

# AGRO PRODUCTIVIDAD

Competitive management  
for export of  
**roses**  
from Mexico to Canada  
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**Agradecimientos:** Son opcionales y tendrán un máximo de tres renglones para expresar agradecimientos a personas e instituciones que hayan contribuido a la realización del trabajo.

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# Some factors affecting the reproductive capacity of hair rams in the American Tropics

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## ABSTRACT

**Objective:** to analyze within scientific databases some factors that affect the reproductive capacity of hair rams in the American Tropics.

**Design/Methodology/Approach:** Scientific databases as Web of Science, PubmEd, Scopus, Redalyc, Scholar Google, Dialnet, SciELO and Latindex containing scientific information were reviewed and analyzed to describe nutrition, libido and semen characteristics in hair rams.

**Results:** nutrition has been reported to affect sperm production, libido, testosterone, and testicular development. Rams start puberty at varying age, therefore with different weight, scrotal circumference, and semen characteristics. In regard to breed, scrotal circumference and semen characteristics are very diverse. Regarding age, semen quality is lower in young rams than in adults. According to the season, there were better semen characteristics in dry seasons in Mexico and in Brazil in the humid season.

**Study limitations/Implications:** information on nutrition, libido, semen characteristics, and scrotal circumference of hair ram breeds is poorly known or non-existent.

**Findings/ Conclusions:** the onset of puberty in hair rams is highly variable. The Blackbelly and Santa Inés rams had the best semen characteristics. Young rams have inferior semen quality. In Mexico, rams have better semen characteristics in the dry season, while in Brazil this occurs in the humid season.

Keywords: nutrition, libido, puberty, breed, age.

## INTRODUCTION

The reproductive efficiency of sheep herds depends, among other things, on the reproductive capacity of rams. So it is of utmost importance that rams are kept in optimal physical and reproductive conditions to improve profitability in the production unit



(Edwards *et al.*, 2015). Rams have very specific characteristics in their sexual behavior. In general, they are seasonal breeders that show along the photoperiod a higher reproductive capacity in the months with shorter days; and lower reproductive capacity during the months with longer days. In the tropical region, this reproductive capacity is related to the availability of food and to some climate conditions (Scaramuzzi *et al.*, 2006). It is reported that the best time for the lamb to start its reproductive life is at one year-old. During this period, both semen quality and libido expression are better (Morón-Cedillo *et al.*, 2012). At this time, the ram has reached adequate growth and development to begin its reproductive activity.

If the reproductive capacity of an ewe is diminished, it affects the reproduction of only that female during an estrous cycle. On the other hand, if the reproductive capacity of a ram is affected, this compromises the reproductive response of all the ewes in the flock that depend on that ram (Cárdenas-Gallegos *et al.*, 2012; Aké-López *et al.*, 2016). An effective way to know if a ram is suitable for breeding is testing it for reproductive capacity and sexual behavior. The reproductive capacity of rams can be evaluated directly with the physical examination of testicles, penis, and observation of libido. Indirectly, it is evaluated by observation of semen characteristics of rams (Aké-López *et al.*, 2017). At present, the reproductive characteristics of some breeds of hair sheep in the American tropics are generally unknown. Due to the above, the objective of this review was to analyze scientific databases to describe some factors that affect the reproductive capacity of hair rams in the American Tropics.

### **Factors Affecting the Reproductive Capacity of Hair Rams**

The reproductive capacity of a ram can change throughout the year and be influenced by multiple factors, including nutrition, libido, puberty, breed, age and season (Sánchez-Dávila *et al.*, 2011; Aké-López *et al.*, 2017). It is important to mention that the information summarized here on nutrition and libido is the result of research generated in wool rams. Something similar can be assumed in hair rams.

#### **Nutrition**

It is now well known that nutrition affects sexual activity, testicular development, sperm quantity and quality in most rams (Joshi, 2022). It has been reported that some variation in feed intake appears to have little effect on testicular endocrine function. However, there is evidence that sperm production is affected by changes in the size of the seminiferous tubules, which affects sperm characteristics in sexually mature rams (Martin *et al.*, 2010). In the case of long-term underfeeding, there are changes in the endocrine function of the testicles which is reflected in a decrease in testosterone synthesis (Martin *et al.*, 2010). It has also been established that the onset of puberty depends on age, body weight and percentage of fat accumulated in animals, which in turn depend on nutrition to activate the neuroendocrine system and with it, the reproductive activity (Plant, 2015; Lea and England, 2018).

The specific importance of energy and protein in sheep feed is vital, because a deficit of these nutrients affects the reproductive functions of prepubertal animals and generates

irreversible damage at the neural and gonad level, unlike adult males (Joshi, 2022). Pang *et al.* (2018) evaluated the effects of energy restriction and compensation on testicular development in sheep. Those authors showed that this restriction induced a decrease in testicular weight and number of sperm cells in the seminiferous tubules. It also caused autophagy and cellular apoptosis in the testicles. However, once the lambs were provided with an energy-balanced diet, there was a compensation of the variables evaluated. On the other hand, Guan *et al.* (2014) showed that sexually active rams, underfed for 65 days, showed reduction in testicular mass and sperm production. This affected sperm quality, as evidenced by a reduction in sperm velocity and DNA damage.

### **Libido**

In the male sheep, libido is referred to reproductive drive and behavior. It is a fundamental characteristic to have good reproductive indicators, since it defines the availability of rams to mate and participate in reproductive activities (Orihuela-Trujillo, 2014). The sexual impulse to mate is influenced by certain factors, among which body condition, time of year, hormones, general health, social interactions and stress are highlighted (Maksimović *et al.*, 2021). Testosterone has among its main functions the regulation of libido; there must be a balanced hormone level for males to have normal sexual behavior (Chacón *et al.*, 2019). Some studies have shown that intramuscular administration of glutamate or glutamate combined with testosterone in Dorper rams improved sexual behavior, with good libido they better displayed their ability to detect and mate with ewes in estrus. This increased the chances of conception and there was also a better reproductive response in the ewes (Calderón-Leyva *et al.*, 2017).

The overall health of the animal is important; for a ram with impaired health, certain diseases and nutritional deficiencies can affect libido (Cruz-Espinoza *et al.*, 2021). Nutritional deficiencies can affect the production of sex hormones. It is recently known that some animals may have a genetic disposition to a stronger or weaker libido (Juengel *et al.*, 2019). In some sheep breeds, libido has a seasonal behavior due to the length of the day and climate conditions; also, it can vary throughout animal reproductive cycle (Alhamada *et al.*, 2017). Environmental conditions such as temperature and irradiation have an impact on libido. Likewise, environmental stress can reduce it, while a comfortable environment can favor it (Maquivar *et al.*, 2021).

It has been reported that other mating experiences and previous interactions with females can influence libido. Negative experiences can decrease the disposition for mating, as well as the presence and behavior of other animals, dominant and subordinate. Social behavior, such as group hierarchy and dominance, can also influence mating readiness (Ungerfeld, 2021). On the other hand, Kumar *et al.* (2017) studied that rams when exposed to multiple stressors generally increased reaction time, and the number of services to the first ejaculation. In addition, the quality of sperm characteristics and testosterone content decreased, and the libido of rams under stress was reduced. So stress reduction is important to maintain the libido required for the reproductive success of rams (Kumar *et al.*, 2017).

### Puberty in lambs

The use of young rams as brood stock in sheep herds occurs every time at younger ages, which causes a reduction in the generational interval (Pacheco *et al.*, 2009). Therefore, it is important for lambs to start puberty as early as possible. Puberty can be defined as the moment in which the lamb is capable of producing male gametes with fertilizing capacity (Zarazaga, 2020). Some data regarding the onset of puberty of hair rams in the literature are shown in Table 1.

The lambs with the longest time until beginning puberty were those of the West African and Santa Inés breeds; those with the less time until puberty were the lambs of the Pelibuey breed. In addition, it can be observed that there is a close direct relationship between age and body weight (Table 1).

### Breed

In the different breeds of rams there is variability in physical and reproductive traits. Specifically, there are notable differences in semen characteristics. Table 2 shows that the rams Katahdin, Dorper (both from Mexico) and Santa Inés (from Brazil) have a larger scrotal circumference compared to the Blackbelly and Pelibuey males (from Mexico), and the Colombian breeds Criollo de Pelo and Katahdin. However, rams of the Santa Inés, Blackbelly and Pelibuey breeds had best semen characteristics. The breed Colombian Criollo de Pelo (OPC) had the smallest scrotal circumference; likewise, together with OPC, Saint Croix rams had the lowest sperm concentrations. Sperm abnormalities were higher in Criollo de Pelo rams from Colombia and Brazilian Somali breed (Table 2).

### Age

As noted above, the use of rams as brood stock in herds occurs at increasingly younger ages; however, those young animals do not have the phenotype and semen characteristics of an adult ram. In general, young rams initiate puberty at six or seven months with a body weight ranging from 25 to 35 kg (Aké-López *et al.*, 2017). Table 3 shows the scrotal circumference and semen characteristics of young and adult hair rams, as reported in the literature.

**Table 1.** Age at puberty, body weight, scrotal circumference, and sperm abnormalities in hair lambs.

Breed	Place	Age (days)	BW (kg)	SC (cm)	C ( $\times 10^6 \text{ mL}^{-1}$ )	SA (%)	Reference
PB	Edo. México, Mexico	143.8 $\pm$ 2.2	33 $\pm$ 3.6	25.7 $\pm$ 1.6	50	18.8 $\pm$ 14.8	Valencia-Méndez <i>et al.</i> (2005).
SI	Espíritu Santo, Brazil	210.8 $\pm$ 50.8	36.3 $\pm$ 9.2	25.2 $\pm$ 3	281.3 $\pm$ 230.3	49.9 $\pm$ 16.5	Alves <i>et al.</i> 2006).
STC	Nuevo León, Mexico	180	34.7 $\pm$ 0.5	27.3 $\pm$ 0.3	126 $\pm$ 200	-	Ledezma-Torres <i>et al.</i> (2022).
OPC	Orinoquia, Colombia	180	15	12	200 $\pm$ 180	18.4	Chacón <i>et al.</i> (2019).
WA	Zulia, Venezuela	330 $\pm$ 0.5	24.2 $\pm$ 0.7	24 $\pm$ 1.4	422.5 $\pm$ 165.2	73.3 $\pm$ 12.1	Rodríguez-Urbina <i>et al.</i> (2001).

BW: Body weight; SC: scrotal circumference; C: sperm concentration; SA: sperm abnormalities; Breed: PB, Pelibuey; SI, Santa Inés; STC, Saint Croix; OPC, Ovino de Pelo Colombiano; WA, West African.



**Table 2.** Average (SE) of scrotal circumference and semen characteristics in adult hair rams of distinct breeds.

Breed	Place	SC (cm)	VOL (mL)	MM (1-5)	IM (%)	C ( $\times 10^6$ mL <sup>-1</sup> )	SA (%)	Reference
PB	Yucatán, Mexico	31.4 $\pm$ 0.2	0.5 $\pm$ 0.0	4.5 $\pm$ 0.1	86.2 $\pm$ 1.5	2963.8 $\pm$ 103	5.6 $\pm$ 1.3	Aguirre <i>et al.</i> (2007); Cárdenas-Gallegos <i>et al.</i> (2012).
SI	Northeast Brazil	34.2 $\pm$ 2.1	0.9 $\pm$ 0.6	4.0 $\pm$ 0.7	77.0 $\pm$ 26.4	3018 $\pm$ 1405	19.4 $\pm$ 9.5	Maia <i>et al.</i> (2011).
STC	Nuevo León, Mexico	32.3 $\pm$ 0.2	1.1 $\pm$ 0.0	2.6 $\pm$ 0.0	52.9	187.5 $\pm$ 8.8	2.1	Santos <i>et al.</i> (2015); Sánchez-Dávila <i>et al.</i> (2019); Sanchez-Davila <i>et al.</i> (2020).
OPC	Sampués, Colombia	27.0	1.2 $\pm$ 0.1	3.7 $\pm$ 0.1	74.0 $\pm$ 2.0	711.8 $\pm$ 133	23.3 $\pm$ 1.9	Carrillo-González y Hernández, (2016).
WA	Zulia, Venezuela	32.1	-	3.9	90.0	3436	5.1	de Cambellas, (1993).
BY	Yucatán, Mexico	32.6 $\pm$ 0.2	0.5 $\pm$ 0.0	4.5 $\pm$ 0.1	84.7 $\pm$ 1.7	3058.7 $\pm$ 114	4.7 $\pm$ 1.4	Cárdenas-Gallegos <i>et al.</i> (2012).
DR	Yucatán, Mexico	34.6 $\pm$ 0.2	0.6 $\pm$ 0.0	4.4 $\pm$ 0.1	83.4 $\pm$ 1.5	2960 $\pm$ 102	12.4 $\pm$ 1.3	Cárdenas-Gallegos <i>et al.</i> (2012).
DR	Northeast Brazil	34.5 $\pm$ 1.2	1.1 $\pm$ 0.2	4.0 $\pm$ 1.4	72.0 $\pm$ 26.8	2250 $\pm$ 426.4	16.6 $\pm$ 7.8	Maia <i>et al.</i> (2011).
KN	Yucatán, Mexico	35.0 $\pm$ 0.2	0.6 $\pm$ 0.0	4.1 $\pm$ 0.1	79.9 $\pm$ 1.6	2572 $\pm$ 105	107 $\pm$ 1.3	Cárdenas-Gallegos <i>et al.</i> (2012).
KN	Orinoquia, Colombia	27.6 $\pm$ 0.2	0.7 $\pm$ 0.1	-	80 $\pm$ 10	1510 $\pm$ 175	-	Chacón <i>et al.</i> (2019).
MN	Caerá, Brazil	-	0.2	1.8	50	520	3.5	Mendes Silva <i>et al.</i> (2010).
SB	Caerá, Brazil	-	0.4	1.9	70	1570	18.0	Silva y Nunes, (1987).

SE: standard error; SC: scrotal circumference; VOL: volume; MM: mass motility; IM: individual motility; C: sperm concentration; SA: sperm abnormalities. Breed: PB: Pelibuey; SI: Santa Inés; STC: Saint Croix; OPC: Ovino de Pelo Colombiano; WA: West African; BY: Blackbelly; DR: Dorper; KN: Katahdin; MN: Morada Nova; SB: Brazilian Somali.

In general terms, the young rams of Pelibuey, Santa Inés, Criollo de Pelo Colombiano and West African have an average scrotal circumference 6.1 cm smaller than adults of the same breed. Within breeds, scrotal circumference in young Pelibuey rams was 2.5 cm smaller than in adults. Meanwhile, in the young rams of Santa Inés, West African and Criollo de Pelo Colombiano, the respective differences in scrotal circumference were 9.0, 8.1 and 4.7 cm smaller in young than adults. The ejaculate volume was similar in all breeds, with no distinction between young and adult rams.

In terms of mass and individual motility between and within breeds, young rams performed better than adults; however, sperm concentration was higher in adults compared to young rams (1435.16 vs. 2193.2 $\times 10^6$  mL<sup>-1</sup>). Finally, young West African and Santa Inés rams had the highest percentage of sperm abnormalities.

**Table 3.** Scrotal circumference and semen characteristics in hair lambs (young) and rams (adult).

Breed	Age	Place	SC (cm)	VOL (mL)	MM (1-5)	IM (%)	C ( $\times 10^6$ mL <sup>-1</sup> )	SA (%)	Reference
PB	Y	Yucatán, Mexico	29.4±0.8	0.63±0.3	4.7±0.1	88±0.8	2535±60.7	3.8±0.9	Aké-López <i>et al.</i> (2016).
	A		31.9±0.8	0.48±0.3	4.4±0.1	84.8±0.8	2849.8±60.7	6.1±0.9	
SI	Y	Northeast Brazil	25.2±3	0.5±0.2	4.2±0.9	76.5±10.3	1340.5±269.6	49.9±16.5	Pacheco <i>et al.</i> (2009).
	A		34.2±2.1	0.9±0.6	4.0±0.7	77±26.4	3018.0±1405	19.4±9.5	Maia <i>et al.</i> (2011).
OCP	Y	Orinoquia, Colombia	22.3	0.5±0.1	-	80±10	430±35	-	Chacón <i>et al.</i> (2019)
	A	Sampués, Colombia	27.0	1.4±0.1	3.7±0.1	74.0±2.0	711.8±133	23.3±1.9	Carrillo-González y Hernández (2016).
WA	Y	Zulia, Venezuela	24.0±1.4	0.4±0.2	1.6±0.2	89.9±8.1	422.5±165.2	73.3±12.1	Rodríguez-Urbina <i>et al.</i> (2001).
	A		32.1	-	-	90.0	3436	5.1	de Cambellas, (1993).

SC: scrotal circumference; VOL: volume; MM: mass motility; IM: individual motility; C: sperm concentration; SA: sperm abnormalities. Breed: PB, Pelibuey; SI, Santa Inés; OPC, Ovino de Pelo Colombiano; WA, West African; Y: young (lambs), 9.0±0.3 months; A: adult (rams), 34.0±6.9 months.

### Seasonal condition

The reproductive activity of sheep breeds in regions near the equator or tropical regions is strongly influenced by temperature, rainfall and availability of forage for food (Scaramuzzi *et al.*, 2006). Table 4 shows semen characteristics of hair rams in different seasons due to humidity conditions, in two studies established in 2012 and 2014.

For the breeds evaluated in Mexico, scrotal circumference was similar in the seasons (Table 4). In the ejaculate volume, the Santa Inés breed (Brazil) had an average of 0.22 mL more than Pelibuey rams (Mexico). Regarding mass and individual motility, Pelibuey rams had better characteristics than Santa Inés rams. In addition, in Pelibuey rams MM and MI were higher in the dry season, the opposite was true in Santa Inés rams, since these

**Table 4.** Seasonal effect on scrotal circumference and semen characteristics of hair rams in tropical regions.

Season <sup>†</sup>	Place	SC (cm)	VOL (mL)	MM (1-5)	IM (%)	C ( $\times 10^6$ mL <sup>-1</sup> )	SA (%)	Reference
Dry	Yucatán, Mexico	33.6±0.2	0.6±0.0	4.5±0.0	85.0±1.4	3295.1±93.9	8.0±1.2	Cárdenas-Gallegos <i>et al.</i> (2012)
Dry	Teresina, Brazil	-	0.7±0.1	3.1±0.3	74.2±4.5	2520±30	29.6±2.6	Frazaõ-Sobrinho <i>et al.</i> (2014)
Humid	Yucatán, Mexico	33.1±0.2	0.5±0.0	4.4±0.0	84.2±1.4	2615.4±90.7	10.1±1.1	Cárdenas-Gallegos <i>et al.</i> (2012)
Humid	Teresina, Brazil	-	0.8±0.1	3.3±0.5	77.4±3.6	2110±40	21.4±3.5	Frazaõ-Sobrinho <i>et al.</i> (2014)

<sup>†</sup> Warm climate, in dry or humid condition. In Mexico, the dry season is observed from spring to early summer, while the humid season occurs from mid-summer to early autumn. On the other hand, in Brazil, the humid season spans from January to April, while the dry season is from August to November. SC: scrotal circumference; VOL: volume; MM: mass motility; IM: individual motility; C: sperm concentration; SA: sperm abnormalities.

variables were higher in the rainy season. Sperm concentration was higher in Pelibuey rams compared to Santa Inés rams in both seasons. Regarding the relationship with the weather condition, in the hot and dry season the sperm concentration was higher than in the rainy season, and this occurred in the two ram breeds (Cárdenas-Gallegos *et al.*, 2012; Frazão-Sobrinho *et al.*, 2014).

On the other hand, sperm abnormalities were higher in Santa Inés (Brazil) than in Pelibuey (Mexico), 25.5 vs. 9.05% in both seasons. In addition, Pelibuey rams had fewer sperm abnormalities in the dry condition than Santa Inés rams; the opposite happened in the rainy season (humid condition). Overall information shown in Table 4 suggests that rams of the two sheep breeds have a good reproductive capacity in the two seasons of the year defined by humidity (Cárdenas-Gallegos *et al.*, 2015).

## CONCLUSIONS

Nutrition affects sperm and testosterone production. In turn, libido is strongly influenced by the presence of testosterone, diseases, length of the day and weather conditions. The onset of puberty in hair rams occurs between 143 and 210 days of age, weight of 15 to 36 kg, scrotal circumference of 12 to 27 cm and with sperm concentrations between 50 and  $281 \times 10^6 \text{ mL}^{-1}$ . Due to the magnitude of variation between breeds, reports were found of rams with scrotal circumferences of 27-35 cm; ejaculate volume (0.2-1.2 mL), mass motility (1.8-4.5); individual motility (52.9-90.0%), sperm concentration of (187.5 to  $3436 \times 10^6 \text{ mL}^{-1}$ ), and sperm abnormalities (2.1-23.2%). With documented evidence that semen quality is lower in young rams compared to adults.

When reproductive and sperm traits were cross-referenced with seasonality, records were found indicating that in the dry season the reported average characteristics are 33.6 cm of scrotal circumference; 0.7 mL of ejaculate volume; 3.8 of mass motility, 79.6% of individual motility; and sperm concentration of  $2907 \times 10^6 \text{ mL}^{-1}$ . While, in the humid season, the average values in the literature are 33.1 cm of scrotal circumference; no changes in ejaculate volume, mass motility, or individual motility; and lower average sperm concentration ( $2362 \times 10^6 \text{ mL}^{-1}$ ).

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# Current perspectives on Long-COVID: a brief review of understanding and management

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## ABSTRACT

**Objective:** Conduct a retrospective analysis of studies compiled in the literature on the current classification of Long-COVID.

**Design/methodology/approach:** A search was conducted in medical information platforms using the keywords: COVID-19, Long COVID, COVID.19 sequelae, SARS-CoV 2, prolonged COVID. Articles published in the chronological period between 2020 and 2023 in both English and Spanish were used. Inclusion criteria were a maximum of five years since publication, review articles, systematic reviews, meta-analyses or clinical practice guidelines. The exclusion criteria were that the articles exceeded the time limit, that they were opinion articles, case reports or trials.

**Results:** There is no homogenized definition or standardized guidelines or norms for the diagnosis of Long-COVID, the epidemiological studies are not completely corroborated and there is a great disparity between the incidence rates estimated in the different research works, same case for treatment.

**Limitations on study/implications:** The lack of homogeneity in the patterns of symptoms, classification and diagnosis by the literature and health officials.

**Findings/conclusions:** A multidisciplinary approach is required, where clinical findings, laboratory and imaging studies are integrated, to homogenize information in search of adequate and timely Long-COVID diagnoses and effective treatments for the benefit of patients.

**Keywords:** SARS-CoV-2, COVID-19, Long-COVID, Viral infection, Sequelae.

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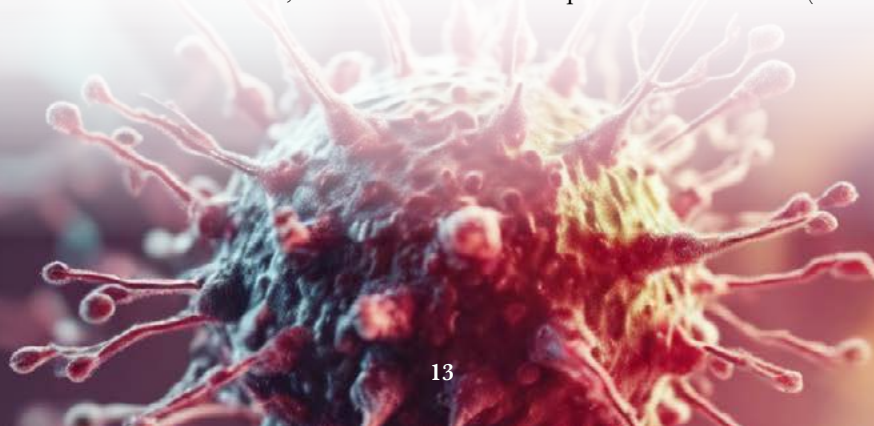
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## INTRODUCTION

Coronavirus disease (COVID-19), caused by the SARS-CoV-2 virus, which emerged in Wuhan, China, has its beginning during December 2019, at the time when cases of pneumonia of unknown cause were detected (Acosta-Morales *et al.*, 2022), which represented the beginning of one of the greatest health challenges of recent times worldwide, as it was declared a pandemic in 2020 (Siddiquea *et al.*, 2021). At



the beginning it was considered as an exclusively respiratory disease, which presented as an influenza picture with alteration of the lower respiratory tract (Gil *et al.*, 2021), however, during the course of this health emergency countless advances in research were made, determining that it is a systemic disease (Anka *et al.*, 2021); where the main extrapulmonary systems affected are the neurological, with symptoms such as anosmia and ageusia, the cardiovascular, with manifestations such as endocarditis and arrhythmias, and the gastrointestinal, with symptoms such as nausea and anorexia (Vázquez *et al.*, 2022).

Today, SARS-CoV-2 disease is recognized as an established and persistent public health problem and no longer as a health emergency (PAHO, 2023), however, it is important to highlight that, after acute infection, a large spectrum of symptoms considered as sequelae of the disease has been reported, the duration of which extends from weeks to months (Raveendran *et al.*, 2021). Some of these symptoms are fatigue, mental fog, myalgia, headache, among others (Ceban *et al.*, 2022; Carfi *et al.*, 2020). The above, takes clinical relevance due to the large number of individuals affected by COVID-19 worldwide, with a total of 774,075,242 reported cases, according to the most current data from the World Health Organization (WHO) (WHO, 2024), where many of these individuals have experienced long-term sequelae associated with SARS-CoV2 infection (Crook *et al.*, 2021). Because of the above, there is still no consensus on appropriate definitions for patient-coined symptomatology referring to the pathological entity whereby persistent symptoms occur in a significant proportion of those who have had COVID-19, whether asymptomatic, mild or severe, persisting beyond the acute phase of infection (Aiyegbusi *et al.*, 2021) or also known as Long-COVID (Long-COVID) (Gonzalez-Hermosillo *et al.*, 2021; Lai *et al.*, 2023).

Because of this lack of standardization in addressing symptoms persisting after acute COVID-19 infection, the aim of the present study focuses on current thinking about Long-COVID, taking into account issues such as definition, epidemiology, clinical manifestations, diagnosis, and treatment.

## **MATERIALS AND METHODS**

A search was performed in the following medical information platforms: Pubmed, Medscape and Google Scholar, where the keywords: COVID-19, Long COVID, COVID.19 sequelae, SARS-CoV 2, prolonged COVID were used and search filters were applied to fit the corresponding inclusion criteria.

Articles published in the chronological period corresponding to the years 2020 to 2023 in both English and Spanish were used; the search for these articles was carried out during the months of December 2023 to February 2024. Different inclusion criteria were used to be considered part of the bibliography, among them are: that they were at least five years old since their publication, that they were review articles, systematic reviews, meta-analyses or clinical practice guidelines. The exclusion criteria were that the articles exceeded the time limit of five years since publication, that they were opinion articles, case reports or trials.



## RESULTS AND DISCUSSION

### Definition

Prolonged COVID (Long-COVID) was the first term referred to by patients to describe the presence of a set of symptoms, over weeks or months after acquiring SARS-CoV-2 infection, without distinction as to whether the symptoms present are new or are the same symptoms that appeared with the acute illness (Raveendran, 2021). Other terms that can be found in the literature in reference to Long-COVID are: Long-COVID-19, acute post-COVID-19, chronic COVID-19, post-COVID fatigue, post-COVID-19 prolonged carrier, post-COVID-19 neurological syndrome, post-acute sequela of SARS-CoV-2 infection, among others (Gonzalez-Hermosillo *et al.*, 2021; Lai *et al.*, 2023; National Institute of Health (NIH), 2023).

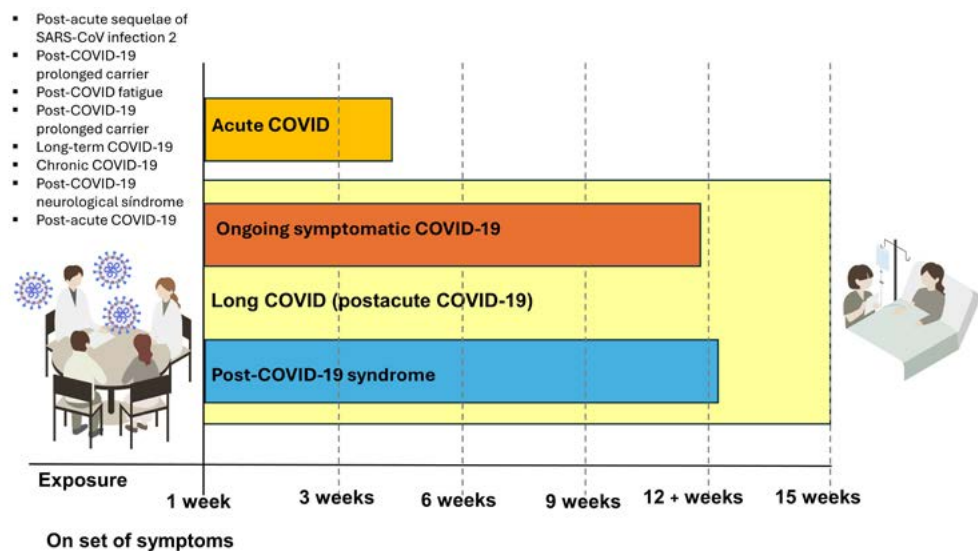
In the same way that there are a large number of terms to try to name this pathology, there are different concepts to try to define it. The WHO defines Long-COVID as a condition characterized by symptoms affecting daily life, such as fatigue, shortness of breath and cognitive dysfunction, which occur after a history of probable or confirmed SARS-CoV-2 infection. Symptoms usually appear three months after the onset of acute COVID-19 symptoms, last at least two months, and cannot be explained by an alternative diagnosis (WHO, 2021). Other studies have concurred in using this three-month time interval in their definitions (Greenhalgh *et al.*, 2020; Chee *et al.*, 2022). Contrary to this, the Center for Disease Control and Prevention (CDC) defines Long-COVID as the development of symptoms that cannot be otherwise explained later than four weeks after acute SARS-CoV-2 infection (CDC, 2023).

Due to the above, establishing a standardized definition has been a matter of debate due to the large number of factors to be considered (Aiyegbusi *et al.*, 2021). Starting with the chronology of the onset of symptoms, which is a relevant point when defining Long-COVID, and as can be noted in the definitions presented above, there is still variation in the range of time that should be considered to speak of this pathology. Also, the set of symptoms that are considered as part of Long-COVID must be defined, as well as the persistence of symptoms due to acute COVID-19 infection. One of the main limitations during the diagnosis of Long-COVID lies in the capacity of viral replication, where its identification is limited to at least three weeks later for its isolation (Terán-Escobar *et al.*, 2023). This becomes relevant in the classification and definition of Long-COVID, since during the clinical assessment it is necessary to clarify whether the symptoms present in the patient are persistent or represent a new symptomatology. In the work of Chent and collaborators, they also emphasize that during the classification of long-COVID it should be categorized as subacute if the symptoms remit in the first 12 weeks, and chronic if they persist beyond that period.

On the other hand, the National Institute for Health and Clinical Excellence of the United Kingdom (NICE), in search of a solution to the problem of the lack of consensus in the definition and terminology of Long-COVID, proposes not to use the terms “chronic” or “persistent” to refer to this entity, because it considers them inadequate. He includes within the term Long-COVID the continuous symptomatic COVID-19 and the post-COVID-19 syndrome. In addition, it suggests using the term acute COVID-19 for signs

and symptoms of COVID-19 for up to four weeks, continuous symptomatic COVID-19 when symptoms last from four to 12 weeks and post-COVID-19 syndrome when they persist for more than 12 weeks in the absence of an alternative diagnosis (NICE, 2021).

NICE’s suggestion to avoid certain terms to refer to Long-COVID represents a promising starting point for standardizing the language used in the scientific literature regarding this condition. In addition, its proposal to classify Long-COVID as “continuous symptomatic COVID-19” when symptoms persist between four and 12 weeks, and as “post-COVID-19 syndrome” when they exceed 12 weeks, bears similarity to the CDC definition, which establishes a period after four weeks of acute SARS-CoV-2 infection. On the other hand, the WHO and several investigators consider a three-month period (equivalent to 12 weeks) in their definitions of Long-COVID (Sreelakshmi *et al.*, 2023; Gaspar *et al.*, 2023). The adoption of these NICE recommendations would contribute to a clearer and more uniform definition of Long-COVID in terms of symptom duration. The advantages of standardizing the definition of Long-COVID are to have precise cut-off points to be able to determine from what time point of persistence in clinical manifestations, a patient can be classified as a probable case of Long-COVID; an ambiguous definition brings with it many repercussions for both health care personnel and patients (Figure 1). On the one hand, there would be a bias in the information to be able to talk about this pathology and difficulties in establishing the diagnosis and on the other hand, patients will remain a long time without knowing their diagnosis, with the psychological and medical consequences that this implies (Chater, 2020; Hassan and Jameel, 2023). The limits and challenges for this approval are the lack of experience in the management of this disease and the increasing information that is being published, with diverse epidemiological data and varied incidence rates (Crook *et al.*, 2021).



**Figure 1.** Temporal classification of different definitions of COVID-19 in week. This figure shows the weeks on the data is based on information provided by NICE. 2021. COVID-19 Rapid Guideline: Managing the long-term effects of COVID-19. NICE Guideline [NG188]. <https://www.nice.org.uk/guidance/ng188> (Retrieved: January 2024).

### **Epidemiology**

The American literature provides estimates suggesting that between seven and 23 million Americans have developed Long-COVID (McCarthy, 2023); worldwide, the estimated number ranges from five (Yong, 2021) to 65 million people facing Long-COVID (Davis *et al.*, 2023). This heterogeneity is probably due to the aforementioned lack of consensus for the definition of this entity, underdiagnosed cases, the presence or absence of verifiable organic damage, as well as the difficulty in establishing whether a patient's current condition derives from COVID-19 or from another entity (Bouza *et al.*, 2021) (Yong *et al.*, 2021).

Worldwide studies have reported various incidence rates of Long-COVID according to different established times of follow-up examinations of patients after presenting with COVID-19. Identifying an incidence rate of 32.6-87% at 60 days after acute SARS-CoV-2 infection (Carfi *et al.*, 2020; Chopra *et al.*, 2021), 96% at 90 days (Davis *et al.*, 2021) and 76% at six months (Huang *et al.*, 2021).

On the other hand, there are findings that relate the severity of acute infection to the probability of developing late symptoms of the disease (Aiyegbusi *et al.*, 2021). In a study conducted in the Netherlands, they estimate that at least 10% of patients with severe SARS-CoV-2 develop Long-COVID (Ballering *et al.*, 2022). According to other research, in the case of people who suffered COVID-19 and did not require hospitalization, they estimate an incidence rate of 10-30% of developing Long-COVID, 50-70% of developing it in patients who required hospitalization for COVID-19 (Bull *et al.*, 2022; Ceban *et al.*, 2022) and 10-20% in patients who received any vaccine for SARS-CoV-2 (Anka *et al.*, 2021; Al-Aly *et al.*, 2022).

Although the findings on Long-COVID are not fully corroborated and there is great disparity between the incidence rates estimated in different research papers, it is clear that a substantial proportion of people who have had COVID-19 will develop this condition (Crook *et al.*, 2021; Katz *et al.*, 2023; Goodridge *et al.*, 2023). This diversity in incidence estimates worldwide reflects the complexity of Long-COVID, being influenced by factors such as lack of consensus on its definition, underdiagnosed cases, and difficulty in determining whether persistent symptoms are attributable to COVID-19 or other disorders. In addition, an association has been observed between the initial severity of acute infection and the likelihood of developing prolonged symptoms. It is crucial to continue research and provide adequate clinical care recognizing the importance of a more complete understanding of Long-COVID starting with the characterization of the disease by identifying its clinical manifestations and thus being able to improve its management and mitigate its impact on public health (Abrignani *et al.*, 2022; Draud *et al.*, 2023).

### **Clinical manifestations**

Based on evidence from various meta-analyses, 60% of survivors of acute COVID-19 infection will develop at least one post-COVID symptom (Fernandez, 2022). The most commonly encountered symptoms have been: dyspnea, cough, fatigue, mental fog, headache, anxiety, palpitations, muscle pain, joint pain, among others (Ceban *et al.*, 2022; Koc *et al.*, 2022). Fatigue is the most commonly reported clinical manifestation at the time

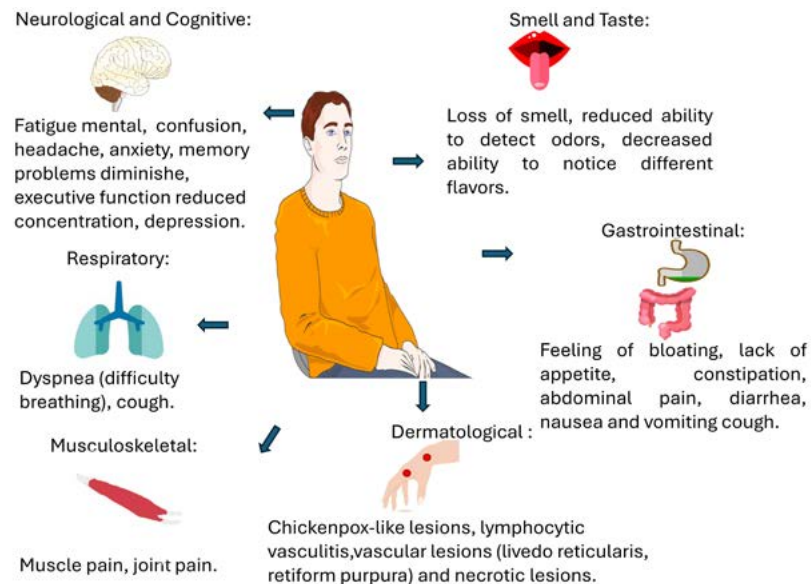
of medical questioning by patients, especially if this was presented as a severe symptom during acute COVID-19 infection (Bouza *et al.*, 2021; Crook *et al.*, 2021; Mansell *et al.*, 2022).

Separating clinical manifestations by apparatus and systems, it was found that patients who were not hospitalized have a higher probability of presenting neuropsychiatric symptoms, such as anxiety and depression, versus patients who were hospitalized during the acute infection (Premraj *et al.*, 2022). Mental fog is one of the main neurological sequelae occurring in patients and is characterized by problems in memory, executive function and concentration (Carfi *et al.*, 2020). Patients are also reported with loss of smell, reduced ability to detect odors and decreased ability to notice different tastes, which has been related to the severity of symptoms and advanced age of the patient (Mansell *et al.*, 2022; Cau *et al.*, 2022).

Within the studies there is little evidence of gastrointestinal complaints (Yang *et al.*, 2022) reported that 15.3% of patients have bloating after each meal and approximately less than 10% may have poor appetite, constipation, abdominal pain, diarrhea, nausea and vomiting (Lai *et al.*, 2023). Regarding the cardiovascular system, several studies show that, while a considerable number of patients with COVID-19 present with myocarditis and elevated serum troponin levels, these symptoms and signs may persist up to 71 days after diagnosis, as well as angina pectoris (Galarza-Vera *et al.*, 2023). Something similar occurs with thrombosis and its complications, which are pathologies reported upon coronavirus infection, since in subsequent controls of these patients after the acute infection, they present dyspnea and chest pain, and in most of the occasions they present thrombotic phenomena in the vascular tree and alteration of vascular perfusion in the pulmonary bed (Acosta-Morales *et al.*, 2022).

In addition, it has been found that some patients may present dermatological manifestations, especially in children, lesions similar to chickenpox, morbilliform exanthema secondary to lymphocytic vasculitis, and vascular lesions such as livedo reticularis, retiform purpura and necrotic lesions (Gutierrez-Bautista *et al.*, 2021).

The range of clinical manifestations evoked by Long-COVID is, moreover, wide (Figure 2). This is a reflection of the great virulence of this coronavirus, although at the beginning of the pandemic it was considered an exclusive disease of the respiratory system, today the metabolic, cardiovascular, neurological and many other complications have shown that it is not a respiratory virus but a microorganism whose virulence is sufficient to cause a change in the human system that lasts over time, even when there are no viral copies in circulation (Gang *et al.*, 2022; Craddock *et al.*, 2022). Long-COVID unfortunately has no single pattern from which it can be suspected, its presentation is varied in both diversity and severity, however, as reviewed, fatigue, as well as mental fog, headache, anxiety, palpitations, muscle pain, joint pain can be strong indicators of its presence. It is emphasized that Long-COVID can enter the list of differential diagnoses with many other pathologies (Turner *et al.*, 2023), the history of SARS-CoV-2 infection and the ruling out of other diagnostic possibilities play a very important role in the characterization of the clinical picture in the diagnostic process.



**Figure 2.** Symptoms and effects associated with a health condition categorized by body systems. This figure represents a visual classification of various symptoms and effects related to a health condition. Symptoms are grouped into specific categories: neurological and cognitive, respiratory, musculoskeletal, gastrointestinal, dermatological, smell and taste.

## Diagnosis

Given that the clinical manifestations are broad and involve different devices and systems, there is no single study that establishes the diagnosis of Long-COVID. A multidisciplinary approach is required, where clinical findings, laboratory and imaging studies are integrated (Gogineni *et al.*, 2022; Hardy-Werbin *et al.*, 2023). Clinicians face a challenge in diagnosing Long-COVID, as it involves an overall process of evaluating symptoms and ruling out other conditions to reach a conclusion. This task is complicated by the existence of multiple definitions and diagnostic standards (Srikanth *et al.*, 2023). However, the same problem of lack of homogenization of information on Long-COVID arises in relation to diagnostic tools, most of which are still under development. To date, no specific biomarkers have been identified for this condition, resulting in a paucity of specific guidelines or standards for its diagnosis (Davis *et al.*, 2023; Morello *et al.*, 2023).

There are proposals about the laboratory studies that should be incorporated along with the radiological findings according to the clinical data of each patient. For the respiratory system, an evaluation with respiratory function tests, chest X-ray at 12 weeks and a high-resolution computed tomography (CT) scan are suggested to complete the evaluation (Cau *et al.*, 2022). For the cardiovascular system, noninvasive studies such as echocardiogram, CT or magnetic resonance imaging (MRI), assessment of D-dimer levels, C-reactive protein, doppler ultrasound to rule out thrombotic lower limb vasculopathy, and angiotomography in patients deemed necessary are required (Raman *et al.*, 2022). NICE guidelines suggest screening for anxiety and depression, and other neurological symptoms, as well as considering a CT, MRI or electroencephalogram study to assess nervous system function (Venkatesan, 2021; Premraj *et al.*, 2022).

The wide variety of diagnostic tools proposed to evaluate each body system reflects, firstly, the great capacity of SARS-CoV-2 infection to generate morbidity in the population (Jaros *et al.*, 2023). Secondly, it demonstrates the complexity of the diagnostic approach to a patient with a history of this infection (Davis *et al.*, 2023; Morello *et al.*, 2023). In a patient who has had confirmation of COVID-19 and who four weeks or more after the acute illness reports fatigue or persistence of neurological, respiratory, cardiovascular or digestive symptoms, in the absence of other causal pathology, Long-COVID can be considered (NICE, 2021), using laboratory or laboratory tests for cases in which their use is warranted. The above is secondary to the scarcity of a diagnostic algorithm stipulated by consensus (Platz *et al.*, 2023), undoubtedly this represents a great challenge for the medical community, since the lack of a clinical practice guideline for this disease, which day by day has better characterization, causes a problem for health care measures, leaving a large proportion of cases undiagnosed (Gray *et al.*, 2023) and, consequently, causing an underreporting in the number of real patients to be able to establish an adequate prevalence and incidences on the one hand, and on the other hand, leaving a part of the population without adequate treatment.

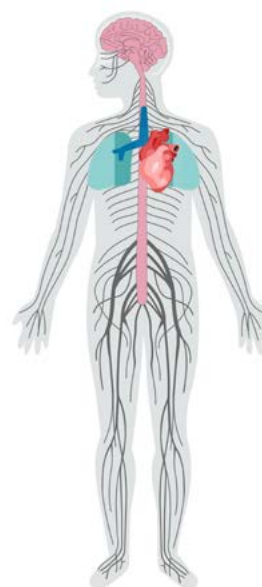
### Treatment

Approaching treatment in a general way, patients may require multidisciplinary care due to the broad spectrum of manifestations involved in Long-COVID, which involves long-term monitoring of the evolution of symptoms to identify possible complications, clinical intervention, and the need for physical exercise, rehabilitation, mental health care, and social service support (Aiyegbusi *et al.*, 2021) (Figure 4).

No specific biomarkers have been identified for this condition, resulting in a paucity of specific guidelines or standards for its diagnosis.

According to the clinical data of each patient:

- Nervous system: CT/MRI/EEG.
- Respiratory system: Chest x-ray, high resolution CT.
- Cardiovascular system: Echocardiogram, CT/MRI, D-dimer levels, C-reactive protein, doppler ultrasound.



**Figure 3.** Diagnostic methods for a condition without specific biomarkers identified. This figure shows the different diagnostic methods used to evaluate various body systems in a condition for which no specific biomarkers have been identified, resulting in the paucity of specific guidelines or standards for its diagnosis. According to the clinical data of each patient.



**Figure 4.** Multidisciplinary care. Comprehensive management of this condition includes long-term monitoring, physical exercise (except in patients with myalgic encephalomyelitis/chronic fatigue syndrome [ME/CFS] where exercise is contraindicated), rehabilitation, and mental health care. Additionally, potential specific treatments are considered.

About possible specific treatments for Long-COVID, one case report observed resolution of Long-COVID after treatment with the antiviral nirmatrelvir/ritonavir (Paxlovid) (Geng *et al.*, 2022), and a study investigating treatment of COVID-19 with Paxlovid showed a 25% reduction in the incidence of Long-COVID (Xie *et al.*, 2022); Paxlovid should be further investigated for the prevention and treatment of Long-COVID (McCarthy, 2023). Naltrexone, an opioid antagonist, at low doses has also shown promise in the treatment of Long-COVID (Pitt *et al.*, 2022). Metformin is under investigation for the general prevention of Long-COVID (Yong *et al.*, 2023).

Due to the lack of evidence that any one drug improves or attenuates the Long-COVID symptom cluster in large-scale, controlled cohort studies (Yong, 2021), most current trials focus on individual Long-COVID symptoms, and medical interventions are aimed at treating these symptoms or isolated organ dysfunction, which may be cardiovascular, respiratory, gastrointestinal, neurological, or psychological (Chee *et al.*, 2023). A small trial of sulodexide, a drug that decreases blood clotting, in people with endothelial dysfunction with which a reduction in symptom severity was observed (Charfeddine *et al.*, 2022). Pilot studies of probiotics indicated potential for alleviating gastrointestinal and non-gastrointestinal symptoms (Thomas *et al.*, 2022; Zhang *et al.*, 2022). Systematic reviews and molecular studies have suggested that histamine-1 and histamine-2 antagonists have broad-spectrum antiviral effects; within these, famotidine, a histamine H<sub>2</sub>-receptor antagonist, has been studied for persistent neuropsychiatric symptoms after acute COVID-19 illness (Alper, 2020; Ishola *et al.*, 2022; Ge *et al.*, 2021).

It has been suggested that drugs used for the management of Long-COVID-like conditions may have the potential to be repurposed for treatment (Yong, 2021). For example, in the case of prolonged fatigue, which is a common manifestation of Long-COVID, due

to the limited availability of specific treatments, the general recommendations made by NICE to manage it are self-care and support (NICE, 2021). However, because myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) has been found to be a condition that can overlap with this prolonged fatigue, the treatment algorithm designed to treat ME/CFS may prove useful in the management of Long-COVID (Crook *et al.*, 2021).

On this line of research of Long-COVID patients with ME/CFS, there are several studies. Exercise has been found to be detrimental for this type of patients (Heerdt *et al.*, 2022) and should not be prescribed as treatment in them (WHO, 2021) (CDC, 2021). Rintatolimod, an experimental drug with immunomodulatory and antiviral properties, appears to have modest to high potential as a treatment option (Yong *et al.*, 2023).

Despite the different lines of research that have been carried out, there are no approved treatments to treat Long-COVID (McCarthy, 2023) due to the great problem it raises in the lack of rigor and homogeneity in its definition, the inclusion/exclusion criteria, the characterization of the pathology and the mechanisms underlying it (Aiyegbusi *et al.*, 2021; Ceban *et al.*, 2022).

## CONCLUSIONS

This review highlights the long-term implication of SARS-CoV-2 infection in the genesis of various clinical manifestations that impact the quality of life of the population that suffered COVID-19. It is still necessary to expand research on this entity, as well as to systematize and deepen the information currently available. In the absence of a standardized definition, epidemiological studies are not completely corroborated and there is a great disparity between the incidence rates estimated in the different research studies, evidencing the clear underreporting of patients who have been affected by this long-term complication of COVID-19. The same obstacle arises when establishing diagnostic criteria, where no specific guidelines or standards for diagnosis have been determined. A multidisciplinary approach is required, integrating clinical findings, laboratory and imaging studies. This interdisciplinary collaboration will broaden the understanding and allow the homogenization of information in the search for adequate and timely diagnosis of Long-COVID and effective treatments for the benefit of patients.

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# Lithium chloride in seed germination and initial growth of Guajillo chili seedlings

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## ABSTRACT

**Objective:** To evaluate the effects of lithium (Li) supplied as lithium chloride (LiCl) on the emergence of seeds and initial growth of chili (*Capsicum annuum* L.) var. Guajillo seedlings.

**Design/methodology/approach:** Guajillo variety chili seeds were treated with five doses of Li chloride (0, 20, 50, 75, and 100  $\mu\text{M}$ ) during the germination phase. The treatments lasted 20 days. In this period, parameters related to seed germination and initial growth of seedlings were evaluated. With the data obtained, analysis of variance and comparison of means test (Duncan) were carried out with the SAS software.

**Results:** The doses of LiCl evaluated did not affect the percentages of germination and relative germination, the coefficient of velocity of germination, the average velocity of germination, and the weights of fresh and dry seedling biomass. Doses of 25 and 50  $\mu\text{M}$  LiCl favored the germination index and the seed vigor index. Likewise, they significantly increased the height of the stem. On the contrary, the 100  $\mu\text{M}$  Li dose significantly reduced the relative radicle growth, the germination index and the stem height.

**Limitations on study/implications:** This study used only a single Li source, so the effects that accompanying anions have, are unknown.

**Findings/conclusions:** Low doses of LiCl have positive effects on shoot growth in the initial phase of seedling growth, without affecting germination parameters.

**Keywords:** Non-essential elements, toxicity, stimulation, growth promotion.

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## INTRODUCTION

Lithium (Li) is an alkaline element of group IA in the periodic table. It is the most electropositive ( $-3.04$  V), the lightest ( $6.94$  g mol<sup>-1</sup>), and the least dense ( $\rho=0.53$  g cm<sup>-3</sup>) metal that exists. These specific properties place it in a strategic position in the world economy (Tarascon, 2010).

Lithium is widely distributed on the planet; its abundance in the lithosphere is 20 mg kg<sup>-1</sup>, in soils from 7 to 200 mg kg<sup>-1</sup>, in surface water from 1-10 g L<sup>-1</sup>, in seawater of 0.18 g L<sup>-1</sup>, and in mineral waters the levels can reach 100 g Li L<sup>-1</sup> (Pais & Jones, 1997; Tanveer *et al.*, 2019).

In higher plants, Li is not an essential element, while its content depends on the levels



Image by Michael Myers at Pixabay

present in the soil, generally trace amounts that vary from 0.002 to 63 mg kg<sup>-1</sup> (Aral *et al.*, 2011; Shahzad *et al.*, 2016; Baran, 2019). Due to its chemical similarity with sodium (Na<sup>+</sup>) and potassium (K<sup>+</sup>), the Li<sup>+</sup> ion is considered an antagonist for both essential nutrients (Pais & Jones, 1997). Its positive effects on higher plants can occur in very small concentration ranges in which it promotes growth. On the contrary, high concentrations negatively interfere with plant metabolism (Aral *et al.*, 2011; Baran, 2019).

Non-essential metals such as Li are becoming an environmental and public health problem in various regions of the world. Its imminent arrival to agricultural soils through irrigation with contaminated water can cause serious problems in crop production and yield (Tanveer *et al.*, 2019).

Knowledge of the impact of Li is essential in crop production systems. To date, there is still little information about its effects on processes as specific as germination and plant growth. Therefore, the objective of this research was to study the effects of Li supplied as lithium chloride (LiCl), on the emergence of seeds and initial growth of chili (*Capsicum annuum* L.) var. Guajillo seedlings.

## **MATERIALS AND METHODS**

### **Seed disinfestation**

Chili seeds of the Guajillo variety were disinfested in a 2% sodium hypochlorite solution for 15 min, then five rinses were performed with sterile distilled water.

### **Treatments and experimental design**

Five concentrations of LiCl were evaluated: 0, 25, 50, 75, and 100  $\mu$ M (LiCl, CAS-No: 7447-41-8, Sigma Aldrich, St. Louis, MO, USA). Each treatment had three replicates. The experimental unit consisted of a Petri dish with 12 seeds, distributed in a completely randomized design within a germination chamber (Thermo Scientific, model 310M, Waltham, MA, USA) at 32 °C. The seeds were kept hydrated with the Li solutions with different concentrations for 20 days.

### **Evaluated variables**

Every 48 h, the number of germinated seeds was recorded for the variables germination percentage (GP), relative germination percentage (RGP), likewise, from germination, the radicle length (RL) was measured. As soon as the shoot emerged, stem height (SH) was measured every 48 h. With the data obtained, the coefficient of velocity of germination (CVG), average velocity of germination (AVG), relative radicle growth (RRG), germination index (GI), and the seed vigor index (SVI) were estimated. After 20 days of treatment, the fresh and dry weights of the seedlings were evaluated. The aforementioned measurements were carried out in accordance with the methodologies described by Anjum *et al.* (2005) and Buendía-Valverde *et al.* (2018).

### **Data analysis**

An analysis of variance and comparison of means were performed with the data using the Duncan test ( $P \leq 0.05$ ), using the SAS software (SAS, 2011).

## RESULTS AND DISCUSSION

### Germination percentage, relative germination percentage, coefficient of velocity of germination, and average velocity of germination

The highest germination percentages occurred in seeds treated with 25 and 50  $\mu\text{M}$  LiCl (84.4%); however, they were not different from those of the control treatment (77.8%). Likewise, although there are no statistical differences in the relative percentage of germination, it can be seen that doses 25 and 50  $\mu\text{M}$  LiCl increased this variable by more than 8% (Table 1).

The coefficient of velocity of germination indicates the speed of germination. Its value increases when the number of germinated seeds increases and the time for germination is reduced (Talská *et al.*, 2020). In this study, it is observed that treatments with some dose of Li present slightly higher values than the control in this variable; however, with no statistical differences (Table 1).

Average velocity of germination was not influenced by the treatments; however, the lowest value was recorded in the control without Li (Table 1).

During the germination process, it has been reported that Li can have positive or negative effects, because the response depends on the concentration applied (Baran, 2019). In this research, germination parameters were generally negatively affected by the 100  $\mu\text{M}$  LiCl dose. On the contrary, the 25 and 50  $\mu\text{M}$  LiCl treatments stimulate parameters related to germination and radicle and shoot growth.

### Relative radicle growth, germination index, and seed vigor index

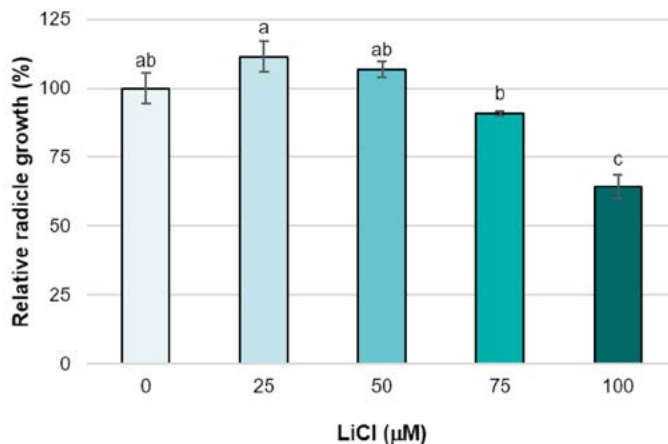
The relative radicle growth was reduced by almost 36% with the 100  $\mu\text{M}$  LiCl treatment compared to the control. Slight, non-significant increases compared to the control were recorded with doses 25 and 50  $\mu\text{M}$  LiCl (Figure 1). Likewise, the germination index decreased by 41.6% with the 100  $\mu\text{M}$  LiCl dose, compared to the control (Figure 2). Regarding the seed vigor index, it was recorded that the 25 and 50  $\mu\text{M}$  LiCl doses exceeded the value obtained with the 100  $\mu\text{M}$  LiCl concentration by 36.1% (Figure 3).

The negative effects of high doses of lithium chloride on germination parameters of Guajillo chili seeds coincide with what was observed in the germination of amaranth (*Amaranthus viridis* L.) seeds treated with 25 to 100 mg  $\text{Li}_2\text{SO}_4 \text{ kg}^{-1}$ . In such study, doses

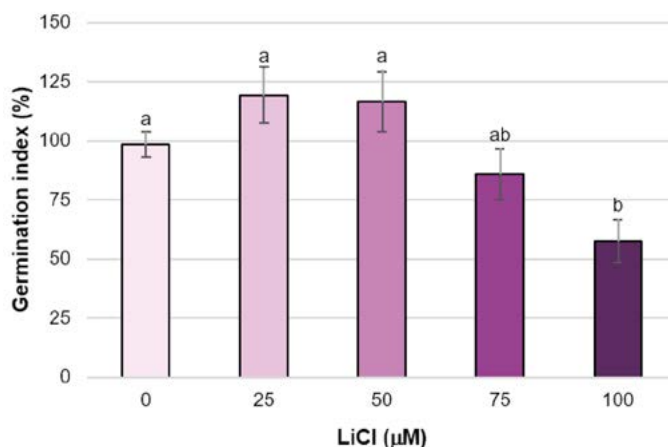
**Table 1.** Germination percentage (GP), relative germination percentage (RGP), Coefficient of velocity of germination (CVG), and average velocity of germination (AVG) of Guajillo chili seeds treated with lithium chloride (LiCl).

LiCl ( $\mu\text{M}$ )	GP	RGP	CVG	AVG
0	77.78 $\pm$ 7.70a	100.00 $\pm$ 9.90a	9.39 $\pm$ 0.90a	0.49 $\pm$ 0.21a
25	84.44 $\pm$ 10.72a	108.57 $\pm$ 13.78a	10.25 $\pm$ 0.20a	0.90 $\pm$ 0.14a
50	84.44 $\pm$ 6.94a	108.57 $\pm$ 8.92a	11.41 $\pm$ 0.40a	0.84 $\pm$ 0.07a
75	73.33 $\pm$ 8.82a	94.29 $\pm$ 11.34a	10.79 $\pm$ 0.69a	0.73 $\pm$ 0.05a
100	68.89 $\pm$ 7.70a	88.57 $\pm$ 9.90a	10.40 $\pm$ 0.41a	0.65 $\pm$ 0.05a

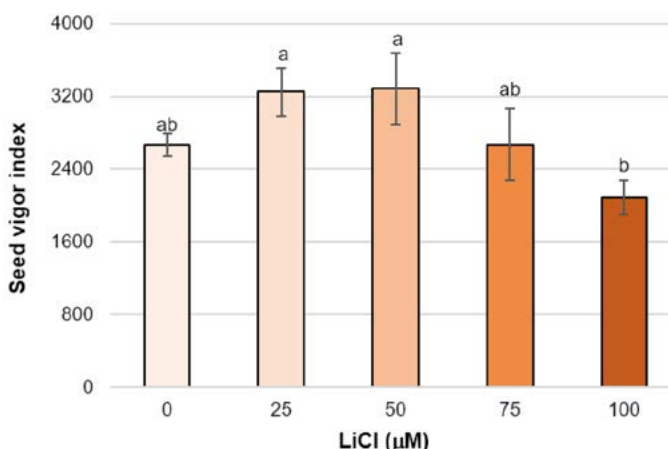
Means  $\pm$  SD with the same letter in each column indicate that there are no statistical differences (Duncan,  $P \leq 0.05$ ).



**Figure 1.** Relative radicle growth of chili seedlings from the treatment of Guajillo chili seeds with lithium chloride (LiCl) during the germination phase. Means  $\pm$  SD with different letters indicate statistical differences (Duncan,  $P \leq 0.05$ ).



**Figure 2.** Germination index in chili seeds treated with lithium chloride (LiCl). Means  $\pm$  SD with different letters indicate statistical differences (Duncan,  $P \leq 0.05$ ).



**Figure 3.** Seed vigor index of Guajillo chili treated with lithium chloride (LiCl). Means  $\pm$  SD with different letters indicate statistical differences (Duncan,  $P \leq 0.05$ ).



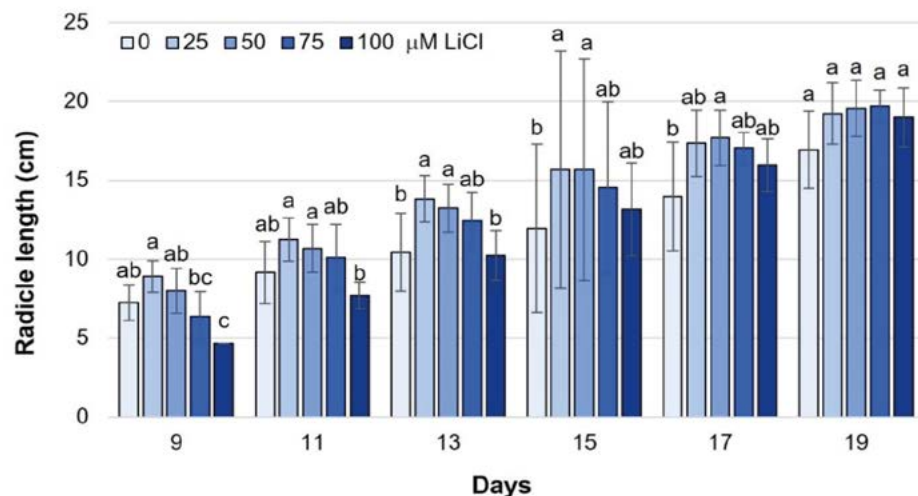
of 50 and 75 mg  $\text{Li}_2\text{SO}_4 \text{ kg}^{-1}$  reduced the germination coefficient rate by 73 and 57%; while, the 75 and 100 mg  $\text{Li}_2\text{SO}_4 \text{ kg}^{-1}$  treatments decreased the germination percentage by 57 and 41%. The germination velocity was reduced with all doses of  $\text{Li}_2\text{SO}_4$ , with the 50 mg  $\text{Li}_2\text{SO}_4 \text{ kg}^{-1}$  concentration being the one that reduced it the most compared to the control (Gayathri *et al.*, 2022). Li *et al.* (2009) observed a significant reduction in the germination rate of Abyssinian mustard (*Brassica carinata*) seeds after exposure to LiCl in a concentration range of 60-180  $\mu\text{M}$  LiCl.

### Radicle length and shoot height

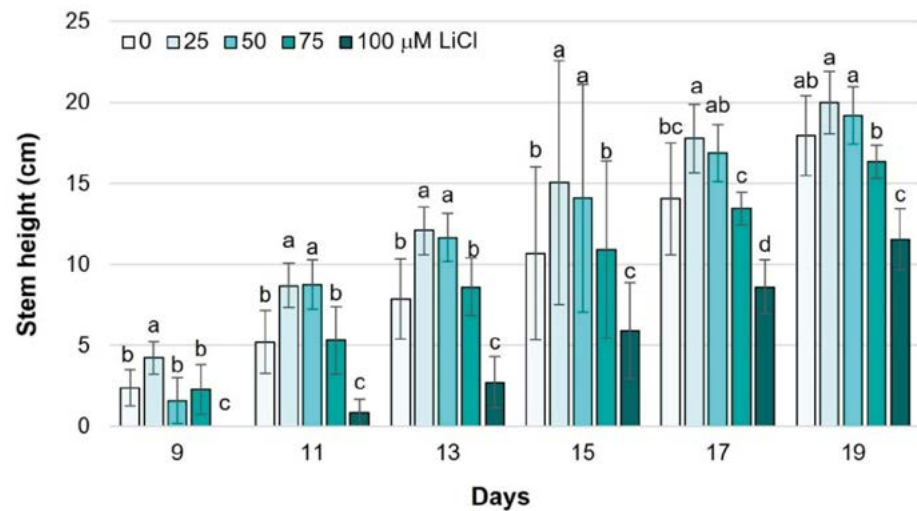
After 13, 15, and 17 days from the start of the test, it was observed that the length of the radicle increased with the 50  $\mu\text{M}$  LiCl dose by 26.7, 31.1, and 26.6%, respectively, compared to the control. In the same way, the 25  $\mu\text{M}$  LiCl dose increased radicle length by 32.3 and 31% after 13 and 15 days, compared to the control. On the contrary, only in the sampling carried out 9 days after the start of the test, did the 100  $\mu\text{M}$  LiCl dose reduce the radicle length by 35.7% with respect to the control (Figure 4).

The stem growth was greater than the control with the 25  $\mu\text{M}$  LiCl dose by 77.1, 67.1, 53.6, 40.8, and 26.4% after 9, 11, 13, 15, and 17 d from the start of the test. Likewise, the 50  $\mu\text{M}$  LiCl concentration increased the stem height by 68, 48.3, and 31.8% after 11, 13, and 15 days after the start of treatments, with respect to the control. On the contrary, the highest evaluated dose (100  $\mu\text{M}$  LiCl) significantly reduced shoot growth in all evaluations carried out compared to the control. This negative effect decreased as time passed, with values of 100, 83.7, 65.4, 44.7, 38.8, and 35.7% on days 9, 11, 13, 15, 17, and 18, respectively (Figure 5).

In amaranth plants, where treatments with doses ranging from 10 to 100 mg  $\text{Li}_2\text{SO}_4 \text{ kg}^{-1}$  increased root length; on the contrary, those same concentrations decreased stem length when treating the seedlings for 21 days (Gayathri *et al.*, 2022). Kalinowska *et al.* (2013) studied the effects of LiCl and LiOH on the growth of butterhead lettuce (*Lactuca*



**Figure 4.** Radicle length of chili seedlings from seeds treated with lithium chloride (LiCl). Means  $\pm$  SD with different letters indicate statistical differences (Duncan,  $P \leq 0.05$ ).



**Figure 5.** Stem height of Guajillo chili seedlings from seeds treated with lithium chloride (LiCl). Means  $\pm$  SD with different letters indicate statistical differences (Duncan,  $P \leq 0.05$ ).

*sativa* var. *capitata*); their results revealed that the reduction in shoot growth is correlated with increasing concentrations from 20 to 100 mg Li dm<sup>-3</sup> with both Li compounds, with a higher inhibition rate in LiOH than in LiCl. Similarly, previous studies in Abyssinian mustard seedlings demonstrated that 60-120  $\mu$ M treatments reduced root growth after 10 d of LiCl exposure (Li *et al.*, 2009). In soybean (*Glycine max*) plants grown in soil, the application of 25 mg Li kg<sup>-1</sup> for 28 d increased plant height by 10% compared to the control. On the contrary, when applying 100 and 200 mg Li kg<sup>-1</sup> the significant reduction of 5 to 55% in plant height was imminent (Shakoor *et al.*, 2023).

The inhibition of growth at high Li concentrations suggests the increase in the production of reactive oxygen species (ROS) (Iannilli *et al.*, 2024). In soybean plants exposed to the Li<sup>+</sup> ion, an increase in the activity of the enzymes superoxide dismutase (SOD) and catalase (CAT) was observed, which protect structures at the subcellular level and prevent oxidative damage caused by ROS (Shakoor *et al.*, 2023). In spinach (*Spinacia oleracea*) leaves, concentrations of 20 to 80 mg Li kg<sup>-1</sup> were observed to cause lipid peroxidation and significant increases in the production of H<sub>2</sub>O<sub>2</sub>, SOD, CAT, and ascorbate peroxidase (APX) (Bakhat *et al.*, 2020).

### Fresh and dry biomass of seedlings

The LiCl concentrations evaluated did not significantly affect the fresh and dry biomass of Guajillo chili seedlings; however, decreases are observed in both variables with the 100  $\mu$ M LiCl dose (Table 2).

In amaranth seedlings treated for 21 days with Li<sub>2</sub>SO<sub>4</sub>, concentrations of 50 to 100 mg kg<sup>-1</sup> increased the total biomass. The application of 10 to 100 mg kg<sup>-1</sup> inhibited root biomass, and treatments with 10, 50, 75, and 100 mg kg<sup>-1</sup> increased shoot biomass (Gayathri *et al.*, 2022). In maize (*Zea mays*) plants grown in Hoagland nutrient solution, there have been significant increases with doses 16 and 32 mg Li dm<sup>-3</sup> in stem biomass,

**Table 2.** Fresh and dry biomass of Guajillo chili seedlings from seeds treated with lithium chloride (LiCl).

LiCl ( $\mu\text{M}$ )	Fresh seedling biomass (mg)	Dry seedling biomass (mg)
0	34.778 $\pm$ 0.210a	3.556 $\pm$ 0.509a
25	30.667 $\pm$ 1.528a	3.500 $\pm$ 0.220a
50	32.333 $\pm$ 2.028a	3.167 $\pm$ 0.083a
75	33.889 $\pm$ 5.394a	3.278 $\pm$ 0.241a
100	26.444 $\pm$ 3.025a	2.722 $\pm$ 0.268a

Means  $\pm$  SD with the same letter in each column indicate that there are no statistical differences (Duncan,  $P \leq 0.05$ ).

while, with the range of 1 to 32 mg Li dm<sup>-3</sup>, the biomass of leaves, roots, and total increased (Antonkiewicz *et al.*, 2017). Negative effects of Li on biomass have also been reported. In butterhead lettuce, treatment with two sources of Li (LiCl and LiOH) at concentrations of 20, 50, and 100 mg dm<sup>-3</sup> decreased the fresh weight of the shoot. The concentration range of 50 to 100 mg dm<sup>-3</sup> decreased the fresh root biomass; however, the doses of 2.5 and 20 mg dm<sup>-3</sup> significantly increased the fresh root weight, suggesting beneficial effects of Li in lettuce growth at low doses (Kalinowska *et al.*, 2013). Likewise, LiCl caused significant reductions in the fresh weight of Abyssinian mustard seedlings in the concentration range of 30 to 120  $\mu\text{M}$  after exposure for 10 d (Li *et al.*, 2009).

## CONCLUSIONS

Doses of 25 to 50  $\mu\text{M}$  improve germination and seed vigor indices, in addition to promoting stem growth. It is concluded that doses less than 50  $\mu\text{M}$  LiCl are positive in the germination and initial growth of Guajillo chili seedlings.

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# Honey as a micro-bacterial agent: identification method of the compounds that inhibit pathogenic bacteria

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## ABSTRACT

**Objective:** To provide an overview of the scientific evidence that supports the use of *Apis mellifera* honey as an antibacterial agent.

**Design/Methodology/Approach:** An exhaustive review of scientific literature was carried out. The collected information included the different honey types that, according to the reports, have antibacterial properties. In addition, the related compounds, the main chromatographic methods used for their identification, and the main pathogens that have been studied were analyzed.

**Results:** The antibacterial properties of honey (especially monofloral honeys) have been widely studied worldwide, focusing on their capacity to inhibit pathogenic bacteria. The different methods used to study honey include agar diffusion, disk diffusion, and broth and agar dilution. These properties have been attributed to honey, as a result of its high sugar content, low moisture content, and acidic pH, as well as the diversity of the chemical compounds —mainly hydrogen peroxide, methylglyoxal (MGO), phenolic acids, flavonoids, peptides, glycopeptides, and different proteins— that were identified by a chromatographic analysis.

**Study Limitations/Implications:** Currently, the honey of bees (*Apis mellifera*) has great potential as an alternative to combat the antibiotic resistance of certain pathogens.

**Findings/Conclusions:** Honey can inhibit both gram-positive and -negative bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterobacter*. These characteristics are the result of the diverse chemical compounds of honey. In addition, these compounds widely change depending on the vegetation that surrounds the hives; therefore, honey from different geographical origins has unique characteristics, in terms of its composition and antibacterial activity.

**Keywords:** *Apis mellifera*, honey, antibacterial property.

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## INTRODUCTION

Honey is a sweet substance produced by bees, from the flower nectar or exudations of live parts of plants, that is transformed and stored in their honeycomb (Martínez-González and Pérez-López, 2013). This natural product consists of approximately 38% fructose, 31% glucose, 10% of different types of sugar, 18% water, and 3% of other compounds —such as

proteins, lipids, amino acids, phenolic compounds, minerals, vitamins, and carotenoids (Hegazi *et al.*, 2014; Bueno-Costa *et al.*, 2016; Visweswara *et al.*, 2016; Deng *et al.*, 2018; Cheung *et al.*, 2019; Leyva-Jiménez *et al.*, 2019).

Honey is very important worldwide and it has been used to treat skin wounds, burns, sores, eye infections, sore throat, and other issues. The properties of honey are the consequence of its high thickness that protects against infections, its enzymatic production of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), its high osmolarity, and its low pH (3.2-4.5). As a whole, these characteristics inhibit the growth of pathogenic bacteria (Mandal and Mandal, 2011). In addition, honey is consumed as a result of its high nutritional value and its antioxidant, bacteriostatic, and anti-inflammatory properties (Alvarez-Suarez *et al.*, 2014); these biological compounds are passed from the nectar to the honey and can be found in high levels (Güneş *et al.*, 2017).

The inappropriate use of antibiotics to treat bacterial infections has produced different degrees of bacterial resistance and, consequently, the use of these products has been limited. *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enterica* are some of the hospital pathogens that cause difficult-to-treat infections; they have developed an increasing resistance to antibiotics, causing a worldwide concern (Da Silva and Mendonça, 2012; Fyfe *et al.*, 2017).

Consequently, finding new alternatives to improve human health is fundamental. Some of these alternatives include the combination of antibacterial formulations aimed to produce more efficient antibiotics or the search for natural alternatives, such as apitherapy. This last alternative offers honey-based treatments and other honeycomb products that can be used to combat several bacterial infections (Estrada *et al.*, 2005; Mandal and Mandal, 2011; Boussaid *et al.*, 2018). Jenkins and Cooper (2012) have reported that the oxacillin and Manuka honey synergy can be used to treat *S. aureus*, which is resistant to oxacillin. In addition, the Manuka honey and rifampicin (antibiotic) combination can be used to inhibit *S. aureus*, which is resistant to methicillin (MRSA). The antibiotic resistance reversion is a consequence of the reduction of the *mecR1* (MRSA resistance gene) caused by honey (Müller *et al.*, 2013).

Consequently, the objective of this review was to gather scientific evidence about the properties of honey produced by bees (*Apis mellifera*) as an antibacterial agent and to identify the main methods used to establish the secondary metabolites involved in the antibacterial potential.

## MATERIALS AND METHODS

A bibliographic review was carried out using the PubMed, Google Scholar, Scopus, and Science Direct databases, in order to gather information about the main methodologies used to evaluate the antibacterial properties of honeys from different geographical origins. In addition, the main secondary metabolites related with the antibacterial activity of honey and the chromatographic methods used to identify them, during the last twenty-five years, were integrated into the review. The search criteria were limited to English, Portuguese, and Spanish and were based on the scientific publications about antibacterial activity of *Apis mellifera* honey, antibacterial metabolites, and antibacterial properties identification

methods. The Mendeley Reference Manager was used to choose and download only those open access publications that included key information.

## RESULTS AND DISCUSSION

The initial search identified 250 publications in the different abovementioned databases. Once all the publications were analyzed, 140 were excluded, because their content was not related to the objective of this study. Most of the chosen publications were experimental qualitative studies, which identified both international and domestic honey compounds and the equipment used for this purpose.

### Methods and techniques used in the identification of antibacterial compounds

As a result of its many compounds, honey is a complex matrix; however, these compounds can be analyzed using chromatographic techniques. Pyrzynska and Biesayga (2009) tested methods and techniques to obtain higher extraction yields for the different chemical structures and functional groups (aldehydes, ketones, carboxylic acid, esters, alcohols, and flavonoids).

The chromatographic analysis requires a representative sample, which must be subjected to a pre-treatment (extraction) to remove any compound that could interfere in the analysis, such as sugars and polar substances. Highly polar compounds (flavonoids and phenolic acid) should be subjected to a derivatization (chemical changes in the analyte or sample) (Ciulu *et al.*, 2016).

Analytical tools, such as the UV-Vis spectrophotometer, the spectrofluorometer, and ready-to-use H<sub>2</sub>O<sub>2</sub> assay kits has been used to measure the H<sub>2</sub>O<sub>2</sub> level of honey (Sowa *et al.*, 2017).

Chen *et al.* (2012) evaluated the H<sub>2</sub>O<sub>2</sub> content of processed (heat treatment) and unprocessed Australian honeys, measuring the absorbency at 560 nm, and found a 0-1,017  $\mu$ M concentration. The correlation of the antibacterial activity was higher in the unprocessed samples, because the compound is sensitive to the heat treatment. Likewise, Sowa *et al.* (2017) determined the H<sub>2</sub>O<sub>2</sub> content of monofloral clover honeys (Poland), using an absorbency of 540 nm. These samples recorded activity against gram-positive bacteria, with 12.5-25% concentrations.

Poli *et al.* (2018) evaluated the H<sub>2</sub>O<sub>2</sub> concentrations of five honey samples, with and without catalase (an enzyme that catalyzes the decomposition of H<sub>2</sub>O<sub>2</sub> into oxygen and water), and they recorded that honey without catalase obtained 7-8% minimum inhibitory concentration (MIC) values, proving its high antibacterial capacity. Meanwhile, when these authors added catalase to the samples, the values increased by 25%. These results were corroborated by Sindi *et al.* (2019), who recorded a 29.4% increase with honey treated with catalase.

The H<sub>2</sub>O<sub>2</sub> assay kits are very reliable, given their sensibility in presence of peroxidase. The reagent of the kit reacts with H<sub>2</sub>O<sub>2</sub>, creating resorufin (a fluorescent red oxidation product). This reaction has been used to detect it in <100  $\mu$ L volumes. Compared with other, cheaper, and easier-to-use colorimeter methods, the main disadvantage of this product is its high cost (Chen *et al.*, 2012; Sowa *et al.*, 2017).

A high-performance liquid chromatography (HPLC) was used to analyze methylglyoxal (MGO) as the quinoxaline content that remained after the *o*-phenylenediamine derivatization. Atrott and Henle (2009) recorded values that fluctuated between 189 and 835 mg kg<sup>-1</sup>, showing a good methylglyoxal-antibacterial activity correlation (12.4 and 30.9%, respectively). For their part, Sultanbawa *et al.* (2015) used an ortho-phenylenediamine derivatization and recorded a lineal correlation between the MGO (279-1,755 mg kg<sup>-1</sup>) content and the *E. coli* and *S. aureus* bacterial inhibition (8-55 mg kg<sup>-1</sup> to inactivate the bacteria).

Cokcetin *et al.* (2016) used an O-(2,3,4,5,6-Pentafluorobenzyl) hydroxylamine derivatization and obtained 1,100 mg kg<sup>-1</sup>, recording a strong correlation between the concentration of the bacterial activity without peroxide and MGO. These results were corroborated by Rückriemen *et al.* (2017).

Other studies have used different methods to identify the phenolic compounds, including a HPLC with a PhotoDiode Array Detector (PDA) and a UV-Vis spectrophotometer. Additionally, they compare the retention time of the analytes with reference standards. Table 1 shows the analytical methods used to identify honey phenolic compounds in different countries. Escriche *et al.* (2014) used a HPLC-PDA to determine the flavonoids and phenolic acids of citrus, rosemary, and honeydew honeys and they identified the following indicators: hesperetin (citrus honey), pinocembrin (rosemary honey), and myricetin and *p*-coumaric acid (honeydew honey). Güneş *et al.* (2017) found caffeic, protocatechuic, and *p*-hydroxybenzoic acids in honeys from Turkey; while the most abundant compounds of these honeys were pinocembrin, chrysin, and galangina.

Apigenin, 4-hydroxybenzoic acid, isorhamnetin, luteolin, and pinocembrin were found in all honey samples from different regions of Algeria (Ouchemoukh *et al.*, 2017). Meanwhile, gallic acid (phenolic acid) and chrysin (flavonoids) were the major compounds found in commercial honeys from different countries (Cheung *et al.*, 2019). For their part, Elrasheid *et al.* (2017) detected vanillic, chlorogenic, syringic acid, and catechin in honey samples from Sudan. These bioactive compounds produce antibacterial, antioxidant, and anti-inflammatory effects (Leyva-Jiménez *et al.*, 2019; Goslinski *et al.*, 2020; Velásquez *et al.*, 2020).

### **Antibacterial properties of honey**

The antibacterial properties of honey are the result of its high sugar content and its capacity to generate H<sub>2</sub>O<sub>2</sub>, produced by the glucose oxidase enzyme synthesized by bees. This compound destroys the essential components of the cells; however, the inhibitory capacity decreases as temperature rises. This phenomenon shows that the antibacterial potential of honey depends on its composition, the nectar source from which bees feed, the harvesting conditions, the pasteurization process, storage time, and temperature of the storing facilities (Fernandes *et al.*, 2020; Goslinski *et al.*, 2020; Velásquez *et al.*, 2020).

The efficiency of H<sub>2</sub>O<sub>2</sub> as an antibacterial agent has been proved; nevertheless, in some honeys, it is related to low pH (3.2-4.5), cytokine release, and several molecules with immunomodulatory and anti-inflammatory properties (Almasaudi *et al.*, 2017; Leyva-Jiménez *et al.*, 2019; Fernandes *et al.*, 2020). For example, Melaleuca honey has an acid



**Table 1.** Main phenolic compounds of honeys from different geographical origins, detected by chromatography.

Country	Secondary metabolites	Honey	Method chromatographic	Reference
New Zealand and y Australia	Myricetin, carysin and chlorogenic, gallic, caffeic, ferulic, <i>p</i> -coumaric, rosmarinic, ellagic, 3,4-dihydroxybenzoic and abscisic acids.	Manuka <i>Leptospermum scoparium</i>	HPLC-DAD, HPTLC	Stanek y Jasicka-Misiak, 2018
Malaysia	Caffeic, chlorogenic, ferulic, <i>p</i> -coumaric, sinapic, vanillic and syringic acids.	<i>Melaleuca alternifolia</i>	RP-UHPLC-ESI-MS	Goslinski <i>et al.</i> , 2021
Poland	Chlorogenic and ferulic acid.	<i>Fagopyrum esculentum</i> , <i>Calluna vulgaris</i> and <i>Tilia</i>	RP-UHPLC-ESI-MS	Goslinski <i>et al.</i> , 2021
Poland	Myricetin, quercetin, naringenin, chrysin and acids: cinnamic, caffeic, abscisic and ferulic.	<i>Robinia pseudoacacia</i> L.	HPTLC	Stanek <i>et al.</i> , 2019
Bangladesh	Catechin, naringin, myricetin, naringenin, hesperetin, kaempferol, apigenin and acids: gallic, chlorogenic, caffeic, coniferous, benzoic and transcinamic.	<i>Brassica nigra</i> , <i>Nigella sativa</i> , <i>Nelumbo nucifera</i>	HPLC-UV	Moniruzzaman <i>et al.</i> , 2014
Chile	Hymenoptaecin, rutin, naringenin, esculetin, scopoletin, and syringic, <i>p</i> -coumaric, and vanillic acids.	<i>Cryptocarya alba</i> , <i>Quillaja saponaria</i>	HPLC-MS-MS	Montenegro <i>et al.</i> , 2008; Velásquez <i>et al.</i> , 2020
China	Quercetin, apigenin, kaempferol, isorhamnetin, chrysin, galangin and protocatechuic, chlorogenic, caffeic, syringic, <i>p</i> -hydroxybenzoic, <i>p</i> -coumaric, ferulic, isoferulic and benzoic acids.	<i>Fagopyrum esculentum</i>	RP-HPLC-UV	Deng <i>et al.</i> , 2018
Spain	Hesperetin, kaempferol, chrysin, pinocembrin, myricetin, naringenin, quercetin, galangin and caffeic, chlorogenic and <i>p</i> -coumaric acids.	<i>Quercus ilex</i> , <i>Quercus robur</i>	HPLC-PDA,	Escriche <i>et al.</i> , 2014
Portugal	Chrysin, galangin, hesperidin, pinobanksin, luteolin, pinocembrin, kaempferol, apigenin and gallic, caffeic, coumaric and ellagic acids.	<i>Eucalyptus</i> spp., <i>Castanea sativa</i> , <i>Rubus</i> spp., <i>Erica</i> spp.	HPLC-DAD Infinity	Silva <i>et al.</i> , 2020
Saudi Arabia	Apigenin, chrysin, galangin, luteolin, myricetin, naringin and 4-hydroxybenzoic, syringic and gallic acids.	<i>Lavandula dentata</i> , <i>Hypoestes forskoolii</i> , <i>Ziziphus spina-christi</i>	HPLC-PDA	Badjah <i>et al.</i> , 2016
Australia	Apigenin, hesperetin, naringenin neoponcirin, narirutin, biochanin and rosmarinic, chlorogenic and caffeic acids.	<i>Eucalyptus marginata</i> and <i>Corymbia calophylla</i>	HPLC-PDA, HPLC-MS/MS	Tang <i>et al.</i> , 2016; Mani <i>et al.</i> , 2021
Türkiye	Apigenin, pinocembrin, chrysin, galangin, genkwanin and gallic, phydroxybenzoic, caffeic, syringic, salicylic, <i>p</i> -coumaric and transferulic acids.	<i>Castanea sativa</i>	HPLC-DAD	Güneş <i>et al.</i> , 2017
Soudan	Catechin and chlorogenic, syringic, caffeic, <i>p</i> -hydroxybenzoic, vanillic, <i>p</i> -coumaric, ferulic and cinnamic acids.	<i>Acacia nilotica</i> , <i>A. seyal</i> , <i>Z. spina-christi</i> , <i>Amaranthus graecizan</i> , <i>Eucalyptus</i> spp.	FTIR-ATR-PLSR, HPLC-DAD	Elrasheid <i>et al.</i> , 2017
Algeria	Gallic acid, and caffeic acid, chrysin, luteolin, pinocembrin, pinobanksin, myricetin, genistein and daidzein.	<i>Eucalyptus</i> , <i>Ziziphus</i> , <i>Euphorbia</i> , <i>Citrus</i> or <i>Hedysarum</i>	UPLC-PDA-MS/MS	Ouchemoukh <i>et al.</i> , 2017
International commercial honeys	Vanillin, syringaldehyde, protocatechualdehyde and gallic, protocatechual, 2,3,4-trihydroxybenzoic, <i>p</i> -hydroxybenzoic, gentisic, chlorogenic, vanillic, caffeic, syringic, <i>p</i> -coumaric, ferulic, synapic, salicylic acids.	<i>Acacia</i> spp., <i>Tilia</i> spp, <i>Leptospermum scoparium</i> , <i>Eriobotrya japonica</i>	RP-HPLC-DAD Infinity	Cheung <i>et al.</i> , 2019

HPLC=High-Performance Liquid Chromatography; PDA=PhotoDiode Array Detector; UV=UV-Vis spectrophotometer; DAD=Diode Array Detector; FTIR=Fourier Transform Infrared Spectroscopy; HPTLC=High-Performance Thin Layer Chromatography; UPLC=Ultra Performance Liquid Chromatography; Ms/Ms (MS-MS)=Tadem Mass Spectrometry; UHPLC=Ultra-High-Performance Liquid Chromatography; RP=Reverse phase; ESI=Electrospray ionization; ATR=Attenuated total reflectance; PLSR=Partial least squares regression.

pH (3.7), perhaps due to the gluconic acid that causes anti-staphylococcal effects (Wen-Jie and Mei-Siew, 2015; Schencke *et al.*, 2016).

The methylglyoxal (MGO) and defensin-1 content of Manuka (*Leptospermum scoparium*) honey results in a high antibacterial activity against *Streptococcus pyogenes*, *Streptococcus mutans*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *S. aureus* (Irish *et al.*, 2011; Habib *et al.*, 2014; Johnston *et al.*, 2018; Matzen *et al.*, 2018). Consequently, Manuka honey is used as an alternative antibiotic to heal tissue and to reduce microbial infections (Johnston *et al.*, 2018).

Revamil<sup>®</sup>, a medical-grade honey, is produced under standardized conditions in greenhouses. Its potent and reproducible antibacterial activity is the result of its osmotic activity, acid pH, and slow and progressive H<sub>2</sub>O<sub>2</sub> release. In addition, its MGO content creates favorable and optimal conditions to heal wounds (Kwakman and Zaat, 2012; Pharma GDD, 2022).

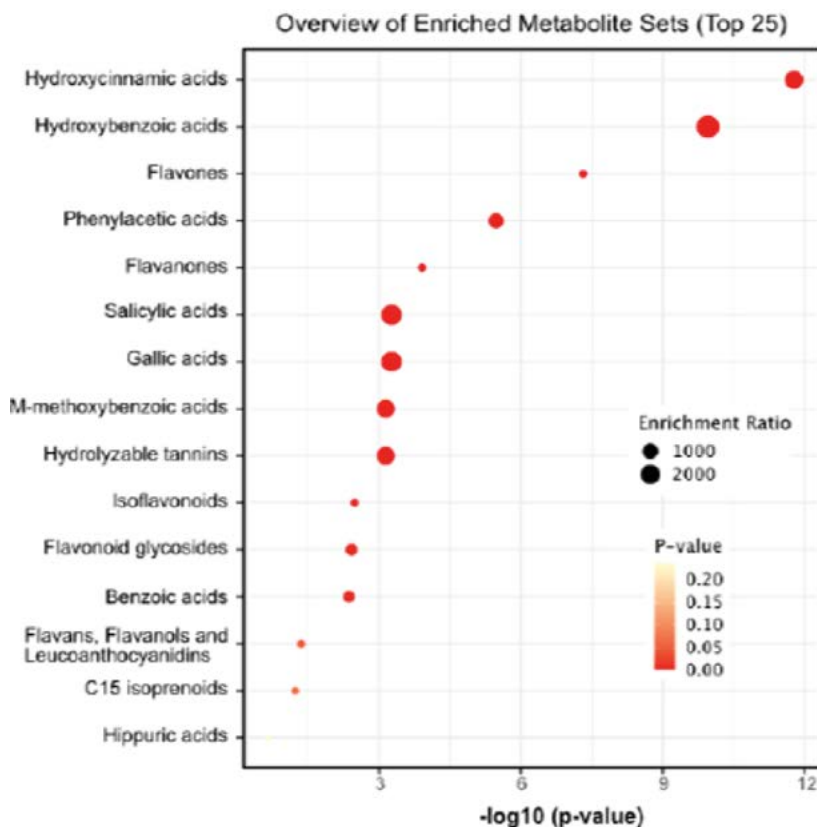
*Leptospermum* honey has high MGO concentrations. Atrott and Henle (2009) and Fernandes *et al.* (2020) have reported up to 800 mg kg<sup>-1</sup> MGO content, which is 100 higher than the concentration found in common honey. MGO concentrations increase as the honey matures. This phenomenon is possibly the consequence of the non-enzymatic conversion of trioses that takes place during long-term storage (up to 120 days, at 37 °C). Meanwhile, its antibacterial effect comes from its capacity to inactivate proteins through cross-linking (Adams *et al.*, 2009).

The physiological wound healing processes can be divided into three phases: inflammatory, proliferative, and remodeling. During the inflammatory phase, honey stimulates the monocytes to release inflammatory cytokines. In the proliferative phase, angiogenesis and fibroplasia take place, along with initial re-epithelialization and contraction of the wound. Finally, during the remodeling phase—when collagen is remodeled and realigned along the tension lines—, apoptosis removes the cells that are no longer needed (Schencke *et al.*, 2016).

Polyphenols are major compounds that have been strongly linked to the antibacterial properties of monofloral honeys. In addition, they can be used to determine the floral and geographic origin of honeys (Iqbal *et al.*, 2015; Albaridi, 2019; Mama *et al.*, 2019; Cebrero *et al.*, 2020) and can be found mainly as flavonoids and phenolic acid and its derivatives (Figure 1) (Cauich *et al.*, 2015; Hossen *et al.*, 2017; Leyva-Jiménez *et al.*, 2019).

The identification and quantification of these compounds are fundamental to understand the bioactivity of honey (Habib *et al.*, 2014). Several authors have proved that darker honeys had high levels of phenolic compounds and, at the same time, high pharmacological potential (Brudzynski and Miotto, 2011; Sant'Ana *et al.*, 2014; Sousa *et al.*, 2016). Although individual phenolic compounds have antibacterial activity, their interaction with H<sub>2</sub>O<sub>2</sub> should be taken into account, because it can promote this effect (Escuredo *et al.*, 2012; Poli *et al.*, 2018).

The high number of peptides help honey bees to protect themselves from diseases that impact the honeycomb. Honey has four antimicrobial peptide (AMP) families: defensins, apidaecins, abaecins, and hymenotaecin.



**Figure 1.** Analysis of the enrichment of secondary metabolites identified to date in honey.

Defensins act against gram-negative bacteria, gram-positive bacteria, and fungi. They are responsible for the destruction of the biofilm formed by the bacteria. Reports indicate that defensins amount to  $0.04\text{--}5.17 \mu\text{g g}^{-1}$  honey (Kwakman and Zaat, 2012; Majtan *et al.*, 2012; Dosler and Karaaslan, 2014; Yi *et al.*, 2014; Valachová *et al.*, 2016). Nevertheless, some studies indicate that some gram-negative bacteria are resistant to defensin-1 (Čeřovský and Bém, 2014). Meanwhile, apidaecin is an antibacterial peptide with a high proline content. Once it enters the bacteria through the lipid bilayer, apidaecin stops the protein expression (Li *et al.*, 2012; Larsen *et al.*, 2019).

Abaecin can be found in western honey bees. It inhibits the growth of both gram-negative and gram-positive bacteria through the permeabilization of the bacteria membrane. Its expression and abundance quickly increase in response to a bacterial infection (Randolt *et al.*, 2008; Danihlík *et al.*, 2015; Larsen *et al.*, 2019). Finally, hymenoptaecin has an abundant glycine content that inhibits the growth of gram-positive and gram-negative bacteria (Erban *et al.*, 2019; Larsen *et al.*, 2019).

### Antibacterial activity of honey

Different protocols have been used to evaluate the antibacterial activity of honey: agar diffusion test, disk diffusion test, broth and agar dilution method, and gradient methods (E-test and spiral plating technique) (Balouiri *et al.*, 2016; Osés *et al.*, 2016). The most used methods to determine the minimum inhibitory concentrations (MIC) required to

stop or eliminate bacterial growth are the disk diffusion, well diffusion, and broth and agar dilution methods. *S. aureus* is usually the bacterium of choice for tests and *in vitro* antibacterial activity, as a consequence of its resistance to high sugar content and acidity levels. In addition, this bacterium is sensitive to the action of H<sub>2</sub>O<sub>2</sub> (Khalil *et al.*, 2014; Osés *et al.*, 2016).

Reproducibility tests are frequently difficult, since the work is carried out with living beings and several methods are used to determine the antibacterial activity of honey (Balouiri *et al.*, 2016; Osés *et al.*, 2016). However, reference material can provide a reliable base for lab work. In addition, reference strains can be used, because mutations can take place or, because depending on the method, the microorganisms can behave differently (Clavijo, 2002; Camaró-Sala *et al.*, 2015; Balouiri *et al.*, 2016; Roshan *et al.*, 2017; Albaridi, 2019; Mama *et al.*, 2019; Rosas *et al.*, 2019).

Chilean honeys, such as the Ulmo (*Eucryphia cordifolia*, Cav.) honey, are drawing attention due to their antibacterial potential. Velásquez *et al.* (2020) evaluated this type of honey using the agar diffusion method to determine their efficiency against *E. coli*, *P. aeruginosa*, and five *S. aureus*-MRSA strains and concluded that their antibacterial activity is mainly the consequence of their phenolic compounds (flavonoids, benzoic acid derivatives, and volatile compounds). Meanwhile, Cebrero *et al.* (2020) used the microdilution technique to evaluate the antibacterial activity of the Peumo (*Cryptocarya alba* (Molina) Looser) and Quillay (*Quillaja saponaria*, Molina) honeys against *S. aureus* and *P. aeruginosa*, pointing out that the antibacterial activity was the result of their hymenoptaecin content.

Roshan *et al.* (2017) analyzed the antibacterial activity in honey from western Australia. The results were compared with the results obtained by Activon, a medical-grade honey made from Manuka honey. The authors proved that six of the samples inhibited the growth of *S. aureus* and recorded larger inhibition areas than medical-grade honey. Meanwhile, Pasiás *et al.* (2018) reported that Greek honeys had high H<sub>2</sub>O<sub>2</sub> and hydroxymethylfurfural (HMF) levels. In addition, their acidity and osmolarity resulted in antibacterial activity against *S. aureus*.

For their part, Leyva-Jiménez *et al.* (2019) used the disk diffusion method to extract phenolic compounds from Iranian honeys, testing them against *E. coli*, *P. aeruginosa*, *S. aureus*, and *Enterococcus faecalis*. The authors concluded that individual and collective phenolic compounds do not have the same potential. These results showed a synergy between the different antibacterial compounds.

Hegazi *et al.* (2017) reported that 20 and 30% concentrations of honeys from Saudi Arabia inhibited *S. aureus*, *S. mutans*, *Klebsiella pneumoniae*, *E. coli*, and *P. aeruginosa* strains, as a result of their flower sources, osmotic properties, pH, H<sub>2</sub>O<sub>2</sub>, flavonoids, and antibacterial volatile substances (*e.g.*, organic acids). Mahendran and Kumarasamy (2015) used the disk diffusion method to evaluate the antibacterial potential of honey collected in Western Ghats, India, during summer and winter, against *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, and *P. mirabilis*. They concluded that the honey samples collected during winter had a higher bactericidal effect than those collected in summer, as a consequence of changes in seasonal flowering.

The eucalyptus honey from the Island of Mauritius is an exceptional case. It showed antibacterial activity against *E. coli*, *P. mirabilis*, *P. aeruginosa*, *Staphylococcus epidermidis* (ATCC 35984), and *S. epidermidis* (ATCC 14990). In addition, Aumeeruddy *et al.* (2019) reported that its phenolic compounds could eliminate cancer cells (MCF-7). Table 2 shows the microorganism inhibited by the different types of honey from several countries.

The processing and storage conditions can impact antibacterial properties of honeys from different geographical and botanical origin against various pathogens, because some of them lose their bactericidal effect under 40-60 °C temperatures (Chen *et al.*, 2012; Pimentel González *et al.*, 2017; Matzen *et al.*, 2018).

### Overview of the antibacterial properties of Mexican honey

Honey plays a very important role worldwide not just as food, but also as a medical-grade product. The five main exporters of honey are China, Argentina, Mexico, Germany, and Brazil (Campos *et al.*, 2018). Beekeeping has a major socioeconomic and ecologic importance in Mexico, because this farming activity generates many jobs (Magaña *et al.*, 2016). Honey is very appreciated in several countries, as a result of its nutritional properties, its perfume, taste, and color; consequently, Mexican honey is currently competing with honey produced in China, Vietnam, Nicaragua, Argentina, Chile, Turkey, and Ukraine (Martínez-González and Pérez-López, 2013; Campos *et al.*, 2018).

In Mexico, researches have included different topics: botanical characterization (Córdova-Córdova *et al.*, 2013), physicochemical properties, volatile compound profiles (Viuda-Martos *et al.*, 2010; Rodríguez *et al.*, 2012), color (Figure 2) (Grajales-Conesa *et al.*, 2018), and antioxidant activity of harvested honey (Leyva-Daniel *et al.*, 2017; Mondragón, 2019). However, few studies have focused on the antibacterial properties of honey and the identification of the main compounds that provide Mexican honey with the said properties (Maddocks *et al.*, 2013).

Ramón-Sierra *et al.* (2020) compared the main phenolic compounds of honey produced by *Melipona beecheii* and *A. mellifera* from Merida, Yucatan. They found that the honey produced by *A. mellifera* had a higher phenolic compound and that 145  $\mu\text{g mL}^{-1}$  (phenolic extracts) and 60  $\mu\text{g mL}^{-1}$  (protein extracts) concentrations recorded antibacterial activities against *E. coli* and *S. aureus*. These results corroborated the findings of Chan-Rodríguez *et al.* (2012).

In addition, other studies have compared Mexican honey with honey from different countries. For example, some studies compare the honey of several honey bees (*Apis*) and the stingless bees from different countries such as Mexico (Jalisco, Chiapas, Quintana Roo, and Yucatan), Paraguay (Asuncion), Australia (Gatton and Brisbane), Philippines (Pili), Thailand (Chantaburi and Bangkok), Japan (Aichi), and Nepal. The evaluations were carried out to determine the effectiveness of those honeys against *Lactococcus lactis* ssp., *L. lactis cremoris* ssp. MAFF 40007, *Lactobacillus casei* JCM 1134, *E. faecalis* IFO 12964, *Enterococcus faecium* IFO 13712, *K. pneumoniae* ssp., and *Staphylococcus* ssp. The honeys from Yucatan and Chiapas (Mexico) recorded promising results in the inhibition of almost all the tested microorganisms, except for *K. pneumoniae* ssp. (Kimoto-Nira and Amano, 2008). Peláez-Acero *et al.* (2021) reported several new techniques to improve honey properties

**Table 2.** Minimum inhibitory concentration (MIC) of honeys from different geographical origins.

Country	floral source	Microorganisms that it inhibits	MIC (%)	Reference
Saudi Arabia	Dharm ( <i>Lavandula dentata</i> L.), Majra ( <i>Hypoestes forskalii</i> (Vahl) R.Br.), Sider ( <i>Ziziphus spina-christi</i> (L.) Willd)	<i>S. aureus</i> , <i>S. mutans</i> , <i>K. pneumoniae</i> , <i>E. coli</i> and <i>P. aeruginosa</i> .	20-30	Hegazi <i>et al.</i> , 2017.
Saudi Arabia	Yemeni Sidr and Mountain	<i>Gram-negative bacteria</i>	40-80	Alqurashi <i>et al.</i> , 2013
Algeria	Multifloral	<i>E. coli</i>	50-70	Bourabah <i>et al.</i> , 2020
Argentina	<i>Rapistrum rugosum</i> (L.) All., <i>Taraxacum officinale</i> F.H. Wigg.	<i>S. flexneri</i> , <i>Salmonella typhi</i> , <i>E. coli</i> .	100	Tejerina <i>et al.</i> , 2020
Australia	Jarraah ( <i>Eucalyptus marginata</i> Donn ex Sm.) y Marri ( <i>Corymbia calophylla</i> (Lindl.) KD Hill & LAS Johnson)	<i>Candida</i> spp. and <i>dermatophytic fungi</i> .	4-32	Irish <i>et al.</i> , 2011.
Chile	Ulmo ( <i>Eucryphia cordifolia</i> Cav.)	<i>P. aeruginosa</i> , <i>S. aureus</i> , <i>E. coli</i> and <i>S.</i> <i>typhimurium</i>	9.4-20	Mullai y Menon, 2007; Sherlock <i>et al.</i> , 2010; Acevedo <i>et al.</i> , 2017; Velásquez <i>et al.</i> , 2020., Olate-Olave <i>et al.</i> , 2021
Denmark	Raspberry ( <i>Rubus idaeus</i> L.), rapeseed ( <i>Brassica napus</i> L.), Linden ( <i>Tilia</i> L.) and Heather ( <i>Calluna vulgaris</i> (L.) Hull)	<i>S. epidermidis</i> and <i>E. coli</i> .		Matzen <i>et al.</i> , 2018.
Ecuador	Multifloral	<i>S. aureus</i>	100	Montero-Recalde <i>et al.</i> , 2018
Egypt	Trefoil ( <i>Trifolium repens</i> L.)	<i>S. typhimurium</i> and <i>E. coli</i> O157: H7	10-20	Mandal y Mandal, 2011.
Ethiopia	Multifloral	<i>S. aureus</i>	70-50	Mama <i>et al.</i> , 2019.
Ethiopia	Tazma mar (melipona), multiflorales	<i>S. aureus</i> , <i>P. aeruginosa</i> and <i>E. coli</i> .	9.38-11.25	Getaneh <i>et al.</i> , 2013.
Greece	Conifers, thyme ( <i>Thymus</i> L.), citrus and multifloral	<i>S. typhimurium</i>	17-24	Voidarou <i>et al.</i> , 2011
India	Multifloral	<i>S. aureus</i> , <i>S. pyogenes</i> , <i>E. coli</i> , <i>P. aeruginosa</i> and <i>P. mirabilis</i>		Mahendran y Kumarasamy, 2015.
Mauritius Island	Eucalyptus ( <i>Eucalypto globulus</i> Labill.)	<i>E. coli</i> ATCC 25922, <i>P. mirabilis</i> ATCC 12453, <i>P. aeruginosa</i> ATCC 27853, <i>S. epidermidis</i> ATCC35984, and <i>S.</i> <i>epidermidis</i> ATCC1499	100	Aumeeruddy <i>et al.</i> , 2019.
Malaysia	Tualang ( <i>Koompassia excelsa</i> (Becc.) Taub.)	<i>E. coli</i> , <i>S. typhi</i> and <i>S. pyogenes</i> .	8.75-25	Mandal y Mandal, 2011.
Mexico	Acaxochitl ( <i>Lobelia laxiflora</i> Kunth), multifloral	<i>B. subtilis</i> , <i>S. aureus</i> , <i>L. monocytogenes</i> , <i>E.</i> <i>coli</i> , <i>S. typhimurium</i> and <i>P. aeruginosa</i> .	55-100	Rodriguez <i>et al.</i> , 2012; Pimentel González <i>et al.</i> , 2017.
New Zealand	Manuka ( <i>Leptospermum scoparium</i> J.R. Forst. and G. Forst.)	<i>S. pyogenes</i> , <i>S. mutans</i> , <i>P. mirabilis</i> , <i>P.</i> <i>aeruginosa</i> , <i>E. cloacae</i> and <i>S. aureus</i> .		Irish <i>et al.</i> , 2011; Johnston <i>et al.</i> , 2018.
Pakistan	Multifloral	<i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. aureus</i> , <i>E. faecalis</i> .	75-100	Mustafa <i>et al.</i> , 2022
Portugal	Multifloral, <i>Leptospermum scoparium</i>	<i>E. coli</i> CECT 434, <i>E. coli</i> EC3a	25	Oliveira <i>et al.</i> , 2017
United Kingdom	Heather ( <i>Calluna vulgaris</i> )	<i>S. aureus</i> , <i>E. coli</i> , <i>E. faecalis</i> and <i>P. vulgaris</i> .	2.5-40	Vučić <i>et al.</i> , 2014.



**Figure 2.** Color range of honey samples from Tabasco, Mexico.

(crystallization). These authors used an ultrasound to study the effect of liquefaction in the phenolic compounds, flavonoids, and antibacterial activity of crystalized honey from three multifloral honeys from Hidalgo. Their antibacterial activities were tested against *S. typhimurium*, *B. subtilis*, *P. aeruginosa*, *L. monocytogenes*, *S. aureus* and *E. coli*. Honeydew honey recorded a higher gallic acid content and some multifloral samples increased their quercetin content; meanwhile, all the samples inhibited the tested microorganisms. In particular, the antibacterial activity against *S. typhimurium* increased by 13%.

Overall, monofloral honeys have a higher inhibition range against pathogens than multifloral honeys (Oelschlaegel *et al.*, 2012; Rodriguez *et al.*, 2012; Escriche *et al.*, 2014; Pimentel-González *et al.*, 2017; Aumeeruddy *et al.*, 2019; Olate-Olave *et al.*, 2021).

### Limitations

Currently, the use of honey for therapeutic effects has increased. It can be used for wound healing and to treat gastrointestinal diseases and eye infections. Nevertheless, evaluating the quality of the available scientific evidence is fundamental. On the one hand, information about the sample size is limited; on the other hand, the methods used to identify the properties of honey and the sample extraction before chromatography are not standardized. In addition, processing methods (pasteurized or unpasteurized) should be taken into account and the range of pathogenic bacteria evaluated should be expanded.

### CONCLUSIONS

The antibacterial activity of honey has been used since ancient times to treat different health problems. Studying honey has never been an easy task: it is a complex system with inherent characteristics and many chemical compounds that change depending on the types of honey and its botanical and geographical origin. Overall, honeys produced from specific botanic sources have a high antibacterial activity, as a consequence of the medicinal properties of the flower source that are transferred to the honey through the nectar.

The efforts of the scientists to determine the underlying mechanisms of the bactericidal effect of honey have paid off. Scientists have proved that the said activity is related to the pH level, the osmotic effect caused by the high sugar content, the hydrogen peroxide content, the methylglyoxal content, the phenolic compounds, and the flavonoids that can work together or individually, depending on the type of honey (floral source, geographical

origin, etc.). All these characteristics help to stop the growth of certain pathogens and, consequently, honey has been used in the health sector to treat several diseases. Nevertheless, the antibacterial activity must be confirmed to prove the medical grade of a given honey. Subsequently, optimal production, handling, harvesting, and storage procedures should be established, in order to guarantee the safety of the product —*i.e.*, honey should be free of pathogens and toxic compounds. Finally, the effects of the infection and disease treatment should be proved through clinical trials.

The protocols used to study the phenolic compounds are different and are related to the detector that will be used with the HPLC. This selection is directly related to the analytes that will be identified and the budget established for the research. New technologies have led to promising results, reducing costs and analysis time; however, new technologies should be focused on the analysis of the metabolites of honey.

Consequently, further research about the different components of honey should be carried out to find new alternatives that reduce or stop antibiotic resistance. These studies should result in the use of honey as a substitute or its combination with classic antibiotics. Nevertheless, establishing standardized methodologies among the scientific community will help to determine the antibacterial activity of honey, facilitating its analysis and expanding the research of this property among honeys of different botanical origins.

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# Association of Hsp70 locus polymorphism with thermotolerance and ailment occurrence in Gulf Creole cattle within intensive systems

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## ABSTRACT

**Objective:** To estimate thermotolerance by analyzing physiological constants and ailment occurrence in Gulf Creole bovine cattle (GCB) and to relate them to Hsp70 locus polymorphism under an intensive production system.

**Design/Methodology/Approach:** Using a 440 bp fragment, we genotyped leukocyte DNA from 60 BCG through PCR-RFLP (Fok I). Physiological variables were estimated at 7-day intervals for four months during the hottest season. The variables considered were respiratory rate (RR) and layer temperature (LT). Environmental variables —temperature and humidity— were also recorded to determine thermal comfort. Using the production system database, we categorized the animals' ailments during the studied period.

**Results:** The Hsp70 gene in GCB is polymorphic. The frequency of the AB heterozygous genotype was 0.77; for the AA homozygous genotype, it was 0.23. We observed a predominance of the A allele (0.61). Data analysis allowed us to find differences in RR in GCB with AA and AB genotypes ( $p < 0.05$ ). LT showed no differences ( $p > 0.05$ ). The genotype did not affect ailment occurrence in GCB ( $p > 0.05$ ).

**Study limitations/implications:** Since the intensive system is dynamic, and the GCB stay period is short, few animals were available for the study.

**Findings/Conclusions:** The Hsp70 gene present in GCB is polymorphic, and the animals are thermotolerant. Their performance regarding the occurrence of clinical conditions is favorable.

**Key words:** Gulf Creole bovine cattle, THI, Heat stress, Ailment, Hsp70, Polymorphism.

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## INTRODUCTION

Environmental factors such as temperature, humidity, precipitation, atmospheric pressure, wind speed, among others, can affect the productive behavior of cattle (Mader



*et al.*, 2010). In tropical and subtropical regions, high environmental temperatures have a negative effect on cattle (Molina-Coto, 2017). Temperature and the temperature-humidity index (THI) determine the thermal comfort of animals and are, therefore, the most studied indicators to measure environmental effects on cattle (Jia *et al.*, 2012).

Numerous genes participating in thermotolerance have been described for different bovine breeds and genetic groups (Sosa *et al.*, 2022; Habimana *et al.*, 2023). Among these genes, the ones producing heat shock proteins (HSP) stand out. Said proteins have cytoprotective functions in the face of stressful changes due to heat (Abbas *et al.*, 2020).

The Hsp70 gene regulates the Hsp70 subfamily synthesis. This sensitive and conserved gene is related to thermotolerance and ailment susceptibility in cattle. These characteristics place it as a candidate gene for selecting animals resilient to adverse climates, with better immune response and greater productive performance (Hassan *et al.*, 2019; Badri *et al.*, 2021). The response to thermal stress can vary significantly between breeds and genetic groups. This variability must be considered when selecting animals for their reproductive and productive performance (meat and milk). Some breeds of Creole cattle, such as the Romosinuano (*Bos taurus*), show resistance to heat stress compared to other taurine breeds not adapted to tropical environments (Hernández-Cerón *et al.*, 2004). Silva *et al.* (2013) suggest that this higher resistance reported in different genetic groups is due to the high expression of genes that are protective against heat shock.

Gulf Creole bovines (GCB) constitute a genetic group originating in the Iberian breeds and located in the Gulf of Mexico. They have considerable rusticity, resilience, and reproductive efficiency. GCB are considered a source of genetic diversity that can contribute to forming herds with better adaptability to the climate of tropical areas (Hernández *et al.*, 2015). However, molecular tools have not been used to assess the adaptation of GCB to the adverse conditions of elevated temperatures derived from variations associated with climate change. Hence, this study aims to estimate thermotolerance by analyzing physiological constants and clinical ailment occurrence in Gulf Creole bovine cattle (GCB) and to relate them to Hsp70 locus polymorphism under an intensive production system.

## **MATERIALS AND METHODS**

### **Ethics statement**

All handling, immobilization, and sampling procedures conducted on the GCB within the livestock production unit (LPU) by the veterinary services were evaluated and approved by the Bioethics Committee with registration number COBIBA012/2022 (School of Veterinary Medicine and Animal Sciences, Veracruz University).

### **Location and description of the study area**

We conducted this study from September to December 2022 in an intensive fattening bovine system located in the municipality of Veracruz, in the state of Veracruz, Mexico, with the following geographic coordinates: 19° 10' 01.9" N and 96° 17' 03.0" W, at an altitude



of 10 masl, in a warm subhumid climate ( $AW_2$ ), with an average annual temperature of  $>18$  °C. The average annual precipitation is  $\sim 1600$  mm with rains in summer (Köppen-Geiger; Beck *et al.*, 2018; Vidal-Zepeda, 2005).

### Sampling conditions and experimental animals

The experiment encompassed 60 bovines that met the criteria of belonging to the GCB genetic group ( $n=35$  males and  $n=25$  females), having an average weight of  $203 \pm 6.5$  kg (mean  $\pm$  SD), coming from various regions of the Gulf and south-southeastern Mexico, and being destined for meat production.

Considering the management conditions of the production unit, we conducted a first selection process when animals from different herds entered the LPU. This first selection aimed to separate the bovines with the highest genotype of taurine breeds meeting the inclusion criteria of phenotypic characteristics (coat color, coat type, and horns), indicated in the method proposed by Sponenberg (1992) and modified by Perezgrovas (2011). This first selection allowed us to characterize the differences between *Bos taurus* and *Bos indicus* by assigning scores (on a scale from 0 to 100) to specific characteristics of the animals, stipulating high values for individuals belonging to the *Bos taurus* genetic group and low values to individuals of the *Bos indicus* group.

The group of bovines with taurine characteristics ( $>75$  points) underwent a second selection to ensure they presented the phenotypic characteristics reported by Hernández *et al.* (2015) for GCB cattle regarding coat color, skin appendages, direction and position of horns, ear size, and head shape. This second selection allowed us to obtain a population of  $n=60$  (GCB).

The selected GCB were housed along with other cattle from different genetic groups in pens  $\sim 37$  m wide and  $\sim 40$  long, with an average capacity of 80 individuals ( $18.5$  m<sup>2</sup>) and  $2.4$  m<sup>2</sup> of shade per animal. Each pen had concrete flooring for the entire area, a 3 m wide and  $\sim 28$  m long feeding trough, and a 1.60 m wide and 4 m long concrete drinking trough, both covered with a concrete roof.

### Obtaining meteorological and physiological variables

Respiratory rate (RR) and layer temperature (LT) were measured individually, in a resting state, during a four-month period (September-December 2022), in 7-day intervals from 1:00 p.m. to 3:00 p.m. To measure RR (breaths/min), we resorted to direct observation of inspiratory movements in the animals' flank region (right or left) (Romo *et al.*, 2022). LT (°C) was obtained using an infrared digital thermometer (Steren, model HER-427) directed toward the frontal region of the animal's head (Dorota *et al.*, 2019).

To establish a relationship between physiological constants and climatic variability, the environmental temperature (°C) and relative humidity (%) values were recorded on clinical evaluation days. Data were obtained from the meteorological station located 10.3 km from the LPU in a straight line (Meteorological Station #309-692 of the Veracruz International Airport, Heriberto Jara Corona). We thus estimated the livestock climatic safety index, known as the Temperature and Humidity Index (THI),

which determines the animals' thermal comfort using the following equation (Valtorta and Gallardo, 2004):

$$THI = (1.8 \times T) + 32 - (0.55 - 0.55 \times HR / 100) \times (1.8 \times T - 26)$$

The THI is a number (in units) used to indicate the lack of comfort caused by the combined effects of air temperature and humidity. Hahn *et al.* (1999) and Nienaber and Hahn (2007) use the results of this equation and consider four categories of THI to evaluate thermal environmental conditions and their impact on animal welfare. THI values  $\leq 74$  are considered comfortable, 75-78 indicate alert, 79-83 indicate danger, and  $\geq 84$  indicate an emergency (Saizi *et al.*, 2019).

### Determination of Hsp70 gene polymorphism

To genotype the Hsp70 gene, we used leukocyte DNA extracted from whole blood per individual and implemented the salt-precipitation method, according to the instructions provided by the manufacturer (Wizard<sup>®</sup> Genomic DNA Purification Kit Promega<sup>®</sup>). Blood was drawn by jugular venipuncture using vacuum tubes with anticoagulant (EDTA 7.2 mg), which were refrigerated at 8 °C until processing. The genotyping technique was Polymerase Chain Reaction - Restriction Fragment Length Polymorphism with restriction enzyme Fok I (PCR-RFLP/Fok I). The amplification was conducted with the commercial kit GoTaq<sup>®</sup> Green Master Mix (Promega<sup>®</sup>), 15  $\mu$ L of Green GoTaq<sup>®</sup> Reaction Buffer (Taq DNA Polymerase, dNTPs [400 mM dATP; 400 mM dGTP; 400 mM dCTP; 400 mM dTTP], 3 mM MgCl<sub>2</sub>), and 1  $\mu$ L/10 pM of each primer commercially designed at UNAM's Institute of Biotechnology (Biotecnologías UNAM<sup>®</sup>) (Forward 5'-CCGGCCTACTTCAACGACTC-3' and Reverse 5'-CAAGCTCCCGTAGCTGAAGA-3') (Grosz *et al.*, 1994) for a fragment of 440 base pairs (bp) (Lamb *et al.*, 2006), 5.0  $\mu$ L of DNA, and nuclease-free water to complete the final volume of 35  $\mu$ L (Lamb *et al.*, 2007). The oligonucleotides sequence corresponds to the Hsp70 gene in band 22 of bovine chromosome 23 (Gene Bank U09861.1, National Center for Biotechnology Information - NCBI). A negative control was used with each assay consisting of the PCR reagent mix without DNA. The PCR amplification protocol was as follows: initial denaturation 94 °C/2 minutes, followed by 35 cycles of 94 °C/30 seconds, 56 °C/30 seconds, 72 °C/30 seconds, and a final extension of 72 °C /10 minutes. The amplification and size of the amplicon was verified by electrophoresis in 2% agarose gel/1X Tris-Borate-EDTA (44.5 mM Tris-HCl, pH 8.0; 44.5 mM boric acid; 1mM EDTA) (Sambrook and Russel, 2001), stained with SYBR Safe Invitrogen<sup>®</sup> (0.8:1,0000), and molecular weight marker pBR322 DNA-MspI Digest (NEB<sup>®</sup>) (Grosz *et al.*, 1994; Lamb *et al.*, 2007). PCR amplification was performed in an MJ Mini thermocycler (Bio-Rad<sup>®</sup>).

Digestion of the 440 bp amplicon was performed with the restriction enzyme Fok I to identify the RFLPs of the Hsp70 gene. According to the amplification and digestion protocol proposed by Lamb *et al.* (2006), the size of the expected fragments is 440 bp for the non-allelic gene fragment (bp) and 171 and 269 bp for restriction sites fragments.

The expected genotypes were homozygous AA of 171 and 269 bp, genotype BB of 440 bp —undigested fragment—, and heterozygous genotype AB of 440, 171, and 269 bp. The digested product was analyzed in 2.5% non-denaturing agarose gel stained with ethidium bromide (0.5 mg/ml), molecular weight marker pBR322 DNA-MspI Digest (Promega®). To visualize the expected fragments, a UV transilluminator was used (Ultra-Lum Spectroline®, 260 nm).

### Categorization of clinical conditions

To quantify and categorize the animals' ailments, we used the information provided by the LPU system database, in which clinical conditions are classified according to the main organ affected —respiratory (BRC), locomotive (LC), and digestive (DG) (Estima-Silva *et al.*, 2020). The four months of the evaluation were considered to determine the clinical conditions present in each studied animal to establish a relationship with the THI of the period and the polymorphism of the Hsp70 gene.

### Statistic analysis

The Hardy-Weinberg Equilibrium test was used to determine the allele frequency. We conducted statistical analyses with the STATISTICA v.7 software (StatSoft, 2008) using main effects variance analyses considering the genotype, gender, and THI categories (Vega-Murillo *et al.*, 2023). Multiple comparisons of means of the clinical variables (RR and LT) were performed using the Tukey test ( $p < 0.05$ ). The statistical model used was the following:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \delta_k + \varepsilon_{ijk}$$

$Y_{ijk}$ =clinical response variables (RR and LT);  $\mu$ =general mean;  $\alpha_i$ =genotype effect (AA, AB, BB);  $\beta_j$ =gender effect (male and female);  $\delta_k$ =THI category effect (comfort, alert, danger, and emergency);  $\varepsilon_{ijk}$ =random error of  $i$ -th genotype, the  $j$ -th gender, and the  $k$ -th THI category.

To analyze the association of genotype, gender, and ailments, we used  $2^k$  contingency tables and the  $\chi^2$  distribution of the non-parametric module of the Statistica v7 software (2004). Regarding the combined effects of genotype and gender, logistic regression was used to estimate the odds ratios and identify whether the latter are risk factors for the occurrence of any clinical condition (Zavaleta-Martínez *et al.*, 2024).

The logistic regression equation was:

$$\log\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 * x_1 + \beta_2 * x_2 + \beta_n * x_n$$

Where “ $p$ ” is the probability of the event occurring (clinical condition: BRC, DG, LC); the independent variables are represented with the letter  $x$ ; the absence of the genotype

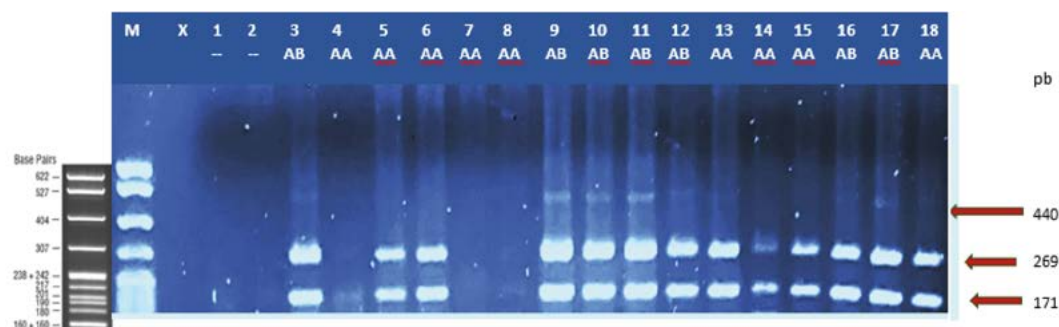
(AA, AB, BB)=0, and the presence=1; the THI that considers thermal comfort and the coefficients associated with each variable are represented with the letter “ $\beta$ ”. The figures were edited with the Sigma Plot V11 software (Systat, 2008).

## RESULTS AND DISCUSSION

### Hsp70 gene polymorphism

The Hsp70 gene was polymorphic in all the specimens evaluated (Figure 1). Two genotypes were identified: homozygous AA and heterozygous AB; the presence of homozygous BB was not reported.

In the analyzed animals (Nn60), the frequency of the A allele was higher (0.89), with expression of heterozygosity for the AB genotype (0.78) and homozygosity for the AA genotype (0.22) (Table 1). These results concur with those reported by Bhat *et al.* (2016) and Li *et al.* (2011) on the presence and frequency of alleles in studies conducted on Tharpakar and Chinese Holstein cattle. The Hardy-Weinberg test indicated that the evaluated population is in equilibrium, with theoretical  $\chi^2$  values of 3.84 and experimental  $\chi^2$  values of 0.885. These values may be due to random crossbreeding with other bovine genetic groups, mainly of zebu origin, with little difference between the observed and expected values of the genotypes. However, we must consider that the animals came from various locations in the Gulf and southern Mexico, which may also lead to said results.

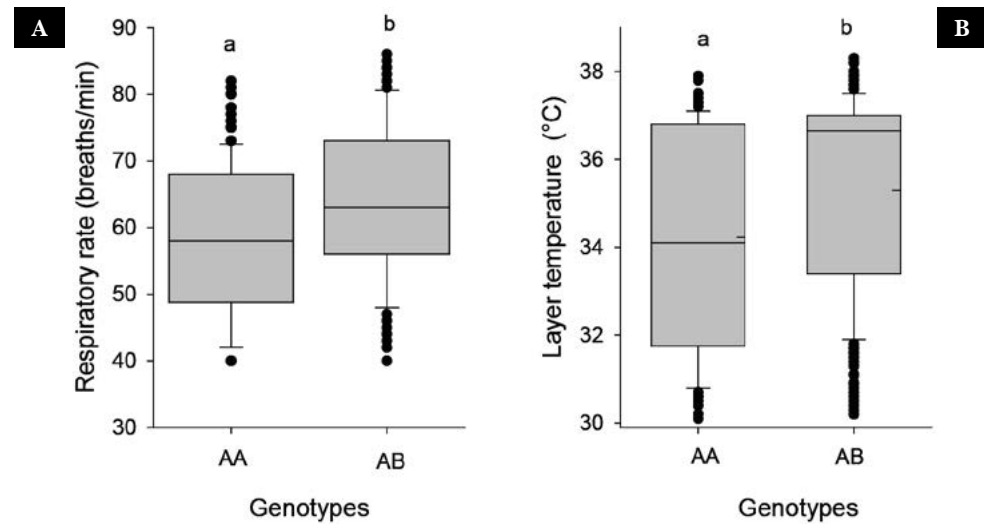


**Figure 1.** Electrophoresis in 2.5% agarose gel stained with EtBr (0.5mg/ml). The fragments of the Fok I digestion belong to the Hsp70 locus (440 bp). The order of the lanes is as follows: Lane M=Low molecular weight marker. Lane X=Negative control. Lanes 1 and 2, no result. Lanes 3-18 correspond to samples AA: 269 and 171 bp; AB: 440, 269, and 171 bp; BB, undigested amplicon, 440 bp not observed.

**Table 1.** Genotypic and allelic frequencies obtained for the Hsp70 gene in Gulf Creole bovine cattle.

Gene frequencies		Allele frequencies	
A	0.89	AA	0.22
B	0.11	AB	0.78
		BB	0.00

N=60; 1 gL;  $\chi^2$  0.8856



**Figure 2.** Box and whisker diagram showing the differences between the clinical variables RR (A) and LT (B) among the GCB genotypes studied. The box shows Q1 (bottom line) and Q3 (top line), the dashed line shows the median, and the wireframe, maximum and minimum non-outlier values. The letters a and b indicate differences between clinical variables after the Tukey post hoc test, indicating statistically significant differences ( $p < 0.05$ ). ANDEVA values for RR were  $F(1, 958) = 46.324$ ,  $p = 0.0001$ ; for LT,  $F(1, 958) = 35.963$ ,  $p = 0.0001$ .

### Environmental and physiological variables

When analyzing the comparison of means, we observed an effect ( $p < 0.05$ ) of the genotypes of the Hsp70 gene on RR (Figure 2A). GCBs with genotype AA presented low RR values compared to those with genotype AB. Likewise, the AA and AB genotypes showed an effect on LT ( $p < 0.05$ ) (Figure 2B).

Previous studies have shown an association between various polymorphisms in the Hsp70 gene locus and thermotolerance in Chinese Holstein and Tharpakar cattle (Li *et al.*, 2010; Basfrico *et al.*, 2011; Liu *et al.*, 2011; Sodhi *et al.*, 2013). When evaluating Tharpakar cattle, Bhat *et al.* (2016) noted that animals with the AA genotype presented low values in RR and LT, followed by the AB and BB genotypes. Their results establish an association between the Hsp70 polymorphism and thermotolerance parameters. Likewise, the A allele positively affected thermotolerance, and the AA genotype was superior, coinciding with the results of our study.

Moreover, when studying zebu cattle (Tharpakar) and their crosses with Creole cattle (Karan), Maibam *et al.* (2017), identified a positive correlation ( $r = 0.86$ ) between layer temperature and the expression of the Hsp70 gene, in addition to finding that skin layer temperature showed changes depending on the season evaluated. However, a study conducted by Prasanna *et al.* (2022) on Sahwal cows and their crosses with Holstein Friesian reached different results: they found no difference ( $p > 0.05$ ) in the physiological parameters between the AA and AB genotypes and did not report the presence of the BB genotype. In this regard, we must point out that heterozygosity is generally greater in organisms living in limiting environments than in those living in favorable environments. A heterozygosity greater than predicted based on the effective

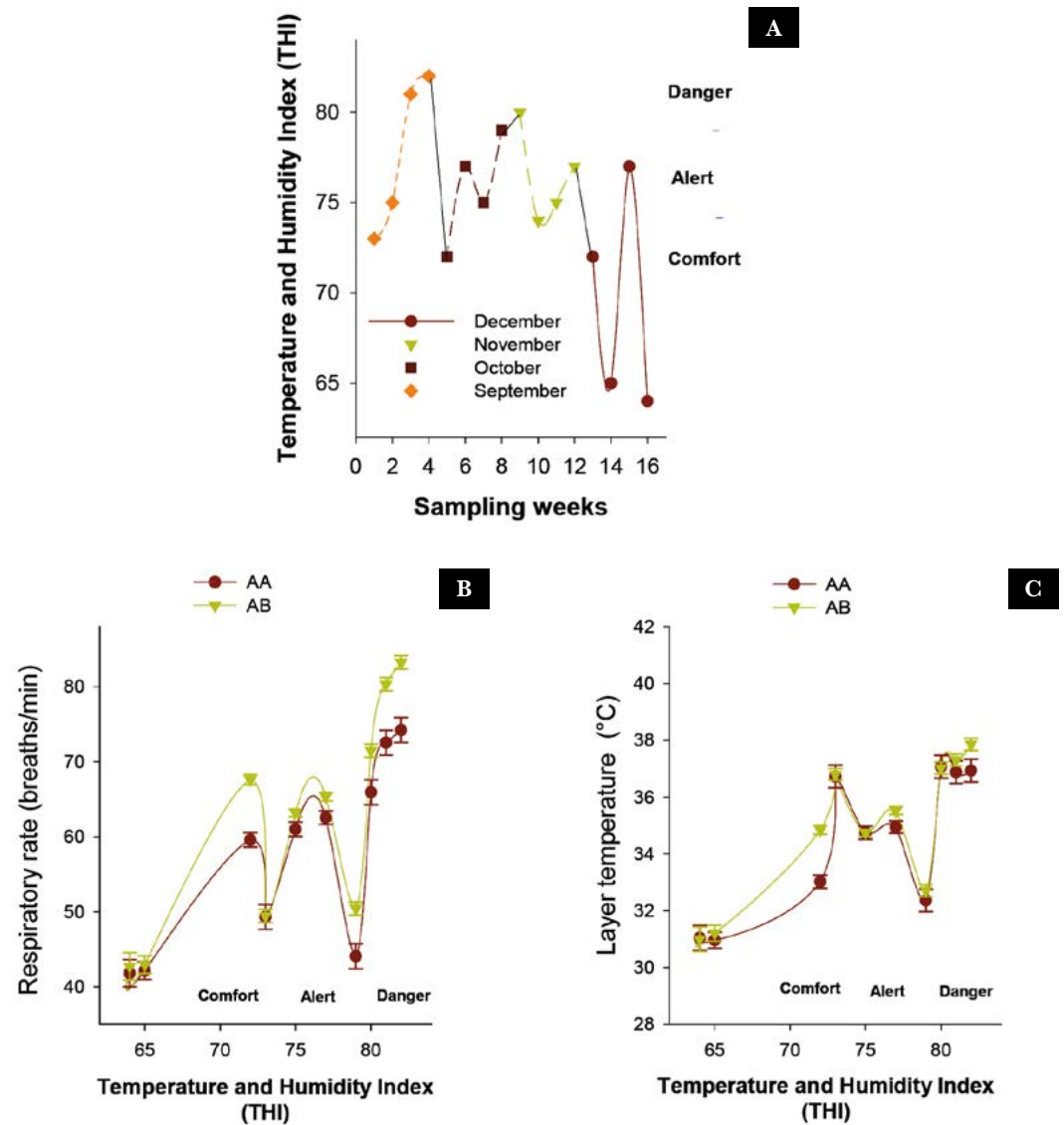
population size can be expected in small populations at the limit of a species' natural distribution due to selection (Liu, 1999). Higher than expected heterozygosity at some loci may indicate selection or an artifact widely observed in *Bos taurus* cattle in temperate regions (Falchi *et al.*, 2023). Small populations maintaining heterozygosity may be under intense selection pressure for heterozygous loci, or the population has recently become small and has not yet lost heterozygosity. In any case, conservation efforts should focus on increasing population size (Mitton, 2000). In local breeds with small population sizes, one of the most relevant problems is the increase in the inbreeding coefficient. High levels of inbreeding lead to reduced genetic diversity and inbreeding depression. Most local livestock breed result from a particular adaptation to environmentally conditioned production systems, and in many cases, no other breed could survive in the same habitat if the local breed became extinct. These local populations may harbor specific genetic variants that must be conserved and used to recover the loss of genetic diversity occurring in major breeds due to intensive selection for production traits (Mastrangelo *et al.*, 2016).

To establish the environmental effects on the GBC, we quantified the degree of comfort in the studied period. The THI was charted as a function of time (sampling weeks), as shown in Figure 3. With these results, we proceeded to analyze the effect of the THI on the physiological constants (RR and LT) in GBC (Figure 3B and 3C). We can see that, as the THI goes from the category of comfort to that of danger, the clinical constants RR and LT increase. Furthermore, the AB genotype shows greater increases in physiological constants than the AA genotype ( $p < 0.05$ ) due to a response mechanism that aims to dissipate the heat absorbed from the environment and to maintain a balanced body temperature (Samara *et al.*, 2016; Hansen, 2020).

A respiratory rate increase in animals is a crucial factor in the thermoregulatory response to heat stress since it helps dissipate heat through evaporative cooling. A lower respiratory rate may indicate better thermotolerance (Das *et al.*, 2016). For its part, an increase in LT is related to vasodilation of the capillary bed of the skin, which facilitates heat dissipation by increasing blood flow on the skin surface (Katiyatiya *et al.*, 2017; Madhusoodan *et al.*, 2019). Since the skin is an organ that comes into direct contact with the external and internal environment, it is also influenced by the characteristics of the coat, such as color, density, and size (Romanello *et al.*, 2018).

### **Aliments**

One of the most salient factors in beef production is animal health since it influences the productive performance of animals and may cause their removal from the feedlots, which impacts profitability (Zhukov *et al.*, 2020). Among the main clinical conditions in bovines at the LPU were respiratory, digestive, and locomotor ailments that cause high rates of morbidity and mortality (Estima-Silva *et al.*, 2020). The etiology of these conditions is multifactorial: pathogens, age, individual susceptibility, and environmental stressors, among others. Stress is a direct contributor, especially in respiratory conditions (Wisnieski *et al.*, 2021), due to the suppression of the immune system induced by high cortisol (Bagath *et al.*, 2019).



**Figure 3.** Climatic variables recorded in the LPU, and physiological variables analyzed in GBC concerning the THI present on the evaluation day. A. Description of the THI depending on the study period. B. Respiratory rate (breaths/min) as a function of the change in the THI and categories. C. Layer temperature (°C) as a function of the change in the THI and categories. The horizontal dashed lines in A denote the change of the THI categories. Dashed vertical lines in B and C denote changes in the THI categories.

Digestive (DG), locomotor (LC), and respiratory ailments (this last one known as Bovine Respiratory Complex, BRC) were reported in the GBC analyzed during the experimentation period at the LPU. When performing a logistic regression analysis to find associations between the effect of genotypes and the THI, only the THI showed an effect on the respiratory condition (BRC) in the GCBs evaluated ( $p < 0.05$ ) (Tables 2 and 3). The increase in environmental temperature and humidity during summer can compromise animals' thermoregulation mechanisms, causing endocrine alterations to facilitate heat loss, which in turn affects immune functions, increasing susceptibility to various pathogens (Vandana *et al.* 2018).

**Table 2.** Logistic regression analysis of the genotype effect on the occurrence of ailments in Gulf Creole bovines.

Indicator variables of the logistic regression equation				
n=60		BRC	DG	LC
Constants	$\beta_0$	$\beta_1$	$\beta_2$	$\beta_3$
Estimate	-0.93	-0.42	0.93	-0.31
Standard Error	0.74	0.36	1.41	0.8
t (56)	-12.66	-1.16	0.66	-0.38
p-level	0.01	0.24	0.5	0.69
-95%CL	-1.08	-1.13	-1.84	-1.89
+95%CL	-0.79	0.28	3.71	1.26
Wald's $\chi^2$	160.34	1.35	0.43	0.15
p-level	0.01	0.24	0.5	0.69
Odds ratio (unit ch)	0.39	0.65	2.55	0.73
-95%CL	0.33	0.32	0.15	0.15
+95%CL	0.45	1.33	41.18	3.54
Odds ratio (range)		0.65	2.55	0.73
-95%CL		0.32	0.15	0.15
+95%CL		1.33	41.18	3.54

BRC=Bovine Respiratory Complex, DG=Digestive, LC=Locomotive.

**Table 3.** Logistic regression analysis of the THI effect on the occurrence of ailments in Gulf Creole bovines.

Indicator variables of the logistic regression equation				
n=60		CRB	DG	LC
Constants	$\beta_0$	$\beta_1$	$\beta_2$	$\beta_3$
Estimate	0.47	1.01	-0.475	-0.7
Standard Error	0.06	0.37	1.41	0.67
t (56)	6.93	2.7	-0.33	-1.03
p-level	0.01	0.006	0.73	0.3
-95%CL	0.34	0.27	-3.25	-2.02
+95%CL	0.6	1.75	2.3	0.62
Wald's $\chi^2$	48.08	7.33	0.11	1.07
p-level	00.1	0.006	0.73	0.3
Odds ratio (unit ch)	1.6	2.76	0.62	0.49
-95%CL	1.4	1.32	0.03	0.13
+95%CL	1.84	5.76	10	1.86
Odds ratio (range)		2.76	0.62	0.49
-95%CL		1.32	0.03	0.13
+95%CL		5.76	10	1.86



## CONCLUSIONS

The Hsp70 gene is polymorphic in Gulf Creole bovines, with a predominance of the A allele. We found an association of the AA genotype with lower respiratory rate values under heat stress conditions, indicative of a favorable thermotolerance genotype in this livestock. Nonetheless, the results must be complemented with more studies in a larger population and with diverse genetic groups.

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# Evaluation of the aquaculture potential of *Dormitator maculatus* (Bloch, 1792) from the Alvarado lagoon, Veracruz, Mexico

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## ABSTRACT

**Objective:** The naca fish, *Dormitator maculatus*, is a species which commercial importance is based exclusively on the extraction of the mature gonads of the females, which reach a high price in the regional market as a gourmet dish. However, there is scarce information regarding its biology.

**Design/methodology/approach:** Their aquaculture potential (survival and growth) was evaluated in a recirculation system, in which the response in captivity of stages of their life cycle (treatments) were tested: T1=fry (8.35±2,83 g), T2=juveniles (17.22±5,13 g) and T3=adults (25.55±6,05 g), in triplicate, for 90 days. Measurements were performed every 15 days, the diet supplied was a commercial balanced feed for tilapia 3.5 mm in diameter (GrowFish<sup>®</sup> Protein 35%, fat 5.5%), at 1.5% of the biomass which was positively accepted.

**Results:** At the end of the experiment, it was observed that Treatment 1 (fry) presented a greater survival and growth. This result may be related to the fact that the species is very resistant to environmental changes in its natural environment.

**Limitations on study/implications:** However, this growth was slow, which may be since *D. maculatus* has a stationary diet.

**Findings/conclusions:** The results encourage more studies on its cultivation, such as the influence of environmental conditions on reproduction, which would help to mitigate the pressure on natural populations.

**Keywords:** recirculation, culture, life stage, survival, growth, gourmet fish.

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## INTRODUCTION

Worldwide, there are many species of fish that are not used massively as food resources, despite having the potential to meet to meet the growing demand sources of protein (Larumbe, 2002). In Mexico, there is a great diversity of native species of crustaceans, mollusks and fish that have been traditionally captured with artisanal fishing. In the State of Veracruz, this activity bases its practice on rustic fishing gear for fish, mollusks and crustaceans (Arreguin-Sanchez, 1986). This is the case of the fishery for the “naca” *Dormitator maculatus* that has been carried out for decades in the Alvarado Laguna. These



fish have a small size (6-12 cm), a discreet appearance, a grayish color and their meat has a flaccid consistency, so they are not used for human consumption. Its use is based exclusively on the extraction of mature gonads of females, which reach a high price in the regional market (Chávez *et al.*, 1997).

The *Dormitator maculatus* species is traditionally exploited in the Alvarado Lagoon System, Veracruz, Mexico. It is a species that has an important ecological role by transforming the energy of detritus into useful forms for organisms of higher trophic levels (Yáñez-Arancibia and Díaz-González, 1976). Its commercial importance lies exclusively in the consumption of the mature gonads of the females known as “roe”, which is highly valued locally. In the Alvarado Lagoon System there are few reports on this resource that provide statistics and volumes of annual catches at the regional or national level. The extraction season takes place from September to October when the mature fish come to spawn in the lagoon (Chávez *et al.*, 1997). It is a fish that is captured seasonally due to its economic importance, which places it among the ten most important species captured in the Alvarado lagoon system (CONAPESCA, 2005). Despite its regional importance due to its commercial value and reported catch volume, to date there is still no basic information on its reproductive biology or farming aspects. Therefore, it is necessary to carry out studies regarding the culture in recirculation systems for this species and to gather scientific information that allows knowing the current situation of its fishery and thus being able to propose management plans for its protection and sustainable use in Alvarado, Veracruz. The objective of this study is to evaluate the aquaculture potential of the Naca *Dormitator maculatus* in a recirculating aquaculture system.

## MATERIALS AND METHODS

### Study area

Alvarado's Laguna (parallels 18° 46' and 18° 42' north latitude and meridians 95° 34' and 95° 58' west longitude) in southeastern Mexico, 70 km from the port of Veracruz (Figure 1). The Alvarado lagoon system is made up of a complex network of



**Figure 1.** Lagoon system of Alvarado, Veracruz (Google Earth, 2017).

more than 28 permanent and temporary lagoons, rivers/streams, which are associated with mangrove vegetation. The main water bodies that converge in the study area are the Papaloapan, Blanco, Acula, Limón, El alacrán, La manta, El Pájaro and Palma Real rivers. The permanent streams are Paso del Burro, Mano Perdida, Puente Amaca and Paso del Zapote; the intermittent stream Caño de Arena and the shrimp lagoons, Buen País, Alvarado, Tlalixcoyan, Las Pintas, Pajarillos, Santacomapan, El Embarcadero, Coyol, María Elvira, El Pájaro, Popuyeca, El Lodo and Coralillo (SEDAP 1997; INEGI 1990 ab). The system extends longitudinally in an east-west direction for approximately 26 km from the point of Isla Vives to the northwestern end of Laguna Camaronera.

### **Experimental design**

An experiment was carried out for 90 days under controlled conditions, where 216 *D. maculatus* fish were used, the tested treatments were: T1=24 fish (fry,  $8.35 \pm 2.83$  g), T2=24 fish (juveniles,  $17.22 \pm 5.13$  g) and T3=24 fish (adults,  $25.55 \pm 6.05$  g), each treatment was tested in triplicate.

### **Experimental organisms (collection, transport and acclimatization)**

The collection of the fish was carried out in the Alvarado Laguna system, Veracruz; Using local fishing gear, they were transported in a rectangular tank with a capacity of 500 L supplied with air with three diffusers to the Applied Aquaculture Research Laboratory (LIAA) of the Technological Institute of Boca del Río (ITBOCA), subsequently, they were measured with a 40 cm ichthyometer (with a precision of 0.1 mm) and weighed with a 5 kg digital scale ( $\pm 2$ g). For their acclimatization, they were introduced into a 3000 L reservoir to observe their behavior and the possible appearance of diseases caused by bacteria and fungi for 40 days in October 2015.

### **Experimental system**

A recirculation system was used that included nine rectangular fiberglass tubs with dimensions of 2.40 m $\times$ 56 cm $\times$ 40 cm, with a capacity of 442 L, which were under controlled conditions in the Laboratory (LIAA). The mechanical filter used was built with oyster shells, 5 mm gravel and 1 mm silica sand, in addition a 2.0 hp Flipperl pump was used to supply a water flow of 3m<sup>3</sup>/h. For water quality monitoring, the following was measured every 15 days: pH, dissolved oxygen (mg L<sup>-1</sup>), salinity (g L<sup>-1</sup>) and temperature (°C) for which a multiparameter probe (YSI 556 MPS) was used, In addition, for the monitoring of ammonium (mg L<sup>-1</sup>), nitrites (mg L<sup>-1</sup>) and nitrates (mg L<sup>-1</sup>), a Nutrafin<sup>®</sup> colorimetry test kit was used.

### **Feeding**

The diet used was commercial balanced feed for tilapia 3.5 mm in diameter (GrowFish<sup>®</sup> protein 35% and fat 5.5%), supplying 50% of the recommended ration (1.5% of the biomass), based on the manufacturer's feeding tables.

### Fish husbandry

To avoid the accumulation of nitrogenous residues, such as feces and uneaten food, the maintenance of the tanks consisted of eliminating them daily by siphoning and general cleaning every 14 days.

### Measuring

The fish were weighed every 15 days using a digital scale with a capacity of 5 kg ( $\pm 2$  g) and the total length was recorded using a 40 cm ichthyometer (accuracy of 0.1 mm); which were made at the beginning of the experiment and after 30 days of cultivation, determining the total length in cm and weight in g.

### Statistic analysis

A statistical method of means (Tukey) with 95% confidence was used. For the normality and homoscedasticity tests, a one-way analysis of variance (ANOVA) was used, later it was analyzed using the statistical package Statistical version 7.

## RESULTS AND DISCUSSION

### Adaptation in captivity

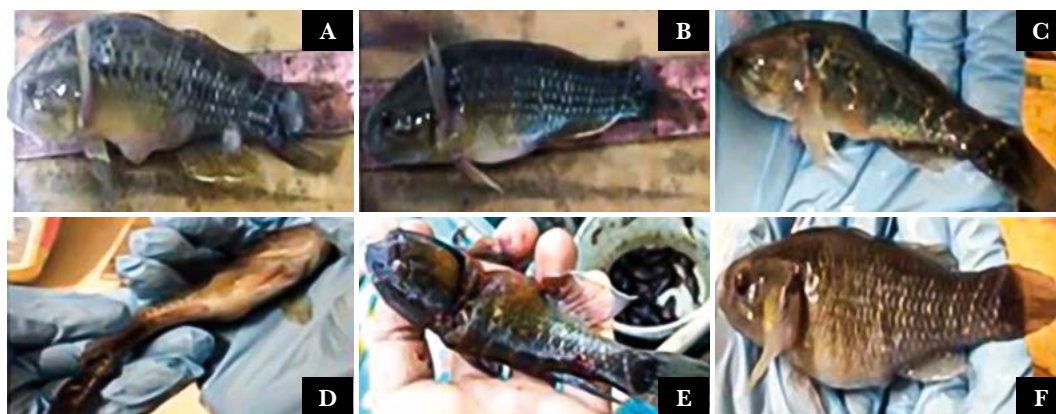
During the quarantine period, some diseases caused by fungi and bacteria occurred, causing the organisms: a) frayed fins, b) small lacerations, c) skin tumors, d) opaque coloration, e) loss of scales, f) skin lesions, this was due to stress (Figure 2).

### Weight gain

The results obtained from the measurements for weight during the experiment did not show significant differences between the treatments, as shown in (Figure 3).

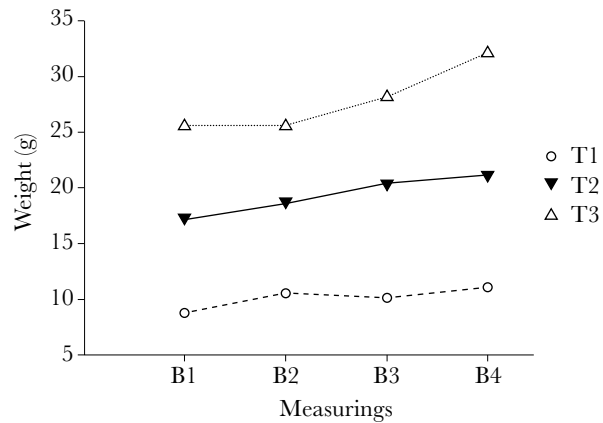
### Growth

It was observed that treatment 1 (fry) obtained a greater favorable response during the experiment compared to the other treatments, as shown in (Figure 4).

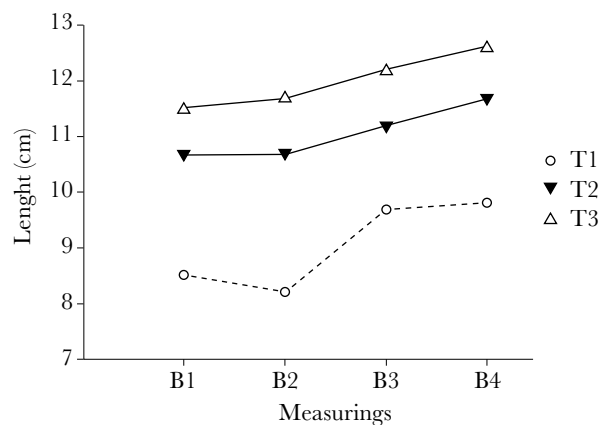


**Figure 2.** A) Frayed fins, B) small lacerations, C) skin tumors, D) opaque coloration, E) loss of scales, F) skin lesions.





**Figure 3.** Measurement results (weight) of the three treatments T1=24 fish (fry), T2=24 fish (juveniles) and T3=24 fish (adults), every 15 days, during the 90 days of the experiment.



**Figure 4.** Measurement results (length) of the three treatments T1=24 fish (fry), T2=24 fish (juveniles) and T3=24 fish (adults), every 15 days, during the 90 days of the experiment.

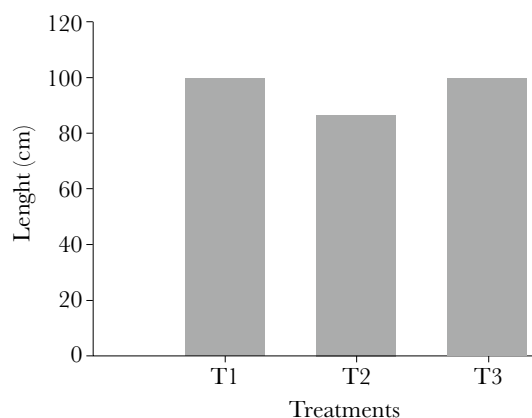
### Survival

During the 90 days of the experiment, survival between treatments was evaluated, observing that; treatment 1 (fry) and treatment 3 (adults) did not show differences, maintaining 100% survival. However, treatment 2 (juveniles) obtained a survival of 96%, which was not significant among treatments as can be seen in (Figure 5).

### Water quality

In this study, the water quality did not present significant variations, since all the conditions were controlled with a recirculation system, the monitoring of the variables was carried out every 15 days and they remained within the standard values for the species (Table 1).

Despite the diseases present at the start of the experiment, all the organisms of the different treatments avidly consumed the commercial diet provided, which was favorable since feed on organic matter in their natural environment. The adaptation of *D. maculatus* was largely due to the good acceptance and consumption of the balanced food during the



**Figure 5.** Evaluation of final survival among treatments T1=24 fish (fry), T2=24 fish (juveniles) and T3=24 fish (adults).

culture, this was favorable for fish development and growth of the in all the treatments, recording a slow but growth.

The lack of significant differences during the measurements for weight during the experiment could probably their seasonal behavior to rise to the surface and feed very slow and scarcely, this agrees with (Castro *et al.*, 2005) who mentions that *D. maculatus* has a similar behavior to *D. latifrons*; The authors also observed that at the beginning of his experiment, they used to appear on the surface to feed, noticing important changes in their behavior over time, since they began to decrease the frequency of going up to the surface, until finally, they stopped doing it. Basically, it is a stationary feeding fish (it feeds at times) with a very slow digestive system, which means that they do not feel hungry all the time (Chang and Navas, 1984).

The greater growth observed in Treatment 1 (fry) suggests that this species easily adapts to artificial feeding during its first stage of life, thanks to its double mandibular and pharyngeal dentition, which allows it to eat different types of food. This agrees with (Larumbe, 2002) who evaluated the growth and weight gain of *D. latifrons* feeding balanced feed and reported that growth in his study was slow. In addition, various investigations indicate that growth is an indicator of the adaptation of the organism to the environment, if this is favorable the fish grows, but when it is subject to variables that cause stress, growth stops. Several factors influence the rate of growth; stand out the stage of development, activity, food availability, photoperiod, salinity and temperature. It should be noted that the relationship between growth and intake rate is not simply linear; in studies on the effect

**Table 1.** Record of water quality in the *D. maculatus* culture recirculation system.

Sample	Temperature (°C)	Salinity (g L <sup>-1</sup> )	DO (mg L <sup>-1</sup> )	pH	Ammonia (mg L <sup>-1</sup> )	Nitrites (mg L <sup>-1</sup> )	Nitrates (mg L <sup>-1</sup> )
M1 (may/23 <sup>th</sup> /2016)	28	20	5.58-7.21	7.97-8.72	0.1	2	0.1
M2 (june/7 <sup>th</sup> /2016)	28	20	3.27-7.3	7.94-8.75	0.1	2	0.2
M3 (june/22 <sup>th</sup> /2016)	28	20	5.35-7.20	7.91-8.70	0.1	3	0.05
M4 (july/7 <sup>th</sup> /2016)	28	20	5.45-7.56	7.95-8.77	0.2	3	0.1

of environmental factors on growth, it is essential to consider this phenomenon (Jobling, 1994). Fish obtain their maximum potential for physiological growth due to a series of conditions: chemical composition of the water (water quality), water temperature, genetic characteristics and physiological state (Hepher and Proginin (1985).

Despite there were not significant differences recorded in survival, it should be noted that there is no reports on survival in captivity at experimental level for this species. In addition, research work on *D. latifrons* and *D. maculatus* is not recent and basically focuses on describing its abundance and the ecological role that these species have in different bodies of water and lagoon systems, and on the physiological characterization to differentiate the *D. maculatus* from *D. latifrons* (Yáñez-Arancibia and Díaz-González 1977; Uribe *et al.*, 1988; Hendrickx *et al.*, 1996; Clive *et al.*, 1995). Perhaps the low mortality during the experiment could be due wáter quality was maintained adequate for the cultivation of the species (temperature of 28 °C, a salinity of 20 g L<sup>-1</sup>, dissolved oxygen 5.27-7.56 mg L<sup>-1</sup>, pH 7.91-8.77, ammonium 0.1-0.2 mg L<sup>-1</sup>, nitrites 2-3 g L<sup>-1</sup>, nitrates 0.05-0.2 mg L<sup>-1</sup>. This agrees with Nicovita (2007), who mentions that the adequate temperature for example will depend on the species to be cultivated.

## CONCLUSIONS

Comparing the data obtained in this experiment, with the theoretical background of commercial species, it was found that the values obtained for *D. maculatus* were positive, because they presented a good response to captivity at the experimental level; the fry showed greater adaptation, growth and survival; since, in the natural environment, they are very resistant to environmental changes. As for weight gain, it was slow, which may be due to their stationary feeding. However, the balanced food was positively accepted, this is since they have double mandibular and pharyngeal dentition, which allows them to eat different types of food. In this sense, it can be concluded that the species does have a favorable aquaculture potential. However, further studies on cultivation are recommended; the influence of environmental conditions on captive reproduction; the identification of the stages in the life cycle and the dissemination of the importance of fishery management among the sectors involved.

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# Impact of the USMCA on corn (*Zea mays* L.) trade dynamics and food security in Mexico

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## ABSTRACT

**Objective:** To determine the impact of corn imports on food security in Mexico by describing the trade dynamics generated by the United States-Mexico-Canada Agreement (USMCA) to highlight the positive effects of foreign trade and free trade policies.

**Design/Methodology/Approach:** The research is based on a quantitative analysis of statistical data on corn involving 27 periods, coinciding with the entry into force of the North American Free Trade Agreement (NAFTA). We used a gravity model with two simultaneously estimated equations—very effective in describing the economies' trade dynamics.

**Results:** The estimate of the simultaneous equation model identified the United States as the country of greatest significance regarding corn trade with Mexico—an important consideration being that corn trade has a major influence on food security.

**Study limitations/implications:** The most relevant limitation was the lack of a unique source for data documentation.

**Findings/Conclusions:** Mexico's government policy aims to guarantee food supply. Yet, in 2020, imports supplied 36% of the national corn consumption. Corn imported from the United States is of the yellow variety; the tariff liberalization of this product as per USMCA and the geographical proximity to Mexico contribute to the imported volumes of yellow corn. As per the measurements provided by the Food and Agriculture Organization of the United Nations (FAO), the physical availability of food—in this case, corn—and the economic and physical access to food are met in Mexico. Nevertheless, food security has not been achieved, since 70% of the supply for consumption should come from national production.

**Keywords:** Gravity models, Agricultural econometrics, Trade dependency, International trade.

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## INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) posits four main dimensions for food security: the physical availability of food, economic and physical access to food, food utilization, and the stability of these three dimensions over time (IICA, 2019). Food security can be linked to foreign trade in Mexico in that the supply strategy regarding some strategic products such as corn, wheat, and beans has shown an increase in imports, particularly yellow corn (Luquez *et al.*, 2022a; Franco *et al.*, 2018, and Moreno *et al.*, 2016). This phenomenon has increased since the entry into force of the North American Free Trade Agreement (NAFTA) (Wise, 2023).

The 1994 NAFTA allowed Mexico preferential treatment in terms of international trade with the United States (US) (Luquez *et al.*, 2022b); the United States-Mexico-Canada Agreement (USMCA) is the new trade agreement between Mexico, the US, and Canada, which replaced NAFTA as of July 1, 2020 (Gobierno de México, 2020).

According to the Food Security Agency (SEGALMEX) (2019), whose goal is to promote agri-food productivity and food distribution for the benefit of the most disadvantaged population in Mexico (SADER, 2019), the country's priority should be food self-sufficiency as regards corn, beans, rice, wheat, and milk.

Soria and Palacio (2014) maintain that sovereignty and food security in Mexico are affected by multiple factors, mainly the world market. This idea runs contrary to the traditional international trade theory, which states that the effects of foreign trade are positive. In the face of the quandary regarding the relationship between these variables, the relevance of corn consumption and production in Mexico, and the country's food self-sufficiency political strategy, it is relevant to analyze the effects of international trade on the physical availability of the grain. Even more so when more restrictive measures have been decreed on transgenic and white corn imports to Mexico, the types of corn purchased abroad (DOF, 2023).

This research aims to determine the impact of corn imports on food security by describing the trade dynamics generated by the USMCA to highlight the positive effects of foreign trade and free trade policies.

The tested hypothesis is that international trade has contributed to improving the physical availability of yellow corn. Hence, the USMCA has generated a stable supply, favoring food security in Mexico since this type of corn is an essential input for animal protein production.

## **MATERIALS AND METHODS**

The research used annual statistical data on corn in Mexico from 1994 to 2020: production and trade volume, in addition to sociodemographic variables from Mexico, the United States, and Canada, such as population and income. These variables were collected from various sources: Trade statistics for international business development TRADEMAP (2023) and FAOSTAT (2023), US Department of Agriculture (USDA) (2023), Servicio de Información Agroalimentaria y Pesquera [Agri-food and fishing information service] (SIAP) (2023), Instituto Nacional de Estadística y Geografía [National Institute of Statistics and Geography] (INEGI) (2023), and World Bank (2023).

A rigorous identification of trade records was made based on the custom tariff classification: 10.05.90 for corn and 10.05.90.03 for yellow corn. The trade tax between Mexico and the US and Canada was also identified—corn imports and exports to/from Mexico are tariff-exempt due to the trade preferences acquired since NAFTA (TIGIE, 2022).

### **Gravity models**

The first researchers to posit gravity models were Isard and Peck (1954), who proved that distance and flows of raw materials are related. For his part, Tinbergen (1963)

proposed that the size of bilateral trade flows between countries can be estimated using the gravity equation derived from Newton's theory of gravitation (Pöyhönen, 1963). These types of models are used to predict trade volume because estimates are obtained through regression models (Appleyard and Field, 2013). Such models have been used to analyze trade around the world (Ávila Aguirre, 2017; Albornoz-Flores and Tonon-Ordóñez, 2020; Alarcón Flores *et al.*, 2021; and Luquez *et al.*, 2022b).

An econometric gravity model is appropriate for explaining and predicting the exchange patterns of goods considering economic and geographical factors, such as those implicit in the USMCA trade agreement (WTO, 2017).

The basic form of the gravity equation, as expressed by the WTO (2017), is “based on the metaphor of Newton's universal law of gravitation, the gravity model of trade predicts that international trade (gravitational force) between two countries (objects) is directly proportional to the product of their sizes (masses) and inversely proportional to the trade frictions (the square of the distance) between them,” such that the size of the masses is homologous to the size of the economy, which is expressed as follows:

$$X_{ij} = G \frac{Y_i E_j}{T_{ij}^{\theta}}$$

Where:  $X_{ij}$ : exports of countries  $i$  and  $j$ ;  $G$ : inverse of world production  $G=1/Y$ ;  $Y_i$ : national production of country  $i$ ;  $E_j$ : aggregate expenditure of country  $j$ ;  $T_{ij}$ : total trade costs between countries  $i$  and  $j$ .

Econometric estimation results usually present a series of inconsistencies, particularly in gravity models. One of the main drawbacks generating inconsistencies is the multicollinearity generated by variables such as the physical distance between economies. Yotov (2012) offers a solution to this problem and postulates that “the effect of distance on international trade between a given pair of countries is estimated relative to the effect of distance on international trade for another pair of countries,” that is, it is assumed to be constant and, for that reason, including distance in the estimation is not necessary; furthermore, distance has ceased to be significant in estimations due to globalization processes (Cairncross, 1997).

### Model formulation

Observing the trade balance behavior led to determining the effect of the USMCA on corn supply. The estimation of the proposed model was conducted using the simultaneous equations method (Gujarati, 2010), which can be expressed as follows:

$$Y_{1i} = \beta_{10} + \beta_{12}Y_{2i} + \gamma_{11}X_{1i} + v_{1i}$$

$$Y_{2i} = \beta_{20} + \beta_{21}Y_{1i} + \gamma_{21}X_{1i} + v_{2i}$$

Where  $Y_1$  and  $Y_2$  are dependent and stochastic variables,  $X_1$  is an exogenous variable, and  $v_1$  and  $v_2$  are the stochastic disturbance terms. One must prove that the stochastic explanatory variables are distributed independently of  $v$ , so that the ordinary least squares (OLS) method can generate optimal results (Gujarati, 2010).

Two equations were constructed. The first one encompasses the effects of Mexico's trade dynamics with the US and Canada. In this equation, the dependent variable refers to Mexico's net imports, including imports from the US and Canada and Mexican exports to said countries. The second equation expresses the simultaneous relationship between trade and country income, meaning the trade balance directly influences the Gross National Income (GNI). The simultaneous equation model proposed to analyze the trade dynamics, and which can be replicated for any product, is:

$$\begin{aligned} LNvcomercialmx = & \alpha_0 + \alpha_1 INBpc_{USA} + \alpha_2 INBpc_{MX} + \alpha_3 INBpc_{CAN} + \alpha_4 PRODpc_{USA} \\ & + \alpha_5 CONSp_{USA} + \alpha_6 PRODpc_{MX} + \alpha_7 CONSp_{MX} + \alpha_8 PRODpc_{CAN} \\ & + \alpha_9 CONSp_{CAN} + v_{ij} \end{aligned}$$

$$\begin{aligned} LNINBpc_{MX} = & \alpha_0 + \alpha_1 INBpc_{USA} + \alpha_2 vcomercial_{MX} + \alpha_3 INBpc_{CAN} + \alpha_4 PRODpc_{USA} \\ & + \alpha_5 CONSp_{USA} + \alpha_6 PRODpc_{MX} + \alpha_7 CONSp_{MX} + \alpha_8 PRODpc_{CAN} \\ & + \alpha_9 CONSp_{CAN} + v_{ij} \end{aligned}$$

The dependent variables are defined as follows:

$LNvcomercial$  is the natural logarithm of the volume of the international trade share in Mexico's apparent national consumption; and  $LNINBpc_{MX}$  is the natural logarithm of the real GNI *per capita* in Mexican prices. The dependent variables are logarithmic so that the estimation analysis can be expressed in percentage terms; these variables are causal to each other, the positive trade balance contributes to the country's income and, in turn, the GNI is a measure of the country's income.

The independent variables are defined as follows:

$INBpc_{USA}$ , GNI at real prices *per capita* in the US;  $INBpc_{CAN}$ , GNI at real prices *per capita* in Canada;  $INBpc_{MX}$ , GNI at real prices *per capita* in Mexico;  $PRODpc_{USA}$ , *per capita* production in the US;  $PRODpc_{CAN}$ , *per capita* production in Canada;  $PRODpc_{MX}$ , *per capita* production in Mexico;  $CONSp_{USA}$ , *per capita* apparent national consumption in the US;  $CONSp_{CAN}$ , *per capita* apparent national consumption in Canada;  $CONSp_{MX}$ , *per capita* apparent national consumption in Mexico; and  $v_{ij}$ , estimation error for each equation.

Simultaneous equation models combine multiple regression and factor analysis, which allows the assessment of dependency interrelations (Cupani, 2012). In this research, the regression model estimation used the three-stage least squares method, which is the most common estimation method in the context of system estimation methods (SEM) (Wooldridge, 2009). Corn production is cyclical because its vegetative period lasts less than



12 months; however, the data examined are annual, so that there are no cycles within the analyzed series, and data seasonality was not a problem within the econometric model.

## RESULTS AND DISCUSSION

### The simultaneous model of corn

Gravity models are widely used to identify whether the implementation of a trade agreement has had a significant impact on trade evolution in specific trade blocs. Moreover, the implementation of this model shows the influence of trade agreements on the growth and rapprochement of economies. Table 1 presents the results of the simultaneous gravity model estimation for the three economies participating in the USMCA and its influence on the corn grain trade with Mexico.

Results show a very high adjustment due to the coefficient of determination for both equations in the model. In the model formulation, codependency was assumed, that is, the dependent variables in both equations are interrelated and mutually affect their values.

**Table 1.** Results of the gravity model with simultaneous equations.

Variable dependiente=Ln volumen comercial México	Coefficiente	Error estándar	Z	p-value
México INB <i>per capita</i>	0.00021	0.00023	9.26000	0.000
EE. UU. INB <i>per capita</i>	-0.00001	0.00007	-2.53000	0.012
Canadá INB <i>per capita</i>	-0.00001	0.00005	-3.47000	0.001
Producción <i>per capita</i> EE. UU.	0.10	0.75	0.13	0.89
CNA <i>per capita</i> EE. UU.	-0.05	0.70	-0.08	0.94
Producción <i>per capita</i> Canadá	1.52	0.85	1.77	0.08
CNA <i>per capita</i> Canadá	-0.83	0.62	-1.34	0.18
Producción <i>per capita</i> México	-22.14	2.26	-9.77	0.00
CNA <i>per capita</i> México	19.29	1.86	10.35	0.00
Constante	14.51	0.27	53.70	0.00
R-cuadrado 0.97	Chi <sup>2</sup> =1024.96		Prob>p=0.000	
Variable dependiente=Ln México INB <i>per capita</i>	Coefficiente	Error estándar	Z	p-value
Volumen comercial México	-0.000001	0.000000003	-6.31	0.000
EE. UU. INB <i>per capita</i>	0.000030	0.000005800	5.13	0.000
Canadá INB <i>per capita</i>	0.000008	0.000003400	2.21	0.027
Producción <i>per capita</i> EE. UU.	0.0041	0.54	0.01	0.994
CNA <i>per capita</i> EE. UU.	0.16	0.50	0.32	0.747
Producción <i>per capita</i> Canadá	-0.77	0.60	-1.21	0.201
CNA <i>per capita</i> Canadá	1.06	0.42	2.49	0.013
Producción <i>per capita</i> México	-20.14	3.72	-5.40	0.000
CNA <i>per capita</i> México	20.29	3.75	5.41	0.000
Constante	7.11	0.21	33.73	0.000
R-cuadrado 0.94	Chi <sup>2</sup> =535.08		Prob>p=0.000	

Source: Own elaboration.

In the first equation, the dependent variable was the logarithm of trade volume. Of the nine explanatory variables proposed, five were significant with 95% confidence. The sign of Mexico's GNI variable coefficient is consistent with the classical theory of international trade and the principle of absolute advantage, described by Adam Smith, which indicates the positive relationship between openness to foreign trade and the global wealth of countries. In this case, GNI is expressed as an approximation of this variable. On the other hand, the negative sign of the US' and Canada's GNIs reveals the effect of export demand in Mexico by its trading partners.

Consumption in the US and Canada was not significant, which shows that Mexico is not a net corn supplier for these countries. Regarding the production variables, Mexico's is the only significant one in the model estimation, which is consistent with the inverse effects of this variable on the net imports of the analyzed product. Moreover, the model's constant shows a positive behavior in the trade volume. Such growth—not explained by the independent variables— can be attributed to factors such as trade preferences within the USMCA and the distance variable between the countries, as Hernández-Pérez (2021) points out when discussing the characteristics of the neoliberal agro-export model balance in Mexican agriculture.

Six out of nine explanatory variables in the second equation were statistically significant. This equation estimates the effects of international trade on the country's GNI. The dependent variable is the natural logarithm of Mexico's GNI, determined by exports. Results show that trade volume influences the dependent variable negatively. In this regard, FAO (1996) mentions that international trade directly affects the population's access to food due to its effects on income and employment. The US' and Canada's income positively influences Mexico's GNI since, by increasing their purchasing power, they can boost their demand for imports and, in turn, increase Mexico's income. Canada's and Mexico's consumption was significant in explaining the behavior of the dependent variable. Both coefficients have the expected sign since they contribute positively to income. That is, an increase in Canadian consumption in the context of the USMCA can lead to an increase in bilateral trade, with lower tariff barriers and regulations that favor the flow of goods and services, benefiting Mexican exports and, therefore, its national income.

According to the model results, Mexico's trade volume is expected to continue growing. Therefore, corn food dependence will also increase. Our research determined that national production is the most efficient way to decrease this dependence, with a negative impact of just over 22% on net imports for each change unit (ton) in corn production. This result leads to a consideration of the particularities of the national productive sector. López-González *et al.* (2018) point out that family production is under threat due to the plurality of farmers' activities. This results in a decrease in yield per unit of corn, which, in addition to generating food insecurity, leads to economic problems and job instability.

### **Yellow corn in Mexican food security**

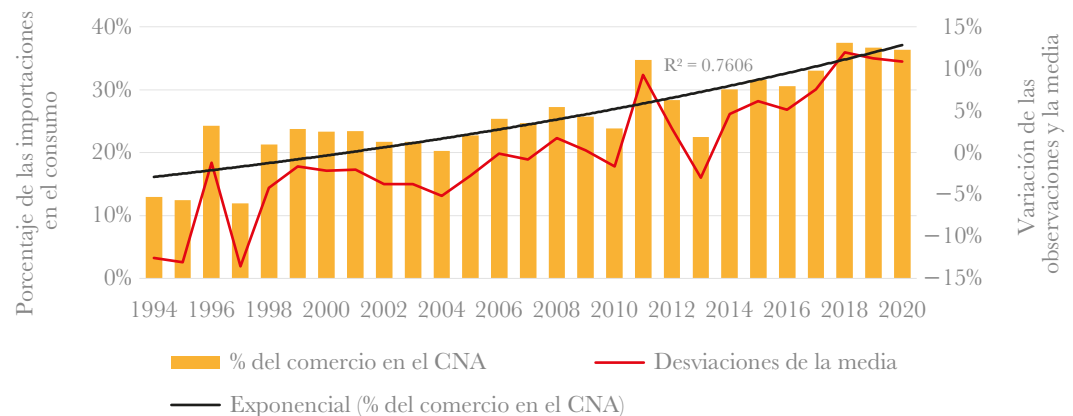
The volume of corn imports in Mexico explains how trade contributes to food security. Trade records analysis must be complementary and differentiate the type

of imported corn to determine its final use in Mexico. Mexican consumers have a deep-rooted gastronomic culture and particular tastes. It is a fact that the Mexican population consumes the corn varieties produced in the country. Hence, imports are predominantly destined for animal diets or industrial processing (IDMAIZ, 2023). Consumption preferences for human use in the country will not change in the short term. Imports confirm the UN's assumption on food security, which postulates that food must be physically available. Compliance with this premise means that trade has contributed effectively to food security.

Figure 1 shows the evolution of corn imports insofar as they contribute to Mexico's consumption. Since the gravity model proved the US to be the most significant country in Mexico's trade flows (more than one-third of the corn consumed in Mexico is imported from that country), the figure shows the commercial records of Mexican imports from the US. The trend line takes an exponential form—a better fit when compared to other functional forms, such as the polynomial form. This line has an  $R^2$  of 0.76, which implies a growth in the share of trade in the country's consumption. Hence, the dependence on imports to satisfy domestic consumption in Mexico is expected to continue growing. This evidence is consistent with the results of the econometric model estimation.

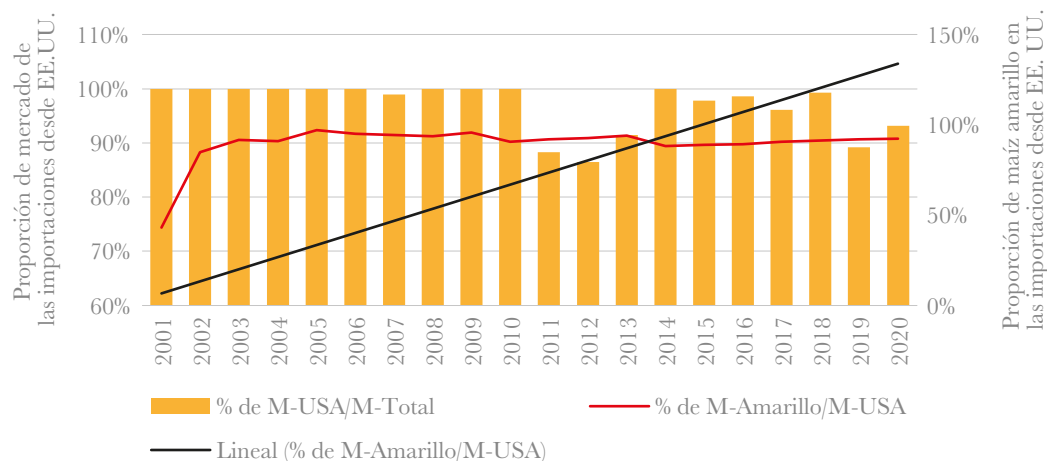
Deviations from the mean in the proportion of trade share growth in national consumption show that growth has occurred rapidly. This trend appears in 10 of the 27 studied periods and can also be identified as the predominant behavior in the last five years. Due to the relevance of imports from the US, the following graph shows the proportion of trade in the total imports of Mexico, as well as the proportion of yellow corn, the variety with the highest import volumes according to Zahniser *et al.* (2019).

Figure 2 shows the importance of the US market for the national food supply. The share of US exports in total imports for the represented period was, on average, 97%, which implies an almost total coverage in the national market. Yellow corn imports account for 87%, a percentage that is growing and reveals Mexico's vulnerability in terms of food



**Figure 1.** Proportion and variation rate of the share of corn trade between the US and Mexico in apparent national consumption.

Source: Own elaboration with data from USDA, SIAP, INEGI, FAOSTAT, and the World Bank.



**Figure 2.** Share of imports from the US in total imports/Share of yellow corn imports in total imports from the US.

Source: Own elaboration using TRADEMAP data.

security for yellow corn. In this regard, FAO states that for food security to exist, national production must satisfy at least 70% of consumption (FAO, 2010).

The trend line has a very steep slope, indicating that the US holds a monopoly on sales to Mexico due to its market share. The remaining corn imports are corn varieties used for extracting high fructose corn syrup—used in the industry—and dry grains. Yellow corn imports are used for animal feed, which is especially important because it has the largest share in the cost structure of livestock production units (Nuñez-Torres, 2017).

## CONCLUSIONS

Guaranteeing food supply by creating programs to ensure food availability is part of the national and international agenda as per the 2030 Sustainable Development Goals. In 2020, one-third (36%) of the Mexican national corn consumption was supplied through imports. Corn imported from the US is of the yellow variety. The USMCA tariff exemption for this product and the geographical proximity between Mexico and the US have contributed to the growth of imported volumes. Therefore, one can conclude that there is food dependency in the case of corn.

International trade, specifically the USMCA, contributes to fulfilling two of the four dimensions of food security highlighted by FAO: physical availability of food and economic and physical access to it—both met through strategies implemented by SEGALMEX. However, to achieve FAO indicators regarding the source of supply for national consumption, strengthening national production is still pending. According to FAO, at least 70% of consumption must be satisfied by national production.

The gravity model determined that the variables impacting the dynamics of international corn trade the most were production, commercial volumes and income, the influence on traded volumes, and the country's purchasing power. According to the estimate's results, national production is the most significant variable and should therefore be encouraged to reduce commercial dependence. Most of the imported corn is not consumed directly



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# Competitive management for export of roses from Mexico to Canada

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## ABSTRACT

**Objective:** To evaluate the behavior of the international market of roses between Mexico and Canada and to propose competitive management strategies that could be implemented in the rural economic units to consolidate the presence of Mexican roses in the Canadian market.

**Design/methodology/approach:** The methodological process to fulfill the objectives of this study uses the deductive approach, since we start from an analysis of the rose market in Canada in order to determine the competitive management strategies. Gathering information was conducted in platforms of agrifood documental information and statistics in the national and international spheres.

**Results:** Mexico has excellent agro-environmental conditions, efficient international logistics, solid infrastructure, a valid trade agreement with Canada (T-MEC), and outstanding experience in agricultural production of roses. In addition, there is a significant demand in the Canadian rose market that has been dominated in recent years and until today by Colombia and Ecuador.

**Limitations on study/implications:** It is a study that could be used as reference for any agricultural producer, rural production society, of international trade company that wants to penetrate the rose market and its exports from Mexico to Canada.

**Findings/conclusions:** Finally, this study evidences the importance of taking advantage of the available commercial opportunities and to promote synergy between the public and private sectors to drive the growth of agroindustry of roses in Mexico and to contribute to economic development of the country. With an intelligent approach and strategic execution, Mexico has the potential of becoming a prominent actor in the export market of roses to Canada in the coming years.

**Keywords:** Agribusiness, social welfare, international trade, cut flowers, competitive management.

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## INTRODUCTION

In recent decades, vegetable products in Mexico have acquired a growing importance, both in the national market and in the international. At present, the global economy and the globalization process have promoted a greater commercial integration between the countries. The main objective of this integration is to maximize the economic benefits through international trade, at the same time generating greater welfare for agroindustrial producers (Tyce, 2020). Egea *et al.* (2021) explain that agricultural production should be connected with science, politics and society, so that it can generate through it a bioeconomy that produces wealth, economic growth, development and employment through conservation, management and reinvestment in ecosystems.

According to the studies performed by Avendaño-Ruiz *et al.* (2023) and Foreign Agricultural Service (2023), the international flower market is dominated primarily by the world's main exporter, the Netherlands, followed by Colombia and Ecuador. In the year 2020, Colombia registered a total export value of approximately US\$ 1,411 million dollars, with an annual mean growth rate of 4.7% for nearly three decades. In order of importance, Ecuador occupies a noted place in this international market. During the period between 1989 and 2020, Ecuador experienced growth of 12%, which demonstrates an outstanding mean annual rate. This growth has consolidated this country as a prominent actor in international flower trade, increasing its competitiveness in face of other international competitors.

According to Darras (2021), the global industry of cut flowers has been facing grave challenges throughout the years, but it still continues to be an important sector of agriculture. Floriculture businesses seek trends and new and innovative niches to increase sales, as well as the implementation of marketing strategies to communicate to the final consumer the practices carried out during cultivation, cutting and post-harvest, which tend towards an ecological profile, low CO<sub>2</sub> footprint, and environmental responsibility. There are diverse factors such as evolution of markets, selection of varieties that adapt to the meta market needs, quality of flowers, optimization of trade, and competitive management which are essential for the sustainable success in this sector (Faust & Dole, 2021).

Throughout the years, the production and commercialization of roses in Mexico has presented a series of obstacles that have prevented its growth and consolidation as a prominent agribusiness. There are several causes identified associated to this predicament, among them the lack of updating in the productive system, lack of knowledge management by those in charge of production, a deficient logistics-distribution system, low level of adoption of administration practices in agribusiness, as well as the lack of knowledge about opportunities in the international market and the basic export process, which has had the consequence of producers not to being able so far to exploit business opportunities in the international market. Therefore, this study approaches a perspective of the importance of international trade as one of the sources of economic development in our country, fundamentally due to the possibilities of market diversification, attaining better prices, addressing the growing demand, and improving the quality of life, which determine a favorable growth potential for this sector in the country.

Floriculture is one of the most profitable agricultural activities that Mexico can have, and the country has the potential to become an important exporter in this sector. Diverse competitive advantages, including the geographic location with commercial ports and terminals in two oceans, border crossings, customs offices, road infrastructure, cargo airports, in addition to proximity to the principal markets, United States and Canada, and the signing of free trade agreements that allow access to international markets (ProMéxico, 2018). Thus, the free trade agreement between the United States, Canada and Mexico (T-MEC) is defined by the internationalization policy of the economy of the commercial block, through a preferential and permanent relationship with a fundamental actor in the global economy, with the aim of gaining access to broader markets, competitiveness, elimination of commercial conditions and barriers,



and technology exchange, a greater growth and economic development (Gobierno de México - SADER, 2019). These references indicate that the country has the experience, the logistic and commercial abilities to occupy an important place in the flower trade at the global level, and in addition they show that there are still many possibilities of growth given that the production level destined to the international market continues to be low.

Therefore, the analysis of competitive management shows great relevance, since it provides an integral view of the internal factors that influence the insertion and positioning of an organization both in the local and international markets (Melo-Perdomo *et al.*, 2018). In a globalized and highly dynamic scenario, the stakeholders of rose agribusinesses face complex challenges that require accurate strategies and a deep understanding of international markets.

This study has the objective of providing the necessary information to serve as a reference to agricultural producers in Mexico, which are considering entering the export market of roses to Canada. This country offers excellent business opportunities for the trade of this product. Therefore, the purpose of this study consists in evaluating the behavior of the international market of roses between Mexico and Canada, and proposing competitive management strategies that could be implemented in the rural economic units to consolidate the presence of Mexican roses in the Canadian market.

## MATERIALS AND METHODS

**Type of research:** Descriptive research will be used.

**Research method:** The methodological process to fulfill the objectives of this study uses the deductive approach, since the starting point is evaluating the behavior of the rose market in Canada and, therefore, proposing competitive management strategies for the consolidation of the export market of roses from Mexico to Canada.

**Search for agricultural information and the demand of the rose market in Canada:** The methodology proposed by Vasco-Leal *et al.* (2022) was followed. The statistical data of roses in Mexico were obtained from sources of information, among others, from the software called *Sistema de Información Agroalimentaria de Consulta* (SIACON). To estimate the potential and value of the rose market in Canada, it was necessary to understand the situation of rose imports in this country, as well as its main trade partners. The statistical information was obtained from the customs duty fraction “060311”, using the international archive of Trade Statistics for International Business Development for the International Development of Companies (<https://www.trademap.org/>).

### SWOT analysis to diagnose the current situation of the export process of roses from Mexico to Canada

According to Ramírez (2017), “The SWOT analysis is a subjective analysis that helps to understand, present, discuss and make decisions. It can be used in any type of decision making”. This analysis is generally presented in matrix form divided into four areas of analysis: Strengths, Weaknesses, Opportunities, and Threats.

## RESULTS AND DISCUSSION

### Agricultural production of roses in Mexico

Table 1 shows that for the year 2021, Mexico had approximately 1,746 hectares of rose crops, which are concentrated especially in the states of Estado de México (863 ha), Puebla (385 ha), Querétaro (125 ha) and Morelos (301 ha). For that same year, the national production of roses in the country reached US\$ 143,923,954, with Estado de México being the top national producer and generating 76.61% of the total economic value of domestic production, followed by the state of Puebla (US\$ 13,765,890), Querétaro (US\$ 11,367,025) and Morelos (US\$ 4,866,189). These results for the year 2021 present a stable trend compared to the year 2018 in the three main producing states of Mexico (Solís-Lozano *et al.*, 2022).

Performing a stricter analysis to understand the dynamics of each of the producing states, the result evidence that Estado de México is the one responsible for 76.61% of the economic value of the production; the main producing municipality is Villa Guerrero (US\$ 58,918,040), followed by Tenancingo (US\$ 27,747,132) and Coatepec Harinas (US\$ 13,998,626), among others. In turn, Puebla contributed 9.56% of the production value in the year 2021, which is distributed mainly in the municipalities of Chiautzingo (US\$ 5,075,089), San Salvador El Verde (US\$ 4,099,761) and Atlixco (US\$ 2,659,916), among others. In Querétaro, the rose production is concentrated specifically in the municipalities of San Juan del Río (US\$ 9,580,227) and Pedro Escobedo (US\$ 1,786,738), which represents 7.90%.

This situation means that there is at least the basic infrastructure, experience and production, so as to have feasibility of volume for a trade process of roses with some type of destiny. According to Velázquez-Torres *et al.* (2021) and De León (2018), the rose agribusiness is of great economic and social importance in the territories, and this is why competitive management strategies should be established by the private sector to obtain better results in the production, business management, economic efficiency, as well as the public sector to facilitate guidelines through public policies and institutional reinforcement that improve the quality of life of the members of the primary sector and their rural communities.

**Table 1.** States that produce roses in Mexico 2021.

Mexican state	Planted area (ha)	Production (gruesa)*	Production value (US\$)	(%)
México	863	7,416,230	110,257,586	76.61
Puebla	385	720,011	13,765,890	9.56
Querétaro	125	573,642	11,367,025	7.90
Morelos	301	696,778	4,866,189	3.38
Jalisco	34	150,498	2,900,478	2.02
Oaxaca	21	12,824	393,548	0.27
Hidalgo	8	7,768	183,333	0.13
Guerrero	7	7,240	135,544	0.09
Tlaxcala	2	2,220	54,361	0.04
Total	1,746	9,587,211	143,923,954	100

Source: Prepared by the authorS with data obtained from SIACON (2023).

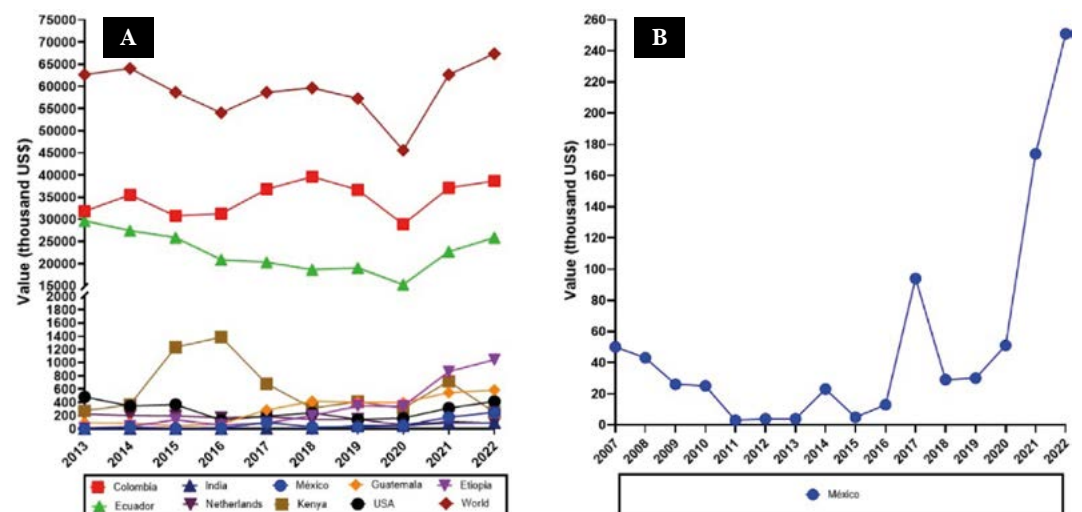
Note<sup>1</sup>: 1 gruesa is 144 rose stems. Note<sup>2</sup>: 1 US\$ dollar = 16.74 MX\$ pesos.

### Behavior of the import market of roses in Canada

In a globalized, highly dynamic scenario with much evolution, the actors from this agribusiness face complex challenges that require accurate strategies and a deep understanding of international trade. In recent years, the international market of roses has experienced a moderate growing trend without significant changes. However, as Figure 1 clearly shows, in the year 2020 there was a decrease in international rose trade due to the COVID-19 pandemic.

Next, Figure 1A presents a series of data obtained from TRADEMAP (2023) which show the behavior of imports in Canada between the years 2013 and 2022, according to the classification of the customs duty fraction 060311. It is estimated that the results from the global rose market for the year 2022 reached approximately US\$ 3,013,231,000 dollars. At the international level, for the year 2022, the main imports in Canada come from Colombia (US\$ 38.7 million) and Ecuador (US\$ 25.8 million). In second place, there are imports from Ethiopia (US\$ 1.0 million), Guatemala (US\$ 584 thousand), United States (US\$ 412 thousand), Mexico (US\$ 249 thousand), Kenya (US\$ 249 thousand), India (US\$ 92 thousand), and the Netherlands (US\$ 88 thousand).

Considering the results, Colombia presents the highest participation in the Canadian market, going from US\$ 31.8 million to US\$ 38.7 million, between the years 2013 and 2022. This behavior responds to the excellent agroclimatic conditions of this country, reflected in products of excellent quality (Rivera, 2021). Likewise, given the experience of Colombian agro-exporters there are standardized negotiation processes, agile commercial solutions, in addition to proximity of producing zones to international airports. In this sense, according to Pinzón-Muñoz *et al.* (2022), Colombia strives to strengthen its competitiveness in the international market. To achieve this, the consolidation of new products and the positioning of the broad variety of flowers available in the flower producing sector are essential, with the objective of successfully entering the international markets. Colombia currently has the



**Figure 1.** Value of imports in Cadada in the rose market. A) Import of roses from the main countries (2013-2022). B) Import of roses from Mexico (2007-2022). Source: Prepared by the authors with data obtained from TRADEMAP (2023).

signature of a Free Trade Agreement with Canada, which benefits it with 0% customs duty in this type of products (Ministerio de Comercio, Industria y Turismo, 2011), aspects that hugely benefit this agribusiness. It should be mentioned that it also has great challenges in logistics and conservation technologies that have not allowed it to consolidate and position itself further in international markets.

For its part, the participation of Ecuador in the years from 2013 to 2022 presents a decrease in the behavior of the market of roses in Canada, going from US\$ 29.7 million in the year 2013, to the lowest sale average of US\$ 15.3 million in the year 2020, until reaching US\$ 25.9 million for the year 2022. The great waver of this country is in relation to the innovation in the final product with special interest in the trade of roses of excellent quality. Flower producers have made investments in improving their knowledge, abilities and capacities, in addition to adopting technologies which benefit the differentiation of the product exported. In a similar way, the great challenges that must be faced are recognized, due to the high production costs and logistic processes for export. For the year 2023, Canada established a customs duty of 10.5% for the entry of flowers from Ecuador (Canada Border Services Agency, 2023), becoming a disadvantage compared to other competitors.

Finally, for the case of the import of roses from Mexico, Figure 1B) evidences the behavior between the years 2007 to 2022. The behavior has been very fluctuating in the years studied, with unprecedented results for the years 2021 (US\$ 174 thousand) and 2022 (US\$ 249 thousand), respectively. These results show the current interest that has been generated by this product in the Canadian market and the business opportunities that can be established benefitting international trade. Mexico has agricultural production, standardized export processes, specialization in logistics, a Free Trade Agreement (T-MEC) which benefits it with 0% of customs duty (SIICEX/CAAAREM, 2023), elements that make Mexico a strategic ally with a promising future in the region.

Without a doubt, this is a great opportunity for agricultural producers of roses in our country, which often express their desire to obtain a better remuneration for their production. However, they face a scarce number of options in terms of trade channels, in addition to lacking counseling regarding foreign trade, which leaves them with very little information available. In their search for new opportunities, they have started to explore foreign markets with the hope of obtaining higher profits thanks to more favorable prices or, alternately, an increase in the volume of sales. It is important to highlight that the export business is not limited exclusively to large companies. Thanks to the advances in information technology, communication and transport, management has become more accessible, regardless of the size of the business, which could doubtless benefit the small-scale rural economic units.

### **Import of roses in the Canadian market (2022)**

For the year 2022, Table 2 shows that Colombia and Ecuador represent 95.8% of the imports of roses under the customs duty fraction 060311. This situation shows a very high penetration into the Canadian market by these two countries, which represents approximately US\$ 64.5 million from a total of US\$ 67.3 million. Meanwhile, Mexico has a very small participation of only 0.4% of the total imports. Although reviewing the growth rate (2022 *vs.* 2021), it presents the highest growth (43%) compared to the other importing

countries, followed in order of growth by the United States (32%), Ethiopia (21%), Ecuador (14%), among others. Another one of the aspects to analyze is the price of the unit value, which was on average for the countries at US\$ 4.57 per dozen; Mexico attained a better quote, receiving payment of US\$ 7.98 and at the same time far exceeding the behavior reached by countries like Colombia, Ecuador and India. This can be verified in Table 2, which could be because of scarcity of product in this lapse of time, production costs, limited offer of the producing countries, increase in demand, and variation in exchange rate, among others.

Until now, we have identified that Mexico has many aspects that can be key to drive this highly competitive national agroindustry. However, the country faces important internal and external factors that will have to be addressed to reach its full potential. Next, Table 3 describes the most outstanding aspects about the strengths, weaknesses, opportunities and threats from the international market of roses between Mexico and Canada, which ought to be analyzed in greater detail for managerial decision making.

### Competitive management for the consolidation of the export market of roses from Mexico to Canada

The consolidation of the export market between Mexico and Canada could have significant benefits for the growth of the agricultural production of roses in the current producing states, as well as for the opening of new cultivation areas in potential states. This could generate productive projects with positive impacts in the economy, society and rural communities, as well as in the creation of opportunities that promote the interest for primary production, the creation of formal employment in rural communities, rural development and generational replacement that attracts the attention of young people. Table 4 shows the proposals for strategies based on competitive management that could be implemented in the rural economic units.

**Table 2.** Import of roses in the Canadian market (2022) –customs duty fraction 060311.

Countries	Value imported in 2022 (thousands of USD)	Import share for Canada (%)	Quantity imported in 2022* (dozen)	Unit value (USD/unit)	Growth rate of imported values between 2021 and 2022 (% p.a.)	Average tariff (estimated by Canada)
World	67,386	100	14,752,069	4,57	8	-
Colombia	38,668	57.4	7,363,203	5,25	4	0
Ecuador	25,886	38.4	6,456,108	4,01	14	10.5
Ethiopia	1,044	1.5	383,776	2,72	21	0
Guatemala	584	0.9	408,189	1,43	7	10.5
USA	412	0.6	31,268	13	32	0
Kenya	331	0.5	44,758	7,40	-54	10.5
México	249	0.4	31,200	7,98	43	0
India	92	0.1	20,200	4,55	4	10.5
Netherlands	88	0.1	9,714	9,06	-17	2.5
Other countries	32	0.1	3,653	-	-	-

\*Note: 1 dozen is 12 units of rose stems. Source: Prepared by the authors with data obtained from TRADEMAP (2023).

**Tabla 3.** Strengths, Weaknesses, Opportunities, Threats Analysis.

Strengths	Opportunities
<ul style="list-style-type: none"> <li>• The country is strategically located close to Canada, which represents competitive advantages in export logistics over other competing countries (Colombia, Ecuador, Ethiopia, Kenya, Guatemala).</li> <li>• Mexico is among Canada’s top 10 trading partners in the import of roses identified with tariff item (060311).</li> <li>• The country has extensive experience in exports, including perishable food products, which facilitates the logistical handling of roses.</li> <li>• By 2023, Mexico has a rose production area of approximately 1,746 ha, which represents growth compared to other years.</li> <li>• Mexico has a long history of rose production, which implies that there is knowledge of agricultural practices, use of technology, and skilled labor, among other things.</li> <li>• Mexico has international logistics infrastructure (airports, highways, seaports, and customs).</li> <li>• Production and labor costs can make the supply price more competitive.</li> <li>• Confidence in trade relations between Canada and Mexico.</li> <li>• Experience in the logistics process of agricultural products between Canada and Mexico.</li> <li>• Public policies that encourage agricultural production and the social welfare of producers.</li> </ul>	<ul style="list-style-type: none"> <li>• Access to the Canadian market through the T-MEC trade agreement.</li> <li>• Productive economic units in the state of Mexico with entrepreneurial capacity and export experience.</li> <li>• Canadian marketers are open to new international suppliers that meet quality, delivery time, and price standards.</li> <li>• The rose-producing states in Mexico offer suitable agro-climatic conditions for the formulation and development of rose growing projects.</li> <li>• Organize groups of rose growers with interest in the export market.</li> <li>• Canadian consumers interested in products free of residues and/or toxic traces.</li> <li>• Fair trade tendency on the part of the specialized niche market</li> <li>• Demand for product at specific seasons (Mother’s Day, Valentine’s Day, Thanksgiving, Christmas) and other holidays.</li> <li>• Attract foreign investment by developing greenhouse projects in the producing areas to increase flower production in the country.</li> <li>• Establish a distribution center specialized in flowers that articulates the national production to Canada, the United States and other countries.</li> <li>• Diversification of agricultural crops that are related to different value chains and have an impact in terms of competitiveness in the markets.</li> <li>• Manage agreements and strategic alliances between the public, private and/or social sectors.</li> <li>• Generate added value through the creation of products such as oils, soaps and perfumes.</li> <li>• Agricultural production of roses in Canada is very limited due to agro-climatic conditions.</li> </ul>
Weakness	Threats
<ul style="list-style-type: none"> <li>• Domestic agricultural production is mainly focused on the domestic market.</li> <li>• In some Mexican states, agricultural producers do not have patents on the materials used, which makes the export process impossible.</li> <li>• Non-compliance with royalty payment requirements for plant varieties marketed under UPOV 78 and the new UPOV 91 revision.</li> <li>• Failure to pay patents to breeders on some occasions, resulting in products that are unattractive to the Canadian market.</li> <li>• Lack of organization and administrative management of production units to comply with quality requirements and export certifications.</li> <li>• Deficiency in logistical infrastructure (cold chain) to ensure that the quality of production meets export requirements.</li> <li>• Aging of agricultural producers and lack of generational replacement to carry out farm work.</li> <li>• Scarce product quality certifications.</li> <li>• Adverse environmental effects due to the irrational use of resources, contamination of water, soil, air, and the disposal of plastic waste, among others, causing environmental alterations.</li> <li>• Risk of contracting diseases due to the misuse of agrochemicals in agricultural production.</li> </ul>	<ul style="list-style-type: none"> <li>• Implementation of attack strategies by other competing countries.</li> <li>• Shift to other types of flowers (Alstroemeria, chrysanthemums, orchids, tulips, gerberas, etc.).</li> <li>• Increase in phytosanitary import requirements by the CFIA (Canadian Food Inspection Agency).</li> <li>• Decrease in the price of flowers in the market.</li> <li>• Higher labor costs.</li> <li>• Exchange rate volatility that destabilizes the market.</li> <li>• Sanctions against growers under Mexico’s plant variety law.</li> <li>• Vulnerability to rising international oil prices, as chemical inputs such as urea, plastics, and freight costs tend to increase in response to higher hydrocarbon prices.</li> <li>• Economic crisis (financial crisis, economic slowdown, pandemic, etc.).</li> <li>• High exposure to uncontrollable factors such as climate change (hailstorms, frost, excessive rainfall, winds), diseases and pests.</li> <li>• Natural disasters (floods, landslides, etc.).</li> <li>• Deficient research and development of new varieties and production techniques at the national level by academia.</li> </ul>

Source: Prepared by the authors.

**Table 4.** Competitive management strategies for the export of roses from Mexico to Canada.

<b>Organization of agricultural producers</b>	Producers should promote the organization of their members in order to have the possibility of obtaining joint benefits, for example, in the processes of purchasing and negotiating agricultural inputs. As well as for access to programs, support and financing to improve their production practices and systems. Finally, they should strive for legal organization, which will allow them to market their products for export.
<b>Administrative organization</b>	The producers must be formally constituted from the administrative, fiscal and legal point of view, in order to be able to exercise the pertinent controls and have certainty of the results of their actions, in order to be able to offer their products at competitive prices and enter the international market.
<b>Information management</b>	It is essential to take advantage of the information available to standardize and guarantee the traceability of processes in areas such as production, packaging and logistics, in order to reduce costs. In addition, this information can be used to forecast and predict market behavior, identify price trends and perform customer analysis, all of which will benefit strategic decision making.
<b>Commercialization</b>	Through negotiation processes, establish favorable commercial agreements with buyers at the final destination. Market research, prospecting, contract farming, market intelligence, consumer behavior, among others.
<b>Commercial missions</b>	Identify potential provinces and territories in Canada with interest in the Mexican product, followed by attending trade events, advertising in specialized magazines, traditional advertising campaigns and social networks, to publicize the Mexican product.
<b>Reinvestment plans in rural economic units</b>	Agricultural producers should work on developing plans for future reinvestment in their production units to help improve productivity through access to training and the use of information technologies.
<b>Technology management</b>	Existing and planned infrastructure, machinery and equipment must be adequate to establish production systems suitable for export. The facilities must comply with a minimum recommended design to be able to apply greenhouse production management techniques that ensure conditions for air circulation, humidity and adequate lighting for the production of high quality roses. Specialized infrastructure is required for post-harvest handling of export roses to preserve, grade, and pack the roses according to international export standards, as well as facilities that comply with occupational health and safety conditions.
<b>Technology for agricultural production</b>	Good agricultural practices should be applied to ensure the production of high quality roses, including fertilization, pest control, pruning, disease control and above all, the indiscriminate reduction of agrochemicals, since export flowers tend to be better accepted when production practices are environmentally friendly.
<b>Quality control</b>	As production processes become more efficient and focused on obtaining products with export characteristics, quality controls will be implemented to select the best flowers for export, taking into account aspects such as color intensity, stem thickness and length, quality of the rose bud, and crop management during harvest to ensure that the flowers are harvested without affecting their physical appearance.
<b>Export logistics</b>	Producers must have a transport system that ensures the export specifications, that there is an adequate cold chain and that the transport for export is adequate. In this aspect, it is also necessary to have the appropriate advice to comply with the characteristics of export logistics.
<b>Certifications</b>	Focus on advancing crop and final product certification, with emphasis on the ecological profile, sustainable production and low CO <sub>2</sub> footprint. As well as sustainable water use, waste management, rational use of agrochemicals and occupational health and hygiene for workers.
<b>Regulations</b>	For imports, it is essential to establish pest and disease prevention processes, as well as adequate post-harvest handling. This must be accompanied by compliance with minimum quality requirements, which include obtaining an import permit, presenting phytosanitary certificates and complying with labeling and labeling standards.
<b>Plant Variety Property Rights UPOV 1991</b>	For marketing purposes, especially for export, agricultural producers will have to abide by the regulations incorporated in the T-MEC regarding intellectual property. Thus, they will have to take into account the provisions of UPOV 1991, which is important in the export of roses from Mexico to Canada because it protects the property rights of breeders and complies with international standards and requirements.

Source: Prepared by the authors.

## CONCLUSIONS

This study assessed in depth the perspectives of export of roses from Mexico to Canada. The results evidence a promising market and a growing demand from the Canadian market, which suggests significant opportunities for Mexican rose producers. In addition, competitive management strategies have been proposed which could strengthen Mexico's position in the export market of roses to Canada. The combination of the quality of Mexican products, the experience of complying with high international standards, the geographic proximity to Canada, the free trade agreement (T-MEC), and the competitive management strategies proposed position Mexico favorably in this market. However, it is essential for agricultural producers and government authorities to work hand in hand to overcome the regulatory and logistic challenges that can emerge in the export process. Finally, this study shows the importance of taking advantage of available commercial opportunities and promoting collaboration between the public and private sectors to drive the growth of the rose agroindustry in Mexico and to contribute to the economic development of the country. With an intelligent approach and a strategic execution, Mexico has the potential of becoming a prominent actor in the export market of roses in Canada for the coming years.

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# The regenerative and multifunctional livestock value network in La Antigua Basin, Veracruz, Mexico

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## ABSTRACT

**Objective:** To identify and to characterize production units (PUs) and economic and non-economic stakeholders that belong in the regenerative and multifunctional livestock value network in La Antigua Basin, Veracruz.

**Design/methodology/approach:** Farmers with regenerative and multifunctional livestock production, who were selected by directed and snowball sampling, were interviewed with semi-structured interviews with key actors, and review of documental information was carried out.

**Results:** Producers who manage their livestock systems based on agroforestry, with water management were identified, both in harvesting, storage, caring for water springs and efficient use; and who, in addition, promote composting of organic residues and grassland management. Livestock feeding is carried out in grasslands with support of electric fencing. In general, the PUs present 20 years since their production began, with a size of 11 ha; decision makers are 57 years old, and education profile of undergraduate and graduate studies, 57% with agrarian sciences orientation; and they employ 4 people. The value network is characterized by non-economic actors, mainly those that complement, who promote regenerative and multifunctional livestock production through consultancy, training and financing of projects.

**Limitations on study/implications:** The need to promote a participative innovation agenda is identified.

**Findings/Conclusions:** In La Antigua Basin, various economic actors were found that apply regenerative and multifunctional livestock production as their productive system, where they have found strengths to maintain or recover their natural resources, and based on the characteristics of their products they recognize the market segments where they must be destined.

**Keywords:** value chain, silvopastoral grazing, agroforestry, rotational grazing, associativity.

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## INTRODUCTION

In La Antigua Basin (Veracruz, Mexico), various agriculture and livestock activities have emerged and evolved, from conventional productive systems to those that seek to be more friendly with nature (Torres-Rivera and Palma-García, 2021), such as regenerative and multifunctional livestock production systems.

Regenerative livestock production has demonstrated benefits such as improvement in the soil structure, increase in organic matter contained, and reduction of erosion, which are reflected in better soil health, more biodiversity by providing habitat for plants and

animals, improved water quality, reduction in nutrient and sediment contamination, climate regulation, reduction in greenhouse gas emissions by sequestering carbon in the vegetation and in the soil (Schreefel *et al.*, 2020; Spratt *et al.*, 2021).

When regenerative livestock production is combined with multifunctional agriculture, the income of livestock farmers increases with the production of quality meat and milk that can be sold at a higher differentiated price, and the conservation of their production units (PUs) is strengthened with a holistic view that allows them to detect new products to be traded (Alquiler *et al.*, 2009). Therefore, regenerative and multifunctional livestock production requires analysis with an approach of economic and social assessment that brings together the views of protagonist actors in the activity and of those who also participate indirectly.

Based on this, the value network analysis proposed by Nalebuff and Brandenburger (2005) is a tool that allows visualizing the interactions of the network's actors: producers-agroindustry, those who complement, competitors, clients and suppliers, in addition to economic and non-economic actors, with the objective of identifying the actions that contribute to the creation of value or wealth and in order to generate strategies to strengthen the production units in the territories (Muñoz and Santoyo, 2011). Thus, the objective of this study was to identify and to characterize production units and economic and non-economic actors that participate in the regenerative and multifunctional livestock value network in La Antigua Basin, Veracruz, Mexico.

## MATERIALS AND METHODS

The study was carried out in La Antigua Basin, Veracruz, which occupies an approximate surface of 2 176 km<sup>2</sup> and originates in the Sierra Madre Oriental at an altitude of 3350 masl. In central Veracruz, it covers 20 municipalities; the municipalities of Calchahuaco and Huatusco include a small portion of La Antigua Basin.

The methodological process consisted of the following phases: 1) PUs with regenerative and multifunctional livestock production were identified and characterized, and their value network was determined through the methodology proposed by Nalebuff and Brandenburger (2005), adapted by Muñoz and Santoyo (2011). The analysis of key actors was carried out to have a view of the environment; stemming from this, the dynamic of the value network was developed through the analysis of the role of those who integrate it. 2) The procurement of information from stakeholders was conducted from June to September, 2023, with interviews performed in situ (livestock ranches, government offices, among others). 3) A questionnaire was applied, which included two sections: i) Profile of the actor: name, date of birth, location, level of education, contact information; and ii) Dynamics of the activity in the network (producers-agroindustry, those who complement, competitors, clients and suppliers): years of experience in production, animal and agricultural species, economic importance of the activity, percentage of income, staff that supports in the activity, data from the farm, type of production, market or markets, advantages and disadvantages both of the activity and of the PU, and opinions about associativity and cooperation.

The systematization and analysis of information from the value network was carried out through qualitative processes, as well as through quantitative processes (Santoyo *et al.*, 2002).

## RESULTS AND DISCUSSION

### Identification and characterization of regenerative and multifunctional livestock production cases

Five cases of PUs with regenerative and multifunctional livestock production were detected in the basin, which have an interrelation of livestock production with agriculture, allowing them to be multifunctional, since they do not depend solely on one type of product to trade (Table 1).

In the first link, generally the PUs practice agroforestry, protection and improvement of the soil, and moisture retention in their plots. All of them carry out water management, both through harvesting, storing and caring for natural springs, and with an efficient use. Animal excretes are used, where wastes are reincorporated into the soil through composting of organic residues and the management of grasslands; this is linked to what was described by Schreefel *et al.* (2020).

On average, PUs started their production  $20 \pm 13.3$  years ago and have  $11 \pm 17.3$  ha, and decision makers have undergraduate and graduate studies; 57% are oriented toward the area of agrarian sciences, age of  $57 \pm 17.3$  years, and employ  $4 \pm 1.7$  people constantly.

In animal species, laying hens stand out in every PU; 50% of them have Mexican Creole hairless pigs or crosses with this breed; 50% have milk-producing goats; and in a smaller proportion there is livestock for meat, Melipona bee, sheep and tilapia. Feeding in every PU is based on grassland rotation with the support of an electric fence, protein banks and use of grains or concentrate as complement; 50% of the PUs use residues or silage.

**Table 1.** Main activities developed in the links of the regenerative and multifunctional livestock value chain in La Antigua Basin, Veracruz, Mexico.

Linkage	Livestock products	Agricultural products
Primary Production	Range-fed pork Range-fed beef Stable-produced goat milk Range-fed chickens for eggs Bee honey Aquaculture	Planting forage and protein banks Coffee Fruit trees: orange, lychee, macadamia, banana Vegetables Aromatic and medicinal plants Spices Production and sale of seeds
Processing	Cuts of meat Sausages Goat milk cheeses: matured, semi-ripe and unconventional Milk cheeses: semi-ripe and fresh Goat-bovine, goat-sheep milk mixture cheeses Recipes made with pork and goat meat Typical goat milk desserts	Preserves: jams, jellies, pickles, and more Dehydrated Essence extracts Primary coffee transformation, coffee roasting and grinding processes Sale of seedlings and cuttings Humus and lixiviate Compost
Market	Products Local: on-site, repeat buyers, referrals, tourists Regional: agroecological markets (Xalapa, Coatepec), specialized stores, social networks State and national: specialized stores, sales through digital platforms Services Farm tours, tastings or product pairings	

All the PUs process part of the production and some systems stand out where 100% of the production is processed for its sale as differentiated meat, transformed into cold meats or regional dishes, and in the case of milk into cheeses from semi-mature to mature with fungus cover. Egg is the only product that is destined to fresh sale or for subsistence.

In terms of agricultural products, a part is sold fresh, such as cherry coffee, macadamia, litchi, and fruits in general. In every PU, at least one product is elaborated as a preserve, such as jam, jelly, pickles, ferments, extracts or dehydrated products; and for coffee, 33% of the PUs applies processing, drying and toasting. As complement to the activity, plants and cuttings are produced, which provide extra income to the PUs for their management or as material to reincorporate into the farms.

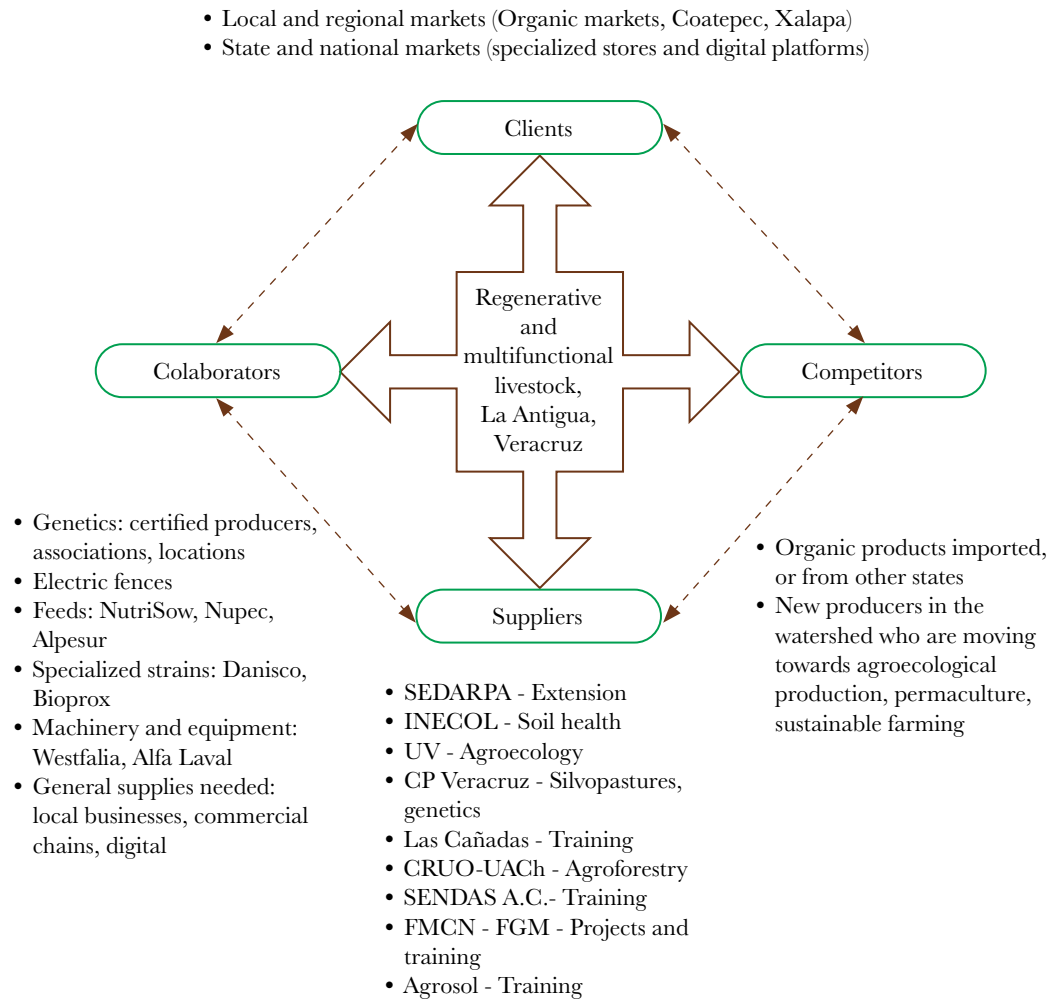
From the total PUs, 66% manage agricultural residues, livestock excretes or other organic residues, through the elaboration and use of composts or vermicompost and the use of lixiviate. In all the PUs with grazing livestock, there is a preference not to use agrichemical products that affect soil biodiversity, and the ecosystem services that macro and microorganisms provide are recognized to reincorporate residues to the soil, as well as the advantages to incorporate nutrients to the soil and air and water infiltration.

In general, the PUs have a well-defined market or segment to which they send their product, and recognize the existence of markets in Xalapa and Coatepec, Veracruz, which add value to the products with agroecological and organic management; however, the PUs also place their products outside the region, in cities such as CDMX, Guadalajara, Monterrey, Puebla, Oaxaca, Estado de México, among others, where they have found allies to enter these markets through specialized trading companies in agroecological or organic products. Of the PUs, 66% receive support from trading companies to place their products, the rest depend on the positioning that they presently have and their products are sold directly to the consumer.

### **The value network**

The regenerative and multifunctional livestock value network in La Antigua Basin, Veracruz, is presented in Figure 1. The five PUs with regenerative and multifunctional production in the basin are identified as trailblazing companies, which are next described in general.

**Agrosol-Educación activa, servicios y producción, S. C. de R. L. de C. V.** Located in Zoncuantla, municipality of Coatepec, Veracruz, is characterized by promoting animal production with grazing with electric fence. Since 1982, Agrosol was defined as a training organization for producers, students and apprentices, through dual education (Dual German training system), with the topics of agroecology, agroforestry, participant research, importance of livestock equipment, and agroecological products as its main themes. It has hired staff, labor from cooperative partners, interns and apprentices. This PU is an example of integration of agricultural, livestock and forestry production, multi-species and multi-plant. The main products that they trade are training (dual training), project elaboration, counseling and facility visits; sale of dairy, meat products and fruit preserves, with their main clients being producers in general, students and research centers.



**Figure 1.** Schematic representation of the regenerative and multifunctional livestock value network in La Antigua Basin, Veracruz. SEDARPA: Secretariat of Agricultural, Rural and Fisheries Development of the state of Veracruz; INECOL: Institute of Ecology; UV: Veracruzana University; CP: Postgraduate College; CRUO-UACH: Eastern Regional University Center-Chapingo Autonomous; SENDAS A.C.: Civil Association Paths and Meetings for Sustainable Autonomous Development; FMCN-FGM: Mexican Fund for Nature Conservation-Gulf Mexico Fund.

**Granja “Don Nelo”.** It is located in Pacho Viejo, municipality of Coatepec, Veracruz. The PU emerged in 2004 with goat dairy livestock, to produce mature cheeses. The milking goats are stabled and fed with fodder banks, primarily mulberry (*Morus alba*), fodder cane (*Saccharum* spp.), cuba 22 grass (*Pennisetum purpureum* × *Pennisetum glaucum*), use of residues from citrus juice-makers, brewery and bakery. As a service, it offers guided visits in the farm, which end with tasting and pairing of their products; another income is from offering training courses inside and outside their facilities.

Its market is regional to national, its clients and referred ones attend the PU directly, and the commercial evolution of the “Don Nelo” Farm has been strengthened by its collaboration with education and research institutions, participation in communication media, and presence in pairing events. This farm is an example of multifunctional

livestock production, since in addition to goats, it produces Mexican Creole hairless pig meat, takes advantage of organic residues to generate compost, leaf fertilizers and biogas, sells seedling and cuttings from their fodder banks, among other products, which agrees with the concept of a multifunctional farm (Torres-Rivera and Palma-García, 2021).

**El Risueño Ganadería Regenerativa.** It is located in the localities of Jalcomulco and Coatepec, Veracruz. The PU started activities in the year 2018, it has Tropical Creole Dairy (TCD) and Romosinuano cattle, Mexican Creole Hairless (MCH) pigs, and Creole laying hens; it has a certificate as supplier of organic products farmed in rotational grazing, management of endemic plant species, restoration of the ecosystem, water harvest and use of electric fencing. El Risueño currently processes beef and pork, it sells specialized cuts, cold cuts and finished meat products. Its market is local to national, with support from specialized stores that trade gourmet and organic products with private customers and in restaurants.

**Tentlanman Chantico cooperative edible forest.** It is a PU organized as a cooperative society, located in Tlanalapan, in Coatepec, Veracruz. It keeps various livestock and agricultural species in 1.4 ha. From its main products, goat milk and unconventional cheeses stand out, as well as vegetable production, hen eggs, coffee, and fruit trees, all produced agroecologically. Its main markets are the agroecological ones in Coatepec and Xalapa. The promotion of the production system is done through visits for the public in general, staff from institutions or organizations.

**Finca Rey Luna.** This PU is found in the locality of Baxtla, municipality of Teocelo, Veracruz, and it has two hectares of space. It self-defines as an agroecological farm, which uses animal species such as sheep, laying hen and tilapia, in addition to cultivating coffee, banana, vegetables, various fruit trees, spices, aromatic plants, among others. The main products it trades are: sheep meat, egg, roasted and ground coffee, and banana, primarily in the agroecological market of Coatepec and with clients that go directly to the farm.

In the surrounding areas of La Antigua Basin there are other PUs that are part of the actors referred, one of them being Las Cañadas, Centro de Agroecología y Permacultura, located in Huatusco, Veracruz. Another one is Mandumed Granja Ecológica, located in Lechuguillas, Vega de Alatorre, Veracruz. Both PUs maintain ties of cooperation and collaboration through the sale or exchange of animals, plant species, ecological technologies, and training.

**Actors who complement:** Among those who complement, a series of institutions, organizations, and other actors were detected, which are recognized by the PUs as actors that promote, drive and provide support to maintain a regenerative and multifunctional livestock production system (Figure 1).

It was possible to perceive recognition among economic and non-economic actors, where they distinguish the advances and evolution of their PUs, as well as the interactions with other actors such as research centers or civil associations. In addition, unspoken associativity by the PUs is observed, where technologies and animal and plant species are shared, among others, which can be improved through plans and strategies with specific actions for cooperation (Granados-Sánchez *et al.*, 2016). Cervantes-Escoto *et al.* (2013) recommend consolidating the articulation of the various actors, to strengthen the



values of reciprocity, solidarity, assertive communication, and trust, and to identify their areas of opportunity and strategies. The relationships between producers and those who complement can also be observed by the actions of technology transference, research studies, and their dissemination (Ireta-Paredes *et al.*, 2020).

To promote regenerative and multifunctional livestock production in La Antigua Basin, the recommendation is to elaborate an innovation agenda, with the collaborative participation of the network's actors (García-Rodríguez *et al.*, 2022), where actors such as those who complement propose ordered actions. For example, the adequate legal figure of the PUs in the basins to decrease the discouraging factors of associativity, due to the requirements and obligations that are acquired and the promotion of inter-institutional collaboration with state programs of Secretariat of Agricultural, Rural and Fisheries Development of the state of Veracruz (SEDARPA) and federal ones from Secretariat of Agricultural and Rural Development (SADER), such as the Milk Technical Support Strategy (EATL) in the central zone of Veracruz and the research institutions.

## CONCLUSIONS

In La Antigua Basin, there are economic actors that apply regenerative and multifunctional livestock production in their systems, where they have found the strengths to maintain or recover their natural resources, and due to the characteristics of their products, they recognize the segments of the market where they should be destined. The value network is characterized by an interaction between owners of the production units and those who complement; through technology transference, counseling, training, and financing of projects, they promote the activity of regenerative and multifunctional livestock production, which allows documenting the degree of connection between actors.

## ACKNOWLEDGEMENTS

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# Characterization of tilapia (*Oreochromis niloticus*) production system in the Zacatecas, Mexico

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## ABSTRACT

**Objective:** To characterize the tilapia (*Oreochromis niloticus*) production system in Zacatecas, Mexico.

**Methodology:** Seventeen Aquaculture Production Units (APU) in Zacatecas were analyzed, using a cross-sectional design and qualitative-quantitative method. The instrument used was a questionnaire, and the sampling was non-probabilistic.

**Results:** Tilapia cultivation is conducted in various types of ponds, with an average of 2340 organisms per pond, yielding a production of 5705.88 kg year<sup>-1</sup>, and a sale price of \$116.47 pesos kg<sup>-1</sup> of fresh gutted fish at the farm gate. Production costs are primarily associated with concentrated feed and electrical energy. Regarding the financial characteristics analyzed, APUs are categorized into four types of production: basic, as a complementary activity with limited economic resources; pre-intermediate, characterized by basic infrastructure and managed by adult aquaculturists (49-55 years old); Intermediate, full-time dedication with average profitability; and high-intermediate, with optimal infrastructure and activity managed by adult aquaculturists aged 30-55 years with a propensity to adopt technological innovation in aquaculture.

**Limitations/ Study Implications:** No major limitations were identified.

**Conclusions:** In general, aquaculturists perceive stagnation in their activity due to lack of promotion, production scheduling, and insecurity. It is necessary to develop an inter-institutional strategy where universities, research institutions, and government agencies participate to promote elements of policy, financing, and training.

**Keywords:** *Oreochromis niloticus*, sustainability, rural aquaculture.

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## INTRODUCTION

One of the most important challenges of the 21st century is the global need for food. In Mexico alone, the Comisión Nacional de Pesca y Acuicultura (National Fisheries and Aquaculture Commission) reports that an additional supply of food from aquaculture will be necessary to maintain the current level of per capita consumption (12 kg/person/year). Therefore, solutions that include sustainable aquaculture (with the implementation of renewable energy systems, bioculture systems, or polycultures) will be required rather than the promotion of a single aquaculture strategy.



Aquaculture is one of the main sources of protein in the human diet (Naor *et al.*, 2021) and is considered one of the least harmful activities to the environment (Goyal *et al.*, 2021). In addition, fish contains a variety of amino acids (leucine, isoleucine, threonine, methionine-cysteine) and fatty acids such as omega-3 (Khanjani *et al.*, 2022) that are essential for the human diet.

Among the main aquaculture crops worldwide are cichlids, such as tilapia, which represent 80% of production (Betanzo *et al.*, 2021) and are the second most cultivated fish, due to their economic viability and pleasant flavor (Le *et al.*, 2022). Their extensive production is attributed to rapid growth, high acceptance of artificial feeding, resistance to stress or diseases, and ability to reproduce in confinement (Mugwanya *et al.*, 2022).

Currently, China is the main producer and exporter of tilapia while Mexico ranks ninth. Furthermore, Mexico is among the main consumers of this product according to CONAPESCA, (2020).

Intensive aquaculture systems provide controlled management involving factors such as feed quality, population density, feeding rate, feeding frequency, and water quality (temperature, dissolved oxygen, pH, salinity, and ammonia) (Khanjani *et al.*, 2022). Tilapia is a cold-blooded fish that requires its body temperature to match the water where it grows, which directly correlates with the growth curve. Under optimal conditions (28 °C to 32 °C) it can multiply its weight tenfold, reaching 500 g fish in six months (Enciso *et al.*, 2019). Therefore, the production of tilapia in a controlled environment represents a complementary development activity for arid areas.

About half of the world's countries have arid and semi-arid regions. In Mexico, 48.3% of its territory are regions that can be used for protein production through aquaculture (Enciso *et al.*, 2019). According to the Instituto Nacional de Geografía y Estadística (INEGI, 2021), 73% of the territory of Zacatecas consists of regions where tilapia represents a viable option for high-protein food production due to: i) the development of various production models, ii) limitations in fresh water supplies, iii) loss of arable land, and iv) soil degradation resulting from erosion and loss of soil fertility.

In Zacatecas, the promotion of aquaculture began in 2003 as a strategy to diversify farmers' income (CONAPESCA, 2020). This strategy aimed to enhance food security by using water resources as a development alternative. The Food and Agriculture Organization of the United Nations (FAO, 2020) states that aquaculture does not directly consume water but utilizes and recycles it, increasing the organic load of the discharged water (eutrophication of water). This approach aligns with sustainable development, which requires a socially acceptable objective for economic growth and the conservation of natural resources, ensuring their availability for present and future generations (FAO, 2020). The objective of this research was to determine the characteristics of the tilapia (*Oreochromis niloticus*) production systems in Zacatecas, by analyzing the technical, financial and social dimensions, as well as meteorological phenomena, to better understand the adaptability of this fish in the region.

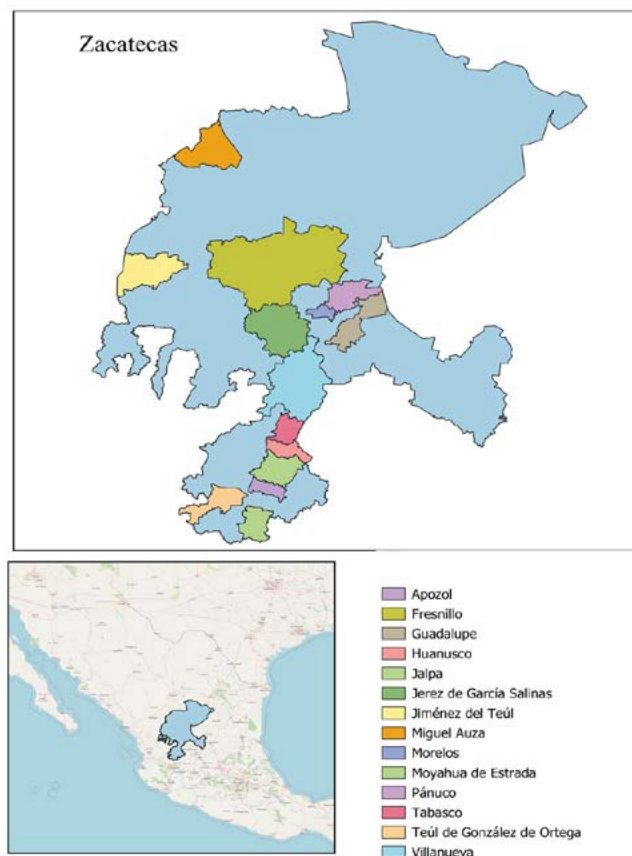
## MATERIALS AND METHODS

### Study site

Zacatecas is located in north-central Mexico ( $25^{\circ} 07' 31''$  N,  $21^{\circ} 02' 31''$  S,  $100^{\circ} 44' 32''$  E, and  $104^{\circ} 21' 13''$  W) at a mean altitude of 2,230 meters above sea level.

The study was conducted in Aquaculture Production Units (APUs), located in various municipalities of Zacatecas (Figure 1) over four months, using a cross-sectional design based on non-probabilistic convenience sampling ( $N=17$ ).

The participating municipalities were Fresnillo ( $23^{\circ} 10' 30''$  latitude and  $-102^{\circ} 52' 06''$  longitude), Moyahua de Estrada ( $21^{\circ} 15' 56''$  latitude and  $-103^{\circ} 09' 57''$  longitude), Jalpa ( $21^{\circ} 38' 11''$  latitude and  $-102^{\circ} 58' 37''$  longitude), Guadalupe ( $22^{\circ} 44' 48''$  latitude and  $-102^{\circ} 31' 08''$  longitude), Teúl de González Ortega ( $21^{\circ} 27' 51''$  latitude and  $-103^{\circ} 27' 43''$  longitude), Pánuco ( $22^{\circ} 52' 33''$  latitude and  $-102^{\circ} 32' 27''$  longitude), Apozol ( $21^{\circ} 28' 15''$  latitude and  $-103^{\circ} 05' 28''$  longitude), Huanusco ( $21^{\circ} 46' 20''$  latitude and  $-102^{\circ} 58' 19''$  longitude), Villanueva ( $22^{\circ} 21' 15''$  latitude and  $-102^{\circ} 53' 01''$  longitude), Jerez de García Salinas ( $22^{\circ} 38' 58''$  latitude and  $-102^{\circ} 59' 24''$  longitude), Morelos ( $22^{\circ} 51' 44''$  latitude and  $-102^{\circ} 36' 34''$  longitude), Miguel Auza ( $24^{\circ} 17' 40''$  latitude and  $-103^{\circ} 27' 05''$  longitude), Jiménez del Teúl ( $23^{\circ} 15' 15''$  latitude and  $-103^{\circ} 47' 54''$  longitude), and Tabasco ( $21^{\circ} 51' 55''$  latitude and  $-102^{\circ} 54' 47''$  longitude).



**Figure 1.** Location of Aquaculture Production Units (APUs, UPAs by its acronym in Spanish) in Zacatecas, Mexico.

### Study participants

Participants were selected through non-probabilistic convenience sampling based on the following inclusion and exclusion criteria: i), being of legal age, ii) gender was not a factor, iii) possessing a minimum of one year experience, iv) having the necessary infrastructure for pond production, and v) having engaged in tilapia production in the previous year (2022). Farmers practicing extensive aquaculture systems or operating outside of Zacatecas excluded from the study.

### Instrument design

The study was conducted in APUs of the state of Zacatecas using a structured questionnaire that included 65 qualitative and 22 quantitative variables, as described by Hernández *et al.* (2002). These variables covered the following attributes: 1) 11 sociodemographic variables, 2) 10 organizational variables, 3) 40 technical-economic productive variables, 4) 12 marketing variables, 5) 2 financing variables, 6) 6 innovation variables, and 7) 6 meteorological phenomena variables.

Based on financial characteristics, a typology of aquaculturists was developed to relate the profitability of their enterprises. The following indicators were considered to parameterize the behavior of sustainable APUs from the financial perspective: a) benefit-cost ratio (B/C) b) internal rate of return (IRR) c) period recovery d) net present value (NPV). These indicators are used to evaluate the financial performance of sustainable APU.

### Instrument application

The research began with a diagnosis of the APUs of the cooperating aquaculturists in Zacatecas. The registry of the Comité de Sanidad Acuícola del Estado de Zacatecas, A.C. (COZAEZ A.C.) served as the basis for non-probabilistic sampling. The aquaculturists were contacted, and appointments were scheduled for interviews. Data was collected over four months, from September 2022 to January 2023.

### Statistical analysis

To identify the “normal” variation in the characteristic of the APUs, the frequency distribution was analyzed by identifying the median value of each characteristic. For characterizing the APUs based on quantitative characteristics, a basic data matrix was constructed to calculate the similarity coefficient by computing Euclidean distances between each characteristic. The goal was to identify APUs with similar financial characteristics to propose common development strategies. The data were analyzed using R programming (R Language 4.3.1. and R studio 2023.06.1 Build 524).

## RESULTS AND DISCUSSION

The results were grouped into seven attributes: sociodemographic, organizational, technical-economic-productive, marketing, financing, innovation, and meteorological phenomena.

### **Sociodemographic attributes**

The study describes the structure of the APUs in 14 municipalities, validating the structure of aquaculture activity in the state of Zacatecas for the year 2022.

Regarding job position, the majority of aquaculturists are owners (70.58%), followed by managers (17.64%) and the rest are technical advisors or administrators (11.78%). APU aquaculturists are adults with an average age of 54 years (minimum=30 years; maximum=68 years). In terms of education, 58.88% have completed secondary and high school, 29.36% hold a bachelor's degree, and only 11.76% have a postgraduate degree (master's). The majority of aquaculturists are men (82.35%) and 88.23% are married. With an average of  $3.4 \pm 1.6$  S.D., financial dependents. They reported an average of 11.52 years  $\pm 2.96$  S.D. years of experience in aquaculture.

### **Organization attributes**

A total of 58.82% of aquaculturists are organized as a cooperative society, while 41.18% are organized as a rural production society. Most APUs (82.35%) are maintained on private property, with the remainder situated on ejidal land. Aquaculturists typically dedicate  $6.1 \pm 1.9$  hours per day to their activities. Additionally, 29.41% of aquaculturists are exclusively dedicated to aquaculture, while the rest work engage in agricultural as a complement to agriculture activities such as farming, livestock, and teaching.

### **Technical-economic-productive attributes**

Production in the APUs is primarily conducted in geomembrane ponds (88.24%) and, to a lesser extent, in concrete ponds (11.76%). Approximately 58.82% of the APUs maintain the temperature with a greenhouse, while the rest produce under open field conditions.

On average, APUs have  $6.1 \pm 3.5$  ponds, each with an average capacity of  $81.9 \pm 7.4 \text{ m}^3$ . Each pond is stocked with an average of 2339.07 organisms, consuming  $5714.5 \text{ m}^3 \pm 4720.9 \text{ m}^3$  of water per production cycle.

The APUs utilize common technologies such as aerator (vanes and blower) (76.47%) to introduce oxygen into the water, motor pump (70.58%), power generators (64.70%), oximeter (64.70%), transformer (52.94%), and thermometer (41.17%).

Regarding problems that affect their aquaculture production, 58.83% of aquaculturists do not identify any issues. However, 17.65% mention the high costs of inputs (fingerlings, concentrated feed, labor, electrical energy), 11.76% cite frequent failures in the supply of electrical energy, and another 11.76% point the shortage of specialized feed within the region and the difficulty in marketing fishing production.

In terms of solutions to technical problems, 88.24% of aquaculturists consider the using of alternative sources of electrical energy as a strategy to lower production costs. Meanwhile, 5.88% mention purchasing equipment with electric storage batteries to cope with frequent electrical failures, and the remaining do not identify a solution to their problems. Additionally, 58.82% of aquaculturists describe UPA production management as conventionally diversified, 23.53% as transition to organic, and 17.65% as diversified organic.

### Marketing attributes

Referring sales, 64.71% of aquaculturists sell their fish fresh (whole gutted fish), 35.29% sell prepared dishes, and 94.12% sell their production in the surrounding communities (local sales). In terms of the type of buyer, 64.71% sell it whole gutted fish to the final consumer, and 35.29% sell cooked fish in restaurants associated with the APUs.

The average production in the 2022 crop cycle was  $5705.88 \pm 2302.84$  kg per year, with a sale price of  $\% 116.47 \pm 20.97$  S.D.

Those responsible for the APUs report no losses, and 35.29% of aquaculturists process part of their production (filleting). Most aquaculturists (94.12%) acquire tilapia fingerlings from the state of Jalisco, while the rest (5.88%) source from Sinaloa. The balanced feed used in tilapia production is purchased from local suppliers (70.59%), foreign suppliers (23.53%), and direct from the factory (5.88%).

### Innovation attributes

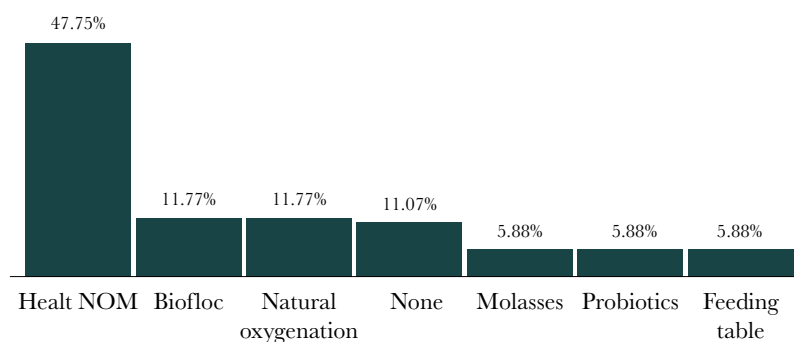
Most of aquaculturists (47.75%) report applying the official Mexican standards (NOM) of aquaculture health in the APUs as the main innovation (Figure 2), which is considered acceptable. Another practice related to water quality, such as biofloc, promotes the rapid growth of tilapia; however, this practice was abandoned due to lack of technical assistance (15.38%).

Regarding the perception in the development of their activity, 58.82% of those responsible for the APUs consider that it stagnant, while 41.17% believe it is declining. Additionally, 88.24% perceive the activity to be in “apparent stability”, and 11.76% view as “deteriorated and unproductive”.

Among the main limitations, 23.53% of those responsible for APUs identify the expansion strategy of their product as a significant constraint (Figure 3).

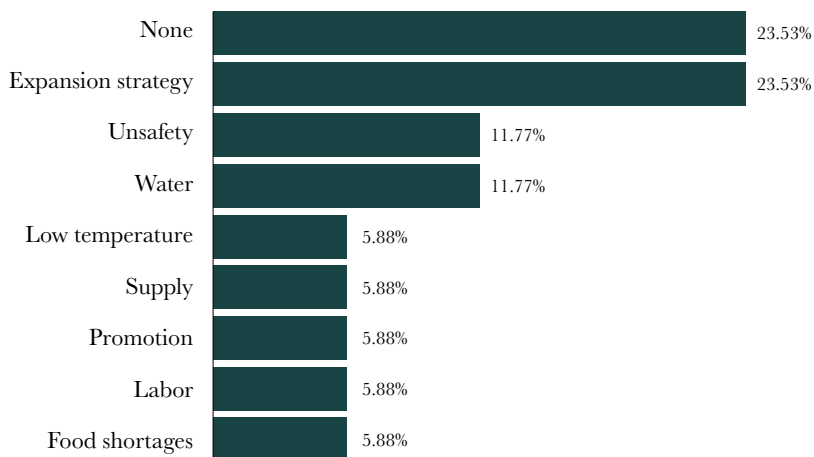
### Attributes related to meteorological phenomena

Regarding the meteorological factors causing cultivation losses (Figure 4), aquaculturists first mention hail (61.53%), followed by strong regional winds (23.09%), and diseases affecting tilapia (15.38 %). According to those responsible for the APUs, the intensity of the meteorological phenomena is mild (52.94%), moderate (23.53%), or critical (5.88%), with

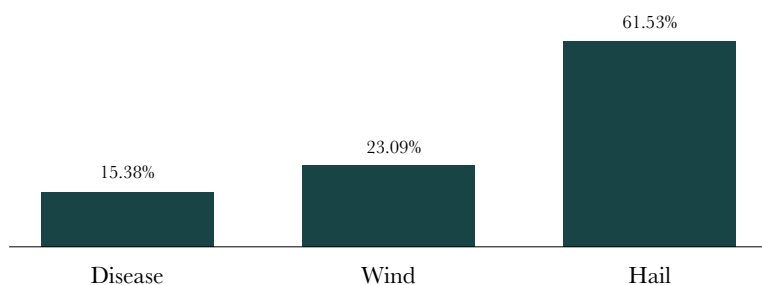


**Figure 2.** Innovations implemented in the productive management of Tilapia (*Oreochromis niloticus*), in Zacatecas.





**Figure 3.** Limitations for Tilapia (*Oreochromis niloticus*) cultivation identified by aquaculturists in Zacatecas.



**Figure 4.** Meteorological factors that cause losses of Tilapia (*Oreochromis niloticus*) harvest in the state of Zacatecas.

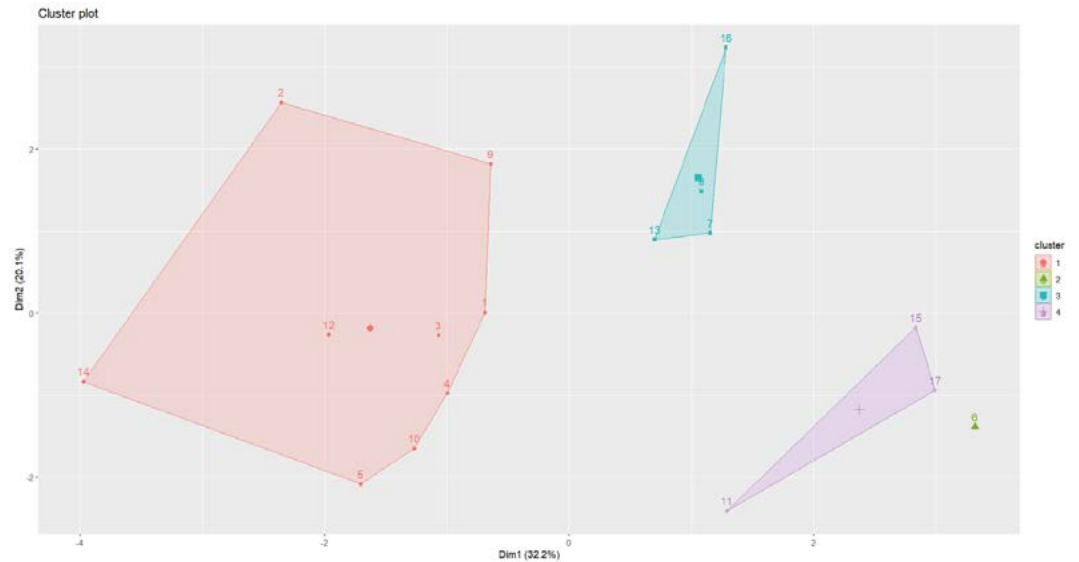
17.65% considering the climate effects not relevant. Concerning the level of damage caused by the climate, those responsible for the APUs consider slight (41.18%), moderate (29.41%), or critical damage (11.76%), while 17.65% consider the damage non-relevant.

**Identification aquaculturists type**

The APUs were classified with the following variables: education level, age, experience in aquaculture, type of production, production volume, sales price, innovation activities, number of direct jobs, and financial indicators such as benefit-cost ratio (B/C), net present value (NPV), internal rate of return (IRR), and payback period.

Four groups of APUs were identified (Figure 5), explaining 53.2% of the total variance. The first dimension explains 30.1% of the variance, defined by B/C, NPV, IRR, and payback period, and places groups 2, 3, 4, and 1 in order of increasing B /C, NPV and IRR, and decreasing payback period. The second dimension explains 23.1% of the variance, dominated by the time dedicated to the activity and sales price, thus describing the ascending order of groups 1, 4, 2, and 3.

Additionally, group one is characterized by low production and profitability. Group two is noted for basic experience and average profitability. Group three is distinguished by full-time dedication by medium profitability and a high payback period- Finally,



**Figure 5.** Profile of the groups extracted from the cluster analysis.

group four is marked by a medium production, high sales price, and medium job creation (Table 1).

In the rural areas of Zacatecas, tilapia production is predominantly managed by men, with 82.35% of aquaculturists being male and 17.65% female. This gender distribution is consistent with regional agricultural trends, as reported by Avadí *et al.* (2022), who found that 60-84% decision-makers in agricultural production are men, often the heads of the families and landowners where APUs are located. The average age of these aquaculturists is 54 years, indicating a mature demographic, as noted by Rachel *et al.* (2022). Most are organized as a cooperative society, reflecting findings by Avadí *et al.* (2022).

Aquaculture in Zacatecas is often a complementary activity to agriculture (47.05%) (Rachel *et al.*, 2022; Berg *et al.*, 2021), contributing between 26% and 51% of the aquaculturists' economic income. This suggests that aquaculture has promoted the diversification of economic activities in semi-arid areas, positively impacting sustainable rural development in the region (García-Mondragón *et al.*, 2013, Montijo *et al.*, 2016).

Most tilapia farming in Zacatecas is conducted in geomembrane ponds with a diameter of 9 meters and an average depth of 1.20 meters. These ponds are typically fed with well water and sometimes aerated using blower-type aerators, along with other equipment such as motor pumps and oximeters. On average, each pond contains 2340, which reach

**Table 1.** Description of the identified clusters.

Aquaculture production units	Members	Distinctive feature
Basic	9	Low production and low profitability
Pre-intermediate	4	Basic experience and average profitability
Intermediate	2	Full-time dedication, average profitability and high recovery period
Intermediate-high	2	Average production, high selling price and average creation

average harvest weight of 0.50 kg per organism with a 95% survival rate. This result is an expected production of 5.90 tons per year<sup>-1</sup>, sold at \$116.47 MXN per kg of fresh gutted fish at the farm gate. This aligns with Vega *et al.* (2010), who reported that low density and minimal management practices reduce risks and enhance the production of high-quality protein. There is a recognized need to add value to the production through processing into frozen products, nuggets, steaks, and others items, allowing access new market niches.

Production costs are mainly attributed to concentrated feed and electrical energy, consistent with studies on aquaculture in southern Mexico (Platas *et al.*, 2018; Delfin *et al.*, 2023), Tanzania (Berg *et al.*, 2021), and Honduras (Lee *et al.*, 2022). Health advice for aquaculturists in APUs is provided by field professionals from the Comité de Sanidad Acuícola del estado de Zacatecas (COSAEZ) highlighting the need for training, technical assistance, innovation extension, and actions that strengthen and integrate the productive chain to enhance competition of APUs. This need is supported by finding from other studies (Bueno *et al.*, 2020; Malcolm *et al.*, 2016; Rachel *et al.*, 2022; Avadí *et al.*, 2022; Guilhermino *et al.*, 2019).

Regarding meteorological factors, APUs are particularly sensitive to hail and strong winds. However, most aquaculturists consider the level of damage to be minor. Additionally, the owners of the APUs do not have insurance coverage, as their minimum profit margin makes them unattractive to insurers. Some aquaculturists supplement their income by operating a restaurant, which can significantly enhance the unit's profit margin.

Based on the characteristics analyzed, APUs are grouped into four types of production: 1) Basic, as a complementary activity with limited economic resources; 2) pre-intermediate, complementary activities with basic infrastructure, managed by mature adult aquaculturists (49-55 years), who view the activity as profitable with long-term growth (Avadí *et al.*, 2022); 3) Full-time intermediate, aquaculturists with experience and average profitability. This group has a higher cultural, allowing access to information systems and the adoption of innovations, consistent with Mogíca *et al.* (2002); finally, 4) High-intermediate, APUs with optimal infrastructure, managed by adult aquaculturists (30-55 years) inclined to adopt technological innovation in aquaculture.

## CONCLUSIONS

Aquaculturists perceive a stagnation in their activity due to the lack of product promotion, production scheduling, and insecurity. These limitations highlight the need to create an inter-institutional strategy involving universities, research institutions, and government agencies that promote policies, financing, and training. The characterization of the tilapia production system in Zacatecas will help develop specific programs tailored to each identified cluster based on the unique characteristics of the APUs. The results can aid in understanding and analyzing the production systems and adaptability of tilapia (*Oreochromis niloticus*), in the Zacatecas region, steering the activity towards profitable and sustainable aquaculture. Thus, aquaculture could become a productive alternative with significant social and economic impact in the state of Zacatecas.

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# Effect of starch from chayotextle (*Sechium edule* (Jacq.) Sw.) and erythritol on the physicochemical and sensorial quality of a yogurt enriched with inulin

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## ABSTRACT

**Objective:** To evaluate the incorporation of chayotextle (*Sechium edule*) root starch and erythritol on the physicochemical quality of a yogurt enriched with inulin.

**Design/methodology/approach:** The following treatments were established: T1: Erythritol 2.1%; T2: Starch 1% + Erythritol 2.1%; T3: Sugar 1.5% + Erythritol 1.05%; T4: Starch 1% + Sugar 1.5% + Erythritol 1.05%; T5: Sugar 3% and T6: Starch 1% + Sugar 3%. The variables nutritional content, pH, total soluble solids, syneresis, and sensorial quality were determined through CATA.

**Results:** The incorporation of chayotextle starch to yogurt favored an increase in the content of non-fatty solids and a decrease in syneresis, while the use of erythritol as sugar substitute decreased the caloric content and of non-fatty solids and increased the pH and syneresis, contrary to the use of sugar. Enriching all the formulations with 5% of inulin favored the stability and increased the dietary fiber in all the treatments. Sensorially, the chayotextle starch combined with sugar favored a greater sensorial acceptance of the yogurt.

**Limitations on study/implications:** To evaluate the texture profile of the yogurt, as well as the use of other stabilizers such as potato, corn, yucca and gums like xanthan, arabic or even carboxymethyl cellulose which are commercial.

**Findings/conclusions:** The addition of erythritol does not substitute sugar as a sweetener from the sensorial point of view. However, nutritionally, it reduces the caloric content in 57%. Inulin favors the dietary fiber content, while the chayotextle starch together with sugar preserved the physicochemical and sensorial characteristics of yogurt.

**Keywords:** *Sechium edule*, chayote, prebiotics, sweeteners, lactic cultures.

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## INTRODUCTION

Yogurt tends to be considered a healthy food since it provides many essential nutrients such as proteins, calcium, phosphorus, riboflavin, thiamine, vitamin



B12, folate, niacin, magnesium and zinc (Fernández *et al.*, 2017); however, some aspects of commercial yogurt can negatively affect health, because many have high amounts of added sugar, low dietary fiber, and artificial coloring (Soukoulis *et al.*, 2007). Nowadays, there is interest in using probiotics and prebiotics (Nyanzi *et al.*, 2021), to improve the nutritional quality of a fermented beverage that can be associated with a better health in general for the consumer (Barengolts *et al.*, 2019). Considering this, consumers are looking for a yogurt that reduces the consumption of added sugars to decrease the caloric content (Nekoucian and Jafarpour 2021). Alternative sweeteners such as stevia, aspartame, acesulfame K and sucralose can replace up to 100% of sugar in yogurt maintaining the flavor and the texture (Chadha *et al.*, 2022). In addition, there are other approaches that include adding prebiotics to yogurt such as inulin, since it favors the presence of soluble fiber that is not hydrolyzed in the human digestive tract and has a positive impact on texture and flavor, improving the sensorial properties of the yogurt when an optimal concentration of 2 to 7% is added (Abbasi Asl *et al.*, 2022; Żbikowska *et al.*, 2020). Sugar alcohols or polyols, also known as polyalcohols such as erythritol and isomaltose have been used as sweeteners in yogurt to reduce the calories and keep the sweetness (Grembecka, 2015). Erythritol is a sugar alcohol that is 60% to 80% sweeter than sugar, although without calories, and it is a food additive considered very safe in many countries (Boesten *et al.*, 2015). Natural coloring derived from plants and fruits are commonly used in yogurt to improve the appearance and the appeal for the consumer (Dias *et al.*, 2020). It has been shown that the pulp of blackberry fruits is rich in anthocyanins with great antioxidant power, an efficient natural coloring for yogurt that favored a pleasant violet pink color that complies with the industry standards (Merino-Peñafiel *et al.*, 2018).

The use of starches from potato (*Solanum lycopersicum*), sweet potato (*Ipomoea batata*) and yucca (*Manihot esculenta*), and modified corn starches (*Zea mays* L.), as stabilizers in yogurt improves the texture, the flavor and reduces the syneresis (Agyemang *et al.*, 2020; Saleh *et al.*, 2020). It has been reported that starches could partially or completely replace powdered skimmed milk in the yogurt, maintaining the quality while reducing the cost (Saleh *et al.*, 2020); however, the effects of the addition of starch depend on the type and amount (Skryplonek *et al.*, 2019). Among the new sources of starch, there is chayotextle root, from the *Sechium edule* plant which is native to Mexico and has been reported as a source of polysaccharides like pectins, arabigans, galactans and arabinogalactans. These polysaccharides are stable even after cooking, in addition to being a good source of starch and fiber (Shiga *et al.*, 2015).

Since there is no information about the use of chayotextle root starch as stabilizer, or about the use of erythritol as sweetener in yogurt elaboration, the incorporation of chayotextle root starch and erythritol as sweetener in a yogurt enriched with inulin was evaluated, on its physicochemical and sensorial quality.

## MATERIALS AND METHODS

### Collection of blackberry fruits for coloring

The blackberry fruits from the species *Rubus Fruticosus* L. were harvested in maturity for consumption in Zitácuaro, Michoacán (19° 25' 43" N and 100° 23' 25" W) at an



altitude of 1940 m.a.s.l., in a Cwb climate according to Koppen and modified by García (2004).

The blackberry fruits were taken to a pressing process, so that the juice that could be obtained from this process could be subjected to reduced evaporation with a pressure of 70 mb at 40 °C in a rotary evaporator (Buchi. R100, Suiza). Lastly, the concentrated juice was stored in refrigeration at 2 °C for its later incorporation into the corresponding treatments.

### **Extraction of chayotextle starch**

The chayotextle was harvested in Irimbo, Michoacán (19° 41' 48" N and 100° 28' 16" W), at an altitude of 2120 m.a.s.l., with Cwb climate according to Koppen and modified by García (2004), and then the starch extraction was carried out according to Román-Brito *et al.* (2020).

### **Elaboration of enriched yogurt**

The yogurt samples were prepared according to what was described by Hashim *et al.* (2009), where each of the treatments was enriched with 5% of inulin and inoculated with starter cultures *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* at 2% (Vivolac, United States).

### **Physicochemical quality: bromatological analysis**

The nutritional analysis of yogurt samples was carried out according to AOAC (2003). The moisture content was determined (method 934.01), lipids determined by Soxhlet extraction (method 920.39), raw protein according to method 955.04 with a conversion factor of 6.25, ash content (method 923.03), and carbohydrate content by difference. The total dietary fiber of the samples was determined with the method 962.09.

### **pH and Total Soluble Solids**

The pH of the samples was determined under room temperature conditions, according to the AOAC method (2011). The content of total soluble solids was measured according to Torrico *et al.* (2019) and expressed in °Brix.

### **Determination of syneresis**

This variable is determined according to Robitaille *et al.* (2009), where a centrifuge was used (Velab, TDL-50B, Mexico) at 2200 rpm during 10 min at 4 °C, where the susceptibility of syneresis was calculated, through the following formula:

$$\text{Syneresis} = (\text{Weight of supernatant}) / (\text{Weight of the sample}) \times 100$$

### **Sensorial analysis by the CATA test**

For this sensorial test of the yogurt variations, the attributes were randomized and n=50 consumers were asked to indicate the sensorial characteristics of the yogurt through the CATA method, according to Ghasempour *et al.* (2020), where the sensorial terms were previously identified by experts of yogurt consumption.

### Experimental design

For the analysis of the physicochemical variables, a completely randomized experimental design was established with a LSD means comparison test with  $P \leq 0.05$ , where 6 treatments were established that consisted in changing the amount of stabilizers such as chayotextle starch and sweeteners. T1: Erythritol at 2.1%; T2: Starch at 1% + Erythritol at 2.1%; T3: Sugar at 1.5% + Erythritol 1.05%; T4: Starch at 1% + Sugar at 1.5% + Erythritol at 1.05%; T5: Sugar at 3%; and T6: Starch at 1% + Sugar at 3%. In the case of the sensorial analysis, the CATA method was used where Cochran's Q test was conducted with a value of  $q \leq 0.05$  and multivariate correspondence analysis. All the statistical analyses were carried out with the R Studio software version 12.0.

### RESULTS AND DISCUSSION

According to Table 1, the treatment that obtained the highest moisture content was T1 where the sweetener was erythritol; contrary to this, T6 with sugar as sweetener resulted in the lowest moisture percentage, and this way the addition of sugar to yogurt increased the viscosity and the excess in air volume incorporated to the yogurt, which indicates a decrease in moisture in addition to higher amounts of sucrose, dextrose and fructose increasing the viscosity from 6 to 8% (Guggisberg *et al.*, 2011). The fat content was not significant, but lower than in other yogurts that present inulin at 7% and a yogurt with modified starches where the fat content was 2.88% and 2.40%, respectively (Iriundo-DeHond *et al.*, 2020). This indicates that the amount of fat in the yogurt depends on the type of milk used, and the addition of fat and sugar (Arriaga *et al.*, 2019). In addition, the starter cultures of yogurt can affect the levels of fat because of their lipolytic activity, especially when they are combined with different amounts of added sugar (Rodríguez-Bernal *et al.*, 2014; Aslam *et al.*, 2015). The highest caloric content is attributed to T6 and the lowest caloric content to T1, and therefore the sugar content of yogurt has a significant impact on its recount of calories and nutritional profile (Güven *et al.*, 2005). Likewise, yogurt, which has starches in its composition, significantly favors the caloric density (Miao *et al.*, 2021). The use of erythritol in yogurt resulted in the lowest percentage of glucid (9.81%), a sugar alcohol low in calories that

**Table 1.** Nutrition content of blackberry yogurt enriched with inulin and chayotextle starch using different sources of sweeteners.

Treatments	Moisture	Fat	Proteins	Carbohydrates	Ash	Dietary fiber	Energy (Kcal)
	%						
T1	80.29 a*	1.41 a	3.47 a	9.81 f	0.66 a	4.37 a	65.79 f
T2	72.94 d	1.55 a	3.22 a	17.40 c	0.64 a	4.25 a	96.43 c
T3	78.29 b	1.40 a	3.41 a	12.25 e	0.49 a	4.16 a	75.26 e
T6	68.26 f	1.54 a	3.64 a	21.65 a	0.62 a	4.30 a	115.00 a
T4	70.61 e	1.65 a	3.57 a	19.31 b	0.67 a	4.19 a	106.40 b
T5	75.22 c	1.70 a	3.59 a	14.62 d	0.65 a	4.24 a	88.07 d

\* Values followed by different letters in the same column showed significant differences ( $p \leq 0.05$ ) according to the LSD test. T1: Erythritol 2.1%; T2: Starch 1% + Erythritol 2.1%, T3: Sugar 1.5% + Erythritol 1.05%; T4: Starch 1% + Sugar 1.5% + Erythritol 1.05%, T5: Sugar 3%, and T6: Starch 1% + Sugar 3%.

is not metabolized by the organism, so it presents lower caloric content than sugar (Regnat *et al.*, 2018).

In the case of the protein content, no statistical difference was found between the treatments. Iriondo-DeHond *et al.* (2020) mention that for yogurt enriched with 7% inulin, the protein content was 2.78% lower than this research study, and it has been shown that the addition of inulin to yogurt favors a higher protein content (Balthazar *et al.*, 2016). Similarly, the dietary fiber content in the treatments did not present statistical differences between treatments, and several studies agree that adding between 1% and 5% of inulin to the yogurt increases its dietary fiber content (Żbikowska *et al.*, 2020).

Table 2 shows the treatments with highest amount of soluble solids (T4, T5 and T6), which is due to the sugar and chayotextle starch, which agrees with Estévez *et al.* (2010). The starches absorb water and swell during the yogurt fermentation, increasing the total solids (Wong *et al.*, 2020). Lower total soluble solids indicate lower sugar content that acts as a load agent in the sample, which is why it will be more diluted (Thun *et al.*, 2022).

In the case of the pH, it was found that treatments T1, T2 and T3 presented similar values, and they were the highest values in pH, where its composition is mostly lower or no amount of sugar, and the sucrose added increases the necessary time for yogurt to reach its breakdown pH, indicating that sugar slows down the rate of lactic acid development since it makes microbial growth slower, and therefore the specific effects depend on the type and amount of sugar or starch added (Estévez *et al.*, 2010).

In the gel syneresis, it was found that the formulations that presented in their composition chayotextle starch favored lower syneresis. This is because this stabilizer functions through its capacity to form gel structures in water, which leaves less water free for syneresis; that is, when starches are added, such as potato and yucca, the separation of serum in the yogurt decreases, since they form a complex with casein that gives more body and more protection against syneresis, and in addition they participate as generators of viscosity and can withstand severe processing conditions of low pH, high heat and extreme shear (Chandan *et al.*, 2017). The inulin that is present in all the formulations presented prebiotic fiber that decreased the syneresis, which resulted in a lower separation of the serum (Rezaei *et al.*, 2014).

**Table 2.** Physicochemical properties for a blackberry yogurt enriched with dietary fiber and chayotextle starch.

Treatments	Total soluble solids °Brix	pH	Syneresis %
T5	5.70 a*	3.77 b	1.20 a
T6	5.53 a	3.76 b	0.59 b
T4	5.60 a	3.75 b	0.66 b
T1	4.80 b	3.83 a	1.26 a
T3	4.50 b	3.86 a	1.70 a
T2	4.80 b	3.86 a	0.23 b

\*Values followed by different letters in the same column showed significant differences ( $p \leq 0.05$ ) according to Tukey's test. T1: Erythritol 2.1%; T2: Starch 1% + Erythritol 2.1%, T3: Sugar 1.5% + Erythritol 1.05%; T4: Starch 1% + Sugar 1.5% + Erythritol 1.05%, T5: Sugar 3%, and T6: Starch 1% + Sugar 3%.

### Sensorial analysis by the CATA Test

Table 3 shows the results from the CATA test (Check All That Apply) where, according to the Cochran test with a level of  $q \leq 0.05$ , the attributes that do not present statistically significant difference between treatments were fermented flavor and the attribute of elasticity. Treatment T6, which corresponded to adding sugar with starch, is the closest to an ideal yogurt, so the addition of sugar and starch has a great impact on the flavor and the quality, since the sensorial acceptability and palatability of the yogurt was improved when sugar was added (Schnettler *et al.*, 2010).

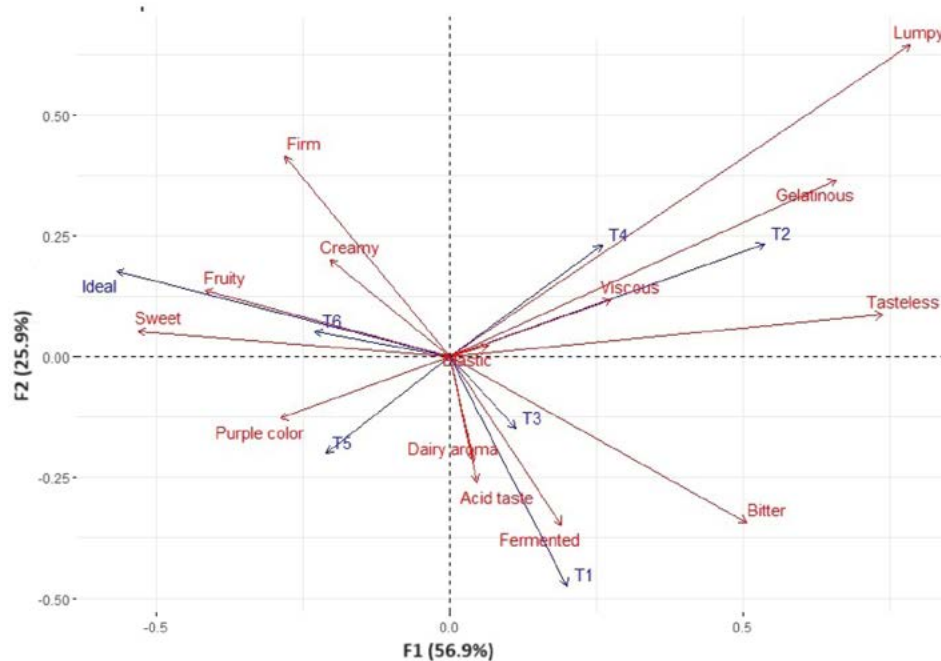
It is important to highlight that the fruity flavor is an important attribute in the ideal product, but none of the treatments proposed presented it. The creamy attribute was highest for treatment T6, with a value that is quite close to the ideal yogurt, and this is because when inulin and sugar are used, they favor a soft and creamy texture (Kamel *et al.*, 2021). The treatment T1 was the farthest from the ideal product because the erythritol decreases the effect of the sweet flavor and does not favor more texture (Akesowan, 2009), attributes that are highly valued in yogurt.

According to Figure 1, for the X axis, the sweet and insipid attributes determined the highest variance and therefore they are associated with the flavor; while for the second axis, the attributes that presented the greatest effect for axis Y were lumpy and firm, which are characteristics of the texture attribute. Therefore, the first two dimensions contributed 82.8% of variability of all the data. Additionally, it can be seen in this test that treatment T6 is the treatment with the most similar characteristics to the ideal yogurt (creamy, fruity, sweet and firm), except for the color, which is considered better in treatment T5, because it has been reported that when sugars such as sucrose, glucose and fructose are added, this

**Table 3.** Results from the sensorial analysis of Cochran's Q test after using the CATA method for a blackberry yogurt enriched with dietary fiber and chayotextle starch.

Attributs	p-value	T1	T2	T3	T4	T5	T6	Ideal
Fruity*	0.00001	0.26 a	0.26 a	0.23 a	0.23 a	0.14 a	0.20 a	0.91 b
Dairy aroma*	0.009	0.86 a	0.63 a	0.77 a	0.80 a	0.77 a	0.57 a	0.57 a
Creamy*	0.00001	0.23 a	0.46 ab	0.46 ab	0.49 abc	0.69 bc	0.86 c	0.91 c
Sweet*	0.00001	0.14 ab	0.09 a	0.31 bc	0.6 cd	0.49 bc	0.31 bc	0.86 d
Firm*	0.00001	0.03 a	0.23 bc	0.14 ab	0.14 ab	0.49 cd	0.37 bc	0.51 d
Bitter*	0.00001	0.43 b	0.29 ab	0.23 ab	0.14 ab	0.14 ab	0.28 ab	0 a
Fermented	0.13440	0.34 a	0.23 a	0.26 a	0.26 a	0.17 a	0.17 a	0.12 a
Acid taste*	0.02799	0.74 b	0.46 ab	0.48 ab	0.53 ab	0.43 ab	0.40 a	0.49 ab
Purple color *	0.00001	0.57 abc	0.26 a	0.34 ab	0.63 bc	0.75 c	0.37 ab	0.83 c
Viscous *	0.001981	0.26 a	0.66 b	0.37 ab	0.26 a	0.31 ab	0.34 ab	0.29 ab
Gelatinous*	0.00001	0.09 ab	0.40 c	0.09 ab	0.23 bc	0.06 a	0.09 ab	0.05 a
Lumpy *	0.00001	0.03 a	0.40 c	0.06 ab	0.03 a	0.09 ab	0.31 bc	0.03 a
Elastic	0.1751	0.17 a	0.40 a	0.25 a	0.31 a	0.29 a	0.17 a	0.26 a
Tasteless*	0.00001	0.29 b	0.51 c	0.31 bc	0.45 c	0.09 ab	0.06 ab	0 a

\* Values followed by different letters in the same column showed significant differences ( $p \leq 0.05$ ) according to the Cochran test. T1: Erythritol 2.1%; T2: Starch 1% + Erythritol 2.1%, T3: Sugar 1.5% + Erythritol 1.05%; T4: Starch 1% + Sugar 1.5% + Erythritol 1.05%, T5: Sugar 3%, and T6: Starch 1% + Sugar 3%.



**Figure 1.** Correspondence analysis using the CATA test, taking into account the attributes that characterize each of the treatments evaluated and an ideal product. T1: Erythritol 2.1%; T2: Starch 1% + Erythritol 2.1%, T3: Sugar 1.5% + Erythritol 1.05%; T4: Starch 1% + Sugar 1.5% + Erythritol 1.05%, T5: Sugar 3%, and T6: Starch 1% + Sugar 3%. Source: Prepared by the authors (2023).

helped to stabilize pigments such as the anthocyanins from blackberries during processing and storage, which gave place to brighter and more vibrant colors that last (Kopjar 2012; Sadilova 2009). However, it can be observed in this study that the absence of erythritol and the addition of sugar positioned them with the highest degree of acceptance by consumers, so sweetness is a factor associated to consumer satisfaction of dairy products (McCain *et al.*, 2018). According to Oliveira *et al.* (2015), strategies are required based on the reduction of sugar depending on the perception of the sweet flavor of those that are based on product acceptance; thus, in a study with dairy desserts, a 20% sugar reduction affected the perception of sweetness through the flavor. Likewise, Andrade-Oliveira *et al.* (2021) showed that it is possible to reduce the yogurt's sweetness by 25% without compromising the perception and the acceptance. Treatments T2 and T4 were located in the quadrant with the least accepted attributes by the consumer, that is, they were described as gelatinous, insipid, lumpy, viscous and elastic; both treatments contain erythritol and starch in their formulation, which, despite being a viable option to decrease the sugar content, do not satisfy the needs of the consumer sensorially.

## CONCLUSIONS

The use of chayotextle starch in yogurt had a positive impact in the content of non-fatty solids and lower syneresis; however, it favors a higher caloric content and in combination with inulin at 5%, it favored the stabilizing effect in all the treatments, increasing the dietary

fiber content and resulting in higher protein level. For the use of erythritol as partial and total substitute of sugar, it decreased the caloric content in 57%; however, sensorially it was not the most accepted, and it was found that the treatment that incorporates chayotextle starch at 1% with sugar at 3% was the most accepted by consumers, where the attributes that most stood out were fruity aroma, creamy texture and sweet flavor, which agree with the attributes of a commercial yogurt.

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# Nutritional and nutraceutical properties of wild cranberry: cahuiche (*Vaccinium leucanthum* Schltld.)

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## ABSTRACT

**Objective:** To determine the physical and chemical characteristics, nutritional and nutraceutical compounds of cahuiche fruits (*Vaccinium leucanthum* Schltld.) at consumption maturity, harvested in five places of the locality of Ciénega Grande, Omitlán, Hidalgo, Mexico.

**Design/methodology/approach:** Cahuiche fruits were harvested with simple randomized sampling, and a proximal and mineral, morphological and chemical analysis (sugars, total soluble solids and acidity) was conducted. For the evaluation of the content of nutraceutical compounds (anthocyanins, phenols, flavonoids and total tannins) and antioxidant activity, six solvents were used with the aim of identifying the one of highest extraction yield, except for the evaluation of vitamin C (ascorbic acid, AA).

**Results:** Cahuiche presented high values (expressed in fresh weight) of Ca (43.24 mg 100 g<sup>-1</sup>), S (28.61 mg 100 g<sup>-1</sup>), protein (1.95 g 100 g<sup>-1</sup>), lipids (1.67 g 100 g<sup>-1</sup>), and raw fiber (6.67 g 100 g<sup>-1</sup>); in addition to high concentrations of anthocyanins (267.50 mg EC 100 g<sup>-1</sup>) and phenolic compounds (407.36 mg EAG 100 g<sup>-1</sup>). The use of methanol-H<sub>2</sub>O-HCl (90:10:1) allowed obtaining the best extraction yields of anthocyanins and total soluble phenols.

**Limitations on study/implications:** Follow-up studies are needed to evaluate the nutritional value and antioxidant compound content of cahuiche products, since their consumption is mostly processed.

**Findings/conclusions:** Cahuiche is an underutilized wild cranberry with high potential for its use as a crop, since it is a species with high nutritional and nutraceutical value.

**Keywords:** extraction solvents, antioxidant activity, phenolic compounds, anthocyanins.

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## INTRODUCTION

*Vaccinium leucanthum* Schltld is a native tree from Mexico whose edible fruit is an underutilized wild cranberry. This species grows in Mexico's temperate forests, such as those in the state of Hidalgo, where it is used in some of its municipalities (Molina-Mendoza *et al.*, 2012; Wilbur and Luteyn, 2008), such as Huasca de Ocampo and Omitlán de Juárez. This fruit is commonly called cahuiche, and it is part of the ancestral diet of the peoples, since it is used for the elaboration of preserves, sweets, jellies, jams and liquors, as well as an ingredient in various dishes such as adobo, mole and salsa, among others.

However, its consumption is local, without commercial exploitation as in other cranberry species, despite its nutraceutical potential; it could be considered a species with functional properties.

Diverse studies point out that the leaves and the fruits of the genus *Vaccinium* provide benefits to health due to their biological activities, such as antioxidant potential, antimicrobial, anti-inflammatory, anti-cancer activity, and glycemic regulation (Fan *et al.*, 2020; Meléndez-Jácome *et al.*, 2021). In addition, the wild species of *Vaccinium* produce fruits with higher nutraceutical value, due to their high content of antioxidant compounds compared to cultivated species (Meléndez-Jácome *et al.*, 2021).

There are few studies about the yields and the extraction capacity of different solvents on the metabolites responsible for the antioxidant activity in cranberry fruits. Some metabolites can interact with the food matrix components, and may be extracted selectively due to greater chemical affinity with a solvent in particular. The recovery of antioxidant compounds of plant materials is generally achieved through different techniques and extraction solvents, considering the chemical composition and the unequal distribution in the plant matrices (Sultana *et al.*, 2009). This is important since it allows establishing appropriate means to evaluate and to quantify antioxidant compounds in *Vaccinium* plant materials, in the most efficient and accurate way.

In this context, Sánchez-Franco *et al.* (2018) reported that the fruits of *V. leucanthum* are an important source of minerals, dietary fiber, protein, phenol compounds, vitamin C and anthocyanins; the latter three metabolites confer to them a high nutraceutical potential. In this study, cahuiche fruits were characterized which were harvested in Huasca de Ocampo, Hidalgo, located at an altitude of 2,100 masl; in contrast, this fruit grows at more than 2,400 masl in Omitlán de Juárez, Hidalgo. These characteristics impact the mineral and phytochemical composition of the fruits, in addition to the genotype, type of soil, environmental conditions (temperature, altitude, radiation, precipitation), nutrition of the plant, season of flowering, degree of maturity at the time of harvest, as well as the post-harvest storage (Zorenc *et al.*, 2016). The objective was to evaluate the physicochemical, nutritional and nutraceutical characteristics of the fruits of *Vaccinium leucanthum* harvested in Omitlán de Juárez, Hidalgo; also, to determine the most effective solvent for the extraction of antioxidant compounds (phenols, flavonoids, condensed tannins, and total anthocyanins).

## **MATERIALS AND METHODS**

### **Biological material**

Fruits of cahuiche (*V. leucanthum*), free of disease and visible damage, were randomly collected in state of consumption maturity, in five places of the locality of Ciénega Grande, Omitlán, Hidalgo (20° 11' 41" N, 98° 39' 54" W, 2637 m of altitude), during the fructification in November, 2022.

### **Taxonomic certification**

In each collection site of "cahuiche" fruits, samples were collected from vegetative and reproductive structures (leaves, flowers and fruits) from wild "cahuiche" plants (*V.*

*leucanthum*) for their taxonomic identification in the Herbarium “Jorge Espinosa Salas”, from the Department of Agricultural Preparatory of Universidad Autónoma Chapingo: record number 36228.

### **Soil characterization of the sampling site**

Soil samples were taken in the various fruit collection points, considering a compound sample for the analysis of the physical and chemical quality of the soil. The results were interpreted according to the reference values by Castellanos *et al.* (2000): sandy loam (58.8% sand, 27.3% loam, 13.9% clay), pH 5.98 (moderately acid), apparent density  $1.05 \text{ g cm}^{-3}$ ,  $0.16 \text{ dS m}^{-1}$  (free of salts), 8.41% organic matter (very high), 22.8 mg of N  $\text{kg}^{-1}$  (medium or adequate), 3.11 mg of P  $\text{kg}^{-1}$  (very low), 854 mg of K  $\text{kg}^{-1}$  (high), 2.96 mg of Ca  $\text{kg}^{-1}$  (medium or adequate), 546 mg of Mg  $\text{kg}^{-1}$  (moderately high), 59.93 mg of Fe  $\text{kg}^{-1}$  (very high), 2.13 mg of Cu  $\text{kg}^{-1}$  (moderately high), 3.48 mg of Zn  $\text{kg}^{-1}$  (moderately high), 29.44 mg of Mn  $\text{kg}^{-1}$  (high), and 1.39 mg of B  $\text{kg}^{-1}$  (medium or adequate).

### **Physical and chemical characterization**

The fruit weight was determined through an electronic scale (Scout Pro SP2001 Ohaus<sup>®</sup>, USA). The equatorial diameter (ED) and the length (L) of the fruit were determined using a digital Vernier (INOX IP54 Caliper, Grass Valley, USA), and the rate between L/ED was considered to determine the shape index. The total soluble solids (TSS, °Brix) were evaluated through a digital refractometer (PAL-1 ATAGO<sup>®</sup>, Japan); the titratable acidity (TA) through the technique described by the Association of Official Analytical Chemists (AOAC, 2005), the content of total soluble sugars through the antrona method described by Witham *et al.* (1971). The rate between TSS/TA was considered to determine the sweetness index.

### **Nutritional characterization**

The percentage of moisture, lipids, raw fiber, ash and total carbohydrates was determined using the methods described by the AOAC (2005). The mineral analysis (P, K, Ca, Mg, S, Fe, Cu, Zn, Mn, B and Mo) was carried out by inductively coupled plasm optical emission spectroscopy (ICP-OES, model 725-ES, Agilent<sup>®</sup>), prior to acid digestion of multiple elements in microwave. The N content was determined through the combustion method by Dumas AOAC (2005).

### **Obtaining plant extracts**

The extracts were prepared separately from six solvent mixtures: water 100%, acetone at 80%, absolute ethanol, methanol at 80%, methanol/H<sub>2</sub>O/HCl (90:10:1), and trifluoroacetic acid (C<sub>2</sub>HF<sub>3</sub>O<sub>2</sub> at 1%).

### **Quantification of nutraceutical compounds and antioxidant activity**

For the quantification of the nutraceutical components and the antioxidant activity, plant extracts prepared with the diverse solvents were used, except for the quantification

of vitamin C, where the extracts were prepared according to what was reported by the two methods for their determination.

The quantification of nutraceutical compounds and antioxidant activity was carried out according to the following methods: 1) Total anthocyanins (TAN): the differential pH method described was used; 2) Total flavonoids: it was determined by the colorimetric method proposed by Chang *et al.* (2020); 3) Total soluble phenolic compounds: it was carried out through the Folin-Ciocalteu method; 4) Condensed tannins: it was quantified through the use of the H<sub>2</sub>SO<sub>4</sub>/vanillin reagent according to what was described by Scalbert *et al.* (1989); 5) Vitamin C: the quantification was done through the volumetric method of the AOAC (2005) and the spectrophotometric method (indophenol-xylene) described by Burdurlu *et al.* (2006); 6) Antioxidant activity: it was determined through the DPPH method (free radical 2,2-Diphenyl-1-picrylhydrazyl), proposed by Brand-Williams *et al.* (1995); ABTS (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), according to that described by Miller *et al.* (1993); and by the method of the ferric reducing ability of plasma (FRAP) method, according to what was described by Benzie and Strain (1996).

### Type of study, experimental design and statistical analysis

The experimental unit was one kilogram of fruit per sampling site, obtained from 10 “cahuiche” trees, with five repetitions defined by the sampling site. The physicochemical and nutritional characterization was a study of descriptive nature. For the nutraceutical characterization, an experimental study under a completely randomized design with five repetitions was carried out, where each solvent used for the phytochemical extraction was considered as treatment. All the data were reported as the mean  $\pm$  standard deviation of five repetitions, values expressed in fresh weight. The data were subjected to analysis of variance, Tukey’s means comparison ( $P \leq 0.05$ ), and Pearson’s correlation analysis through the Statistical Analysis System software (SAS version 9.2).

## RESULTS AND DISCUSSION

According to the morphological characterization, cahuiche fruits are very small round globular berries, given their diameter  $< 7$  mm and fruit weight  $< 0.18$  g (Table 1), characteristic contrary to what is found in fruits of other cranberry species. According to Frías-Ortega *et al.* (2020), for example, the fruits of *V. corymbosum* L., the most commercial blueberry species in the world, can reach medium ( $\geq 12$  mm -  $< 16$  mm) and large ( $\geq 16$  mm)

**Table 1.** Physical and chemical characteristics of cahuiche (*Vaccinium leucanthum*) fruits at consumption maturity, harvested in Ciénega Grande, Omitlán, Hidalgo.

Morphological parameters	Values	Chemical parameters	Values
Fruit weight (g)	0.12 $\pm$ 0.06	Total soluble solids (TSS, °Bx)	16.25 $\pm$ 1.01
Equatorial diameter (mm)	6.01 $\pm$ 1.04	Titrate acidity (TA, % citric acid)	1.49 $\pm$ 0.14
Pole diameter (mm)	5.62 $\pm$ 0.92	Total soluble sugars (% glucose)	13.51 $\pm$ 2.04
Shape index	0.93 $\pm$ 0.02	TSS / TA ratio	9.09 $\pm$ 1.24

Data are expressed as mean  $\pm$  standard error of five repetitions. Values reported in fresh weight (fw).

sizes. This could be explained because cahuche does not receive agronomic management, since it is a wild plant; therefore, it can be subjected to prolonged periods of stress, factor that can condition the size of the fruit, since during the fruit's filling and maturation, the water content is essential to achieve a greater weight gain (Bryla and Strik, 2007).

The total soluble solids (TSS) and titratable acidity (TA) were slightly higher than those found by Sánchez-Franco *et al.* (2018) in *V. leucanthum* (TSS=14.67±0.38 °Bx and TA=1.06±0.02%) cultivated in another municipality of the state of Hidalgo, Huasca de Ocampo. This could be associated with differences in the soil between the two municipalities, since Heeb *et al.* (2005) refer that an increase in sugars and organic acids in fruits that are cultivated in soils with higher content of inorganic nitrogen ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) is common; in this sense, it is important to point out that according to the soil analysis performed, the cahuche that was harvested for this study grew in soils with adequate nutritional availability of inorganic N for the plant.

The results from the nutritional analysis indicated that cahuche is a fruit with high contents of carbohydrates, raw fiber, lipids, nitrogen, Ca, Mg and S (Table 2). In this regard, Sánchez-Franco *et al.* (2018) reported only lower values of protein (1.07%), lipids (0.40%) and energetic value (78.77 kcal 100 g<sup>-1</sup>). Compared to other cranberry species, Karlsons *et al.* (2018) found higher concentrations of P (19.3±2.2 and 16.5±3.3 mg 100 g<sup>-1</sup>) and K (81.6±6.6 and 110.8±8.9 mg 100 g<sup>-1</sup>) in fresh fruits of *V. corymbosum* and *V. myrtilillus*, respectively, compared to cahuche. The differences observed between the species of *Vaccinium* reported in other studies could be explained by the different edaphoclimatic conditions of the sites where the plants grew, factors that influenced the nutritional quality of the fruits (Perez-Lainez *et al.*, 2019).

The analysis of nutraceutical metabolites showed that cahuche is an excellent source of total anthocyanins (TA), total flavonoids (TF) and total soluble phenolics (TSP) (Table 3), as well as vitamin C (volumetric volume: 23.69±3.95 mg EAA 100 g<sup>-1</sup>; spectrophotometric method: 41.33±6.89 mg EAA 100 g<sup>-1</sup>). The presence of condensed tannins in the cahuche fruit was not detected. Cahuche, in comparison to other berries, presented

**Table 2.** Nutritional compounds of cahuche (*Vaccinium leucanthum*) fruits at consumption maturity, harvested in Ciénega Grande, Omitlán, Hidalgo.

Variable	Values	Variable	Values
Moisture (%)	66.84±1.92	Ca (mg 100 g <sup>-1</sup> )	43.24±5.09
Protein (%)	1.95±0.15	Mg (mg 100 g <sup>-1</sup> )	16.09±0.86
Lipids (%)	1.67±0.23	S (mg 100 g <sup>-1</sup> )	28.61±1.35
Ash (%)	0.20±0.02	Fe (mg 100 g <sup>-1</sup> )	0.80±0.08
Carbohydrates (%)	22.67±1.46	Cu (mg 100 g <sup>-1</sup> )	0.13±0.01
Crude fiber (%)	6.67±0.75	Zn (mg 100 g <sup>-1</sup> )	0.15±0.04
Energetic value (Kcal 100 g <sup>-1</sup> )	113.49±7.79	Mn (mg 100 g <sup>-1</sup> )	1.80±0.77
N (mg 100 g <sup>-1</sup> )	250.66±29.27	B (mg 100 g <sup>-1</sup> )	0.30±0.04
P (mg 100 g <sup>-1</sup> )	14.73±1.66	Mo (mg 100 g <sup>-1</sup> )	0.12±0.11
K (mg 100 g <sup>-1</sup> )	37.74±10.90	-	-

Data are expressed as mean±standard error of five repetitions. Values reported in fresh weight (f.w).

**Table 3.** Nutraceutical compounds in cahuiche (*Vaccinium leucanthum*) fruits harvested at consumption maturity, using six solvents for extraction.

Solvent	TA (mg CE 100 g <sup>-1</sup> f.w.)	TF (mg QE 100 g <sup>-1</sup> f.w.)	TSF (mg GAE 100 g <sup>-1</sup> f.w.)
Water	3.37±0.92 e	46.08±3.58 c	126.21±8.28 c
Acetone 80%	162.84±33.45 cd	98.95±9.89 a	356.61±34.60 ab
Ethanol	135.49±5.90 d	116.06±19.91 a	295.12±32.01 b
Methanol 80%	188.70±33.78 bc	94.08±4.42 a	348.82±31.64 ab
Methanol / H <sub>2</sub> O / HCl (90:10:1)	267.50±21.24 a	71.14±8.44 b	407.36±13.59 a
C <sub>2</sub> H <sub>5</sub> F <sub>3</sub> O <sub>2</sub> al 1%	220.84±8.53 b	53.49±3.98 bc	317.78±28.18 b
HSD	46.33	22.83	62.75

Data are expressed as mean±standard error of five repetitions. Values reported in fresh weight (f.w.). Different letters in the same column indicate significant statistical differences (Tukey  $P \leq 0.05$ ). HSD: honest significant difference, TA: total anthocyanins, TF: total flavonoids, TSF: total soluble phenolics, CE: cyanidin-3-glucoside equivalents, QE: quercetin equivalents, GAE: gallic acid equivalents.

higher concentrations of anthocyanins (red pigments) and flavonoids, although with values closer to other species of phenolic compounds: *V. corymbosum* (29.72 mg EC 100 g<sup>-1</sup>, 47.53 mg QE 100 g<sup>-1</sup>, 305.38 mg GAE 100 g<sup>-1</sup> and 73.21 mg EAA 100 g<sup>-1</sup>); other berries such as strawberry (16.03 mg EC 100 g<sup>-1</sup>, 38.17 mg QE 100 g<sup>-1</sup>, 621.92 mg GAE 100 g<sup>-1</sup> and 90.13 mg EAA 100 g<sup>-1</sup>); raspberry (14.69 mg EC 100 g<sup>-1</sup>, 9.61 mg QE 100 g<sup>-1</sup>, 357.83 mg GAE 100 g<sup>-1</sup> and 92.17 mg EAA 100 g<sup>-1</sup>), and blackberry (58.61 mg EC 100 g<sup>-1</sup>, 87.03 mg QE 100 g<sup>-1</sup>, 850.52 mg GAE 100 g<sup>-1</sup> and 52.41 mg EAA 100 g<sup>-1</sup>) (De Souza *et al.*, 2014). Sánchez-Franco *et al.* (2018) reported higher values for cahuiche of vitamin C (102.3 mg EAA 100 g<sup>-1</sup>), total phenolic compounds (1090.3 mg GAE 100 g<sup>-1</sup>) and flavonoids (112.0 mg QE 100 g<sup>-1</sup>), than those found in this study.

Regarding the antioxidant activity, capacity of a substance to inhibit the oxidation of biomolecules (lipids, proteins and nucleic acids) and to avoid the alteration of the cellular functions of the organism, Table 4 shows the antioxidant capacity of the cahuiche fruit determined by three methods. The results showed that wild fruits of *V. leucanthum* have a higher antioxidant capacity than blueberry (*V. corymbosum*) (588 μM TE 100 g<sup>-1</sup> p.f.), cherry (883 μM TE 100 g<sup>-1</sup> p.f.), raspberry (627 μM TE 100 g<sup>-1</sup> p.f.), strawberry (787 μM TE 100 g<sup>-1</sup> p.f.), and blackberry (1323 μM TE 100 g<sup>-1</sup> p.f.) by the ABTS method (De Souza *et al.*, 2014). Sánchez-Franco *et al.* (2018) reported lower antioxidant activity by the DPPH (1.29 mM TE 100 g<sup>-1</sup> p.f.) and ABTS (1.03 mM TE 100 g<sup>-1</sup> p.f.) methods in cahuiche collected in another municipality of Hidalgo.

In general, the differences found in the contents of nutraceutical components and antioxidant activity between the various studies of cahuiche and other species of berries, could be because of the genetic variability between species and genotypes, state of maturity, edaphoclimatic conditions of the place of origin, as well as the techniques for extraction, preparation and analysis of these metabolites (Perez-Lainez *et al.*, 2019).

In most of the extracts, the antioxidant activity observed in cahuiche was higher with the ABTS method (Table 4). According to Floegel *et al.* (2011), the differences found in the antioxidant activity determined by various methods can be due mainly to the polarity of the antioxidants present in the matrix of the fruit and the chemical action

**Table 4.** Antioxidant capacity of cahuiche (*Vaccinium leucanthum*) fruit pulp harvested at consumption maturity, using six solvents for the extraction.

Solvent	AA by DPPH (mM TE 100 g <sup>-1</sup> )	AA by ABTS (mM TE 100 g <sup>-1</sup> )	AA by FRAP (mM TE 100 g <sup>-1</sup> )
Water	0.63±0.15 e	1.21±0.08 d	0.65±0.15 d
Acetone 80%	2.71±0.32 ab	6.39±1.09 a	3.80±0.63 bc
Ethanol	2.51±0.35 b	4.71±0.84 b	3.35±0.69 c
Methanol 80%	2.82±0.21 a	6.33±0.62 a	4.03±0.47 b
Methanol / H <sub>2</sub> O / HCl (90:10:1)	2.04±0.23 c	3.30±0.40 c	5.13±0.16 a
C <sub>2</sub> HF <sub>3</sub> O <sub>2</sub> al 1%	1.77±0.13 d	3.10±0.21 c	3.76±0.38 bc
HSD	0.21	0.72	0.64

Data are expressed as mean±standard error of five repetitions. Values reported in fresh weight (f.w). Different letters in the same column indicate significant statistical differences (Tukey P≤0.05). HSD: honest significant difference, AA: antioxidant activity, ET: trolox equivalents.

foundation of each of the methods. The ABTS assay is based on the generation of a free radical that allows quantifying this biological activity both in hydrophilic and lipophilic antioxidant systems, so it has been reported that this method in most of the fruits allows detecting higher antioxidant capacity; in contrast, the DPPH assay is the most effective one for hydrophobic systems. On the other hand, the FRAP method acts only by the transference mechanism of an unpaired electron, while ABTS acts by transference of an unpaired electron as well as the transference mechanism of a hydrogen atom (Fonseca-García *et al.*, 2014).

Regarding the solvent for extraction, it has been described that ethanol is the most effective solvent to recover phytochemicals, specifically anthocyanins (Nistor *et al.*, 2021); however, in this study the use of ethanol only allowed extracting 49% less of anthocyanins compared to the use of methanol/H<sub>2</sub>O/HCl (90:10:1), mixture that allowed obtaining the best extraction yields of anthocyanins and total soluble phenolics (Table 3), and the highest value of antioxidant capacity by the FRAP method (Table 4), nearly double compared to other methods.

Pearson's correlation analysis (Table 5) indicated that: 1) anthocyanins presented a positive correlation with the high antioxidant activity detected by FRAP in those extracts where CH<sub>3</sub>OH/H<sub>2</sub>O/HCl was used as a solvent; 2) the highest antioxidant activity detected by the ABTS assay in acetonic extracts was highly correlated to the presence of total soluble phenolic compounds, since this solvent allowed extracting higher amounts of these metabolites (Table 4), in concentrations that are statistically equal to those obtained through extraction with methanol/H<sub>2</sub>O/HCl (90:10:1) and methanol at 80% (Table 3); and 3) through the DPPH assay, the highest antioxidant capacity was observed in methanol extracts at 80%, strongly associated to the higher concentration of flavonoids compared to other extracts (Table 4). This brings to light the importance of continuing to study the effect of the solvent in the evaluation of the antioxidant capacity of foods through various methods such as those evaluated here, since each food is a complex and different matrix; also, the polarity of each solvent can affect the transference of electrons and the transference

**Table 5.** Pearson's correlation coefficients between the phytochemical compounds and antioxidant activity for the various solvents used for the extraction of nutraceutical compounds of cahuiche (*Vaccinium leucanthum*).

Variable	H <sub>2</sub> O	Acetone 80%	Ethanol	CH <sub>3</sub> OH 80%	CH <sub>3</sub> OH / H <sub>2</sub> O / HCl	C <sub>2</sub> H <sub>5</sub> F <sub>3</sub> O <sub>2</sub> 1%
DPPH/TA	0.29	0.73	0.03	0.68	-0.13	-0.52
DPPH/TF	0.76	0.57	0.24	0.99**	0.66	-0.99**
DPPH/TSF	0.90*	0.98**	0.47	0.54	0.40	-0.90*
ABTS/TA	0.13	0.74	-0.67	0.44	0.98**	-0.66
ABTS/TF	0.64	0.57	-0.49	0.99**	0.51	0.29
ABTS/TSF	0.81	0.98**	-0.26	0.28	0.75	-0.15
FRAP/TA	0.27	0.48	0.85	0.66	0.99**	0.96**
FRAP/TF	0.74	0.81	0.94*	0.99**	0.60	0.73
FRAP/TSF	0.88*	0.99**	0.99**	0.52	0.81	0.95*

Note: \*P≤0.05 (significant); \*\*P≤0.01 (highly significant). TA: total anthocyanins, TF: total flavonoids, TSF: total soluble phenolics. ABTS, DPPH and FRAP: methods for determining the antioxidant activity.

of hydrogen atoms, key aspects in the measurements of the antioxidant capacity in foods (Pérez-Jiménez & Saura-Calixto, 2006).

## CONCLUSIONS

Cahuiche is an underused wild cranberry with high potential for its exploitation as a crop, since it is a species with high nutritional and nutraceutical potential whose consumption could provide benefits to the health of the inhabitants where this plant is located, due to its high contents of fiber, protein, Ca, S, lipids, anthocyanins and other phenolic compounds; however, it is recommended to conduct more studies for its use in the food industry and the effect of its transformation on its nutraceutical potential. The solvent used for extraction is determinant to achieve better yields for the quantification and analysis of phenolic compounds, anthocyanins, flavonoids and the measurement of antioxidant activity. The values of antioxidant capacity in *Vaccinium* species should only be compared when the measurements have been carried out by the same solvent method. In cahuiche, the best extraction solvents were methanol-H<sub>2</sub>O-HCl (90:10:1), methanol at 80%, and acetone at 80%.

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# Common water-hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) used to treat leachate

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## ABSTRACT

**Objective:** To evaluate the phytoremediation capacity of *Eichhornia crassipes* (common water-hyacinth) and *Pistia stratiotes* (water lettuce) exposed to water polluted with leachate from the El Guayabo landfill, located in Medellín, Veracruz, Mexico.

**Design/Methodology/Approach:** The phytoremediation experiment lasted 21 days. Diluted leachate (30%) and six plants were used, with 3:1 (TA) and 1:1 (TB) ratios for water lettuce and common water-hyacinth. Water turbidity, nitrite, and chemical oxygen demand (COD) were the response variables used to evaluate pollutant removal. A WGZ-200 turbidity meter was used to measure turbidity (NTU), following the NMX-AA-038-SCFI standard (2001). Nitrite was measured based on the NMX-AA-099-SCFI standard (2006), while COD was determined according to the NMX-AA-030/2-SCFI standard (2011).

**Results:** Compared with control, a high percentage of turbidity and nitrite removal (>90%) was observed. The reduction of COD fluctuated from  $29.87 \pm 3.90$  to  $31.08 \pm 4.75\%$ , while control recorded  $18.80 \pm 4.65\%$ .

**Study Limitations/Implications:** The biological material variables were difficult to control under *ex situ* conditions.

**Findings/Conclusions:** Common water-hyacinth and water lettuce can remove chemical pollutants from water polluted with leachate. Compared with TE, TB (T2) recorded significant differences regarding COD; consequently, the best treatment to reduce pollutants is TB (T2).

**Keywords:** physicochemical analysis, landfill, phytoremediation.

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## INTRODUCTION

Mexico generates about 120,128 t of urban solid waste (USW) per day. An average of 84% of the total USW is collected; consequently, the domestic coverage reaches 100,751 t per day (1). The waste sent to landfills or final disposal sites generates leachate (liquid pollutants resulting from decomposition). Leachate is a combination of waste and elements dragged within the waste itself. The amount of leachate generated is directly related to several factors, including precipitation, evapotranspiration, surface runoffs, infiltration, and waste compaction degree (2).

Phytoremediation—a simple, economical, and environmentally-friendly method—has been implemented during the last years to treat leachate. It consists of using plants to remediate or mitigate water pollution (3).

Common water-hyacinth and water lettuce develop in nutrient-abundant waters (phosphorous, potassium, and nitrogen) and, as a result of their high growth rates, are considered as invasive aquatic weeds (4). However, they have important characteristics that can be used to restore water bodies, including the absorption of pollutants from their environment. Since macrophytes can assimilate water pollutants, they have been used to detect and remove pollutants from domestic and industrial sewages (5). According to several studies, water lettuce is a plant used for bioremediation, because it has a 15% potential of removing pollutants. For its part, the common water-hyacinth has similar morphological and pollutant-capture characteristics.

Therefore, the phytoremediation capacity of *E. crassipes* (common water-hyacinth) and *P. stratiotes* (common lettuce) exposed to water polluted with leachate was evaluated. The objective was to prove that the combined use of these plants can remove leachate from polluted water.

## MATERIALS AND METHODS

The leachate was collected in 20 L demijohns from the leachate lagoon of the El Guayabo landfill, Medellín, Veracruz, Mexico. Subsequently, the demijohns were transported to the lab, where they were stored and kept at room temperature.

The common water-hyacinth and water lettuce were obtained from the artificial wetland located within the facilities of the Tecnológico de Boca del Río, Veracruz, Mexico. Once the healthy plants were collected, they were washed and distributed in the different treatments.

The plants were placed in 40 L plastic containers (55×38×33 cm). Three treatments were carried out with 30% leachate. Treatment A (TA) consisted of 75% water lettuce + 25% common water-hyacinth, while treatment B (TB) was made up of 50% water lettuce + 50% common water-hyacinth. Control (TE) consisted of 30% leachate, without plants.

The study lasted 21 days and water turbidity, nitrite, and COD were analyzed. A WGZ-200 turbidity meter was used to determine the nephelometric turbidity units (NTU), following the NMX-AA-038-SCFI standard (2001). Nitrite was measured based on the NMX-AA-099-SCFI standard (2006). Fifty mL of the filtered and diluted sample were poured into a flask; 1.0 mL of sulfonamide solution was added to the flask and the mixture was vigorously shaken. Subsequently, 1.0 mL of N-(1-Naphthyl) ethylenediamine dihydrochloride dilution were added. The mixture was allowed to rest for 10 minutes and then absorbency was measured at 543 nm. Meanwhile, the COD was determined according to the NMX-AA-030/2-SCFI standard (2011): 2.0 mL of the leachate diluted (1:25) sample were poured into glass tubes with screw caps. Afterwards, 1.0 mL of digestion solution and 2.0 mL sulfuric acid solution were placed in a digestion bale, at 150 °C for 2 h. Subsequently, a spectrophotometric reading (620 nm length) was carried out at room temperature. A calibration curve, built with potassium biphthalate,

was used for the calculation. Removal efficiency was calculated with the following equation:

$$R = (Ic - Fc) / Ic \times 100$$

Where  $R$  is the pollutant removal (%),  $Ic$  is the initial concentration of the pollutant, and  $Fc$  is the final concentration.

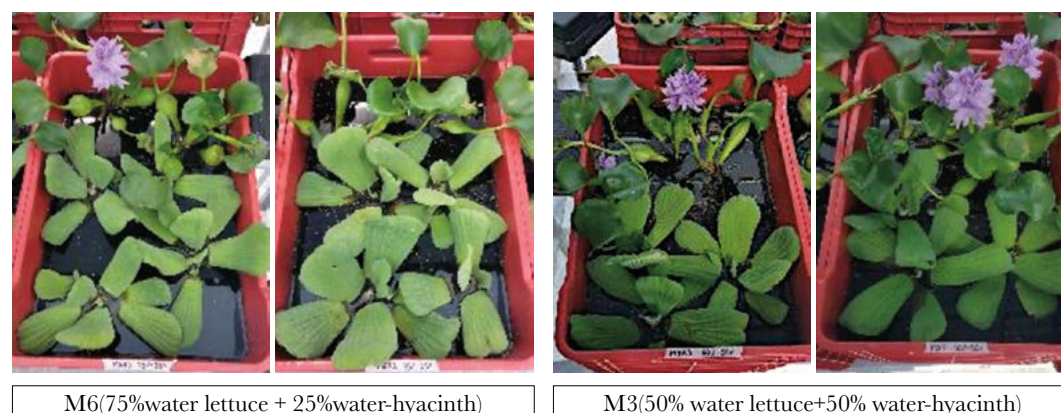
## RESULTS AND DISCUSSION

The treatments were kept under the following average environmental conditions: 25.08 °C temperature, 53.73% relative humidity, 1,418 lux light intensity, and 2,758.34 mW/m<sup>2</sup> irradiance. In average, the plants selected had a 100-g fresh weight, 12-cm long leaves, and 20-cm long roots.

After 21 days, the plants of both treatments with 30% leachate (TA and TB) were able to adapt to the conditions between day 3 and 4 (Figure 1). These results match the findings of Carrion (6), who compared the *Pistia stratiotes* and the *Azolla filiculoides* species and determined that the treatment was efficient after 21 days.

Other authors have reported that *P. stratiotes* was the most efficient macrophyte, because it reduced the turbidity by 2.80 NTU, reaching a 98.92% removal. These results match the findings of this study. *P. stratiotes* can easily adapt and have the capacity to live in different nutrient-saturated water bodies. Coronel-Castro (7) compared *Eichhornia crassipes* and *Lemna minor* and recorded that the former removed turbidity by >75%, proving that it removes more pollutants than the latter, as a consequence of its root system, whose associated microorganisms enabled the removal of organic compounds and the reduction of levels of physical parameters.

In order to evaluate the effectivity of plants in water polluted with leachate, the initial sample (30% leachate) was subjected to a physicochemical analysis. The results obtained were 28.586 NTU, 913.2 mg/L, and 65.934 mg/L for turbidity, COD, and nitrite, respectively. These data were used to determine the pollutant removal percentage of the treatments.



**Figure 1.** Common water-hyacinth and water lettuce treatments.

Table 1 shows the results after 21 days. Turbidity removal ranged from  $91.90 \pm 1.82$  to  $95.02 \pm 3.34\%$  in TA and TB, proving plant activity. Meanwhile, control recorded  $71.45 \pm 0.58\%$  turbidity removal. The same phenomenon happened with COD: the results fluctuated from  $29.87 \pm 3.90$  to  $31.08 \pm 4.75\%$ , proving the removal of chemical pollutants from water polluted with leachate; meanwhile, control recorded  $18.80 \pm 4.65\%$ . These findings match the reports of Vásquez (8) and Sayago (9). The former author experimented with wastewater from mines, while the latter used wastewater from tanneries. Both authors used combined or one-species treatments with *Pistia stratiotes* L. and *Eichhornia* in their evaluations. They pointed out that good results can be obtained for the removal of biological and chemical oxygen pollutants, even using artificial wetlands.

Abbas (10) proved the effective phytoremediation of leachate using water lettuce and common water-hyacinth for a 15-day period. The results of this study match Abbas and Camacho (11). The latter author carried out a similar experiment using *Eichhornia crassipes* to treat wastewater and recorded a significant reduction of COD in the evaluated treatments.

Finally, nitrite values ranged from  $94.54 \pm 1.54$  to  $96.60 \pm 0.51\%$  and recorded a higher reduction percentage than control ( $83.22 \pm 0.51\%$ ).

Nitrogen absorption was the result of the plant's biological pathway (ammonification, nitrification, denitrification, nitrogen capture, biomass assimilation and fixation, and nitrate de-assimilation reduction). Denitrification is the main nitrogen removal mechanism of the artificial wetlands (12).

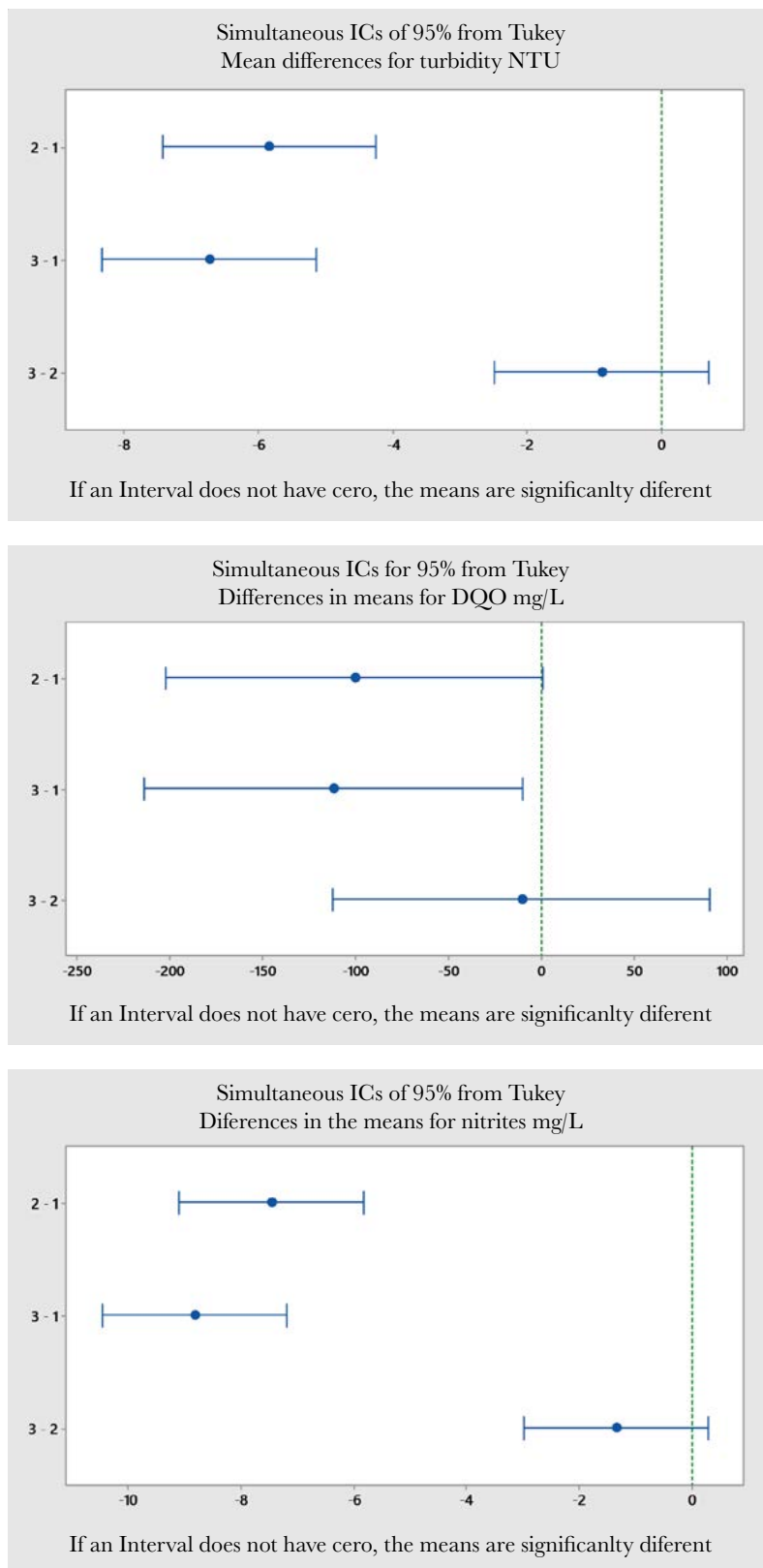
The statistical analysis of the results —through the Student's t-test and the Tukey's Multiple Comparison Test— indicated that TA and TB did not record significant differences regarding turbidity, nitrite, and COD parameters (Figures 2a, 2b, and 2c); however, control recorded significant differences. Except in the case of COD (Figure 2b), TA did not record significant differences regarding TE. Nevertheless, TB was significantly different than TE. Consequently, TB was the best treatment for pollutant reduction.

## CONCLUSIONS

The *Pistia stratiotes* and *Eichhornia crassipes* phytoremediation treatments can tolerate a 30% landfill leachate. The treatment with a 50:50 ratio of *Pistia stratiotes* and *Eichhornia crassipes* (TB) increased the efficient removal of pollutants from a 30% diluted leachate. The efficiency of *Pistia stratiotes* and *Eichhornia crassipes* as phytoremediation plants reduced pollutants from the leachate of the El Guayabo landfill.

**Table 1.** Mean values of the turbidity, COD, and nitrite parameters and percentage of removal per treatment ( $\pm$ SD).

Treatments	Turbidity (NTU)	Removal (%)	COD (mg/L)	Removal (%)	Nitrite (mg/L)	Removal (%)
TE	$8.16 \pm 0.16$	$71.45 \pm 0.58$	$741.56 \pm 42.47$	$18.80 \pm 4.65$	$11.06 \pm 0.34$	$83.22 \pm 0.51$
T1	$2.32 \pm 0.52$	$91.90 \pm 1.82$	$640.44 \pm 35.64$	$29.87 \pm 3.90$	$3.60 \pm 1.02$	$94.54 \pm 1.54$
T2	$1.42 \pm 0.95$	$95.02 \pm 3.34$	$629.33 \pm 43.33$	$31.08 \pm 4.75$	$2.24 \pm 0.34$	$96.60 \pm 0.51$



**Figure 2.** Comparative analysis of the parameters per treatment: a) turbidity; b) COD; and c) nitrite.

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# Pathogen Index in Freshwater drum (*Aplodinotus grunniens* R.) from the Usumacinta River in Southeastern Mexico

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## ABSTRACT

**Objective:** Determine infestation rates and identify parasites in the freshwater drum (*Aplodinotus grunniens* R.) from the Usumacinta River.

**Design/methodology/approach:** A total of 17 specimens were reviewed during the months of April-May 2022 and dissected in the Aquaculture Health Laboratory of Universidad Juárez Autónoma de Tabasco, Ríos Division, for the search and extraction of pathogens under conventional methods and techniques.

**Results:** Two pathogen groups were recorded and taxonomically classified: Nematodes: larvae of *Contracaecum* sp. (n=139) and adult *Rhabdochona* sp. (n=9) and Trematodes: metacercaria of *Austrodiposmotum* sp. (n=19) and *Clinostomum* sp. (n=1). The larvae of *Contracaecum* sp. presented the highest infestation rates with a Prevalence (P) of 41.1%, Mean Intensity (MI) of 19.8, and Abundance (AB) of 8.1. The lowest rates were for the metacercaria of *Clinostomum* sp. with a P of 5.8%, MI of 1.0, and AB 0.05.

**Limitations on study/implications:** From a parasitic zoonosis point of view freshwater drum is a potential disease vector due to the consumption of raw or undercooked fish.

**Findings/conclusions:** The parasitofauna recorded in freshwater drum from the lower basin of the Usumacinta River includes 4 species of parasites belonging to two groups: Trematodes and Nematodes.

**Keywords:** fish; freshwater; parasites; indices; infestation.

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## INTRODUCTION

In the Mexican southeast, *Aplodinotus grunniens* occurs in the Usumacinta River, where it supports an artisanal fishery (Hernández-Gómez *et al.*, 2019). It is among the 57 species of fishing importance in the Usumacinta River basin, and the species with high commercial value (Mendoza-Carranza *et al.*, 2018). For the freshwater drum (*A. grunniens* R.) which belongs to the Sciaenidae family, there are studies describing pathogens including those reported in the United States as monogenean (Platyhelminthes) species of *Microcotyle*



*spinicirrus* (Remley, 1942). In Mexico, Pérez-Ponce *et al.* (2013) recorded *Diplostamenides spinicirrus* in the Falcón Reservoir, Tamaulipas. Similarly, in his study, Escobar-González (1997) reported the presence of two monogenean species (*Microcotyle spinicirrus* and *Lintaxine cockeri*), one metacercaria (*Diplostomum* sp.), three genera of nematodes (*Rhabdochona* sp., *Spinitectus* sp., and *Contracaecum* sp.), one hirudineo (*Illinobdella moorei*), and one copepod (*Ergasilus* sp.). However, the scarce information on parasitic studies regarding the freshwater drum in southeastern Mexico led us to focus this research on parasite infestation rates in freshwater drum of the middle basin of the Usumacinta River, providing information that will be relevant for future research.

## MATERIAL AND METHODS

### Study site

This study was conducted in the lower basin of the Usumacinta River in Tenosique, Tabasco, Mexico, El Recreo community at (17° 42.718' N and 91° 49.116' W), where freshwater drum specimens were obtained from April-May 2022, directly from commercial coastal fishery.

### Biometrics of specimens

Specimens were obtained from coastal fishing. Their total length was recorded in cm with an ichthyometer of 100 cm  $\pm$  1 mm and total weight in grams (g) with a digital balance of 5000g with  $\pm$  0.1g accuracy. The specimens were transported in coolers to the Aquaculture Health Laboratory of the Ríos-UJAT Division. A total of 17 specimens were reviewed following the criterion proposed by Moravec (1994), which indicates that when reviewing 15 fish and the prevalence is still low, this is the proportion of parasitized hosts expected for such a species of helminths.

Ectoparasites and endoparasites were searched for and extracted using brushes and fine needles following the criteria proposed by Margolis *et al.* (1982) and Lamothe-Argumedo (1997). For this purpose, a MOTIC BA<sup>®</sup> 300 microscope and a Zeiss<sup>®</sup> Stemi DVA stereomicroscope were used. An Excel spreadsheet database was created to record the information. Extracted pathogens were preserved based on the techniques proposed by Caspeta-Mandujano *et al.* (2009) for each group of parasites.

### Fixation, staining, and clearing of parasites

In trematodes, the fixation technique was used with 70% alcohol; they were also stained with Gomari's trichrome, but previously dehydrated in gradual alcohols, and cleared with clove oil to observe their internal organs and structure. These parasites were fixed with Hycel Canada Balm. In the case of nematodes, warm 4% formalin was used to spread them; they were preserved in vial bottles and cleared using Amman's Lactophenol technique to observe their morphological characteristics and record their measurements. Pathogens were measured in millimeters (mm) following the criteria and measurement characteristics proposed by Caspeta-Mandujano *et al.* (2009). All parasites were photographed with a MOTICAM<sup>®</sup> 2300 camera, 3.0. megapixels adapted to a

Zeiss<sup>®</sup> Stemi DVA stereomicroscope and a MOTIC BA<sup>®</sup> 300 microscope with MOTIC IMAGES PLUS 2.0 ML<sup>®</sup> software.

### Taxonomic classification of parasites

The classification of parasites was based on the criteria by Eiras *et al.* (2000), Vidal-Martínez (2002), and Caspeta-Mandujano *et al.* (2009).

### Infestation index

The indices proposed by Margolis *et al.* (1982) were calculated:

$$\text{Mean Intensity (MI)} = \frac{\text{Number of parasites}}{\text{parasitized fish}}$$

$$\text{Abundance (AB)} = \frac{\text{Total number of parasites collected}}{\text{Total number of hosts in the sample}}$$

$$\text{Prevalence (\%)(P)} = \frac{\text{Number of infected hosts}}{\text{Number of examined hosts}} \times 100$$

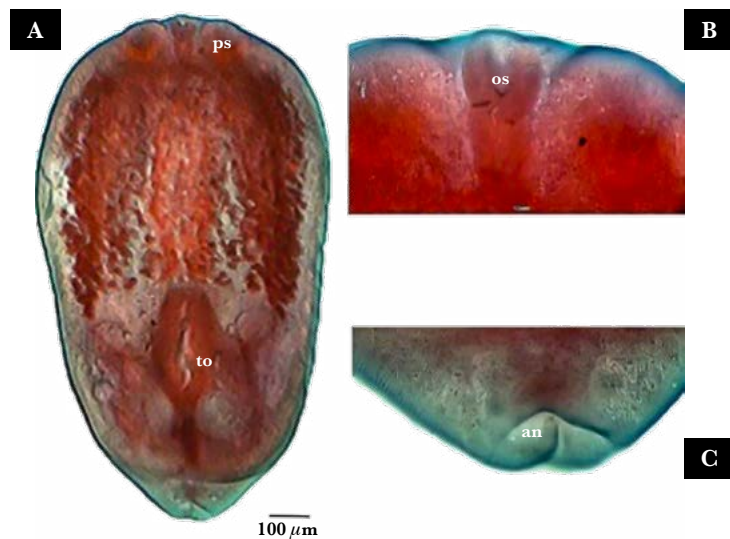
## RESULTS AND DISCUSSION

A total of 17 specimens of freshwater drum were analyzed, showing a total length of 27.8 to 36.9 cm ( $31.7 \pm 2.27$  cm) and a total weight of 207 to 685 g ( $377.4 \pm 142.7$  g). A total of 178 parasites were categorized in two groups of pathogens: Trematodes: 19 metacercariae of *Austrodiplostomum* sp. and one of *Clinostomum* sp.; Nematodes: 139 larvae of *Contracaecum* sp. and nine adults of *Rhabdochona* sp.

### Metacercariae of *Austrodiplostomum* sp.

Located in the vitreous humor of the eye of freshwater drum, they had a small and oval body of 1.4 mm (0.9-2.3 mm) (Figure 1A). A small, spherical subterminal oral sucker 0.1 mm (0.3-0.7mm) and an oval pharynx 0.06 mm (0.03-0.09 mm) can be noticed (Figure 1B). Two lateral pseudo suckers were observed, each next to the oral sucker. An oval tribocytic organ 0.3 mm (0.1-0.6 mm) can also be seen in the posterior half of the body (Figure 1).

The record of metacercariae in freshwater drum, is new for the Usumacinta basin in southeastern Mexico. However, they have been reported as *Diplostomum* sp., for this same species of fish in the Salinillas lagoon, Monterrey, Nuevo León (Escobar-González, 1997). In addition, Moreno-Moreno (1993) recorded *Diplostomum* (*Austrodiplostomum*) compactum in 649 fish from the Manuel Moreno Torres hydroelectric dam, Chiapas, including freshwater drum. Salgado-Maldonado (2006) reported metacercaria in fish from the state of Chiapas, including freshwater drum. In other countries such as Canada and the United States, specifically northern US, two metacercariae have been recorded for this same species of fish: *Diplostomum* sp. and *D. spathaceum* (Leno and Holloway, 1986; Wyatt, 1997), as well as *D. spathaceum* on the island of western Lake Erie, Ohio (Vendeland, 1968).



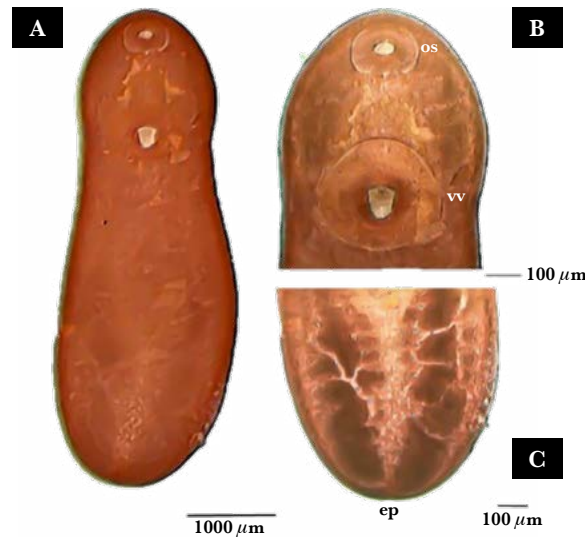
**Figure 1.** Metacercaria of *Austrodiplostomum* sp. A) Full view, pseudo sucker (ps); tribocytic organ (to). B) Anterior view showing the oral sucker (os) and pharynx (ph). C) Posterior view showing the anal opening (an).

In addition, in southeastern Mexico, they have been recorded in native freshwater cichlid fish by Vidal-Martínez *et al.* (2002). The taxonomic analysis of the metacercaria obtained in the present study shows similarities with the species described by Escobar-González (1997), with *Diplostomum spathaceum* in freshwater drum as described by Leno and Holloway (1986), and with the species described by Vidal-Martínez *et al.* (2002), but in cichlid fish. On the other hand, they differ with the species *Austrodiplostomum compactum* with respect to the measure of the oral sucker, which may be due to the fixation or assembly method of the metacercaria (Albuquerque *et al.*, 2017).

#### **Metacercaria of *Clinostomum* sp.**

Infesting the base of the gills, this metacercaria presented a yellow coloration and morphologically displayed a large and oval body of 5.6 mm, flattened dorsoventrally. In the anterior part of the metacercaria, a small and rounded oral sucker of 0.42 mm is visible. In addition, they are distinguished by having a circular ventral sucker larger of 0.93 mm than the oral sucker (Figure 2).

This metacercaria was identified within the genus *Clinostomum* (Rudolphi, 1814) and is considered a new contribution regarding *A. grunniens* infestation. In the state of Tabasco, metacercariae of *C. complanatum* have been recorded infesting in various habitats the bodies of other fish species such as *Centropomus parallelus*, *Mayaheros urophthalmus*, *Herichthys pearsei*, *Petenia splendida*, and *Theraps synspilum* (Salgado-Maldonado *et al.*, 2005). Also in Tabasco, Vidal-Martínez *et al.* (2002) report this metacercaria in cichlid fish. Betanzos (2019) reported metacercariae of *C. marginatum* in *Rhamdia laticauda* of the Jataté River, Ocosingo, Chiapas. In addition, it was recorded in *Astyanax aeneus* in the state of Morelos (Caspeta-Mandujano *et al.*, 2009). The morphometric measurements of the metacercariae of *Clinostomum* recorded in the present study are similar to the records of *C. complanatum* reported by Vidal-Martínez *et al.* (2002) for cichlid fish, but their total body length is



**Figure 2.** Metacercaria of *Clinostomum* sp. A) Ventral view. B) Anterior view of metacercaria, oral sucker (os), and acetabulum or ventral sucker (ac). C) Posterior view of the body, excretory pore (ep).

slightly larger, as well as the total length and width of the oral sucker of the metacercariae identified by Caspeta-Mandujano *et al.* (2009).

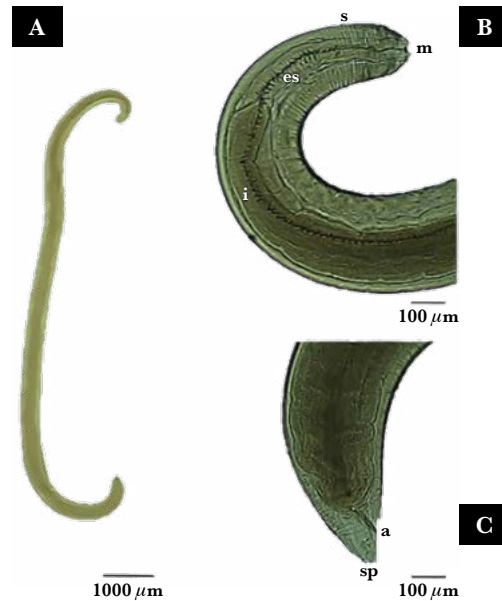
#### **Larvae of *Contracaecum* sp.**

A total of 139 larvae of this nematode were found in the mesentery of freshwater drum, displaying an elongated body with pink coloration of 17.8 mm, a rounded anterior termination, and a small ventral tooth. A cuticle covered with transverse striations is most evident at the anterior and posterior ends of the larva. They showed a rounded terminal mouth of 0.05 mm and a nerve ring of 0.13 mm located above the esophagus (Figure 3).

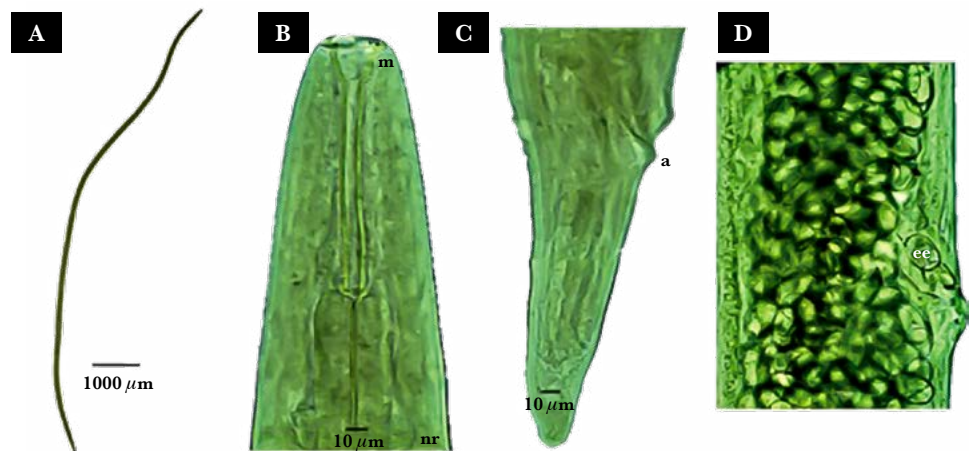
In Mexico, these larvae have been recorded in various hosts such as *Algansea lacustris*, *Atractosteus tropicus*, *Cichlasoma urophthalmus*, and *Poecilia petenensis*, as reported by Caspeta-Mandujano (2010). It has been reported in *Cichlasoma friedrichsthalii*, *C. helleri*, *C. managuense*, and *C. urophthalmus* (Vidal-Martínez *et al.*, 2002) for southeastern Mexico, as well as in other freshwater species such as *Petenia splendida* and *Centropomus parallelus* (Salgado-Maldonado *et al.*, 2005), and in Argentina in species of the Scienidae family such as *Cynoscion guatucupa* (Galeano, 2017). According to their morphometry, *Contracaecum* sp. larvae differ from *Contracaecum* larvae Type 1 recorded by Vidal-Martínez *et al.* (2002) and Caspeta-Mandujano (2010) in having a larger size. However, they are similar to the *Contracaecum* sp. larvae reported by Escobar-González (1997) for a freshwater drum but are different from the one recorded by Pardo *et al.* (2008), Pardo *et al.* (2009), and Galeano (2017) in other fish species.

#### **Adult nematodes of *Rhabdochona* sp.**

Adult nematodes of *Rhabdochona* sp. (n=9) (Figure 4) with thin, spherical, white, and elongated bodies of 9.92-16 mm were collected in the intestine of freshwater drum. An oral and rounded opening of 0.025-0.06 mm can be seen in the anterior part. Females were



**Figure 3.** Larva of nematode *Contracaecum* sp. A) Full view of larvae. B) Anterior part of the body, striations (s), mouth (m), intestine (i), esophagus (es). C) Posterior view, anus (a), and spicule (sp).



**Figure 4.** Adult nematode of *Rhabdochona* sp. A) Dorsal view. B) View of anterior region, mouth (m), nerve ring (nr). C) Posterior view, anus (a). D) Lateral view, mid region with embryonated eggs (ee).

observed in a state of pregnancy, with eggs being 0.015 to 0.035 mm long and 0.015 to 0.022 mm wide. Escobar-González (1997) reported nematodes in the Salinillas Lagoon, Nuevo León, for the host under study. In the state of Chiapas, the species *Rhabdochona kidderi* is reported as infesting *Rhamdia laticauda* and *Theraps irregularis* (Betanzos, 2019). In addition, these nematodes were described in the Atlas of helminth parasites of cichlid fish of southeastern Mexico as *R. kidderi* and *R. kidderi texensis* (Vidal-Martínez *et al.*, 2002). In addition, Caspeta-Mandujano *et al.* (2009) and Caspeta-Mandujano (2010) reported the species of this family in Mexico in freshwater fish such as *Rhabdochona acuminata*, *R. xiphophori*, *R. lichtenfelsi*, and *R. mexicana*.

The species studied here, *Rhabdochona* sp., presented morphometric measurements like the species reported by Escobar-González (1997) for *freshwater drum* in the state of Nuevo León, but different from larvae recorded by Vidal-Martínez *et al.* (2002) and Caspeta-Mandujano *et al.* (2009) for freshwater cichlid fish and marine fish, respectively.

### Infestation rates

Larvae of nematodes of *Contraecaecum* sp. had the highest Prevalence (P) with 41.1%, a Mean Intensity (MI) of 19.8, and an Abundance (AB) of 8.1. The metacercaria of *Clinostomum* sp. presented the lowest rates of infestation with 5.8% (P), an MI of 1.0, and an AB of 0.05. Rates in adult nematodes are noted in Table 5. The prevalence recorded for *Austrodiplostomum* sp. is lower than the one recorded for *Diplostomum* sp., reported in freshwater drum by Escobar-González (1997) (Table 1).

Metacercariae of *Clinostomum* sp. can present a biological cycle similar to the one for *C. complanatum* (Rojas-Sánchez *et al.*, 2014) and produces the disease called Clinostomiasis in fish. Humans can act as accidental hosts for the consumption of parasitized, raw or undercooked fish fillet; once in the person's throat, they can produce laryngopharyngitis and in extreme cases, asphyxia (Ad Hoc Group, 2021). Cases of human laryngeal infection by *C. complanatum* have been recorded in Korea and Japan (Chan-Woong *et al.*, 2009; Hara *et al.*, 2014; Hyun *et al.*, 2018). The metacercariae of the genus reported in this study showed a prevalence lower than the species *C. marginatum* and *Clinostomum* sp. reported by Betanzos (2019) and Murrieta-Morey *et al.* (2022) (Table 2).

Nematode *Contraecaecum* sp. represented the largest sample of pathogens in freshwater drum in this study. However, these rates were lower than the ones recorded by Escobar-González (1997) in this same species, as well as in other host fish such as *Hoplias malabaricus*, *Rhamdia quelen*, *Ophiogobius jenynsi*, and *Odontesthes bonariensis* (Pardo *et al.*, 2008; Mancini

**Table 1.** Comparison of *Austrodiplostomum* sp. infestation rates in freshwater drum, number of parasites (NP), Mean Intensity (MI), Abundance (AB), and Prevalence % (P).

Pathogen/ species	NP	MI	AB	P (%)	Host	Author
<i>Austrodiplostomum</i> sp.	19	3.10	1.10	35.2	<i>A. grunniens</i>	Present study
<i>Diplostomum</i> sp.	-	-	0.66	66.6	<i>A. grunniens</i>	Escobar-González (1997)
<i>D. spathaceum</i>	260	2.60	2.57	99.0	<i>A. grunniens</i>	Leno and Holloway (1989)

NP: number of parasites, MI: Mean Intensity, AB: Abundance, P Prevalence %.

**Table 2.** Comparison of *Clinostomum* sp. infestation rates in freshwater drum, number of parasites (NP), Mean Intensity (MI), Abundance (AB), and Prevalence % (P).

Pathogen/ species	NP	MI	AB	P (%)	Host	Author
<i>Clinostomum</i> sp.	1	1.00	0.05	5.80	<i>A. grunniens</i>	Present study
<i>C. marginatum</i>	42	3.50	42.0	80.0	<i>R. laticuada</i>	Betanzos (2019)
<i>Clinostomum</i> sp.	222	13.87	11.1	80.0	<i>Apistogamma</i> sp.	Murrieta-Morey <i>et al.</i> (2022)
	55	2.89	1.66	57.5	<i>Cichlasoma amazonarum</i>	
	44	11.0	8.80	80.0	<i>Pterophyllum scalare</i>	

NP: number of parasites, MI: Mean Intensity, AB: Abundance, P Prevalence %.

*et al.*, 2014) (Table 3). From a parasitic zoonosis point of view, this nematode species places freshwater drum as a potential transmitter of Anisakiasis, as it happens with the white snook *Centropomus undecimalis* due to the recorded prevalence (68%) (Vergara-Flórez and Consuegra, 2021). The most widely represented group in Mexico by ichthyozoonotic species is the nematodes (Rojas-Sánchez *et al.*, 2014). Ingestion of dead larvae or their remains can produce allergies (Ad Hoc Group, 2021).

The P reported in this study for adult nematodes of *Rhabdochona* sp. is lower than the one mentioned by Escobar-González (1997) of 100%. However, the rate is slightly higher than the one recorded by Betanzos (2019) for *Rhabdochona kidderi* in the host *Rhamdia laticauda* (Table 4).

Infectious transmissions from animals to humans are known as zoonoses. Particularly, those caused by the consumption of raw fish are termed ictiozoonoses (Rojas *et al.*, 2014; Garrido-Olvera *et al.*, 2023). As mentioned earlier in this study, helminths (larvae of *Contracaecum* sp. and *Clinostomum* sp.) have been recorded which can cause health issues in humans upon ingesting raw or undercooked fish containing these larvae. For example, ceviche, a typical dish in some tropical regions. Although these helminths have not yet been reported to affect humans in Mexico, they could become a public health problem in the future, as these parasites may cause diseases with gastrointestinal symptoms and systemic allergies (*e.g.*, some Anisakis species) and be responsible for Halzoun syndrome and laryngopharyngitis (*e.g.*, some *Clinostomum* genus species) (Quijada *et al.*, 2005; Sereno-Uribe *et al.*, 2022). This issue might be underestimated due to the lack of awareness of these diseases in public institutions and the shortage of specialized medical professionals (Rentería-Altamirano and Díaz-Vélez, 2021). For these ictiozoonoses, the most effective way to prevent infection is to “avoid consuming raw, undercooked, smoked, or salted

**Table 3.** Comparison of *Contracaecum* sp. infestation rates in freshwater drum, number of parasites (NP), Mean Intensity (MI), Abundance (AB), and Prevalence % (P).

Pathogen/ species	NP	MI	AB	P (%)	Host	Author
<i>Contracaecum</i> sp.	139	19.8	8.10	41.1	<i>A. grunniens</i>	Present study
<i>Contracaecum</i> sp.	-	52.6	-	100	<i>H. malabaricus</i>	Pardo <i>et al.</i> (2008)
	-	15.7	13.7	87.5	<i>R. quelen</i>	Mancini <i>et al.</i> (2014)
	-	8.2	7.6	93.3	<i>O. jenynsii</i>	
	-	23.8	21.6	90.9	<i>O. bonariensis</i>	

NP: number of parasites, MI: Mean Intensity, AB: Abundance, P Prevalence %.

**Table 4.** Comparison of *Rhabdochona* sp., infestation rates in freshwater drum, number of parasites (NP), Mean Intensity (MI), Abundance (AB), and Prevalence % (P).

Group/ species	NP	MI	AB	P (%)	Host	Author
<i>Rhabdochona</i> sp.	9	1.80	0.50	29.4	<i>A. grunniens</i>	Present study
	-	-	-	100		Escobar-González (1997)
<i>Rhabdochona kidderi</i>	7	2.33	0.47	20	<i>R. laticuada</i>	Betanzos (2019)
	32	5.33	32	60	<i>T. irregularis</i>	

NP: number of parasites, MI: Mean Intensity, AB: Abundance, P Prevalence %.



fish.” The primary prevention mechanism is through health education of the population (Quijada *et al.*, 2005).

## CONCLUSIONS

The parasitofauna recorded in freshwater drum from the lower basin of the Usumacinta River includes 4 species of parasites belonging to two groups: Trematodes (metacercaria of *Austrodiplostomum* sp. and metacercaria of *Clinostomum* sp.) and Nematodes (larva of *Contracaecum* sp. and adult *Rhabdochona* sp.). The larva of *Contracaecum* sp. is the most representative genus infecting the mesentery, while the metacercaria of *Austrodiplostomum* sp. is the most representative one infecting the vitreous humor. The genus *Clinostomum* sp. and the metacercaria of *Austrodiplostomum* sp. are reported for the first time in gills and in the humor, respectively. From a parasitic zoonosis point of view, freshwater drum is a potential disease vector due to the consumption of raw or undercooked fish.

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# Infiltration in three soil management for soybean growing under rainfed agriculture

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## ABSTRACT

**Objective:** To study infiltration parameters (infiltration rate, cumulative infiltration, saturated hydraulic conductivity, and sorptivity), on the basis of three soil management treatments (subsoiling, ploughing, and harrowing), for soybean (*Glycine max*) growing, under rainfed agriculture, during three discontinuous years (2020, 2022, and 2023).

**Design/Methodology/Approach:** The experiment was carried out in the region of Tapachula, Chiapas, Mexico. Each treatment was established in 0.50 ha, with independent plots. Two infiltration tests were made per treatment in 2020 and 2023, using cylindrical infiltrometers for 450 minutes in average. During 2020, 2.0 m × 1.50 m soil profiles were made at a depth of 1.50 m to detect the plough layer. Based on this information, the subsoiling depth (0.70 m) was planned. Additionally, three soil samples were extracted at depths of 0-20 cm and 20-40 cm to analyze their physical and chemical properties.

**Results:** Based on their physical properties, texture, organic matter, and soil conditions, the initial moisture and infiltration parameters (2020) were calculated to compare them with the final results (2023).

**Findings/Conclusions:** The following infiltration parameters had a marked variability in the subsoiling, ploughing, and harrowing soil management systems, for soybean growing under rainfed agriculture: infiltration rate, cumulative infiltration, saturated hydraulic conductivity, and sorptivity. Infiltration parameters were higher with subsoiling than with the ploughing and harrowing systems.

**Keywords:** parameters, cumulative infiltration, ploughing layer.

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## INTRODUCTION

Infiltration is the movement of water from the topsoil to the subsoil layer (Hillel, 2003; Brutsaert, 2005); therefore, physical infiltration ( $q_0$ ) is the time-dependent  $i(t)$  downwards flow of water. Doubtlessly, infiltration is the only source of water for plants and aquifer recharge and is therefore one of the most important issues for agriculture and related sectors (Ahuja, 1974; Alley, 2009). Nevertheless, the soil compaction resulting from the intensive and prolonged use of agricultural machinery reduces water infiltration, increases resistance to root penetration, severely impacts water and nutrient absorption, and restricts plant growth, among other negative results (Bengough *et al.*, 2011; Whalley *et al.*, 2005; Whitmore and Whalley, 2009).

Subsoiling currently contributes to the sustainable improvement and minimizes the compaction of soils (Flower and Lal, 1998; Antille *et al.*, 2015; Shaheb *et al.*, 2021; Antille *et al.*, 2015). Additionally, subsoiling increases infiltration rate and cumulative infiltration, in comparison with traditional ploughing (Desale *et al.*, 2012). Therefore, this research analyzed infiltration in moist soils from the region of Tapachula, Chiapas, México used to grow soybean (*Glycine max*). Soil preparation in this region has caused soil compaction (Motavalli *et al.*, 2003; Botta *et al.*, 2004; Harper *et al.*, 2008; Botta *et al.*, 2016; Ewetola *et al.*, 2022). Soy has been grown under rainfed agriculture conditions for 45 continuous years and is currently grown in 14,000 ha. However, soil has always been prepared under high moisture conditions, resulting in high compaction levels for most of these soils (Alonso *et al.*, 2023). Therefore, the aim of this research was to analyze the infiltration parameters (basic infiltration, cumulative infiltration, and saturated hydraulic conductivity) in three soil management systems used to grow soybean under a rainfed agriculture.

## MATERIALS AND METHODS

The experiment was established for three discontinuous years (2020, 2022, and 2023) in the San Antonio plot, Tapachula, Chiapas, Mexico (14° 45' N and 92° 23' W, at 16 m.a.s.l.). The climate is warm subhumid, has an average temperature of  $28 \pm 1$  °C, and a mean cumulative annual precipitation of 1,110 mm.

According to its texture, the soil is loamy, has a pH of 6.5, is slightly acid, and has as 2.5% organic matter content. The experiment consisted of three 1.5-ha treatments (each one measuring 0.5 ha) and was made up of independent plots: 1) subsoiling (SUB), plus one harrowing and mechanized sowing; 2) ploughing (PLO), plus one harrowing and mechanized sowing; and 3) harrowing (HAR), with two harrowings and mechanized sowing.

Soil preparation for soybeans was carried out every year, on the second fortnight of July, with variable sowing dates during this observation period (July 10-22). Based on previous studies, the subsoiling treatment was carried out during the dry season (April 2022-2023), in order to break the compaction recorded at a depth of  $\approx 35$  cm, before the rainy season. The purpose of such practice was to generate friability and to increase infiltration and to compare this system with the ploughing and harrowing treatments.

The plant material sown consisted of the Huasteca 100 soybean variety. The abovementioned preliminary studies consisted of three 1.5-m wide  $\times$  2.0-m long soil profiles at a depth of 1.5 m in April 2020 and April 2023. A ploughing layer was detected and used to define the subsoiling depth. Likewise, three soil samples were extracted at a depth of 0-20 cm and 20-40 cm and subsequently dried for eight days in the shade. The samples were then sieved with a 2-mm mesh. An 800-g portion of each sample was weighted and sent to the laboratory for its physical and chemical analysis.

The initial moisture parameters (2020) were calculated and later compared with the experiment period (2023). The following moisture parameters were calculated: volumetric moisture content to field capacity ( $\Theta_{CC}$ ), permanent wilting point ( $\Theta_{PMP}$ ) saturated moisture content ( $\Theta_s$ ), infiltration rate ( $q_0$ ), saturated hydraulic conductivity ( $K_s$ ), and apparent density ( $D_a$ ).

A brief description of the physical-mathematical models that rule soil infiltration is shown below. *In situ* infiltration was measured using infiltrometers (Bouwer, 1986); two 8-hour long ( $\approx 480$  min) infiltration tests were made for each treatment (SUB, PLO, and HAR). These tests were carried out in 2020 and 2023. Based on field studies, infiltration parameters were calculated with the following equations:

Infiltration rate:  $q_0 = dI / dt$ ;  $\text{cm min}^{-1}$  (Equation 1), where the 0 subscript is the water inflow from the topsoil;  $Z=0$ . This magnitude approaches a constant value throughout time ( $q_0$ );  $t=0$ ,  $q_0 \rightarrow \infty$  (Equation 2), when  $t \rightarrow \infty$ ,  $q_0 = \text{constant}$ ; theoretically,  $q_0 \approx Ks$ , where  $Ks$  ( $\text{cm min}^{-1}$ ) is the saturated hydraulic conductivity (Equation 3). For a prolonged time, the infiltration rate will have a cumulative infiltration connotation (cm),  $I(t) = \int_0^t q_0(t) dt$  (Equation 4).

One of the objectives of the infiltration analysis was to deduce some parameters, including saturated hydraulic conductivity ( $Ks$ ) (Chow *et al.*, 1988) —a key parameter for the appropriate design of irrigation systems. Other objectives included the description of hydraulic properties and the water balance on soil surface (Campbell, 1985; Hillel, 2003; Lal y Shukla, 2004; Morbidelli *et al.*, 2011; Van Looy *et al.*, 2017).

Cumulative infiltration ( $I(t)$ ) was calculated with only the first three terms of the Philip infiltration equation (1957):  $I(t) = C_1 t^{1/2} + C_2 t + C_3 t^{3/2} + \dots$  (Equation 5), where  $C_1$ ,  $C_2$ ,  $C_3, \dots C_m$ ; are the equation's parameters and  $t$  is time. Kutilek and Krejča (1987) suggested using Equation 5 to determine the adjustment coefficients and Equation 6 to estimate saturated hydraulic conductivity ( $Ks$ ):  $Ks = (3C_1 * C_3)^{1/2} + C_2$  (Equation 6), where  $C_1$  estimates sorptivity ( $S$ ) and  $C_3$  and  $C_2$  are the equation's parameters. Based on the analysis of field information through the abovementioned numeric process, the  $q_0(t)$ ,  $I(t)$ , and  $Ks$  infiltration parameters were determined for each of the soil management systems under study. Numeric estimations and adjustments were carried out with the CurveExpert v. 2.6 software.

## RESULTS AND DISCUSSION

Table 1 shows the initial moisture parameters of the soil and its physical and hydraulic characteristics before the research period (April 2020) and before the soil management systems were established in the experiment site.

The initial referential parameters (infiltration rate ( $q_0$ ), available moisture ( $HD = I(t)$ ), and saturated hydraulic conductivity ( $Ks$ )) were compared with the 2023 measurements (Table 2) for each of the soil management systems (SUB, PLO, and HAR) to determine the impact of the proposed treatments on the hydraulic parameters of infiltration. The results of the comparison of these parameters ( $q_0$ ;  $\text{cm min}^{-1}$ ,  $I(t)$ ; cm,  $Ks$ ;  $\text{cm min}^{-1}$ ) (Table 1) were compared with the results for the same parameters after a two-year observation (Table 2) —with the exception of mean sorptivity ( $S$ ), which was only measured in 2023—, revealing that the initial infiltration rate ( $q_0$ ) was 380%, 312%, and 356% higher under the subsoiling, ploughing, and harrowing systems. Meanwhile, cumulative infiltration [ $HD = I(t)$ ] in subsoiling, ploughing, and harrowing exceeded the initial parameters by 277%, 190%, and 241%; however, the parameter was 80% lower for the ploughing and harrowing systems.

**Table 1.** Physical and hydraulic parameters of the loamy soil from the experimental site located in San Antonio, Tapachula, Chiapas (initial period: 2020).

Parameters	Dense compaction	Hard compaction	Average
$\theta_{PMP}$ (cm <sup>3</sup> cm <sup>-3</sup> )	14.8	14.8	14.8
$\theta_{CC}$ (cm <sup>3</sup> cm <sup>-3</sup> )	28	27	27.5
$\theta_s$ (cm <sup>3</sup> cm <sup>-3</sup> )	40.6	35.2	37.9
Da (g cm <sup>-3</sup> )	1.57	1.72	1.64
HD (cm m <sup>-1</sup> )	13.25	12.17	12.71
q <sub>0</sub> (cm min <sup>-1</sup> )	0.016	0.016	0.016
Ks (cm min <sup>-1</sup> )	0.088	0.025	0.056

Based on these results, infiltration was positively impacted by the subsoiling system in relation to the conventional management systems (ploughing and harrowing) used to prepare the soil for the soybean cultivation, under a rainfed agriculture system in the study region.

In a similar experiment, Singh *et al.* (2019) evaluated three subsoiling treatments in Punjab, India, and reported that the reduction of the apparent density improved the physical and hydraulic properties of the soil—a situation that increased porosity and infiltration rate. Likewise, they concluded that implementing subsoiling practices at a depth of 1-1.5 m every three years strengthens crop yield. Similar research works have proved that the infiltration rate was 1.7 and 2.4 higher with a subsoiling treatment than in soils without subsoiling (Solhjou and Niazi, 2001; Heidari *et al.*, 2008).

After a 10-year comparative study in Brazil with a light use of chisel plough and subsoiling, Peixoto *et al.* (2012) recorded that the latter improved the physical properties of the soil and increased soil yield during the first five years of production. For their part, Singh and Hadda (2014) evaluated the effect of three subsoiling treatments (2012-2013) in the physical and hydraulic properties of soils with high compaction levels and determined that subsoiling resulted in an increase of the infiltration rate and cumulate infiltration.

After a 37-year research in western Tennessee (USA) with no-till farming and reduced tillage, Nouri *et al.* (2018) reported a remarkable increase in soil yield and infiltration. Regarding the physical properties of the soil quality indicators of static and dynamic spheres (soil hydraulic parameters), Lovino *et al.* (2013) and Lozano *et al.* (2016) reported

**Table 2.** Initial and final hydraulic parameters between soil management treatments after a two-year observation (2022-2023), in San Antonio, Tapachula, Chiapas, México.

Parameters	Initial average	Subsoiling	Ploughing	Harrowing
S (cm min <sup>-1/2</sup> )		1.92	1.56	1.28
q <sub>0</sub> (cm min <sup>-1</sup> )	0.016	0.061	0.050	0.057
I(t) (cm)	12.71	33.69	24.17	30.58
Ks (cm min <sup>-1</sup> )	0.056	0.060	0.045	0.045

that, as a result of subsoiling, dynamic indicators predicted soil yield and infiltration with greater efficiency.

Likewise, Sivarajan *et al.* (2018) and Nouri *et al.* (2018) confirmed that subsoiling effectively eliminates the soil compaction caused by the conventional systems used to prepare agricultural land. Overall, subsoiling enhanced the physical and hydraulic properties of the soil—including the internal flow of water in the root area of crops, which is associated with a higher infiltration than with ploughing and harrowing (Desale *et al.*, 2012; Avila *et al.*, 2020; Zibilske y Bradford, 2007; Mohanty *et al.*, 2007). Undoubtedly, these results will help to achieve a sustainable agriculture (Shaheb *et al.*, 2021).

## CONCLUSIONS

Soil cultivation under rainfed agriculture recorded highly variable infiltration parameters (infiltration rate, cumulative infiltration, saturated hydraulic conductivity, and sorptivity), regarding the soil management systems under evaluation. Infiltration parameters recorded better results with subsoiling than with the ploughing and harrowing systems.





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# Moth trapping of males using *Diatraea* spp. (Lepidoptera: Crambidae) pupae in sugarcane plantations

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## ABSTRACT

**Objective:** To standardize the moth trapping method through the use of Delta traps with *Diatraea* pupae to lure males.

**Design/Methodology/Approach:** The following lure types were placed inside Delta traps: two female pupae (T1), three female pupae (T2), and two female pupae with one adult female (T3). The response variable was the number of male moths captured per trap. An analysis of variance and Tukey's test ( $p \leq 0.05$ ) were performed.

**Results:** Statistical differences ( $p \leq 0.05$ ) were observed between T3 and T1/T2: T3 trapped the lowest number of specimens (0.94 adults/trap) from May to July 2022. Likewise, June recorded the highest abundance of *Diatraea* moths (3.05 adults/trap). Nevertheless, T1 captured the highest number of specimens (4.16).

**Study Limitations/Implications:** The use of commercial pheromones has been established as a useful moth trapping method in the case of stem borers. In recent years, no favorable results have been observed with the use of pheromones of the *D. saccharalis*, *D. grandiosella*, and *D. considerata* species in Delta traps. These findings could be indicative of the presence of another *Diatraea* species.

**Findings/Conclusions:** The use of two *Diatraea* pupae per trap attracts male moths. This method would help to detect and regulate moths, with the aim of interrupting their life cycle.

**Keywords:** Sex pheromones, pest, stem borer.

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## INTRODUCTION

The stem borer is widely spread across the American continent and is a major pest of corn (*Zea mays* L.) and sugarcane (*Saccharum* spp.) (Lastra and Gómez, 2006). Various species of stem borers (*Diatraea*, *Eoreuma loftini*, and *Elasmopalpus lignosellus*) have been reported in Mexico; in their larva stage, they cause yield losses of 2 to 10 tons of sugarcane per hectare (Rodríguez and Vejar, 2008). Different species of genus *Diatraea* have been reported, increasing the complexity of their management. The damage they cause can be direct and is reflected in dead hairs or “pelillos” (primary shoots), stems, and bored buds, potentially reducing field yields by up to 50% (CONADESUCA, 2021).

Meanwhile, the damage caused by borer larvae to juice quality can be worsened by the proliferation of fungi and bacteria, which enter the plant through the cavities made by the larvae from within the stem. Among the fungi reported are *Colletotrichum falcatum*, *Fusarium*, and *Nigrospora* (Joyce *et al.*, 2016).

The use of synthetic pheromones has successfully managed stem borer populations. Nevertheless, a low number or no specimens of the *Diatraea* species (*D. saccharalis*, *D.*

*grandiosella*, *D. considerata*) have been trapped in recent years. In contrast, the use of live insects in pupal and adult stages has successfully determined the minimum number of individuals required to trap male moths for each study region (Assis *et al.*, 2022). The objective was to determine the number of pupae per trap required to trap *Diatraea* male moths in sugarcane plantations.

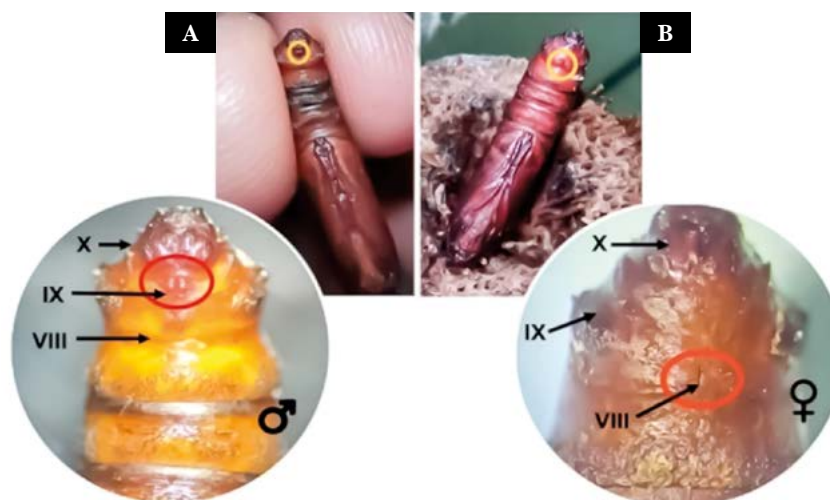
## MATERIALS AND METHODS

**Study area.** The study was conducted in the *ejido* Palmillas, municipality of Yanga, Veracruz, Mexico, part of the sugarcane supply area of the Central El Potrero sugar mill (18° 49' 08.9" N and 96° 46' 38.8" W).

**Biological material.** Larvae of the genus *Diatraea* were collected in the Palmillas study area, before the start of the experiment. They were subsequently transferred to the laboratory of Applied Microbial Biotechnology at the Colegio de Postgraduados - Campus Córdoba. A rearing of *Diatraea* larvae was established inside the facilities, under aseptic and controlled conditions, with a 12:12 (light:dark) photoperiod at  $28 \pm 2$  °C (Lastra and Gómez, 2006). Each larva was placed in a separate 500-mL transparent plastic container along with a 10-cm long cane stalk. The larvae were kept under rearing conditions, until they reached the pupae stage. The specimens were then sexed (Butt, 1962) and selected for reproduction (female and male). Only the female pupae were used in the field attraction evaluation (Figure 1).

### Treatment installation

The experiment was set up in the first week of May 2022, when the crop was six months old (ratoon cycle 2: R2). The 5-ha productive area was divided into three plots, according to the proposed experimental design. The following lures were placed within the traps: two female pupae (T1), three female pupae (T2), and two pupae and one female adult (T3), as the dependent variable. The traps were placed 26 m apart from each other and the distance between repetitions measured approximately 60 m.



**Figure 1.** Sexed pupae of *Diatraea*: A) male pupa and B) female pupa.

A trap was installed for each treatment, which consisted of a Delta-type trap (29 cm deep × 21 cm wide × 17 cm high) with a sheet impregnated with an Adhequim<sup>®</sup> 100 organic polymer. A Reyma<sup>®</sup> 4-ounce transparent plastic container, covered with anti-aphid mesh, was placed in the center of the trap, where the live insects were deposited (Assis *et al.*, 2022).

### Determination of response variables

The moths caught in the Delta traps were monitored weekly. The polymer sheet was replaced every 15 days. Captured male moths were recovered from the trapping film with a paintbrush and white gasoline. Male moths were placed in 50-mL Corning<sup>®</sup> plastic centrifuge tubes and kept refrigerated at  $-20\text{ }^{\circ}\text{C}$  for later identification.

### Experimental design and data analysis

A randomized complete block design with three replications was established. The treatment consisted of a two-factor factorial design: 1) the effect of the traps with different female pupae and 2) the time (months) during which the measurements were taken.

The response variable was the number of male moths captured per trap. The captures were recorded every 15 days and monthly averages were calculated. The resulting data were subjected to the Shapiro-Wilk normality test and Bartlett's test for homogeneity ( $p \leq 0.05$ ). To determine the treatment effect, an analysis of variance (ANOVA) under the proposed design and Tukey's test ( $p \leq 0.05$ ) were performed in Minitab v. 21 for Windows.

## RESULTS AND DISCUSSION

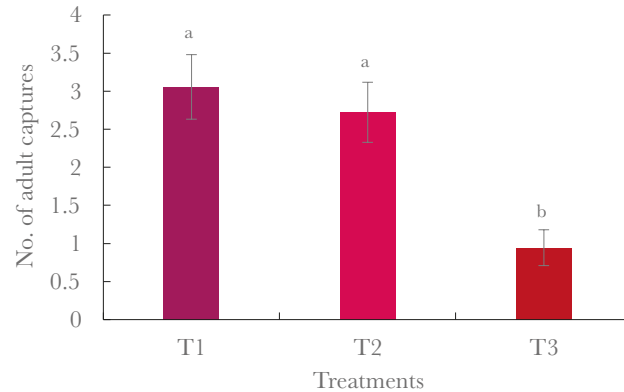
The ejido Palmillas —where the experiment was conducted— had a history of high percentages of damage ( $>5\%$ ) caused by *Diatraea* (Cruz-Tobón *et al.*, 2023). Prior to the experiment, traps with synthetic hormones were set up to lure *D. saccharalis*, *D. grandiosella*, and *D. considerata*. Traps with live insects (natural pheromone) were also prepared. Traps with synthetic pheromone did not capture moths of any of the above-mentioned species, whereas the trap with live insects successfully captured male moths (Assis *et al.*, 2022).

No statistical differences were detected between T1 and T2 ( $p \leq 0.05$ ), with average captures of 3.05 and 2.72 adults/trap, respectively. Meanwhile, T3 had significant differences with respect to the rest of the treatments, with 0.94 adults/trap captured in the treatment with two pupae and one adult. In conclusion, the addition of a female adult moth has an adverse effect, reducing moth trapping by 300%. Consequently, T1 with two female pupae could be the best treatment for subsequent evaluations with pupae (Figure 2).

Previous studies have reported successful results using Delta traps with three pupae and three newly emerged female moths (Butt, 1962) as luring strategies for the management of *Diatraea* spp., populations.

Assis *et al.* (2022) and other authors have recommended setting traps with two female moths 500 m apart from each other. The adults trapped with this device will help to initiate the release of parasitoids.

Species of genus *Diatraea* account for high percentages of the damage caused to sugarcane plantations. Therefore, interrupting the life cycle of male moths and limiting the frequency of male-female copulation is an alternative for the regulation of the stem



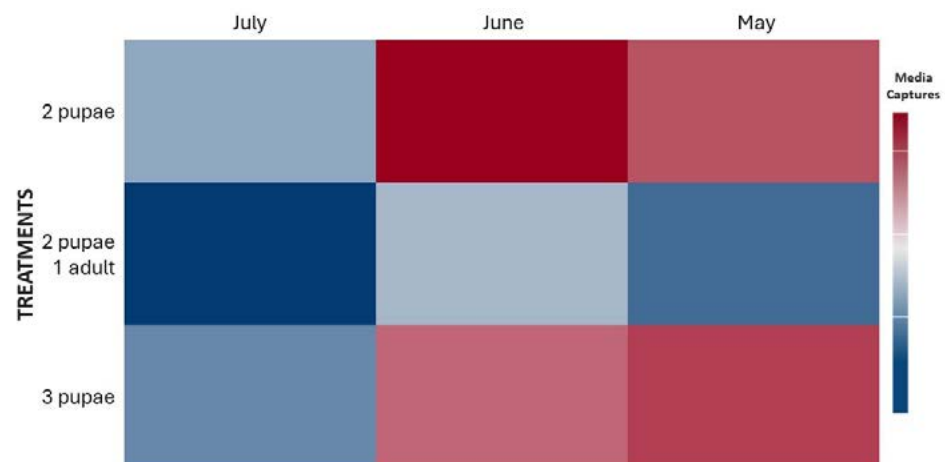
**Figure 2.** Number of adults per treatment. The bars show the mean  $\pm$  standard error. Means with different letters are significantly different (Tukey,  $p \leq 0.05$ ).

borer larvae of a given species, reducing the oviposition of viable eggs. As a consequence of the trapping of male moths, less larvae will emerge. Subsequently, the positive relationship between the presence of trapped moths and the percentage of damage caused by larvae detected per hectare could be estimated. Wilson *et al.* (2012) used the same method to control *E. loftini*.

Regarding the time effect, significant differences were observed in July, when less *Diatraea* adults were recorded (one adult per trap) than in May and June, when three individuals were recorded per trap (Figure 3).

The heat map shows the relationship between the treatment and time variables, using color gradient (number of trapped specimens) (Wilkinson and Friendly, 2009; Akers, 2015). In conclusion, the treatment with two female pupae (T1) had the highest average number of captures and the highest presence of male moths occurred in June.

In other words, the increased abundance of adult *Diatraea* populations is related to two main factors: 1) the age of the crop and 2) the developmental stage of the insect. In the first case, the presence and abundance of *Diatraea* is associated with young crops with succulent



**Figure 3.** Heat map (Minitab version 21).

and fibrous stems which allow their first-instar larvae to bore and penetrate the plant (Cruz-Tobón *et al.*, 2023). In the second case, *Diatraea* need in average 42-60 days to complete their cycle, according to Gómez and Vargas (2014). Therefore, the damage produced by the larvae must have occurred from April to May and adults were observed in June, which matches the findings of this study. Damage can also be the result of the agronomic management of the crop or environmental factors, such as humidity, precipitation and temperature (Pannuit *et al.*, 2015; Carbognin *et al.*, 2023).

## CONCLUSIONS

Traps with two pupae enable the capture of male moths. In addition, this strategy can determine the presence of *Diatraea* species and manage their populations by facilitating an efficient and specific trapping. The timing and distribution of traps are fundamental for the regulation of *Diatraea* populations and are an efficient management strategy. In conclusion, this study determines the possibility of further evaluations about the reach of pheromones as a live lure and specifies the distance and number of traps per hectare.

## ACKNOWLEDGEMENTS

The authors would like to thank the Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHCyT), and the Colegio de Postgraduados - Campus Córdoba for the financial and logistical support for the research. They would also like to thank the state small sugarcane producer of the CNPR and the Colegio de Postgraduados - Campus Córdoba for the *Analysis of productivity factors in the field and in the factory: improvement strategies for the sugar agroindustry* project.

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# Interaction between arbuscular mycorrhizal fungi and rhizobacteria in chili (*Capsicum annuum* L.) plants infected with phytopathogens that cause damping off

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## ABSTRACT

**Objective:** To determine the effect of single or combined inoculations of three rhizobacteria strains and a mycorrhizae consortium in chili seedlings infected with phytopathogens that cause damping off.

**Design/methodology/approach:** Guajillo chili seeds were used in this experiment. In addition, three growth-promoting rhizobacteria strains and an arbuscular mycorrhizae fungal consortium were used as beneficial microorganisms, while *Fusarium* spp. and *Rhizoctonia* spp. were used as phytopathogens. The following variables were evaluated 53 days after emergence: dry weight (mg plant<sup>-1</sup>), leaf phosphorous and nitrogen content (mg plant<sup>-1</sup>), and the percentage of total mycorrhizal colonization (%). Each variable was subjected to a one-way ANOVA. Afterwards, the post hoc test of the Tukey method was used to compare the results, considering that P<0.05 values were significant.

**Results:** Overall, the single or combined inoculation of the B8, B14, B23, and *Glomus* spp. (Zac-19) strains efficiently controlled damping off. These treatments recorded a higher dry matter production and nutrient content than chili plants infected with phytopathogens to which no beneficial microorganisms were applied.

**Limitations on study/implications:** The seedlings could have been transplanted into larger containers.

**Findings/conclusions:** The beneficial microorganisms had a positive effect on the growth and health of the guajillo chili seedlings. Consequently, their use is a biotechnological alternative to control damping off.

**Keywords:** *Capsicum annuum*, damping off, PGPR, mycorrhizae, nutrient content.

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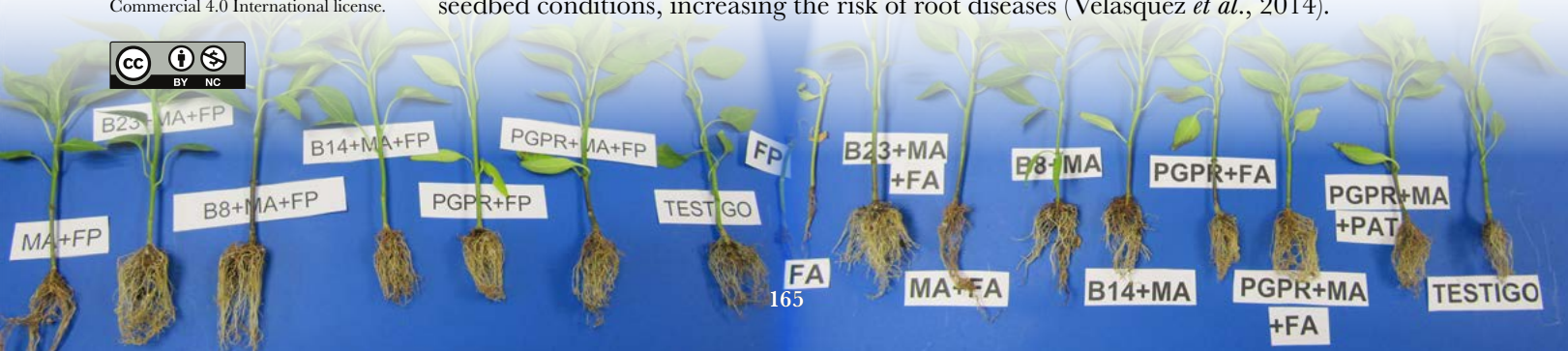
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## INTRODUCTION

From the cultural, agronomic, nutritional, and economic points of view, ancho, jalapeño, serrano, and mirasol (guajillo in its dry form) chilies and red pepper are the most important cultivars in Mexico since they represent 70 to 80% of the domestic production (Aguirre-Mancilla *et al.*, 2017). In the Potosino Plateau, commercial varieties of chili seedlings are produced under soil (seedbed) and greenhouse (in styrofoam trays with substrate) conditions. The disinfection of soil and seeds is not a common practice under seedbed conditions, increasing the risk of root diseases (Velásquez *et al.*, 2014).



The main root disease of seedbed chili is damping off (Ita *et al.*, 2021). This disease has been reported in several states, including San Luis Potosí (Rodríguez *et al.*, 2007; Anaya-López *et al.*, 2011; Montero-Tavera *et al.*, 2013). In Oaxaca, this disease causes significant economic losses up to 76%, particularly during the germination and seedling stages (Pérez-Acevedo *et al.*, 2017).

Damping off is caused by a complex group of pathogenic soil fungi (Reyes-Tena *et al.*, 2021). Producers use fungicides to control them; however, the excessive use of these substances impacts human health, pollutes the environment, and promotes resistance among phytopathogens (Lamichhane *et al.*, 2017).

The use of natural enemies (mainly antagonist microorganisms) stands out as an ecological and sustainable alternative for the control of phytopathogens. Duc *et al.* (2017) mentioned that the inoculation of beneficial microorganisms is an alternative to improve the resistance of the chili crops to biotic stress. For example, the inoculation of arbuscular mycorrhizae fungi and actinomycetes had synergetic effects, including growth promotion and bioprotection against chili blight (*Phytophthora capsici*) (Reyes-Tena *et al.*, 2017).

Therefore, the objective of this study was to determine the effect of single and combined inoculation of three plant growth-promoting rhizobacteria (PGPR) and an arbuscular mycorrhizae (MA) consortium on growth promotion and nutrient content of chili plants infected with pathogenic fungi that cause damping off.

## MATERIALS AND METHODS

**Obtaining the seedlings.** Guajillo chili seeds from the previous year were provided by producers from Villa de Ramos, San Luis Potosí. The seeds were disinfected with 70% alcohol and 10% commercial bleach for 5 and 15 minutes, respectively. Subsequently, they were rinsed three times with distilled water. The seeds were sown in 200-hole Styrofoam seedling trays; each hole had 6 g of substrate (peat moss-perlite in a 1:1 ratio). Substrate was previously twice-sterilized at 121 °C for 1 h., at a 3-day interval between each other.

**Microorganisms and treatments.** The beneficial microorganisms consisted of three strains of *Pseudomonas chlororaphis* (B8), *Pseudomonas* sp. (B14), and *Bacillus* sp. (B23). Hernández-Hernández *et al.* (2018) reported that these strains produce indole-3-acetic acid and phosphorus solubilization. In addition, the Zac-19 mycorrhizae consortium (MA) — made up of the *Glomus claroideum* (Schenck & Sm.), *G. diaphanum* (Morton & Walker), and *G. albidum* (Walker & Rhodes) morphospecies— was used. This AM was provided by the Laboratorio de Microbiología de Suelos, Colegio de Postgraduados - Campus Montecillo. The phytopathogens used were *Fusarium* spp. and *Rhizoctonia* spp. (R) strains. They were classified according to the color produced in the culture medium: yellow phytopathogen (FA), orange phytopathogen (FN), and purple phytopathogen (FP) (Hernández-Hernández *et al.*, 2018).

**Inoculation.** Seven days after emergence (DAE), 1 mL culture of each bacteria strain (with 10<sup>9</sup> CFUs [colony forming units]) were inoculated at the base of the stems of the chili seedlings. The arbuscular mycorrhizal fungi (AMF) treatments were inoculated following the method described by Chamizo *et al.* (2009). Four days later, two 5 mm wide agar discs with mycelia from the pathogen strains were placed in the crown of each seedling. The



experiment was carried out under greenhouse conditions, for 53 DAE. The seedlings were irrigated every 72 hours with 50 ml of a Long Ashton 50% phosphorus nutrient solution (Hewitt, 1966). The activation of a wet wall and the watering the soil were used to keep a <math><30\text{ }^\circ\text{C}</math> temperature inside the greenhouse.

The following variables were determined at 53 DAE: dry matter of the aboveground section (mg); leaf phosphorus and nitrogen ( $\text{mg plant}^{-1}$ ), and the percentage of total mycorrhizal colonization (%).

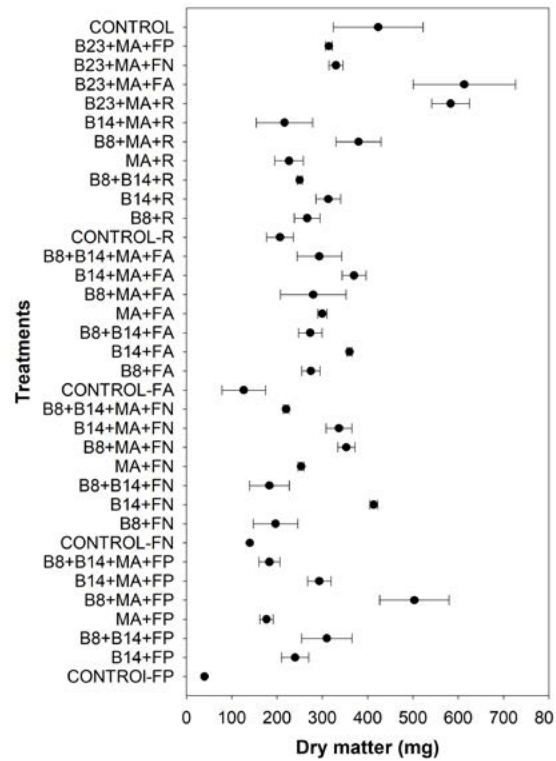
**Statistical analysis.** The assumption of normality and homogeneity of variance were evaluated for each dependent variable, based on the Kolmogorov-Smirnov test and Bartlett's test. Therefore, the comparisons were carried out with a one-way analysis of variance (ANOVA). Afterwards, the post hoc test of the Tukey method was used to compare the results, considering as significant those values with  $P<0.05$ ; the information was presented as error bars.

## RESULTS AND DISCUSSION

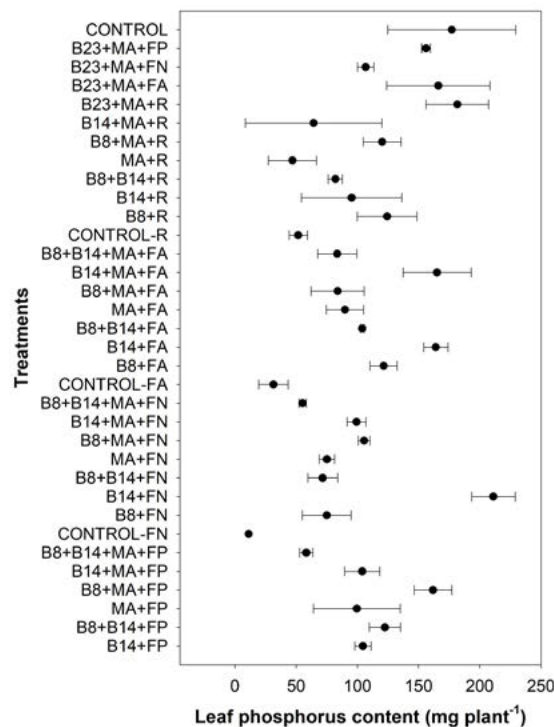
**Dry matter.** Significant differences ( $F_{34, 0.05}=7.84$ ,  $p<0.01$ ) were detected on dry matter production among treatments. The highest values were found in the B23+MA+FA ( $613.33\pm 112.89$ ) treatment, followed by B23+MA+R ( $583.33\pm 41.77$ ) and B8+MA+FP ( $503.33\pm 76.67$ ) (Figure 1). On the other hand, the lowest dry weight was recorded with the purple *Fusarium* negative control (CONTROL-FP, Figure 1). Therefore, the PGPR+MA co-inoculation seems to provide an effective biocontrol.

On this regard, El-Feky *et al.* (2019) reported that pepper plants infected with *F. solani*, *F. oxysporum*, and *F. moniliformis* and inoculated with *P. fluorescens* (P2) and *B. subtilis* (B1) had a significantly higher dry weight than their negative control. Raio and Puopolo (2021) reported that *P. chlororaphis* is a species with a high microbial competitiveness in the soil, which can effectively control several phytopathogens. Hyder *et al.* (2021) mention that the inoculation with *Bacillus* spp. and *Pseudomonas* spp. suppresses the damping off caused by *Pythium myriotylum*, as a consequence of the promotion of defense mechanisms and the growth of chili plants. Meanwhile, Bilgili and Gldr (2018) determined that the single or combined inoculation of three different *Glomus* species significantly increased the dry weight of *Capsicum annuum* plants infected by *F. oxysporum*. For their part, Leos-Escobedo *et al.* (2019) pointed out that the HMA+PGPR (*Pseudomonas* sp. and *Bacillus* sp.) co-inoculation significantly increased dry weight and diminished the damage caused by *P. capsici* to 23 chili cultivars. In this context, Reyes-Tena *et al.* (2017) suggested that the potential synergy between beneficial microorganisms diminishes the damage caused by the *P. capsici* infection to chili plants. Consequently, biological control is an alternative measure for the management of the diseases caused by plant pathogens, given its potential biofertilizing effect which stimulates plant growth (El-Feky *et al.*, 2019).

**Leaf phosphorus content.** Significant differences ( $F_{33, 0.05}=4.52$ ,  $p<0.01$ ) were detected among the treatments (Figure 2). The highest leaf phosphorus (P) values were recorded by the following treatments: B14+FN ( $211.07\pm 17.86$ ), B23+MA+R ( $181.64\pm 25.54$ ), and absolute control ( $177.09\pm 52.25$ ). On the other hand, the lowest values were reported by the negative controls: FA ( $31.41\pm 11.92$ ) and FN ( $11.20\pm 0.01$ ).



**Figure 1.** Comparison of the different treatments, based on the mean  $\pm$  standard error (SE) of the dry weight response variable. The *post hoc* test of the Tukey method was used for contrasting purposes, considering as significant those values with  $p < 0.05$ .

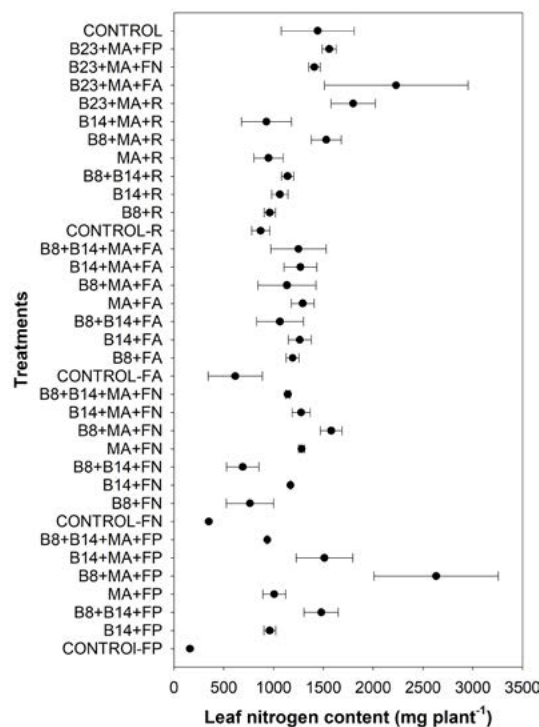


**Figure 2.** Effect of the individual or combined (PGPR and HMA consortium) inoculation on leaf P content in chili plants infected with the fungi that cause damping off (53 DAE).

AMF are widely known to improve the growth of plants, mainly through a greater phosphate nutrition (Thilagar *et al.*, 2015). Meanwhile, PGPRs promote plant growth, both directly —through the solubilization of phosphorus and the control of plant hormone levels— and indirectly —through the inhibition of the development of phytopathogens— (Rodríguez *et al.*, 2006; Emmanuel and Babalola, 2020). Kim *et al.* (2010) reported that the combined inoculation of *Methylobacterium oryzae* strains and AM fungi resulted in significantly higher nitrogen (N) accumulation in the roots and shoots of red pepper plants compared to uninoculated controls.

**Leaf nitrogen content.** Significant differences ( $F_{34, 0.05} = 4.13, p < 0.01$ ) were detected between treatments (Figure 3). The highest values were obtained with the following treatments: B8+MA+FP ( $2632.00 \pm 624.14$ ), B23+MA+FA ( $2230.33 \pm 719.86$ ), B23+MA+R ( $1799.00 \pm 222.72$ ), and B8+MA+FN ( $1578.83 \pm 107.72$ ). On the other hand, the lowest values were recorded by the FN ( $350.00 \pm 0.01$ ) and FP ( $160.00 \pm 0.01$ ) negative controls. The combined inoculation of PGPR+AMF does not only promote the resistance to root diseases, but it also increases the nutrition content of nitrogen, phosphorus, potassium, calcium, magnesium, iron, and zinc (Weng *et al.*, 2022). Meanwhile, red peppers inoculated with rhizobacteria (*Azotobacter chroococcum*+*Azospirillum lipoferum*) absorb more N, while plants inoculated with mycorrhizal species (*Rhizophagus irregularis*+*Funneliformis mosseae*) absorb more P (Sini *et al.*, 2024).

**Total mycorrhizal colonization.** Differences between treatments ( $F_{18, 0.05} = 2.70, p < 0.01$ ; Table 1) were determined based on the comparison of their total mycorrhizal colonization (MC) percentage. The highest values were obtained with the following



**Figure 3.** Effect of the individual or combined (rhizobacteria and mycorrhizal consortium) inoculation on N content of chili seedlings infected with fungi species that cause damping off (53 DAE).

**Table 1.** Mycorrhizal colonization (MC) in chili seedlings infected with root phytopathogens and inoculated with three strains of rhizobacteria (B8, B14 and B23) and the mycorrhizal consortium Zac-19 (MA), the average and its respective standard error ( $\bar{x} \pm S.E.$ ). Multiple comparisons were carried out using the post hoc test of the Tukey, considering that  $P < 0.05$  values were significant.

Treatments	$\bar{x}$ (%)	$\pm S.E.$	Post hoc Tukey
MA+FP	79.07	2.98	abc
B8+MA+FP	60	14.72	abc
B14+MA+FP	74.26	7.58	abc
B8+B14+MA+FP	52.32	1.76	bc
MA+FN	75.55	3.06	abc
B8+MA+FN	67.5	11.08	abc
B14+MA+FN	70.09	16.72	abc
B8+B14+MA+FN	75.19	1.52	abc
MA+FA	89.44	6.24	ab
B8+MA+FA	63.44	16.73	abc
B14+MA+FA	59.58	12.27	abc
B8+B14+MA+FA	75.56	5.34	abc
MA+R	97.96	0.98	a
B8+MA+R	73.33	3.06	abc
B14+MA+R	67.04	2.13	abc
B23+MA+R	39.63	2.25	c
B23+MA+FA	51.3	7.49	bc
B23+MA+FN	57.41	3.96	abc
B23+MA+FP	83.69	9.82	abc

treatments: MA+R ( $97.96 \pm 0.98$ ), MA+FA ( $89.44 \pm 6.24$ ), and B23+MA+FP ( $83.69 \pm 9.82$ ).

The MC percentage of the AMF-PGPR inoculation surpassed the AMF treatment in guajillo chili plants infected by *P. capsici* (Leos-Escobedo *et al.*, 2019). Under natural conditions, AMF can be found in the same ecological niche and colonization site as soilborne pathogens.

## CONCLUSIONS

The beneficial microorganisms used in this study had a positive effect on the growth and health of guajillo chili plants. Seedlings coinoculated with *Pseudomonas chlororaphis* (B8) and *Bacillus* sp. (B23) combined with the Zac-19 mycorrhizae consortium accumulated a higher amount of dry biomass than seedlings that were only infected by *Fusarium* spp. isolates. The same trend was observed regarding the content of P and N on leaves.

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# Vanilla (*Vanilla planifolia* Jacks ex. Andrews) marketing process in the Sierra Nororiental of the State of Puebla

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## ABSTRACT

**Objective:** To analyze the marketing process of vanilla and to identify its limitations in the Sierra Nororiental of the State of Puebla, Mexico, in order to propose timely actions that will benefit regional producers who work in small plots.

**Design/Methodology/Approach:** A mixed method questionnaire was applied to n=68 key participants of the municipalities of Huehuetla, Caxhuacan, Ixtepec, Hueytlalpan, Olintla, Jonotla, Cuetzalan del Progreso, Tuzamapan de Galeana, and Tlatlauquitepec. All the municipalities are located in the Sierra Nororiental of the State of Puebla, Mexico.

**Results:** The commercialization of the product is a major problem for producers. Although they have the required experience from the sowing to the harvesting of the product, intermediaries pose an important problem. Producers are not organized and, consequently, they lack the conditions to receive specific training for each link of the chain. There are no leaders that can influence, encourage, or inspire producers to achieve common objectives. Public support should be permanent and must encompass inclusion, training, support, technological exchange, and validation of the local lore.

**Study Limitations/Implications:** The lack of a producer register for the vanilla production chain in the study area limited the number of participants of the survey.

**Findings/Conclusions:** A producer organization would rearrange the vanilla value chain, facilitate the decision-making process, and propose long-term public policies. Leaders would encourage the members of the group, organizations, families, and communities to achieve common objectives. In addition, they would identify talents for the local and short-, medium-, and long-term development.

**Keywords:** Process, marketing, vanilla, Sierra Nororiental.

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## INTRODUCTION

In Mexico, small-scale farmers are a major agricultural subsector. The Mexican agricultural industry is a highly competitive sector. This situation contrasts with most of the small rural economic units, because the area they work in is not enough to meet the food demand (small farms) (Muñoz *et al.*, 2017; Ocampo *et al.*, 2006).

The marketing process is fundamental to benefit agricultural producers; however, the greatest profits in this link of the chain are usually obtained by the intermediaries. Meanwhile, producers require more support, training, and follow-up during the whole process.

A public policy aimed to benefit small-scale producers—through funding and training about each link of the chain—is required. This policy must be particularly focused on commercialization. In addition, producers should receive professional support, in order to achieve a community development that can improve their quality of life.

Commercialization is a strategic stage of the production chain, in which psychology and certain standards and strategies should be applied to promote the growth of a given company. It is made up of four elements: when (the right time to carry out a given activity); where (geographic location and amenities); whom (target niche market); and how (promotion, commercialization, and sale strategy) (Rizo *et al.*, 2017; Hernández, 2007).

According to Chayanov (1974), the production units of the different chains usually function under a rural trade system, which includes the consumption of local produce, the sale of the surplus, and the purchase of goods that the producers do not grow, but do consume. This commercial system is mainly directed to local and regional markets and, consequently, generates local and regional employment.

Many commercialization systems include small-scale producers, who usually lack sale strategies, distribution networks, and appropriate roads. These factors prevent producers from being part of a fair market.

Commercial intermediaries are a major barrier in the value chain. This situation is the consequence of the lack of organization and ties between producers in each stage of the local, regional, and domestic production. An example of this phenomenon is the scarce or non-existent integration of the processes in the value chain. This integration would add value to the products as a promotion and commercialization strategy, both in local and regional markets.

Maize (*Zea mays* L.) and bean (*Phaseolus vulgaris* L.) are usually considered a major part of food security; however, over the past 15 years, a new commercial agriculture has focused on the generation of local economies, with crops such as vanilla (*Vanilla planifolia* Jacks Ex. Andrews). The inclusion of this crop is a feasible alternative for government programs aimed at local and regional economies, specifically in the indigenous municipalities of the Totonacapan region of Puebla and Veracruz, Mexico.

Vanilla (*Vanilla planifolia* Jacks Ex. Andrews) (Orchidaceae) is a very important economic crop in the Totonacapan region. From its establishment, this orchid has a three-year production cycle. Its fruit (silique) is used to extract essences, internationally and commercially known as vanilla (Kelso-Bucio *et al.*, 2012).

From 2017 to 2021, the average world production of vanilla beans amounted to 6,961 t. The country with the highest production is Madagascar (43.9% of the total world production), followed by Indonesia (23.4%), and Mexico (7.8%) (SMATTCOM, 2023).

The Totonacapan region covers the north of the State of Puebla and the north center of the State of Veracruz. This region is considered the center of origin and the main producer of vanilla (Cervantes *et al.*, 2019).



From 2017 to 2023, about three thousand producers harvested 546 t of vanilla beans (SMATTCOM, 2023). This figure accounts for a 4.3% annual growth rate, placing Mexico as the third world producer of vanilla.

Therefore, the objective of this study was to analyze the commercialization process of vanilla in the Sierra Nororiental of the State of Puebla, Mexico, in order to identify limiting factors and to propose actions aimed to benefit producers.

## MATERIALS AND METHODS

The study was carried out in the Región II Sierra Nororiental (SNOP), of the State of Puebla. The region includes the following municipalities: Huehuetla, Ixtepec, Caxhuacan, Hueytalpan, Olintla, Jonotla, Cuetzalan del Progreso, Tuzamapan de Galeana, and Tlatlauquitepec. The climate ranges from temperate to warm and the area has many water bodies.

A non-probabilistic sampling technique, known as convenience sampling, was applied, given the lack of records or register of vanilla producers in the municipalities under study. Subsequently, small-scale producers were approached, in order to identify individuals whose profile would allow them to provide information.

The initial data was complemented with a snowball or chain-referral sampling (Mendieta, 2015). The size of the sample was  $n=68$  vanilla producers, who are native and neighbors of the municipalities under study. Each municipality has a maximum of 20 producers.

The demographic characteristic of the producers and the current situation of the crop were determined through a structured survey consisting of qualitative and quantitative questions. The information was complemented with official documentary information from the Sistema de Gestión de Apoyos al Campo (SIGA) of the Secretary of Rural Development of the government of Puebla. The survey was conducted from May to June, 2023. The data were analyzed with SPSS Statistics version 24. The variables were related with the overall data of the producers: production volume (kg) of the green vanilla fruit per producer, the gross and net profit per producer, the final destination, quality, and origin of the product, and the public or private external actors during the last stage of the vanilla production chain (commercialization).

## RESULTS AND DISCUSSION

Huehuetla recorded the highest percentage of producers (37%) among the municipalities included in the survey. Women producers account for 15% of that percentage and they are in charge of most of the activities in small-scale production units. These findings show that women are the driving force behind the family economy in this municipality (BANCO MUNDIAL, 2017). In addition, they are in charge of the commercialization of the product, particularly, green vanilla.

Forty-two percent of the interviewees did not finish elementary school. This situation puts them at disadvantage during the negotiation stage. Additionally, they face obstacles regarding new techniques for vanilla cultivation and the acquisition of other skills, particularly commercialization (Baria *et al.*, 2012). Fifty-four percent of the producers are

indigenous and they only speak their native language (Totonaco). This factor also limits the understanding of and the adaptation to the current conditions of the region.

The commercialization of their product is another limitation (Figure 1). Producers have valuable experience from the sowing to the harvest of vanilla; however, intermediaries are a major problem for its marketing process. Producers claim that production units are not organized and, consequently, they cannot request technical training from the government for each link of the vanilla production chain. The interviewees understand the production process; however, they have no management, promotion, and sale skills regarding their harvests. Rivas *et al.* (2018) have suggested that federal or state supports are not enough without training, follow-ups, and organization skills. The first link of the production chain has 68 producers. Five of these producers are also collectors, while one is also an intermediary. Commercialization and consumers are the last link of the chain.

Regarding the vanilla production volume, 40% of the producers obtained  $\leq 50$  kg of green vanilla per unit ( $< 1,000$  m<sup>2</sup> plots) and per production cycle, under traditional agriculture systems. However, 15.4% of the producers recorded a 500 kg yield per production unit, during the 2022 agricultural cycle. Consequently, these producers work in larger plots or have adopted technified production systems.

Limitations related to lower yields are linked to the supply and demand, because producers are not able to meet the domestic and international market demand. Consequently, they are forced to comply with the conditions imposed by the intermediaries, in order to sell their products.

Regarding this situation, producers said that an organization would be fundamental to propose actions to the authorities. As mentioned by Ferrando (2015), these actions should include a permanent government support and the development of public policies that include training, support, technological exchange, and validation of the regional Totonacapan lore.

Ninety percent of the producers are aware of the production process —*i.e.*, they have identified the most important requirements that their product must comply with and the quality characteristics demanded by the vanilla market (color, size, and quantity) for a successful commercialization. These characteristics allow producers to obtain profits or, at least, a fair price for a sustainable quality.

Undoubtedly, the organization of vanilla producers in the Totonacapan region would be fundamental for local and regional growth and development. Producers are convinced that, only through an organization, they will improve their quality of life, drive the development in their communities, and guarantee their permanent inclusion.



**Figure 1.** Links of the vanilla value chain identified in the Totonacapan region of the State of Puebla (Figure developed by the authors, 2023).

The producers lack the motivation to establish new vanilla plantations or to accept the technification of the existing plots. Likewise, this lack of organization limits their inclusion in the decision-making process. All the producers (100%) believe that this situation definitely leaves them outside the participation and the local development.

### **Price and final destination of processed and non-processed vanilla**

Vanilla is an annual crop whose production requires special cultural labors that producers have been carrying out for centuries. The special reproduction of vanilla makes it a highly delicate crop, because it requires manual pollination. Consequently, fecundation requires a very skillful and specialized labor force to obtain good quality fruits in the right quantity to satisfy the market demand.

According to Cofece (2016, quoted by Astudillo *et al.*, 2020), vanilla is commercialized as a fresh product —*i.e.*, most farmers sell their produce directly to the intermediary. Meanwhile, 64% of producers sell their products in local markets, while the rest use the services of five intermediaries or they process their product and sell it to the highest bidder. Other producers add value to their harvest making crafts. In this regard, producers are aware of the urgent need to organize themselves and said that they should participate in the gastronomic, make-up, and medicinal markets. Nevertheless, this objective requires training and knowledge to detect which talented individuals among their own ranks would be good managers, promoters, and negotiators. They said that, once they are organized, they should discuss the possibility of creating a research area.

In 2022, the price of fresh vanilla (vanilla green silique) fluctuated between MXN\$500.00 and MXN\$1,000.00. However, the final destination of this production is unknown, because the intermediaries have monopolized the main market. Producers said their harvests are collected and distributed through several channels to the pharmaceutical industry, the European gastronomic (gourmet) industry, and the domestic and international make-up industry.

More than half of the interviewees (53.8%) pointed out that the plots used to grow vanilla are small and, consequently, they obtain a small production. However, they earn an average of MXN\$300.00 per kilogram of green vanilla —*i.e.*, this is a highly profitable crop (Rodríguez *et al.*, 2023).

In addition, producers mentioned the importance of vanilla in the region, the lore inherited from their ancestors, the fact that Totonacapan is its center of origin, and the optimal conditions of the region to allow a good development of the plant. These producers said that it is well worth the effort to maintain and elevate the production of this crop. Given the implication of the processes, most interviewees do not add value to vanilla. Nevertheless, they agree that they should be first organized, before they can explore new courses, workshops, and training that would help them in all the processes of the vanilla production chain.

Meanwhile, 1% of the interviewees received technical support, particularly regarding the processing of vanilla, in one or more occasions. Producers said that a permanent tie with government organisms should be generated as fast as possible, because these organisms are committed to support and train regional producers. The interviewees also mentioned that

some state government organisms support them through programs that include production modules, vanilla plants, and supplies; however, these programs should receive technical support and be subject to follow-up, evaluation, and monitoring mechanisms during the whole process.

Producers identified a series of obstacles that can be overcome through a guided organization process. Consequently, they have repeatedly demanded a higher commitment, regulation, and full responsibility from the state authorities regarding shared works. In addition, the interviewees have proposed programs based on their own needs and the different types of crops they grow. These programs should include: their role as a development, training, and follow-up tool in the different links of the production chain, a regional vocation diagnosis, development and community growth strategies, and commercialization. The interviewees pointed out that an inter-agency representation committee is required. This committee would be the communication bridge between the three levels of the government and would watch over the appropriate application of the programs designed for the community.

### **Limitations**

The interviewees are convinced of the benefits and advantages of the crop. However, most of the producers said they feel insecure, because their plots have suffered acts of vandalism, causing losses in their harvests and discouraging them. Producers mentioned that this situation is part of the limitations they face; this problem is particularly important for farmers whose productive units are far from their homes. Once again, they pointed out the need to organize themselves as cooperatives and to be registered before the authorities, in order to demand strategies aimed to protect their crops. The acts of vandalism have forced 80% of producers to carry out premature harvesting, which reduces the quality of vanilla, diminishing its economic value.

Intermediaries have always been a major problem for the local and regional commercialization of vanilla. Regarding this situation, 54.4% of the interviewees have confirmed the importance of creating an organization of producers to collectively design strategies. For example, they would like to participate in the decision-making process and to play a key role in the setting of the price of their produce. In addition, they mentioned that a cooperative would allow them to own the brand, production, and commercialization of vanilla. A cooperative would also benefit the members in all the links of the value chain (Jurado, 2014).

### **Participation of external actors**

Producers pointed out their satisfaction with the participation of public, academic, and private organisms during the last five years (2018-2023). They understand their participation as a social development and source of income strategy for the families that grow vanilla, particularly in the Totonacapan region, Puebla.

During this period, the increasing government support for the recovery of the agroindustry has included technological programs, equipment, agricultural supplies, training, and exchange of experiences between producers. Most of the interviewees maintain that, in

theory, the state support programs are dazzling, because they promise additional economic support that would undoubtedly provide better life conditions for the families and improve local growth, consequently, driving local and regional development (Quevedo *et al.*, 2021). Nevertheless, most of the producers also are dissatisfied regarding other government services, such as technical support, follow-up, training, and commercialization of their harvests. Meanwhile, producers said that public and academic organisms are important; however, they are concerned about the companies and intermediaries that subjugate them and, without lifting a finger, obtain the highest profits (60%). This information matches the reports of Méndez *et al.* (2019). Finally, producers maintained that, if they were organized, they could improve and design better commercialization, add value, service management, collection, and price analysis strategies, before they offered their products to the market. The lack of such an organization reinforces the limitation implied in selling a fresh product, when producers have no other options.

## CONCLUSIONS

Small-scale producers of the Región II Sierra Nororiental of the State of Puebla, have a comprehensive experience and lore about the processes involved in vanilla production. Their needs include training, follow-up, and organization. Communication is limited, because they mainly speak their native language. The creation of an organism that represents and takes care of the needs of these specific groups is fundamental. Producers require a timely follow-up, carried out by specialists that support them to understand the marketing process. In addition, they require a permanent training regarding organization, leadership, and visibility improvement. Setting up a cooperative would allow the producers to go beyond the local market and the intermediaries. The analysis of the current procurement mechanisms would allow the development of strategies to overcome the limitations, including the payment time and the communication dynamics. In addition, it would improve the active relationship between actors from the vanilla value chain.

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# Trends and evolution in the scientific research of *Sechium edule* (Jacq.) Swartz

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## ABSTRACT

**Objective:** Analyze the scientific production on *Sechium edule* by using bibliometric and computer tools to identify trends about its research, as well as the key factors and areas of opportunity that require scientific focus  
**Design/methodology/approach:** The “bibliometrix” package was used to extract, debug and select publications from the Scopus platform. The bibliometric analysis was divided into several sections: the first included an analysis of authors (publication count, H-index); analysis of keywords, as well as trends and areas of opportunity.

**Results:** It has found 385 documents from 1976 to 2024, with an annual growth rate of 5.12%, 1448 researchers involved, 13,411 references, 12.2 average articles per year published and an average citation of 15.46. 1248 keywords were found, Mexico is the country with the highest number of corresponding authors and with the highest scientific productivity.

**Limitations on study/implications:** Publications that were not in Scopus or that are in other indexing times were discarded.

**Findings/conclusions:** Current trends are related to biomedical, pharmaceutical applications through the study of cucurbitacins. Genetic and molecular issues are gaining momentum and more research related to bioprospecting is expected. This information is essential for planning future research and ensuring the continuous and cohesive advancement of the scientific knowledge of chayote.

**Keywords:** Bibliometrics, biomedical applications, bioprospecting, cucurbitacins.

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## INTRODUCTION

Bibliometrics is an essential tool in the field of scientific research for carrying out a metric analysis of academic production. It details the trends, impact and collaborations in a specific area of knowledge (Öztürk *et al.*, 2024). Bibliometric studies analyze and evaluate scientific knowledge in different areas of science, such as agriculture and agronomy. The scope of bibliometrics is to denote the current state of knowledge and



future perspectives: the number of publications, authorship, co-authorship, citations, H-index and factor analysis.

The application of bibliometric studies in agriculture has revealed important patterns in research, such as increasing attention towards sustainability and adaptation to climate change, innovation in cultivation and soil management techniques, as well as the development of pest-resistant crops and diseases. These trends reflect global priorities to improve food security and environmental sustainability in response to contemporary challenges. Bibliometric studies identify the leading institutions and countries in agricultural research, as well as the researchers with the most visibility and influence, which facilitates the formation of effective collaboration networks and the transfer of knowledge (Safruddin *et al.*, 2024).

In agriculture, bibliometric studies focus on research on climate change as well as its effects on the phenological stages of crops (Wu *et al.*, 2023); extracts or secondary metabolites of fruits and vegetables (Çelik, 2024); smart agriculture and irrigation (Pang *et al.*, 2023); use of biofertilizers (Coutiño-Puchuli *et al.*, 2023 among many other studies. Bibliometric studies are valuable due to their multidisciplinary nature that for agriculture covers fields of knowledge ranging from biotechnology, genetics to agricultural economics and environmental sustainability Agronomy, for its part, focuses on the science and technology of the production and use of plants for food, fuel, fiber and land restoration.

Bibliometric analysis in these fields not only helps to map the current landscape of the research, but also facilitates the identification of key areas of scientific growth, gaps in knowledge and potential opportunities for interdisciplinary collaboration. Therefore, for the purposes of this scientific review, a bibliometric study is essential to identify the aspects related to research. the trend, evolution, areas of opportunity and knowledge gaps around chayote (*Sechium edule*) as a crop of nutritional, economic, pharmaceutical and industrial importance (Cadena-Iñiguez *et al.*, 2007).

The aim of this research is to analyze the scientific production on *Sechium edule* through the use of bibliometric and computer tools to identify trends in its research development as well as the key factors and areas of opportunity that require scientific focus.

## MATERIALS Y METHODS

A search was carried out in Scopus by entering the keywords “*Sechium edule*” omitting the word “chayote” because in other regions globally the fruit may have different names. The digital platform used for this search was Scopus because its complexity, robustness and prestige for access to scientific literature and search offers high quality, wide coverage of scientific journals and indexing of citations, which is essential for an accurate analysis. bibliometric.

The database included other species related to the genus and family Cucurbitaceae. The data were exported from Scopus in comma separated values (CSV) format in order to facilitate management and analysis. The column headings included author names, title of the work, year of publication, keywords, affiliations, abstract, names of the magazines or publishers, among others. For the analysis, the Rstudio program (R Core Team, 2023) and



the “bibliometrix” library (Aria & Cuccurullo, 2017) were used, which is important for the extraction and elimination of duplicates or errors when importing the information.

The bibliometric analysis was divided into several sections, including an author analysis (publication count, H-index); keyword analysis, as well as trends and opportunity areas.

## RESULTS AND DISCUSSION

### Author analysis

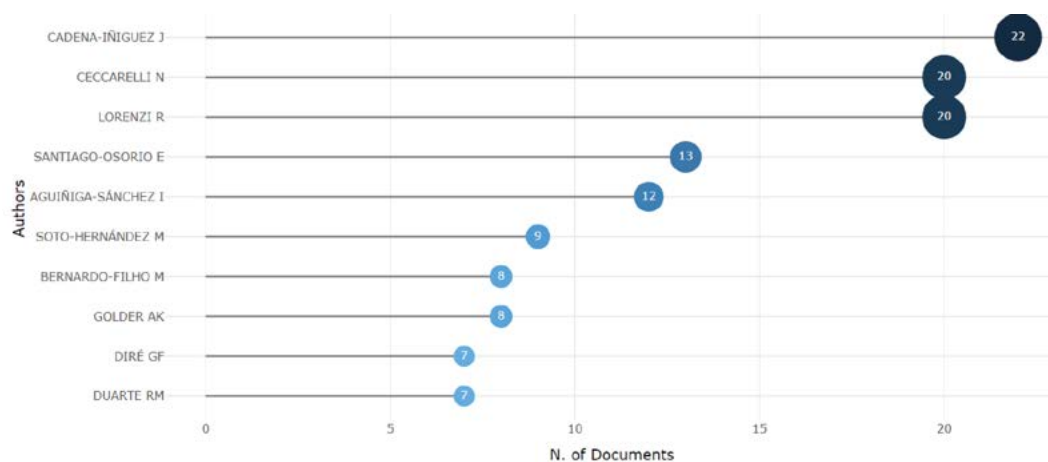
The search carried out in Scopus presented a total of 385 academic documents from 1976 to 2024, with an annual growth rate of 5.12%. The total number of publications consists of 346 scientific articles, 2 book chapters, 19 conference papers, 1 conference review, 1 data paper, 4 notes and 12 reviews.

For these publications, there are a total of 1448 researchers linked to them, of which 16.1% are international collaborations. There is a record of 13,411 references, an average of 12.2 articles published per year and an average citation of 15.46. 1,248 keywords were found, this suggests the plasticity and diversification of research in *S. edule*.

Mexico is the country with the highest number of corresponding authors with a total of 70 documents, followed by India with 49. The entities with the highest number of publications are the Universidad Nacional Autónoma de México with 51, the Sichuan Agricultural University with 40 and the Universidad Veracruzana with a total of 31. Regarding the analysis of authors, Figure 1 shows the list of the main authors in the field of study of *S. edule*, highlighting Mexican scientists.

### Keyword analysis

In the search carried out in Scopus, *S. edule*, as the opening scientific name in such analysis, stands out with 12%. Current bioprospecting studies have gained great popularity in recent years, since keywords stand out, such as plant extracts, fruits, unclassified drugs, chemistries, medicinal plants, flavonoids, apoptosis, among others. This suggests advances in pharmaceutical, nutraceutical research and biomedical applications (Figure 2).



**Figure 1.** Authors with the highest number of publications on the Scopus platform.

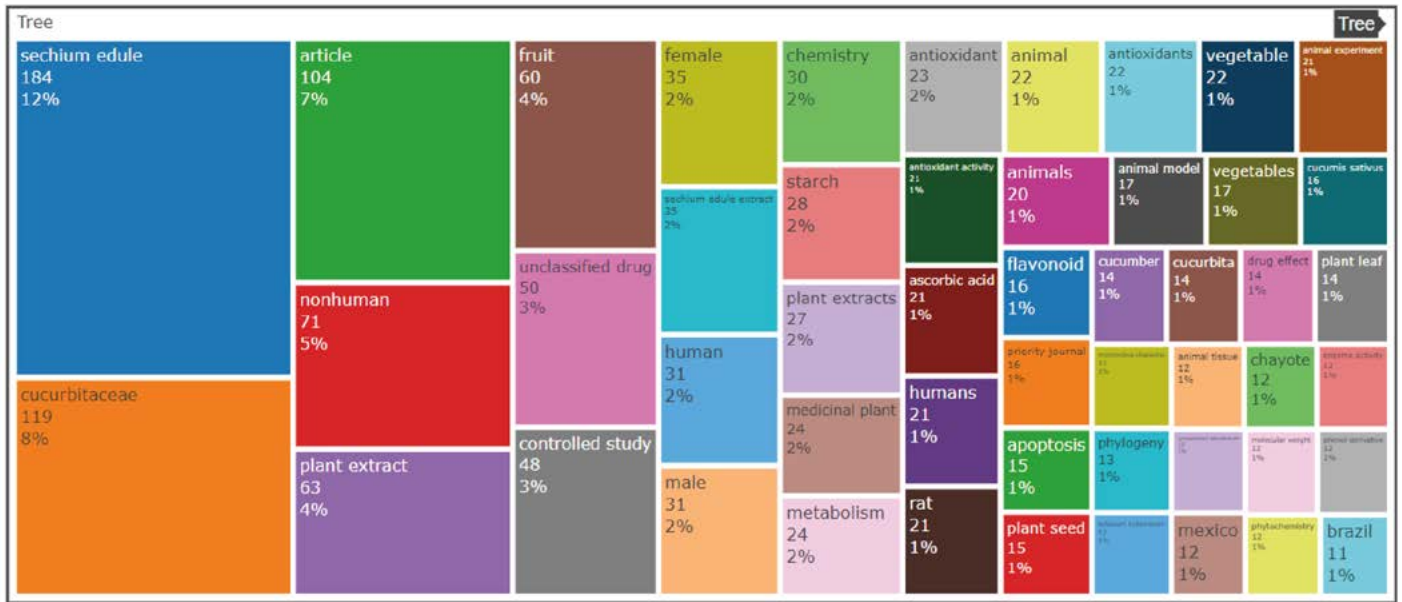


Figure 2. Panel graph with the main keywords from the Scopus database.

Figure 3 shows a network of keywords interconnected by lines that represent the occurrence of these words in scientific publications. Each of the nodes are the keywords and the size is proportional to the frequency of appearance of each keyword based on the database analyzed. The cluster in red focuses on chayote and its related studies. Keywords such as “nonhuman”, “plant extract”, “antioxidant”, and “apoptosis” suggest investigations into the use of chayote extracts in non-human models, possibly exploring their antioxidant properties and effects on apoptosis (programmed cell death). The

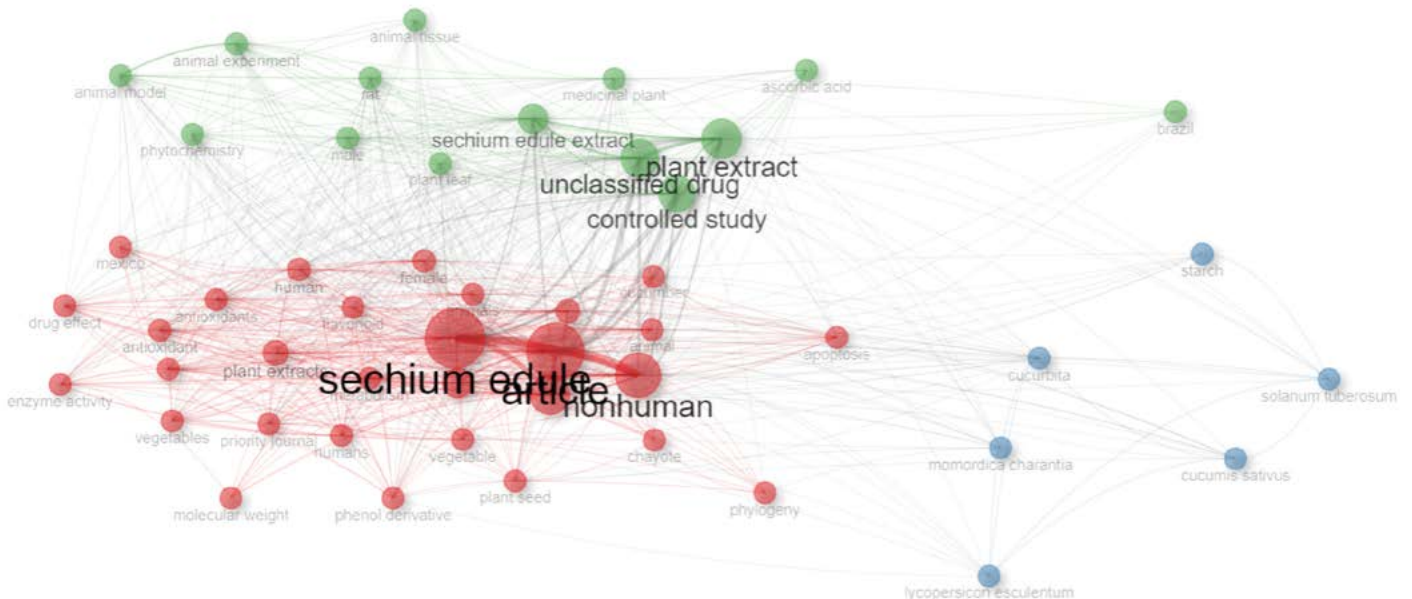


Figure 3. Keyword connectivity, cluster and importance of nodes based on their publication frequency.

connections in which there is a greater density indicate a high degree of interrelation between these themes, showing that studies on chayote are frequently related to research on its biological and chemical effects. The green cluster seems to focus on the use of medicinal plants and their extracts; Keywords such as “animal experiment”, “medicinal plant”, “ascorbic acid”, and “animal tissue” suggest studies focusing on animal experiments and the use of medicinal plants, possibly including ascorbic acid (vitamin C) and its effects on animal tissues. The cluster with the nodes in blue covers different species of the Cucurbitaceae family, such as “*Cucurbita*”, “*Solanum tuberosum*”, “*Momordica charantia*”, and “*Cucumis sativus*”, indicating a focus on agronomic and botanical studies focused on starch and other nutritional properties.

Figure 4 shows an analysis of quadrants that indicate the distribution of topics in a research field based on two axes: the degree of relevance (centrality) and the degree of development (density). In the Motor Themes quadrant, it determines that studies on chayote and its use in non-human models are both central and well developed. This suggests that this topic is crucial in current research, with a greater number of publications and a high degree of internal cohesion. In the Basic Themes quadrant, there are themes that are relevant but could benefit from further internal development. Research in the Cucurbitaceae family, on the topic of chemistry and metabolism, is crucial, but could require more studies to consolidate its internal cohesion. In the Niche Themes quadrant, themes are represented that, although not central to the field as a whole, are highly developed and cohesive. Seed physiology, amino acid sequences and genetic studies are specialized areas with a high degree of internal development. In the Emerging or declining themes quadrant, there is the theme of action Potential, this indicates an area that is currently of low relevance and under development; It may represent an emerging topic that has not yet gained significant

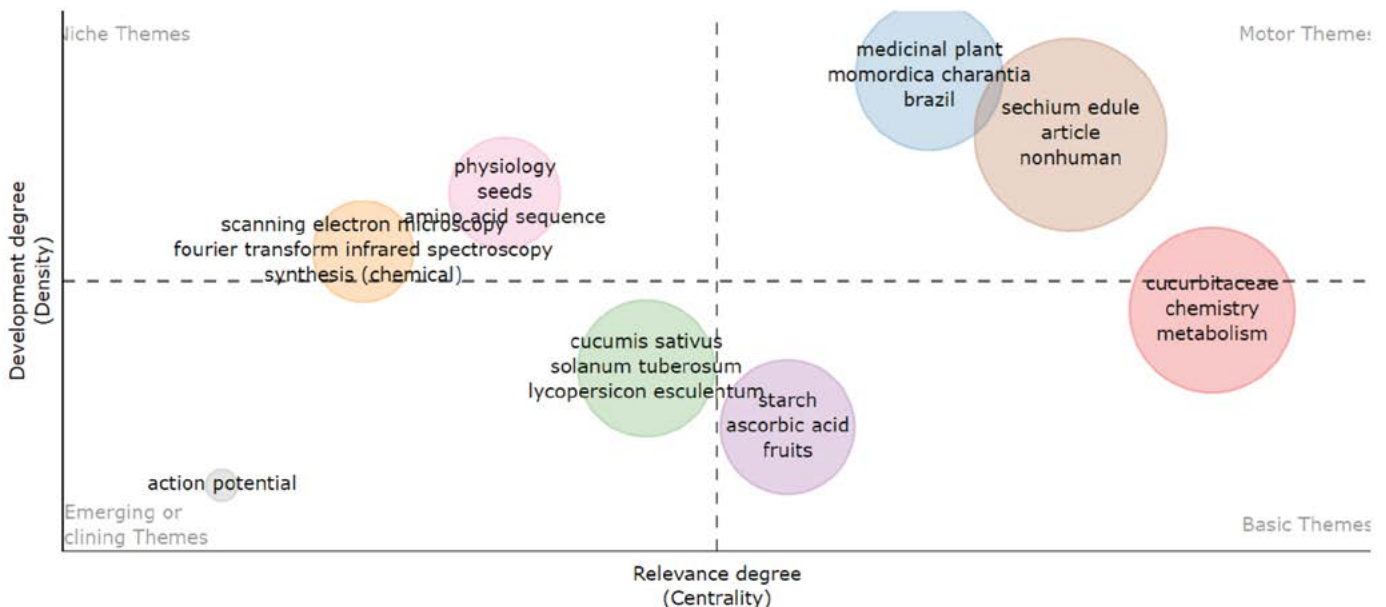
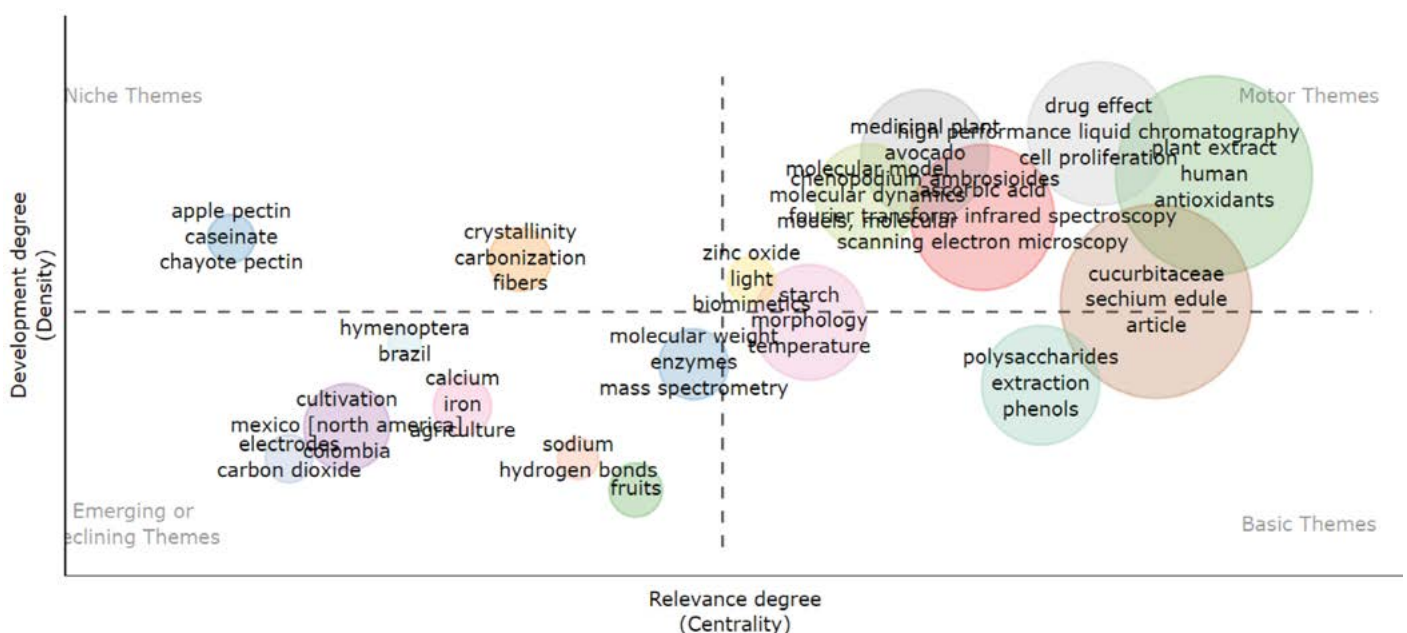


Figure 4. Factor analysis of keywords on the trend in scientific research of *Sechium edule*.

traction in the literature or could be in decline, however, it could be taken up again if bioprospecting studies can be configured.

Figure number 5 shows the areas of opportunity, those with a future perspective such as Drug Effect, Liquid Chromatography, Plant Extract, Human, Antioxidants. Those areas indicate that studies on the effects of drugs, liquid chromatography, plant extracts and antioxidants in humans are both central and well developed. These topics are key in current and future research, a greater proportion of publications and a high degree of internal cohesion are evident. The “Basic Themes” quadrant highlights themes that are fundamental pillars in current research, however they need greater depth and cohesion in their development. Areas of study such as Cucurbitaceae, *Sechium edule*, polysaccharides, phenols, molecular dynamics, zinc oxide, biokinetics, morphology, and analytical techniques such as mass spectrometry are crucial for the advancement of scientific knowledge. Encouraging additional research into these topics will help consolidate their theoretical and practical foundations, enabling the development of innovative applications and technological solutions that can have a significant impact on various industries. Prioritizing these topics on research agendas can lead to important discoveries and technological advances that will benefit multiple sectors. The Niche Themes quadrant represents research areas that have a solid and developed knowledge base but are not fundamental to the field in general. These topics are valuable for their specific applications and for researchers specializing in these areas, although their broader impact and relevance may be limited.

Documentary and experimental research on *S. edule* is very extensive, although publications were only searched in Scopus, there is information in scientific journals indexed from other platforms and for other purposes, for example in scientific dissemination.



**Figure 5.** Factor analysis of keywords on the future dynamics and areas of opportunity in scientific research of *Sechium edule*.

## CONCLUSIONS

This strategic bibliometric analysis provides a broad overview of the structure and dynamics of research in the field of chayote study. Identify major, core, niche, and emerging themes. It allows researchers and scientific policymakers to conduct their efforts effectively, prioritizing important research areas, supporting the development of innovative topics. This information is essential for planning future research and ensuring the continued and cohesive advancement of scientific knowledge of *S. edule*.

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# Profitability of the Production of Sotol (*Dasyliirion* spp.) Seedlings Grown under Greenhouse Conditions

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## ABSTRACT

**Objective:** Sotol (*Dasyliirion* spp.) is a native plant of the Chihuahuan desert, used in the production of alcoholic beverages. The Mexican Council of Sotol (CMS) and the Certification Council of Sotol (CCS) supervise the compliance with the NOM-159-SCFI-2004 official Mexican standard; these organizations also fulfil other functions. The objective was to carry out an economic and financial feasibility analysis of the greenhouse production of sotol seedlings grown under seedbed conditions.

**Design/Methodology/Approach:** The production cost data were obtained from a module established in the municipality of Meoqui, Chihuahua, Mexico.

**Results:** Producers registered in the CMS and in the CCS—and who are interested in establishing commercial sotol plantations—provided direct empirical information. In addition, the Net Present Value (NPV), Internal Rate of Return (IRR), and the Benefit-Cost Ratio (B/C) financial variables were determined. The investment project is feasible, because it recorded a 1,483,396.12 NPV, a 59% IRR, and 1.55 B/C ratio.

**Findings/Conclusions:** A potential demand and a profitable production of sotol seedlings grown under greenhouse conditions in Chihuahua.

**Keywords:** plant, alcoholic beverage, sereque, financial evaluation.

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## INTRODUCTION

Mexico is a major producer of plant-based alcoholic beverages: tequila (*Agave tequilana* L.), mezcal (*Agave angustifolia* L.), and bacanora (*Agave angustifolia*). In addition, *Dasyliirion* spp. is used to produce an alcoholic beverage known as sotol. Therefore, *Agave* and *Dasyliirion* plants are highly appreciated, as a result of the economic benefits they generate for the country. In 2023, 359.9 million tequila liters were produced, out of which, 214.9 million were exported (Forbes staff, 2023).

Sotol belongs to the Asparagus family (Asparagaceae) and is native to the Chihuahuan desert, located in northern Mexico and the southeastern USA. The most common genus *Dasyliirion* used for sotol production is *D. wheeleri*; however, species such as *D. cedrosanum*, *D. leiophyllum*, and *D. texanum* are also used for this purpose. Sotol plants grow in arid and semi-arid ecosystems, where the annual precipitation rate ranges from 300 to 500 mm. Bogler (1994) mentions that the genus *Dasyliirion* was an important part of the diet of the original peoples that inhabited the northern desert of the Americas, while other authors report that it was used in adornments or buildings. Spaniards introduced the distillation process to the Americas. This technique was first applied to Agaves and the result was mezcal. Subsequently—and as a consequence of the similarities between *Agave* and *Dasyliirion*—, sotol plants were also subjected to this process.

The plants of the Agavaceae family are still widely used and they have been the subject of many studies (Espinoza-Andrews *et al.*, 2021). For example, it is well documented that the genus *Agave* reaches its maximum sugar and fructose concentration at 2-4 years old. However, in the case of *Agave tequilana*, Arrizon *et al.* (2010) proved that 4.5-32.5 kg agave hearts can reach their maximum sugar and fructose concentration after 4 years. Although Mexico obtained a designation of origin (DO) in 2002—specified in the NOM-159-SCFI-2004 official Mexican standard (DOF, 2004)—, the information about the whole sotol cycle is very scarce. This standard has doubtlessly favored the production, commercialization, and industrialization of this species.

Unlike tequila and mezcal (which are produced with cultivated plants), sotol is produced with wild plants—*i.e.*, no domesticated species are used for this process (Michael, 2023). Consequently, the production, commercialization, and industrialization of sotol is no match for tequila and mezcal. Nevertheless, the production of sotol is increasing and, therefore, further research is required to domesticate this species. Seedling production is the first stage in the development of a technological package for the sotol plant. Some producers are already growing seedlings. Consequently, the objective of this research was to analyze the profitability of the sotol (*Dasyliirion* spp.) seedling production, under greenhouse conditions. This information could be useful for those producers interested in growing sotol seedlings as the first stage of the process, before planting them in the fields.

## MATERIALS AND METHODS

This study was carried out with the support of producers from the Nuevo San Lucas community, municipality of Meoqui, Chihuahua, Mexico. The following financial variables were used for the profitability analysis: Net Present Value (NPV), Internal Rate of Return (IRR), and Benefit-Cost Ratio (B/C). The NPV is used to determine how to invest; it is supported by the actual comparison of the payments and collection of a given investment or project. This process determines the profits and the losses of an investment. The IRR is a profitability indicator of projects or investments: if the IRR is high, the profits will also be high. Therefore, this indicator supports the decision-making process regarding the investment. Finally, the B/C Ratio is the global ratio between the costs and the benefits during a given period (Baca-Urbina, 2013).



The total cost of the production was based on the calculation of the fixed costs and the variable costs (Atheam *et al.*, 2021; Bragg, 2020) of the following seven activities: 1) soil preparation; 2) sowing; 3) irrigation and fertilization; 4) nutritional analysis; 5) pest and disease control; 6) weed control; and 7) harvest and packaging. Soil preparation included the inherent expenditures of the conditioning, which facilitates germination or the establishment of the seedling and the subsequent development of the plant. In addition, other soil preparation expenditures must be taken into account: soil identification; weed removal; aeration and loosening of the soil; soil levelling; furrows and placement of wood and stakes; soil fertilization and enrichment (substrates); and equipment rental (bobcat). Sowing included the selection of the type of plants and the time of planting, the organization of the sowing, and the inspection of the seed. Irrigation and fertilization took into account the application of fertilizers and amendments and the purchase of soluble monopotassium phosphate, liquid vermicompost, potassium nitrate, calcium nitrate, magnesium nitrate, and urea. The nutritional analysis diagnosed the problems and recommended a fertilization treatment. In addition, soil preparation was taken into account. Pest and disease control was carried out using natural enemies. Micronutrient chelate and fungicides were purchased. A bi-weekly manual weeding was quoted. Finally, the production at the moment of the harvest and the moment when the seedling was ready for delivery were estimated for the harvest and packaging variable, during the final stage. A one-year cost forecast was based on the monthly work activities, starting in January. In addition, a five-year cost forecast was carried out, considering a 12% annual inflation.

## RESULTS AND DISCUSSION

Table 1 shows the main variable costs: irrigation labor (MXN \$22,125.00), sowing (MXN \$17,755.00), and soil preparation (MXN \$15,500.00). According to the recommendations of BTC Bank (2023), variable costs increase or diminish depending on production. Meanwhile, fixed costs do not change depending on production —*i.e.*, they do not change based on production level. The highest fixed costs for this activity included: administrative fees (MXN \$105,792.00) and electricity (MXN \$36,000.00).

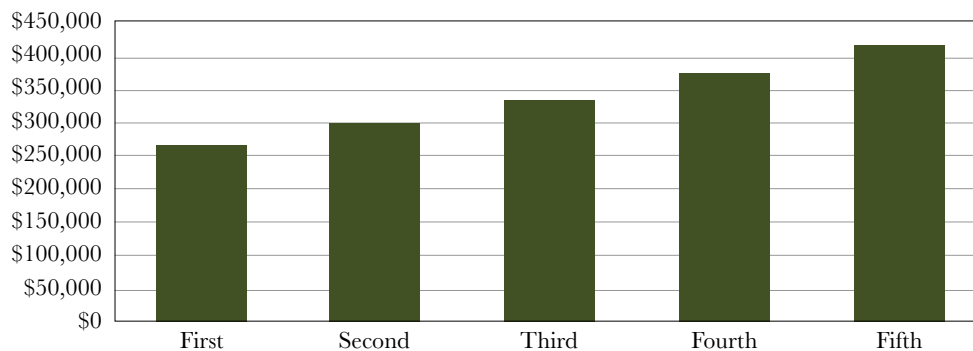
Likewise, Table 1 shows that the biggest expenses were recorded during the first two months of the activity: MXN \$59,451.00 (first month) and MXN \$29,253.33 (second month). For reference purposes, the total cost of the production of sotol seedlings is MXN \$263,723.99. If a total of 240,000 seedlings were produced, the resulting unit cost would be MXN \$1.10. BTC Bank (2023) clearly specifies that some agricultural production elements (*e.g.*, soil, sunlight, heat, and rain) are provided by the environment and consequently do not require financial management.

Figure 1 shows the five-year cost forecast for the production of sotol seedlings (annual inflation: 12%). On the fifth year, the approximate cost would be MXN \$414,994.00. Since today's Mexican peso or American dollar has a greater purchasing power, a given investor understands that a current investment will generate more money than the amount that could be received a year from now. This situation exemplifies the relationship between money at different points in time.

**Table 1.** Variable and Fixed Costs (1<sup>st</sup> year).

Concept	Cost
Variable	mexican pesos (\$)
Land preparation	15,500.00
Nutritional analysis	2,200.00
Sowing	17,775.00
Fertilization	8,340.00
Fungicides	2,199.99
Water	2,592.00
Labour - Risks (3 dairy risks from \$1.50 to \$50.00 p/ho)	22,125.00
Labour - Gas charge and discharge	3,400.00
Labour - Weeding and fallow	6,000.00
Labour - Fertilization	8,000.00
Subtotal	88,131.99
Fixed	
Module Instalations	11,500.00
Labour - sowing	2,500.00
Greenhouses and bleaches structures manteinment	8,800.00
Greenhouses belts manteinment	6,000.00
Electric energy	36,000.00
Use of pump, nebulizer, tank	4,000.00
Others	1,000.00
Administrator fees	105,792.00
Subtotal	175,592.00
Total	263,723.99

Elaboración propia (2023).



**Figure 1.** Five-year cost forecast to produce sotol seedlings.

In December, production amounted to 240,000 seedlings. The sale price per seedling amounted to MXN \$1.70, resulting in an income of MXN \$408,000. Fisher *et al.* (2014) mentioned that seedling production is the easiest stage of a greenhouse business, although they specified that the main objective is to guarantee a profitable production within a highly competitive industry.

The NPV, IRR, and B/C ratio were the financial variables used to determine the result of the profitability analysis (Table 2). The five-year forecast was determined based on the initial investment, income, and costs, using a readjustment rate. Table 2 shows the NPV value (1,483,396.12); the NPV parameter is one of the traditional methods for the evaluation of investment projects (Brotons, 2017). López-Marín *et al.* (2020) studied the greenhouse production of chili peppers and reported a NPV of €178,394 —a higher figure than the normal or traditional NPV. As a rule, a given investment with a positive NPV should be accepted, while a negative NPV should be rejected (Cruz and Singerman, 2019). In other words, a positive NPV guarantees that income levels will be higher than production costs. Consequently, this study recommends investing in the production of sotol seedlings.

Meanwhile, IRR is also an advantageous method used to evaluate a given investment project. This method does not require a discount rate, since the internal rate of return is intrinsic to the investment under evaluation (Ross *et al.*, 2005). Table 2 specifies the IRR value for this study (59%); therefore, the average annual return of this sotol seedling production project will be MXN \$59 for every MXN \$100 invested. This percentage is like the 53% IRR reported by Sengar and Kothari (2008), who concluded that cultivating roses was a highly attractive project from the financial point of view. Overall, a >20% TAS is acceptable for any investment project, while any investment project with a <5% TAS should be reconsidered. Finally, Miller *et al.* (2017) and other researchers proved the financial advantages of calculating the NPV and TAS values for the greenhouse production of hydroponic lettuce and tomato.

This study reported a 1.55 B/C ratio, which can be considered acceptable for this kind of investment project. Nevertheless, some greenhouse crops can have a higher B/C ratio. For example, Sengar and Kothari (2008) reported a very high B/C ratio (4.5) for the cultivation of roses, owing to the price of roses in the market.

**Table 2.** Results of the profitability analysis, using the NPV, IRR, and B/C ratio financial variables.

Years	Income (mexican pesos \$)	Cost (mexican pesos \$)	Cash Flow (mexican pesos \$)	Interest rate $1/(1+t)^N$	Updated income (mexican pesos \$)	Update expenses (mexican pesos \$)
0		253,800.00	253,800.00	1.00		
1	408,000.00	263,723.99	109,523.99	0.79	323,809.52	209,304.75
2	456,960.00	295,370.87	52,065.14	0.63	\$611,640.21	395,353.42
3	511,795.20	330,815.37	894,675.71	0.50	\$867,489.71	560,730.02
4	573,210.62	370,513.22	1,838,370.26	0.40	1,094,911.49	707,731.44
5	641,995.90	414,974.80	2,895,370.26		1,297,064.18	838,399.37
Total	2,591,961.72	1,929,192.25	824,786.68		4,194,915.11	2,711,519.00
<b>Financial indicators</b>						
NPV	1,483,396.12					
ROI	59%					
B/C	1.55					

NPV: net present value, ROI: internal rate of return, B/C: benefit/cost.

## CONCLUSIONS

There is economic and financial viability of sotol seedling production under greenhouse seedbed conditions. Economic profitability indicators, such as NPV of 1,483,396.12, IRR of 59% and a B/C ratio of 1.55 confirm that the project is acceptable and profitable. Given the demonstrated economic potential, it is suggested to promote and expand sotol seedling production in greenhouses. It is crucial that regulatory agencies, CMS and CCS, continue to support producers by implementing training programs and providing resources for the establishment of new commercial plantations.

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# Comparison of three commercial feeds in the fattening of Yorkshire pigs (*Sus scrofa domesticus*) in the starter and growth

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## ABSTRACT

**Objective:** To assess the efficiency of three commercial feeds for pig fattening.

**Design/Methodology/Approach:** The study was conducted in the pig unit of the Centro de Bachillerato Tecnológico Agropecuario No. 90. Thirty-three pigs were randomly assigned to one of the following three treatments (11 specimens per treatment): 1) Sabamex<sup>®</sup>; 2) Campeón<sup>®</sup>, and 3) Ganador<sup>®</sup>. In the starter phase, the three feeds were provided *ad libitum* for four weeks. The same feeds were offered from the fifth to the eighth week (growth phase). The response variables were analyzed in a completely randomized design for each phase. The impact of sex differences on weight gain was analyzed using Student's t-test.

**Results:** All variables assessed recorded a difference ( $P < 0.05$ ) between treatments. In the initial four-week period, a statistically significant difference ( $P < 0.05$ ) was observed in the daily weight gain per animal per day variable. The same difference ( $P < 0.05$ ) was observed in the subsequent four weeks of growth (end stage) for the same variable. Additionally, sex differences had an impact on weight gain ( $P < 0.05$ ).

**Study Limitations/Implications:** Pigs should not be fattened in autumn and winter, due to the adverse effects of low temperatures. The Campeón<sup>®</sup> feed is the most profitable and advisable option.

**Findings/Conclusions:** Pigs that were fed on Sabamex<sup>®</sup> and Ganador<sup>®</sup> had a better performance than pigs that were fed on Champion<sup>®</sup>.

**Keywords:** economic analysis, feed efficiency, weight gain, cost benefit, sex differences.

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## INTRODUCTION

The pork population and production in Chihuahua are not sufficient to meet the demand, as a result of the limited number of pork producers in the state. According to the USDA, the annual per capita consumption of pork in Chihuahua is 15 kg (approximately 53,348 tons, which increases proportionally with population growth). Consequently, in order to meet the demand, a significant portion of the pork consumed in Chihuahua is imported (González-Vejar and Levario-Quezada, 2013).

The municipalities of Bachiniva, Carichi, Cuauhtémoc, Cusihiuriachi, and Riva Palacio, located within the Cuauhtémoc district, account for 24% (2,157 t) of the state's total pork production (SIAP, 2015). The production of high-quality pork in the municipality of Cuauhtémoc is limited by the rudimentary and informal way in which this activity is typically conducted: pigs are fed on leftovers from greengrocers and restaurants, among other sources (González-Vejar and Levario-Quezada, 2013). The Mennonite community from the municipality of Cuauhtémoc is engaged in the fattening of pigs. In this community, pigs are fed on yellow corn and whey, employing an empirical approach. This lack of balanced diets results in an inefficient pig fattening production system. Consequently, fatty meat is the result of a prolonged feeding period (González-Vejar and Levario-Quezada, 2013). Pigs are one of the most productive species and, with an adequate diet, gains are achieved within a 5-month period (Magaña-Magaña *et al.*, 2018). The parameters used for the assessment of production systems include feed consumption, feed efficiency, weight gain, and cost per kilogram of meat produced (Benitez-Meza *et al.*, 2015). The efficient feeding of pigs is a fundamental aspect of a piggery, as it not only affects the productive yields of the pigs, but also the profitability of the farm. In fact, feeding accounts for 80-85% of total production costs (Campadal, 2009).

The objective of this study was to assess the nutritional value of three pig feeds widely used by regional producers. These commercial feeds (Sabamex<sup>®</sup>, Campeón<sup>®</sup>, and Ganador<sup>®</sup>) were selected based on their ability to meet the nutritional requirements of pigs and to improve productive parameters. The nutritional requirements of initiation and growth were used as the basis for the assessment. At least one of the feeds (treatments) is higher than the others in at least one of the productive parameters.

## MATERIALS AND METHODS

The study was conducted in the pig unit of the Centro de Bachillerato Tecnológico Agropecuario No. 90 of Ciudad Cuauhtémoc, Chihuahua, located at 2,098 m.a.s.l. The average annual temperature is 14 °C. According to the Köppen climate classification modified by García (2004), the climate is classified as BS<sub>1</sub> KW (W)(e<sup>1</sup>): a dry temperate climate with summer rains, a frost-free period of 208 days, and an average annual rainfall of 450 mm. The minimum and maximum temperatures were recorded on the coldest days with a ThermoPro TP50 temperature and humidity monitor.

The 33 pigs were subjected to internal and external deworming with Ivermectin and ADE (1.0 mL per 33 kg live weight). During an eight-week period, the animals were weighed on a weekly basis. The following variables were recorded: feed intake, feed efficiency (feed consumed to produce 1.0 kg of body mass), weight gain (daily weight gain), and cost per kilogram of meat produced.

In average, the initial weight of the 33 pigs was 19.21 kg (starter phase). The pigs were randomly distributed into one of three treatments: Sabamex<sup>®</sup>, Ganador<sup>®</sup>, and Campeón<sup>®</sup>. *Ad libitum* feed —with a protein level guaranteed by the manufacturer— was provided in 5-space automatic feeders. The starter feeding (provided until the end of the fourth week) consisted of Sabamex<sup>®</sup> (18% protein), Campeón<sup>®</sup> (18% protein), and Ganador<sup>®</sup> (17% protein).

The growth feed provided in the fifth week of the study consisted of Sabamex<sup>®</sup> (16% protein), Campeón<sup>®</sup> (16% protein), and Ganador<sup>®</sup> (14.5% protein). Each treatment was administered to the 11 experimental units. Each unit was comprised of seven male pigs and four sows. At the start of the fifth week of the experiment, the 33 animals entered the growth phase with an average initial weight of  $16.36 \pm 4.58$  kg (Sabamex<sup>®</sup>),  $19.5 \pm 4.35$  kg (Campeón<sup>®</sup>), and  $21.72 \pm 4.41$  kg (Ganador<sup>®</sup>).

Table 1 shows that the Sabamex<sup>®</sup> feed had a guaranteed analysis. This feed was fully provided once the suckling pigs were weaned (at a weight of 7-8 kg) and until the pigs reached an average weight of 20 kg. The pigs had free access to fresh and clean water throughout the study period.

Table 2 shows the results of the guaranteed analysis of the Sabamex<sup>®</sup> feed for the growth phase. Pigs had ad libitum access to the full feed once they reached 18 kg live weight and until they weighted 40 kg. They were also offered fresh, clean water without restriction.

Table 3 shows the guaranteed analysis for the Campeón<sup>®</sup> balanced feed for starter pigs; Table 4, the guaranteed analysis of Campeón<sup>®</sup> balanced feed for growing pigs; Table 5, the guaranteed analysis of the Ganador<sup>®</sup> balanced feed for growing pigs; and Table 6, the guaranteed analysis of the Ganador balanced feed for growing pigs. All balanced foods were offered as recommended by the manufacturer.

### Experimental Design

The statistical model used in the study was a completely randomized design with a significance level of  $\alpha=0.05$ . The Duncan's test, available in the SAS statistical software (SAS, 2002), was used for the comparison of means. The experimental period lasted from October 20 to December 15, comprising four weeks for the starter phase and four weeks for the growth phase. The same statistical model was employed to assess the impact of

**Table 1.** Guaranteed analysis of Sabamex<sup>®</sup> balanced feed (Reg. SAGARPA-7031-010) for starter pigs.

Concept	%	Concept	%
Minimum Protein	18.0	Nitrogen free extract	64.5
Minimal Fat	3.5	Minimum Calcium	0.7
Maximum Fiber	6.0	Minimum Phosphorus	0.6
Maximum Ashes	8.0	Lysine	1.1
Maximum Humidity	12.0	Minimal Methionine	0.3

**Table 2.** Guaranteed analysis of the Sabamex<sup>®</sup> balanced feed for growing pigs.

Concept	%	Concept	%
Minimum Protein	16.0	Nitrogen free extract	63.0
Minimal Fat	3.0	Minimum Calcium	0.7
Maximum Fiber	6.0	Minimum Phosphorus	0.5
Maximum Ashes	6.0	Lysine	0.75
Maximum Humidity	13.0	Minimal Methionine	n/s

n/s=not specified on the product label.

**Table 3.** Guaranteed analysis of the Campeón® balanced feed (Reg. SAGARPA A-7297-015) for starter pigs.

Concept	%	Concept	%
Minimum Protein	18.0	Nitrogen free extract	56.0
Minimal Fat	2.0	Calcium	n/e
Maximum Fiber	5.0	Phosphorus	n/e
Maximum Ashes	7.0	Lysine	n/e
Maximum Humidity	12.0	Methionine	n/e

n/s=not specified on the product label.

The animals had *ad libitum* access to the feed and water.

**Table 4.** Guaranteed analysis of Campeón® balanced feed (Reg. SAGARPA A-7297-009) for growing pigs.

Concept	%	Concept	%
Minimum Protein	16.0	Nitrogen free extract	57.5
Minimal Fat	1.5	Calcium	n/s
Maximum Fiber	5.0	Phosphorus	n/s
Maximum Ashes	7.0	Lysine	n/s
Maximum Humidity	12.0	Methionine	n/s

n/s=not specified on the product label.

The animals were provided *ad libitum* access to the diet and water.

**Table 5.** Guaranteed analysis of the Ganador® balanced feed (exempt from registered before the SADER).

Concept	%	Concept	%
Minimum Protein	17.0	Nitrogen free extract	n/s
Minimal Fat	3.5	Calcium	n/s
Maximum Fiber	6.0	Phosphorus	n/s
Maximum Ashes	8.5	Lysine	n/s
Maximum Humidity	12.0	Methionine	n/s

n/s=not specified on the product label.

The animals were provided with *ad libitum* access to the diet and water.

**Table 6.** Guaranteed analysis of the Ganador® balanced feed (Reg. SADER A-0544-926) for growing pigs.

Concept	%	Concept	%
Minimum Protein	14.5	Nitrogen free extract	n/s
Minimal Fat	3.0	Calcium	n/s
Maximum Fiber	7.5	Phosphorus	n/s
Maximum Ashes	7.5	Lysine	n/s
Maximum Humidity	12.0	Methionine	n/s

n/s=not specified on the product label.

The animals were provided with *ad libitum* access to the diet and water.



the treatments on each test phase (starter and growth) and for each variable (productive parameters) under study. The Student's t-test was used to assess the sex differences.

## RESULTS AND DISCUSSION

### Weight gain and consumption

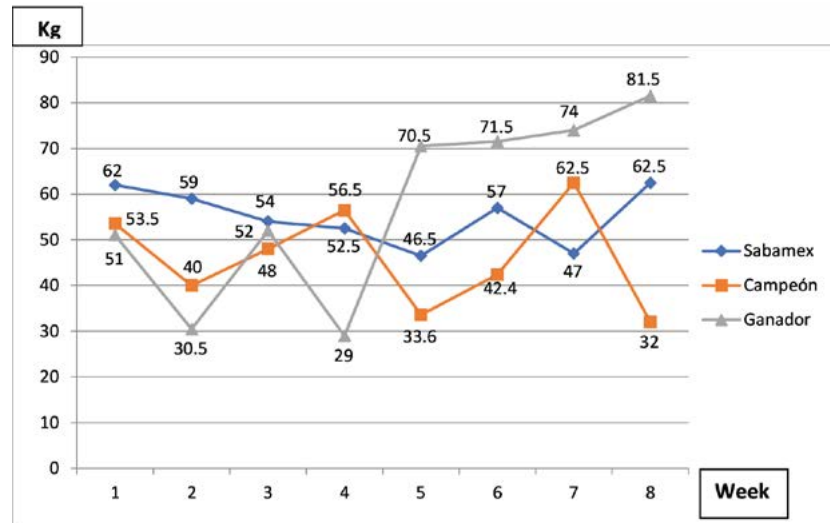
In the initial four-week testing period, a statistically significant difference ( $P < 0.05$ ) with probability  $F = 0.01225147$  was observed in the daily weight gain per animal per day variable between the Sabamex<sup>®</sup> ( $0.738 \pm 0.138$  kg/day), Campeón<sup>®</sup> ( $0.641 \pm 0.176$  kg/day), and Ganador<sup>®</sup> ( $0.527 \pm 0.234$  kg/day) treatments. The results were like the findings of Valdes and Arcilla (2014) and Shimada (2015) (Table 1). In the initial stage of the study, Sabamex<sup>®</sup> recorded the highest daily weight gain, followed by Campeón<sup>®</sup> and Ganador<sup>®</sup>. This discrepancy may be attributed to the quality and interaction of the ingredients of Sabamex<sup>®</sup>, as well as the quantity prepared, resulting in enhanced feed-to-meat conversion, regardless of the analysis guaranteed by the manufacturer. According to the manufacturers, the ingredients used to prepare each feed are not identical across all diets. Consequently, the chemical and nutritional analyses of these feeds may differ, potentially contributing to the variations in weight gain observed in the starter phase.

During the final four weeks of the growth phase, a significant difference ( $P < 0.05$ ) was again observed in the daily weight gain per animal per day variable between the Sabamex<sup>®</sup> ( $0.690 \pm 0.102$  kg/day), Campeón<sup>®</sup> ( $0.553 \pm 0.182$ ), and Ganador<sup>®</sup> ( $0.956 \pm 0.068$  kg/day) treatments (Table 7).

In this growth phase, Ganador<sup>®</sup> showed the highest weight gain, due to its higher daily intake ( $2.358 \pm 0.395$  kg) compared to Sabamex<sup>®</sup> ( $1.858 \pm 0.248$  kg) and Campeón<sup>®</sup> ( $1.855 \pm 0.332$  kg). These figures suggest that the greater the intake, the greater the weight gain. One of the most plausible explanations for the higher intake of Ganador<sup>®</sup> at this phase would be its higher palatability (Table 7). At the end of the 8-week trial (starter and growth), weight gain showed variability among treatments ( $P < 0.05$ ). The average weight gain for each treatment was:  $41.72 \pm 5.67$  kg (Ganador<sup>®</sup>),  $40.09 \pm 7.06$  kg (Sabamex<sup>®</sup>), and  $33.18 \pm 9.13$  kg (Campeón<sup>®</sup>) (Table 7, Figure 1).

No statistical differences ( $P > 0.05$ ) were reported among the treatments regarding the mean daily intake of pigs during the eight-week testing period (probability  $F = 0.075394747$ ). The mean daily intake of the Sabamex<sup>®</sup>, Campeón<sup>®</sup>, and Ganador<sup>®</sup> treatments was  $1.656 \pm 0.326$ ,  $1.661 \pm 0.351$ , and  $1.969 \pm 0.411$  kg, respectively (Table 7). However, no differences between the consumption of the feeds were reported. At the end of the two phases, a trend indicated that Ganador<sup>®</sup> was consumed in greater quantity, which may explain the weight gain at the end of the eight-week period. The results showed a reduction in weight gain, except for Ganador<sup>®</sup>, which had similar results to those previously reported by Rostagno *et al.* (2011), Church (2009), and Shimada (2015).

In the wake of the eight-week trial period, the feed efficiency of the three treatments was determined to be statistically equivalent ( $P > 0.05$ ). The mean consumption required to achieve a 1.0 kg weight gain was  $2.345 \pm 0.557$  kg (Sabamex<sup>®</sup>),  $2.905 \pm 0.758$  kg (Campeón<sup>®</sup>), and  $2.834 \pm 0.857$  kg (Ganador<sup>®</sup>). The three treatments showed a greater feed efficiency than the efficiency reported by Church (2009) and were comparable with the findings of



**Figure 1.** Weight gain per week and treatment in pigs fed with Sabamex<sup>®</sup>, Campeón<sup>®</sup> and Ganador<sup>®</sup> in Cuauhtemoc, Chihuahua, Mexico.

Castellanos (2022a, 2022b; Table 7). In the initial four-week testing period, no notable difference ( $P > 0.05$ ) was identified for this variable among the Sabamex<sup>®</sup> ( $1.991 \pm 0.499$  kg), Campeón<sup>®</sup> ( $2.336 \pm 0.326$  kg), and Ganador<sup>®</sup> ( $3.232 \pm 1.069$  kg) treatments. However, in the final four weeks of testing, a significant difference ( $P < 0.05$ ) was observed among treatments, with a feed intake per kg of meat produced of  $2.708 \pm 0.375$  (Sabamex<sup>®</sup>),  $3.475 \pm 0.609$  (Campeón<sup>®</sup>), and  $2.437 \pm 0.391$  kg (Ganador<sup>®</sup>) (Table 7). The results suggested that Sabamex<sup>®</sup> (followed by Ganador<sup>®</sup>) was the treatment with the highest feed intake per kg of meat produced. Both feeds obtained better results than the treatments reported by Taipe-Cando (2023).

### Effect of sex differences on weight gain

The weight gain of males ( $40.28 \pm 8.78$ ) was 10.6% (probability  $F = 0.01347312$ ) higher than the weight gain of females ( $36 \pm 6.17$  kg) ( $p < 0.05$ ). Castellanos (2021) has also reported that male pigs reach a higher weight than females. Large biotypes record the highest rate of weight gain, and, within these, males gain more weight than females. At the same age than other animals, the larger or taller specimen reaches a higher weight —*i.e.*, it has a higher rate of daily weight gain and reaches a higher maximum weight. From a physiological perspective, weight gain is the result of the accumulation of protein, fat, and water over time. The animal's protein mass increases in proportion to its weight, even under variable feeding conditions (Di Marco, 2007).

**Economic analysis.** Table 8 shows the findings of the economic analysis of the study. The cost of the Campeón<sup>®</sup> treatment is lower than other treatments, amounting to \$13.52 Mexican pesos per kg of meat produced. Consequently, the cost-effectiveness of this treatment is greater than Sabamex<sup>®</sup> and Ganador<sup>®</sup>. Table 9 shows the profits and profitability of the fattening pig test over the course of eight weeks, encompassing the starter and growth phases. Once again, the profits (\$4,635 Mexican pesos) and profitability

(30.20%) of the Campeón<sup>®</sup> treatment were higher than with the other treatments. In conclusion, fattening pigs with the Campeón<sup>®</sup> feed has a greater economic viability than the Sabamex<sup>®</sup> and Ganador<sup>®</sup> feeds.

## CONCLUSIONS

During the eight-week testing period, pigs fed on Sabamex<sup>®</sup> (40.09±7.06 kg) and Ganador<sup>®</sup> (41.72±5.67 kg) recorded higher results ( $P<0.05$ ) in the weight gain variable than Campeón<sup>®</sup> (33.18±9.13 kg). Regarding the feed consumption per pig per day variable, no statistically significant difference was observed between the Sabamex<sup>®</sup> (1.656±0.326 kg), Campeón<sup>®</sup> (1.661±0.351 kg), and Ganador<sup>®</sup> (1.969±0.411 kg) commercial brands. Furthermore, there was no difference ( $P>0.05$ ) in the efficiency of the feed consumed to gain one kilogram among the three treatments: Sabamex<sup>®</sup> (2.345±0.557 kg), Campeón<sup>®</sup> (2.905±0.758 kg), and Ganador<sup>®</sup> (2.834±0.857 kg). Regarding sex differences, males gained 10.6% more weight (40.28±8.78 kg) than the females (36±6.17 kg). The cost of Campeón<sup>®</sup> was lower (\$13.52 per kg of meat produced). The highest profits (\$4,635 Mexican pesos) and profitability (30.20%) were obtained with this feed. Therefore, producers of fattening pigs with an 8-week period of growth are advised to provide the Campeón<sup>®</sup> feed *ad libitum*, using the starter feed provided for 4 weeks, followed by the growth feed for the remaining 8 weeks.

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# Mineral Profile in Soil and Forages of Rangelands of the Huasteca Potosina, Mexico

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## ABSTRACT

Mineral imbalances in soils and forages may cause suboptimal production of forage biomass and livestock.

**Objective:** To determine the concentrations of minerals in soil and forage during the dry and wet seasons in the Huasteca Potosina.

**Materials and Methods:** Samples from the soil and the main forage species consumed by livestock were collected in 17 production units (PU). The P content in soil and forage was determined by colorimetry, while the Ca, Mg, Cu, Fe, Zn, Mn, and Co content was established by atomic absorption spectrophotometry, and flame photometry was used to calculate Na and K content. The data were subjected to an analysis of variance; the effect of PU, season, and interactions was considered as fixed effect. Means were compared using Tukey's test.

**Results and Discussion:** P and Cu content was below the minimum critical level required for adequate plant growth. Furthermore, the P, Mg, K, Na, Cu, Co, and Zn concentration in forage failed to meet the minimum requirements for livestock.

**Conclusions:** The mineral imbalances in the grazing areas and the forages do not meet the minimum mineral requirements of dual-purpose cattle in the Huasteca Potosina.

**Keywords:** Ruminant nutrition, forage quality, mineral requirements.

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## INTRODUCTION

The Mexican territory covers 1,964,375 km<sup>2</sup>, approximately 51% of which are arid and semi-arid, 19% warm sub-humid, 17.9% temperate, 6.9% warm and semi-warm humid, and 2.5% semi-warm sub-humid zones (SEDARH, 2007). Mexico has 33,356,369 heads of cattle, out of which 92.7% are used to produce meat. Fifty percent of these heads are located in the humid and dry tropic (SIAP, 2021). San Luis Potosí has 1,020,109 heads of cattle, 51.4% of which are found in the Huasteca (SIAP, 2021). These herds are managed

in meadows with cultivated, introduced, and natural pastures whose mineral deficiencies could be reflected in suboptimal production of livestock (McDowell and Arthington, 2005). Poor absorption by the grasses resulting from a low mineral content (N, P, S, Cu, and Na) in the soil limits the growth of grasses and results in nutritional deficiencies in the livestock (McDowell, 1985; Coates *et al.*, 2019). In extensive livestock farming, livestock nutrition depends on the nutrients it obtains from the forage. In turn, nutrient concentration and mineral balance depend on the soils in which the forage plants grow. Therefore, the study of soil fertility —understood as the capacity to supply adequate quantities of nutrients to satisfy the requirements of plants (Kemp *et al.*, 1999)— is an important factor for the development of an adequate management program for livestock herds. The availability of nutrients and their ratios mainly depend on the type of soil, cation exchange capacity (CEC), pH, and organic matter (OM).

CEC is mainly influenced by OM, texture, and type of clay. It varies horizontally and vertically, with clear variation between horizons (Porta *et al.*, 2003). Ca is the dominant exchangeable cation (60-85%), followed by Mg (5-30%), K (2-6%), and Na (2-6%) (Bohn *et al.*, 1979; Mengel and Kirkby, 1987). With an acidic pH, Al ions ( $Al_3^+$ ,  $Al(OH)_2^+$ ) decrease the availability of phosphate, sulfate, and molybdate and restrict nitrification and decomposition of soil organic matter; the effect is more severe with lower pH (Osorio, 2012). Another limiting factor is the high concentration of Al, Mn, and Fe that interact with P and Se (Whitehead, 2000; McDowell, 1985). As the pH in the soil increases, the availability and absorption of Fe, Mn, Zn, Cu, and Co decreases, while Mo and Se increase in the forage (McDowell, 1985; Suttle, 2022). Vegetable crops maintain an active growth with a 4.0-8.5 pH; however, some are sensitive to extreme values (Whitehead, 2000). Therefore, pH determines the bioavailability of minerals and largely regulates the nutritional value of grasses (Toledo and Schultze-Kraf, 1982).

OM is important for crop sustainability: it improves the soil's physical (texture, structure, bulk density, and water retention capacity), chemical (nutrient availability, cation exchange capacity, reduced aluminum toxicity, and allelopathy), and biological (nitrogen-mineralizing bacteria, nitrogen fixation, mycorrhizal fungi, and microbial biomass) characteristics (Fageria, 2012). A high OM percentage can result in high Mo and S concentrations (Suttle, 2022) and low Cu availability for the plant (Haynes, 2005); conversely, a low OM percentage tends to cause low I concentrations (Suttle, 2022).

Soil productivity tends to fluctuate over time, due to changes in its physical, chemical, and biological fertility (Feng *et al.*, 2021). Both water scarcity and excess cause mineral deficiencies. In the first case, a decrease in the solubility of elements reduces their availability in root systems (Kawas and Houston, 1990). In the second condition, high humidity solubilizes and leaches out the minerals away from the perimeter of root absorption (Velasco, 1992). In the Huasteca Potosina, the intense use of natural resources by agricultural and livestock production systems and the climatic conditions suggests that, from the point of view of the nutritional requirements of the livestock, the soils and forage plants in the area have mineral deficiencies and imbalances. The objective was to determine the concentration of minerals in soil and forages of the Huasteca Potosina, during the dry and wet seasons.

## MATERIALS AND METHODS

### Characteristics of the Study Area

The study was conducted in the municipalities of Tamuín, Ébano, and San Vicente Tancuayalab, San Luis Potosí. The area is located in the province of Llanura Costera del Golfo Norte, subprovince of Llanuras and Lomeríos (Instituto Nacional de Estadística y Geografía (INEGI, 2002). The dominant landforms are alluvial plains with hills (57.8%), alluvial floodplains (19.3%), valleys with plains (7.3%), alluvial plains (6.5%), and typical hills (4%), among others. The altitude varies from 820 m (Abra-Tanchipa mountain range) to 5 m (alluvial plains) (INEGI, 2002). In the plains, valleys, and hills with gentle slopes the predominant soils are Pellic Vertisol (Vp), Calcaric Phaeozem (Hc), and Eutric Fluvisol (Je). Meanwhile, hills are characterized by Calcaric Regosol (Rc), Calcic Cambisol (Bk), and Haplic Kastanozem (Kh). Lithosol (I), Rendzina (E), and Calcaric Regosol (Rc) are found in rugged areas. The dominant climates are semi-warm humid (A)C(fm) (48.3%), warm sub-humid (A)(C)w<sub>0</sub> (44.6%), and temperate sub-humid Aw<sub>0</sub> (6.2%). The average annual precipitation ranges from 900 to 1,500 mm and the average annual temperature is  $25 \pm 1$  °C (INEGI, 2023). The most abundant vegetation types are secondary, forests, and low, medium, and high jungle. The main land uses are agriculture (32.8%), cultivated grassland (28%), and induced grassland (0.5%) (INEGI, 2021).

### Location of the Sampled PUs

The number of PUs to be sampled was determined through the stratification of the study area, using cartographic information at a 1:250,000 scale. These maps included pedological, geological, and physiographic information, as well as data about land use, vegetation, climate, and flood-prone areas (SEDARH, 2007). In addition to this cartography and fieldwork, the ArcGIS 9.2 geographic information systems were used, based on the resulting landscape units delimited in ArcView 2.3. Those units were based on the 1:250,000 cartography developed by INEGI (2007), which includes pedological, topographic, geomorphological, hydrological, and geological data, as well as information on land use, vegetation, and flood-prone areas. Seven PUs used to produce dual-purpose cattle were located in Tamuín, six in Ébano, and four in San Vicente Tancuayalab.

### Sample Collection and Preparation

Samples of the soil and plant species available to livestock were collected from each PU, in the dry (April and May) and wet (August and September) seasons. Soil samples were collected from the top 20 cm of each site and were divided into 10 subsamples. In total, 35 samples were collected in the dry season and 40 in the wet season. Forage plant species were sampled collecting 500 g of fresh matter, using the simulated grazing technique (hand plucking). The soil samples were air-dried, ground, and sieved with a 2 mm mesh. The forage samples were dried at 60 °C in a forced-air oven until they reached a constant weight; they were subsequently ground and passed through a 1 mm diameter sieve and stored in plastic containers, until they were analyzed in the laboratory.

### Laboratory Analysis

The soil was analyzed following the NOM-021-SEMARNAT-2000 official Mexican standard (SEMARNAT, 2002). The exchangeable bases (Ca, Mg, Na, and K) were determined with 1N ammonium acetate at pH 7.0 as a saturating solution, while the diethylenetriaminepentaacetic acid technique (DTPA) and the Olsen method were used to establish microminerals (Cu, Fe, Zn, and Mn) and phosphorus (P), respectively. The technical interpretation was based on NOM-021-SEMARNAT-2000 (SEMARNAT, 2002). Forage minerals were extracted according to Allan (1970). The P content was determined by colorimetry in a Thermo Scientific™ GENESYS™ UV-visible spectrophotometer (Fisher Scientific Inc.). The Ca, Mg, Cu, Fe, Zn, Mn, and Co content was determined by atomic absorption spectrophotometry (Perking Elmer 3110 spectrometer) and the Na and K content was calculated by flame photometry (Corning 410 flame photometer).

### Statistical Analysis

The mineral content of soil and forage was analyzed based on a completely randomized design, considering the effect of PU, season, and interactions as fixed effects, and applying an analysis of variance through the GLM procedure (SAS, 2021). The means were compared with Tukey's test.

## RESULTS AND DISCUSSION

The forage plant samples were collected during the dry season (50) and during the wet season (49). The predominant forage species in the area were *Cynodon nlemfuencis* (31.3%), *Brachiaria* spp. (14.1%), *Rhynchelytrum repens* (13.1%), and *Cynodon dactylon* (9.2%); the remaining 32.3% included, in varying rates, *Leucaena leucocephala*, *Panicum maximum*, *Pennisetum purpureum*, *Saccharum officinarum*, *Digitaria eriantha*, *Sorghum vulgare*, *Zea mays*, *Guazuma* sp., *Acacia* sp., and *Prosopis* sp. Additionally, three and five PUs used *Leucaena leucocephala* and *Saccharum* spp. as forage, respectively.

### Soil Mineral Content

Table 1 shows that the concentration of P (14.74 ppm) and Cu (2.87 ppm), in soils where the forage consumed by livestock grows, did not reach the minimum levels required for good plant development (Castellanos *et al.*, 2000). The availability of P in the soil-plant-animal system plays a fundamental role in its productivity, since P deficiency reduces forage growth and quality (Quintero and Boschetti, 2001). Furthermore, an  $\approx 12$  ppm P concentration in the soil improves phosphate fertilization (Benavidez *et al.*, 2000), while 25 ppm allow alfalfa crops to reach maximum yields (Vivas *et al.*, 1996; Berardo and Marino, 2000).

PU had an effect ( $P < 0.01$ ) on the K in the soil: K concentration ranged from 230 ppm (PU 15) to 836 ppm (PU 14). Likewise, the content of Mg, Fe, Zn, and Mn had  $P \leq 0.056$ , 0.053, 0.070, and 0.080 trends, respectively. Season had an impact on Fe and Mn: concentrations were higher in the wet season ( $P < 0.05$ ).

The interaction between PU and the season had an effect ( $P < 0.05$ ) on the Ca and Na concentration: higher Ca contents were found in PUs 6 and 14 during the dry season,



**Table 1.** Mineral concentration in the soils where the forage consumed by the dual-purpose livestock from 17 production units grows, in the Huasteca Potosina, Mexico.

Production units (PU)	Macrominerals (mg kg <sup>-1</sup> )					Microminerals (mg kg <sup>-1</sup> )			
	Ca	P	Mg	K	Na	Cu	Fe	Zn	Mn
1	8609 <sup>ab</sup>	19.29	458	424 <sup>bc</sup>	299 <sup>b</sup>	3.51	38.57	4.94	49.69
2	9599 <sup>ab</sup>	14.44	378	435 <sup>bc</sup>	243 <sup>b</sup>	1.43	22.75	3.29	25.72
3	9879 <sup>ab</sup>	5.30	313	557 <sup>abc</sup>	337 <sup>ab</sup>	3.18	30.14	5.49	33.32
4	8520 <sup>ab</sup>	34.79	464	383 <sup>bc</sup>	188 <sup>b</sup>	1.41	7.86	2.66	27.11
5	11096 <sup>a</sup>	24.24	398	610 <sup>ab</sup>	424 <sup>ab</sup>	2.85	15.20	2.26	31.81
6	10472 <sup>ab</sup>	8.93	485	528 <sup>abc</sup>	418 <sup>ab</sup>	3.66	28.98	2.94	31.36
7	11806 <sup>a</sup>	19.12	312	477 <sup>abc</sup>	317 <sup>b</sup>	1.71	17.75	1.80	24.89
8	10119 <sup>ab</sup>	12.38	531	679 <sup>ab</sup>	515 <sup>ab</sup>	3.85	14.00	2.85	29.05
9	10048 <sup>ab</sup>	15.95	253	387 <sup>bc</sup>	233 <sup>b</sup>	2.47	30.70	3.46	25.39
10	9574 <sup>ab</sup>	8.63	328	439 <sup>bc</sup>	280 <sup>b</sup>	2.60	19.34	2.32	17.24
11	10786 <sup>ab</sup>	27.70	436	540 <sup>abc</sup>	311 <sup>b</sup>	2.18	34.30	2.98	31.97
12	12223 <sup>a</sup>	12.15	436	553 <sup>abc</sup>	388 <sup>ab</sup>	2.17	18.58	2.25	26.98
13	10480 <sup>ab</sup>	26.64	383	568 <sup>abc</sup>	334 <sup>ab</sup>	5.28	37.34	4.61	20.42
14	10818 <sup>ab</sup>	13.51	782	836 <sup>a</sup>	692 <sup>a</sup>	2.18	15.35	2.67	29.14
15	6336 <sup>b</sup>	7.76	498	230 <sup>c</sup>	190 <sup>b</sup>	2.79	11.83	2.32	25.34
16	10776 <sup>ab</sup>	10.59	644	494 <sup>abc</sup>	519 <sup>ab</sup>	2.02	8.81	2.34	27.22
17	10743 <sup>ab</sup>	9.00	683	418 <sup>bc</sup>	262 <sup>b</sup>	4.18	25.73	3.10	48.24
SEM <sup>1</sup>	879	7.46	117	72	73	0.92	8.30	0.92	7.39
Season:									
Dry	9450 <sup>x</sup>	17.86	401	481	335	2.88	16.85 <sup>x</sup>	3.68	26.24 <sup>x</sup>
Wet	10860 <sup>y</sup>	12.12	471	516	356	2.86	30.54 <sup>y</sup>	2.91	33.95 <sup>y</sup>
SEM <sup>1</sup>	199	2.18	35	21	19	0.27	2.26	0.28	1.96
production units (PU)	0.001	0.205	0.056	0.001	0.001	0.153	0.053	0.070	0.080
Se	<0.001	0.081	0.092	0.517	0.884	0.979	0.001	0.084	0.046
PU*Se	0.006	0.481	0.511	0.260	0.036	0.440	0.816	0.868	0.114
means	10217	15.91	439	500	347	2.79	24.30	3.26	30.44
critical level <sup>2</sup>	1500	25	200	200	70	3.0	9.0	2.0	12.0

abc, xy=Mean values in the same column with different letters are different ( $P \leq 0.05$ ).

<sup>1</sup>SEM (EEM)=standard error of the mean. <sup>2</sup>Critical level=minimum critical level of minerals in soils required for plant growth (Castellanos *et al.*, 2000).

while the Na content was higher in PUs 1, 5, 6, 7, 8, 13, 14 and 16, during the dry season.

### Mineral Content in Forage

The diversity and predominance of the forages found in the study area did not enable the evaluation of the independent effect of the PUs. The P and K content was higher ( $P < 0.01$ ) in the wet season. Underwood (1999) reported higher P concentrations in the same season in northern Australia and southern Africa. Meanwhile, the higher K content in forages in the wet season differs from the findings of Almaráz *et al.* (2007). High Fe contents ( $P < 0.05$ )

in the dry season were also recorded by Domínguez-Vara and Huerta-Bravo (2008). The P, Mg, K, and Na content in forages does not meet the minimum requirements for livestock (Puls, 1994; NRC, 2001). Mn and Fe at soil level possibly reduced the absorption by the forages, hindering the quantification of Cu and Co (Ungerfeld, 1998) (Table 2).

Table 2 shows that only Ca and Mn had an adequate range, Fe level was in excess, and the rest of the minerals were below the appropriate concentration for dual-purpose cattle (Puls, 1994; NRC, 2001). The effect ( $P < 0.01$ ) of the interaction between PU and season on the Mn content in the forage was caused by its higher concentration in PUs 3, 5, 7, 8, and 16, during the dry season. The main forage species and their mineral profiles in the dry and wet seasons, were: *Cynodon nlemfluencis* (31.3%), *Brachiaria* spp. (14.1%), *Rhynchelytrum*

**Table 2.** Mineral concentration in the forage consumed by dual-purpose livestock from 17 production units in the Huasteca Potosina, Mexico.

Production units	Macrominerals (%)					Microminerals (ppm)		
	Ca	P	Mg	K	Na	Fe	Zn	Mn
1	0.65	0.08	0.06	0.17	0.10	142	28	35
2	0.14	0.06	0.03	0.13	0.07	201	20	25
3	0.13	0.07	0.04	0.10	0.06	151	17	33
4	0.35	0.07	0.05	0.13	0.06	125	26	21
5	0.20	0.06	0.06	0.14	0.07	89	31	36
6	0.30	0.07	0.05	0.12	0.09	163	29	20
7	0.24	0.61	0.03	0.07	0.05	82	17	30
8	0.42	0.04	0.04	0.07	0.08	138	16	27
9	0.27	0.06	0.03	0.15	0.08	196	25	28
10	0.23	0.05	0.03	0.16	0.09	115	22	33
11	0.29	0.07	0.04	0.18	0.10	91	29	40
12	0.24	0.06	0.03	0.15	0.10	177	22	32
13	0.26	0.11	0.03	0.15	0.12	163	27	62
14	0.39	0.11	0.06	0.17	0.15	177	20	44
15	0.44	0.15	0.08	0.28	0.17	61	34	77
16	0.38	0.07	0.05	0.14	0.07	92	19	35
17	0.26	0.04	0.03	0.14	0.07	119	18	22
SEM <sup>1</sup>	0.11	0.01	0.01	0.03	0.02	35	5	9
Season:								
Dry	0.33	0.06 <sup>y</sup>	0.05	0.13 <sup>y</sup>	0.08	149 <sup>x</sup>	26	30
Wet	0.27	0.07 <sup>x</sup>	0.04	0.17 <sup>x</sup>	0.09	114 <sup>y</sup>	20	36
SEM <sup>1</sup>	0.024	0.002	0.003	0.009	0.006	9	5	2
production units	0.090	0.058	0.256	0.210	0.064	0.160	0.679	0.072
Se	0.397	0.003	0.172	0.003	0.875	0.035	0.127	0.071
PU*Se	0.714	0.544	0.705	0.334	0.765	0.237	0.507	0.009
Average	0.30	0.07	0.04	0.14	0.08	131	23	33
Adequate concentration <sup>2</sup>	0.30-0.50	0.25-0.30	0.10-0.20	0.90-1.40	0.10-0.16	15-100	30-75	15-40

xy=Mean values in the same column with different letters are different ( $P \leq 0.05$ ).

<sup>1</sup>SEM (EEM)=standard error of the mean. <sup>2</sup>Adequate concentration in forage for livestock (Puls, 1994).

*repens* (13.1%), and *Cynodon dactylon* (9.1%). Other species were found in smaller quantities (32.3%), including *Leucaena leucocephala*, *Panicum maximum*, *Pennisetum purpureum*, *Saccharum officinarum*, *Digitaria eriantha*, *Sorghum vulgare*, *Zea mays*, *Guazuma* sp., *Acacia* sp., and *Prosopis* sp. Some of these forages are used to feed livestock in both seasons of the year (Table 3).

**Table 3.** Mineral concentration in the forages used to feed dual purpose cattle in the Huasteca Potosina, Mexico, in both seasons of the year.

Forage	Wet season									Dry season						
	%					mg kg <sup>-1</sup>				%					mg kg <sup>-1</sup>	
	Ca	P	Mg	K	Na	Fe	Zn	Mn	Ca	P	Mg	K	Na	Fe	Zn	Mn
Star grass ( <i>Cynodon nlemfluencis</i> )	n=17									n=14						
Average	0.22	0.08	0.03	0.18	0.09	152	20	43	0.23	0.06	0.04	0.13	0.09	155	22	27
Road grass ( <i>Rhynchelytrum repens</i> )	n=7									n=6						
Average	0.25	0.07	0.03	0.17	0.10	89	20	34	0.35	0.03	0.03	0.10	0.06	125	31	39
Brizantha ( <i>Brachiaria brizantha</i> )	n=6									n=8						
Average	0.08	0.07	0.03	0.14	0.08	152	31	24	0.32	0.06	0.06	0.14	0.08	129	32	29
Bermuda grass ( <i>Cynodon dactylon</i> )	n=6									n=3						
Average	0.27	0.09	0.04	0.22	0.12	102	21	61	0.19	0.02	0.02	0.12	0.06	212	21	22
Guinea grass ( <i>Panicum maximum</i> )	n=3									n=2						
Average	0.17	0.07	0.03	0.13	0.07	108	19	30	0.33	0.07	0.07	0.14	0.13	109	22	37
Sugar cane ( <i>Saccharum officinarum</i> )	n=1									n=4						
Average	0	0.03	0.05	0	0.02	33	8	15	0.38	0.06	0.05	0.14	0.11	117	26	23
Leucaena ( <i>Leucaena leucocephala</i> )	n=3									n=4						
Average	1.17	0.10	0.07	0.17	0.12	143	27	51	0.62	0.10	0.07	0.16	0.08	182	35	54
Mulatto grass ( <i>Brachiaria hibrido</i> )	n=1									n=2						
Average	0	0.04	0	0	0	73	10	18	0.32	0.06	0.06	0.11	0.07	145	40	45
Pangola grass ( <i>Digitaria eriantha</i> )	n=1									n=1						
Average	0	0.02	0.02	0.12	0.06	58	8	11	0.19	0.02	0	0.12	0.06	189	18	16
Sorghum ( <i>Sorghum vulgare</i> )	n=1									n=1						
Average	0.29	0.07	0.05	0.36	0.20	40	22	49	0	0.05	0.04	0.17	0.08	228	16	14

n=number of samples analyzed.

### CONCLUSIONS

The concentration of P and Cu in the soil was below the minimum critical level for adequate plant growth. The mineral concentrations in the forage were below the recommended requirements for livestock in both seasons of the year, except for Fe and Mn. Along with these mineral imbalances, the failure to meet the minimum requirements that grazing areas must have for dual purpose cattle in the Huasteca Potosina may affect livestock health and result in suboptimal production.

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# The Sociocultural Dimension of the Maize Value Chain in Chiapas, Mexico

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## ABSTRACT

**Objective:** To analyze the sociocultural dimension of the maize value chain in the Frailesca region, Chiapas, Mexico, in order to contribute to a comprehensive understanding of the system.

**Design/Methodology/Approach:** The research was exploratory and descriptive, combining quantitative and qualitative methods. The value chain and systems analysis approaches were integrated. Two types of information were used: primary data, through semi-structured interviews with actors in the production chain, and secondary data, through available official statistical information.

**Results:** The main results indicate that traditional management, product use, and technological modernity influence marketing channels in a non-linear manner. The production link is based on family labor; the transformation and consumption links are based on local food culture. The sociocultural factor is a determinant of the maize agroecosystem value chain in Chiapas.

**Study Limitations/Implications:** It is necessary to consider sociocultural aspects in the development programs for maize production and consumption in La Frailesca, with an approach that goes beyond value chain analysis and recognizes networks and production-consumption systems. Analyzing the value chain of agricultural product use and transformation from a scientific research perspective contributes to identifying critical limitations among actors and their relationships for the improvement of the agroecosystem.

**Findings/Conclusions:** The production link is based on family labor and small production systems with diverse potentials in the four municipalities studied. The transformation and consumption links are driven by local food culture, creating feedback that also affects the production sector. The maize value chain in the Frailesca region is characterized by encompassing family and local domains, with significant sociocultural influence, where the value addition flow is far from linear. Although not hegemonic, the sociocultural factor plays a predominant role in the maize agroecosystem value chain in La Frailesca.

**Keywords:** *Zea mays* L., ethnobotany, tradition, value chain, self-consumption.

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## INTRODUCTION

Globally, in 2022, approximately 206 million hectares of maize (*Zea mays* L.) were planted, producing about 1,160 million tons of grain (FAO, 2022). In Mexico, maize is the most important crop, not only as a staple food but also for its economic, social, and political significance. In terms of planted area, with 5.8 million hectares, it ranked 8<sup>th</sup> in the world, after the United States, China, and Brazil. In terms of production, approximately 26.5 million tons placed it 7<sup>th</sup>, following the United States, China, Brazil, Argentina, India, and Ukraine. This was the lowest production in four years, as in 2021 it was 27.5 million tons (FAO, 2022).

Mexican producers establish the maize agroecosystem across a wide range of altitudes and climatic variations, from sea level to 3,400 meters above sea level (CONABIO, 2020). Within this great variability of environments, indigenous and peasant farmers, through their knowledge and skill in crop management, have successfully adapted and maintained an extensive diversity of native maize varieties (González *et al.*, 2013; Coutiño *et al.*, 2015; Vázquez *et al.*, 2018; Guevara *et al.*, 2019).

The uses and values of communities extend across multiple aspects of Mexicans' survival strategies. Maize grain is used to prepare various foods, with tortillas and their variations being the most notable (Cadena *et al.*, 2012). The annual *per capita* consumption is 346.5 kg (SADER, 2022). Authors such as Barros (2009) and Esteva and Marielle (2003) have highlighted aspects of using the entire maize plant, including roots and stalks, which serve as fertilizer or fuel. The stubble is used as forage, the cane (stem) is used in house construction, pasture fences, and other parts of the plant are used to make figures, medicines, wrappings, fertilizer, fuel, and refreshing or alcoholic beverages.

The maize leaf serves as a wrapper for tamales, for making ritual or artisanal objects, and as containers. The corncob husk, or "olote," is used as fuel and animal feed, as a tool for husking corn cobs, polishing wood and pottery pieces, or as stoppers or lids for containers. Maize is also employed for medicinal purposes, curing various illnesses of the "body and soul," according to regional traditions.

In order for the diversity of maize products and by-products to reach consumers, they go through a series of value aggregation stages known as the Value Chain (Neven, 2015), which includes, basically, primary production, marketing, and consumption (USAID, 2019). Within this chain, social actors and economic agents coexist, each with diverse capacities to organize and coordinate the flow of added value until consumption (Sandoval, 2015).

The analysis of the value chain for the use and transformation of agricultural products, from a scientific research perspective, contributes to the task of identifying critical limitations in actors and their relationships, aimed at improving the system. This is verified by revitalizing distribution channels and developing producers' and transformers' vision through exploring new alternatives in the mobility of goods and services.

In La Frailesca, most research or technological and market development efforts have largely focused on maize agro-productivity (Hellin *et al.*, 2013; Caballero *et al.*, 2017; Delgado *et al.*, 2018; Guevara *et al.*, 2021). Meanwhile, aspects related to distribution, transformation, and consumption have not been sufficiently studied.

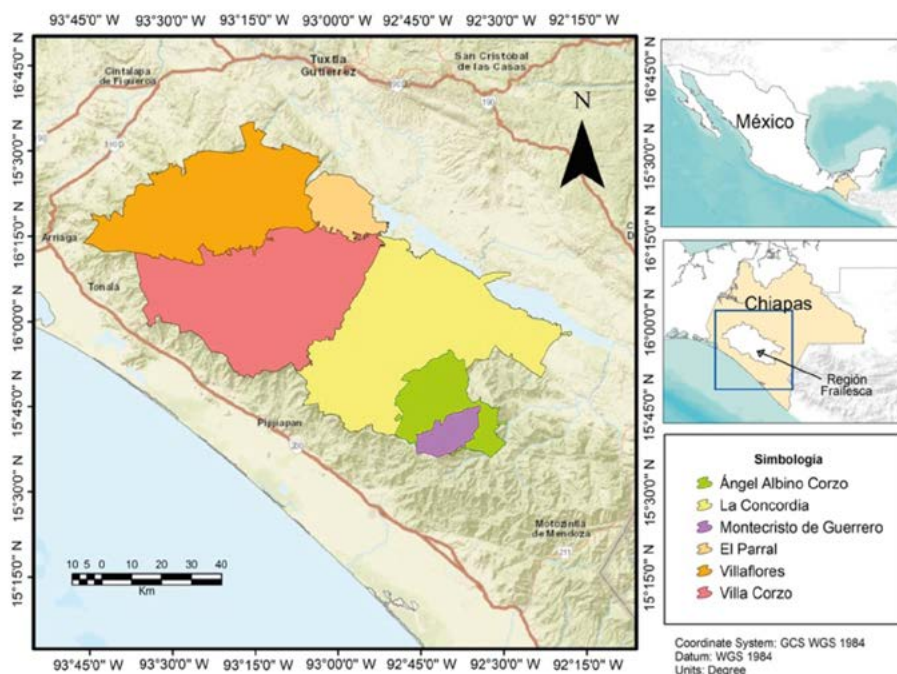


The Frailesca region is the second largest region by land area in Chiapas, comprising the municipalities of Villa Corzo, Villaflores, El Parral, Ángel Albino Corzo, Montecristo de Guerrero, Capitán Luis Ángel Vidal, and La Concordia. Its territory covers 798,023.9 hectares, representing 10.7% of the state's surface area. In 2022, 60,285 hectares were dedicated to maize cultivation, producing 30.3% of the state's volume and occupying 50.9% of the total planted area (SIAP, 2022; CEIEG 2022). Due to the importance of maize cultivation, Frailesca has been recognized as the 'granary of Chiapas', where farmers select varieties of maize, both native and hybrid, aiming for productivity and adaptability (Delgado *et al.*, 2018). Building upon the prior mentioned, the analysis focused on the sociocultural dimension within the productive relationships of the maize value chain links in the Frailesca region, aiming to thoroughly understand the system and identify factors influencing the behavior, trends, and configuration of its links.

## MATERIALS AND METHODS

The Frailesca region, Chiapas, is located in the Pacific Coastal Plain and the Central Depression of Chiapas (Figure 1); the main soil types are lithosol, regosol, and agrisol. The predominant climate is warm sub-humid, followed by a semi-warm humid climate, both with summer rainfall (CEIEG, 2022).

The research was conducted from January to December 2021 and geographically covered 36 localities across the municipalities of El Parral, Villaflores, Villa Corzo, and La Concordia. Data was obtained from surveys conducted with 292 producers, 202 transformers-traders, and 259 consumers within the maize value chain in Frailesca, focusing on municipalities known for maize production (INEGI, 2018).



**Figure 1.** La Frailesca region in the state of Chiapas and its municipalities.

The research was exploratory and descriptive, employing a combination of quantitative and qualitative methods. For its design and implementation, value chain and systems analysis approaches were integrated (López, 2009). In this case, all components of the maize value chain, from production to consumption, were considered.

In the analysis, both primary and secondary information was utilized. Primary information was gathered through the application of semi-structured interviews with stakeholders involved in the links of the production chain, predefined from the theoretical perspective of the value chain approach (Table 1).

The fieldwork for data collection was based on the methodologies of ethnobotanical exploration (Hernández X., 1985) and ethno-agronomy (Guevara-Hernández, 2007), including visits to communities to look for key informants. Secondary information was obtained from available official statistics, sourced from databases of SADER, INEGI, SIAP, and SIACON. The representation of chain components was developed through the perceptions of interviewees and their relationships with other actors involved in the production, distribution, transformation, and consumption of maize and its derivative products.

### Analysis of the information

Descriptive statistics and frequency analysis were used. A t-test was applied to determine the difference between acquisition prices via long or short value chains, and analysis of

**Table 1.** Variables used in the analysis of the maize value chain.

Link	Variable	Source of information
Primary producer	Annual production by municipality (t)	SIAP (2020) and INEGI (2020)
	Sample annual production (t)	Interview with the producer
	People you feed	Estimated according to Funes (2009)
	People he/she feeds	Estimated according to Funes (2009)
	Energy availability	Estimated according to Funes (2009);
	Production (t)	SIAP (2019)
	Maize yields ( $t\ ha^{-1}$ )	
	Production value (\$)	
	Type of labor	Interview with the producer
	Production destination	Interview with the producer
	Production scale	Interview with the producer
	Planted area, sample (ha)	Interview with the producer
	Area by municipalities (ha)	Source: SIAP, 2019.
	State surface area (ha)	SIAP (2020); INEGI (2020)
Local marketer/ transformer	Destination of national corn production in Mexico	SADER-SIAP (2019)
	Destination of local production	Interview
	Local corn products	Interview
	Unit production cost	Interview
	Selling price (one ton)	Interview
	Benefit cost ratio	Interview
Criteria for price formation.	Interview	
Consumers	Derived products consumed	Interview
	Frequency of consumption	Interview

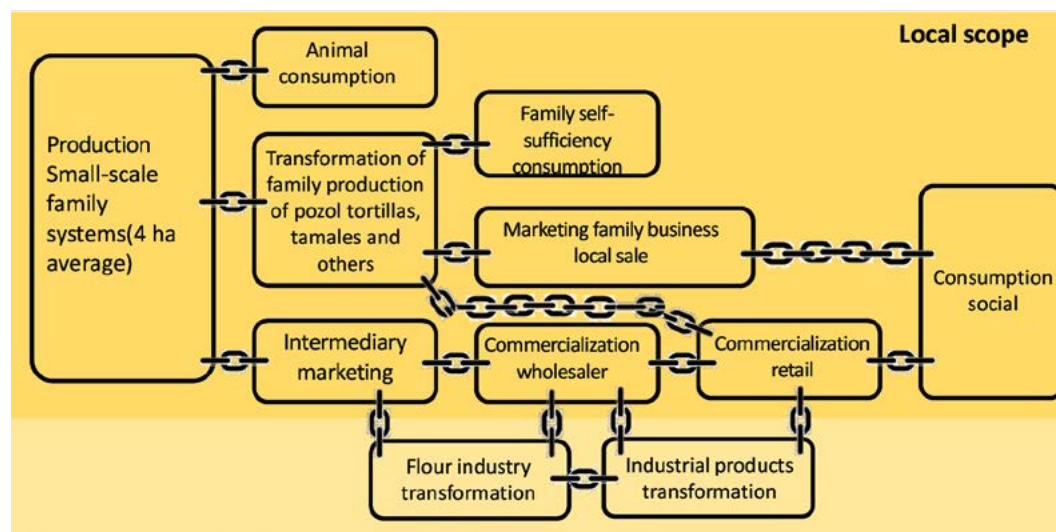
variance was conducted using a general linear model to analyze differences in cost-benefit relationships among different actors in the chain. Associations between categorical variables were analyzed using Simple Correspondence Analysis (Hoffman and Franke, 1986). The associated variables included: municipalities, type of labor, destination of production, forms of preparation, and frequency of maize consumption as a human food. To facilitate graphical interpretation of factorial dimensions, variable scores were adjusted with linear or quadratic vectors. STATISTICA<sup>®</sup> software (data analysis software system), version 8.0 (StatSoft, 2007), was used.

## RESULTS AND DISCUSSION

**Representation of the value chain.** In the Frailesca region, the maize value chain consists of the classic links: production, transformation, commercialization, and consumption (Casanova *et al.*, 2019 and Isaza, 2010). Based on farmers' perceptions, particularities were identified in the production link, highlighting family labor in various processes within the chain as a whole (Figure 2). Thus, production occurs on small plots (around four hectares), and a significant portion of maize and its by-products are used for self-sufficiency, including the raising of domestic animals. The grain is primarily marketed locally, either within the same community or municipal head, with the involvement of local and regional intermediaries.

The family and local nature of consumption foster interactions among the links of production, transformation, and consumption that are culturally defined by the uses and values attributed to maize by the community, in such a way that feedback is generated within the chain in the opposite direction of the value-added flow, leading to the co-evolution of its components.

In the context of agricultural development, the term “co-evolution” was coined by Norgaard (1994), Vara-Sánchez and Cuéllar-Padilla (2013), who described how co-



**Figure 2.** Representation of the maize value chain and its links and dynamic interactions in La Frailesca region (Source: own elaboration).

evolutionary processes have implications on biological resources, their natural and socio-economic environments. In the case of Mexico, the cultural mega-diversity is consistent with the genetic and usage diversity of the crop (Guevara *et al.*, 2019). In this case, the value chain serves as an analytical framework to understand the socio-economic environment of the crop.

In Frailesca, the boundaries of the agri-food system associated with maize are defined by the level of local consumption of the product. Therefore, the local culture not only sets guidelines for each added value but also influences agronomic practices in production and crop improvement.

The analysis of federal and state statistics allows us to understand that, based on maize cultivation, there is an energy availability of 277% for the inhabitants of La Frailesca. This can be interpreted as the fact that the region produces five times more maize than required to meet the energy needs of its population (Table 2).

The region meets local needs and exports maize to the rest of the state and country. However, not only the local culture may be dictating guidelines in the evolution of the value chain, as there is demand beyond the theoretical limits of the local agri-food system. In this regard, Casanova *et al.* (2019) emphasized the importance of context and demands on the performance of value chains.

The mentioned demand does not necessarily impose the same guidelines as those emerging from Frailesca and Chiapanecan culture. Thus, it is a value chain influenced by multiple cultural vectors. One of these is biocultural memory, and another could be modernity. These two vectors are not, in themselves, dichotomous categories. Instead, they intertwine to generate oscillating trends (Toledo and Barreras, 2008; Muradian *et al.*, 2012).

From the perspective of economic and social development research, different analytical lenses can be identified to influence these trends and prioritize one of these vectors (biocultural or modernist). In this regard, authors such as Muradian *et al.* (2012) emphasized two fundamental ones. On the one hand, economic and innovation approaches highlight the role of the market, mechanisms of income creation, and the distribution of benefits along the chain, as well as technological change and knowledge management. On the other hand, some approaches emphasize the social and cultural role in explaining the configuration of actors and the social construction of the meaning and attributes of products or use value.

**Table 2.** Analysis of maize production and theoretical self-sufficiency capacity in La Frailesca region, Chiapas.

Ambit	Surface <sup>(1)</sup> (ha)	Annual production <sup>(1)</sup> (t)	People you feed <sup>(2)</sup>	People that feeds/ha	People <sup>(1)</sup>	Energy availability (%)
State	798,023.9	353,585.65	1,269,511	1.59	5,543,828	23%
La Frailesca	60,285	227,261.70	815,958	13.54	294,812	277%
Coverage <sup>(3)</sup>	7.55%	64%			5%	

<sup>(1)</sup> Sources: SIAP (2022) and INEGI (2021).

<sup>(2)</sup> Own calculation based on an energy requirement per person of 1022 Mcal/year; energy contribution of maize (3,300 kcal/kg dry weight). According to Funes-Monzote's proposal (2009) for energy analyses.

<sup>(3)</sup> Proportion of La Frailesca region relative to the state indicator.

Use value is a concept derived from Adam Smith's theory of value, which states that, each good or service has the primary characteristic of satisfying a specific need (Economipedia, 2020). When applied to value chains, this concept helps to unravel the underlying relational models (Valdés, 2017). These models are based on frameworks of motivations or modes of justification assumed by the involved parties. Muradian *et al.* (2012) described these relationships as grammars of value or regimes of justification. Justifications explain how social links evolve within a production network (Cabrera, 2018).

A study related to value chains (Fair Trade Coffee; Raynolds, 2009) identified at least three analytical models: a) one that incorporates considerations for local development as part of business practices, b) another that adopts quality as the main driving force of relationships, and c) the third that is founded on the interest in traceability as a strategy to meet new hygienic-sanitary quality control requirements of markets. However, in the case of the maize agroecosystem value chain in the Frailesca region, these models do not seem sufficient to understand the relationships and frameworks of motivations mentioned. "Comercio Justo" (Fair Trade) is a socio-economic innovation that challenges modernity, while the case of Frailesca maize follows more traditional channels, such as bartering.

Thus, this discussion highlights the biocultural vector to understand the relationships between actors in value chains for this case study. Toledo and Barreras (2008) noted that societies, like individuals, have memory. This attribute allows them to remember past events, and when there is an intersection between the biological and the cultural, biocultural memory results, which is a form of social memory, whose essence is traditional knowledge. These same authors placed Mexico among the top ten countries with the greatest biocultural wealth. This assertion is based on the country's biological, linguistic, and agricultural diversity, as well as the prevalence of indigenous, peasant, and rural populations. Regarding biological diversity, the nation ranks fourth among megadiverse countries (Sierra *et al.*, 2014) and fifth in linguistic diversity (Hernández and Maya, 2016).

On the other hand, the modernity vector corresponds to a sort of ideology of progress and development, which considers what it calls 'pre-modern knowledge' to be insufficient. This ideology confers upon itself a universal and totalitarian reach (Morin, 2016). Under this premise, traditional culture is often commodified by attempting to reflect in 'values' and quantify the qualitative aspects behind sociocultural dimensions, which would be termed exchange value (Economipedia, 2020).

From the relationship between both vectors, the trends of the maize agri-food chains and the co-evolutionary dynamics of their links in the Frailesca region emerge. Biocultural memory tends to favor family consumption and direct channels between producers and consumers, while the modernity vector encourages the participation of intermediaries, the milling industry, and exports.

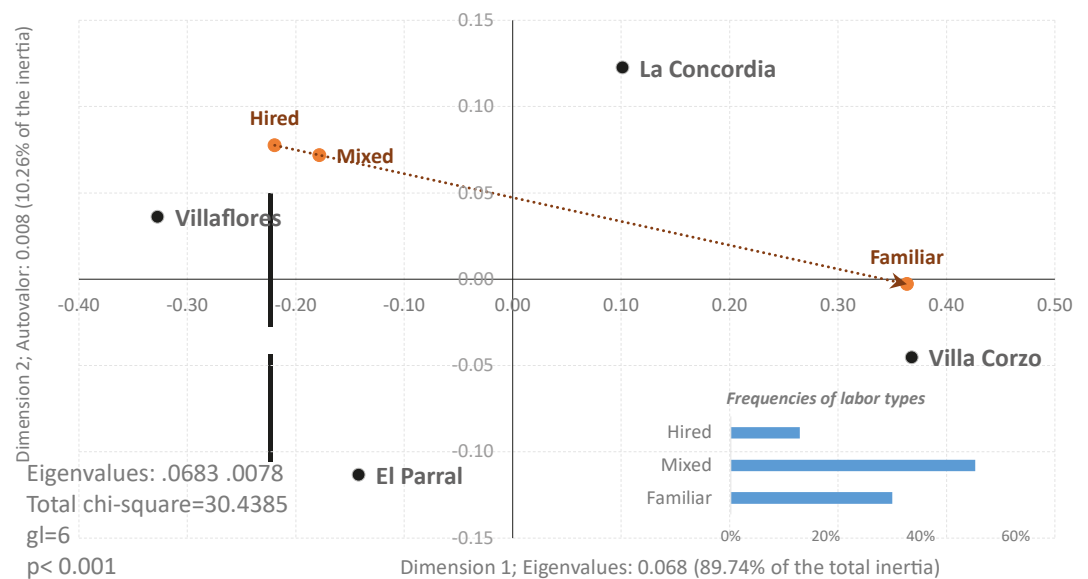
Not necessarily are both vectors at odds. Rather, they are sources of dialogue and enriching interactions in the processes (Solleiro *et al.*, 2014). The traditional knowledge that supports biocultural memory is a living, resilient entity that reconfigures itself through praxis in continuous learning cycles (La O *et al.*, 2018). Therefore, it is ready to co-evolve with the value chain itself.

In terms of the productive link, the potential varies among the municipalities of the region. Villaflores, La Concordia, and Villa Corzo stand out for their production and yield (Table 3), where agriculture is the main economic activity, as more than 50% of the economically active population is directly involved in this primary sector (SIAP, 2018).

In the Frailesca region, three types of labor are prominent in maize production: hired, family, and mixed (Figure 3). Overall, family and mixed labor predominates. Mixed labor refers to family labor supplemented by temporary external hires for specific tasks like planting and harvesting. The figure shows that hired labor, though less frequent, is significantly associated ( $p \leq 0.0001$ ) with the municipalities of Villaflores, La Concordia, and El Parral, falling within the general range between family and mixed labor, whereas Villa Corzo is more strongly associated ( $p \leq 0.0001$ ) with family labor. This suggests that production in Villaflores is somewhat more oriented towards sales, possibly due to larger production units.

**Table 3.** Maize production and income in the municipalities of La Frailesca region of Chiapas. Source: SIAP, 2022.

Municipality	Sown area (ha)	Production (t)	Yield ( $t\ ha^{-1}$ )	Production value (\$ Mexican Pesos)
Captain Luis Angel Vidal	1,132	1,634.38	1.44	6,696.22
El Parral	2,611	9,623.76	3.69	49,139.54
Angel Albino Corzo	3,802	10,151.34	2.67	53,746.88
La Concordia	18,895	75,580.00	4.00	397,773.76
Monte Cristo de Guerrero	880	2,367.20	2.69	12,503.05
Villaflores	22,531	90,004.32	3.99	475,839.09
Villa Corzo	10,434	38,261.70	3.67	193,001.91



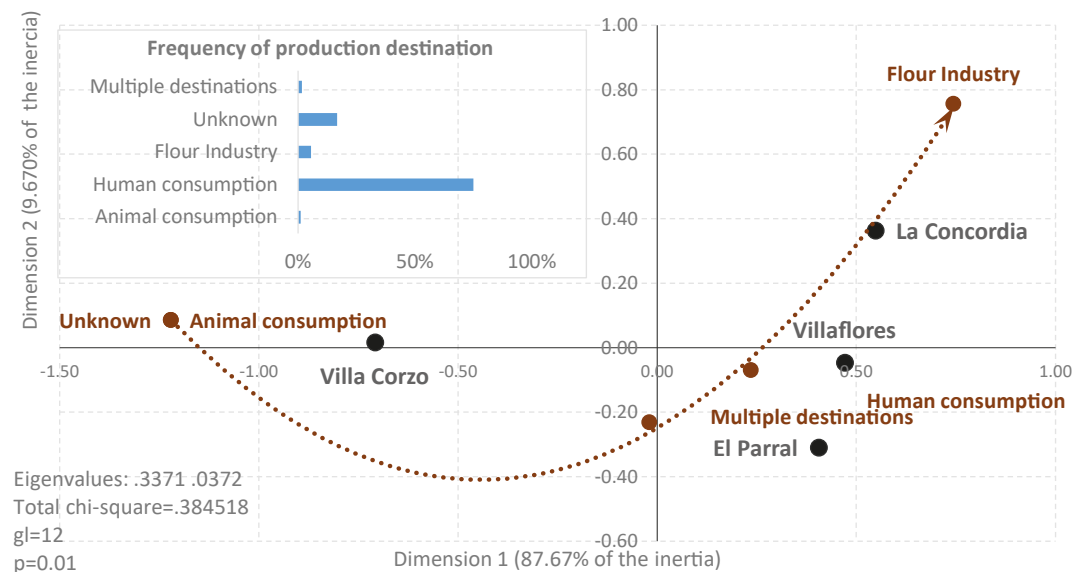
**Figure 3.** Type of labor involved in maize production in La Frailesca region. Relationships of municipalities with the type of labor vector, through simple correspondence analysis.

Family labor transforms maize cultivation into a space for the construction and socialization of traditional knowledge. It is called construction because collective productive activity is a source of continuous experiential learning cycles that influence the knowledge, beliefs, and skills of the group of people involved. Each experience is given meaning through the mental filters of culture, which can modify the practice itself to generate new cycles where learning is consolidated or events are “reinterpreted” (Pérez *et al.*, 2015).

In other words, the process is understood as socialization because it involves a social construction, wherein tacitly the entire preceding set of beliefs, knowledge, and skills is made available to those involved. According to Toledo and Barreras (2008), this entails the combination of kosmos (system of beliefs) –corpus (system of knowledge), which give meaning to a praxis (system of practices). This is the concrete mechanism through which the productive link of the value chain begins to receive cultural feedback, an effect that manifests in the reverse direction of the value flow within the chain, influenced by the patterns set by consumer culture.

In the value chain of the maize agroecosystem in the study area, various destinations for the grain were identified, but human consumption dominated in all municipalities (Figure 4). The milling industry was particularly present in La Concordia, while animal consumption was evident in Villa Corzo. Throughout the commercialization process, sales through intermediaries predominated.

Product destinations, in this case maize during the period 2015-2019, are indicators of the balance among channels of the agri-food chain (Table 4). According to national statistics, just over a third of the production flows through the channels of the milling industry at the national level. This turned out to be the main destination for the grain; however, livestock consumption and self-supply are also important destinations, which run through family consumption channels, together representing around 70%.



**Figure 4.** Destination of maize production in La Frailesca. Relationships of municipalities with the destination vector, through simple correspondence analysis.

**Table 4.** Destination of national maize production in Mexico, during the period 2015-2023.

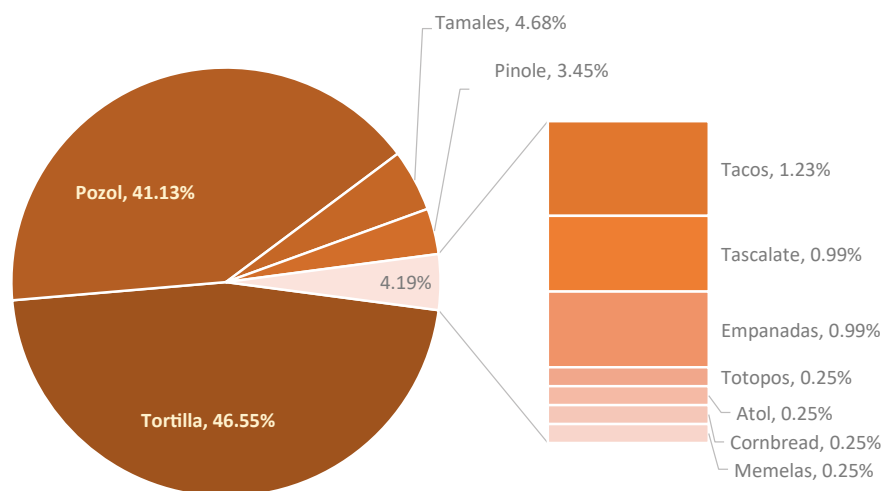
Year	Offer (t)	Production (t)	Grain consumption		
			Flour mill (%)	Livestock (%)	Human (%)
2015	1,647	22,255	21.18	18.12	52.73
2016	1,843	22,335	18.02	18.75	52.37
2017	1,528	24,468	20.11	18.26	50.89
2018	2,106	24,384	20.72	19.23	51.68
2019	2,485	25,433	21.24	19.46	51.80
2020	3,296	23,575	19.48	19.97	52.65
2021	1,765	24,294	20.70	18.17	54.55
2022	2,159	24,564	20.69	18.11	54.55
2023	1,915	23,115	21.32	17.44	56.01

Source: f <. Data presented in thousands of tons (t).

Source: SADER-SIAP (2019 and 2023). Data presented in thousands of tons (t).

The local transformation-commercialization channel was the most relevant in the value aggregation flow, highlighting products such as tortilla, pozol, and tamal (Figure 5). This channel has three pathways: direct chain, short chain, and long chain. In this order, the number of participating actors increases. The increase in the number of actors, ‘links’ in a chain, leads to greater specialization and more extensive biophysical circuits (Ferrer *et al.* 2020). In this case, it is observed that locally produced maize ‘returns’ after passing through the milling industry and other local transformation processes that generate derived products and by-products, adding a significant burden of mobilization or transportation costs to the transformation expenditure structure.

The direct chain represents 45% of the transformer-commercializers studied. These are generally primary maize-producing families that reach consumers with locally or traditionally transformed products. This responds to survival strategies, as maize production



**Figure 5.** Main local products derived from maize transformation in La Frailesca.

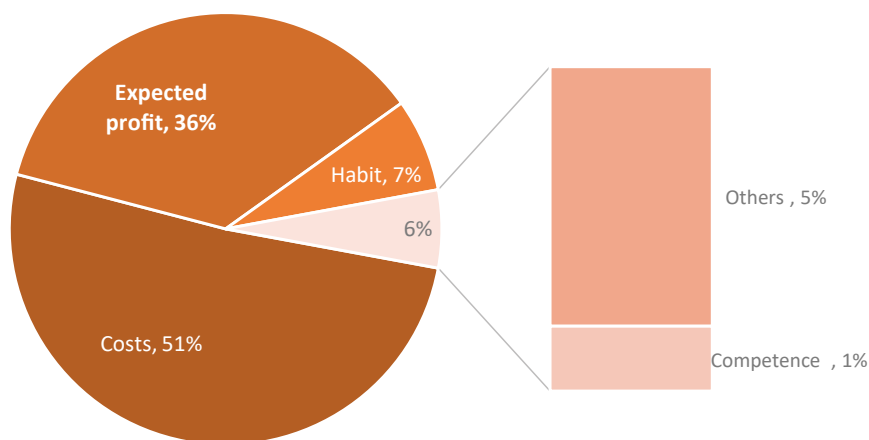


occurs under rain-fed conditions and only occupies one-third of the year. The producer-transformer has a competitive advantage over others because they self-supply their own raw material while stabilizing their income. The transformation and commercialization links generate a better cost-benefit relationship than primary production (Table 5). In this sense, products like pozol and tortilla stand out with statistically significant differences ( $p < 0.001$ ) compared to primary production. This is another positive effect for the producer that extends to these links (Analuisa *et al.*, 2022).

The short chain represents 40% of the sample. These are local transformer-commercializers who buy directly from producers. The long chain represents 15% of the sample and includes intermediaries, industry players, and wholesale or retail marketers. The long chain involves an increase of \$1.89 Mexican pesos in the retail price of maize, rising from \$6.12 to \$8.10 Mexican pesos per kg (significantly higher,  $p < 0.001$ , when applying the *Student's t*-test,  $df = 98$ ).

The local price of maize is relevant because input costs are the primary criterion for setting prices of products or by-products derived from maize by transformer actors, accounting for 51% (Figure 6). The second criterion is expected profit. This latter criterion is more complex as it includes production costs plus an expectation of the profit margin to be achieved (Nahuel and Padilla 2017).

Understanding consumption patterns was relevant as it was found that the main daily forms of maize consumption are as tortillas and pozol, the latter being a beverage



**Figure 6.** Criteria for price determination in local products derived from maize in La Frailesca. Source: Authors' elaboration based on the study sample.

**Table 5.** Profitability and distribution of economic benefits among primary maize producers and maize transformers.

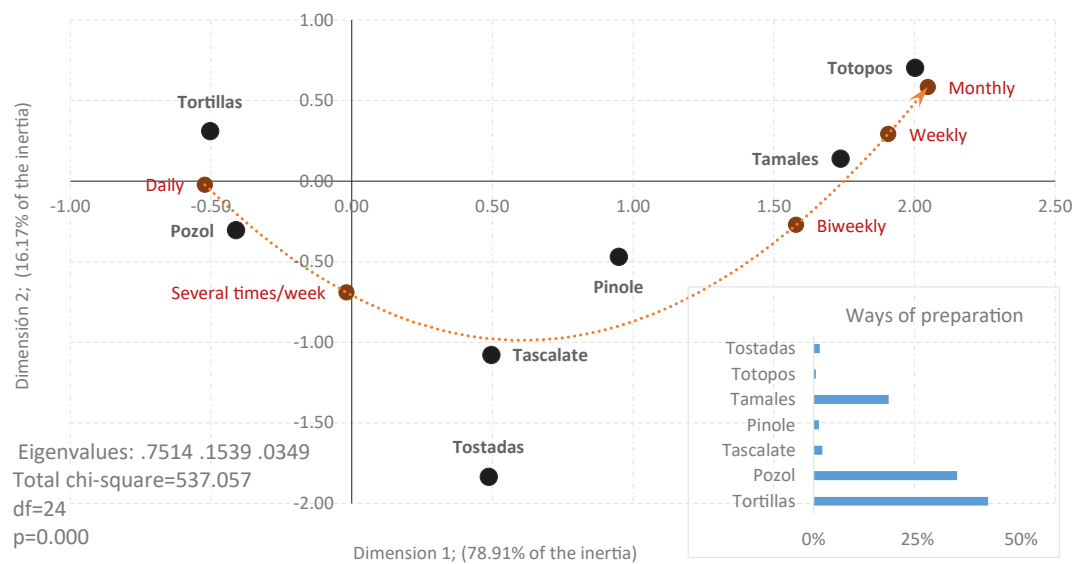
Variable	Producers		Local marketing transformers					
	Maize (t)		Tortilla (kg)		Pozol (450 ml)		Tamales (unit)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Unit production cost (per ton)	\$2,481.82	230.40	\$ 5.33	0.86	\$4.57	0.76	\$3.70	0.82
Sales price (per ton)	\$4,273.37	277.02	\$11.54	1.12	\$10.26	1.06	\$6.95	0.75
Benefit cost ratio (Sig. $p < 0.001$ )	1.73 <sup>c</sup>	0.14	2.20 <sup>ab</sup>	0.25	2.27 <sup>a</sup>	0.23	1.94 <sup>bc</sup>	0.30

made from maize dough and ground cocoa, among other ingredients (Ordoñez, 2017). Additionally, maize is consumed in tamales once a week or every fifteen days, and irregularly as pinole (derived from the Nahuatl word pinolli, a pre-Hispanic food made from toasted maize flour sweetened with sugar or piloncillo); or as tascalate (also known as taxcalate), a typical beverage from Chiapas made with toasted maize, cocoa beans, annatto, sugar, and cinnamon. All these ingredients are ground to create a powder, which when mixed with cold water or milk, results in a refreshing drink (Ordoñez, 2017). Finally, maize is also consumed in the form of fried or baked tortillas, called tostadas and totopos (Figure 7).

The transformation-consumption pattern, represented in Figure 5, defines a shared habit that transcends time and prevails despite modernity’s new trends. This is interpreted as part of traditional food culture and is one of the aspects that characterize Mexico as one of the world’s most important centers of biocultural memory (López and García, 2017).

The transformation of the mentioned products is carried out in an artisanal and semi-mechanized manner. For this purpose, utensils commonly found in households are used, such as: pots (painas), manual or electric mills, tin spoons, presses, metal griddles (comales), molds, wood-fired stoves, and to a lesser extent, gas stoves. It is confirmed that maize is a raw material with multiple uses, where nixtamalization forms the cultural basis for grain transformation for food. This process involves placing maize grains in hot water with a bit of lime, allowing it to soak for several hours. Finally, the liquid is drained, and the remaining solids are ground to obtain maize dough (Perales, 2012).

Paredes *et al.* (2009) pointed out that when nixtamalized maize is ground, it loses its structure because the components of the grain have been modified. The resulting dough from grinding consists of fragments of the germ, residues of the pericarp, and endosperm



**Figure 7.** Traditional forms of maize food use in La Frailesca region. Relationships with the frequency consumption vector through simple correspondence factorial analysis.

held together by partially gelatinized starch, and emulsified proteins and lipids. Maize is deficient in the amino acids lysine and tryptophan; however, through this process, the availability of most essential amino acids is increased, which undoubtedly represents one of the greatest contributions of Mesoamerican biocultural memory to the world.

In this way, although the term ‘chain’ suggests a flow that links connections, it is actually a value aggregation flow that is far from being linear, like in a literal chain. Taking into account the above, García *et al.* (2009) compare it to a web of relationships, where actors with high organizational power and influence could dominate processes and impose their influence over the less powerful. Moreover, it implies that there are relationships in multiple directions, channels, and dimensions.

The chain approach can also provide a methodological framework for regional development management and the design of public policies (Pietrobelli and Staritz, 2017; García *et al.*, 2009). In other words, it demonstrates that such an approach is an additional tool to enable multi-actor processes, as mapping chains necessarily requires identifying and interacting with stakeholders and allows for gathering relevant information about the relationships established among them. This involves an inclusive process of citizen management that requires building awareness regarding these relationships.

## CONCLUSIONS

Cultural feedback have been described, guided by consumption traditions in the Frailesca region, where the influence of multiple trends in this area is recognized. Tradition (referred to in the study as biocultural memory) and modernity are lines of thought that constantly interact for the functioning and balance of marketing channels (lightly addressed in the study). The production link is based on family labor and small-scale production systems with diverse potentials in the four municipalities studied. Transformation and consumption links are guided by local food culture and produce feedback that also impacts the productive sector.

The maize value chain in the Frailesca region is characterized by encompassing the familial and local realms, with significant sociocultural influence, where the value aggregation flow is far from linear. While not hegemonic, the sociocultural factor plays a predominant role in the maize agroecosystem value chain of Frailesca.

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