996), thus, as inoculum sources, their relevance in terms of disease spread should not be underestimated; 2) due to their popularity as ornamental palms and their known association with *H. crudus* (Howard and Mead, 1980), *Roystonea* spp. have the potential of introducing group 16SrIV phytoplasmas to new areas by means of unrestricted movement of plant material within and between countries, whether directly (through diseased palms) or indirectly (through healthy palms carrying infective vectors); 3) as evidenced by Narvaez *et al.* (2006), some palm species in the Yucatan Peninsula can harbor subgroup 16SrIV-A phytoplasmas without showing symptoms of infection, therefore, the occurrence of this phenomenon should also be examined for *Roystonea* palms.

CONCLUSION

Royal palms have shown evidence of susceptibility to the LY pathogen throughout the Caribbean Basin. In light of this, their potential role as long-term phytoplasma reservoirs should be examined in order to better comprehend this disease's pathosystem.

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Colegio de Postgraduados

Current and potential demand of fertilizers in Mexico

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ABSTRACT

Objective: To diagnose the market situation of fertilizers through the statistical analysis of the main market variables, considering the importance of fertilizers as a strategic input for increasing productivity in the agricultural sector.
Methodology: The growth of the apparent domestic consumption of fertilizers for the 2007-2020 period, the variables that determine it, and the current and potential demand per state in 2019 were estimated.
Results: During the period under analysis, fertilizer consumption grew at an annual rate of 4.8%, reaching 6.3 million tons in 2020. Almost 75% of consumption was supplied by imports and only 25% was supplied by domestic production. If 100% of the sown area was fertilized, the potential demand for fertilizers would exceed 8 million tons.
Implications: The country's heavy dependence on fertilizer imports means that the Mexican agricultural sector is vulnerable to a potential increase in fertilizer prices, as a result of the crisis that the global agrochemicals market is currently facing.
Conclusions: Given Mexico's dependence on fertilizer imports, the domestic industry must be strengthened, in order to increase production and satisfy a greater percentage of the domestic demand.

Keywords: fertilizers, apparent national consumption, potential demand, imports.

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INTRODUCTION

Rapid population growth is a global problem that has required innovation and growth in agricultural activity, because this sector is responsible for generating the food required by the population. In this context, chemical fertilizers take center stage. Since the Green Revolution, chemical fertilizers have been complemented by genetically modified seeds and the use of mechanical means to modernize agricultural activity (Picado, 2008). Chemical fertilizers are also responsible for a 40% increase in food production over the last 40 years (FAO, 2011).

According to Medina *et al.* (2018), yields in grain maize crops to which fertilizers have been applied have increased by 29.3% with respect to fertilizer-free crops.

Image by Samuel Faber at Pixabay

In terms of production, the boom of the chemical fertilizer industry in Mexico started in the 1970s and 1980s. In 1978, this industry was nationalized and became Fertilizantes Mexicanos (FERTIMEX), a public company which promoted fertilizer production, marketing, distribution, and supply, through a single price policy for the whole country. In 1992, FERTIMEX was privatized and the fertilizer industry —which until that point had been ruled by national policies— began a new stage that, to put it succinctly, consisted of the gradual reduction of agrochemical production and the constant growth of imports (CE, 1990).

Chemical fertilizers are indispensable inputs for agricultural activity; therefore, they represent a significant percentage of production costs. However, despite their importance, there are few studies that analyze their behavior.

Fertilizer market analysis is important for the following reasons: a) fertilizers are one of the main inputs for the increase of agricultural productivity; b) they are the main instrument for the increase of agricultural production, lowering the dependence on food imports; c) the dependence of this market on imports makes it vulnerable to the likely increase in international prices; and d) the current crisis in fertilizer exporting countries (*e.g.*, Ukraine and Russia) will have a strong impact on the international market.

Considering their importance as an essential input for the increase of productivity in the agricultural sector, the objective of this work was to estimate the current demand for fertilizers in Mexico. The potential demand for fertilizers was also quantified to measure the challenge that the Mexican agricultural sector would face if the input was used on 100% of the sown agricultural area.

MATERIALS AND METHODS

The apparent national consumption of fertilizers was estimated in order to determine the research objectives. In economic terms, apparent national consumption (ANC) is the amount of goods that the market requires or demands. It is the main indicator used to analyze demand.

Afterwards, the annual data from the following indicators were used to calculate the ANC of fertilizers for the 2007-2020 period: a) total production of chemical fertilizers, taken from the Monthly Survey of the Manufacturing Industry (INEGI, 2021); and b) total imports and exports of chemical fertilizers, taken from the Online Tariff Information System (SIAVI) of the Ministry of Economy (SIAVI, 2021).

According to Miranda (2005), the ANC of year a was calculated using the following formula:

$$ANCa = Pa + Ma - Xa \qquad (1)$$

Where Pa is the domestic production of chemical fertilizers in year a; Ma are the imports of fertilizers in year a and; Xa is the national exports of fertilizers in year a.

To estimate the demand by state, the fertilized area by state for the year 2019 —obtained from the Agrifood and Fisheries Information Service (SIAP)— was taken as a weighting factor (SIAP, 2021).

The following procedure was used to determine the fertilizer demand by state. First, the average consumption of fertilizers per hectare in year *a* (AFC*a*) was obtained, dividing the apparent national consumption of fertilizers (ANC*a*) by the fertilized national area (FNA*a*) and using the following formula:

$$AFCa = \frac{ANCa}{FNAa} \tag{2}$$

Subsequently, the following formula was used to determine the potential fertilizer demand per state, multiplying the average consumption of fertilizers per hectare by the total area sown in each state:

$$PDFSi = AFCa \times TSASi \tag{3}$$

Where *PDFSi* is the potential demand of fertilizers in the state *i* and *TSASi* is the total area sown in the state *i*.

RESULTS AND DISCUSSION

Table 1 shows the apparent national consumption. The data suggest that the ANC of chemical fertilizers in Mexico had an average annual growth of 68.3% during the 2007-2020 period (from 3,754 to 6,319 thousand tons), representing an average annual growth of 4.8%.

Factors that could explain such an impressive growth in consumption are related to the use of the input to increase productivity in the agricultural sector. Increasing the production of the agricultural sector is a challenge that must be met through the increase in yields and it requires an increased use of fertilizers.

The analysis of the behavior of the variables that determine apparent national consumption leads to the following observation: during the 2007/2009-2018/2020 period, production grew by 65.4%, from 1,458 to 2,411 thousand tons (an average annual growth of 4.7%). During the same period, imports grew by 61.5%, from 2,885 to 4,658 thousand tons (an average annual growth of 4.5%). Fertilizer exports grew by 27.5%, from 587 to 751 thousand tons (an average annual growth of 2.2%). The above data reflect the country's heavy dependence on fertilizer imports, which leaves the country vulnerable to the behavior of the global agrochemicals market.

The growing behavior of the ANC of fertilizers during the 2007-2020 period and its related factors can help to explain the behavior of fertilizer demand during the last few years, which can be attributed to changes in the said factors. As the main input for agriculture, the increase in arable land has been a determining factor; while 17.9 million ha were sown in 2018, by 2021 this area had increased to 20,665 thousand ha (SIAP, 2022).

Experience shows that farmers in developed countries are hardly affected by the increase in nitrogen fertilizer prices, while farmers in developing countries would face

Table 1. Apparent national consumption of fertilizers in Mexico (2007-2020) in thousands of tons.

Year	Production	Imports	Exports	Consumption
2007	985	3,270	286	3,969
2008	1,427	2,735	688	3,474
2009	1,963	2,649	792	3,820
2010	2,163	2,807	677	4,293
2011	2,062	3,772	270	5,564
2012	2,102	3,385	1,015	4,472
2013	2,734	3,275	988	5,021
2014	2,721	3,615	926	5,411
2015	2,608	3,655	784	5,479
2016	2,458	4,209	742	5,924
2017	2,522	4,491	716	6,296
2018	2,400	5,055	792	6,664
2019	2,505	4,090	809	5,786
2020	2,329	4,830	651	6,507
Average 07/09	1,458	2,885	589	3,754
Average 18/20	2,411	4,658	751	6,319
GR (%)	65.4	61.5	27.5	68.3
AAGR (%)	4.7	4.5	2.2	4.8

GR (TC)=Growth rate; AAGR (TCMA)=Average annual growth rate.
Source: Table developed by the authors based on data from INEGI (2021) and SIAVI (2021).

lower availability and would be forced to reduce their application. For example, from 2008 to 2009, the use of nitrogen fertilizer in Africa fell by 13%. International reference prices for fertilizers increased throughout 2021, with many estimates reaching all-time highs. The price of nitrogen fertilizers has recorded the most significant increase. The price of urea, a major nitrogen fertilizer, more than tripled in the last 12 months (FAO, 2022).

Variations in the price of the inputs used in the technological package (including fertilizers) have a strong impact on the adoption rate of the input. A decrease/increase in the price of these variables would increase/decrease the adoption rate and reduce/increase the gap between total observed consumption and potential consumption (García *et al.*, 2018).

Table 2 shows the production of fertilizers in Mexico in 2021 per agrochemical group. During that year, the country produced 2.08 million tons, out of which 46.7% were phosphate fertilizers, 26.9% acid fertilizers, and 26.4% nitrogen fertilizers. Therefore, Mexico specializes in the production of the said group of fertilizers.

Apparent national consumption suggests that demand is highly dependent on imports. Table 3 shows fertilizer imports and exports by group. In 2019, Mexican imports reached 4.83 million tons. Considering the different groups of imported fertilizers, the most important are: nitrogenous fertilizers (61.5% of the total imports), followed by complex fertilizers (28.9%) and potassium fertilizers (6.2%).

Table 2. Production of fertilizers in Mexico (2021) in tons.

Group	Production	%
Nitrogenous	548,869	26.4
Phosphatide	972,310	46.7
Acids	561,013	26.9
Total	2,082,192	100.0

Source: Table developed by the authors based on data from INEGI (2022).

Table 3. Imports and exports of fertilizers in Mexico (2019) in thousands of tons.

Group	Fertilizer	Imports		Exports	
		Volume	%	Volume	%
Nitrogenous	Urea	1,911	39.6	14	2.2
	Ammonium sulfate	280	5.8	1	0.1
	Ammonium nitrate	20	0.4	1	0.2
	Sodium nitrate	3	0.1	0	0.0
	Others	756	15.7	2	0.3
	Total	2,970	61.5	18	2.8
Phosphatide	Super phosphates	6	0.1	89	13.6
	Others	3	0.1	0	0.0
	Total	9	0.2	89	13.6
Potassium	Potassium chloride	299	6.2	5	0.8
	Potassium sulfate	0	0.0	0.00	0.0
	Others	156	3.2	0.5	0.1
	Total	455	9.4	6	0.9
Complex	Di-ammonium phosphate	249	5.2	149	22.9
	Mono-ammonium phosphate	156	3.2	347	53.3
	NPK	332	6.9	24	3.8
	Others	659	13.6	18	2.7
	Total	1,396	28.9	539	82.7
Grand total		4,830	100.0	651	100.0

Source: Table developed by the authors based on data from SIAVI (2021).

Although Mexico depends on imports to supply apparent domestic consumption, it exports a considerable volume of fertilizers. However, foreign sales have decreased significantly in recent years (Table 1). Table 3 details fertilizer exports by agrochemical group and shows that Mexico exported 651 thousand tons in 2019.

The foreign sales of fertilizers per agrochemical group were divided as follows: 82.7% were complex fertilizers, 13.6% phosphate fertilizers, 2.8% nitrogen fertilizers, and only 0.9% potassium fertilizers.

Table 4 shows the results of the calculation of potential consumption at the state level. The demand for fertilizers in Mexico is directly related to the arable and fertilized land.

Table 4. Current and potential demand of fertilizers by state (2019). Thousands of hectares and tons.

State	Area			Consumption	
	total	fertilized	%	actual	potential
	hectare			thousands of tons	
Aguascalientes	128	107	83.6	42	50
B. California	180	161	89.4	63	70
B. Cal. Sur	41	38	92.7	15	16
Campeche	340	276	81.2	108	133
Chiapas	1,360	772	56.8	301	531
Chihuahua	1,036	1,002	96.7	391	404
Cd.de México	16	15	93.8	6	6
Coahuila	252	140	55.6	55	98
Colima	162	120	74.1	47	63
Durango	576	365	63.4	142	225
Guanajuato	948	830	87.6	324	370
Guerrero	902	656	72.7	256	352
Hidalgo	529	222	42.0	86	207
Jalisco	1,650	1,306	79.2	509	644
México	747	696	93.2	272	291
Michoacán	1,119	1,057	94.5	412	437
Morelos	137	133	97.1	52	54
Nayarit	370	250	67.6	98	144
Nuevo León	330	61	18.5	24	129
Oaxaca	1,254	610	48.6	238	489
Puebla	939	768	81.8	300	366
Querétaro	137	124	90.5	48	53
Q. Roo	118	92	78.0	36	46
San Luis Potosí	638	295	46.2	115	249
Sinaloa	1,059	1,044	98.6	407	413
Sonora	603	599	99.3	234	235
Tabasco	266	184	69.2	72	104
Tamaulipas	1,326	850	64.1	332	517
Tlaxcala	235	232	98.7	91	92
Veracruz	1,515	1,053	69.5	411	591
Yucatán	699	72	10.3	28	273
Zacatecas	1,051	703	66.9	274	410
National	20,665	14,832	71.8	5,786	8,061

Source: Table developed by the authors based on data from SIAP (2021), INEGI (2021) and SIAVI (2021).

In 2019, 20.67 million ha were sown in Mexico, 14.83 million ha of which were fertilized, indicating a 71.8 % use rate of fertilizers. This rate varies in the different states of the country.

In 2019, the states with the highest percentage of the total sown area were: Jalisco (8.0%), Veracruz (7.3%), Chiapas (6.6%), Tamaulipas (6.4%), and Oaxaca (6.1%) (Table 4).

The states with the highest fertilized area in relation to their sown area were: Sonora, Tlaxcala, Sinaloa, Mexico City, Morelos, Chihuahua, Michoacán, State of Mexico, Baja California, and Queretaro, all of which fertilized more than 90% of their sown area the said year.

The states with the highest fertilizer consumption in 2019 were: Jalisco (8.8%), Michoacán (7.1%), Veracruz (7.1%), Sinaloa (7.0%), Chihuahua (6.8%), and Tamaulipas (5.7%).

Table 4 shows the potential demand for fertilizers. If the entire sown area were to be fertilized, more than 8 million tons of fertilizers would be needed. This would be a great challenge, considering the dependence of the country on imports and the situation of the international fertilizer market. The main potential consumer states would be Jalisco, Veracruz, Chiapas, Tamaulipas, Oaxaca, and Michoacán, which together would account for 39.8% of the total potential demand (Table 4).

CONCLUSIONS

As a result of its use as one of the main inputs for increasing productivity, the apparent national consumption of fertilizers has recorded a strong growth during the last few years. Such growth has been closely related to a sharp increase in agrochemical imports, in response to the decrease in the domestic production available for national consumption. As a consequence of the increased dependence on imports, the fertilizer industry has become vulnerable to changes in the international prices resulting from fluctuations in the international fertilizer market. The consumption of fertilizers currently amounts to almost 6 million tons of fertilizers and the efforts to fertilize the entire national agricultural area represent a huge challenge for the fertilizer industry, which must supply the potential demand (estimated at just over 8 million tons). Policies that contribute in the short term to increase fertilizer production must be implemented; otherwise, in the future, the situation will be increasingly difficult for national producers, who will face rising prices of one of the main inputs used to increase agricultural productivity.

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Colegio de Postgraduados

Physicochemical characteristics of milk marketed by family production units in the Atoyac River Basin in Puebla and Tlaxcala, Mexico

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ABSTRACT

Objective: To determine the physicochemical characteristics and possible adulteration of raw milk marketed in dairy family systems located in the Atoyac River Basin in Puebla-Tlaxcala.

Design/methodology/approach: The physical characteristics of milk were determined: pH, acidity and freezing point; and chemical characteristics: fat, protein, lactose, minerals, non-fat solids, total solids and water content. This milk is produced and marketed by 264 family production units to three cheese producing companies in the Atoyac River Basin in the states of Puebla and Tlaxcala. The data were analyzed by company and subsequently grouped with the Cluster technique of the SAS software.

Results: Significant differences ($p<0.05$) were found in the physical and chemical characteristics of the milk marketed by the producers with the three processing companies. In general, the milk is acid and adulterated with an average of 11.49% water; this results in a loss of $0.67/liter and in a dilution of its components, particularly protein and lactose, which leaves it outside the parameters established by normativity. The multivariate analysis generated four groups of producers and suggests the possibility that a group of producers adulterates with water, but also adds some kind of compound to increase the fat content to mask the adulteration.

Limitations on study/implications: The study of this topic, where the economic interests of the primary actors in the production chain can be affected, is delicate and difficult to carry out; however, it is necessary in order to know the quality of the products that are generated and their implication in the economy and the health of consumers.

Findings/conclusions: This study showed that the milk marketed by producers to processing companies does not comply with current regulations due to adulteration with water and the possible addition of compounds that replace fat; therefore, verification programs for compliance of the regulations are required, hoping with this that producers will receive a fair price for their production and will not have the need to adulterate the milk.

Keywords: milk adulteration, cheese companies, dairy regulations.

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INTRODUCTION

Within the food industry in Mexico, dairy production is the third of highest economic importance (CANILEC, 2021), contributing 24.3% of the agriculture and livestock GDP, which corresponds to 1.23% of the total national GDP. In the last year, the national milk production was 12.8 million tons (SIAP, 2021), giving it 16th place globally; however, this volume only covers 69% of the national demand, which is why there are imports of powdered milk and other derivatives, unfortunately affecting the price of milk paid to the producers (Ángeles *et al.*, 2004; Espinoza-Arellano *et al.*, 2019).

Dávalos (2020) shows a structural polarization of national dairy production, highlighting that 73.95% of the 95,887 production units have less than 20 cows and concentrate barely 28% of the dairy cattle, while 4.4% of the units have more than 50 cows and have 48.4% of the 2,274,366 dairy cattle in the country.

Strictly speaking, the first stratum cited above corresponds to family dairy production, characterized by being part of a diversified peasant economy with low-production animals (Abrego, 2011), deficient facilities (Botero *et al.*, 2012), and the lack of an organizational structure to sell or transform their production, so that they are dependent on intermediaries, who fix the price and the conditions of milk trade.

On the other hand, the families devoted to this activity, in addition to being vulnerable to the imports and the inequality there is between the price of raw milk paid and the price of processed milk and substitutes exhibited in commercial centers (Munguía, 2015), have been scarcely benefitted with government programs, among them the guaranteed prices announced in the present administration, where one of the conditions to fulfill is the quality standards of milk determined by the normativity (NMX-F-700-COFOCALEC-2012).

Traditionally, in the commercial producer-intermediary relationship, the quality of the milk in terms of its physical characteristics, chemical composition, bacteria load, and content of adulterating agents, has been scarcely valued and of low interest (Bernal *et al.*, 2007), which is partly because there is not a differential payment treatment based on quality, and on the other hand, because the normativity established by regulating institutions for quality control is voluntary and without supervision.

Facing this scenario, the producers can resort to adulterating practices with the aim of increasing the yield and economic value of the milk (Rodríguez-Pérez, 2011), with the addition of water being the most common practice. In this sense, the objective of this study was to determine the physical and chemical characteristics of milk traded by family production units located in the Atoyac River Basin in the states of Puebla and Tlaxcala, and to identify the economic effect of a possible adulteration.

MATERIALS AND METHODS

Study zone

This study was carried out in family type dairy farms, located in the Atoyac River Basin in the states of Puebla and Tlaxcala, which are part of the list of intermediary clients who collect milk to deliver it to three cheese producing companies located in Santa Ana

Xalmimilulco, Huejotzingo, Puebla. Traditionally, milk is collected by intermediaries, called "boteros", who make verbal agreements with the producers to collect milk every morning from the two milking events (afternoon and morning). They deliver it to processors, which are cheese companies that process on average 30,000.00 liters per day to elaborate fresh cheese, such as Oaxaca, Panela and Ranchero cheeses, in addition to semi-mature cheeses that are distributed locally and regionally.

Milk was sampled from 264 production units, distributed in 12 municipalities located in the Center-West region of the state of Puebla and in the South of the state of Tlaxcala. The cows are Holstein breed, fed with cut fodders produced in small irrigation areas, farm residues, and balanced meals.

Sampling

By duplicate, 100 mL of raw milk were collected from the mixture of the morning milking and the afternoon milking from the prior day. It was taken from the container where the total production from each farm was placed, at the time of being delivered to the "botero". To obtain a representative sample, the milk was previously mixed with a 52-point homogenizer, making circular movements by one minute. The samples were placed in sterile Dorninc tubes labeled under the NOM-109-SSA1-1994 and transported in an ice box at a temperature of 4 °C to the laboratory, for their corresponding analysis within the next two hours.

Laboratory analyses

The analyses of the samples were conducted in the shelf life laboratory of the Universidad Tecnológica de Huejotzingo. A Lactoscan, LA, was used with 4 lines × 16 characters LCD screen, Milkotronic Ltd, 8900, Nova Zagora Bulgaria, where the physical variables (acidity, pH, and freezing point) and the chemical variables (content of protein, fat, lactose, minerals, non-fatty solids, total solids and water) were determined by triplicate. Based on the results obtained on water content in the samples, an additional variable referring to the economic profit of producers or the loss by collectors, considering a price of $8.20/liter of milk (guaranteed price), which was called "economic loss". In addition, a correction was made to estimate the real content of the milk components, subtracting the amount of water added and adjusting for each component with a significance level of 5%.

To contrast the results, the NMX-F-700-COFOCALEC-2012 was consulted, which regulates the quality of raw milk (Table 1).

A classification analysis was also conducted, based on the physical and chemical characteristics of milk, which had the purpose of grouping producers with homogeneity between individuals and heterogeneity between the different resulting groups, performed with the Cluster method of the SAS software, version 2003. The information from the classification analysis came from the review and selection of variables. Then, a variance analysis between defined groups was carried out; again, with the Proc-GLM procedure of the statistical package SAS.

Table 1. Data from NMX-F-700-COFOCALEC-2012.

FAT (%)	Class A ≥ 3.2 Class B 3.1 minimum Class C 3.0 minimum
Protein (%)	Class A ≥ 3.1 Class B 3.0 to 3.09 Class C 2.8 to 2.99
Lactose (%)	4.3 to 5.0
Minerals (%)	Less than 1%
Non fat solids (%)	8.3 minimum
Total solids (%)	10.5 and 15.5%
Added water (%)	------------
Cryoscopic point (°C)	Between −0,510 (−0,530) and 0,536 (−0,560)
pH	6.5 to 6.8
Acidity (%)	0.13 to 0.17

RESULTS AND DISCUSSION

Analysis by processing company

The results obtained evidenced significant differences ($p<0.05$) in the physicochemical characteristics of the milk sold by family production units to the three processing companies. Table 2 shows the values found for each variable, where it can be seen that regarding the normativity table (Table 1), the pH in companies 2 and 3 is found within the acceptable ranges, although not in company 1 which is slightly lower and statistically different ($p<0.05$); however, the average value of pH in the milk is acceptable. The acidity and the freezing point are outside the values specified in the norm, indicating that there is adulteration with water in the milk that the three companies acquire and, on the other

Table 2. Physicochemical characteristics of raw milk traded by family production units and collected in three cheese companies of the Atoyac River Basin.

Variable	ET 1	ET 2	ET 3	MEAN	LSD
	(n=14)	(n=75)	(n=175)		
pH	6.33b	6.67a	6.56a	6.58	0.17
Acidity (%)	0.21b	0.22b	0.25a	0.24	0.03
Cryoscopic point (°C)	−0.47a	−0.47a	−0.45a	−0.46	0.03
Fat (%)	3.49a	3.5a	2.82b	3.05	0.53
Protein (%)	2.61b	2.76a	2.65ab	2.68	0.12
Lactose (%	3.91b	4.15a	3.98ab	4.03	0.18
Minerals (%)	0.61a	0.62a	0.59a	0.6	0.03
Non fat solids (%)	7.33a	7.57a	7.26a	7.35	0.32
Total solids (%)	10.81a	11.07a	10.09b	10.4	0.6
Added water (%)	6.83b	8.67b	13.07a	11.49	4.01

Samples with the same letter in the lines are statistically similar, according to Fisher's means comparison (α=0.05); PC=Processing company; n: Number of sample.

hand, there are acidification problems that reduce their hygienic quality, with a higher notoriety of company 3, which has a statistically higher value ($p<0.05$) than the two other companies.

Concerning the chemical composition of milk, the average values indicate that regarding the normativity, the milk is normal in fat but diluted in protein, lactose and minerals; therefore, it is milk low in non-fatty solids and slightly low in total solids. In the analysis by company, the fat in 1 and 2 is within the parameters marked by the normativity and without difference between them, although with statistically higher values ($p<0.05$) than those of company 3, which does not comply with the norm. For the three companies, protein and lactose contents in the milk are outside and lower than the parameters established, with company 1 being still more remarkable, where the lowest and statistically different values were found ($p<0.05$). Regarding minerals and non-fatty acids, no differences were observed between companies. Meanwhile, water addition was identified as a common practice for milk from the three companies, with this habit being even more evident for company 3, which is statistically higher.

In the estimations carried out with corrected data for the amount of water added and specified in Table 3, it can be seen that all the components increase considerably and comply with the normativity, except the non-fatty acids from company 1, which are statistically the lowest ($p<0.05$) compared to companies 2 and 3. This is a similar trend to the previous table, the content of fat and total solids did not show differences between processing companies, although their values were within the normativity. In function of the water content, it was found that company 3 has an economic loss per liter of milk received, statistically higher ($p<0.05$) than those of the other companies, which show similar losses between them.

The classification carried out with the Cluster analysis (Figure 1) resulted in 4 groups of milk that producers in the study zone traded, and the grouping was basically given by the fat content and the added water content (Table 4). The first group included 18.18% of the producers, which correspond to the highest in fat content and in total solids, but with deficient levels of protein, lactose and minerals, even outside the official norm. Group 2,

Table 3. Chemical composition and economic loss of raw milk, corrected by water content in family production units, which is collected by three cheese companies from the Atoyac River Basin.

Variable	ET 1 (n=14)	ET 2 (n=75)	ET 3 (n=175)	MEAN	LSD
Fat (%)	3.76^{a}	3.86^{a}	3.26^{a}	3.46	0.63
Protein (%)	2.81^{b}	3.03^{a}	3.05^{a}	3.03	0.03
Lactose (%)	4.2^{b}	4.55^{a}	4.59^{a}	4.55	0.04
Minerals (%)	0.66^{b}	0.68^{a}	0.68^{a}	0.68	0.01
Non fat solids (%)	7.87^{b}	8.29^{a}	8.35^{a}	8.31	0.07
Total solids (%)	11.63^{a}	12.15^{a}	11.61^{a}	11.77	0.59
Loss ($/L)	0.4^{b}	0.5^{b}	0.76^{a}	0.67	0.23

Means with equal letters in a line are statistically equal, according to Tukey's means comparison ($\alpha=0.05$); PC=Processing company; n: Number of sample.

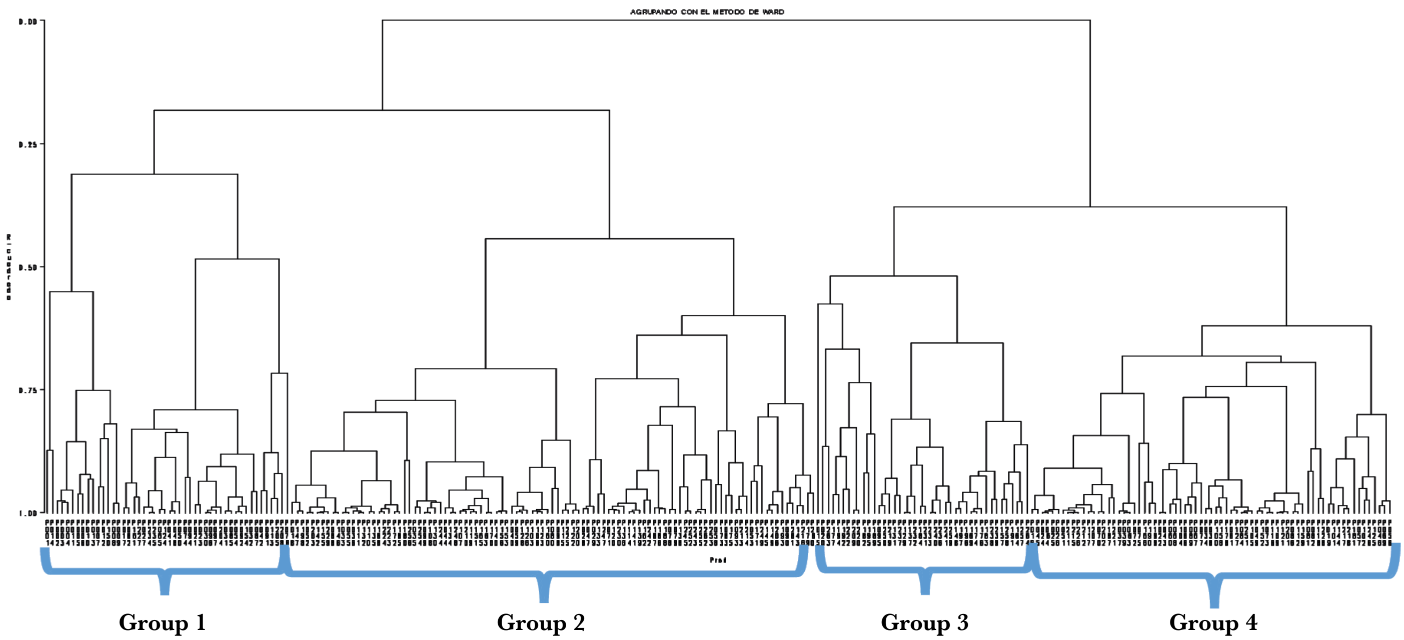

Figure 1. Dendrogram of 264 producers classified by the characteristics of raw milk they trade in the Atoyac River Basin.

which included 39.01% of the producers, stands out because it is the one that adulterates the milk the most with water and, therefore, all the components are diluted and fall outside the parameters established by the norm. Group 3 was made up by 15.90% of the producers and corresponds to those with the lowest levels of fat and with problems of hygienic quality due to its low level of pH and high acidity. Finally, group 4 includes 26.91% of producers that add the least amount of water and, therefore, of highest content of non-fatty solids and its components.

Table 4. Chemical composition of the raw milk traded by family production units from the Atoyac River Basin grouped by multivariate analysis.

Variable	G 1	G 2	G 3	G 4	Mean	LSD
	(n=48)	(n=103)	(n=42)	(n=71)		
pH	6.58^{a}	6.68^{a}	6.19^{b}	6.67^{a}	6.58	0.22
Acidity (%)	0.23^{b}	0.22^{b}	0.32^{a}	0.23^{b}	0.24	0.04
Cryoscopic point (°C)	−0.45^{b}	−0.43^{a}	−0.47^{b}	−0.49^{c}	−0.46	0.03
Fat (%)	4.31^{a}	2.76^{bc}	2.47^{c}	2.96^{b}	3.05	0.67
Protein (%)	2.63^{b}	2.54^{d}	2.75^{b}	2.88^{a}	2.68	0.15
Lactose (%)	3.95^{c}	3.81^{d}	4.13^{b}	4.32^{a}	4.03	0.23
Minerals (%)	0.59^{c}	0.56^{d}	0.61^{b}	0.64^{a}	0.6	0.03
Non fat solids (%)	7.25^{c}	6.95^{d}	7.53^{b}	7.87^{a}	7.35	0.41
Total solids (%)	11.05^{a}	10.47^{b}	10.06^{b}	10.08^{b}	10.4	0.76
Water (%)	10.08^{b}	16.98^{a}	10.22^{b}	5.21^{c}	11.49	5.06

Means with equal letters in a line are statistically equal, according to Tukey's means comparison (α=0.05); G=Group; n: Number of sample.

Table 5 presents the values of the components corrected by subtracting the content of water. It can be seen that in the milk produced, with the exception of the fat content which is very high in group 1 and very low in group 3, the other components were within the acceptable ranges indicated by the normativity. On the other hand, the same table specifies the economic profit that producers obtain from adding water, or the loss of the companies, showing that group 2 is where producers obtain a higher profit per liter of milk.

Fulfilling the parameters established by the norm is one of the challenges that family production units face. Statistically, there are differences between companies and between the groups resulting from the multivariate analysis in the pH of raw milk, and it seems like there is a positive association with acidity; however, Negri (2005) mentions that there is not necessarily an association between both variables, and relates the pH with the stability of milk in the presence of industrial thermal treatments. On the other hand, Rodríguez-Pérez *et al.* (2011), in a study where water addition to raw milk was controlled, found that adding 20%, the pH increases a percentage unit and technically has a probable repercussion on the loss of the acid-base regulating capacity of the components of raw milk.

Concerning the acidity, in general the milk collected has high values and outside the normativity, which can be attributed to the lack of hygiene in milking (Cervantes *et al.*, 2011) and inappropriate storage that allows the development of the bacteria load, problem that increases later due to bad transport conditions (without refrigeration equipment) and the time that the collection by "boteros" takes; however, in the study zone this quality can be favorable because this milk is destined to elaborate string cheese or Oaxaca cheese, where the acid milk is used, and therefore eases for the companies to advance in their transformation processes. Naturally this action can bring consequences in the shelf life of the products and in the health of consumers, because it is a product made with unpasteurized milk.

WingChing and Mora (2014) mention that the freezing point of the milk corresponds to the temperature at which it freezes, and in which the liquid part and the solutes are in balance; they report that this parameter changes proportionally in function of the amount of water added up to 15% and, on the other hand, that the minimal addition of 1% of water to the milk

Table 5. Correction in the chemical composition of raw milk and economic loss from the addition of water in family production units in the River Atoyac Basin grouped by multivariate analysis.

Variable	G 1 (n=48)	G 2 (n=103)	G 3 (n=42)	G 4 (n=71)	Media	DMS
Fat (%)	4.85[a]	3.32[b]	2.77[c]	3.11[cbc]	3.46	0.79
Protein (%)	2.92[c]	3.06[a]	3.06[a]	3.03[b]	3.03	0.03
Lactose (%)	4.39[c]	4.60[a]	4.61[a]	4.56[b]	4.55	0.05
Minerals (%)	0.66[b]	0.68[a]	0.68[a]	0.67[a]	0.68	0.01
Non fat solids (%)	8.07[c]	8.38[a]	8.39[a]	8.31[b]	8.31	0.1
Total solids (%)	12.93[a]	11.71[b]	11.17[c]	11.43[cbc]	11.77	0.76
Loss ($/L)	0.82[b]	1.39[a]	0.83[b]	0.42[c]	0.67	0.29

Means with equal letters in a line are statistically equal, according to Tukey's means comparison ($\alpha=0.05$); n: Number of sample.

dilutes their components. The results obtained in this study show the considerable presence of water in the milk and are comparable to those reported by Barham *et al.* (2014) in Pakistan, where they found values between −0.534 and −0.441 and agrees with what was reported by Escoto *et al.* (2013) in the dairy basins of the state of Hidalgo, where they also mention that adulteration is more accentuated in small-scale livestock farmers.

Regarding the chemical composition, the results in Table 2 show a quite marked dilution in protein and lactose, and therefore in non-fatty solids, due to the addition of water, in agreement with a growing proportion between processors, 6.83, 8.67 and 13.07% for companies 1, 2 and 3, respectively; these values agree with those reported by WingChing and Mora (2019) in a specific study where different percentages of water were added intentionally to milk from Holstein cows.

It can also be seen that fat is only diluted in company 3, so it is possible that some producers who sell to companies 1 and 2 adulterate milk with fat substitutes. This possibility is marked more in Table 3, where group 1 is formed by making the adjustments by removing the added water with 18.18% of producers whose milk averages 4.85% of fat, abnormal value that considerably exceeds the minimum fixed in the normativity and is very high for this type of farm.

Milk adulteration with water is the most common practice in many countries and responds to different needs, among them the high demand and the limited offer of milk as in China (Gale and Hu, 2007), although the one most disseminated is to increase the volume and to obtain higher income (Bernal *et al.*, 2007), which causes for components to be diluted and for there to be the need to add other adulterants that substitute or mask them.

To increase the fat content in milk adulterated with water, plant or animal fat may be used (Bernal *et al.*, 2007) or vegetable oil (Barham *et al.*, 2014); to trick the value of protein or nitrogen component, urea can be used (Zhao and Zhang, 2019) accompanied by surfactant products to make it foamy (Sadat *et al.*, 2006); on the other hand, to falsify the protein content, a compound called melamine can be added (Wu *et al.*, 2009). To simulate lactose, sugar is a cheap source of sweetener, and therefore, it can be assumed that sugar from sugarcane is added diluted into the raw milk to improve the flavor (Chanda *et al.*, 2012).

Milk is also adulterated with starch, wheat flour and rice flour, which help to increase the non-fatty solids (NFS) content and viscosity of the milk. Other components are caustic soda, sodium carbonate and bicarbonate, to neutralize the pH and the acidity of the milk (Fakhar and Law Walker, 2006; Afzal, 2011). On the other hand, the addition of hydrogen peroxide is very common to minimize microbe growth and control the degradation of milk (Paixao and Bertotti, 2009), although beneficial microorganisms are harmed for the elaboration of cheeses and their byproducts.

The consequences of intentionally adding substances that are not allowed and some with toxic effects such as melanin bring with it serious health problems (Lam *et al.*, 2008). In China, they also add synthetic powders to increase the protein value, and milk traders dilute the milk (Gale and Hu, 2007).

According to Lateef *et al.* (2009), milk adulteration can be with water, urea, formalin, hydrogen peroxide and sugarcane. Díaz *et al.* (2002) and Gutiérrez *et al.* (2009) evaluated adulterations of milk and dairy products with animal and plant fats in pasteurized and ultra-pasteurized milks. Some other countries are also suffering from this unethical activity. In general, the results obtained by groups of milk corrected with water, when compared with norm NMX-F-700-COFOCALEC-2012, indicate that the physicochemical composition of the raw milk from the Atoyac River Basin fulfill the parameters established. These results are highly important for producers since with them the processor and the consumers can be guaranteed a product of quality and high nutritional value; however, the price at which it is paid and the lack of normative vigilance makes it possible for the producer to adulterate with water and add other components in order to mask this action, which demerit the value of the production and can cause health problems in the consumers.

CONCLUSION

Based on the results found, it can be concluded that milk produced by family production units in the Atoyac River Basin complies with the parameters of the current normativity; however, due to the deficient management, it is sold acidified, and it is adulterated with water on average with levels of 11.49%, with which transforming companies loose on average $0.68/liter of milk. Significant differences were observed in the physicochemical characteristics of milk that is sold to the three companies studied, where adulteration with water is common in the three cases, causing for the components to be diluted, particularly protein and lactose, which mostly make up the non-fatty solids. The multivariate analysis generated four groups of producers, with one of them standing out, where when correcting the components from the addition of water, the possibility of adding fat to mask water addition can be seen.

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Social interactions and business portfolio among vegetable producers in central Mexico

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ABSTRACT

Objective: To analyze the social interactions and business portfolio of vegetable producers in central Mexico.
Design/methodology/approach: Work was conducted with 16 small-scale vegetable producers. Semi-structured questionnaires and periodic monitoring were used in the field to collect data. A Social Network analysis was carried out to understand the social interactions between producers, and the Boston Consulting Group (BCG) and Ansoff matrix was used for the business portfolio.
Results: The study found that the products with potential in the market were lettuce and nopal. Broccoli and squash represent low sales and low utility. Producers with a higher degree of centrality grow lettuce, broccoli and squash, so we suggested developing strategies for introducing nopal.
Limitations on study/implications: It was necessary to develop into market and consumers analysis.

Keywords: Social Network analysis, Boston Consulting Group and Ansoff matrix.

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INTRODUCTION

Mexico produces a large variety of vegetables which, in addition to generating foods, also represent an important source of employment. These productive systems belong to small-scale agriculture, contributing to the agriculture and livestock production economy of the country with 54% of food production and 80% of hired and paid employment (SADER, 2022a).

According to SADER (2022a), small-scale agriculture includes farmers from 0.2 ha with irrigation to 5 ha rainfed, characterized using family labor, limited access to resources, and production directed to auto-consumption. Small-scale production is conceptualized as a "way of life", as well as generator of territorial development (Santos-Barrios *et al.*, 2017). It represents an opportunity to make local economies more dynamic, especially when specific policies are combined (Robles Berlanga, 2016).

Approximately 20% of Mexican families reside in rural zones (INEGI, 2020). Small-scale farmers in our country contribute 40% of the foods that we consume, contributing to the welfare and food sovereignty of the population. These production schemes allow creating the conditions to produce healthy foods that contribute to the diet and the family economy (SADER, 2022b).

In Estado de México, it is estimated that the surface planted with vegetables is 32,333.24 ha, representing 4.87% of the total national vegetable surface, of which 11,550.56 ha are cultivated under irrigation conditions and 20,782.68 ha are rainfed; this activity is developed in 70 municipalities of the state (SECAMPO, 2022). Horticulture is considered one of the most profitable activities, where 2,129 inhabitants are occupied and receive financial income (PDM 2022-2024).

The vegetables are used as food, in the cosmetics industry, in traditional medicine, and in the transformation industry (CEDRSSA, 2020), among others.

In the municipality of Xonacatlán, 58.57% of the territory has agricultural use, primarily for grain production, followed by perennial crops and to a lesser extent the cultivation of vegetables (PDM, 2019-2021). This activity is relatively recent and has taken place thanks to the collective work among vegetable producers, fostering a positive interaction between them and other actors involved in the productive activity (Gutiérrez, 2018).

The existing relationships between collectives are based on principles and historical experiences that can be the result of bonds of trust created during years or via kinship, geographical proximity, grocer's activities, technical assistance, and sales; these allow projecting development strategies to improve the socio-productive and economic activities in horticulturists (Rousseau *et al.*, 1998).

For its part, the Social Network analysis allows exploring the structure of relationships between individuals, groups and organizations, both inward and towards the context where they develop (Sanz, 2003). Thus, the Social Network analysis (SNA) was adopted as a methodological tool to study the productive structure of vegetables (group and individual) based on the existence of information flows (Velázquez and Aguilar, 2005).

Figueroa *et al.* (2012) evaluated and determined the organization and the value of trust in businesses and vegetable producers' networks. Pérez *et al.* (2017) evaluated the behavior of poultry production agglomerations, the various forms of relating, such as friendship, kinship, solidarity and culture; as well as the attributes generated around the 11 groups of women devoted to this activity. Santos-Barrios *et al.* (2021) assessed the socio-productive relationships of small-scale pork producers and their weight in the reproduction and continuity of the system.

Vegetable production in the municipality is primarily lettuce, nopal, broccoli, spinach, squash, and chives, among others. This portfolio of products fulfills two functions mainly: to satisfy family consumption and to generate complementary financial income.

The business portfolio is established as a basic input for the analysis, commercial positioning and strategy design (Villavicencio- Gutiérrez, 2018). The Boston Consulting Group (BCG) matrix allows deciding what is necessary to invest, maintain or abandon in the Strategic Business Units (SBUs) (Castellanos, 2015). The Ansoff matrix allows identifying new opportunities for growth regardless of the size or sector of the activity.

This matrix bases the business opportunities on the markets and the products (Kotler and Keller, 2012).

From this the importance of establishing the social relationships and the social structure of vegetable producers, as well as outlining commercial strategies based on their business portfolio to maintain their productive systems and, with this, to contribute to the territorial development of the zone.

MATERIALS AND METHODS

Study zone

The study was conducted in the localities of Santa María Zolotepec, Mimiapan, Col. Emiliano Zapata and Tejocotillos in the municipality of Xonacatlán, located in Valle de Toluca. The predominant economic activity in the municipality is the manufacture of stuffed toys. The predominant climate is temperate-subhumid, with mean annual temperature of 12.4 °C (Atlas de Riesgo Municipal, 2019), mean altitude of 3050 meters above sea level (INEGI, 2020). It borders north with the municipalities of Otzolotepec and Jilotzingo; east with Jilotzingo, Naucalpan de Juárez and Lerma; south with the municipality of Lerma; west with the municipality of Otzolotepec.

Vegetable production in Xonacatlán is a relatively new activity, started 10 years ago with the conformation of the Local Agency of Rural Producers (Agencia Local de Productores Rurales, ALPR) of Xonacatlán. Presently the municipality is known for the production of nopal, spinach, broccoli, chives, chard, lettuce, beet, cilantro, tomato, green tomato, epazote and cucumber.

In this zone, the production of vegetables is carried out in two cycles: 1) spring-summer, when the vegetables harvested are the ones sensitive to frosts, such as tomato, squash, cucumber, broccoli, chili pepper, tomato and nopal; and 2) fall-winter, with the harvest of those that tolerate lower temperatures, such as the case of broad-leaf vegetables like spinach, chard, lettuce, beet, radishes and chives. There are two types of facilities: 1) open-air, whose vegetable patches are characterized by surfaces that range from 500 m^2 to 2 hectares where seasonal vegetables are produced, rainfed irrigation is used, and mostly organic and natural fertilizers are used; and 2) greenhouse, productive units that range from 100 to 250 m^2 where covered facilities are used with controlled climates and vegetables of various cycles and broad leaf are used. There are mixed productive units (greenhouse and open-air).

Data collection

Semi-structured interviews were applied to all the members of the production units. The sample was 16 vegetable producers, which represent 57.1% of the total recorded in the current register of the municipality of Xonacatlán. From the 16 producers, 11 belong to a social group under the scheme of the Local Association of Rural Producers (ALPR), and the remaining 5 are independent producers. Four producers are open-air, seven greenhouse and five mixed systems.

The interviews gathered information related to the characteristics of the producer (age, locality, schooling, members of the family, recipients of social programs, migration, additional activities to farming, income, and family expenses) and of the productive unit (crops planted, type of production, geographical proximity, surface, place of trade, suppliers, inputs, assistance institutions, training). Information was gathered on social relationships and trust among producers. The compilation of a list of cases (producers) and connections (bonds) was considered, according to Scott (2013).

Data analysis: Social Network Analysis (SNA)

The existing structures between actors and their relationships were examined, where interactions were seen as a set of points and lines (Dettmer, 2019). For González and Basaldúa (2007), a network is the result of the relationship between human groups that have two or more people, with the purpose of helping themselves, doing business or conducting any activity with common interests. It should be said that the social networks are made up of: nodes (actors), bonds (social relationships/interactions) and flows (direction in which social relationships move).

The links visualized were: 1) Trust, when two actors know each other and share information; 2) Kinship, blood relationship or family bonds between producers; 3) Purchases, interaction that are produced by trade exchanges between counterparts; 4) Procurement, interactions between producers and suppliers of farming inputs; 5) Commercialization, relationships between the producer and those that purchase their products, including bartering; 6) Public institutions, interactions between vegetable producers and institutions.

The data were organized into symmetrical and asymmetrical matrices. The first are square and express a homologous relationship of bonds (Lugo Morín *et al.*, 2010). The second ones are articulated through public institutions. In each matrix, the "0" indicated the absence of relationship and the "1" presence of the relationship. To identify the producers (actors) within the SNA and to protect their identity, each was assigned a code using letters.

The level of participation was determined by two centrality measurements:

1) Degree of centrality (number of actors to which an actor is directly connected). It is expressed under the following formula (De la Rosa *et al.*, 2005).

$$d_i = \sum_{j \in V} A_{ij}, \forall_i \in V$$

Where: A_{ij} = Matrix that connects the nodes i and j; and d_i is the centrality (degree).

2) Degree of intermediation or betweenness (possibility of a node or actor to intermediate the communications between pairs). The formula (Álvarez and Aguilar, 2005) to calculate such a measurement is:

$$g_k = \sum_{i<k<j} \frac{g_{ikj}}{g_{ij}}, \forall_k \in V$$

Where: g_k=degree of intermediation; g_{ij}=number of geodesic distances from node i to node j; g_{ikj}=number of bonds between i and j and which go through k (de la Rosa *et al.*, 2005). The data were analyzed with UCINET version 6.7 for Windows (Borgatti *et al.*, 2002).

Boston Consulting Group Matrix

It was used to calculate the relative market quota and the market's annual growth rate from the business economic units (BEUs) as a criterion to make investment decisions (Kotler and Keller, 2012). It is made up of four quadrants: i) Star quadrant, product that tends to grow rapidly and has participation in the market; ii) Interrogative quadrant, representing the strategic business division, has low participation in the market, but has possibility of rapid growth; iii) Cow quadrant, strategic product in the market with low growth and high participation in the market; and iv) Dog quadrant, strategic product with low participation in the market and low growth rates. The life cycle of the product was considered to determine the strategies for each product, according to the following: 1) Interrogative=Introduction; 2) Star=Growth; 3) Cow=Maturity; and 4) Dog=Decrease.

Ansoff Matrix

The Ansoff Matrix allowed identifying growth opportunities regardless of the size or sector of activity, which in the case studied are of family type. The matrix was based on the business opportunities detected in the markets and the products. The market was a short-circuit commercialization street market. The products were lettuce, nopal, broccoli and squash.

The following was considered: i) whether greater market participation could be attained with the current products and markets (strategy for market entry); ii) whether new markets can be found or developed for the current products (strategy of market development); iii) the possibility of developing new products of interest for current markets (strategy of product development); and iv) whether there are opportunities to develop new products for new markets (diversification strategy) (Kotler and Keller, 2006).

RESULT AND DISCUSSION

The average age of producers is 46±11 years with secondary education and seven years of experience in vegetable production. Likewise, Santos Barrios *et al.* (2021) consider backyard pork breeding as a family economic activity, and vegetable production in Xonacatlán is similar to this concept. Each family production unit has on average five members. Of the families, 69% have extra income from non-agricultural employment; 50% of the families participate in social assistance programs (Table 1).

Of the orchards and greenhouses, 62% have masculine leadership and the remaining feminine. Despite the important role of rural women, decision making is still concentrated in men, which agrees with what was reported by Ruiz-Torres *et al.* (2017) in the case of dairy farms and with Bain *et al.* (2018) with regards to the involvement of women in milk production. Women's empowering and their inclusion in decision-making areas will

Table 1. Social programs which benefit vegetable producers.

Social program	Percentage of horticultors	Origin of the program
Scholar breakfast	12.5	State program
Strong families	12.5	State program
Youth builders	6.3	State program
Elder pension	6.3	State program
Pink card	12.5	State program

contribute to breaking barriers about their visibility in access to productive resources and the decision regarding these (De la O Campos, 2015; Kidder *et al.*, 2014).

Social Network Analysis (SNA)

The social networks that are directly related to the production of vegetables are based on the trust built through kinship links, economic activity, type of production, proximity, and sale or barter of inputs. Two factors are added to these trust bonds: geographical proximity (neighbors), belonging to the same formal ALPR union. For Santos (2018), trust is understood as a social relationship that consists in the ability to open to others and represents the strength of the bonds.

The general network of vegetable producers in the municipality of Xonacatlán is concentrated in 30.48%, which indicates that the information is distributed through several central actors and far from behaving like a star network (Figure 1). For García Hernández (2013), the presence of several "star" actors benefits the direction and organization of activities given their experience and knowledge for the resolution of problems.

The actors Ga, Ro and L have, each, thirteen significant relationships within the network (Figure 1), which means that they connect with 86.66% of the actors in the network,

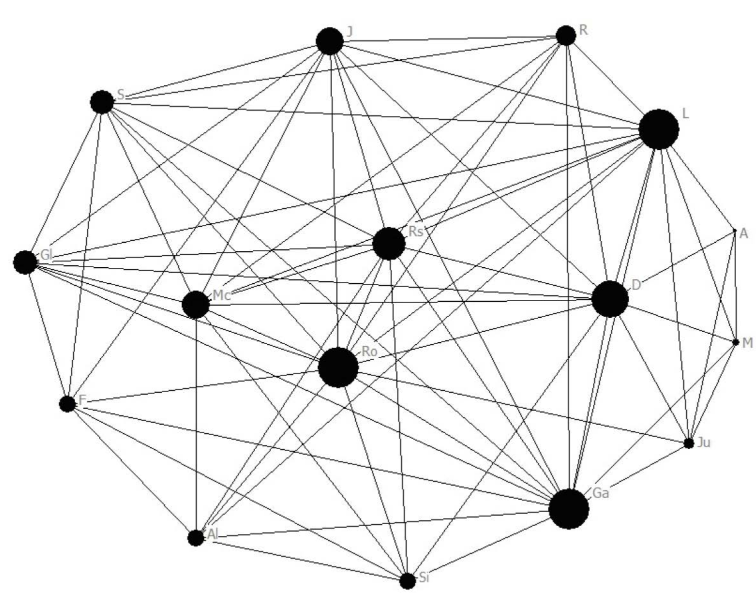

Figure 1. Horticulturist Degree.

produce in greenhouse and open-air, and trade their products in the street market and on the farm. D, Rs, J, Mc, S and Gl connect with more than 60% of the network. L and Ga have a greater capacity of connecting with those that do not connect with one another, given their degree of intermediation that represented 11.54% and 11.48%, respectively. The intermediation actors, in addition to giving information to socially distant actors, can capitalize the data that flow through them (Aguilar Gallegos *et al.*, 2016).

The information that is exchanged between producers has to do primarily with the productive activity. Relationships of kinship were observed (sister-sister, mother-son, father-son and uncle-niece) which influence the sub-networks created for the exchange of technical consulting (Figure 2). Ga and Ro, in addition to sharing kinship bonds, are the actors with highest degree of centrality, which is why they have been recognized as experts in the productive system. The nodes Ju, A, M and D are connected, in addition to kinship bonds (M and A), to the geographical proximity that productive systems have between them.

The type of programs and the relationships with actors are presented in Table 2 and Figure 3, respectively.

Three state institutions are the ones that have the highest number of connections with the producers (Figure 3). ICAMEX connects with 75%, SEDAGRO with 75%, and Ayuntamiento Municipal with 68%.

The social networks between institutions and producers agree with the hybrid networks described by Senesi *et al.* (2013), which allow connecting several actors by the same objective, sharing resources and improving the profitability of the business; however, the risk lies in the institutions restricting the development of each productive unit.

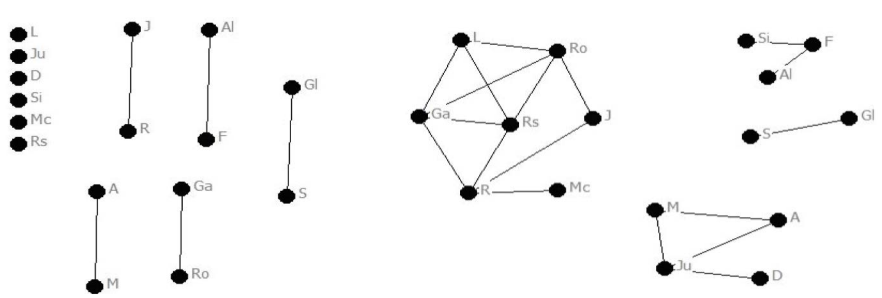

Figure 2. Kinship networks (left); technical consulting (right).

Table 2. Institutions and programs.

Institutions	Program
SADER	Fertilizers and catastrophic insurance
ICAMEX	Technical assistance and training.
SEDAGRO	Technical assistance, agricultural inputs, infrastructure, and machinery
City Hall	Business licenses Rural infrastructure
Local Delegation	Local workshops and exhibitions
Private institutions	Agricultural machinery, materials, and inputs

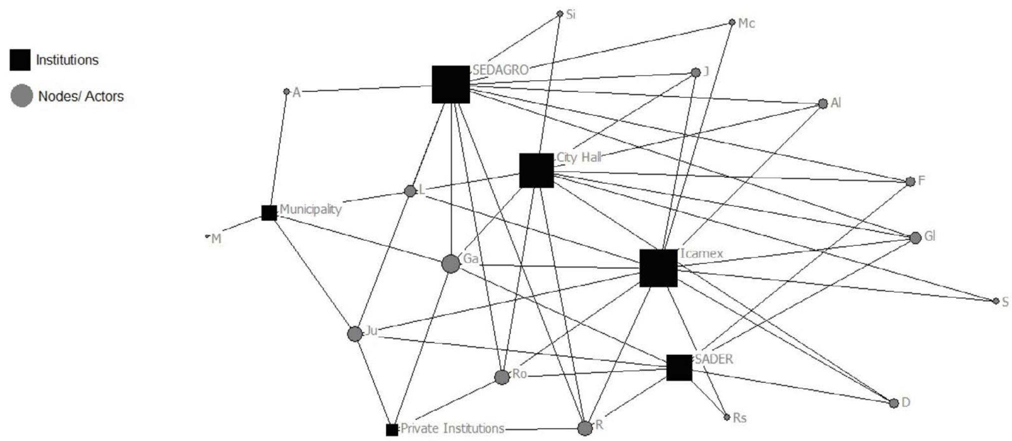

Figure 3. Degree between horticulturists and public institutions.

Boston Consulting Group Matrix

The results from the BCG matrix show that the BEU is located in the interrogative and dog quadrants (Figure 4).

Market growth in the nopal and lettuce BEUs are above average. To approach a star product, the companies must invest significantly in them, since they have a high potential (Moose and Reeves, 2022). The broccoli and squash BEUs are below the average in the dog quadrant, have low participation and low growth, and are essentially useless and should be paid off; it is unlikely that their current position will generate profit (Moose and

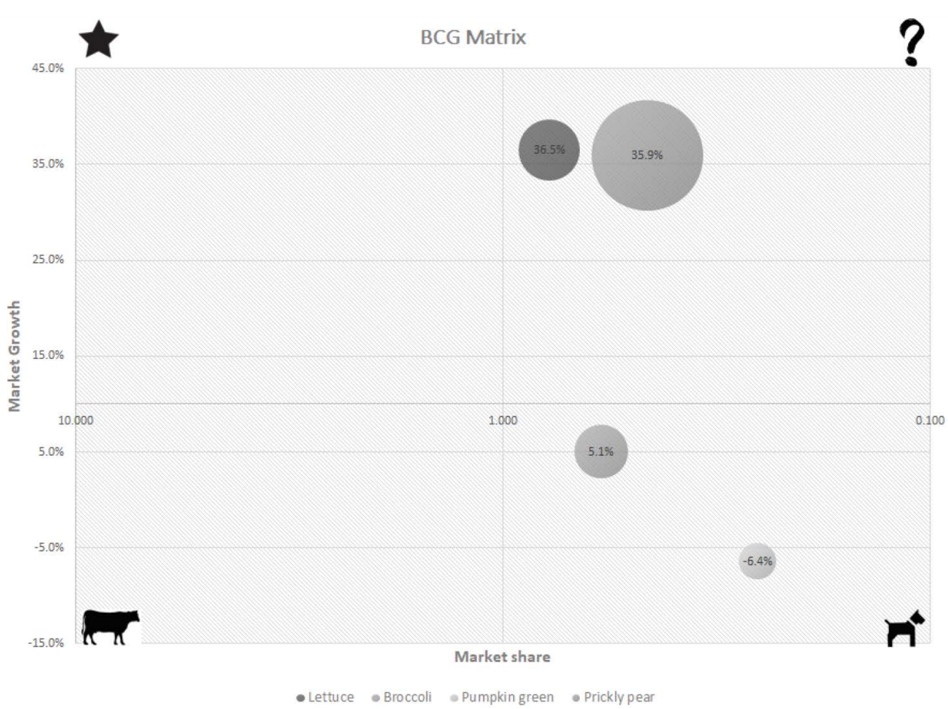

Figure 4. BCG Matrix

Reeves, 2022). Because of their cultural importance and since they are growing, strategies to reposition them are proposed.

When it comes to the relative market share (Table 3), lettuce (.78) is the product closest to 1.0. The relative market share represents the market participation of a company in relation to its largest competitor; if it is higher than 1.0 it surpasses the leader, and under 0.5 it is twice smaller than the leader (Kotler and Keller, 2012).

Nopal (.46) is twice smaller, compared to the leader producer, Otumba (SIACON, 2022). The nopal BEU represented the product with highest proportion in sales volume $454,000.00 in the year 2021. The broccoli BEU (.59) is twice smaller, compared to the production from the leader, Valle de Chalco (SIACON, 2022).

To become star products, the BEUs should concentrate the development of entrepreneurial abilities that allow them to improve their competitive position. The strategy of differentiation and positioning of a company ought to change as the product, the market and the competitors change throughout the product's life cycle (PLC) (Kotler and Keller, 2012).

The lettuce and nopal BEUs are in the introduction stage, their sales and benefits are low, and their main distribution mode is a short trade circuit through the market of local products in Xonacatlán; however, these BEUs are increasing their participation in the market. They require commercial technical investment and communication, as well as applying expansion strategies.

The broccoli and squash BEUs are inside the dog quadrant which indicates that the products are decreasing, there is low sale and a decrease in the utilities; however, they are products with cultural importance and value.

Ansoff Matrix

From the four options set out, it is suggested to focus on the market entry and market development strategies that imply a lower risk.

Table 3. Relative market share.

Product	Market share
Lettuce	0.78
Broccoli	0.59
Pumpkin	0.25
Nopal	0.46

Table 4. Xonacatlán BEU Ansoff Matrix.

	Current products	New products
Current markets	Market uptake - Market share increase	Product development - New products in the same market (chives, Swiss chard)
New markets	Market development - Expand the market for the current UEN, (geographical expansion, define the market segment, capture of new segments)	Diversification - Vertical integration (forward, backward)

Marketing strategies in declining phase. In this phase, the following is proposed:

- Product strategy: emphasizing the added value of family production, considering their cultural and nutritional value, underlying their benefits (type of agriculture and form of farming).
- Price strategy: maintaining prices in agreement to the market.
- Distribution strategy: the Xonacatlán street market represents an opportunity to position these products.
- Advertising strategy: retaining loyal clients, emphasizing nutritional and cultural advantages of the products.

CONCLUSIONS

Lettuce and nopal are products with market potential; broccoli and squash represent low sales and low utility. The producers with highest centrality (L, Ro and Ga) cultivate lettuce, broccoli and squash, which is why the importance of broccoli and squash should be reassessed. Nopal is a product with great market potential, so it is suggested to seek strategies for its introduction through pre-existing interactions in the general network, placing interest on the three producers of social relevance (L, Ro and Ga).

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