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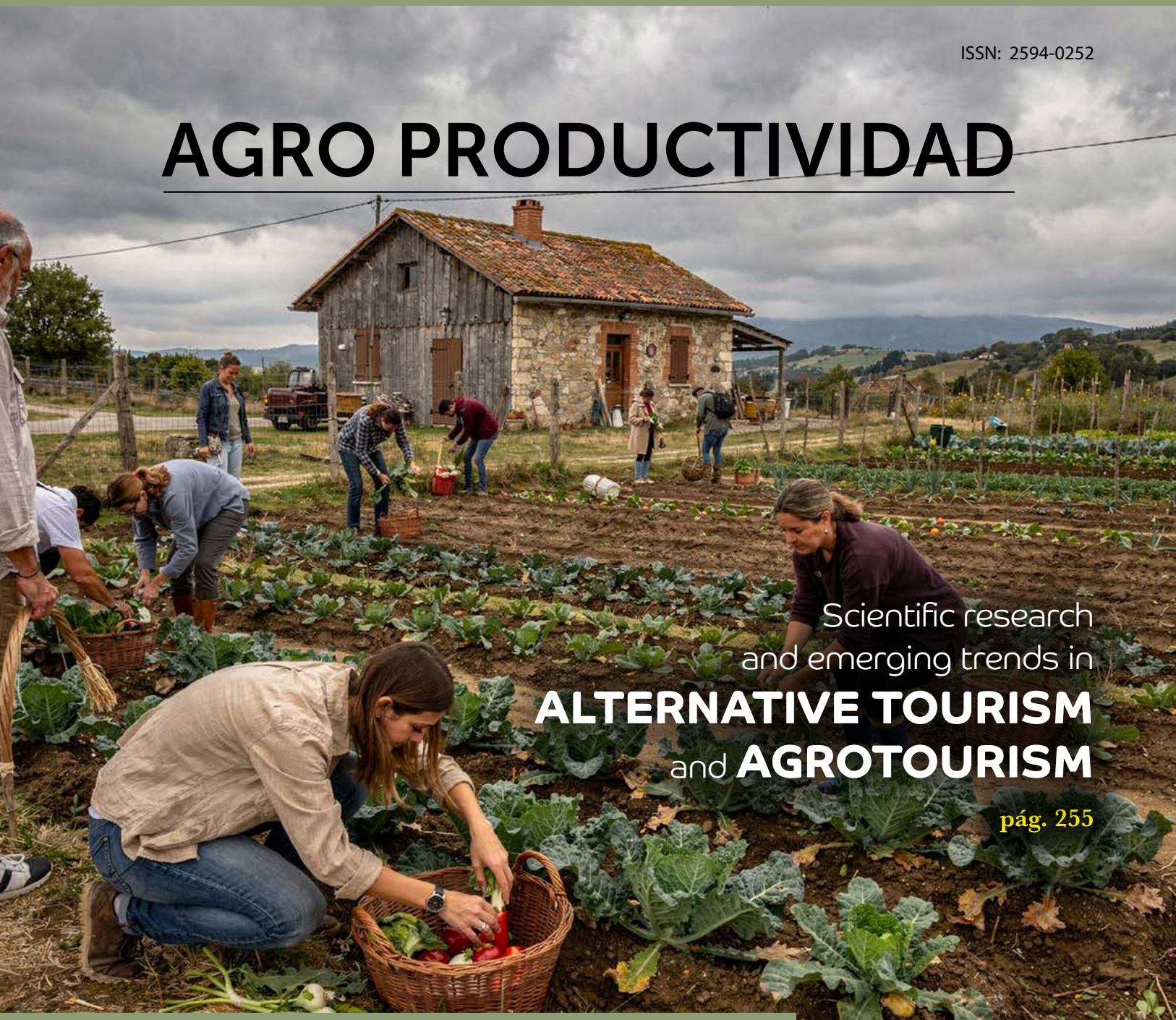

Scientific research and emerging trends in ALTERNATIVE TOURISM and AGROTOURISM

pág. 255

Año 18 • Volumen 18 • Número 12 • diciembre, 2025

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Contenido

Año 18 • Volumen 18 • Número 12 • diciembre, 2025

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The role of strategy in organizational performance: a case study of an avocado producer organization

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ABSTRACT

Objective: to conduct an internal and external diagnosis of groups of avocado producers —which for reasons of confidentiality are hereinafter referred to collectively as The Organization— in order to identify problems and opportunities through a problem tree, to determine their type of strategy designed, its implementation and its consequences on organizational performance.

Design/methodology/approach: problems were identified through 25 semi-structured interviews with the actors involved in the value network and the analysis of the environment was performed. The prevailing strategy type was determined and an ERRC matrix [Eliminate-Reduce-Raise-Create] was created to reformulate a new strategy.

Results: a value network analysis of The Organization highlighted the relevance of non-partner suppliers, almost as relevant as partners. We found The Organization has two sales channels of similar importance for avocado sales, the fresh market and processed avocado; the latter has registered an eight-fold growth plus in the period 2013-2022. Despite this, a vision prevails that bets on the first channel despite lacking the capabilities to position that market with an advantage, in addition to the intense competition that exists among 97 packing houses. In order to leave a red ocean, The Organization should leverage through their experience and strengths by designing a value-added product for Mexico's national market.

Limitations/implications of the study: the implementation of a new line of business with a differentiated product would allow The Organization to participate in a blue ocean.

Findings/conclusions: the people who lead The Organization do not systematically implement the analysis of their environment in order to formulate a strategy that considers internal opportunities and capabilities.

Keywords: strategy, processed avocado, added value.

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INTRODUCTION

Strategy is a concept widely used in various disciplines, but when it is used excessively, its meaning can be lost. Pérez and Massoni (2009) proposed a new strategic theory from communication to be applied in the different areas of human activity. This theory is human-centered and based on the qualities that come from our strategic and relational capacities, as well as on the recognition of our differential plurality, both cultural and experimental.

The theory is proactive, it seeks to make better decisions in the face of uncertainty. That is, in the face of the conflicts and opportunities of a changing environment, as a legacy of the scientific revolution of the 20th century.

Freedman (2016) agreed with this proposal when he stated that using strategy means "to have the ability to observe the world and analyze it in the short term, also the ability to foresee the long-term consequences and, most importantly, to identify the causes rather than the symptoms." In addition, he stated that people are not natural strategists, it takes a deliberate and thoughtful effort to achieve strategy. Porter (2011) mentioned that the challenge, either to develop a strategy or to reconstruct it clearly, is organizational and depends on leadership. It is essential to have leaders capable of making decisions, because strategy is the essence of general management. Defining and communicating the company's unique position through key activities, such as choosing what not to do (trade-offs), is just as important as choosing what will be done; in addition to integrating value-creating activities.

In this regard, Kim & Mauborgne (2005) stated that when a company decides to compete in an existing market space, in order to try to beat the competition, it opts for a red ocean strategy. Whereas, if you choose to create a space by making the competition lose relevance, you opt for a blue ocean strategy. With this in mind, the issue raised was addressed with a case study of a 70-year-old avocado producer organization located in Michoacán, a Mexican state which is global leader in avocado production. According to Statista (2022), the value of this industry in 2021 was 13.97 billion USD dollars and is projected to reach 26.04 (billion USD) by 2030.

Mexico is the center of origin of avocados and the leading producer in the world, 2.9 million tons (Megagrams, Mg) obtained in 2023 (SIAP, 2024). It is also the leading exporter with 1.3 million Mg shipped to the United States (US), which is the most important trading partner, 89% of its avocado imports came from Mexico in 2023 according to Hass Avocado Board (HAB, n/d.). Also, according to the Bank of Mexico, the value of Mexican exports was 3.153 billion dollars that year.

In this case study, The Organization analyzed faces the challenge of aging and loss of its corporate base. The objective of the research was to carry out an internal diagnosis, through the value network, and external, through the analysis of the environment, to identify the central problem, its effects and causes, to determine the type of strategy (design and implementation) and the consequences on organizational performance.

MATERIALS AND METHODS

The information was collected between 2023 and 2024. Interviews with 25 key actors in the value network were conducted through semi-structured questionnaires. Databases on avocado deliveries were analyzed with descriptive statistics in Microsoft™ Excel®. The analysis of those involved was done under the value network approach (Nalebuff & Brandenburger, 1996) to know the weaknesses and strengths of The Organization. The analysis of the environment, proposed by Osterwalder & Pigneur (2011), was performed to have an external diagnosis of The Organization, that is, to identify its opportunities and threats. To this end, databases from FAO (2024), Agronometrics (2024) and HAB (n/d.)

were consulted to assess Mexico's position in the production and marketing of avocados worldwide, and in a trade relationship with the US.

The determination of the core problems of The Organization was accomplished by design-thinking (Brown, 2008) *i.e.* the appreciative inquiry of perceived problems (Cooperrider *et al.*, 2008). Also, through the problem tree (Muñoz Rodríguez, 2010), that explores the perceptions regarding problems of the actors involved, by performing an analysis of the environment. Finally, the situation of the strategy in The Organization was determined by creating an ERRC matrix [Eliminate-Reduce-Raise-Create] (Kim & Mauborgne, 2005).

RESULTS AND DISCUSSION

Value network of The Organization

The Organization is made up of 188 members; it is dedicated to the collection and sales of avocados to the national and international markets, as well as the processing of the fruit and its marketing to the Netherlands. Total supply area is 530 ha with a 10 Mg ha^{-1} average yield in the orchards.

In 2022 the organization recollected 5477 Mg of avocado; 52% from 145 members and 48% from 81 non-members (business associates). Of the total collected, 80% was Hass variety avocado for fresh sale, the rest included Hass for processing, organic Hass and creole Hass. Half of the total sales (260 million MXN) corresponded to the fresh market and the other half to the processed market. In the fresh avocado line, 85% was sold to Mexico's domestic market and 15% to the international market; whereas in the processing channel, practically everything was sent to the client and complement associate to the Netherlands (Figure 1).

That latter client has shared multiple benefits with The Organization, including training in the development of products with high added value, flexible and stable payments, favorable commercial agreements and the promotion of obtaining certifications. In addition, the volume of avocado processed to that customer grew considerably, from 160 Mg in 2013 to 1580 Mg in 2022.

Figure 1. The value network of The Organization (Nalebuff & Brandenburger, 1996). Data source: SENASICA (2024) and The Organization. [z]ASIAM – Association of Avocado Industrializers of Mexico A.C.; y Miscellaneous Certifications include SMETA, Kosher, Halal, BRCGS, PrimusGFS, Metrocert, GLOBALG.A.P. GRASP, and SRRC. [x]FIRA – Trusts Instituted in Relation to Agriculture.

Defining the Problem/Opportunity

To better understanding each of the problems perceived by the actors and located at the level of effects in the problem tree, a description of each one is included.

i) There is a perception about "*the members sell the good fruit to outsiders and the bad fruit to The Organization*";
ii) Overprotection of partners, by paying for quality not produced nor delivered. From August 2021 to April 2022, it was estimated that the leakage of avocado produced by the partners and not delivered to The Organization reached 46% of what was produced. Considering that the price paid for fresh avocado to non-members was 36% higher compared to the price paid to members, it is then presumed that partners delivered the best quality fruit to other companies. Likewise, 52% of this fruit acquired from partners was finally reclassified and processed, however, they were paid MXN$ 2 per kg more than the established price. This is evidence of preferential treatment to the partner by the Organization, which complies to demands for a higher price, due to fear of losing more members;
iii) Purchase prices are not attractive for members with fruit that has US-exportation quality; although the estimated harvest of US-certified fruit in the period described above was 731 Mg, only 68 Mg were exported, thus only 9.3% of the avocado with that quality was used for what was intended. This means that The Organization stops serving the market with the most lucrative prices, MXN$ 68 per kg in 2022, versus the client with the second-best price (MXN$ 58 per kg);
iv) Wasted high-value assets; just the membership paid to the Association of Avocado Producers and Packers Exporters of Mexico – APEAM to export fruit to the United States did cost 7 MMXN (millions of Mexican pesos). Moreover, a maximum of 57% of the installed capacity of the packaging is used, plus the 67% of the processor unit;
v) Perception of immobility in the leading managers; it was documented that, in approximately 30 years, almost half of The Organization's span, only two families have held the position of presidents of the board in The Organization.

All the above translates into the decrease and aging of the Organization's corporate base, with a decrease of 52% in 33 years (1990-2022). From the detailed analysis done for the period 2015-2022, it was found that 75% of the separations of members were due to voluntary resignation or expulsion, with a net loss of 53 members in just eight years. This decline has resulted in the aging of the current membership in the face of little generational renewal. Thus, according to INEGI (2019), generational change does not occur in Mexican agriculture, since 46% of the heads of rural economic units in Mexico are over 61 years-old. In The Organization under study, this situation is even more serious, with 59% of members over 61 years-old.

The causal reasons for the perceived problems, located at the root level in the problem tree, are described below.

i) Dominance of a vision according to which the present and future of The Organization is in the fresh market, which is increasingly competitive. According to FAO (2024) world avocado production was 716 353 Mg in 1965, compared to 8.6 million Mg in 2020, which represents an increase of 1112% in 55 years. Although Mexico also experienced significant growth, its share of trade in the Americas has declined from 45% to 28%, and in the world from 49% to 40% during the same period (Figure 2).

This reduction is linked to strong competition with other producing countries. In the third quarter in 2020, 79% of US imports came from Mexico; but, in the same quarter in 2022, US imports from Mexico were only 69%. Peru increased its share from 18% to 27% (HAB, n/d.). Also, Imbert (2021) noted the trend of a production larger than the demand for the fruit; he projected a gap of 100 000 Mg in 2021, which could widen to 150 000 Mg in 2024. It should be observed, however, that at the end of 2023 this scenario had not occurred. Since in the US, prices by origin ranged between USD 2 and USD 4 from 2014 to 2023 (Agronometrics, 2024).

ii) Mexico's national market for fresh avocado is poorly institutionalized; Sales operations in cash are dominant, with verbal dealings, consequent conflicts, and delay in commercial trials; all of which means high receivables. For example, from 2001 to 2013, 5 MMXN of receivables were accumulated that reached 8 MMXN in 2022. On the contrary, with the Dutch customer, these types of accounts are null (non-existent).
iii) A deficient management system for internal information to assess the attractiveness of markets and channels, and deficient capacities for prospecting and managing the fresh market channel (Table 1).

Thus, it can be seen that the main channels that The Organization has are processed avocado and fresh avocado for the city of Torreon (Coahuila) Mexico, although they have the smallest margin. On the other hand, the most attractive channels are the United States and Monterrey (Nuevo Leon) Mexico.

Although the latter are the most lucrative markets, they represent a red ocean strategy, as these involve competing for suppliers and customers with the 97 packing houses authorized to send product to the US, in addition to Mexico's national market (SENASICA, 2024).

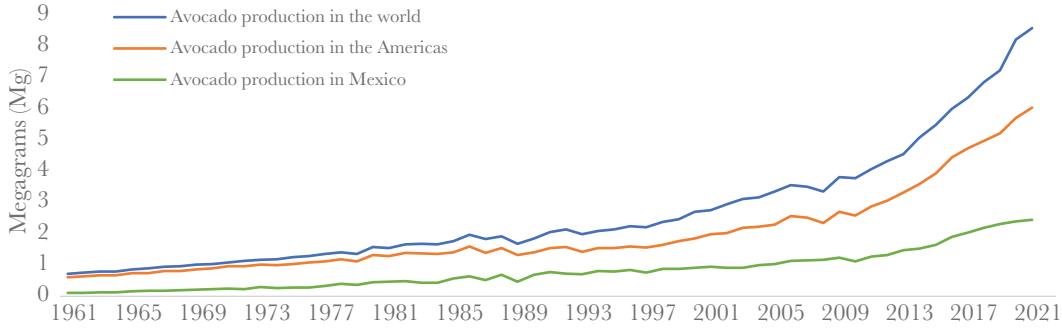

Figure 2. Mexico's participation in avocado production in the Americas and in the world. Source: (FAO, 2024).

Table 1. Margin by sales channel in 2022.

Destination of the fruit by sales channel	Volume per sales channel (%)	Margin (MXN$) per kilogram
Processed	34 (1) [y]	6.65 (4) [x]
USA	1 (5)	27.21 (1)
Spain FOB[z]	8 (4)	12.46 (3)
Monterrey	14 (3)	20.48 (2)
Torreon	25 (2)	4.79 (5)
Others[w]	18	

[z] FOB – free on board ("Free Associate"). [y] The number in parentheses indicates the percentage from highest (1) to lowest (5). [x] The number in parentheses reports the gross margin from highest (1) to lowest (5). [w] CDMX: 4%, Estado de México: 3%, local: 2%, maquila: 4%, Puebla: 4%. Source: data from The Organization for this study.

The sum of these three causal reasons explains why The Organization has decided to prioritize the processed product line, which grew 834% between 2013 and 2022. On the contrary, the avocado line destined for fresh market decreased by 35% in the same period (Figure 3).

Therefore, it is considered that the experience in the industrialization of avocado and the HPP technology (high-pressure cold pasteurization) they have, both represent a strength that the Organization should take advantage of with greater emphasis. Since these products could be sold as premium quality, all-natural and with high added value. Such characteristics would increase the chances for The Organization to differentiating from other companies, and to participate in a blue ocean strategy (Tonello-Carole *et al.*, 2017). Then, in terms of the problem-tree analysis, it was concluded that the organization has a problem in this lack of capabilities, but also an opportunity for further positioning a value-added product (Figure 4).

Definition of the strategy

According to the different scholars of the strategy, The Organization under study does not have a strategy that fully takes advantage of its capabilities. Although it has managed to survive for 70 years, it has done so at the cost of losing more than half of its corporate base, with the consequent aging of the members that remain.

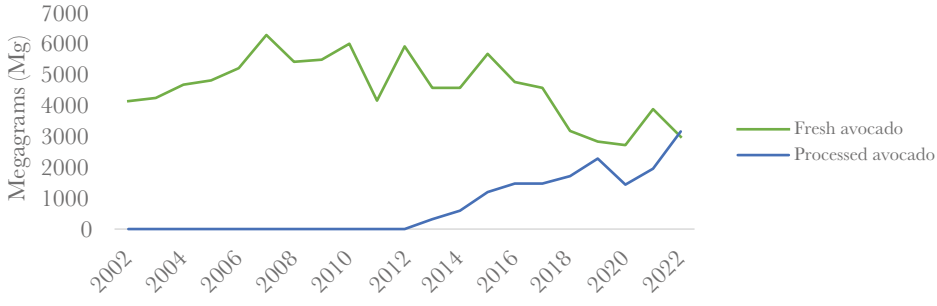

Figure 3. Destination of avocados by business line (2002-2022). Source: data from The Organization.

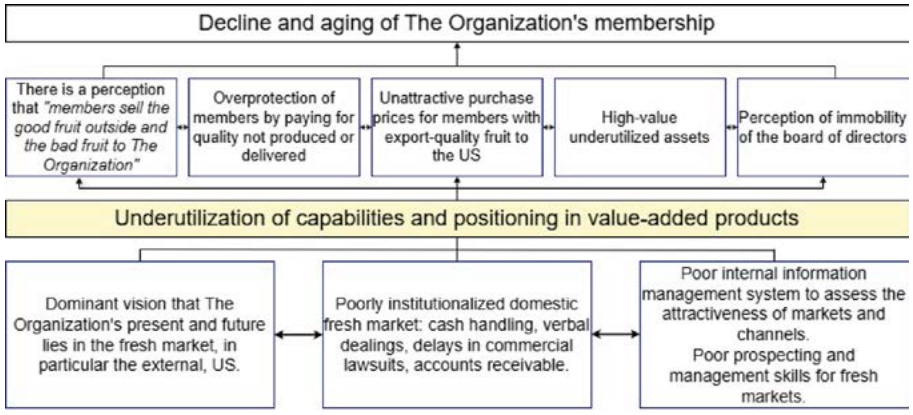

Figure 4. Problem-tree of The Organization of avocado producers; based on Muñoz Rodríguez (2010).

In order to endure, according to Muñoz Rodríguez *et al.* (2010), a company must be fully aware of the competition that operates in the environment in which it operates, as well as of the changes in the needs of partners and customers to maintain the ability to differentiate their products continuously. Kim & Mauborgne (2005) stated that a blue ocean strategy is characterized by creating and capturing new demand; building spaces without competition in the market, and breaking with the dilemma of differentiation and low price.

Therefore, using the ERRC matrix as a strategic analysis tool, the proposal is to eliminate the ambiguity in the commercial mix of volume of fresh produce from the city of Torreón (Coahuila), Mexico.

This sales line generates a lower margin than processed avocado. Furthermore, the company seeks to create a processed product for the Mexican domestic market, initially destined for retailers and subsequently for the HORECA channel (hotels, restaurants, and cafes). Finally, the company seeks to reduce partner losses by giving them the flexibility to deliver export fruit to the highest bidder and by giving the organization the flexibility to deliver quality fruit suitable for processing. The company also seeks to increase the number of avocado suppliers for this new market (Figure 5).

Figure 5. ERRC [Eliminate-Reduce-Raise-Create] Matrix of The Organization; based on Kim & Mauborgne (2005).

CONCLUSIONS

The case analyzed represents a good example of how the people who lead an organization do not deploy their abilities to observe the world and analyze it. That is, they do not identify the causes other than the symptoms of the problems, in order to formulate a strategy that considers the opportunities and inherent capabilities of the Organization.

Because of this, they allow themselves to sail against the current in a red ocean of intense competition, which ends up compromising the performance of the Organization, and even their very existence.

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Colegio de Postgraduados

The plant microbiome as a driver for business models in agriculture that reach unicorn status

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ABSTRACT

Objective: to analyze the business model of the agricultural company based on the plant microbiome that is the first to achieve the Unicorn designation.

Design/Methodology/Approach: this paper reports a single case study by applying the deviant case technique. This approach was chosen because Indigo Agriculture (Indigo Ag) is paramount as a company specialized in agricultural technology that has achieved the top position on the list of the 50 most disruptive companies in the world.

Results: this analysis of the business model reveals how the company has overcome the adversities of agricultural activity, transforming disadvantages into advantages. This was achieved through the convergence of technologies such as computational biology and machine learning. As well as building alliances with universities, seed production companies, microorganism breeding companies; with granting of guarantees for farmers through shared risk and technical support. It is also notable that they preserved the identity of those sustainably produced grains through direct links between farmers and buyers.

Limitations/Implications of the study: business confidentiality and lack of public financial data limited this study to a qualitative analysis based on secondary sources, such as industry reports and documented interviews with experts. This limitation restricts the financial evaluation of the profitability and economic efficiency of Indigo Ag's business model.

Findings/Conclusions: innovations developed by Indigo Ag are centered on plant microbiome. However, there are several additional elements that explain the high performance and rapid positioning of the company.

Keywords: regenerative agriculture, unicorn company, plant microbiome, business models.

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INTRODUCTION

Agriculture faces the structural challenge of increasing food production without compromising environmental sustainability. For decades, the increase in agricultural yields has been based on the intensive use of chemically synthesized inputs, which has generated adverse effects on ecosystems and human health. Added to this is the pressure to reduce emissions from the food system, which is responsible for about a third of greenhouse gases (Crippa *et al.*, 2021).

In this context, biotechnology innovations are emerging that promise to transform the way food is produced, including the use of the plant microbiome. This is composited of bacteria, fungi, archaea and viruses that inhabit the rhizosphere, endosphere and phylosphere of plants, and whose joint action favors growth, health and resilience of agricultural crops (Olanrewaju *et al.*, 2024).

The agronomic potential of the microbiome has raised the interest of the industry, driving the formulation of biofertilizers, biopesticides, and biostimulants. This trend is reflected in the growth of the agricultural microbes market, which is identified as one of the most dynamic areas within biological inputs for agriculture (Batista & Singh, 2021).

However, despite its scientific relevance, little is known about the conditions that allow this type of innovation to scale into viable and sustainable business models. Understanding how a plant microbiome-based company managed to position itself in the market can offer key guidance for entrepreneurs, policymakers, and research institutions interested in accelerating the transition to regenerative agriculture.

This study aimed to analyze the business model of the agricultural company based on the plant microbiome that was the first to achieve the Unicorn designation.

MATERIALS AND METHODS

The study was conducted through a unique case study design, which comprises the in-depth analysis of a case whose own value lies in qualities that allow a greater understanding of the phenomenon under study (Heale & Twycross, 2018). This method has been used to analyze companies that achieve outstanding performance levels and that manage to complete highly challenging processes. The case analyzed is the U.S. company Indigo Agriculture, which was listed as the first unicorn company in agricultural technology (Chandaria *et al.*, 2021). According to Brown & Wiles (2020), a unicorn company is one that reaches a value of a billion dollars in less than 10 years without operations in the stock market.

Case selection is a crucial stage of research based on unique case studies. To this end, the deviant cases technique was used, which consists of identifying the case whose behavior in one or more variables of interest is significantly different from the usual behavior within a group (Bennett, 2022). Since there is no registry that integrates information on companies based on plant microbiome, we began by making a list of companies that are more frequently mentioned in scientific articles. Articles search was done in Scopus considering publications between 2011 and 2021 that contained the keywords: company OR business OR industry AND "plant microbiome" OR "microbial inoculant".

A total of 60 papers including articles and scientific reviews were collected. After the detailed review, five companies referred to in these articles were identified. To increase the number of companies, the snowball method was used, that is, citations were identified within the paragraphs in which those companies were mentioned. The citations corresponded to news and other scientific articles that mentioned some other companies based on plant microbiome. In the end, a total of 20 companies was identified (Table 1).

The companies most frequently mentioned in the documents were Indigo Agriculture, with four mentions in Batista & Singh (2021); Fox (2015); Singh, (2017) and Waltz (2017); and the company New Leaf Symbiotics. This shows us that the scientific community has identified certain plant microbiome companies stating them as new players in the agriculture inputs industry.

Table 1. List of agriculture companies based on plant microbiome.

Name	Year Founded	Country	Website
Aphea Bio	2017	Belgium	https://aphea.bio/
Joyn Bio LLC	2017	USA	https://joynbio.com/
Boots Biome Inc.	2016	USA	https://boostbiomes.com/
Biome Makers Inc.	2015	USA	https://biomemakers.com/
Bioconsortia	2014	USA	https://bioconsortia.com/
Indigo Ag	2014	USA	https://www.indigoag.com/
BioAg	2013	Denmark	https://biosolutions.novozymes.com/en/bioag
Zymergen	2013	USA	https://www.zymergen.com/
Agbiome	2012	USA	https://www.agbiome.com/
Adaptive Symbiotic technologies	2011	USA	https://www.adsymtech.com/
Pivot Bio Inc.	2011	USA	https://www.pivotbio.com/
Symborg	2010	Spain	https://symborg.com/es/
Plant Response	2008	USA	https://plantresponse.com/
BioWish Technologies international Inc.	2007	USA	https://biowishtechnologies.com/
Concentric Ag	2007	Canada	https://www.concentricag.com/
Marrone Bio innovations- Syngenta	2006	USA	https://marronebio.com/
Intrinsyx Bio	2005	USA	https://intrinsyxbio.com/
NewLeaf Symbiotics Inc.	1999	USA	https://www.newleafsym.com
Certis Biologicals	1996	USA	https://www.certisbio.com/
Rizobacter	1983	Argentina	https://www.rizobacter.com/es

In this study, Indigo Agriculture (Indigo Ag) was selected as the deviant case because it is paramount among the identified companies as it is the only one, for now, to achieve the unicorn company designation. This recognition was granted in 2019 by Consumer News and Business Channel (CNBC), a leading global company in business news. The Disruptor 50 list measures disruption based on qualitative and quantitative information related to scalability, user growth, use of innovative technologies, and the size of the industry that is disruptive.

As a result of the recognition that Indigo Ag has achieved, a significant amount of information about the company has emerged. For this reason, Indigo Ag is also a convenient case study, as it offers opportunities for access to information in sufficient quantity and quality, which is crucial to successfully conduct research based on case studies.

Data collection and analysis

The research was conducted using the principle of constant comparison, which means that the data were collected and analyzed simultaneously. The information on the case of the company Indigo Ag was collected through the compilation and review of documentary sources. The information is concentrated in the period 2016-2022 and the sources include

the information published by the company itself on its website (https://www.indigoag.com/), as well as scientific articles, international reports, documented interviews, statistics, news and two case studies prepared by Harvard Business School.

On the other hand, the business model analysis was performed based on the Business Model Canvas (BMC) by Osterwalder & Pigneur (2010). BMC integrates nine modules from which the business logic of a company can be understood; 1) Customer segments, 2) Value propositions, 3) Channels, 4) Customer relationships, 5) Revenue streams, 6) Key resources, 7) Key activities, 8) Key partnerships, and 9) Cost structure (Figure 1). It should be noted that, due to business confidentiality and the absence of accessible public financial data, it was not possible to provide in this study specific details on the modules of revenue streams and cost structure of the business model of Indigo Ag.

The company, as a private entity, do not disclose detailed financial information, which is common in the biotechnology sector and in companies with innovative business models. Given this context, the analysis of revenue streams and costs focused on secondary sources such as industry reports, previous case studies, and documented expert interviews, which provide an overview of their business strategies, albeit without precise financial figures. This qualitative approach limits the scope of the evaluation of the profitability and economic efficiency of the company, and is recognized as an inherent limitation of this research. The constant comparison principle was developed by classifying the information within the nine modules of the BMC framework, using adaptive analysis as the available data were revised.

RESULTS AND DISCUSSION

The BMC framework helps understanding how a company creates, delivers and captures value. The nine building modules of the business model of Indigo Ag are described below.

Customer segments

The market segment was identified primarily as farmers who purchase microbial products from Indigo Ag to inoculate their seeds. The first customers were farmers growing cotton in West Texas, who had the problem of water stress due to lack of water in the production area. Similarly, wheat farmers also relied on Indigo Ag's microbial products to counteract water stress issues (Sutherland *et al.*, 2019). Other customers were corn farmers in central Kansas, where in 2017 they received only 50% of the average rainfall; according to internal reports, sowing Indigo Ag's products increased their yields. Finally, farmers growing rice, soybeans, sunflower and cover crops are also customers.

Another type of customers are those agribusinesses that buy farmers' crops through the Marketplace. In 2017, partnership was announced with the client Grain Craft, the largest milling company in the U.S. that sells its premium flour in bulk and in bags for the bakery industry, pizzas and tortilla retail shops. For that customer, Indigo Ag hired wheat farmers who can produce huge harvests under a more sustainable production system.

In 2018, Indigo Ag announced a partnership with the Better Cotton Initiative (BCI), a global organization that has the world's largest cotton sustainability program. Within the framework of this partnership, Indigo Ag supports cotton farmers that ensure their

production is done under BCI criteria. On the other hand, in 2019, Indigo Ag announced a partnership with Anheuser-Busch, the leading brewer in the U.S. The partnership involved Indigo Ag farmers delivering 44 905 metric tons (Megagrams, Mg) of sustainably produced rice, through reducing water and nitrogen use.

A third group of customers are buyers of carbon credits, which have raised international interest. In February 2021, companies such as Maple Leaf Foods, Epiphany Craft Malt, The North Face, Cool Effect, Barclays, JPMorgan Chase, Dogfish Head Craft Brewery, New Belgium Brewing, Givewith, IBM, Shopify, and Boston Consulting Group committed to acquiring carbon credits to promote sustainability. Carbon credits are paid to farmers who comply with regenerative practices in relation to the amount of carbon sequestered in the soil. Indigo Ag recently made public such monitoring, reporting and verification process (Brummitt *et al.*, 2024).

Value propositions

Indigo Ag was founded in 2014 in Boston, Massachusetts, USA. This company generates value propositions based on "a better way to feed the planet" in alliance with farmers and buyers through the development of biological innovations such as the plant microbiome. As well as the use of data science and advisory services that improve profitability for farmers, environmental sustainability and consumer health (Iansiti *et al.*, 2019). In business models it is important to generate value for all stakeholders such as farmers, consumers, as well as for the environment and society (Baldassarre *et al.*, 2017).

According to the information reviewed on the company's website, Indigo Ag offers products and services under a sustainable and innovative approach. It currently offers products for crops such as cotton, wheat, corn, rice, soybeans, sunflower and cover crops. These microbial products meet objectives such as improving efficiency in nutrient absorption, providing resistance to drought and tolerance to high temperatures.

The information on the website reveals that the services offered by Indigo Ag are based on the advisory services by Indigo Agronomists for the adoption of regenerative agriculture practices, who through data captured in the field and satellite images formulate recommendations to improve agricultural productivity, as well as grain quality. The GeoInnovation team is in charge of data analysis through Atlas Indigo, a platform that integrates data from remote sensing, ground equipment, historical and meteorological data to study the dynamic variables that affect crop productivity.

With this technology, Indigo Ag has been able to characterize local soil conditions, draw field boundaries, and understand differences in crop yields across regions. In addition, with this data analysis, Indigo Ag is able to provide a real-time market view, helping farmers to adjust their harvest times. This business model shows that integrating services with the product is often attractive to farmers. It is relevant because training in regenerative practices can have positive effects on efficient carbon sequestration (Burns, 2021). For this goal, the support of agronomists is important for farmers supported by carbon credits.

The service offered by Indigo Atlas benefits farmers, traders, investors, buyers, grain traders, government agencies, and other industry participants.

Channels

In the case of inputs, Indigo Ag uses the established channel of the seed producers, who agree to impregnate their seeds with microbial consortia and then market those to farmers through their distribution network, seeking to maintain a direct relationship with the producer (Iansiti *et al.*, 2018).

In the case of grains produced by Indigo farmers, the most important channel is the Marketplace, which allows companies to contact farmers directly to market grains and fibers (Anshari *et al.*, 2019).

With this platform, Indigo Ag has managed to make innovation that prevents farmers from depending on larger collection companies and intermediaries, thus Indigo Agriculture manages to reach agribusinesses directly. Another key channel for Indigo Ag is their website, where Indigo Transport is located, a platform with which the logistical details are defined, for the collection and delivery of the crops.

Customer Relationships

Due to rising input costs, low sales prices of crops, and negative climate impacts, farmers face the greatest risk within agrifood chains. Therefore, Indigo Ag developed a model to reduce risk through microbial products and data analysis. Their model consists of sharing risks and rewards. In the first launch of its product Indigo Cotton® Indigo Ag did not charge upfront for microbial treatments. Instead, the payment was agreed as a fixed amount per acre after harvest, provided that increases in production were in fact achieved (Iansiti *et al.*, 2018). To improve production, Indigo Ag offers technical support through Indigo Agronomists, who work with farmers throughout the season using data to make better decisions about implementing regenerative and agronomic practices.

An important aspect that benefits the relationship with farmers is that Indigo Ag conducts commercial trials, where environmental conditions are realistic and not controlled as in small trials. Benefit is supported on the greater confidence that farmers have in the results obtained from trials under actual conditions.

On the other hand, through the Marketplace platform, Indigo Ag manages to de-commoditize sustainably produced grains by preserving their identity throughout the marketing chain. Through that platform, farmers have access to a large number of buyers, who can select the best grain supplier according to their needs. In this way, the best customers are obtained for each production. Another advantage for farmers is that access to the Marketplace is free and includes follow-up with grain marketers. Finally, by using the platform, farmers have protection of their transactions through insurance.

Revenue streams

As it was discussed in Customer Relationships, Indigo Ag started by sharing risks and profits with farmers, *i.e.*, the company did not charge upfront for microbial treatments, but tied income to the results obtained by farmers. For example, in the case of cotton, a charge of USD10 per acre (24.71 USD per hectare) was proposed, as long as an increase was achieved in yield, higher than 30 pounds (13.60 kg) of fiber (14.2% of added value)

(Kephart *et al.*, 2018). Although the pay-for-performance structure has been a part of Indigo Ag's strategy, the company has also adopted other business models.

Subscription model: Indigo Ag has created a subscription-based model where farmers periodically pay for the use of services and products. That model is linked to crop yield, is also called pay-for-results. This means that farmers pay based on the productive benefits they obtain, such as increased yield or improved soil health.

Sales of biological products: a key aspect of Indigo Ag's revenue is the sale of its microbial products such as biofertilizers, biopesticides and biostimulants for corn, cotton, wheat and soybeans. These products are sold through a network of distributors in various states in the United States.

Data-driven services, analytics and consulting: another segment of Indigo Ag's revenue comes from the digital services it offers to farmers and agribusiness. These services include soil microbiome analysis, yield projections, and data-driven recommendations. Data are collected in the field through sensors and stored in Indigo Ag platforms that allow them to obtain other sources of income.

Key resources

Through exploring the company's website, it was identified that one of Indigo Ag's base technologies is the computational biology platform, through which DNA sequencing is performed to identify microbes and classify the effects on plants. In addition, the company developed a satellite imagery and artificial intelligence platform, Indigo Atlas Insights©, with which automatic learning is implemented on satellite, meteorological and historical data that are recorded through remote sensors, and ground and meteorological equipment. Atlas Insights© enables the development of models that assess crop health, productivity, and other factors at different scales, field, county, state, and nationwide.

In regard to human resources, Indigo Ag had 25 employees with doctoral degrees and 17 with master's degrees in 2016, with whom it created a multidisciplinary team highly specialized in the different areas of the company such as business, agriculture, academic researchers and science (Iansiti *et al.*, 2018).

Key activities

With the visits and review of the information on the company's website, it was recognized that the development of Indigo Ag's microbial products requires certain key activities (Figure 1). The first is the identification of outstanding plants in adverse environments. It is hypothesized that those plants have a microbiome that allows them to withstand high temperatures, saline soils and present greater resistance to pests and diseases. Once such a plant is identified by field scientists, it is extracted from the soil to be taken to the laboratory where a sequencing of its endophytic microorganisms is performed. Another way Indigo Ag obtains endophytic microorganisms is through the payment of licenses to universities that have already performed the sequencing. Among those universities, the University of Texas and the University of Arizona are included in the US; also, the University of Saskatchewan in Canada; and Flinders University, in Australia.

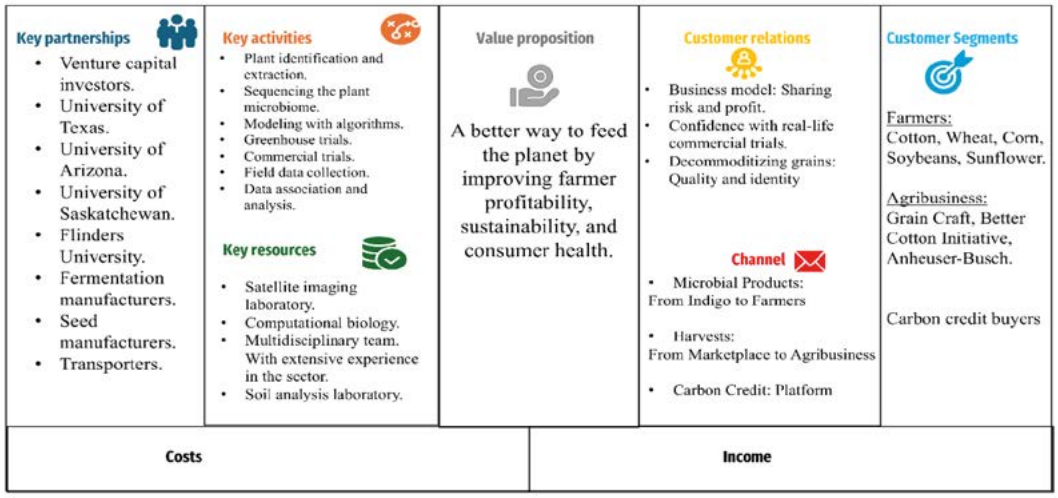

Figure 1. The Indigo Agriculture business model; based on the Business Model Canvas (BMC) by Osterwalder & Pigneur (2010).

After obtaining the endophytic microbes, with the support of computational biology and machine learning, modeling is done with algorithms that predict in which environments microorganisms can express their potential to improve productivity. Subsequently, microorganisms are reproduced through high-precision fermentation to establish trials with greenhouse seedlings, then on soils under cover, and finally on a commercial scale.

Key partnerships

A key part of Indigo Ag's success are the venture capitalists, thanks to whom funding for the research and development of microbial products was achieved. Major investors include Insignia-Flagship, Alaska Permanent Fund, Biallier Gifford, Activant Capital, Investment Corporation of Dubai, FedEx and Pacific Western Bank. Thus, between February 2016 and August 2020 Indigo Ag managed to raise a total of 1.2 billion dollars from series B to F, which represents one of the largest private equity financings in the agricultural technology sector (Manning, 2016) and a growing valuation of the company by investors.

Also relevant for Indigo Ag is the partnership made with universities to access strains already studied and expand the diversity of microorganisms. In addition, alliances with universities allow tests and trials to be implemented, which even are consolidated in partner research that favors the continuous improvement of Indigo Ag's microbial products.

Similarly, alliances are made with companies for the large-scale reproduction of microorganisms whose potential has been validated. Finally, the partnership that Indigo Ag has with seed producing companies allows for a more direct relationship for microbial treatments to be applied to seeds that will be sown by Indigo Ag's partner farmers.

Cost structure

As mentioned above, a major component of Indigo Ag's business model is technological innovation that involves high costs in research and development (R+D). The development of biological products such as biostimulants, biofertilizers and biopesticides requires

significant investment, in laboratories, field trials and validation. According to MacDonald *et al.* (2023), companies responsible for agriculture biotechnology are used to allocate close to 10% of their annual revenue to R+D in their first years of operation.

Once their products reach the commercial scale, those costs related to production and scalability play the most important role. Since infrastructure is required to reproduce and process microorganism at a large-scale, this is the most crucial stage in bio-inputs elaboration (Lorenzoni *et al.*, 2024). As indicated by Kumawat *et al.* (2021), optimizing the supply chain and production capacity contributes to lowering the costs of agriculture microbial products. This is how Indigo Ag has achieved economies of scale, which has allowed the company to reduce manufacturing costs as it increases production.

There are also costs in logistics, which are related to products distribution. For Indigo Ag these costs are significant, because biological products have a limited life, therefore special care is necessary in storage and transport.

The business model of a *unicorn* company, based on plant microbiome, at the service of agriculture

In general, farmers are described in the literature as actors with a low level of education, reduced entrepreneurial skills and a highly reluctant attitude to innovate and take risks (Pindado & Sánchez, 2017). In turn, agricultural units are characterized by their small scale and high geographical dispersion. Likewise, agricultural production depends on biological processes that are characterized by being uncertain and requiring long execution times (Arafat *et al.*, 2020); defining situations that makes experimentation difficult. Such adverse characteristics, added to climate and economic challenges faced by farmers, mean that agriculture is usually considered an unattractive activity for the development of high technologically-disrupting businesses.

However, Indigo Ag's business model (Figure 1) provides insight into how a plant microbiome-based company can overcome the adversities inherent in agriculture and position itself as a disruptive company that has achieved unicorn designation. First, the use of technologies such as computational biology and machine learning for modeling, as well as the partnership with universities to expand the spectrum of microorganisms and with companies for mass production via precision fermentation, allows reducing the times and costs of experimentation and product development.

As a second point, Indigo Ag shares the risk with the farmer, offers proven products on a commercial scale and provides technical support through its agronomists. In this way, the company reduces the risk aversion of innovating on the part of farmers. Finally, for Indigo Ag's business model, the geographical dispersion of farmers does not represent a disadvantage. On the contrary, the diversity of environments allows the company to feed their data management platforms, as well as training their modeling algorithms.

In February 2017, the California-based VentureBeat published a note asking why there were no unicorn companies in agriculture. The portal found the explanation in the adversities usually documented in published papers on agricultural units; and about

stakeholders managing and operating those units. Seven months later, in September 2017, Indigo Ag was announced on local news portal as the new unicorn company in Boston (The Boston's Globe, 2017).

This study allows us to understand how a company manages to overcome the adversities inherent to agricultural activity and even manages to convert disadvantages into advantages. Indigo Ag achieved this through the convergence of technologies that accelerate R+D exponentially; through the construction of alliances, the granting of guarantees for farmers, and the preservation of the identity of sustainably produced grains.

CONCLUSIONS

The case study of the company Indigo Ag shows that the strategic integration of microbial biotechnology, digital traceability and risk management schemes can generate scalable business models even in traditionally adverse sectors such as agriculture. Their approach on the plant microbiome, complemented by institutional alliances and access to big capitals, has allowed the company to overcome structural barriers, thus positioning as a unicorn company.

The development of microbiome-based companies requires more than scientific innovation. This requires business ecosystems capable of directly linking producers with differentiated markets, accelerating technology adoption processes and attracting investment aimed at economic, social and environmental impacts. There are still gaps in knowledge about the potential of the plant microbiome to transform agrifood systems, so it is recommended to advance research that evaluates new business models and their contribution to the transition to regenerative agriculture.

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Effect of lignin concentration on CO_2 emissions in forest soils of the Sierra Nevada

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ABSTRACT

Objective: To evaluate the effect of lignin concentration in leaf litter on the mineralization rate and cumulative mineralization in forest soils.

Design/methodology/approach: Twenty-five grams of soil from an *Abies religiosa* forest in the Sierra Nevada were incubated with increasing concentrations of lignin from leaf litter and branches, at 60% humidity and 35 °C. CO_2 was captured in a 0.5 N NaOH and 0.5 N barium chloride solution, and titrated with 0.5 N H_2SO_4. A completely randomized experimental design with two factors was used. Mineralization rate and cumulative mineralization were determined. Linear regression analysis and ANOVA were performed using the SAS OnDemand for Academics statistical package.

Results: CO_2 emissions followed a linear model for both lignin and soil levels, with mineralization rates ranging from 12.06 mg CO_2 day^{-1} to 33.68 mg CO_2 day^{-1}. There were highly significant differences between lignin concentrations. The interaction between plot and lignin concentration also showed statistically significant differences. A positive and highly significant relationship was found between soil nitrogen and phosphorus content.

Limitations on study/implications: It is recommended to consider climate as a year-round source of variation in CO_2 emissions, as well as its interaction with leaf litter quality and microbial activity.

Findings/conclusions: CO_2 emissions exhibit a linear and positive trend with increasing lignin concentration. Both mineralization rates and cumulative mineralization show statistically significant differences across lignin levels and total nitrogen content, as well as in the interaction between plot and lignin concentration.

Keywords: Organic soil carbon, recalcitrant carbon, C:N, humus.

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INTRODUCTION

Mineralization is the biological transformation of organic compounds into simple inorganic forms. The mineralization of soil organic carbon is the main cause of carbon dioxide (CO_2) emissions into the atmosphere. This process is influenced by temperature, moisture, soil characteristics, microbial diversity and structure, enzymatic activity, and type of vegetation. Temperature is one of the most influential factors in mineralization,

as increases or fluctuations in temperature affect the mineralization rate (Huang *et al.*, 2019) (1).

CO_2 release into the atmosphere in forest systems originates mainly from the mineralization of organic carbon compounds contained in leaf litter, such as sugars, proteins, phenols, hydrocarbons, and glycerides. The proportion of these carbon forms varies across plant structures and species, including cellulose, hemicellulose, and lignin, which are components of the cell wall. Lignin accounts for 15% to 40% of the total leaf litter although in some cases it may range from 4% to 50%. It is an extremely flexible molecule with a variable structure (Krishna & Mohan, 2017) (2). This biopolymer, composed of phenolic heteropolymers, is the most abundant and recalcitrant form of carbon on Earth, resistant to microbial degradation, and the main source of humus. Lignin contributes to the stabilization and cycling of soil organic carbon (Wang *et al.*, 2019) (3). In addition, lignin plays an important role in the biogeochemical cycles of other elements, as well as in maintaining soil fertility. In this context, lignin and cellulose are the main organic components involved in the mineralization of leaf litter (Li *et al.*, 2016) (4). The amount and composition of lignin released into forest soils from organic residues are influenced by environmental conditions such as temperature, moisture, solar radiation, and the mineralization rate (Stutz *et al.*, 2019) (5). Lignin is a complex organic molecule that represents approximately 20% of plant residue composition. It is resistant to enzymatic degradation, meaning that the lignin content in plant tissue is negatively correlated with its decomposition. Its content, composition, and structure vary among plant species. The quality of this plant material influences its decomposition dynamics and, consequently, the stabilization of organic matter in the soil. Decomposition models for plant residues suggest that recalcitrant carbon compounds with high lignin content decompose more slowly than residues rich in soluble sugars. The biochemical decomposition of recalcitrant carbon forms is more sensitive to temperature fluctuations compared to labile carbon forms. Moreover, the enzymatic kinetics involved in the decomposition of recalcitrant compounds increase at higher temperatures, as greater energy is required to activate the associated biochemical processes (Stewart *et al.*, 2015) (6). Among the variables correlated with litter mineralization, the carbon-to-nitrogen (C:N) ratio, the lignin-to-nitrogen (L:N) ratio, and cellulose content stand out. These factors have been used as predictors of organic residue decomposition (Talbot & Treseder, 2012) (7). The C:N ratio is one component among a set of variables that significantly influence the mineralization of organic residues in the soil. However, there is a linear relationship between mineralizable nitrogen and lignin concentration, making the lignin content in organic materials an indicator of residue mineralization when applied to soil (Hernández-Mendoza *et al.*, 2007) (8). This ratio has been used as an indicator of nitrogen levels in the surface layers of forest soils and of mineralization; however, it is influenced by the lignin and nitrogen content of leaf litter and other residues from different plant species. Species with high lignin content and low nitrogen decompose more slowly (Cools *et al.*, 2014) (9). This biochemical process is limited by the lability of carbon and the availability of nitrogen (Fujii *et al.*, 2020) (10).

Forest soils are the main terrestrial reservoir of organic carbon (40%); however, their storage capacity is finite, as the mineralization of organic carbon is estimated to

occur after the maximum accumulation of organic carbon in the form of humus in the organic horizon of mineral soils (Prescott & Vesterdal, 2021) (11). Based on the above, the objective of this research was to estimate the effect of lignin concentration on the mineralization rate and accumulated mineralization of organic carbon in forest soils.

MATERIALS AND METHODS

Soil samples were collected at a depth of 0 to 10 cm in the oyamel fir forest (*Abies religiosa*), located at 19° 25.609' N latitude and 98° 45.785' W longitude, on Mount Tláloc in the Sierra Nevada, eastern State of Mexico. The altitude ranges from 3,093 to 3,489 meters above sea level. The climate is semi-cold, with an annual average temperature between 5 °C and 12 °C. Annual precipitation ranges from 800 to 1,200 mm. pH, Soil Organic Carbon (SOC), Organic Carbon (OC), Soil Organic Matter (SOM), Cation Exchange Capacity (CEC) were determined (Table 1).

Soil incubation was carried out in airtight 200 ml jars. Twenty-five grams of soil (air-dried in the shade and sieved to 2 mm) were placed in each jar, adjusted to 60% of its water holding capacity, and incubated at 35 °C. The leaf litter incorporated into the soil consisted of a mixture of needles and branches, ground and sieved through a 40-mesh sieve. To determine lignin concentrations, a weighted average was used, based on neutral detergent fiber and acid detergent fiber analysis values of the leaf litter samples (Table 2). The amount of leaf litter mixture incorporated into the soil was estimated based on the annual leaf litterfall at each sampling plot (P1: 3.0 t ha^{-1} $year^{-1}$; P2: 3.9 t ha^{-1} $year^{-1}$; P3: 2.5 t ha^{-1} $year^{-1}$; P4: 2.6 t ha^{-1} $year^{-1}$; and P5: 2.5 t ha^{-1} $year^{-1}$).

CO_2 emissions were captured using a 0.5 N NaOH solution. In each jar, a test tube containing 5 mL of the NaOH solution was placed and replaced at each measurement. CO_2 readings were taken daily during the first four days, every two days for the

Table 1. Characteristics of the soils used for determining the carbon mineralization rate in forest soils.

Plot	Altitude (m)	pH	SOC	OC	SOM	CEC meq 100 g^{-1} S	N	P
			%				mg kg^{-1} S	
P1	3489	5.8	4.2	7.4	12.8	34.8	18.8	6.2
P2	3360	6.1	3.0	4.6	7.8	37.3	19.5	6.4
P3	3359	6.4	2.0	6.5	11.2	38.0	21.2	13.0
P4	3359	6.2	2.5	4.4	7.6	37.5	23.4	21.8
P5	3093	6.2	2.3	4.0	6.9	36.7	30.5	17.4

SOC: Soil Organic Carbon; OC: Organic Carbon; SOM: Soil Organic Matter; CEC: Cation Exchange Capacity; N: Nitrogen; P: Phosphorus.

Table 2. Characteristics of needles and branches of *Abies religiosa* used in the incubation.

Litterfall	Litterfall dry matter (g)	Ashes	Cellulose	Hemicellulose	Lignin	Total nitrogen	L:N
		%					
Needles	97.3	5.1	21.8	14.5	11.6	1.2	9.8
Branches	96.1	4.4	28.9	13.1	21.9	0.8	28.2

L: Lignin; N: Nitrogen.

following eight days, then every three days for six days, and finally on day 28 after the start of the incubation. For the quantification of the recovered CO_2, the NaOH solution was mixed in an Erlenmeyer flask with 2.5 mL of 0.5 N barium chloride solution to precipitate the adsorbed CO_2. Two drops of phenolphthalein were added, and the solution was titrated with 0.5 N H_2SO_4. A completely randomized experimental design with two factors (plots and lignin concentrations) and three replicates was used. The mineralization rate and cumulative mineralization were determined from the data. These variables were analyzed using linear regression, analysis of variance (ANOVA) and multiple mean comparison (Tukey). The relationships between variables were evaluated through linear correlation (Pearson). The statistical package SAS OnDemand for Academics was used.

RESULTS AND DISCUSSION

Based on the analysis of variance of the linear regression of lignin concentrations and incubation time, it was determined that CO_2 emissions fit a linear model with an alpha of 0.01 (Table 3) for all lignin levels and each soil evaluated, indicating that lignin mineralization under these conditions is independent of soil characteristics.

Table 3. Analysis of variance of the linear regression of lignin concentrations on CO_2 emissions after 28 days of incubation.

Source of variation			Model	Error	Total
Degrees of freedom			1	40	41
Sum of squares (% Lignin concentration in litterfall)	P1	0	642623**	19389	662011
		10.6	941876**	15763	957639
		13.6	830375**	89561	919936
		23.3	662203**	38007	700210
	P2	0	595126**	24731	619857
		11.8	525190**	4353.16	529543
		14.8	695654**	19051	714705
		22.2	418158**	19935	438093
	P3	0	1483852**	18077	1501930
		12	1568789**	18627	1587415
		14.5	2028942**	18150	2047091
		20.8	1485147**	45441	1530588
	P4	0	1842007**	17077	1859084
		12.1	2605616**	4929.58	2610546
		14.5	2381299**	16876	2398175
		23.1	2194099**	56817	2250917
	P5	0	2279219**	42675	2321894
		11.5	2767987**	35590	2803577
		13.6	3263165**	43630	3306796
		20.2	3125949**	63182	3189131

*Significant=0.05; **Highly significant=0.01; ns: not significant.

The linear regression analysis determined that CO_2 emissions show a linear trend between the lignin concentrations added to each soil and the CO_2 recovered in the five soils evaluated after 28 days of incubation, with mineralization rates ranging from 12.06 mg day^{-1} of CO_2 to 33.68 mg day^{-1} of CO_2 (Table 4). Similarly, the coefficient of determination ranged from 0.90 to 0.99, indicating that more than 90% of CO_2 emissions depend on the incubation time.

The linear behavior is independent of the added lignin concentration, since when comparing the CO_2 emissions from soils with 0% lignin, similar rates are observed among the applied concentrations (Figure 1). Klotzbücher *et al.* (2011) (12) mention that lignin mineralization in soil mainly depends on the inputs of labile carbon and other soluble forms that provide energy to the microbial biomass.

The analysis of variance for the effect of plot and lignin concentration on the organic carbon mineralization rate and cumulative organic carbon mineralization indicates that there are highly significant differences between the individual factors for both the mineralization rate and cumulative mineralization. Meanwhile, for the interaction between plot and lignin concentration, highly significant differences were found in the mineralization rate and significant differences in cumulative mineralization (Table 5).

The multiple mean comparison analysis of the plot effect on the mineralization rate indicates that the highest rate occurred at plot 5, with 31.45 mg day^{-1} of CO_2, which

Table 4. Lignin mineralization rates at 28 days of incubation.

Plot	Lignin concentration in litterfall (%)	Lignin mineralization rate (mg day^{-1})	r^2
1	0	14.95	0.97
	10.6	18.09	0.98
	13.6	16.99	0.90
	23.3	15.17	0.95
2	0	14.38	0.96
	11.8	13.51	0.99
	14.8	15.55	0.97
	22.2	12.06	0.95
3	0	22.71	0.99
	12	23.35	0.99
	14.5	26.56	0.99
	20.8	22.72	0.97
4	0	25.30	0.99
	12.1	30.10	1.00
	14.5	28.77	0.99
	23.1	27.62	0.97
5	0	28.15	0.98
	11.5	31.02	0.99
	13.6	33.68	0.99
	20.2	32.96	0.98

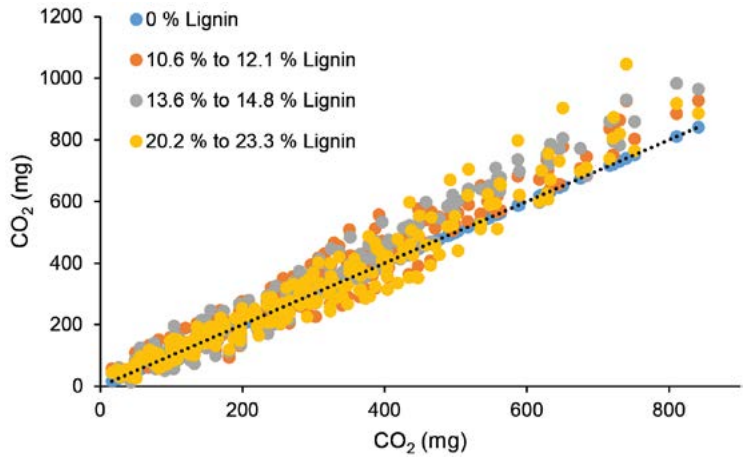

Figure 1. Relationship between CO_2 emissions and lignin concentration in the soil.

Table 5. Analysis of variance for the effect of plot and lignin concentration on the organic carbon mineralization rate and cumulative organic carbon mineralization.

SV	DF	Lignin mineralization rate (mg day^{-1})		Cumulative mineralization (g)	
		SS	F-value	SS	F-value
Plot	4	2690.80	286.13**	67.80	173.46**
LC	3	86.57	12.27**	2.24	7.63**
Plot-LC	12	75.98	2.69**	3.82	3.25*
Error	40	94.04		3.91	
Total	59	2947.40		77.76	
R^2	0.97			0.95	
CV	6.76			7.11	

SV: Source of variation; DF: Degrees of freedom; SS: Sum of squares; LC: Lignin concentration in litterfall; CV: Coefficient of Variation; *Significant=0.05; **Highly significant=0.01; ns: not significant.

corresponds to a total nitrogen content of 30.5 mg kg^{-1} S. In contrast, at plot 2, the mineralization rate was 13.88 mg day^{-1} of CO_2, with a nitrogen content of 19.5 mg kg^{-1} soil (Table 6). These CO_2 emissions suggest a relationship between nitrogen content and the mineralization rate, showing that lower soil nitrogen content is associated with lower mineralization rates.

Regarding cumulative mineralization, statistical differences were observed among the plots, with P5 showing the highest CO_2 accumulation and P2 the lowest, with 5.98 g and 2.95 g, respectively. Based on these data, and considering that soil nitrogen content plays a role in the mineralization process through the C:N ratio, both the rate and cumulative mineralization are influenced by the nitrogen content in the soil. This, in turn, determines the activity of microorganisms involved in mineralization, as residues with a low C:N ratio and high N content undergo faster mineralization. This is because microorganisms use nitrogen for cell growth and reproduction. It has been estimated that when the C:N ratio exceeds 25, nitrogen in the soil tends to become immobilized (Pei *et al.*, 2019) (13).

Table 6. Multiple mean comparison of the plot effect on the rate of organic carbon mineralization and cumulative organic carbon mineralization.

Plot	Lignin mineralization rate (mg day^{-1})	Cumulative mineralization (g)
P5	31.45 a	5.98 a
P4	27.95 b	5.04 b
P3	23.84 c	4.41 c
P1	16.30 d	3.59 d
P2	13.88 e	2.95 e
MSD	1.79	0.36

MSD: Minimum Significant Difference; Means with the same letter are not significantly different from each other (P. 0.05 ANOVA followed by Tukey test).

The addition of lignin to the soil showed statistically significant differences in both the mineralization rate and cumulative mineralization, compared to soils without lignin addition. The highest mineralization rate and cumulative mineralization were observed with the addition of 13.6% to 14.8% lignin (Table 7). Based on the CO_2 emissions determined in this experiment, a linear pattern was observed across all simulations. However, the statistical differences found in the mineralization rates indicate an influence of the amount of lignin present in the soil on its resistance to microbial mineralization. This is because carbon quality affects both the composition of microbial communities and the total microbial biomass. For example, in the presence of recalcitrant forms of carbon, such as lignin, fungi tend to dominate (Ali *et al.*, 2018) (14).

The resistance of lignin to mineralization is due to the fact that it is a form of carbon that provides little energy to microorganisms, as it is protected by cellulose compounds. Microorganisms expend more energy breaking down this structure than the energy they gain from its mineralization (Rinkes *et al.*, 2016) (15).

The multiple comparison analysis indicates that the interaction Plots-Lignin Concentration shows statistically significant differences between the mineralization rate

Table 7. Multiple comparison of means for the effect of lignin concentration on the organic carbon mineralization rate and cumulative organic carbon mineralization.

Lignin concentration in litterfall (%)	Lignin mineralization rate (mg day^{-1})	Cumulative mineralization (g)
LC3	24.31 a	4.63 a
LC2	23.22 ab	4.50 a
LC4	22.11 bc	4.34 ab
LC1	21.10 c	4.11 b
MSD	1.5007	305.96

LC1: 0% Lignin; LC2: 10.6%-12.1% Lignin; LC3: 13.6%-14.8% Lignin; LC4: 20.2%-23.3% Lignin; MSD: Minimum Significant Difference; Means with the same letter are not significantly different from each other (P. 0.05 ANOVA followed by Tukey test).

and the accumulated mineralization (Table 8). The highest mineralization rates ranged between 33.68 and 30.10 mg CO_2 day^{-1} in the interactions P5-LC3, P5-LC4, P5-LC2, and P4-LC2, which correspond to the plots with the highest nitrogen content of 23.4 mg kg^{-1} soil and 30.5 mg kg^{-1} soil, respectively. This suggests that nitrogen content has a greater influence on the mineralization rate than lignin concentration. This condition is similar for accumulated mineralization.

The correlation analysis (Table 9) suggests a positive and highly significant relationship between total soil nitrogen, extractable phosphorus, and both the mineralization rate and cumulative mineralization, with coefficients of 0.83, 0.87; 0.86, and 0.79, respectively. This is because high levels of soil nitrogen stimulate microbial respiration (Ma *et al.*, 2020) (16). Furthermore, the mobilization and mineralization of organic compounds of C, N, and P are highly correlated; that is, immobilization and mineralization have a strong relationship with microbial activity (Brödlin *et al.*, 2019) (17).

Table 8. Multiple comparison of means for the effect of the plot and lignin concentration interaction on the rate of organic carbon mineralization and accumulated organic carbon mineralization.

Plot-LC	Lignin mineralization rate (mg day^{-1})	Plot-LC	Cumulative mineralization (g)
P5-LC3	33.68 a	P5-LC3	6.37 a
P5-LC4	32.96 ab	P5-LC4	6.32 a
P5-LC2	31.02 abc	P5-LC2	5.90 ab
P4-LC2	30.10 abc	P5-LC1	5.33 bc
P4-LC3	28.77 bcd	P4-LC2	5.31 bc
P5-LC1	28.15 cd	P4-LC3	5.13 bc
P4-LC4	27.62 cde	P4-LC4	4.97 bcd
P3-LC3	26.56 cdef	P3-LC3	4.87 cd
P4-LC1	25.30 def	P4-LC1	4.76 cd
P3-LC2	23.35 ef	P3-LC2	4.46 cde
P3-LC4	22.72 fg	P3-LC4	4.15 def
P3-LC1	22.71 fg	P3-LC1	4.14 def
P1-LC2	18.09 gh	P1-LC2	4.07 def
P1-LC3	16.99 h	P1-LC3	3.79 efg
P2-LC3	15.55 hi	P1-LC4	3.49 fgh
P1-LC4	15.17 hi	P2-LC1	3.31 fgh
P1-LC1	14.95 hi	P1-LC1	3.02 gh
P2-LC1	14.38 hi	P2-LC3	2.98 gh
P2-LC2	13.51 hi	P2-LC4	2.77 h
P2-LC4	12.06 i	P2-LC2	2.76 h
MSD	4.7427	MSD	0.9669

LC1: 0% Lignin; LC2: 10.6%-12.1% Lignin; LC3: 13.6%-14.8% Lignin; LC4: 20.2 %-23.3% Lignin; MSD: Minimum Significant Difference; Means with the same letter are not significantly different from each other (P. 0.05 ANOVA followed by Tukey test).

Table 9. Correlation of soil characteristics with litter mineralization.

Correlation	pH	SOC	OC	SOM	CEC (meq 100 g^{-1} S)	N	P
		%					
Lignin mineralization rate (mg day^{-1})	0.53	−0.65	−0.47	−0.46	0.32	0.83**	0.87**
Cumulative mineralization (g)	0.42	−0.55	−0.44	−0.42	0.19	0.86**	0.79**

SOC: Soil Organic Carbon; OC: Organic Carbon; SOM: Soil Organic Matter; CEC: Cation Exchange Capacity; N: Nitrogen; P: Phosphorus; *Significant $\alpha = 0.05$; **Highly significant $\alpha = 0.01$; ns: not significant.

CONCLUSIONS

CO_2 emissions show a positive linear trend in relation to the concentration of added lignin. Mineralization rates and cumulative mineralization exhibit statistically significant differences due to the addition of increasing levels of lignin to the soil. Plots with higher total nitrogen content present higher mineralization rates and cumulative mineralization compared to plots with lower total nitrogen content. Significant differences exist in mineralization rate and cumulative mineralization in the interaction between plots and lignin concentration.

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Colegio de Postgraduados

Evaluating perception of animal welfare among owners of family farming units on dry tropical lands

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ABSTRACT

Objective: To evaluate the perception of animal welfare (AW) among owners of family farming units (FFUs) through selected livestock management practices.

Design/ Methodology/ Approach: A semi-structured questionnaire was applied to survey a sample of 80 FFU owners. The survey collected data on demographics, AW perception, feeding practices, infrastructure, animal handling and use of preventive veterinary care.

Results: Among respondents, 92.5% were male and 7.5% female. 51.2% were over 60 years old, 36.2% were between 40 and 59 years old. Around 80% had 20 to 40 years of livestock-rearing experience. Educational attainment was low; 80% had no schooling, only 20% had an average of 4.3±5 years of schooling. In terms of species, 47.5% raised small ruminants (sheep and goats) and 52.5% managed cattle; in a complementary way all raised other species (such as pigs, poultry, or equines) within mixed production systems. Notably, 98.8% of respondents were unaware of AW principles; none had received technical assistance or training related to AW. Feeding practices primarily relied on grazing native pastures, with 20% providing feed supplements (1-4 kg) during the dry season. Water sources included the river (53%) and deep wells (47%). Only 37.5% had basic infrastructure for animal handling, while 62.5% has none. A 33% of respondents practiced preventive veterinary care, whereas 67% only provided veterinary care to animals when symptoms of sickness appeared.

Limitations/Implications of the study: Traditional production systems in place pose a significant barrier to the implementation of effective AW measures.

Findings/Conclusions: There is not AW perception in the FFUs evaluated. A critical need for outreach, education, and training programs is emphasized, in order to equip FFU owners with knowledge and tools to implement animal welfare standards.

Keywords: Cattle, animal handling, resources, feed.

INTRODUCTION

Animal welfare (AW) is an aspect of great importance, which increases worldwide and has generated concern in the sectors dedicated to animal husbandry or production. Animal welfare brings about major changes in the way animals are kept and treated in farms, slaughterhouses, and other production systems (Broom, 2016). Animal welfare is currently considered a priority in the scientific field, in response to some of the concerns related to livestock production (FAO, 2008; Rosas *et al*., 2019).

The success of any livestock production system depends fundamentally on the personnel in charge of handling the animals, who are responsible for their welfare and productivity (Losada-Espinosa, 2020). Because of this, the World Organisation for Animal Health (2023) designated animal welfare as "*the physical and mental state of an animal in relation to the conditions in which it lives and dies*". WOAH (2023) also stated that it is a complex and multidimensional issue addressed from scientific, ethical, economic, cultural, social and political aspects. Moreover, Valadez *et al.* (2018) indicated that the factors that influence animal welfare also have an impact on human health; Hence the concept of One-Health, that promotes a comprehensive and collaborative approach, recognizes the link between human, animal and environmental health.

In the dry tropics lands of the state of Guerrero, there are many families that are dedicated to livestock activity with semi-extensive systems, where there are no studies that show the knowledge that producers have about animal welfare. Despite livestock farming is an activity that owners have developed for a long time. For this reason, the objective of the study was to evaluate the perception that the owners of family farming units have about animal welfare through the management practices they implement under tropical conditions, in the state of Guerrero, Mexico.

MATERIALS AND METHODS

Description of the study area

The study was established during the dry season (January-May) in the towns Morelita and Tiringeo, of the municipality Tlapehuala (Guerrero), Mexico. Both towns are located within the geographical coordinates 18° 14' 14" N and 100° 32' 22" W, at 240 m altitude. Climate is classified as warm sub-humid (AW_0), with an average temperature 35 to 45 °C, and 750 mm of average rainfall per year.

Population and sample size

In the study communities, 100 owners of family farming units (FFUs) were identified, the equation described by Rojas (2013) was applied to obtain the sample size:

$$n = \frac{N \times Z_a^2 \times p \times q}{d^2 \times (N-1) + Z_a^2 \times p \times q}$$

In a population size of 100 owners, with 95% confidence level and 5% margin of error; sample size n=80 FFU owners.

Data collection and statistical analysis

A semi-structured questionnaire was applied personally to the producers. The survey structure was segmented into demographic data (gender, age, level of schooling, years dedicated to livestock activities), knowledge about animal welfare (AW), animal feeding, infrastructure in the production units, livestock management, application of preventive veterinary medicine. Questions in the survey were open, we sought to generate conversation with the interviewees. Data collected were analyzed using descriptive statistics, it is included here their graphic analyses in figures.

RESULTS AND DISCUSSION

Data showed that 92.5% of FFU owners belong to the male gender and 7.5% to the female gender. About 51.25% are over 60 years old, 36.25% between 40 and 59 years old; more than 80% of the respondents said that have been engaged in livestock farming for about 20 to 40 years. However, most of the producers do not have any degree of schooling; only 20% reported that had an average 4.3 ± 5.0 years of schooling, a percentage represented by the youngest producers in the study.

The age of the producers and educational attainment are considered fundamental factors that limit training and the adoption of cutting-edge technologies in the production units (Fuentes *et al.*, 2012; Salas *et al.*, 2013). For example, Villanueva *et al.* (2022) reported that producers in Puebla, with a higher level of education, do a better management in their production units by implementing better management systems.

Another finding; of the total number of production units, 38 breed small ruminants (goats and sheep) and other 42 breed cattle. In most of the production units, owners breed other species (pigs, poultry, equines or canines). A notable 98.8% is unaware of the aspects of animal welfare (AW) and only 1.2% have any idea about welfare in animal handling.

It is remarkable that none of the producers have received advice or training regarding animal welfare; however, 80% have unconsciously applied some intuitive principles of animal welfare that they consider important. Such as better feeding practices, water supply, and general care of their animals. The lack of training and scarcity of economic resources are a set of aspects that limit proper development of the family farming units (Juárez *et al.*, 2014).

Of the producers surveyed, 85% consider this issue of animal welfare as important, also showing interest in receiving training (Figure 1).

Animal feeding

The main source of feed in all production units is through grazing on native pastures or maize (*Zea mays*) harvest residues. Only 15% add feed supplements to the diet of their animals in the dry season every year, offering 1 to 4 kg of supplement per animal. Those owners indicated they do this due to the low availability in quantity and nutritional quality of the forage offered (Figure 2).

Juárez *et al.* (2014) reported that 98% of producers implement a traditional grazing system in their production units in a micro-watershed of the Michapa river, where animal

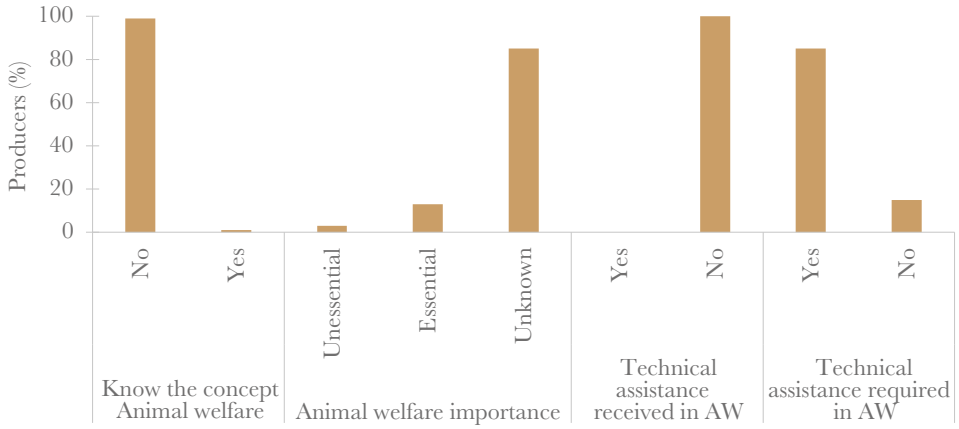

Figure 1. Perception about animal welfare (AW) among the owners of family farming units in the localities Morelita and Tiringueo, municipality of Tlapehuala (Guerrero), Mexico.

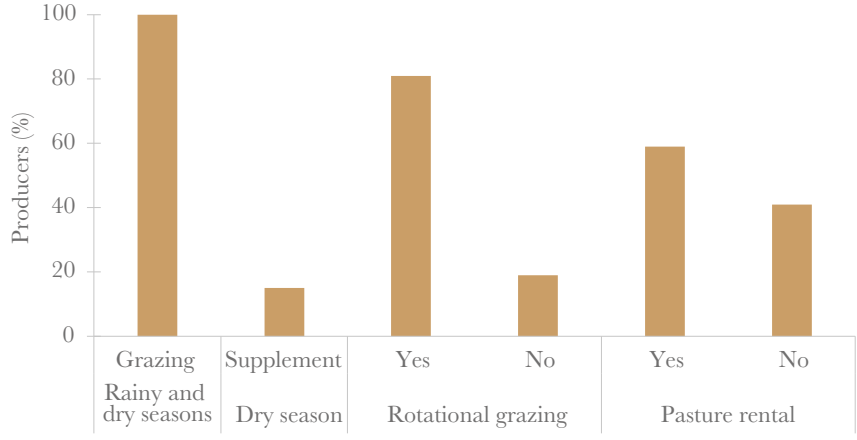

Figure 2. Animal feeding used at family farming units (FFUs) in the localities Morelita and Tiringueo, Tlapehuala (Guerrero) Mexico.

feed is mainly based on grazing and only 1% offered balanced feed. Therefore, it is considered that the use of supplementation is added according to the availability of existing forages in a region (Vilaboa and Díaz, 2009).

About 59% of producers also have reported that they need to rent grazing areas, to store forages or by-products, or they need to plant species for cutting fodder (Sheen and Riesco, 2002). Seasonal variations and irregular rainfall affect forage production in pastures, which consequently affect animal productivity and animal welfare in production systems (Gallo & Tadich, 2018).

The main source of water used for the animals is the river. About 53% of the owners made journeys of 1.5 to 3 km to get water during the dry season; they had water available for a short time, only once a day; while the other 47% transported water to grazing areas, or used a deep well. During the rainy season there are more water sources available, such as streams, dams, embankments, or water channels. Thus, the animals have more water available at a short distance, even in the same grazing areas.

Animal handling

About 62.5% of the producers do not have a corral for animal handling, only 37.5% have a rustic corral for cattle management. Producers said they don't have the financial resources to build more sophisticated facilities (Figure 3).

Due to the grazing system used in the production units, the animals remain most of the time in grazing areas. This allows them to use some areas for resting, such as the natural shade provided by trees (Damián *et al.*, 2022). Ceballos and Tarazona (2023) indicated that facilities are of great importance for animal handling, because infrastructure facilitates handling, reduce the time for activities, provide better animal welfare conditions, and decrease the risk of accidents or injuries to both animals and the personnel responsible of animal handling.

Animal health

Only 33% of familiar farming units implement vaccination programs on a regular basis to prevent diseases. That activity is accomplished through local veterinary services. The other 67% do not use veterinary medicine programs on their animals, they only attend to their animals when an illness occurs. Owners stated that they consider it less expensive to care for a single animal. This shows the lack of knowledge available to producers. In addition, the average age of producers is a limiting factor to generate changes in this type of production units. The results obtained by Vilaboa and Diaz (2009) showed the opposite, indicating that 88% of producers evaluated in Veracruz implemented preventive health campaigns, such as vaccination and deworming.

About 58% of the producers do control ectoparasites, mainly according to the presence of flies (*Haematobia irritans*). During the last three years, the death of 43 bovines, 33 goats and 27 sheep has been recorded due to different causes. It is very likely that producers need training to implement prevention measures. This is key since owners only seek advice from a veterinarian when an animal is already sick, and they do not observe healing after several days.

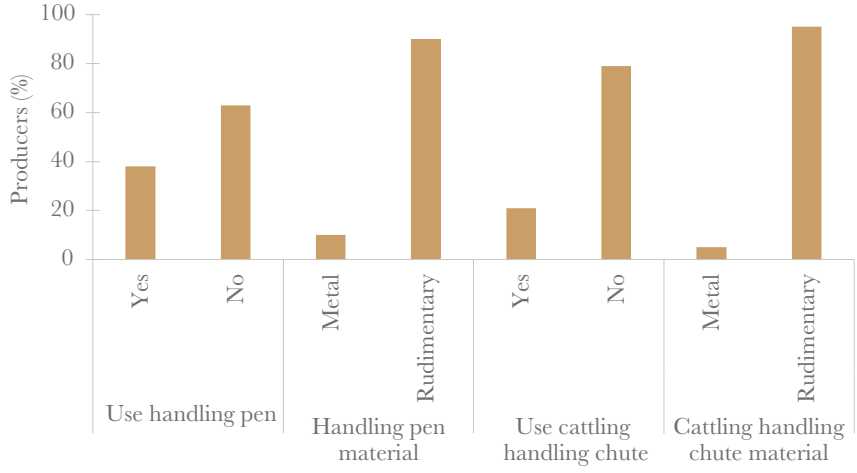

Figure 3. Facilities for livestock management implemented by producers in the localities Morelita and Tiringueo, Tlapehuala (Guerrero), Mexico.

CONCLUSIONS

In the family farming units evaluated there is no perception of animal welfare. Therefore, management practices that are developed lack elements that provide welfare. We consider it is necessary to provide training to support the implementation of elements for animal welfare, towards a better development of the family farming units.

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Colegio de Postgraduados

Characterization of regional substrates for the production of plants in containers

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ABSTRACT

Objective: To evaluate the physical and chemical properties of waste from the oil palm industry (empty fruit bunches and palm kernel shell charcoal), from the forestry industry (*Cedrela odorata* sawdust and *Eucalyptus* spp. bark), and from the agroindustry (sugarcane bagasse, cocoa pod husk, and coconut fiber), in order to determine their potential as components of regional substrates.

Design/Methodology/Approach: A completely randomized design was used for the experiment. Seven regional substrate treatments with three replicates were used to evaluate the response variables. An analysis of variance (ANOVA) and Tukey's multiple comparison test ($p \leq 0.05$) were used to analyze the results in the InfoStat v. 2020 statistical software.

Results: Regional substrates had similar characteristics —and even a higher concentration of nutrients— than the commercial substrate, which was mainly based on sphagnum peat moss. Substrate S5 —eucalyptus (*Eucalyptus* spp.) bark:cocoa pod husk:cedar (*Cedrela odorata*) sawdust (3:1:1)— had more variables that were statistically similar to the commercial substrate, while S4 —cocoa pod husk:cedar sawdust:palm kernel shell charcoal (3:1.5:0.5)— stood out for its higher concentration of micronutrients. The results identified sustainable and accessible options that meet the recommended criteria for plant production in containers.

Study Limitations/Implications: This study only took into account the characterization of regional waste and substrates; consequently, its effects on future plant production should be evaluated.

Findings/Conclusions: The substrates were sustainable and affordable and met the recommended criteria for the plant production in containers.

Keywords: organic waste, sphagnum peat moss, physical and chemical properties, nursery, recycling.

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INTRODUCTION

Peat moss is an organic, natural, and renewable material, widely used in horticulture and agriculture due to its fiber structure and porosity that favor water retention and drainage [1]. Peat moss mainly comes from peatlands in the northern hemisphere, where anaerobic

decomposition of plant material has created large deposits. However, its extraction implies the drainage of wetlands, resulting in the subsequent loss of ecosystem services [2].

A wide range of waste with the potential to become substrates can be found in southeastern Mexico. The valuation of this waste offers an alternative to mitigate environmental impacts. Waste from fishing, agricultural, forestry, poultry, and cattle-raising activities (and its resulting inputs) can be classified as special waste [3]. This type of waste comes from production processes and is not considered dangerous or part of urban solid waste, because it is not produced by large urban solid waste generators.

One of the main types of waste of the oil palm (*Elaeis guineensis* Jacq.) agroindustry is the empty fruit bunches. This fibrous and lignocellulosic material is the waste of the processing of fresh bunches and each tonne of raw palm oil causes 350 kg of dry waste [4]. The bunches are first introduced into a sterilizer, where they are subjected to high temperatures and moisture in order to facilitate the loosening of the fruits. Afterwards, a threshing machine separates the fruits from the bunches, leaving aside the empty bunches [5]. Another by-product is palm kernel shell charcoal. This hard and compact biomass is obtained from the husk that protects the kernel that contains the palm oil [6]. During this production process, the nuts are crushed to divide the kernels from the husk, which is usually used as a combustion energy source [4]. In the sugarcane (*Saccharum officinarum* L.) agroindustry, sugarcane bagasse is a fibrous waste resulting from the extraction of the juice from cane stalks. This material is mainly made up of raw fiber and neutral and acid detergent fibers [7]. Cocoa pod husk is the waste produced by the cacao (*Theobroma cacao* L.) agroindustry. It accounts for 60-70% of the dry weight of cocoa pods. It is composed of cellulose (35%), hemicellulose (11%), lignin (14.6%), and pectin (6.1%) [8, 9]. Cocoa producers usually throw this waste out in their growing areas. In time, soil microorganisms degrade cocoa pod husks, releasing organic matter back to the soil as nutrients and minerals, starting once again the production cycle [10]. Cedar (*Cedrela odorata* L.) sawdust is a by-product of the timber industry. It has abundant cellulose (45-50%), lignin (23-30%), and hemicellulose (20-30%) [11]. In Mexico, *Cedrela odorata* is the second most popular timber species, after *Eucalyptus* spp., with a 37,296 ha established area in commercial forest plantations. Cedar also classifies second in Tabasco, with 3,211 ha established areas [12]. Its importance in the forestry industry offers opportunities to add value to its waste. Eucalyptus (*Eucalyptus* spp.) has the largest established commercial plantation area in Mexico: 48,523 ha. Out of this total, 21,757 ha are located in Tabasco [12]. This tree is mainly used to produce cellulose and, to a lesser degree, lumber. One of the initial processes of eucalyptus exploitation is debarking, which produces large quantities of waste (bark). Eucalyptus bark is a valuable resource for the circular economy of the forestry industry [13]. Coconut (*Cocos nucifera* L.) fiber is another valuable resource. It is made up of the coconut external shell and an intermedium layer that surrounds the fruit. The white meat inside the coconut shell is known as copra and it has a high commercial value. The coconut fiber by-product is obtained during the copra exploitation from the mesocarp and the short fibers of the shell [14]. Tabasco has the fifth largest planting area (12,644 ha) used for the exploitation of copra [15]. Coconut fiber has favorable properties for the production of plants; however,

its effects on the growth, development, yield, and quality of high value crops has not been studied in depth yet [16].

Substrates are made up of solid materials. They are different from the natural soils. They can be made up of minerals, organic matter, and synthetic or waste products. Substrates are used in containers in order to support and anchor to the plant root system. This material can be used pure or mixed. Its main purpose is to guarantee appropriate oxygen and moisture conditions, as well as to provide nutrients required to achieve an optimal plant growth. The composition of these substrates can include porous, low fertility, and lifeless materials that favor aeration and water balance in the root environment [17, 18, 19]. The quality of substrates is fundamental for plant production, because they must provide the physical, chemical, and biological conditions, as well as the essential nutrients, required to achieve the appropriate plant development [20]. In order to evaluate the suitability of the substrate formulas, the following properties were analyzed: apparent density (AD), total porosity (TP), aeration porosity (AP), water retention porosity (WRP), pH, electrical conductivity (EC), organic matter (OM), organic carbon (OC), carbon:nitrogen ratio (C:N), and total nutrient of the substrate [19, 21, 22]. These parameters determine nutrient availability and the development of seedlings, contributing to more sustainable and accessible agricultural practices for the production of plants in containers. Therefore, the objective of this study was to characterize local organic waste and the resulting regional substrates, evaluating their usefulness in the production of plants in containers.

MATERIALS AND METHODS

Study Area

Waste was collected from the Prolade S.A.P.I. de C.V. oil palm extractor and the Presidente Benito Juárez (IPBJ) – Impulsora sugar mill, located in the municipality of Huimanguillo, Tabasco. In addition, wood waste was collected from the "Maderas COCONÁ" lumberyard and cocoa waste came from the Ranchería Nicolás Bravo cocoa plantation, both located in the municipality of Teapa, Tabasco. Finally, the processing and characterization of the waste and the substrates were carried out in the Laboratorio de Ciencia Animal and the Laboratorio Central in the Área de Instrumentación Analítica of the Colegio de Postgraduados - Campus Tabasco.

Organic Waste

The waste evaluated as potential components of regional substrates were: oil palm empty fruit shells, palm kernel shell charcoal, sugarcane bagasse, cocoa pod husk, cedar sawdust, eucalyptus bark, and coconut fiber. In addition, COSMOPEAT® peat moss —the main component of the commercial control substrate— was partially and totally replaced in the regional substrate treatments. The waste was open-air dried, chopped, and sieved in a 5.0 mm mesh before it was processed.

Physical and Chemical Properties of Waste and Substrates

The physical and chemical properties chosen to characterize the waste and substrates were based on previous studies that proved their important role in the quality evaluation

of substrates used in the production of plants in containers [21, 23, 24, 25]. The values of each property were determined based on the averages from the three replicates established for each type of organic waste and substrate.

The apparent density (AD) of the organic waste was determined following the NMX-FF-109-SCFI-2008 Mexican standard, which establishes the quality characteristics and specifications of vermicompost [26]. The procedure consisted of drying the sieved sample (5.0 mm mesh) in an oven, at 70±5 °C for 24 h. Five-point-cero and 10 g (W) of the samples were used. The sample was poured into a 100 mL graduated cylinder, wrapped in a dampened cloth over a firm base. The cylinder was hit twenty times on the side, at 10-20 cm height, with a frequency of one blow per second. Subsequently, the final volume (V) of the sample was measured and the following calculation was made:

$$AD = \frac{W(\mathrm{g})}{V(\mathrm{cm}^3)}$$

The process described by Landis *et al.* was used to determine the total porosity (TP), aeration porosity (AP), and water retention porosity (WRP) of the substrates [27]. The different substrates were placed in 160 mL sealed cells. Water was slowly poured into each substrate, until it was completely saturated (the surface became bright). The total water added to the substrates was recorded (total pore volume). Subsequently, the seal of each cell was removed and the freely-drained water was collected. The volume of drained water was measured (aeration pore volume). The TP, AP, and WRP were determined with the following formulas:

$$TP(\%) = \frac{total\ volume\ of\ pores(\mathrm{mL})}{container\ volume(\mathrm{mL})} * 100$$

$$AP(\%) = \frac{volume\ of\ aeration\ pores(\mathrm{mL})}{container\ volume(\mathrm{mL})} * 100$$

$$WRP(\%) = total\ porosity(\%) - aeration\ porosity(\%)$$

A Hanna® Instruments handheld potentiometer and conductivity meter was used to measure the pH and electric conductivity (EC) of organic waste and substrates, with a solution volume ratio of 1:2 (substrate:distilled water) [28, 29].

The calcination method was used to determine organic matter (OM) and organic carbon (OC) [26]. One-point-five grams of sample were weighted and placed in a previously dried and weighted porcelain crucible. Subsequently, the crucibles were placed for three hours in a muffle that had been previously warmed until it reached 550 °C. Afterwards, the crucibles were cooled in a desiccator and weighted. The ash OM, and OC percentages were calculated. The 1.724 factor proposed by Van Bemmelen was used to convert OM into OC [30].

$$Ash\% = \frac{Weight\ of\ the\ crucible\ with\ ashes\left(\mathrm{g}\right) - Weight\ of\ the\ crucible}{sample\left(\mathrm{g}\right)} * 100$$

$$OM\% = 100 - Ash\%$$

$$OC\% = \frac{OM\%}{1.724}$$

Nitrogen (N) concentration was determined with the semimicro Kjeldalh method, with sulfuric-salicylic acid for the digestion [31]. Zero-point-fifteen grams of the sample were crushed and sieved with a 2.0 mm diameter sieve (mesh 10). The percentages of OC and N were divided to determine the carbon:nitrogen ratio (C:N).

The nutrient concentration of the substrates was measured by wet digestion with the perchloric acid ($HClO_4$) and nitric acid (HNO_3) digestion mix (2:1 ratio) [32]. The extracts were read in a PerkinElmer Inc. AANALYST™ 700 high-performance atomic absorption spectrometer, using the Syngistix™ for AA v. 3.0.3 software.

Substrates

Seven substrates were prepared, including a control. Control was a commercial substrate with a higher proportion of peat moss and it was used in the nursery of Prolade S.A.P.I. de C.V. This material was partially or totally replaced in the other substrates. The substrates were prepared mixing the previously open-air dried waste, sieved in a 5.0 mm mesh. Twenty L of each substrate were prepared for their characterization.

Experimental Design and Statistical Analysis

The experimental design was completely randomized and included 7 regional substrates treatments, with three replicates. They were used to evaluate the responses of the abovementioned variables. An analysis of variance (ANOVA) and Tukey's Multiple Comparison Test were used to analyze the results in the InfoStat v.2020 software [33].

Production Costs of Regional Substrates

The expenses associated with the preparation of regional substrates for the production of plants were taken into account. This analysis included two main components: local waste costs and the costs of preparing the substrates. Local waste costs included the costs of the materials available in the region. Data was gathered about the price per unit of volume of each waste, depending on its availability and transportation to the processing site and manpower. The costs associated with the activities required to prepare the substrates included the collection, cleaning, processing, and mix of waste. The parameters used for this calculation were working hours and hourly rate. The costs in local currency were estimated through the sum of these two elements. This approach resulted in a valuation of the technical feasibility for the implementation of regional substrates in the production of oil palm seedlings.

Structure of the Unit Cost of the Materials Used in the Experiment

This section briefly describes the materials and the conditioning (preparation) process that generated the various substrates used in this research.

a) **Empty fruit bunches**: Provided by the Prolade S.A.P.I. de C.V. oil palm extractor. It was delivered as dry bunches, open-air dried under a shade. It was minced with a Siemens™ meat mincer (5 HP and 220 V). A worker sieved it through a 5 cm mesh for three hours. One worker operated a mini skid steer to load the waste into the container, which was then transported 9.1 km to the nursery.

b) **Oil palm kernel shell charcoal**: Provided by the Prolade S.A.P.I. de C.V. oil palm extractor. Two working days were required to load and unload the charcoal (cost of the working day: MXN$281.00 (USD$16.72)). It was transported in a 1 tonne pick-up truck. This waste was processed with a Siemens™ meat mincer (5 HP and 220 V). One worker sieved it through a 5 cm mesh for three hours.

c) **Eucalyptus bark**: Provided by Tecnotabla by PROTEAK. It was loaded into a dump truck and transported 47.1 km to the nursery, where a worker sieved it through a 5 mm mess for three hours.

d) **Cedar sawdust**: Collected fresh, no later than 3 months after it was produced at a timber shop in Teapa, Tabasco. It was transported 121 km to the nursery, where one worker sieved it through a 5 cm mesh for 3 hours.

e) **Cocoa pod husk**: Collected from a cacao plantation harvest, located in Ranchería San Nicolás, municipality of Teapa, Tabasco. The husk was stored in sacks for six months after the harvest. It was transported 121 km to the nursery and was open-air dried under a shade, before it was sieved using a 5 cm mesh.

f) **Coconut fiber**: Bought online. It consisted of 50% coconut fiber and 50% coconut bran. The 20 L were decompressed after the package was opened. No additional processing was required and it was transported 19.7 km to the nursery.

g) **Peat moss**: Bought online. It recorded a 0.11 g cm^{-3} AD and 45-50% moisture content. It did not require additional processing and it was transported 19.7 km to the nursery.

h) **Perlite**: Bought online. It had a 34-65% porosity, 63% water retention, and 1.5-2.3 mm granules. It did not require additional processing and it was transported 19.7 km to the nursery.

i) **Vermiculite**: Bought online. Granules with fine and coarse particles. It did not require additional processing and was transported 19.7 km to the nursery.

RESULTS AND DISCUSSION

Physical and chemical properties of organic waste

Table 1 shows the physical and chemical characteristics of the diverse types of organic waste. The AD of peat moss (0.11 g cm^{-3}) is statistically similar to the AD of empty fruit bunches and eucalyptus bark. The AD of peat matches the results of Estrada-Botello *et al.* [34] (0.12 g cm^{-3}), although the value of empty fruit bunches in their study (0.24 g cm^{-3}) was higher than the value reported in this research (0.14 g cm^{-3}). Oil palm kernel shell

Table 1. AD, pH, EC, OM, and OC value of the evaluated waste.

Organic wastes	AD g cm^{-3}	pH	EC dS m^{-1}	OM	OC	N	C/N
				%			
Peat moss	0.11^{d}	5.23^{e}	0.80^{c}	69.10^{g}	40.08^{g}	0.83^{d}	48.06^{d}
Empty fruit bunches of palms	0.14^{d}	7.67^{a}	1.16^{b}	91.68^{cd}	53.18^{cd}	2.01^{b}	26.62^{e}
palm kernel shell charcoal	0.48^{a}	7.49^{a}	0.39^{d}	95.47^{b}	55.38^{b}	0.78^{d}	71.31^{c}
sugarcane bagasse	0.08^{e}	6.28^{bc}	0.38^{d}	91.10^{d}	52.84^{d}	0.42^{e}	127.82^{b}
Cocoa pod husk	0.34^{b}	5.42^{de}	1.27^{b}	75.15^{f}	43.59^{f}	2.58^{a}	16.68^{e}
Coconut fiber	0.07^{e}	6.03^{bc}	3.28^{a}	94.52^{bc}	54.82^{bc}	0.48^{e}	115.41^{b}
Eucalyptus bark	0.14^{d}	6.34^{b}	0.34^{d}	82.92^{e}	48.10^{e}	1.06^{c}	44.61^{d}
Cedar sawdust	0.17^{c}	5.83^{cd}	0.35^{d}	98.53^{a}	57.17^{a}	0.19^{f}	297.91^{a}

AD: apparent density. pH: potential of hydrogen. EC: electric conductivity. OM: organic matter. OC: organic carbon. N: nitrogen. C:N: carbon:nitrogen ratio. Means that share the same letter in the same column are not significantly different ($p>0.05$).

charcoal had the greatest AD among all the waste types (0.48 g cm^{-3}) and consequently a greater weight. For its part, cocoa pod husk had a 0.34 g cm^{-3} density, which makes it the second heaviest type of waste. These findings are different from the values reported by Sánchez-Hernández *et al.* [35] (0.676 g cm^{-3}) and Estrada-Botello *et al.* [34] (0.27 g cm^{-3}) for this type of waste in Tabasco.

Meanwhile, coconut fiber and sugarcane bagasse were the waste types with the lowest ADs, indicating that they are lighter than the other analyzed materials. Sánchez-Hernández *et al.* [35] reported a significantly higher AD (0.42 g cm^{-3}) for sugarcane bagasse, which could be attributed to differences in waste management.

The pH values of the analyzed waste have significant differences. The pH values of oil palm kernel shell charcoal (7.49) and empty fruit bunches (7.67) were close to neutrality. Cocoa pod husk and peat were the most acid waste. A clear variability can be seen in the pH results of cocoa pod husk (5.42) reported by other authors. Sánchez-Hernández *et al.* [35] recorded a 6.4 pH, suggesting a slightly acid original environment. For their part, Estrada-Botello *et al.* [34] reported a significantly higher pH (7.4), which indicates that cocoa pod husk waste was practically neutral. Likewise, Palma-López *et al.* [36] recorded a 6.9 pH a greater alkaline level than the results of this research. According to the NOM-021-SEMARNAT-2000 official Mexican standard [37], peat moss, cocoa pod husk, eucalyptus bark, coconut fiber, sugarcane bagasse, and cedar sawdust had a moderately acid pH (5.1-6.5), while empty fruit bunches and oil palm kernel shell charcoal had a moderately alkaline pH (7.4-8.5). These differences can be explained by several factors, including cultivation conditions, cocoa pod husk management, and the geographical origin of the samples. The variable pH of cocoa pod husk depends on regional agricultural practices —*e.g.*, the application of Bordeaux mixture (calcium and sulphur) as a health control measure.

Eucalyptus bark and cedar sawdust recorded the lowest EC values: 0.34 dS m^{-1} and 0.35 dS m^{-1}, respectively. Oil palm kernel shell charcoal (0.39 dS m^{-1}) and sugarcane bagasse (0.38 dS m^{-1}) had statistically similar values. For its part, coconut fiber (3.28 dS

m^{-1}) stood out as the waste with the highest salt concentration. The EC of coconut fiber (3.28 dS m^{-1}) was significantly higher than the results of Gayosso-Rodríguez *et al.* [38], who recorded a 1.49 dS m^{-1} EC for this material, indicating potential differences in its origin or in the preparation of the samples.

The N concentrations of the waste recorded a clear variability (0.19-2.58%). Cocoa pod husk and empty fruit bunches had the highest N concentrations, which explains their low C:N ratio. In contrast, cedar sawdust recorded the lowest N concentration and the highest C:N ratio (297.91) among the evaluated materials. This result suggest that this type of waste has a limited mineralization capacity. Consequently, cedar sawdust is not be the best material for the application of N to the substrate. Regional waste had higher organic matter values than peat, indicating a higher carbon availability (Table 1). This high content of organic matter can improve the quality of substrates. Meanwhile, N is an essential structural constituent of all proteins and chlorophyll [39]. Its presence in substrates is key to guarantee the adequate development of plants. The C:N ratio plays a key role in the regulation of the decomposition rate of organic matter and the availability of nutrients in the substrate. The most popular waste used for composting (*i.e.*, empty fruit bunches and cocoa pod husk) recorded the lowest C:N ratio, while fresh cedar sawdust had the highest value among the evaluated waste.

Physical and Chemical Properties of Regional Substrates

Substrate formulations based on regional materials are a sustainable alternative for the production of plants in containers. Several local materials were chosen as substrate components in this study (Table 2). The ratios are connected to the substitution of the components of the commercial substrate, according to their function.

Seven substrates were formulated (control and S1-S6). The control substrate was evaluated as a commercial substrate, given its high ratio of peat moss. This commercial substrate is currently used in "La Razón" nursery of Prolade S.A.P.I. de C.V. to produce oil palm seedlings in the pre-nursery stage. A higher ratio of regional waste was used in substrates S1-S6, complemented with lower volumes of other materials, in order to improve physical and chemical properties and to favor the adequate growth and development of plants grown in containers (cells).

Table 2. Materials used in the formulation of seven substrates and their volumetric ratio.

Substrates	Peat moss	Perlite	Vermiculite	Empty fruit bunches of palms	Cedar sawdust	palm kernel shell charcoal	Cocoa pod husk	Eucalyptus bark	Coconut fiber
Control	3	0	0	0	0	0	0	2	0
S1	1	0.5	0.5	3	0	0	0	0	0
S2	0	0	0	3	1	0.5	0.5	0	0
S3	1	0	0	0	3	0.5	0.5	0	0
S4	0	0	0	0	1.5	0.5	3	0	0
S5	0	0	0	0	1	0	1	3	0
S6	0	0	0	0	1	0	1	0	3

Tables 3 and 4 show the physical and chemical characteristics of the substrates. S4 was the formulated substrate with the highest AD. Additionally, only substrates S1 and S6 recorded statistically similar AD. Abad *et al.* [17] reported that plants grown on the open air must be cultivated in substrates with 0.50-0.75 g cm^{-3} AD. Meanwhile, AD in nurseries (where wind is not a limiting factor) can be as low as 0.15 g cm^{-3}. The AD of the substrates formulated for this study are lower than the recommended density.

TP was statistically similar in the control and S4. Both substrates recorded the highest values, which complied with the recommended values (RV). Meanwhile, substrates S1, S2, S4, S5, and S6 had statistically similar AP values. S3 recorded the lowest AP, while the control had the highest value and was the only substrate to reach the RV. The PA values of the substrates were below the recommended range (20-35%). However, Bowman and Paul and Cabrera [40, 41] suggest that the optimal AP ranges from 10 to 20% although these values belong to substrates commonly used to produce ornamental plants in pots. Regarding the capacity of substrates to retain water in their pores after irrigation, all the evaluated substrates fall within the recommended WRP values (25-55%) [27]. WRP varied between formulated substrates. S4 recorded the highest WRP (49.80%), surpassing the control and the remaining substrates. All substrates complied with the RV for WRP.

Out of the seven substrates evaluated, only two (S1 and S2) had a high pH (>7), according to the classification proposed by Warnecke and Krauskopf [28]. This phenomenon could be attributed to their higher ratio of empty fruit bunches (Tables 2 and 3). The pH of the other substrates fell within the 5.0-6.5 range recommended by Warnecke and Krauskopf [28] and Landis *et al.* [27]. Meanwhile, the EC level of the waste with greater ratio in the substrate diminished when it was mixed with other materials, consequently falling within the value recommended (<1.00 dS m^{-1}) by Landis *et al.* [27]. The EC value of S6 was

Table 3. Physical and chemical characteristics of the evaluated substrates.

Substrates	AD g cm^{-3}	TP	AP	WRP	pH	EC dS m^{-1}	OM	OC	C/N
		%					%		
Control	0.11^{d}	66.46^{a}	20.6^{a}	45.86^{ab}	6.13^{d}	0.36^{c}	68.65^{e}	39.82^{e}	40.28^{b}
S1	0.13^{cd}	58.59^{b}	19.70^{ab}	38.89^{cd}	7.21^{b}	0.81^{b}	66.43^{e}	38.53^{e}	26.56^{d}
S2	0.16^{b}	50.20^{e}	16.77^{ab}	33.43^{d}	7.60^{a}	0.78^{b}	83.22^{c}	48.27^{c}	26.85^{d}
S3	0.11^{b}	53.54^{cd}	13.54^{b}	40.00^{bc}	6.59^{c}	0.25^{c}	89.31^{a}	51.81^{a}	67.61^{a}
S4	0.31^{a}	66.46^{a}	16.67^{ab}	49.80^{a}	5.49^{f}	0.72^{b}	74.58^{d}	43.26^{d}	26.73^{d}
S5	0.16^{bc}	51.41^{de}	16.77^{ab}	34.65^{cd}	5.87^{e}	0.22^{c}	85.56^{bc}	49.63^{bc}	35.26^{c}
S6	0.13^{cd}	54.95^{c}	17.07^{ab}	37.88^{cd}	5.82^{e}	1.08^{a}	87.10^{ab}	50.52^{ab}	40.66^{c}
RV		60-80	20-35	25-55	5.5-6.5	<1.0			50-70

AD: apparent density. TP: total porosity. AP: aeration porosity. WRP: water retention porosity. pH: potential of hydrogen. EC: electric conductivity. OM: organic matter. OC: organic carbon. (C/N): carbon:nitrogen ratio. Control (experimental control): peat moss:eucalyptus bark (3:2). S1: empty fruit bunches:peat moss:perlite:vermiculite (3:1:0.5:0.5). S2: empty fruit bunches:cedar sawdust:palm kernel shell charcoal:cocoa pod husk (3:1:0.5:0.5). S3: cedar sawdust:peat moss:palm kernel shell charcoal:cocoa pod husk (3:1:0.5:0.5). S4: cocoa pod husk:cedar sawdust:palm kernel shell charcoal (3:1.5:0.5). S5: eucalyptus bark:cocoa pod husk:cedar sawdust (3:1:1). S6: coconut fiber:cocoa pod husk:cedar sawdust (3:1:1). RV: recommended values for substrates used to produced forest species in trays [27, 19]. Means that share letters in the same column are not significatively different ($p>0.05$).

slightly higher (1.08 dS m^{-1}). However, according to Abad *et al.* [42], the acceptable content of soluble salts in substrates can reach up to 1.5 dS m^{-1}. For their part, Warnecke and Krauskopf [28] pointed out that the adequate EC range in substrates fluctuates between 1.0 and 2.0 dS m^{-1}.

OM and OC contents were similar between control and S1. However, the other substrates recorded a higher OC content. S3 recorded the highest OC content, although it also had the lowest N content (Table 4) and the highest C:N ratio among all the substrates. This situation would limit the absorption of N by the plants. All the substrates complied with the C:N ratio values included in the criteria set forth by Landis *et al.* [27]. With a balanced C:N ratio (50:70), microorganisms can efficiently mineralize organic matter, releasing essential nutrients that are then absorbed by the plants. S4 recorded a higher micronutrient and OC content than the control; among all the evaluated substrates, it had the lowest pH (5.49). For their part, S5 and S6 had statistically similar pH values (5.87 and 5.82, respectively). Additionally, these two substrates recorded a higher micronutrient content than control. Given its impact on nutrient availability (particularly micronutrients), pH is a critical factor in mineral soils. Under extreme pH conditions, certain nutrients can become hard to reach or even toxic. Organic soils have a greater availability of nutrients at a lower pH (≈5.5.) than mineral soils, which have a maximum pH availability of ≈6.5 [27]. This difference could explain the higher concentration of micronutrients and OM in S4 —whose pH is closer to the optimal level for organic soils. Controlling pH in organic crop environments is of great importance.

Substrates S1, S2, S4, S5, and S6 had a higher N and K concentration than the control. For its part, the higher acidity of S4 was likely the cause of its micronutrient concentration —the highest among all substrates.

Economic evaluation of the production of regional substrates

Table 5 shows the costs associated with organic waste. Costs were calculated based on an exchange rate of MXN$16.80 pesos per US dollar (April 2024).

Table 4. Average nutrient concentration in the evaluated substrates.

Substrates	N	P	K	Mg	Ca	Fe	Mn	Zn	Cu
	(%)					(mg kg^{-1})			
Control	1.00^{e}	0.33^{ab}	0.12^{b}	2.20^{a}	0.87^{c}	8713.64^{b}	168.04^{de}	24.42^{e}	10.32^{f}
S1	1.46^{c}	0.44^{a}	0.72^{a}	2.13^{a}	1.19^{b}	8557.40^{b}	171.88^{d}	26.88^{e}	47.65^{b}
S2	1.80^{a}	0.29^{bc}	0.76^{a}	0.32^{c}	1.32^{a}	4699.88^{c}	151.66^{ef}	35.45^{d}	49.91^{b}
S3	0.76^{f}	0.09^{d}	0.21^{b}	0.68^{b}	0.64^{d}	4399.22^{c}	140.30^{f}	23.66^{e}	17.015^{e}
S4	1.63^{b}	0.17^{cd}	0.23^{b}	0.37^{bc}	0.73^{d}	14793.21^{a}	632.29^{a}	114.17^{a}	55.82^{a}
S5	1.41^{c}	0.12^{d}	0.15^{b}	0.25^{c}	0.94^{c}	8024.80^{b}	338.15^{c}	61.13^{c}	28.83^{d}
S6	1.25^{d}	0.13^{d}	0.74^{a}	0.32^{c}	0.67^{d}	8773.42^{b}	394.99^{b}	82.96^{b}	39.70^{c}

N: nitrogen. P: phosphorous. K: potassium. Mg: magnesium. Ca: calcium. Fe: iron. Mn: manganese. Zn: zinc. Cu: copper. Control (experimental control): peat moss:eucalyptus bark (3:2). S1: empty fruit bunches:peat moss:perlite:vermiculite (3:1:0.5:0.5). S2: empty fruit bunches:cedar sawdust:palm kernel shell charcoal:cocoa pod husk (3:1:0.5:0.5). S3: cedar sawdust:peat moss:palm kernel shell charcoal:cocoa pod husk (3:1:0.5:0.5). S4: cocoa pod husk:cedar sawdust:palm kernel shell charcoal (3:1.5:0.5). S5: eucalyptus bark:cocoa pod husk:cedar sawdust (3:1:1). S6: coconut fiber:cocoa pod husk:cedar sawdust (3:1:1) Means that share letters in the same column are not significatively different ($p>0.05$).

Table 5. Costs of organic waste substrate (including costs of the waste, transportation to the nursery and preparation).

Organic wastes	Cost per liter (MXN L^{-1})	Cost per liter (USD L^{-1})
Empty fruit bunches of palm	$0.03	0.0018
Palm kernel shell charcoal	$1.39	0.0827
Eucalyptus bark	$0.71	0.0422
Cedar sawdust	$2.50	0.1488
Cocoa pod husk	$7.79	0.4636
Coconut fiber	$7.75	0.4613
Peat moss	$12.62	0.7511
Perlite	$13.55	0.8065
Vermiculite	$22.65	1.3482

Exchange rate: USD$1 = MXN$16.80 (April 2024).

Unit Cost per Liter of Regional Substrates

The study cost included the abovementioned materials and the values of the substrate mixes. This analysis recorded a variability in the prices of substrates (Table 6). With a higher empty fruit bunches ratio, S2 was the most economical substrate, at a cost of MXN$1.44 L^{-1}. In contrast, the control —which had more peat moss than S1-S6— was the most expensive substrate, at a cost of MXN$7.86 L^{-1}. The prices of substrates S1 (MXN$6.16 L^{-1}) and S6 (MXN$6.71 L^{-1}) were closer to the prices of the control substrate.

Table 6. Costs of the regional substrates used in the experiment at Huimanguillo, Tabasco.

Substrates	Control	S1	S2	S3	S4	S5	S6
Cost per liter (MXN L^{-1})	$7.86	$6.16	$1.44	$4.94	$5.56	$2.48	$6.71
Cost per liter (USD L^{-1})	$0.4678	$0.3666	$0.0857	$0.2940	$0.3309	$0.1476	$0.3994

Control: peat moss:eucalyptus bark (3:2). S1: empty fruit bunches:peat moss:perlite:vermiculite (3:1:0.5:0.5). S2: empty fruit bunches:cedar sawdust:palm kernel shell charcoal:cocoa pod husk (3:1:0.5:0.5). S3: cedar sawdust:peat moss:palm kernel shell charcoal:cocoa pod husk (3:1:0.5:0.5). S4: cocoa pod husk:cedar sawdust:palm kernel shell charcoal (3:1.5:0.5). S5: eucalyptus bark:cocoa pod husk:cedar sawdust (3:1:1). S6: coconut fiber:cocoa pod husk:cedar sawdust (3:1:1). Exchange rate: USD$1 = MXN$16.80 (April 2024).

Seedling production depends on two key factors: the cost and the availability of the materials required to formulate the substrates [43]. An ideal substrate must have characteristics that favor the species to be produced and should be easily available in the vicinity of the nursery, in order to minimize labor and costs [44]. Therefore, in the case of the regional substrates analyzed in this study, S2 had the highest ratio of empty fruit bunches and S5 had the highest ratio of eucalyptus bark. Both are the most inexpensive substrates, due to their regional availability.

CONCLUSIONS

The organic waste available in Tabasco has the potential to be used as substrate in the production of plants grown in containers. The physical and chemical properties of the

substrates are closely related to the characteristics of each type of waste, which depend on its origin and handling.

Some properties of the evaluated substrates are similar to the characteristics of the commercial substrate (peat:eucalyptus bark (3:2)) that was used as control for this study. Specifically, S5 (eucalyptus bark:cocoa pod husk:cedar sawdust (3:1:1)) had the highest number of variables that where statistically similar to the commercial substrate. Additionally, S1 (empty fruit bunches:peat:perlite:vermiculite (3:1:0.5:0.5)) and S4 (cocoa pod husk:cedar sawdust:palm kernel shell charcoal (3:1.5:0.5)) recorded a higher concentration of micronutrients than the commercial substrate. Choosing to use regional substrates is mainly based on the ease with which the waste they are composed of can be acquired, as well as their physical and chemical properties, which can be applied for the production of plants in containers.

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The cultivation, uses, and festivities related to native maize (*Zea mays* L.) in the community of La Virgen, Salvatierra, Guanajuato

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ABSTRACT

Objective: To generate knowledge about the use of native maize and to identify the challenges, opportunities, and strategies for its conservation in an agrarian community.

Design/Methodology/Approach: A mixed-method approach was applied. The qualitative approach included participatory workshops, group discussions, and field visits. The quantitative approach involved a questionnaire applied to 40 key stakeholders which covered socioeconomic aspects, agricultural practices, and conservation strategies. Data were analyzed using basic statistical methods.

Results: Three types of native maize were identified: white, red, and black. White maize is the most widely cultivated and consumed, followed by red and black maize. Seeds are inherited from one generation to another, reflecting continuity in traditional practices. Recurrent droughts have limited grain production. Maize plays a fundamental role in local festivities, such as the Misa del Buen Temporal (Mass for Good Weather), although the abandonment of the Eménguaro feast highlights changes in community identity.

Study Limitations/Implications: The results are applicable to a single community.

Findings/Conclusions: The conservation of native maize reflects resistance to social pressures and current consumption trends. Although festivities linked to maize have undergone transformations, this crop remains important in the local diet. The findings highlight the importance of developing local strategies that integrate lore and agroecological approaches.

Keywords: maize, conservation, traditions, Salvatierra.

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INTRODUCTION

Mexico is the center of origin and diversification of maize (*Zea mays* L.). It concentrates the greatest diversity of maize races in the world (CONABIO, 2020a; Torres Morales *et al.*, 2022). In addition to its nutritional value, maize is a cultural symbol and holds historical significance due to its uninterrupted cultivation over approximately 350 generations (CIMMYT, 2020; SADER, 2016; Sánchez, 2014).

Maize contributes significantly to food self-sufficiency and is predominantly produced and preserved within smallholder family units operating under rainfed agricultural systems (Ramírez-Maces *et al.*, 2023). These households produce nearly 70% of the food consumed in Mexico (Hernández & Alcaraz, 2020; Ruiz-Serrano *et al.*, 2022; SEMARNAT, 2017).

Mexico records 64 recognized maize races (CONABIO, 2020b; Rangel-Lucio *et al.*, 2021; SADER, 2023), which are the result of biologically-induced variability, shaped by rural and Indigenous populations. Over the past 10,000 years, these communities have selected seeds according to their cultural and culinary preferences. More than 700 traditional maize-based dishes have been documented, characterized by a wide range of colors and flavors that contribute to the collective identity of several territories (Jönsson, 2024).

Due to the diversity of native varieties, maize remains a staple food in the state of Guanajuato (Baez Montes *et al.*, 2012). Fifteen native maize races have been reported in the region; *Cónico Norteño*, *Celaya*, and *Elotes Occidentales* are the most prevalent and widely used varieties (Aguirre *et al.*, 2000; Lazos & Chauvet, 2012; Ortega *et al.*, 2014).

Peciado-Ortiz *et al.* (2009) documented the presence of the *Cónico Norteño* and *Bolita* races in the municipality of Salvatierra, Guanajuato. However, both CONABIO (2020c) and Peciado-Ortiz *et al.* (2009) report that *Celaya* is currently the only remaining native race, raising concerns about the loss or replacement of local maize diversity.

The Bajío region of Guanajuato is one of the most productive areas for grain and vegetable cultivation under intensive agricultural models (Cárdenas-Bejarano *et al.*, 2023). However, this productivity is also linked to significant environmental costs, accounting for at least 80% of deforestation and biodiversity loss in various areas (Reyes Palomino and Cano-Ccoa, 2022). These intensive models have replaced and transformed traditional agricultural systems, despite their ecological, social, and cultural consequences (Gil-Méndez and Vivar-Arenas, 2015). This transition entails the substitution of native varieties with high-yield crops, even though native varieties remain fundamental to rainfed agricultural systems.

In recent years, climatic events and increasing migration processes have further threatened the production and conservation of these genetic resources (Segura-Nieto and Cueva-Torres, 2012). This study explores how local agricultural practices and lore contribute to the preservation of native maize genetic resources. It also examines the impact of agricultural modernization and the erosion of traditional practices. The objective was to generate knowledge about the current use of native maize within rainfed production systems in a rural community.

MATERIALS AND METHODS

This study was conducted in the community of La Virgen, municipality of Salvatierra, state of Guanajuato, Mexico (100° 54' 22.304" W, 20° 08' 29.974" N).

The mixed-method approach chosen for this research incorporated both qualitative and quantitative components. The qualitative approach included participatory workshops, focus group discussions, and field visits to maize cultivation plots. These methods enabled an in-depth exploration of traditional agricultural practices, the perceptions of farmers, and the challenges related to the conservation of native maize.

The quantitative component involved a structured survey with 40 key stakeholders —80% of the active *ejidatarios* (communal landholders) who grow maize. The questionnaire collected information on socioeconomic characteristics, risks and opportunities associated with native maize production, technical management practices, and conservation strategies. The data were analyzed in Excel spreadsheets (Microsoft Office, 2016) using frequency distributions, percentages, and trends.

Maize ears from the native types were collected and their morphometric traits were characterized, including ear length, number of rows, grains per row, grains per ear, ear weight, and the weight of 100 grains. The data obtained from the ears were subjected to an analysis of variance (ANOVA). When significant differences were found ($P<0.05$), mean comparisons were conducted using Tukey's test.

RESULTS AND DISCUSSION

Characteristics of the *Ejidatarios*

The results provided a detailed overview of native maize production and conservation, as well as the agricultural practices and their connection to local traditions. In average, the interviewees were 65 years-old, with an age range of 26 to 88 years. This demographic trend involves a critical issue: while older adults play a key role in maintaining agricultural knowledge and traditions, the aging farming population poses a significant risk to the continuity of native maize cultivation and the related sociocultural practices. In the territory of Salvatierra, climatic, economic, and productivity-related risks are major drivers of youth migration, because younger generations migrate to meet basic needs and pursue alternative economic opportunities (Gómez and Tacuba, 2017).

Seventy percent of the interviewees were men and 30% were women. Nevertheless, women play a crucial role, both at home and in the maize plots. Women possess deep knowledge about the cultivation, processing, and culinary use of native maize (Maldonado and García, 2023). In fact, women have been the main pioneers in the conservation of maize. Every day, they are in charge of nixtamalization, grinding, and preparing food (mainly tortillas). Their involvement in food production contributes not only to food security of their households, but also to the diversification of their income (FAO, 2024).

Types of Maize

Ejidatarios differentiate between native maize varieties by the color of their grain. These varieties do not have specific names: they are identified solely by color, reflecting a simplified classification of local native maize. White maize is cultivated by 49% of the *ejidatarios*, followed by red maize (39%) and black maize (13%) (Figure 1). The cultivation of each type is closely tied to family culinary preferences, as well as to certain properties that extend its storage potential. White maize is more resistant to insect damage during storage.

Table 1 shows the characteristics of the ears from the three native maize types. Ear length was the only trait that showed statistically significant differences among the three types. Red maize had the longest ears; their length was statistically similar to the length of the ears of white maize. Although the remaining traits did not show significant differences,

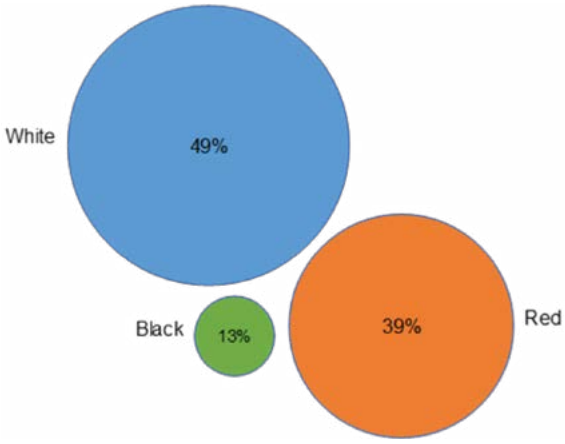

Figure 1. Types of native maize grown in La Virgen, Salvatierra, Guanajuato, Mexico.

Table 1. Morphological characteristics of ears from native maize grown in La Virgen, Salvatierra, Guanajuato, Mexico.

Type of maize	Length (cm)	Number of rows	Grains per row	Grains per ear	Ears weight (g)	100 grains weight (g)
White	15.1 ab	10.7	31.3	309.3	134.9	33.8
Red	17.8 a	8.5	29.3	248.5	129.0	44.3
Black	13.6 b	10.7	28.8	314.5	115.5	34.8

Different letters show significant differences between treatments ($P<0.05$).

white and red maize recorded more grains per ear, greater ear weight, and greater weight of 100 grains. Although the ears were not classified by race, previous studies have reported the presence of the *Cónico Norteño* and *Bolita Cónico* in the municipality of Salvatierra (Peciado Ortiz *et al.*, 2009).

Origin of the Seeds

Three sources of origin for the maize seeds grown in the community were identified (Figure 2). Seeds are mainly inherited from family ancestors, which allows the continuity and preservation of native maize varieties. No cases of replacement or introduction of other maize types were reported. Just like in other regions of the country, this seed reproduction strategy contributes to the safeguarding of native seeds and involves approximately three million farming families in Mexico (Orozco-Ramírez *et al.*, 2017).

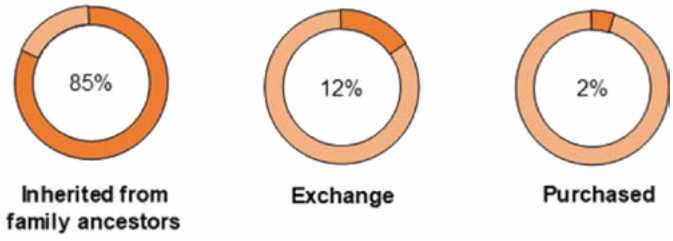

Figure 2. Origin of native maize seeds grown in La Virgen, Salvatierra, Guanajuato, Mexico.

The second alternative for the acquisition of seed is the exchange within the same community. Although this alternative is less popular, it promotes, facilitates, and ensures access to seeds for cultivation. This mechanism supports the conservation of the deeply-rooted biocultural heritage of Mesoamerican agriculture (García and Giraldo, 2021).

A smaller proportion of participants purchased seeds in the locality, perhaps due to the drought conditions experienced in the community over the past five years. *Ejidatarios* noted that 2023 was a particularly dry year, during which most of them were unable to harvest. In response to such adverse events, the local exchange or purchase of seeds has become a viable mean to ensure seed availability, thereby promoting their reproduction and conservation (Flores-Pérez *et al.*, 2024). However, in several regions of Mexico, extreme climate events have severely impacted grain production, threatening household food security and accelerating migration processes (Guzmán, 2021).

Uses of Maize in Daily Life and Festivities

At least six different forms of maize consumption were identified within the community (Figure 3). White maize is the preferred type and is primarily used to make tortillas, but it is also used to prepare atole and tamales. Five uses were reported for red maize, the second most cultivated variety, mainly in the preparation of pozole and for consumption as roasted or boiled elotes (young corn cobs). This preference is linked to its longer ears and larger grains (Table 1); additionally, it is the only variety used for pinole.

Black maize only has two main uses (tortillas and atole), which explains its low production levels. *Ejidatarios* reported that the cultivation of black maize has declined in recent years. This decline is attributed to limited access to black maize seed and to the lack of interest in the cultivation of diverse maize types. Likewise, most *ejidatarios* avoid mixing different grain types during the milling process, because it could alter the desired characteristics of the final product.

In addition to daily consumption, *ejidatarios* reported various celebrations where maize consumption plays a fundamental role (Figure 4). Maize is used in at least eight cases: five national festivities, two local celebrations, and daily consumption. Unlike

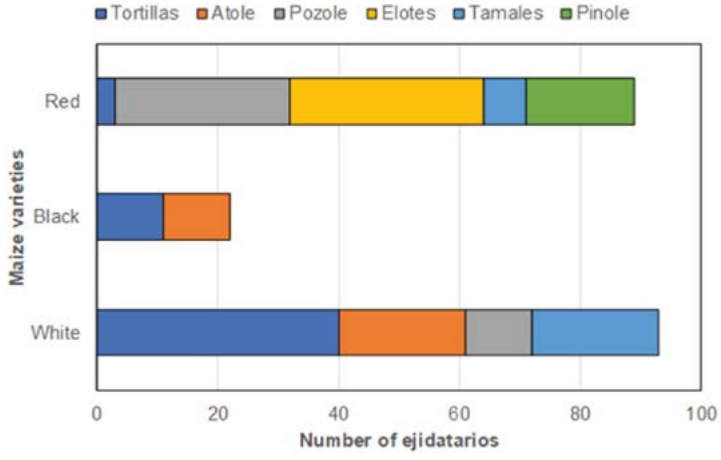

Figure 3. Common uses of native maize varieties in La Virgen, Salvatierra, Guanajuato, Mexico.

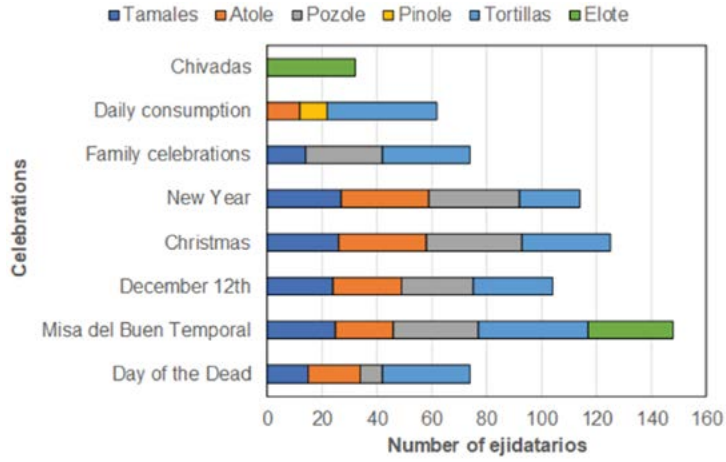

Figure 4. Uses of maize in daily consumption and festivities in La Virgen, Salvatierra, Guanajuato.

other maize-based foods, the consumption of *elotes* and *pinole* is essential during specific celebrations.

The celebrations where the greatest number of *ejidatarios* consume maize in up to four different ways are the Misa del Buen Temporal, Christmas, and New Year's.

Regarding *elotes*, they are mainly used for *chivadas* (a local term referring to roasted corn cobs). This event takes place during the last weeks of August and throughout September, often simultaneous with the feast day of Saint Michael the Archangel on September 29th. During this time, families gather near their fields to share food and roast *elotes* —mostly red maize varieties, which are usually the sweetest.

Pinole tends to be part of the daily diet for some individuals, predominantly older adults. Younger generations have just begun to consume this maize product. Initial discussions with community members revealed two main celebrations where native maize varieties play a significant role.

The first of these celebrations, which unfortunately is no longer held, is the Eménguaro festival, celebrated on September 21st. This event consisted of a pilgrimage from the community of La Virgen to San Miguel Eménguaro, one of the main communities in the municipality, due to its historical and cultural significance. San Miguel Eménguaro ("Place of Early Maize" in Purépecha) is remarkable for the influence of agricultural traditions, especially maize production.

During this festival, the participating communities, including La Virgen, would offer their harvests, flowers, and food as signs of gratitude and devotion. However, a dispute between the two communities led to "*La Virgen distancing itself from Eménguaro*," marking a deep shift in cultural identity and traditional practices. After this break-up, La Virgen dropped "de Eménguaro" from its name and became "La Virgen," thereby ending its participation in the pilgrimages. This change not only caused a rupture in inter-community relations, but also resulted in a significant loss of rituals and cultural expressions related to native maize.

The second festivity, which is still celebrated to this day and where maize plays a significant role, is the "Misa del Buen Temporal." This event features the greatest variety

of maize uses (Figure 4). The celebration takes place on November 2nd to honor the Señor del Socorro and is an expression of gratitude and faith for the harvests of that year. During the festival, the population participates in processions to the main churches, where special masses are held. However, this celebration has deeper roots in the San Juan neighborhood, where five arches are constructed with a Christ figure in the center, decorated with all the harvest products found in the municipality, with colored maize (black and red) playing a prominent role.

In conclusion, despite changes and the loss of certain celebrations, such as Eménguaro, native maize continues to hold a place in the culture and traditions of the community. The continuation of celebrations like the Misa del Buen Temporal underscores the desire of the people of La Virgen to maintain their connection with their culture and spirituality.

No municipal program is specifically focused on the promotion and conservation of native maize. However, maize-related gastronomy is promoted in certain events. The first is the tamale and atole fair held in February. This fair offers a wide variety of traditional tamales and beverages from the region. In addition, this fair includes live music, talks with tamale makers, and tastings of tamales and atoles prepared with maize dough in various flavors.

The second event is the "Festival de la larga y la quesadilla," held in June, where local women gather in the municipal seat to prepare *largas* and quesadillas, which are consumed with typical local stews. However, *largas* are no longer prepared according to the original recipe. The traditional method to prepare this dish has changed; consequently, what is currently offered is closer to a taco, reflecting a cultural transformation in the region.

Furthermore, the dough used to prepare *largas* and quesadillas usually comes from local tortilla shops, which often use hybrid maize rather than native varieties. These events promote local gastronomy, boost the local economy, and are a fundamental part of the culture and collective identity (Baez Montes *et al.*, 2012; Rangel-Lucio *et al.*, 2021; Torres *et al.*, 2024). Encouraging the production, consumption, and dissemination of native maize consumption practices in the territory is essential for its ongoing contribution to local nutrition (Venegas-Martínez *et al.*, 2024). Beyond its role as food for the community of La Virgen, native maize is a key element in local culture. It faces major challenges related to climatic phenomena, migration, and limited incentives for its production and conservation. Nevertheless, resistance processes have enabled its continuity (Guzmán, 2021). Programs and research work could address the challenges and provide effective conservation strategies, based on the contribution of native maize to the local economy and gastronomy.

The conservation of native maize within the community of La Virgen reflects resistance to social pressures and current consumption trends. Although production and consumption practices have been maintained primarily by older *ejidatarios*, agroecological strategies should be promoted to encourage not only the preservation of maize, but also its appreciation as an essential part of local identity.

CONCLUSIONS

Although festivals, events, and traditions linked to maize have undergone transformations and losses, they show the importance of maize, not only as a food source, but also as a symbol of community cohesion. The predominance of white maize reflects both practical and cultural decisions, while the decline of black maize indicates a trend toward a simplified crop selection. Local fairs and events offer valuable opportunities to recover, preserve, and promote native maize.

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Current asparagus (*Asparagus officinalis* L.) production and vermicompost usage in Atenco, State of México, and potential of leachates for rhizome rot control

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ABSTRACT
Objective: To describe the current, agronomic, and socioeconomic situation of the production of asparagus (*Asparagus officinalis* L.) and the use of vermicompost, and to determine if vermicompost leachates can suppress asparagus rhizome rot caused by *Fusarium proliferatum*, in the municipality of Atenco, State of Mexico.
Design/Methodology/Approach: Asparagus producers from the municipality of Atenco were surveyed. Additionally, two greenhouse experiments were carried out using soil from the same area. A completely randomized design was used to evaluate 14 treatments. Non-parametric statistical tests were used to analyze the results.
Findings/Conclusions: Although it faces organizational and technical limitations, asparagus is currently a profitable crop for the producers of Atenco. The intensive use of agrochemical inputs characterizes the production system. However, an increasing number of producers have begun to explore and implement the use of biological amendments as a strategy to transition toward a more sustainable production model. Vermicompost leachates made from sheep and cow manure had the potential to promote asparagus growth in the absence of pathogens; however, they did not reduce the rhizome damage caused by *F. proliferatum*.

Keywords: Vermicompost leachates, *Fusarium proliferatum*, asparagus, rhizome rot.

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INTRODUCTION

Asparagus (*Asparagus officinalis* L.) is a valuable crop grown worldwide. It is valued for its nutritional properties and economic profitability. Mexico ranks third in global asparagus production and holds a significant share of the world export market, with the United States as the primary destination (SIAP, 2024). Asparagus production faces various agronomic challenges, including the rhizome rot caused by fungi of the genus *Fusarium*. Rhizome rot

impacts crop development and yield. Water stress and nutritional deficiencies increase the susceptibility of asparagus crops to this phytopathogen, posing a significant challenge for producers due to its detrimental effects on crop development and yield (Elmer, 2001; González-Cruces *et al.*, 2024).

Sustainable crop management strategies are required to tackle these challenges. Donohoe (2018) proposed the use of organic amendments, such as vermicompost and its by-products. Vermicompost leachates —by-products of the vermicomposting process— contain a high concentration of nutrients, humic and fulvic acids (Ávila, 2015), growth-promoting bacteria (Gudeta *et al.*, 2021), and bioactive compounds that promote plant growth. Vermicompost leachates are known to enhance soil health and suppress diseases caused by soilborne pathogens (Nadana *et al.*, 2020). While these leachates have demonstrated beneficial effects in various crops, including tomato and eggplant (Ollen, 2016; Sundararasu and Alagarmalai, 2014), their efficacy against asparagus rhizome rot has not yet been investigated.

The municipality of Atenco, State of Mexico, has adopted asparagus production as a strategy to diversify its agricultural sector, particularly on the reclaimed soils of the former Lake Texcoco. In this context, the integration of agroecological practices —such as the application of vermicompost leachates— offers a sustainable approach to enhance crop productivity and mitigate the impact of rhizome rot. This study describes the current situation of asparagus production in the municipality of Atenco. It includes the results of two experiments focused on whether or not vermicompost leachates from different organic matter sources can suppress the infection caused by *Fusarium proliferatum*, one of the causal agents of asparagus rhizome rot (Elmer, 2001; Baayen *et al.*, 2000; Hamel *et al.*, 2005).

MATERIALS AND METHODS

Questionnaire

A 63-item questionnaire was administered to a randomized sample of eight asparagus producers in the municipality of Atenco. This sample represented 50% of the total producers in the area. The questionnaire was divided into the following thematic sections: personal and demographic information, plot characteristics, general asparagus cultivation practices, management of asparagus rhizome rot and the use of vermicompost leachates, production and harvesting costs, and commercialization.

Experimental evaluation of vermicompost leachates

Two experiments were conducted to evaluate the potential of vermicompost leachates from sheep, horse, and cow manure to control *Fusarium proliferatum*, the causal agent of asparagus rhizome rot.

Vermicompost leachates production

Vermicomposting beds with a 3% slope for drainage and collection were used to produce the leachate (Domínguez and Pérez, 2011). Redworms (*Eisenia foetida*) were fed with pre-composted sheep, cow, and horse manure in separate beds. The beds were stirred

and irrigated during the six-week pre-composting process to eliminate potentially toxic components for the worms and ensure the quality of the leachate (Acosta *et al.*, 2013).

Establishment of the experiments

Fourteen treatments (Table 1) were established in two independent experiments to evaluate the effect of the application of vermicompost leachates (VCL) before and after the transplant of asparagus plants, with and without inoculation of *Fusarium proliferatum*. The experiment also included two control treatments: one inoculated but without VCL application, and a non-inoculated control that received neither inoculation nor VCL.

The treatments were applied based on a completely randomized experimental design with ten repetitions. The experiment was conducted under growing chamber conditions, with semi-controlled temperature, inside a greenhouse located at the Colegio de Postgraduados - Campus Montecillo, Texcoco, State of Mexico.

Sowing and leachate application

Seeds of the Sulken genotype of *Asparagus officinalis* L. were used in the experiment. Seeds were disinfested with 5% sodium hypochlorite and rinsed with sterile distilled water (Gómez, 2020). Subsequently, they were hydrated for 24 hours. Sowing was carried out in 200-cavity trays with peat and vermiculite substrates (2:1) and sterilized three times at 15 lb/in^2 for one hour (Nongthombam *et al.*, 2022). Three months after germination, the seedlings were transplanted into 2-L plastic bags.

Two phases of leachates were applied as follows: pre-transplant (20 mL per cavity) and post-transplant (200 mL per plant per month, for six months). Control treatments only received distilled water.

Table 1. Treatments applied to asparagus plants (*Asparagus officinalis* L.) in two independent experiments.

Treatment	Source of vermicompost leachate	Inoculation with Fusarium proliferatum	Pre-transplant application (in germination trays)	Post-transplant application
PHI	Horse	YES	YES	YES
HI	Horse	YES	NO	YES
PHNI	Horse	NO	YES	YES
HNI	Horse	NO	NO	YES
PSI	Sheep	YES	YES	YES
SI	Sheep	YES	NO	YES
PSNI	Sheep	NO	YES	YES
SNI	Sheep	NO	NO	YES
PCI	Cow	YES	YES	YES
CI	Cow	YES	NO	YES
PCNI	Cow	NO	YES	YES
CNI	Cow	NO	NO	YES
IC	INOCULATED CONTROL			
NIC	NON-INOCULATED CONTROL			

* Six applications were made per treatment, one each month.

Inoculation

The treatments were inoculated with *Fusarium proliferatum* strain P3RD, reactivated in PDA medium with oxytetracycline. After 10 days of growth in Petri dishes, a suspension was prepared with Tween 80 (0.01%) and adjusted to 1×10^6 conidia/mL. A sterile syringe was used to apply 8 mL of this suspension to the rhizome of each plant, immediately after the foliage was pruned (Miguel *et al*., 2018; González-Cruces *et al*., 2024). Foliage dry weight (FDW), root and crown dry weight (RCDW), and total spear number (TSN) were evaluated 60 days after the inoculation. Measurements were taken in the field and the laboratory using a measuring tape and an OHAUS® precision electronic scale. A forced air oven was used to dry the samples at 70 °C for 72 h (Quevedo *et al*., 2017).

Re-isolation of *Fusarium proliferatum*

To re-isolate and identify *Fusarium proliferatum* from the inoculated plants, rhizome fragments were collected, surface-disinfected, and sown in a PDA medium. The mycelium was purified after several days of incubation under continuous light (Figure 1). Fungal structures were observed under the microscope to identify the pathogen, following the recommendations of the handbook published by Leslie F. and Summerell (2006).

Statistical analysis

Statistical analysis was performed using R (v.4.3.2) (R Core Team, 2020). The Shapiro-Wilk test was used to evaluate data normality (Shapiro and Wilk, 1965). The nonparametric Kruskal-Wallis (Kruskal and Wallis, 2012) and Mann-Whitney U (Mann and Whitney, 1947) tests were applied to evaluate the planned comparisons between treatments (Table 2).

RESULTS AND DISCUSSION

Situation of the asparagus production in Atenco

In the municipality of Atenco, asparagus is cultivated by *ejidatarios* (*ejido* landowners), in plots of 1 to 6 ha. All producers started with one hectare and have gradually expanded

Figure 1. Mycelium growth from a root infected with *Fusarium proliferatum* (A). Mycelium sown using the carnation leaf method (B).

Table 2. Planned comparisons to analyze the effects of different treatments applied to asparagus plants (*Asparagus officinalis*) in two experiments involving plants with and without inoculation with *Fusarium proliferatum*, treated or not with vermicompost leachates, derived from three sources of pre-composted manure (horse, cow, and sheep).

	Comparisons	Description	Statistical test
1	IC *vs*.. NIC	Inoculation effect	Mann-Whitney U
2	IC *vs*.. PHI, PSI, PCI	Pre-transplant control effect of VCLs	Kruskal-Wallis.
3	IC *vs*. HI, SI, CI	Post-transplant control effect of VCLs	Kruskal-Wallis.
4	PHI, PSI, PCI *vs*. PHNI, PSNI, PCNI	Effect of inoculation with pre-transplant application of VCLs	Mann-Whitney U
5	HI, SI, CI *vs*. HNI, SNI, CNI	Effect of inoculation with post-transplant application of VCLs	Mann-Whitney U
6	PHI, PSI, PCI *vs*. HI, SI, CI	Effect of VCLs between pre- and post-transplant application in the presence of the pathogen	Mann-Whitney U
7	PHNI, PSNI, PCNI *vs*. HNI, SNI, CNI	Effect of VCLs between pre- and post-transplant application in the absence of the pathogen	Mann-Whitney U
8	PSI, SI *vs*. IC	Effect of sheep-derived VCL in the presence of the pathogen	Mann-Whitney U
9	PSNI, SNI *vs*. NIC	Effect of sheep-derived VCL in the absence of the pathogen	Mann-Whitney U
10	PHI, HI *vs*. IC	Effect of horse-derived VCL in the presence of the pathogen	Mann-Whitney U
11	PHNI, HNI *vs*. NIC	Effect of horse-derived VCL in the absence of the pathogen	Mann-Whitney U
12	PCI, CI *vs*. IC	Effect of cow-derived VCL in the presence of the pathogen	Mann-Whitney U
13	PCNI, CNI *vs*. NIC	Effect of cow-derived VCL in the absence of the pathogen	Mann-Whitney U
14	PSI, SI, *vs*. PHI, HI	Effect of sheep- *vs*. horse-derived VCL in inoculated plants	Mann-Whitney U
15	PSNI, SNI, *vs*. PHNI, HNI	Effect of sheep- *vs*. horse-derived VCL in non-inoculated plants	Mann-Whitney U
16	PSI, SI, *vs*. PCI, CI	Effect of sheep- *vs*. cow-derived VCL in inoculated plants	Mann-Whitney U
17	PSNI, SNI, *vs*. PCNI, CNI	Effect of sheep- *vs*. cow-derived VCL in non-inoculated plants	Mann-Whitney U
18	PHI, HI, *vs*. PCI, CI	Effect of horse- *vs*. cow-derived VCL in inoculated plants	Mann-Whitney U
19	PHNI, HNI, *vs*. PCNI, CNI	Effect of horse- *vs*. cow-derived VCL in non-inoculated plants	Mann-Whitney U

VCL=Vermicompost leachate.

their growing areas. Given the lack of formal producer associations in the area, individual producers undertake their own crop management and commercialization.

Sulken and Early California are the most commonly used seed varieties because they have adapted to the local soil. Producers mainly use chemical fertilization, although some of them combine chemical fertilizers with agroecological inputs (vermicompost), beneficial microorganisms, and commercial organic products. Arancon *et al.* (2003) and Lazcano

and Domínguez (2010) have reported significant improvements in horticultural crops by using vermicompost. However, no experimental demonstration about the benefits or improvements of such a combination for asparagus cultivation has been reported for Atenco yet. Brandenberger *et al.* (2015) emphasized that proper irrigation and fertilization management are key to maximizing asparagus yield. Practices such as the use of organic amendments can also improve soil quality and extend the productive life of crops.

The main diseases identified by producers in Atenco are rhizome rot, Cercospora leaf spot, and rust. Grasshoppers are the most common pest in the area. Chemical fungicides are used for disease management; however, some producers are initiating preventive processes with vermicompost leachates and microorganisms, and they have reported that this method is quite effective. According to Altieri and Nichols (2012), agroecology seeks to optimize agricultural systems not only in terms of production, but also in terms of their social and ecological impact. The adoption of agroecological practices (*e.g.*, the use of leachates and beneficial microorganisms) aligns with the strategies followed by some Atenco producers to improve soil fertility and crop health.

Asparagus is harvested manually. First, spears with closed, flawless bracts are selected. Then, the product is classified by size (pencil thin, small, standard, and jumbo), hydrated, and transported for its commercialization to the Central de Abastos in Mexico City. Production is higher in summer (up to 7 t/ha) than in winter (1.5-2 t/ha). However, winter is a more profitable season, because asparagus is sold at higher prices. Although some producers harvest asparagus twice a year, they agree that three harvests in two years are the best choice. Drost (2023) reported that asparagus productivity depends on the development of plant structures during the previous harvest cycle. In this sense, less frequent harvests give plants more time to replenish their reserves and strengthen their root system.

Producers are doing their sales. No middlemen are involved in this process. Despite challenges such as water access, high input costs, and labor shortages, producers believe that asparagus is a resilient and profitable crop because it has adapted to saline soils and adverse climatic conditions; due to its high quality, it has good marketing potential. Although producers are not organized, the construction of agroecological cooperation networks, to improve market access and strengthen the resilience of production systems, is still possible (Rosset and Altieri, 2017).

Establishment costs of asparagus crops

Currently, 16 producers grow asparagus at approximately 22 ha in Atenco. It takes two years to obtain the first harvest. Land preparation includes mechanized leveling, subsoiling, harrowing, plus furrowing or bed preparation. Costs of these tasks are shown in Table 3.

In addition to the mechanized work, essential activities for crop establishment and management are manually performed, including transplanting, irrigation, weed control, pest and disease management, amendment or nutrient incorporation, harvesting, and packaging. The shortage of agricultural workers is a common problem among producers. In 2024, the National Agricultural Council (CNA) reported a considerable shortage of agricultural workers. Table 4 shows the cost and number of days required to establish and maintain the crops.

Table 3. Mechanized labor costs for the establishment of the asparagus crop in the municipality of Atenco, State of Mexico.

Activity	Quantity	Unit cost ($)	Subtotal ($)
Land leveling*	1	7,000.00	7,000.00
Subsoiling	1	2,500.00	2,500.00
Manure incorporation	1	800.00	800.00
Harrowing**	3	1,100.00	3,300.00
Furrowing or bed shaping	1	1,200.00	1,200.00
Cutting of old shoots***	1	1,300.00	1,300.00
Total cost of the mechanized work process			16,100.00

*Leveling is carried out in an average of 10 hours, depending on the condition of the land.
**One harrowing is performed before and two after the leveling.
***Cutting of old shoots is performed prior to harvest.
Source: M. Méndez, Personal communication, March 20, 2025.

Table 4. Costs and number of workdays for transplanting, maintenance, and first harvest of the asparagus crop.

Activity	Workdays ha^{-1}	Workers	Unit cost ($)	Subtotal ($)
Transplanting and maintenance				
Transplanting	1	5	350	1,750
Nutrition	3	1	350	1,050
Pest and disease control	3	1	350	1,050
Irrigation	18	2	350	12,600
First harvest (20 to 30 days)				
Weeding	1	3	350	1,050
Harvest	25	2	350	17,500
Trimming and packing	25	2	350	17,500
General crop management				
Packing facility worker*	313	1	350	109,550
Total workdays and cost	389			162,050

*Required for daily crop management.
Source: A. Gonzalez, Personal communication, March 20, 2025.

The estimated total cost of crop establishment is MXN 248,150.00 per hectare. This cost includes daily wages, mechanized work, and the purchase of seedlings (MXN 50,000.00), plus agricultural inputs such as fertilizers, fungicides, pesticides, and herbicides (MXN 20,000.00). The first harvest yields approximately 7 t ha^{-1}, generating an estimated gross income of MXN 490,000.00. These data indicate that, during the first production cycle, recovering the initial investment and obtaining a significant net profit is possible (M. Méndez, personal communication, March 20, 2025).

Experimental results

The Shapiro-Wilks test for normality was used to evaluate the RCDW, TSN, and FDW data (Shapiro and Wilk, 1965). The results indicated that all variables had a nonparametric

distribution (P=0.0001 to 0.0431). Table 5 shows the results of the statistical comparisons between treatments for the two experiments.

The effect of the *Fusarium proliferatum* inoculation without VCL (IC *vs.* NIC) in both experiments significantly reduced RCDW, indicating a lower accumulation of underground biomass in infected plants (Figure 2). A significant FDW decrease in inoculated plants was recorded in Experiment 1 (E1). A similar trend was observed in Experiment 2 (E2). However, TSN did not have significant differences in either experiment, suggesting that the infection had a lower impact on the spear production capacity.

In both experiments, the effect of pre-transplant VCL application did not improve the development of the plants inoculated with *F. proliferatum*, compared with the inoculated control (IC *vs.* PHI, PSI, PCI). RCDW and TSN did not have significant differences. In addition, biomass values were within the range of the inoculated control (Figure 3). RCDW and TSN did not record significant differences: the values found in the leachate treatments were within the control range, indicating the absence of protective effects on the root system or the shoot. Furthermore, a significant reduction in FDW was recorded in the plants treated with VCL in both experiments, suggesting that the treatment may have accentuated the negative effects of the pathogen on aerial biomass.

Table 5. Significance of the differences between Experiments 1 and 2, resulting from the application of the Kruskal-Wallis and Mann-WhitneyU statistical tests to the TSN, FDW, and RCDW variables, in different comparisons between treatments.

Comparisons		Experiment 1			Experiment 2		
		TSN	**FDW**	**RCDW**	**TSN**	**FDW**	**RCDW**
1	IC *vs*.. NIC	0.2676	0.0258	0.0058	0.101	0.0887	0.0101
2	IC *vs*.. PHI, PSI, PCI	0.0051	0.0003	0.0099	0.2098	0.0087	0.0603
3	IC *vs*. HI, SI, CI	0.1459	0.1008	0.4495	0.9251	0.1008	0.4495
4	PHI, PSI, PCI *vs*. PHNI, PSNI, PCNI	0.0001	0.0001	0.0001	0.0008	0.0001	0.0001
5	HI, SI, CI *vs*. HNI, SNI, CNI	0.0022	0.0019	0.0054	0.0498	0.0001	0.0001
6	PHI, PSI, PCI *vs*. HI, SI, CI	0.0664	0.0004	0.0398	0.1157	0.0003	0.0102
7	PHNI, PSNI, PCNI *vs*. HNI, SNI, CNI	0.6718	0.6788	0.9293	0.4692	0.1332	0.4125
8	PSI, SI *vs*. IC	0.0619	0.0193	0.3125	0.7572	0.3438	0.6121
9	PSNI, SNI *vs*. NIC	0.6559	0.4154	0.7248	0.4229	0.1589	0.1589
10	PHI, HI *vs*. IC	0.0335	0.031	0.0642	0.6116	0.1081	0.3903
11	PHNI, HNI *vs*. NIC	0.8593	0.0114	0.0005	0.6223	0.5971	0.3008
12	PCI, CI *vs*. IC	0.0556	0.0038	0.0808	0.309	0.2095	0.2339
13	PCNI, CNI *vs*. NIC	0.025	0.0476	0.1655	0.7455	0.9817	1
14	PSI, SI, *vs*. PHI, HI	0.2527	0.7043	0.3387	0.9675	0.2672	0.8708
15	PSNI, SNI, *vs*. PHNI, HNI	0.9783	0.1676	0.0883	0.6997	0.2182	0.7555
16	PSI, SI, *vs*. PCI, CI	0.692	0.5591	0.492	0.353	0.0501	0.9675
17	PSNI, SNI, *vs*. PCNI, CNI	0.0661	0.3103	0.5427	0.6281	0.0742	0.2107
18	PHI, HI, *vs*. PCI, CI	0.5715	0.9328	0.8108	0.7138	0.5158	0.6255
19	PHNI, HNI, *vs*. PCNI, CNI	0.1494	0.6455	0.2554	0.875	0.489	0.3317

FDW=Foliage dry weight. RCDW=Root and crown dry weight. TSN=total spear number.

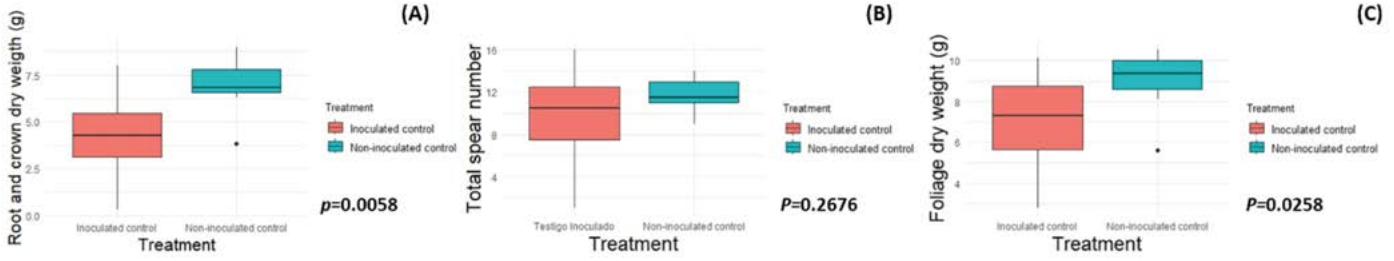

Figure 2. Effect of *Fusarium proliferatum* inoculation on asparagus (*Asparagus officinalis*) plants. RCDW (A), TSN (B), and FDW (C) comparison between IC and NIC. The treatments were compared with the Mann-Whitney U test. Experiment 1. Similar results in Experiment 2.

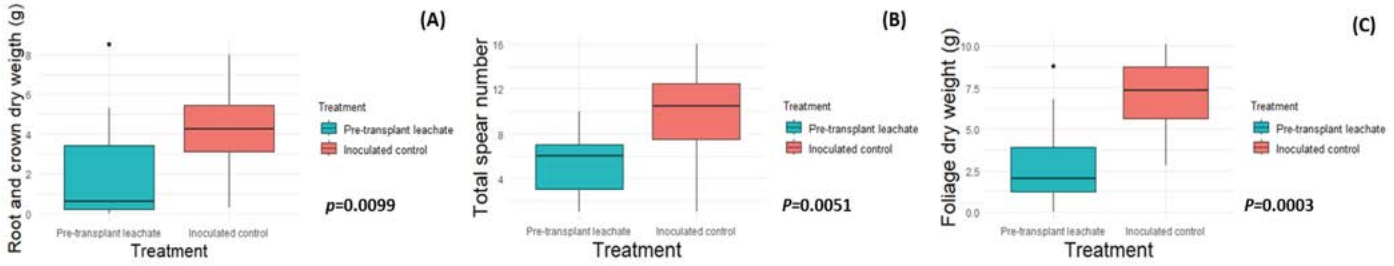

Figure 3. Effect of the pre-transplant VCL (sheep, horse, and cow) application on the RCDW (A), TNS (B) and FDW (C) of asparagus (*Asparagus officinalis*) plants inoculated with *Fusarium proliferatum* and compared with inoculated treatments without VCL application (PSI, PHI, and PCI *vs.* IC). Experiment 1. Similar results in Experiment 2.

In both experiments, the pre-transplant VCL application in non-inoculated plants, the accumulation of root and crown biomass, foliage dry weight, and shoot production were significantly higher than in plants treated with the same leachates but inoculated with the pathogen (PHI, PSI, PCI *vs.* PHNI, PSNI, PCNI) (Figure 4). These results indicated that the pathogen infection had a negative impact on the overall development of the plants, despite the application of pre-transplant leachate.

Plants that were inoculated and treated with leachates post-transplant also recorded a higher RCDW, FDW, and TSN reduction in both experiments than non-inoculated plants treated with VCL (HI, SI, CI *vs.* HNI, SNI, CNI) (Figure 5). These results indicated that inoculation affected plant growth and productivity, and that the inoculation harmed the overall development and productivity of the plants. The post-transplant leachate

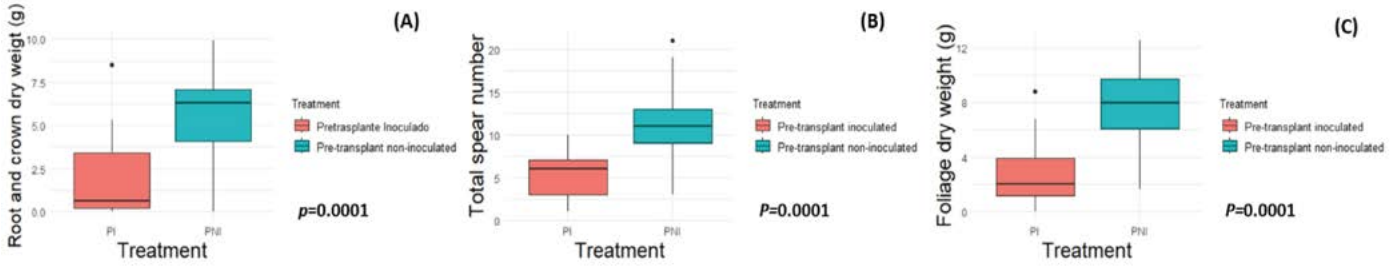

Figure 4. Effect of *Fusarium proliferatum* inoculation on asparagus (*Asparagus officinalis*) plants treated with a VCL pre-transplant application. Comparison of inoculated and non-inoculated plants (PHI, PSI and PCI *vs.* PHNI, PSNI, and PCNI) in RCDW (A), TNS (B) and FDW (C). Experiment 1. Similar results in Experiment 2.

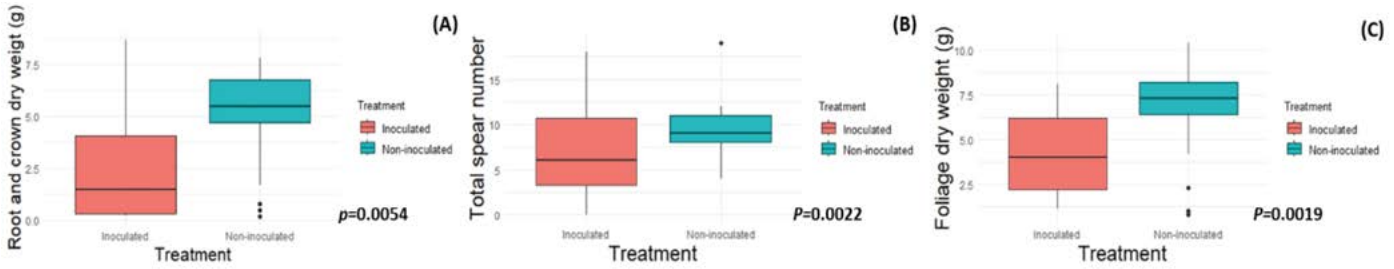

Figure 5. Effect of *Fusarium proliferatum* inoculation on asparagus (*Asparagus officinalis*) plants treated with post-transplant VCL applications. Comparison between inoculated and non-inoculated plants, treated with VCL (HI, SI, CI *vs.* HNI, SNI, CNI) on RCDW (A), TSN (B) and FDW (C). Experiment 2. Similar results to Experiment 1.

application did not counteract the negative effects of *F. proliferatum* under experimental conditions either.

In both experiments, plants inoculated and treated post-transplant with leachate (HI, SI, CI) showed a significantly higher accumulation of RCDW and FDW than those treated with pre-transplant VCL applications (PHI, PSI, PCI). Statistically significant differences were recorded (Figure 6). No significant TSN differences were recorded in both experiments. This indicated that the period of VCL application produced differences in biomass accumulation, but not in spear production under conditions of pathogen infection.

Compared with the inoculated control (PSI, SI *vs.* IC), the application of sheep VCL to the inoculated plants of E1 did not improve RCDW or TSN (Figure 7). However, a significant reduction in FDW was recorded. This indicated that this treatment did not

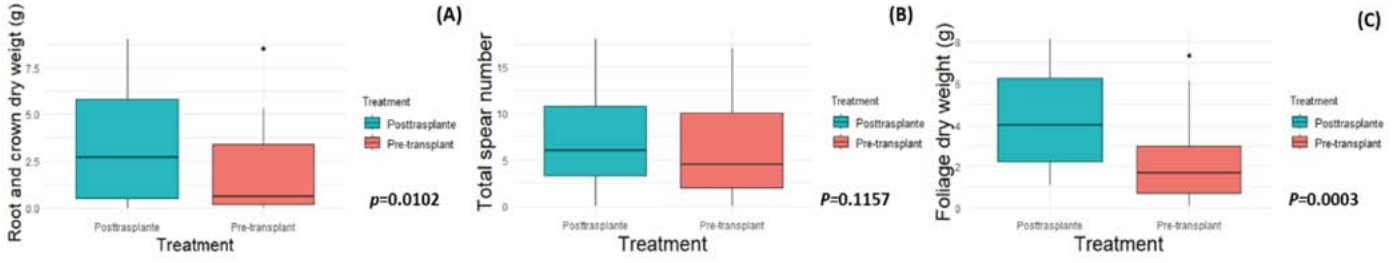

Figure 6. Effect of pre-transplant and post-transplant VCL application on the growth of asparagus (*Asparagus officinalis*) plants inoculated with *Fusarium proliferatum* (PHI, PSI, PCI *vs.* HI, SI, CI) in RCDW (A), TSN (B), and FDW (C). Experiment 2. Similar results to Experiment 1.

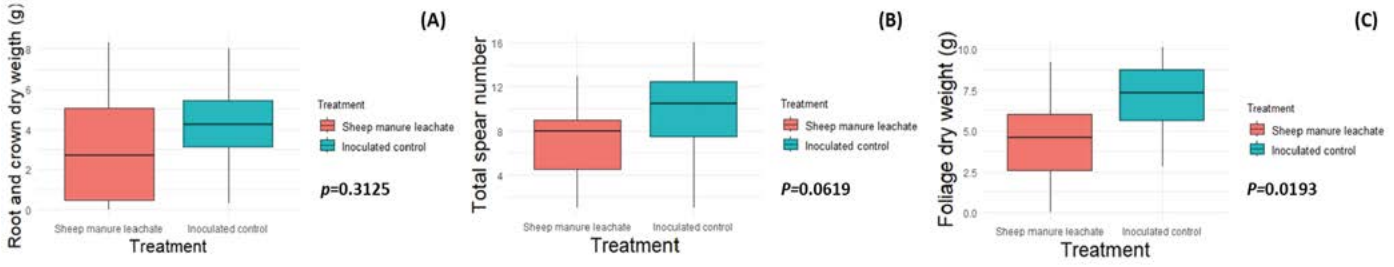

Figure 7. Comparison of the effect of pre-transplant and post-transplant sheep VCL application on asparagus (*Asparagus officinalis*) plants inoculated with *Fusarium proliferatum* and inoculated control (PSI and SI *vs.* IC). RCDW (A), TSN (B), and FDW (C) were evaluated. Experiment 1.

mitigate the impact of the infection. This situation suggests that the VCL source did not provide a protective effect against the pathogen.

The application of horse VCL to inoculated plants (PHI, HI *vs.* IC) did not significantly improve RCDW in E1. However, compared to the inoculated control (data not shown), a significant reduction in FDW and TSN was recorded. This result indicated that the horse VCL treatment did not protect against the pathogen; instead, it negatively impacted foliage accumulation and spear production. Applying horse VCL to non-inoculated plants (PHNI, HNI) significantly reduced RCDW and FDW, compared with the non-inoculated control (NIC). No significant TSN differences were recorded.

Compared with the inoculated control (PCI, CI *vs.* IC), the application of cow VCL to inoculated plants did not record significant RCDW or TSN differences in E1. However, FDW was significantly lower (data not shown). Compared with the non-inoculated control (PCNI, CNI *vs.* NIC), the application of cow VCL to non-inoculated plants did not record significant RCDW differences. However, TSN and FDW differences were recorded: plants treated with cow VCL recorded lower values than those of NIC. These results suggest that the application of cow VCL did not promote better aerial biomass development and better spear production. No significant differences were recorded in the evaluation of the remaining planned comparisons (3, 7, 9, and 14-19 in E1; 3 and 7-19 in E2) (Table 2) for any of the variables (Table 3).

Several studies have documented the positive effects of VCL on disease suppression and plant growth improvement. However, the results of this study indicated that, under our experimental conditions, VCL made from sheep, horse, and cow manure did not significantly protect the asparagus plants grown in Atenco soil against *Fusarium proliferatum*. In contrast, Mupambwa *et al.* (2024) reported an improved germination, root growth, and nutrient availability in crops treated with VCLs from small ruminants. The lack of protective effects in the asparagus plants recorded in this study could be related to the variability of the microbiological and chemical VCL composition, which could have been influenced by the type of manure used and the conditions under which the vermicompost was established (Sarker *et al.*, 2021). Zamora *et al.* (2017) documented that certain leachates can increase the severity of fungal infections, depending on their origin and microbiota. Another important factor could have been the interaction of the leachate with native soil microbiota, which may have limited the establishment of beneficial VCL microorganisms, resulting in a reduced suppressive potential (Birinchi *et al.*, 2010; Zhao *et al.*, 2019). Additionally, the availability of nutrients in the VCLs may not have been enough to cover the nutritional demand under biotic stress conditions (Ávila *et al.*, 2015). Therefore, the lack of protective effect could be the result of the combination of these factors. Consequently, optimizing VCL, from its preparation to its application in the plants, is fundamental.

CONCLUSIONS

Asparagus cultivation in Atenco is a profitable activity, despite the organizational and technical limitations for yield improvement and expansion of the growing area. According to the producers, agroecological practices (*e.g.*, VCL and beneficial microorganism application) improve soil health and crop resilience; nevertheless, the adoption of these

agricultural practices is limited. In this study, no protective effect was recorded with the application of the three different types of VCL in the soil of Atenco. However, sheep and cow leachates had some potential to promote asparagus growth in the absence of the pathogen. Further research is required under field conditions to validate the efficacy of VCL against diseases caused by *F. proliferatum* and other pathogens. This is particularly important when VCL is used in combination with other biological control strategies.

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Gibberellin spraying in the production of male flowers of squash (*Cucurbita pepo* L.) and their postharvest quality

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ABSTRACT

Objective: To evaluate the effect of gibberellic acid (GA_3) spraying on the production of male flowers and their physical and chemical quality for fresh sale of pumpkin (*Cucurbita pepo* L.).

Methodology: A pumpkin crop was established under open-air conditions, where two GA_3 sprays were applied at doses of 0, 30, 60, and 90 mg L^{-1} during the first 45 days after planting. The number of male and female flowers accumulated over 31 days after GA_3 application was recorded, and selected physical and chemical quality parameters were determined. A randomized complete block design with four blocks was used.

Results: The proportion of male to female flowers increased from 2:1 at 0 mg L^{-1} to 4:1 at 90 mg L^{-1} during the evaluation period. Chlorophylls, carotenoids, phenolic compounds, antioxidant activity, and vitamin C were not negatively affected by GA_3 spraying.

Limitations/Implications: This experiment should be replicated under different climatic conditions and with other varieties.

Conclusions: GA_3 spraying increased the proportion of male flowers without affecting the quality of pumpkin flowers.

Keywords: Phenolic compounds, carotenoids, vitamin C.

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INTRODUCTION

Squashes (*Cucurbita* spp.) have been part of the diet of the Americas for more than 1,000 years and are traditionally included in the milpa production system, associated with maize, beans, and chili, which were the dietary staples of Mesoamerican civilizations. Today, squash remains an important crop in traditional Mexican agriculture, a region recognized as the center of origin and diversity for four of the five species of the genus: *Cucurbita argyrosperma*, *Cucurbita ficifolia*, *Cucurbita moschata*, and *Cucurbita pepo* (Basurto-Peña *et al.*, 2010). In 2023, Mexico cultivated 77,745.98 ha of squash for seed, zucchini, and other edible squashes, producing 744,295.5 t, with a market value of 6,163,102 thousand pesos (Servicio de Información Agroalimentaria y Pesquera [SIAP], 2024).

The cultivated *Cucurbita* species intended for human consumption exhibit great diversity in fruit size, shape, color, and ornamentation. Their fruits are not bitter and

can be consumed along with their seeds, flowers, and shoots (Eguiarte *et al.*, 2018). In particular, squash flowers are a popular ingredient in the USA, Asia, and Europe due to their appealing yellow color, soft texture, and delicate flavor. In Mexico, they are extremely popular and are sold in local markets, mainly as an ingredient in soups or incorporated into various dishes such as tamales, tacos, quesadillas, and stuffed peppers, among others (Mulík & Ozuna, 2020).

One hundred grams of squash flowers contain 93.2% moisture, 46 mg of carbohydrates, 18.1 mg of protein, 5 mg of fat, 15.9 mg of ash, and 10.5 mg of fiber (Sotelo *et al.*, 2007). Beyond their culinary qualities, squash flowers have beneficial properties for human health. Each 100 g provides 11 mg of Na, 18.2 mg of K, 17.6 mg of Ca, 17.4 μg of phenolic compounds, 17.1 μg of flavonoids, 10.3 mg of anthocyanins, an antioxidant activity of 51.6% (DPPH), and between 15.2 and 45.8 mg of carotenoids. The phytochemical compounds present make squash flowers a functional food, as some of these molecules have demonstrated antimicrobial activity (Ghosh & Sing, 2021; Biezanowska-Kopec *et al.*, 2022).

The sexual expression of *Cucurbita pepo* is monoecious, with large, showy orange flowers. Female flowers are produced in lower proportion than male flowers. At the beginning of the crop cycle, only male flowers are produced; later, male and female flowers are produced alternately, and finally, female flowers dominate (Costa-Silva *et al.*, 2019). In Morelos, Mexico, there is a market for squash flower production, where it is desirable to maintain or increase the proportion of male flowers for a longer period.

Hormones play a key role in regulating sex expression in cucurbits. In general, ethylene and auxins promote feminization, whereas gibberellins induce masculinization (Rudich, 1990). Gibberellins act as regulators of plant growth and development, promoting cell elongation, seed germination, flowering transition, pollen development, pollen tube growth, and fruit development (Taiz *et al.*, 2023). In Morelos, Mexico, no specific studies have been conducted to determine the appropriate gibberellin application rates to maintain a higher production of male flowers relative to female flowers in squash crops. Likewise, the quality, functional metabolites, and antioxidant activity of flowers treated with gibberellic acid have not been evaluated. Therefore, this study assessed the effect of different GA_3 doses on the number of male and female flowers, as well as on the quality, functional metabolites, and antioxidant activity of squash flowers.

MATERIALS AND METHODS

The study was conducted on a 0.4 ha area located in Cuentepec, Temixco, Morelos, Mexico (18° 86′02.7" N, 99° 32′63.8" W, 1,390 m a.s.l.), which has a warm-subhumid climate. The average annual temperature ranges from 20.7 °C to 26 °C, with an annual rainfall of 891.9 mm (Díaz *et al.*, 2008). *Grey zucchini* squash seeds (Seminis, USA) were used as plant material.

Experimental Design and Agronomic Management

Sowing was carried out in furrows spaced 80 cm apart, with a distance of 50 cm between plants. Two seeds were sown per planting hole, and 20 g of fertilizer (NPK 18-4.5-

3) was applied midway between the plants. A randomized complete block design was used, consisting of four blocks. The four treatments were randomized within each block.

The treatments consisted of the application of GA′ (Biogib® 10 PS, Arysta). Four treatments were evaluated: Treatment 1 served as the control, with no GA_3 application; Treatments 2, 3, and 4 consisted of two foliar sprays (at 30 and 45 days after sowing) with 30, 60, and 90 mg L^{-1} GA_3, respectively. The solution was prepared by dissolving the product in tap water, and applications were performed in the morning at 9:00 a.m. The experimental unit consisted of three rows, each 2 m long; the central plant in each row was sampled, and three plants were evaluated per experimental unit. Blocks were separated by one row.

Agronomic management included treating the seeds with a solution of N-(trichloromethylthio) cyclohex-4-ene-1,2-dicarboximide (Captan® WP, ADAMA) for 12 h prior to sowing. A second fertilization was applied 30 days after sowing with 30 g of NPK 80-40-20. Preventive pest control was carried out using Betacyfluthrin + Imidacloprid (Muralla Max® 300 OD, Bayer®), and preventive disease control was performed with Quintozene 30% + Thiram 30% (Interguzan 30-30®, AgroIQC). After 20 days of sowing, Methomyl 90% (Matador® 90 PS, ADAMA) was applied for the control of fruit borer (*Diaphania nitidalis*, *Diabrotica* spp.). Foliar fertilizers Foltron® (Arysta) and later Bayfolan® (Bayer) were applied weekly during the first 25 days after sowing.

Evaluated Variables

The number of male and female flowers was recorded at 38, 46, 54, 62, and 70 days after sowing. Flower counts were performed daily over a 31-day period on three plants. Flower size and peduncle length were measured for each flower using a 30 cm ruler with a precision of 0.01 mm.

The total chlorophyll and carotenoid contents were determined following the methodology proposed by Rodés and Collazo (2006). One gram of squash flower tissue was homogenized with 5 mL of 80% acetone using an UltraTurrax (IKA®, USA). An additional 5 mL of acetone was added during homogenization, and the mixture was then filtered using filter paper. After filtration, absorbance readings were taken with a spectrophotometer (HACH DR 5000R, USA) at 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 440.5 nm for carotenoids. Pigment concentrations were calculated using the following formulas:

$$Ca\left(\text{mg L}^{-1}\right) = 12.7(A663) - 2.69(A645)$$

$$Cb\left(\text{mg L}^{-1}\right) = 22.9(A645) - 4.68(A663)$$

$$Ccar\left(\text{g L}^{-1}\right) = 4.695(A440.5) - 0.268(Ca + b)$$

Where: *Ca*=chlorophyll a concentration, *Cb*=chlorophyll *b* concentration, and *Ccar*=total carotenoid concentration. A663, A645, and A440.5=absorbance readings at 663, 645, and 440.5 nm, respectively.

The concentration of phenolic compounds was determined using the Folin-Ciocalteu method (Singleton *et al.*, 1999). One gram of flower tissue was placed in a test tube and homogenized with 20 mL of distilled water using an Ultra Turrax (IKA®, USA R), then filtered. A 0.5 mL aliquot of the filtrate was mixed with 2.5 mL of Folin-Ciocalteu reagent (1:10). After 5 minutes, 2 mL of sodium carbonate (7.5% w/v) were added, and the mixture was allowed to stand for 2 hours. Absorbance was then measured at 760 nm using a spectrophotometer (HACH DR 5000®). Results were expressed as mg gallic acid equivalents (GAE) per 100 g of fresh weight.

Using the same extract employed for phenolic determination, antioxidant activity was assessed through the FRAP, ABTS, and DPPH methods. The ferric reducing antioxidant power (FRAP) was determined following the methodology developed by Benzie and Strain (1996). The FRAP reagent (TPTZ, FeCl′, and acetate buffer) was prepared, and 1.8 mL of FRAP solution were mixed with 140 μL of distilled water and 60 μL of sample. The reaction mixture was incubated for 30 minutes at 37 °C, and absorbance was then measured at 593 nm. Results were expressed as mg ascorbic acid equivalents (AAE) per 100 g of fresh weight.

For the DPPH method, the procedure proposed by Brand-Williams, Cuvelier, and Berset (1995) was followed. One gram of pulp was homogenized with 10 mL of distilled water and filtered. From the filtrate, 0.10 mL was mixed with 3 mL of a methanolic DPPH solution (6.1×10^{-5}′ M, Sigma-Aldrich, USA) and allowed to react for 30 minutes in the dark. The change in absorbance was measured at 517 nm. Antioxidant activity was determined using a standard curve of ascorbic acid, and results were expressed as mg ascorbic acid equivalents (AAE) per 100 g of fresh weight.

For the ABTS method, the ABTS reagent (Sigma-Aldrich®, USA) was prepared at 7 mM and potassium persulfate ($K_2S_2O_8$) at 2.45 mM. Both solutions were mixed at a 1:1 ratio and allowed to stand for 16 hours, then diluted with 20% ethanol until reaching an absorbance of 0.7 ± 0.02 at 734 nm. Subsequently, 3 mL of ABTS solution were mixed with 50 μL of sample and allowed to react for 15 minutes. Absorbance was measured at 734 nm. Results were expressed as mg ascorbic acid equivalents (AAE) per 100 g of fresh weight, obtained from a calibration curve (Re *et al.*, 1999).

The vitamin C content was determined following the methodology proposed by Jagota and Dani (1982), a colorimetric technique for vitamin C estimation using the Folin-Ciocalteu reagent. One gram of sample was homogenized with 4 mL of 10% (w/v) trichloroacetic acid (TCA) and placed in an ice bath for 5 minutes. The mixture was then centrifuged at 9,464 × *g* for 20 minutes at 4 °C. For the reaction, 0.5 mL aliquots of the supernatant were mixed with 1.5 mL of double-distilled water and 200 μL of Folin reagent, and the mixture was allowed to react in darkness for 15 minutes. Absorbance was measured at 760 nm. Vitamin C content was estimated using a standard curve prepared with ascorbic acid, and total concentration was expressed as mg g^{-1} of fresh weight.

Data Analysis

Data were analyzed and graphed using SigmaPlot v.14 (Systat Software, Inc., San José, California, USA). A one-way analysis of variance (ANOVA) was performed, followed by

mean comparisons using Tukey's test. Simple correlations were evaluated using Pearson's method (Systat Software, Inc., San José, California, USA).

RESULTS AND DISCUSSION

Control plants produced approximately 21 male flowers and 10 female flowers over 31 days, resulting in a male-to-female flower ratio of 2:1 (Figure 1). The application of gibberellic acid increased the number of male flowers and reduced the proportion of female flowers, with total flower production ranging from 32 to 35 when GA_3 was applied at 30-90 mg L^{-1} (Figure 1). Male flowers increased by 4-7, while female flowers decreased by 2-3 as the GA_3 dose increased from 30 to 90 mg L^{-1} (Figure 1). The male-to-female flower ratio was 3:1 when 30 or 60 mg L^{-1} of GA′ was applied and 4:1 at 90 mg L^{-1}. These results indicate that GA_3 application significantly increased the number of squash flowers and enhanced the proportion of male flowers.

Authors such as Babhare *et al.* (2020) reported that foliar application of GA_3 increased male flowers in papaya; however, they also noted that the male-to-female:hermaphrodite flower ratio remained unchanged. In the present study, it is evident that GA_3 application significantly increased the number of male flowers, making it a viable option for maintaining squash plants with male flower production for sale. Gupta and Chakrabarty (2013) reported that GA_3 treatments promote male sexual tendency in cucumber (*Cucumis sativa*), both in gynoecious and hermaphrodite lines, although the exact mechanism of action is not yet understood. Future studies should evaluate a greater number of applications to maintain the flowering period with the observed ratios and achieve higher benefits.

Flower size did not show significant differences among treatments during the sampling periods, except on day 60, when plants treated with 30 mg L^{-1} GA_3 had significantly larger flowers compared to the control plants and those treated with 60 or 90 mg L^{-1} GA_3

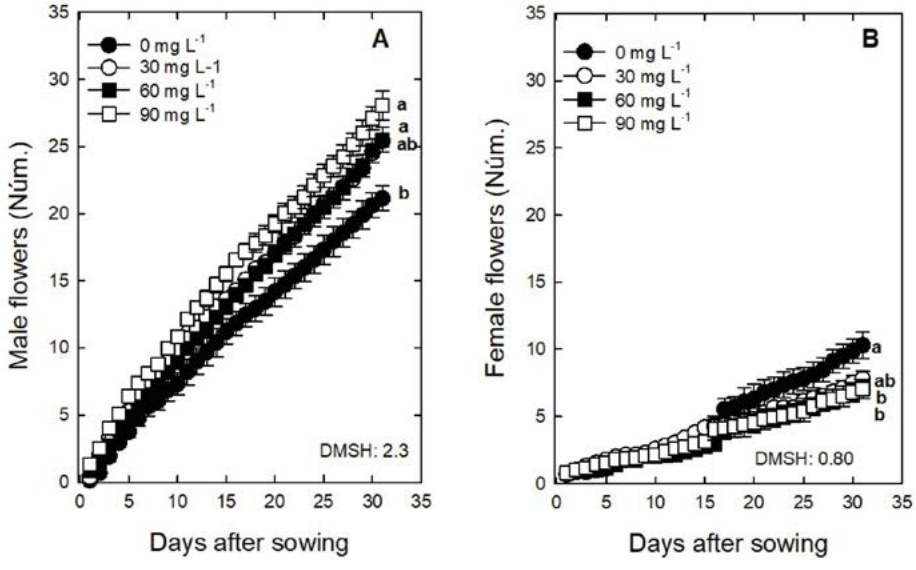

Figure 1. Number of male (A) and female (B) squash flowers accumulated during the evaluation period. Each point represents the mean of three observations ± standard error. Different letters among treatments indicate significant differences according to Tukey's test ($p \leq 0.05$). HSD: Honest significant difference.

(Figure 2A). Flower dimensions increased from initial values of 65-73 mm to 80-88 mm (Figure 2A).

The peduncle showed the most significant increase. When 90 mg L^{-1} GA′ was applied, peduncle length ranged from 104.1 to 126 mm, whereas in control plants it ranged from 76 to 99 mm (Figure 2B). Han *et al.* (2014) reported that GA_3 application in papaya increased the length of male flower peduncles, attributing this to a transient effect rather than a trait controlled by genes involved in gibberellic acid metabolism. However, in the present study, peduncle length remained significantly greater with GA_3 application, suggesting a residual effect of at least 32 days on this morphological characteristic of squash flowers.

Total chlorophyll concentration decreased in all squash flowers as sampling dates progressed, with initial values ranging from 11.6 to 13.3 mg g^{-1} fresh weight, declining to 2.5-4 mg g^{-1} fresh weight in the last sampling (Figure 3A). GA_3 application significantly delayed the decrease in total chlorophyll, which was evident on days 54 and 70 (Figure 3A). Janowska and Jerzy *et al.* (2003) reported that applying a 300 mg L^{-1} GA_3 solution to calla lily leaves prevents chlorophyll degradation.

Total carotenoids decreased from initial values of 5.5 and 8 mg g^{-1} fresh weight to 1.5 and 1.8 mg g^{-1} fresh weight by the end of the evaluation period (Figure 3B). Aquino-Bolaños *et al.* (2013) reported that in squash flowers, carotenoid concentration decreases from 75 to 42 mg 100 g^{-1} fresh weight during eight days of storage at 5 °C. GA_3 application maintained total carotenoid concentration for a longer period, with the greatest effect observed at 30 mg L^{-1} GA_3 (Figure 3B). It has been reported that in 'Kinow' mandarins, GA_3 application at 25 mg L^{-1} helps to maintain total carotenoid content for a longer time (Talat *et al.*, 2020).

Phenolic compound content decreased significantly during the evaluation period in all treatments, except in plants sprayed with 90 mg L^{-1} GA′ (Figure 4A). At the beginning of

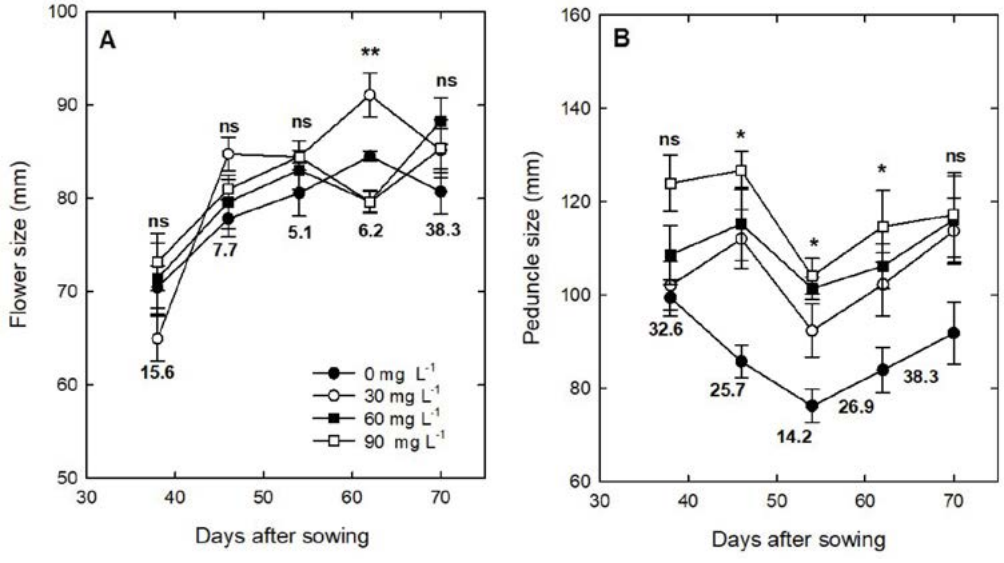

Figure 2. Flower and peduncle size in squash following GA_3 application. Each point represents the mean of three observations ± standard error. The HSD value is shown for each sampling.

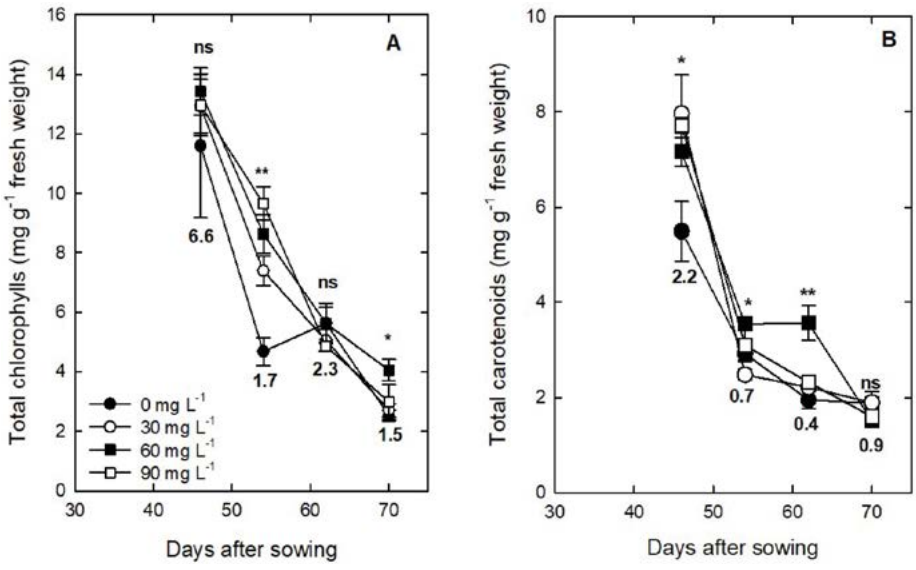

Figure 3. Chlorophyll (A) and total carotenoid (B) content in squash flowers following GA_3 application. Each point represents the mean of three observations ± standard error. The HSD value is shown for each sampling.

the evaluations, values ranged from 21 to 65 mg GAE 100 g^{-1}, decreasing to 15-19 mg GAE 100 g^{-1} by the last sampling. Differences were detected only at the beginning and end of the evaluations, with no clear trend observed (Figure 4A). Aquino-Bolaños *et al.* (2013) reported that phenolic compound concentration in squash flowers decreased from initial values of 334 mg 100 g^{-1} to 238 mg 100 g^{-1} after eight days at 5 °C. Toro-Vélez *et al.* (2022) indicated that phenolic content in squash flowers can decrease by up to 50% during postharvest.

Vitamin C content showed a decrease during the samplings at 46 and 54 days, and later increased in the final samplings (Figure 4B). Significant differences were detected only in the initial sampling, where higher GA_3 concentrations corresponded to higher vitamin C content. No significant differences were observed among treatments in the subsequent samplings, although flowers without GA_3 application had the lowest ascorbic acid concentration (Figure 4B). Aquino-Bolaños *et al.* (2013) report that vitamin C in squash flowers decreases after harvest from 16.51 mg 100 g^{-1} to 9.09 mg 100 g^{-1} during eight days of storage at 5 °C. There are no reports on the effect of gibberellins on vitamin C content in plants; however, in squash flowers a notable effect was the increase in its concentration, indicating that future studies should investigate this relationship in more detail.

Antioxidant activity measured by the DPPH and FRAP methods decreased during the sampling period (Figure 5A and C), whereas antioxidant activity measured by the ABTS method increased (Figure 5B). No significant effect of GA_3 application or control treatment was detected for any of the methods evaluated. Antioxidant activity ranged from 20 to 120 mg AAE 100 g^{-1} for the DPPH and ABTS methods, and from 200 to 600 mg 100 g^{-1} for FRAP, subsequently decreasing to 36 mg AAE 100 g^{-1} (Figure 5A-C). Aquino-Bolaños *et al.* (2013) reported that postharvest squash flowers exhibit a decrease in antioxidant

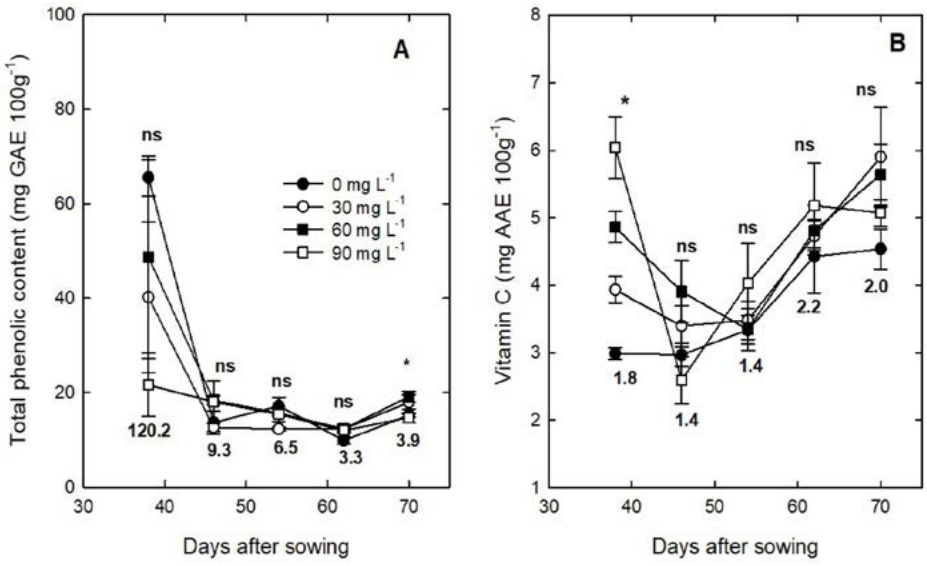

Figure 4. Total phenolic (A) and vitamin C (B) content in squash flowers following GA_3 application. Each point represents the mean of three observations ± standard error. The HSD value is shown for each sampling.

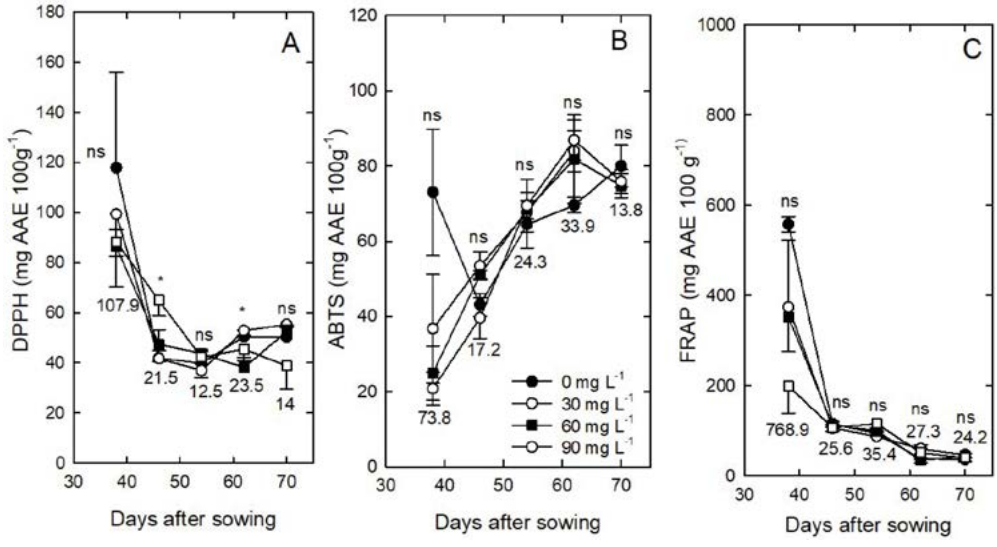

Figure 5. Antioxidant activity of squash flowers measured by DPPH (A), ABTS (B), and FRAP (C) following GA_3 application. Each point represents the mean of three observations ± standard error. The HSD value is shown for each sampling.

activity from 62 mg AAE 100 g^{-1} to 37.8 mg AAE 100 g^{-1} after 8 days at 5 °C. Morittu *et al.* (2019) indicated that antioxidant activity in squash flowers, as measured by FRAP and ABTS, is substantial, and that the flowers also display antidiabetic activity.

A strong positive association was detected between total carotenoid content and antioxidant activity measured by FRAP (r=0.67***), and a negative association between total carotenoids and antioxidant activity measured by the ABTS method (r=0.71***). Phenolic compounds were positively and highly significantly associated with antioxidant

activity measured by DPPH and FRAP (r=0.72*** and r=0.88***, respectively). These results indicate that, in squash flowers, phenolic compounds and carotenoids contribute the most to antioxidant capacity, compared to vitamin C.

CONCLUSIONS

GA_3 application at doses of 30-60 mg L^{-1} increased the number of male flowers without affecting morphological quality, functional molecules, antioxidants, or antioxidant activity.

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Colegio de Postgraduados

Determinants of the mean rural price of Maradol papaya (*Carica papaya* L.)

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ABSTRACT

Objective: To analyze the factors influencing the low Farm Gate Price (FGP) faced by Maradol papaya producers in Villa de Tututepec, Oaxaca, a municipality that accounts for 40.9% of the state's production and 11.5% of the national production, yet whose FGP is 20.7% lower than the national average, ranking 167th among the 229 papaya-producing municipalities distributed across 19 states in Mexico.

Design/methodology/approach: A total of 155 commercialization units were georeferenced, and their effects on the FGP were analyzed using cartography in QGIS, distance measurement, and a bubble chart in Python that related FGP, production volume, infrastructure, and state-level competition.

Results: Limited commercialization infrastructure and long distances to major markets reduce the FGP received by producers. Although greater infrastructure availability can improve sales conditions, its effect may be moderated by high local competition. Furthermore, a high production volume, without adequate logistical support, tends to saturate the market and exert additional downward pressure on prices.

Limitations on study/implications: The lack of specific data on transportation conditions limited the construction of a more robust econometric model and reduced the significance of the variables analyzed.

Findings/conclusions: Strengthening logistical infrastructure and improving proximity to distribution channels could significantly increase the income of Maradol papaya producers in Villa de Tututepec.

Keywords: Farm Gate Price (FGP), commercialization infrastructure, logistical accessibility, geographic information systems (GIS).

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INTRODUCTION

For farmers, the price received for their production constitutes virtually the only determinant of their agricultural income (Timmer, 1983). In this sense, the Farm Gate Price (FGP) constitutes a key measure to evaluate the primary profitability of crops. In addition, it allows identifying structural faults in the market, such as insufficient infrastructure, high transport costs, or intermediation problems, factors that can distort the prices paid to the producer. In the case of perishable products, the FGP acquires special relevance, since it determines the income that producers obtain before the product loses value due to its fast decomposition. In this context, Maradol papaya (*Carica papaya* L.) is especially vulnerable

due to its short post-harvest life and the need for efficient logistical management to preserve its quality and value.

Mexico occupies an outstanding place in the global agricultural economy. According to recent data, in 2023, it was consolidated as the ninth largest global exporter of agrifood products (Morales, 2024) and the twelfth in terms of total agricultural production (SENASICA, 2016). In this context, Maradol papaya stands out as a crop of strategic importance, not only because of its production volume, but also for its role in foreign export. According to data from FAOSTAT (2024), Mexico is the third global producer of papaya, but it leads world exports of this fruit, which evidences a high exporting efficiency. Within the segment of "Edible fruits", papaya occupies the thirteenth place in value of national exports, according to the International Trade Center (ITC, 2024), with the Maradol variety being the most representative in the country.

Based on data consulted in SIACON (SIAP, 2024), Oaxaca led Maradol papaya production in Mexico in 2023, with 318,322.7 tons, equivalent to 28% of the total national production. Villa de Tututepec de Melchor Ocampo concentrated 40.9% of the state production and 11.5% of the national production, positioning itself as the main producing municipality in the country from among 229 municipalities. However, the FGP in Villa de Tututepec was $5,558.35 pesos MX per ton, 20.7% lower than the national average of $7,013.11 pesos MX, which shows a contradiction between the high production volume and the low price, attributable to structural factors that affect the income of local producers. Given this panorama, it is essential to analyze statistically and geographically the conditions of Maradol papaya producing municipalities, to identify the factors that explain the differences in farm gate prices. Understanding these elements will allow proposing strategies to improve the profitability of producers and to consolidate the exporting leadership of Mexico in this crop.

MATERIALS AND METHODS

The study was conducted under a quantitative, descriptive and comparative approach, with the objective of identifying the factors that impact the low levels of farm gate prices (FGPs) in the municipalities where Maradol papaya is produced, with special emphasis on the case of Villa de Tututepec, Oaxaca. To achieve this purpose, statistical and geographic analysis tools were applied, using primary and secondary information.

The database was built from records of the Agrifood and Fishing Information Service (*Servicio de Información Agroalimentaria y Pesquera*, SIAP) from 2023, which provided information about the production (ton) and the FGP ($/ton) of 229 producing municipalities distributed in 19 states of Mexico. Box diagrams were elaborated with these data to analyze the dispersion of the FGP, and dispersion graphs to explore their relationship with the production volume.

In addition, a bubble and dispersion graph was elaborated using Python code executed in Google Colab, with the objective of examining the interaction between FGP, production volume, commercial infrastructure (measured as the sum of packaging units and main supply centers in each state), and state competition (defined as the percentage of producing municipalities compared to the total of municipalities in each state).

To delve into the analysis of commercial infrastructure and its relationship with logistical distances, 155 packaging units and supply centers in the country were identified, using official sources such as the Directory of Certified Suppliers from SENASICA, the National Directory of Exporters from the Ministry of Agriculture, the Directory of Supply Centers from the Ministry of Economy, as well as complementary searches in Google Maps and social networks. Each packaging unit and supply center was georeferenced, which, in combination with the data of state FGP, allowed elaborating a map in QGIS software that represented the location of commercial infrastructure and its spatial relationship with the levels of FGP of each producing state. This representation facilitated the identification of zones with logistics advantages, access gaps to infrastructure, and strategic zones for the commercialization of Maradol papaya. Likewise, geopositioning data of the packaging and distribution centers, together with a representative sample of 38 municipalities (two for each state: the one with the highest and the one with the lowest FGP), were used to calculate the distances from each packaging unit and/or commercial center towards the producing municipalities of the sample. Then, the average of the distances corresponding to each municipality was obtained, thus generating the variable "average distance to local infrastructure".

Additionally, considering that wholesalers from Mexico City's Supply Center (CEDA-CDMX) tend to assume the transport costs, according to interviews conducted with producers from Villa de Tututepec, the distances from each municipality of the sample to the CEDA-CDMX were also measured. Both measurements of distance were later compared with the respective averages of municipal FGPs, with the aim of analyzing their possible effect on the producer's price.

RESULTS AND DISCUSSION

Despite its leadership in production volume, Villa de Tututepec presents a considerably low FGP compared to the 229 Maradol papaya producing municipalities in Mexico. This contradiction is evidenced in the box diagram, where the inter-quartile range of the FGP is found between $5,478.5 and $7,300 pesos MX, covering 50% of the municipalities. The median, of $6,619.23 pesos MX, confirms that Villa de Tututepec is among the municipalities with lower FGP than this value.

The extreme values of the diagram correspond to the highest and lowest FGPs recorded in Altamira, Tamaulipas ($12,540.88) and Mocorito, Sinaloa ($3,045.00), respectively. Villa de Tututepec, with a FGP of $5,558.35, is below the national average ($7,011.51) and slightly over the first quartile, within the second quartile of municipalities with the lowest prices.

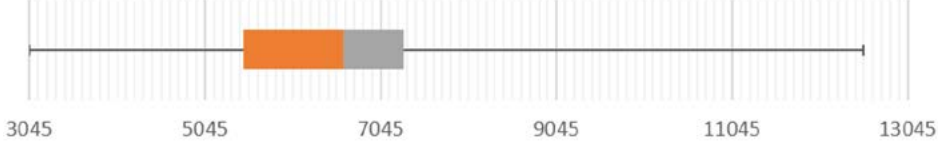

Figure 1. Variability of the Maradol papaya in municipalities of Mexico.

Analyzing the factors that impact the differences in FGP between the municipalities is essential. In the first place, it is suggested that a higher production volume could pressure the prices to decrease. The dispersion graph shows that the municipalities with lower production tend to obtain higher prices, while those with higher offer show lower FGPs. Although Villa de Tututepec is the principal producer, it presents a lower FGP than the national average, which evidences that a high volume does not ensure better commercial conditions.

The inverse relationship between the offer and the FGP indicates that a higher production volume does not always imply better prices for the producer. However, reducing the productive volumes would not be an adequate strategy, because these high volumes are derived mainly from the high crop yields in the region, which reached 107.24 ton/ha in 2023, figure 102.7% higher than the national mean (52.9 ton/ha). This outstanding yield reflects the high agricultural potential of the zone, previously identified by Espinosa Trujillo *et al.* (2018), who emphasized that the climate conditions in Oaxaca offer a better productive potential (14.6%) for papaya in comparison to states like Campeche (9.8%), Michoacán (5.6%) or Colima (0.4%), which suggests the intervention of other structural factors in the determination of the price.

When the average state FGP was considered as the representative indicator of the prices received by the municipalities within the local market, and when analyzing their relationship with their respective variables of commercial infrastructure, production volume, and market competition, a broader panorama about the formation of farm gate prices is revealed. Although the individual analysis of each variable shows low correlations, their joint interaction gives a better understanding of the factors that impact the behavior of the FGP.

In the bubble graph, Oaxaca is found in the lower left corner, with a Farm Gate Price (FGP) of approximately $6,056/ton, reflecting high production volumes, but minimal commercial infrastructure, which limits its capacity for negotiation and reduces the price received. Colima is found towards the middle right part of the graph, with a FGP of $7,721/ton, characterized by a very high commercial infrastructure (60 units), but affected by high competition between municipalities, which decreases the positive impact of said infrastructure in the prices. On the other hand, in the upper left part, Tamaulipas and the State of Mexico stand out, with FGPs of $12,112/ton and $8,914/ton, respectively, where the highest prices are found despite their low levels of production, competition and infrastructure. These patterns show that, in addition to productive and commercial factors,

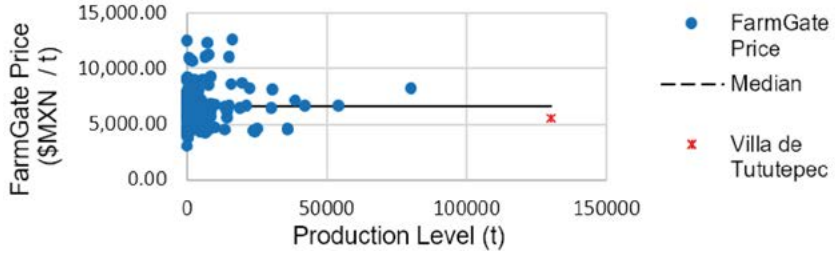

Figure 2. Relationship between the Maradol papaya offer and the FGP per municipality.

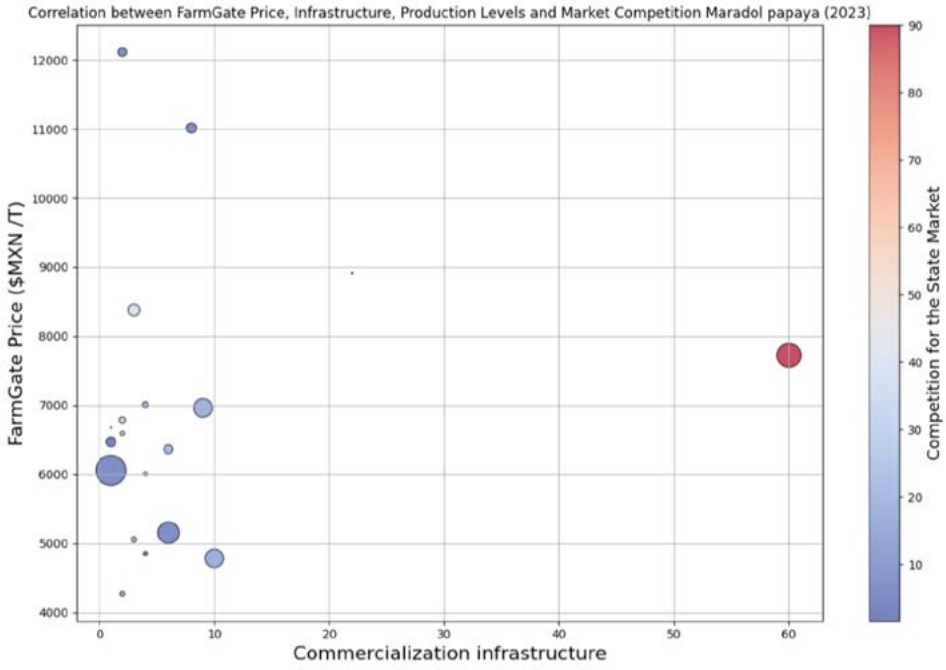

Figure 3. Relationship between FGP, infrastructure, production and state competition for Maradol papaya (2023).

the geographic location and proximity to strategic markets carry out a defining role in the formation of the farm gate price of Maradol papaya. In this context, the importance of spatially locating each state according to its distribution of FGP and commercial infrastructure emerges, as is presented in the following map.

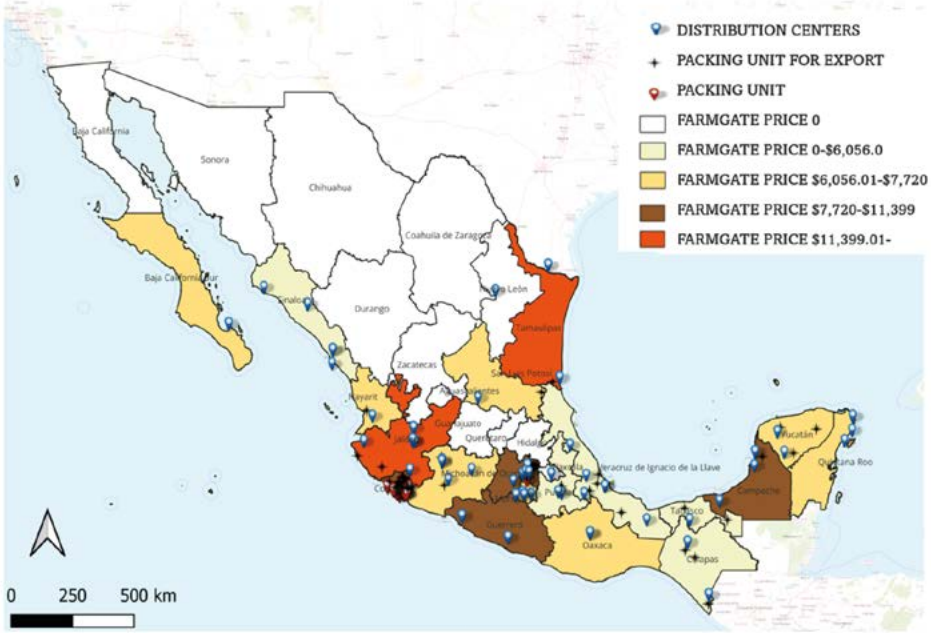

Figure 4. Relationship between commercial infrastructure and papaya FGP in Mexico.

Table 1. Relationship between the average FGP and the distance to commercial centers.

Distance Type	Range	Interval (km)	Average Farmgate Price ($MXN/ t)
Local infrastructure	Short	0-190	7,105.10
	Medium	190-380	6,976.80
	Long	>380	6,949.4
CEDA-CDMX[1]	Short	0-600	7,209.01
	Medium	600-1200	7,055.01
	Long	>1200	6,305.50

[1] CEDA-CDMX=Mexico City Central Wholesale Market.

The map reaffirms that Tamaulipas and State of Mexico achieve the highest FGPs despite their limited infrastructure, production and competition, thanks to their strategic location. Tamaulipas, with only four commercialization units, benefits from its proximity to the United States, which in 2023 absorbed 99.9% of the Mexican exports of papaya (ITC, 2024). Similarly, the State of Mexico, with high population concentration and located in the area of influence of the CEDA-CDMX, the largest wholesale market in the world (CCA, 2021), attains high prices despite its low production, supported by its reliance on external supply, dynamic that also benefits highly producing and neighboring states, such as Colima.

These geographic patterns confirm that, although the infrastructure does not determine by itself the price to the producer, its availability and strategic location can favor better commercial conditions. To delve into this relationship, a group of 38 producing municipalities was analyzed, which were selected for their extreme prices (two per each producing state), where a negative correlation between the distance to local infrastructure or to the CEDA-CDMX and the Farm Gate Price (FGP) was evidenced, highlighting the key role of the territorial component in the formation of agricultural prices.

The table shows that the producing municipalities located at less than 190 km from the local infrastructure or at less than 600 km from the CEDA-CDMX show the highest FGPs, of more than $7,000/ton. In contrast, those located at a distance of more than 1,200 km present decrease by up to 10% compared to the national average, confirming that the proximity to stockpiling and distribution centers favors better prices for the producer. Although in Villa de Tututepec producers do not assume the total cost of transportation directly, the distance impacts the price received, since the buyers adjust the value offered to compensate for the logistical expenses, estimated in 30 pesos per kilometer in 2023 according to interviews with local producers.

This pattern responds not only to the increase in the transport costs associated with longer distances, but also to the participation of more intermediaries in the national chain. Granados Ramírez *et al.* (2015) document a similar phenomenon in Veracruz, where the lack of logistical infrastructure transfers the transport and storage of papaya to the entrepreneurial sector, favoring higher margins of profit for the intermediaries. In Villa de Tututepec, a comparable dynamic is observed: the buyers, in addition to adjusting the price by distance, apply discounts from physical damage to the fruit during transport, reducing

even more the producer's income. Although the distances vary, the CEDA-DCMX is the main sales destination in both cases: Veracruz is located at around 380 km (six hours away) and Villa de Tututepec at 727 km (10.5 hours), which reinforces the idea that the distance and the lack of local infrastructure increase reliance on intermediaries and reduce the profitability of the producer.

Pindyck and Rubinfeld (1998) suggest taking advantage of economies of scope (ES) in transport, reducing costs when combining loads of different products with the same destination. This strategy could be viable in Villa de Tututepec, where the producers, in addition to Maradol papaya, grow lime, watermelon, corn, coconut, banana and mango, which increases the potential to consolidate mixed shipments towards CEDA-CDMX.

To measure the economy of scope (ES) in transport, the cost of sending two products separately is compared with the cost of transporting them together. If the joint cost is lower, there is an advantage in logistical, operative or infrastructure efficiency (Pindyck and Rubinfeld, 1998). The ES is calculated as:

$$ES = \left(C\left(q_1\right) + C\left(q_2\right) - C\left(q_1, q_2\right)\right) / C\left(q_1, q_2\right)$$

where: $C\left(q_1\right)$ and $C\left(q_2\right)$ are the individual costs of transport, and $C\left(q_1, q_2\right)$ the combined cost.

In Villa de Tututepec there are structural limitations to achieve economies of scope, since papaya, transported in bulk and with minimum packaging, is vulnerable to damage and requires conservation temperatures between 10 and 13 °C (Shakila and Anburani, 2010). This makes its logistical compatibility with other local crops such as lime or watermelon difficult, which demand lower temperatures (5-10 °C) and use refrigerated transport in boxes or sacks (AgroMarket, 2003).

Although papaya can be transported together with compatible crops such as mango (AgroMarket, 2003), interviews with producers and transporters indicate that the cost of the freight towards CEDA-CDMX or Puebla is the same for both products ($22,000 per shipment). Therefore, when the formula for economy of scope is applied, the result is zero, which reflects absence of logistical savings when combining them.

However, specific conditions are identified where a positive economy of scope could be generated in transport. According to local testimonies, in 2023, the cost of sending a Torton-type truck —three-axle, with approximate size of 6.50×2.50×2.40 meters (FAW México, s.f.)— was $22,000, regardless of the volume of the loads shipped and who pays for it, although 13 and 16 tons were considered the standard capacity of the truck and the minimum volume that transporters and stockholders require for the shipment payment to be convenient. Although these trucks can withstand up to 20 tons (FAW Mexico, s.f.), when exceeding 16 tons, the transporter applies an additional charge of $1,000 for each extra ton. Given that some papaya harvests do not always reach the 13 tons required, completing the load with compatible products such as mango would allow taking better advantage of the capacity available. In a scenario of combined load —13 tons of papaya and 7 of another fruit—, the total cost of transporting both products in a joint manner would reach $29,000,

while conducting two separate shipments it would cost $44,000. The economy of scope (ES) is assessed as:

$$ES = \left(\left(\$22{,}000 + 22{,}000\right) - \$29{,}000\right) / \$29{,}000 = 0.517 = 51.7\%$$

of savings, which shows a significant improvement in the logistical efficiency under controlled conditions.

If the cost of $29,000 were assumed by an alliance of producers, the unitary transport from Tututepec to CEDA-CDMX would be approximately $1,450 per ton. This would allow them to trade their papaya at similar prices to those found in the State of Mexico, where in 2023 the farm gate price was $8,914.30 per ton. In comparison, Villa de Tututepec received $5,558.35, reflecting a loss of $3,355.95 per ton when the stockpiler pays for the shipment. Thus, even when paying for transport, producers could significantly improve their income, representing a profitable opportunity.

Nevertheless, this strategy implies logistical and sanitary risks. According to FAO and WHO (2005), joint transport, even between compatible producers, can cause cross-contamination, physical damage, acceleration of maturation from exposure to ethylene, and even total rejection of the load when there is deterioration. In addition, it could complicate the unloading and classification, increasing time and operative costs.

Despite the challenges, sharing transport units continues to be viable in Villa de Tututepec, not only because most of the producers handle surfaces of 3 to 5 hectares in the same scale of production, which allows them to reach considerable volumes, but also because they can become consolidated with other producers from the same municipality to form joint loads. This highlights the need to improve packaging, going from bulk shipping to more streamlined systems that, in addition to protecting the quality of the fruit, reduce damage during transport. Likewise, it is essential to establish local packaging centers that decrease the reliance on external infrastructure. These actions would not only improve the price for producers but would also open access to markets of higher value, including direct exports currently limited by logistical and commercial presentation deficiencies.

CONCLUSIONS

The low Farm Gate Price (FGP) of Maradol papaya producers in Villa de Tututepec is the result of structural factors that limit their commercialization, among them the scarce infrastructure for packaging and distribution, the distance from main supply centers, and local competition. Although the municipality presents high productive yields, the lack of adequate logistics restricts its competitiveness. The results emphasize that improving packaging, establishing stockpiling centers, and optimizing transport through consolidated loads are key strategies to strengthen the commercial position, reduce damage, and gain access to markets of higher value. The conclusion is that logistical restructuring is essential to increase the income of producers and to consolidate their competitiveness in the Maradol papaya market.

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Vicia faba L. microgreens: effect of the variety and substrates on the morphological characteristics, photosynthetic pigment content, and soluble protein

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ABSTRACT

Objective: To evaluate the effect of variety and substrates on the morphological characteristics, photosynthetic pigment content, and soluble protein of fava bean (*Vicia faba* L.) microgreens cultivated in a prototype growth chamber.

Design/Methodology/Approach: Microgreens from two local fava bean varieties (T-5 and T-12) were cultivated in aluminum trays under 8/16 h light/dark cycles using 30 W LED bulbs. The substrates evaluated were sand and coconut fiber (CF). The microgreens were harvested eight days after emergence, and morphological variables (fresh weight, height, root length), photosynthetic pigment content, and soluble protein were determined.

Results: The chamber prototype maintained stable environmental conditions (temperature 20-25 °C, relative humidity 65-88%, luminosity 165-206 μmol m^{-2} s^{-1}). Variety T-5 showed higher fresh weight (2.44 g/plant) and height (19.86 cm) in CF compared to sand. Both varieties exhibited greater root length in CF. Only variety T-12 showed higher chlorophyll a (119.47 μg/g FW) and total chlorophyll (171.24 μg/g FW) content in CF. Soluble protein was higher in T-5 cultivated in sand (69.67 mg/g DM). Dry matter showed no significant differences between substrates (10.84-11.91%).

Limitations/Implications: The results are limited to the substrates and varieties evaluated; future studies could include more substrates and adjustments to the photoperiod.

Findings/Conclusions: The morphological and biochemical characteristics of fava bean microgreens were significantly influenced by the variety × substrate interaction, highlighting the importance of selecting the appropriate substrate according to the variety to optimize production and nutritional quality.

Keywords: sand, coconut fiber, fava bean, microgreens, growth chamber prototype.

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INTRODUCTION

The growing demand for alternative foods to combat chronic noncommunicable diseases, such as obesity, diabetes and cardiovascular disease, has motivated research into

functional foods (OMS, 2021). Among these, microgreens have gained relevance because of their enhanced organoleptic and nutritional profiles (Šamec *et al.*, 2018). These are harvested in early development stages, generally between 7 and 21 days after germination, which makes them ideal for urban and controlled production systems due to their short cycle and low space requirements (Xiao *et al.*, 2012; Yeargin *et al.*, 2023).

A defining factor in the production of microgreens is the selection of substrates, which directly influence the yield and the biochemical and physiological quality. Organic substrates, such as compost, have resulted in significant increases in biomass compared to inorganic substrates (Bilalis *et al.*, 2025). Mixtures such as coconut fiber (CF) and vermiculite (1:1) favor the germination and accumulation of bioactive compounds in Brassicaceae (Pant *et al.*, 2023). Likewise, commercial substrates with CF, perlite and peat improve the yield in species such as radish and mizuna, thanks to their high water retention and nutritional contribution (Alloggia *et al.*, 2025). Light regulated through LED systems is another key factor to optimize physiological processes in controlled environments (Pant *et al.*, 2023; Hamilton *et al.*, 2023).

Although species from the Brassicaceae family are the ones most used in the production of microgreens, legumes such as chickpeas, lentils and mung beans have gained importance due to their nutritional attributes (Gunjal *et al.*, 2024). In this context, fava bean (*Vicia faba* L.) emerges as a promising species to produce microgreens, not only because of its conventional nutritional value, but also because of the significant improvement in its bioactive profile during germination. Studies such as that from Wei *et al.* (2022) show that a short germination significantly increases the content of soluble proteins and essential amino acids, while reducing anti-nutrients such as phytic acid. This profile is enriched with the increase of antioxidant activity, associated with the biosynthesis of phenolic compounds during germination, as reported by Okumura *et al.* (2016), who identified phenols in fava bean sprouts with high capacity to neutralize free radicals.

Despite the recognized nutraceutical potential of *Vicia faba* L. sprouts and the documented influence of substrates in microgreens from other species, little is known about the effect of alternative substrates on fava bean microgreens, particularly in their morphological characteristics, photosynthetic pigment concentration, and soluble protein content. Therefore, the objective of this study was to evaluate the effect of two substrates (sand and coconut fiber) on these parameters in two local varieties of *Vicia faba* L. cultivated in a growth chamber prototype, with the aim of optimizing their production and nutritional quality.

MATERIALS AND METHODS

Plant material and growth substrates

Seeds from two local varieties of fava bean (*Vicia faba* L.) were used: T-5 ("Cochinera") and T-12 ("Criolla Amarilla"), from the municipality of Tlahuapan, Puebla, Mexico. These varieties were selected due to their morphological variability and contrasts in biomass yield, according to what was previously reporte by Fuentes-Herrera *et al.* (2025).

The substrates evaluated were sand (obtained from San Antonio Cacalotepec, Puebla) and coconut fiber (CF; Eco Sustrato Orgánico®). The sand was sifted with a No. 12 sieve

(1.4 mm holes) to homogenize the particle size, and the impurities were eliminated. Both substrates were sterilized in autoclave at 125 °C during 30 minutes and saturated with tap water before sowing.

The seeds were disinfected through immersion in sodium hypochlorite solution at 100% during 3 minutes, followed by five rinses with distilled water.

Experimental design and cultivation conditions

Sowing was conducted in perforated aluminum trays (38×29×6.5 cm) which were divided into two sections (19×14.5 cm), each containing a different substrate (CF or sand). Three replicates per variety were used. The sowing density was 90 seeds/section for T-5 (15×6 arrangement) and 76 seeds/section for T-12 (11×6 arrangement + 1 row of 10). The treatments were randomized within each tray to eliminate potential effects from position.

Cultivation was carried out in a chamber prototype built with a transparent plastic box, made of virgin high-density polypropylene (HDPE), with measurements 52×34×18 cm, in which the aluminum tray was placed with the substrates and the seeds. To maintain the relative humidity inside the chamber, white cotton pads moistened were placed around the plastic box, which were regularly sprayed in the morning, at the beginning and end of the light period. The lighting conditioning in the growth chamber prototype was established with two wood structures. Each was built with four 68 cm high beams, with a 65 cm long by 20 cm wide board placed on the upper part, where three LED 30 W light bulbs were installed in a circuit (2700 lumens, 6500 K), placed in a series and equidistant (16.5 cm between them). Once the microgreens emerged, they were subjected to a photoperiod of 8/16 h (light/dark), which was controlled by a switch (Figure 1a).

The prototype with the growth trays was placed in a dark room, and the environmental conditions (temperature, relative humidity, and luminosity) were monitored every 30 minutes using a datalogger (HOBO, MX1105).

Harvest and morphological measurements

The days until emergence were recorded for each variety and replicate; the growth measurements of microgreens were taken on harvesting day. The harvest was conducted once the plants had passed the sprout stage and presented two true leaves emerging, which was 8 days after emergence (Figure 1b), taking as the first day of emergence when there were 50% of the seeds sprouted; this was recorded daily to determine the percentage of germination.

Measurements of morphological characteristics were recorded in 20 microgreens completely randomly from each variety, substrate and replicate at the time of harvest. The plant height was recorded in centimeters (cm) with a Vernier (Digimatic Caliper, Mitutuyo), taking as reference from the start of the epicotyl to the apical part of the sprout growth. The root length was measured in cm from the start of the epicotyl down, taking the longest root and the width of the epicotyl in millimeters (mm). The shoot fresh weight was measured in grams (g) on an analytical balance (A&D, GR-202), after removing any remaining seed coat and the roots.

Figure 1. Growth chamber prototype: a) Adaptation of the structure for illumination with tray and substrates, b) Fava bean microgreens during growth.

After recording the measurements and weight of the microgreens from each substrate and replicate, a portion was used for some fresh measurements such as percentage of humidity and photosynthetic pigments. The other portion of the microgreens was processed through freeze-drying for soluble protein analysis.

The percentage of humidity (%H) of each substrate and each repetition was determined by four replicates. The determination was made in an infrared radiation thermobalance (OHAUS, MB 45); based on the %H of each sample, the percentage of dry matter was obtained (%DM).

To freeze-dry the microgreens, they were cut into small pieces (0.5 cm^2 approximately), wrapped in aluminum foil and frozen at −50 °C. The samples were kept frozen for at least 24-48 h before starting the freeze-drying process. A Labconco freezer was used (FreeZone Triad) with the following parameters: collector temperature of −80 °C and vacuum pressure of 0.220 mbar; at −80 °C, the process lasted 36 to 48 hours until reaching a humidity of 5-10%. Later, the freeze-dried samples were ground with an electrical mill (Krups GX4100) and sifted with a number 35 sieve with particle size 420 μm. Once the samples were ground, they were stored in sealed amber containers and kept frozen at −20 °C.

Determination of photosynthetic pigments

The quantification of chlorophyll a and b, carotenoids, and xanthophylls was carried out according to the method described by Lichtenthaler (1987) with some adaptations according to Viveros (2024). For the extraction, six samples of 400 mg of fresh microgreens of the two varieties were randomly used from each substrate and each replicate, cut into small slices (3-5 mm), which were placed in 2 mL Eppendorf tubes with 1600 μL of acetone at 80%. During this process, the tubes with the samples were kept in ice. Then, the samples were placed in an ultrasonic bath (Ultrasonic Cleaner, model AS5150B) at 4 °C with ice, for 3 minutes, with 6 pulses of 30 seconds and 10 seconds of rest between each pulse (at power 7 and 6 of degasification). Later, the extracts were kept under refrigeration at 4 °C for 24 h. Next, the tubes were placed again un the ultrasonic bath for 30 s (at power 7 and 6 of degasification) and centrifuged at 2350 *g* at 4 °C

for 5 minutes. Finally, absorbance at 470 nm, 646 nm and 663 nm was read on the supernatant with a spectrophotometer UV/VIS (Evolution 300, Thermo-Scientific). With the absorbance data, the concentration of chlorophylls (chl a, chl b), xanthophylls and carotenoids (Cx + c) was calculated according to the equations from Lichtenthaler (1987). The concentrations were expressed in μg/g FW.

Determination of soluble protein

To elaborate extracts, 10 mg of freeze-dried sample were weighed in Eppendorf tubes and 1 mL of deionized water was added; during this process, the tubes were kept in ice. Then, the tubes were agitated in Vortex (Genie 2 Digital de Scientific Industries) for 10 seconds, to later centrifuge them in a microcentrifuge (Hettich, MIKRO 200R) for 10 minutes at 1960 *g* at 4 °C. The supernatant was used as extract.

The quantification of soluble protein was carried out according to the Bradford (1976) method. On a 96-well plate (Costar), 50 μL of diluted or standard extract and 200 μL of Bradford reagent were placed (Sigma Aldrich. Catalogue B6916); the plate was shaken for one minute, and it was left resting for 5 min in the dark. Deionized water was used as control. Then, the absorbance readings were conducted in a multimode microplate reader (Varioskan Flash, Thermo Scientifc) at a wavelength of 595 nm. The calibration curve was prepared with a stock solution of 1mg/mL Bovine Serum Albumin (BSA) in a range of 0 to 250 μg/mL. The concentration of soluble protein was calculated based on the curve equation and expressed in mg/g DM.

Statistical analysis

Prior to the analysis of variance, the assumptions of normality and homogeneity of variances were verified. The normality of the residues was evaluated through the Shapiro-Wilk test, and the homogeneity of variances through the Levene test. All the variables analyzed (fresh weight, height, root length, sprout width, dry matter, photosynthetic pigments, and soluble protein) fulfilled both assumptions ($p \geq 0.05$), except for the variables fresh weight and carotenoids + xanthophylls, which do not fulfill the assumption of homogeneity of variances ($p \leq 0.05$). However, given that the ANOVA is robust in the presence of mild violations of these assumptions and given the balanced design of the experiment, the parametric analysis was started.

To determine the effect of the variety × substrate interaction, analysis of variance (ANOVA) was conducted as well as Tukey's means comparison with a level of significance $p \leq 0.05$ (PROC GLM; SAS Institute Inc., 2002).

RESULTS AND DISCUSSION

Environmental conditions for growth

Recent studies have documented the different ways in which microgreens can be cultivated, and under quite varied conditions, such as open or closed spaces, like greenhouses, whether in a hydroponics system with inert substrates, on soil, or in a soil-less medium (Arya *et al.*, 2022; Seth *et al.*, 2025). Although growing microgreens is a relatively simple and quick process, it is important to control and evaluate the environmental conditions for

each species, to improve their growth in terms of productivity, nutritional and functional quality (Franks and Richardson, 2009).

For this study, a prototype of growth chamber was designed and tested, through which there was a constant supply of light in a light/dark cycle of 8/16 h. The recording of the environmental variables within the growth chamber of microgreens of the two fava bean varieties since sowing on July 23 until October 11, showed that, in general, the temperature, relative humidity and luminosity showed low variation throughout the period and between replicates. For example, in variety T-5, cultivated from July 23 to August 21, 2024, the temperature was kept at 22.9 °C on average, the range per day in the replicates varied between 20.4 and 25.7 °C, with a coefficient of variation (CV) under 1% (Figure 2a). The relative humidity showed an average of 77.2%, and the variation per day within the replicates was 60.9 to 88.4%, with a CV under 2% (Figure 2b). The luminosity showed an average of 9841 lux, with a variation in the replicates per day of 8915 to 11281 with a CV below 5% (Figure 2c).

In variety T-12, the environmental conditions also remained without much variation. Since sowing on September 1 until harvesting on October 11, 2024, the temperature was kept at 21.9 °C on average, with a variation per day in the replicates between 20 and 25.3 °C, and CV under 8% (Figure 2a'). The average relative humidity was 78.2%, where the CV was under 1%, with a variation per day in the replicates between 67.4 and 88.2% (Figure 2b'). The average luminosity was 9742 lux with a variation per day in the replicates of 8999 to 10975 lux and a CV lower than 3% (Figure 2c').

The light regime during cultivation of microgreens is an important parameter, given that in addition to supplying the energy for photosynthesis (Delian *et al.*, 2015), it performs a fundamental role in various physiological and biochemical processes. It is known that light conditions, in terms of spectral quality (wavelength) and light intensity (quantity), can influence both the morpho physiology and the biosynthesis of phytochemicals of the sprouts and micro-vegetables (Santin *et al.*, 2022). In this study, the prevailing light intensity ranged between 8957 lux (165 μmol m^{-2} s^{-1}) and 11128 lux (206 μmol m^{-2} s^{-1}). In this regard, some authors suggest preferably using artificial light, such as light from light emitting diodes (LEDs) to cultivate microgreens in controlled environments (Seth *et al.*, 2025), such as the type of light that was used in this study.

Percentage of germination

Microgreens of the two varieties cultivated in the CF and sand substrates presented percentages of germination greater than 90%. Variety T-5 showed 96% in the sand substrate, the plants emerged at 5 days, and the time from sowing to harvesting was 13 days (Figure 3). This same variety in the CF substrate showed 95% germination, 7 days until seed emergence, and 15 days from sowing to harvesting of the microgreens. Meanwhile, variety T-12 exhibited a slightly lower germination percentage; for example, in the sand substrate they showed 93% and in CF 92%, and the seeds in both substrates showed on average the same days until emergence (4 days) and the same time from sowing until harvesting of the microgreens (14 days) (Figure 3).

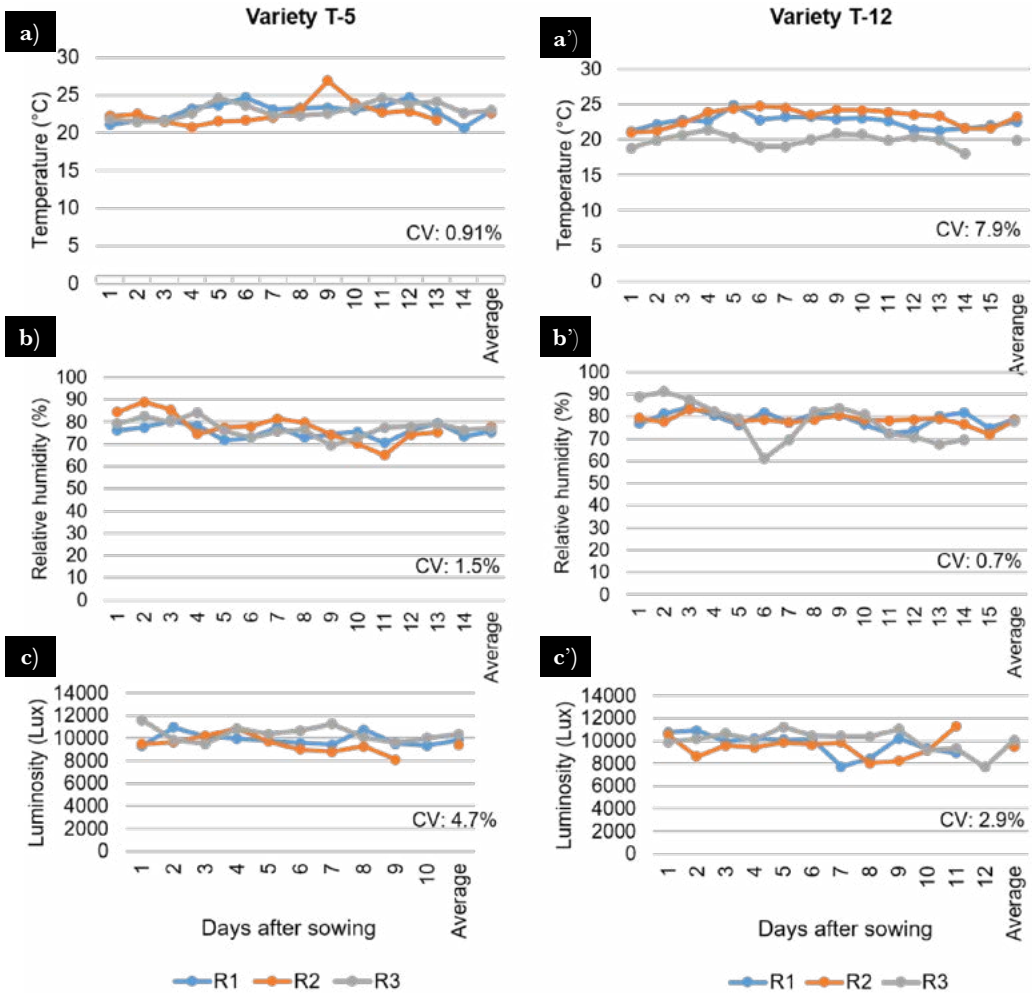

Figure 2. Environmental conditions found in the growth chamber during the growth of microgreens of variety T-5 (a-c) and variety T-12 (a'-c') with a light/dark cycle of 8/16 h per day. a) and a') Temperature; b) and b') Relative humidity; and c) and c') Luminosity per day of the replicates, and average at the end of the period when the microgreens harvest was conducted.

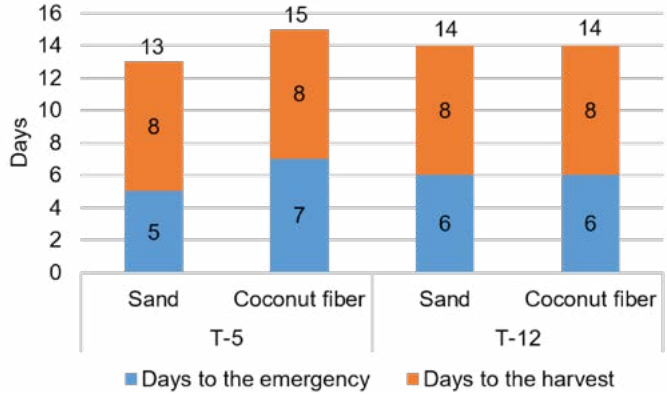

Figure 3. Days until emergence and days until harvest of fava bean microgreens, varieties T-5 and T-12, cultivated in a sand and coconut fiber substrate.

In both varieties, the days until harvesting to obtain microgreens were short. In this regard, Bewley *et al.* (2013) mention that, in cultivating sprouts or microgreens, where the production cycle is short and high productivity is sought in a short time, achieving quick and homogeneous germination becomes essential to obtain quality products and reduce losses.

Effect of the variety and the substrate on the growth and accumulation of biomass in fava bean microgreens

Although some variables deviated from normality, the ANOVA is considered robust to such deviations given the sample size (>30 per group). The interaction between variety × substrate showed a significant effect ($p \leq 0.05$) on the morphological variables and the fresh weight of fava bean microgreens (Table 1). In variety T-5, the CF substrate promoted a higher fresh weight (2.44 g/plant), sprout height (19.86 cm), and root length (12.65 cm) compared to sand (1.96 g, 16.37 cm and 10.17 cm, respectively). On the contrary, in variety T-12, only the root length was significantly higher in CF (12.13 cm *vs.* 10.46 cm in sand), while the fresh weight and height did not differ between substrates. These results suggest a differential response of the varieties to the type of substrate, possibly related to their capacity for morphological adaptation and efficiency in resource absorption.

The longer root length observed in CF for both varieties is consistent with what was reported by Di Gioia *et al.* (2017), who emphasize that a well-developed root system improves the absorption of water and nutrients, which can translate into better aerial growth. In addition, the presence of longer and less ramified roots in CF (Figure 4a) compared to those in sand (Figure 4b), suggests differences in the root architecture influenced by the physical properties of the substrate, such as porosity and humidity retention.

The absence of differences in the sprout width between substrates suggests that this variable is more influenced by genetics than by the growth medium. Köpke and Nemecek (2010) describe that, in legumes, characteristics such as stem thickness is associated with the production of aerial biomass and can be indicative of the productive potential of the plant.

Regarding dry matter (DM) content, no significant differences were observed between substrates for any variety, with values between 10.84% and 11.91%. These

Table 1. Weight, morphological characteristics, and dry matter of fava bean microgreens of varieties T-5 and T-12 cultivated in two substrates.

Variable	T-5		T-12		HDS
	Coconut fiber	Sand	Coconut fiber	Sand	
Weight (g)	2.44a±0.29	1.96b±0.20	2.52a±0.35	2.41a±0.37	0.15
Sprout height (cm)	19.86a±3.19	16.37b±2.48	17.70b±3.58	16.80b±3.22	1.49
Sprout width (mm)	3.93b±0.47	4.00ab±0.40	4.17a±0.49	4.10ab±0.56	0.23
Root length (cm)	12.65a±3.01	10.17b±2.33	12.13a±2.31	10.46b±2.36	1.19
Dry matter (%)	10.84a±1.08	11.89a±1.33	11.30a±1.81	11.91a±1.04	2.03

Mean values±standard deviation. MSD=minimum significant difference; a different letter in the same line marks significant differences according to Tukey's test ($p \leq 0.05$).

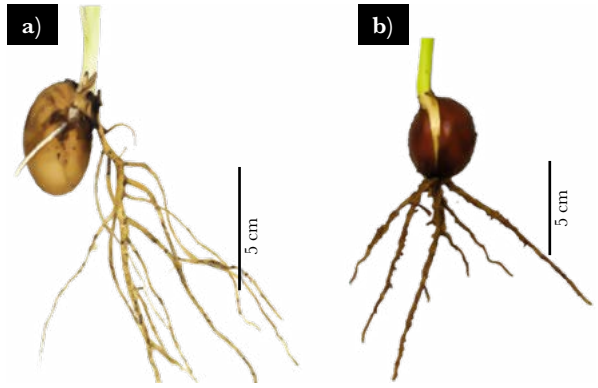

Figure 4. Roots of fava bean microgreens 8 days after emergence. a) Roots of seeds cultivated in coconut fiber substrate, b) Roots of seeds cultivated in sand substrate.

values are notably high in comparison to those reported for other microgreens, such as those from Brassicaceae, which tend to fluctuate between 4.5% and 7.4% (Bafumo *et al.*, 2024). This reflects the potential of legumes, such as fava beans, to accumulate dry biomass and compounds of nutritional value in early stages of development (Mangla *et al.*, 2025).

These findings emphasize the importance of selecting substrates that favor root development and productivity, especially in controlled production systems where efficiency in the use of resources is critical (Rouphael *et al.*, 2018). Future studies could explore the combination of substrates or the use of conditioners to further optimize the growth and nutritional quality of fava bean microgreens.

Effect of the interaction variety x substrate in photosynthetic pigments and soluble protein

Results from this study show a significant effect ($p \leq 0.05$) of the variety × substrate interaction on the content of photosynthetic pigments and soluble protein in fava bean microgreens (Table 2, Figure 5). This interaction highlights the importance of considering both the genotype and the growth medium to optimize the nutritional and functional quality of microgreens.

Table 2. Photosynthetic pigment content of fava bean microgreens of the varieties T-5 and T-12 cultivated in coconut fiber and sand.

	T-5		T-12		
Variable	**Coconut fiber**	**Sand**	**Coconut fiber**	**Sand**	**MSD**
Total Chlorophyll (μg/g FW)	176.92a±14.44	180.28a±25.81	171.24a±30.01	112.86b±17.89	23.48
Chlorophyll a (μg/g FW)	125.05a±8.41	125.38a±11.10	119.47a±13.99	85.42b±13.91	11.96
Chlorophyll b (μg/g FW)	51.86a±11.04	54.90a±15.99	51.77a±18.05	27.43b±6.83	14.32
Chlorophyll a/b	2.51b±0.50	2.44b±0.63	2.46b±0.50	3.25a±0.75	0.59
C+x (μg/g FW)	34.66a±2.82	32.41ab±6.27	34.24a±3.65	28.31b±7.65	5.01

Mean values±standard deviation. C+x=: carotenoids + xhanthophylls. MSD=Minimum significant difference. Means with the same letter per line are not significantly different between substrates according to Tukey's test ($p \leq 0.05$).

Variability in the photosynthetic pigment content

The photosynthetic pigment content showed a differential response between varieties and substrates. While variety T-5 did not show significant differences in the concentration of chlorophylls between substrates, variety T-12 cultivated in CF showed significantly higher values of chlorophyll a (119.47 μg/g FW) and total chlorophyll (171.24 μg/g FW), compared to cultivation in sand (85.42 and 112.86 μg/g FW, respectively) (Table 2). This difference represents an increase of 39.6% in chlorophyll a, and 51.6% in total chlorophyll for T-12 in CF. The a/b chlorophyll rate, which ranged between 2.42 and 3.45, suggests a photosynthetic adaptation to the light intensity used, consistent with what was reported by Wojdyło *et al.* (2020) and Lichtenthaler (2007), who mention that this proportion reflects the cultivation conditions, where high values indicate exposure to high light intensity.

The absence of significant differences in carotenoids and xanthophylls between substrates agree with studies such as Amitrano *et al.* (2024), who observed that these secondary pigments are less variable in the presence of changes in the substrate, possibly due to their stabilizing role in the photosynthetic apparatus under stress conditions.

Influence of the substrate in the accumulation of soluble protein

The soluble protein content showed a marked influence of the substrate depending on the variety. Variety T-5 cultivated in sand presented a significantly higher content (69.67 mg/g DM) than in CF (49.9 mg/g DM), which represents a reduction of 28.4 % in the latter substrate (Figure 5). On the contrary, variety T-12 did not show significant differences between substrates, although a slightly higher trend was observed in CF (62.65 mg/g DM vs. 58.36 mg/g DM in sand).

These results can be attributed to differences in the absorption efficiency of nutrients and water between substrates, as well as the capacity of each variety to modulate its nitrogenated metabolism in response to the medium. Mangla *et al.* (2025) point out that legumes, such as fava bean, have a high potential to accumulate proteins in early stages,

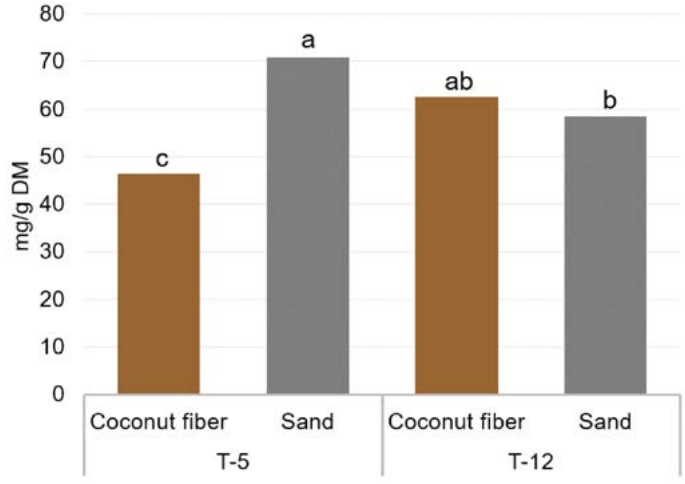

Figure 5. Soluble protein content of fava bean microgreens of varieties T-5 and T-12 cultivated in coconut fiber and sand substrates. Bars with the same letter are equally significant according to Tukey's test ($p \leq 0.05$).

although this accumulation is subject to abiotic factors such as availability of nutrients and water in the substrate. Since it has lower water retention capacity, sand could induce a slight water stress that favors the synthesis of soluble proteins as a mechanism for osmotic adjustment, especially in sensitive genotypes such as T-5.

Substrate selection is crucial to maximize the nutritional quality of microgreens, particularly in legume species such as fava bean, which show a high genetic variability in response to the growth medium. This study's findings agree with Kyriacou *et al.* (2019), who reported wide variation in the content of chlorophylls and other bioactive compounds in microgreens of different species and cultivation conditions.

The 8/16 h light/dark cycle used in this study may not be optimal for the synthesis of soluble proteins. Silva *et al.* (2025) showed that longer light cycles (12/12 or 16/8) significantly increase the protein content in kale microgreens, which suggests that adjustments in the photoperiod could improve the protein quality of fava bean microgreens.

The interaction variety × substrate is a determinant factor in the accumulation of photosynthetic pigments and soluble protein in fava bean microgreens. While CF favored the content of chlorophylls in variety T-12, sand promoted a higher accumulation of soluble protein in variety T-5. These findings underline the need to select specific combinations of variety and substrate, to optimize the production of microgreens with high nutritional and functional value.

CONCLUSIONS

The growth chamber prototype proved to be an effective tool to keep the environmental conditions stable (temperature, relative humidity, and luminosity), providing a viable controlled environment for the production of fava bean microgreens, which validates its use for future studies and applications for urban or precision agriculture.

A significant interaction was confirmed between the fava bean variety and the type of substrate, which determined the growth, development and biochemical profile of the microgreens. This emphasizes that the plant's response is not independent from the substrate, but rather that it critically depends on the genotype; thus, generalizations should be avoided, highlighting the need to select specific combinations.

For variety T-5, the CF substrate was better to promote morphological characteristics such as fresh weight, height, and root length. However, although the sand substrate induced a higher content of soluble protein, this suggests that the selection of the substrate for this variety should prioritize the objective of production: biomass (CF) or protein quality (sand).

For variety T-12, CF significantly favored the biosynthesis of chlorophyll a, and total chlorophyll, as well as a greater root length. The soluble protein did not show differences between substrates. Therefore, for this variety, CF presents as the most adequate substrate to improve both the development and the photosynthetic pigment content.

The high percentage of dry matter (10.84-11.91%) compared to other microgreens, together with the high contents of soluble protein, place *Vicia faba* L. microgreens as a promising functional food, rich in compounds of nutritional value.

The main contribution of this study lies in proving, with scientific evidence, that the optimization of the production of fava microgreens requires a joint selection of genotypes and substrates. These findings are relevant for farmers, plant breeders, and the food industry, by providing specific criteria to maximize the yield and the nutritional quality in controlled agriculture systems, thus contributing to the diversification of nutritious and sustainable food sources.

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Scientific research and emerging trends in alternative tourism and agrotourism

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ABSTRACT

Objective: To map the scientific production on alternative tourism and agrotourism in databases like Scopus and Google Scholar during the 1991-2025 period.

Design/methodology/approach: The bibliometric analysis of alternative tourism is essential to highlighting the trends and impact of this sector in both academic and practical settings. By studying scientific production, citations, and collaboration networks in this field, emerging research areas, knowledge gaps, and the most relevant topics of interest were identified. This study analyzes the scientific production on alternative tourism and agrotourism through a bibliometric approach, using tools such as VOSviewer and Scopus databases.

Results: Significant growth in publications was identified since the 1970s. Alternative tourism promotes a positive impact on local development, environmental sustainability, and the cultural strengthening of rural communities. The bibliometric analysis identified 363 publications in Scopus, demonstrating academic interest in this field. Among the topics related to alternative tourism are sustainable development, climate change, and environmental impact, highlighting the need to integrate climate change as a transversal axis in tourism policies.

Limitations on study/implications: The presence of agrotourism publications outside of Scopus limits knowing the exact value of scientific production during the 1991-2025 period.

Findings/conclusions: Alternative tourism and agrotourism are key elements for the resilience and sustainable development of natural spaces.

Keywords: agro-ecotourism, climate, rural areas.

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INTRODUCTION

The World Tourism Organization (UNWTO) conceptualizes alternative tourism as a way of traveling that includes recreational activities directed at interacting with the natural environment and cultural manifestations, seeking the preservation of environmental resources. This touristic approach is characterized by its proposal, design and execution based on sustainability criteria, positioning itself as an alternative to large-scale conventional tourism, according to what the UNWTO establishes (2024).

Alternative tourism encompasses various forms (ecotourism, agrotourism, and rural tourism) for enjoying enriching experiences in harmony with the natural and socio-cultural environment, as opposed to massive traditional tourism (Dorta and de la Caridad 2022). This type of tourism is associated with the search for less popular destinations, which are more agreeable to coexisting in daily and community life, and local cultures, fostering a greater social, environmental and economic impact. García de Fuentes and Cervera (2020) maintain that alternative tourism emerges in the decade of the 1970s and acquires great importance since the 1990s, when it reached annual growth rates of 20% globally, becoming the tourism segment of greatest growth.

As one of the modalities of alternative tourism, agrotourism has a great social and cultural importance, since it offers new opportunities for participation, decision-making, access to resources and new technologies, employment generation, diversification of sources of personal and community income, strengthening of agricultural culture, and all of this has an effect on the quality of life of people and families.

Agrotourism has been developed in Mexico as a touristic modality, integrated by fundamental components to promote sustainability and decrease poverty levels, through the implementation of initiatives that drive its development in zones that are at a disadvantage, local and indigenous communities. Some studies point to the lack of updating in the trends of the research topic and that it has experienced changes in response to contemporary socioeconomic demands (Jiménez Bulla 2019).

Bibliometry is a tool that allows exploring the current state of a field of study through the quantification of global scientific production. In this analysis, the trends in research about alternative tourism are evaluated, identifying the main themes, authors, institutions, and relevant journals, as well as the evolution in the field in recent years.

It is considered that bibliometric mapping is the most adequate research approach to synthesize the findings of studies, as described by Zupic and Cater (2015). On the other hand, the studies and publications related to the topic can be measured clearly (Cobo *et al.*, 2011), giving us a measure of the importance and relevance of the topic of study today, and detecting the main authors who are working with the topic of interest, the main collaborations between authors and countries, among other aspects. Bibliometric analysis measures the scientific production of a specific topic. This analysis is based on gathering and analyzing bibliographic information, with the aim of measuring the influence and impact of scientific publications on alternative tourism. The databases are selected by the number of journals and the theme, in addition to these databases having broad international recognition and reach (Zhu and Liu, 2020; Duque and Duque, 2022). Therefore, the objective of this study was to map the scientific production of the topic of alternative tourism and agrotourism in databases such as Scopus and Google Scholar during the 1991-2025 period.

MATERIALS AND METHODS

A documentary exploration was conducted using the Scopus platform (http://www.elservier.com/es-mx/solutions/scopus). The search criterion was based on the broad catalogue of periodical publications with high impact index. The search term used was Agroturismo, without applying additional filters, with the purpose of obtaining an integral

view of agrotourism in the different thematic classifications that Scopus offers to organize contents. The time range covered from 1991 to March 26, 2025, and 308 published documents were identified. The bibliographic data were exported in CSV format including all the information available on the platform for each publication.

Through the Bibliomerix package in R 4.2.0, the following variables were analyzed: annual distribution of publications, categories of predominant documents (scientific articles, reviews, books, book chapters, brief communications, and other types of documents), disciplines with greatest productivity, temporal evolution of the most studied areas, countries with most production, most productive journals, authors with largest number of studies and citations, and articles of highest impact. The graphs of temporal production, evolution of the three main disciplines and distribution by documentary types, were generated using Sigmaplot version 10.0. The visualization of networks with key terms was constructed from the keywords assigned by the authors, where the frequency determined the size of the nodes. Both the network of key terms and the network of international collaboration were developed in VOSviewer version 1.6.19. The thematic map was generated using the biblioshiny function of the Bibliometrix package in R version 4.2.0.

RESULTS AND DISCUSSION

The bibliometric analysis in the study field of agrotourism has evolved considerably, with a predominant focus on the relationship between "agrotourism" and "rural tourism", although as pointed out by Sánchez-Oro *et al.* (2022), these concepts are not synonyms but rather represent different touristic manifestations, where "agrotourism represents a specific modality within the broad spectrum of rural tourism, characterized by its direct connection with productive agricultural activity". This conceptual distinction is fundamental to understanding the complexity of the phenomenon and its theoretical and practical implications.

Scientific production at the global level

According to the study conducted, the temporal trends and increase in the concept of agrotourism show a field of study in clear growth, with a global annual growth rate in scientific production of 6.68%, with a modest start in the 1990s (1-2 articles/year) and sustained growth in 2000-2010 (3-8 articles/year). An important increase is seen since 2015 (10+ articles/year) and an increase in publications in recent years (2019-2025). The highest peak in production was in the years 2023 (47 articles) and 2024 (55 articles). This increase indicates that agrotourism and alternative tourism in rural ecosystems is a robust field of study, with growing importance in the academic-scientific scope.

Keywords and topics in trend

The significant presence of terms such as "Rural development", "Sustainable development", and the coexistence of "Agriculture" and "Tourism" in keywords reflects the interdisciplinary and multidimensional nature that this field has acquired. As described by Moral-Cuadra *et al.* (2023), "agrotourism of the 21st century has evolved toward a triple-

impact model where economic profitability is balanced with environmental conservation and social justice". That is, a change in paradigm is foreseen which transcends the traditional view of agrotourism as a merely complementary economic activity to position it as an integral catalyst of sustainable territorial development.

Figure 1 shows the word cloud of keywords related to agrotourism, where the correlation of countries or terms can be seen such as rural areas, agricultural development, ecotourism, agrotourism, and agriculture as central words of touristic development.

The most relevant results of the bibliometric analysis were that the keywords and the main topics addressed, according to the most frequently used keywords by authors, are related to Agrotourism (with a total of 122), rural tourism (32), tourism (23), agrotourism (19), and rural development (17). At the same time, from the keywords plus (Keywords-Plus) derived from the titles of the references, the ones that stand out are tourism development (29), tourism (24), agriculture (20), rural area (18), and sustainable development (16). The coincidence between both sets of keywords shows consistency in the field approach toward topics of rural development, sustainability, and the interaction between agriculture and tourism.

In the indicator of the word "agrotourism", it was seen that the main keywords that coincide are related to tourism development and sustainable development (Figure 2). This agrees with Martínez-González *et al.* (2023), since they suggest that recent studies demonstrate that environmental sustainability has become an intrinsic component of modern agrotourism. Providers of agrotourism services are adapting their business models not only to mitigate environmental impacts, but also to take advantage of the growing interest of tourists in experiences that combine sustainable agricultural production with practical ecological knowledge, thus creating a virtuous cycle between the offer of rural tourism and the visitor's environmental awareness.

When it comes to the main publications found in Mexico related with the topics, the ones that stand out are sustainable development, sustainability, perception, tourism, rural

Figure 1. Word cloud of keywords related to agrotourism.

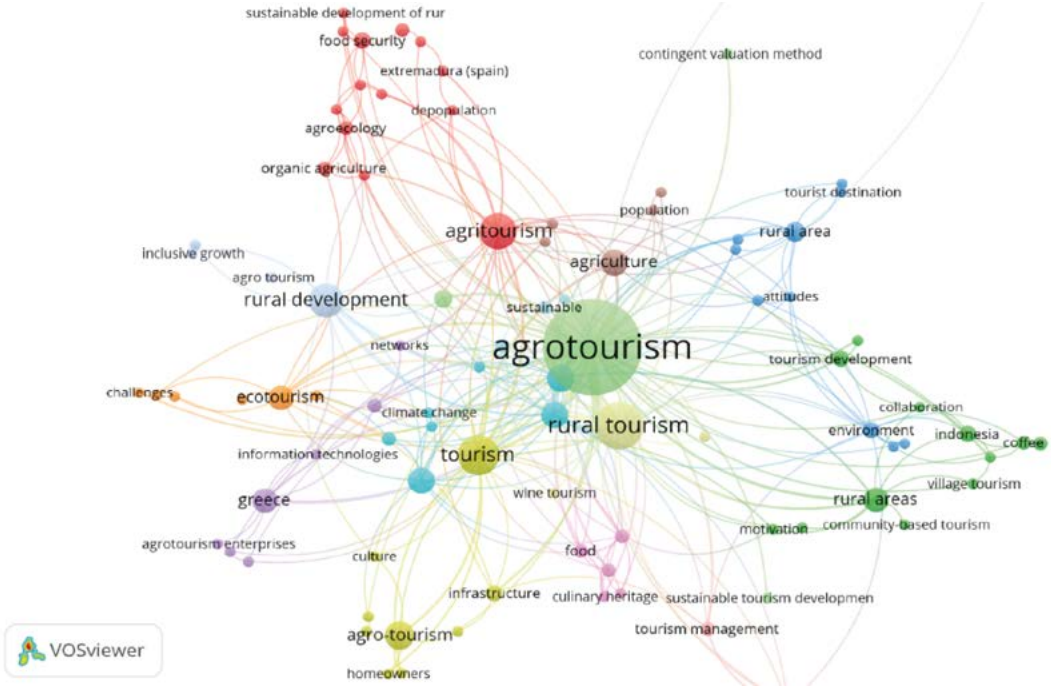

Figure 2. Network of keywords related to agrotourism.

tourism, ecotourism, tourism development, tourism management, and rural tourism where the relationship between countries and the keywords is indicated. This shows a gap of knowledge of specific topics on agrotourism, or tourism that more directed at agroecosystems and with an approach toward sustainable rural development and its socioeconomic impacts.

Collaboration between countries

Figure 3 shows the countries with highest number of publications. When the geographic distribution of the publications is analyzed, it is observed that it is distributed in a geographically uneven manner, with the highest number being from Indonesia (70 articles, 33.49%), Poland (15 articles, 7.18%), Greece (14 articles, 6.70%), Rumania (12 articles, 5,74%), and Spain (10 articles, 4.79%).

Concerning international collaboration, the general rate of international collaboration is low (9.92%). Indonesia shows the lowest international collaboration (just 7.14%), while

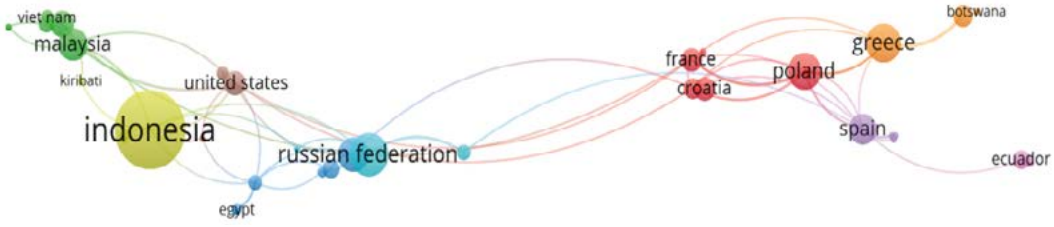

Figure 3. Roster of countries where the highest number of studies published were found.

Ecuador and Saudi Arabia show the highest rates (50%). On the other hand, it was found that the impacts per country are related with the United Kingdom, which has the highest impact from citations (106.25 citations/article), France (64.00 citations/article), and Brazil (57.00 citations/article); Indonesia also stands out, which despite its volume has low citation (2.31 citations/article). It is notable that Indonesia is the most productive country, which suggests a strong interest in the industry of agrotourism due to its contribution to the local economy.

In the academic panorama of agrotourism, researchers with different profiles of scientific impact stand out. On the one hand, authors like Sugihardjo lead in production volume with six articles, followed by a group consisting of Indonesian researchers (Lestari E., Mulyo J.H., Rusdiyana E., Setyowati R., and Widiyanto) with five publications each, integrating a productive nucleus in Southeastern Asia. This pattern contrasts with what was identified by Maroto-Martos *et al.* (2020), who mention that "scientific production on agrotourism is characterized by a marked geographic concentration in Europe and North America, with emerging research poles in Asia that are redefining the traditional balance of knowledge in this field". However, when impact by citation is considered, a different scenario emerges where western authors like Sharpley R. (418 citations) and Love D.C. (302 citations) stand out, demonstrating what Sánchez-Sánchez and Ramírez-Hurtado (2021) describe as "a persistent gap between production volume and scientific impact, where the traditional centers of knowledge maintain hegemony in influence despite the growing geographic diversification of the research".

When analyzing the fractioned productivity that weighs collaboration, researchers like Evgrafova L.V. (2.17), Hooper J. and Šimková E. (2.00 each) stand out, followed by

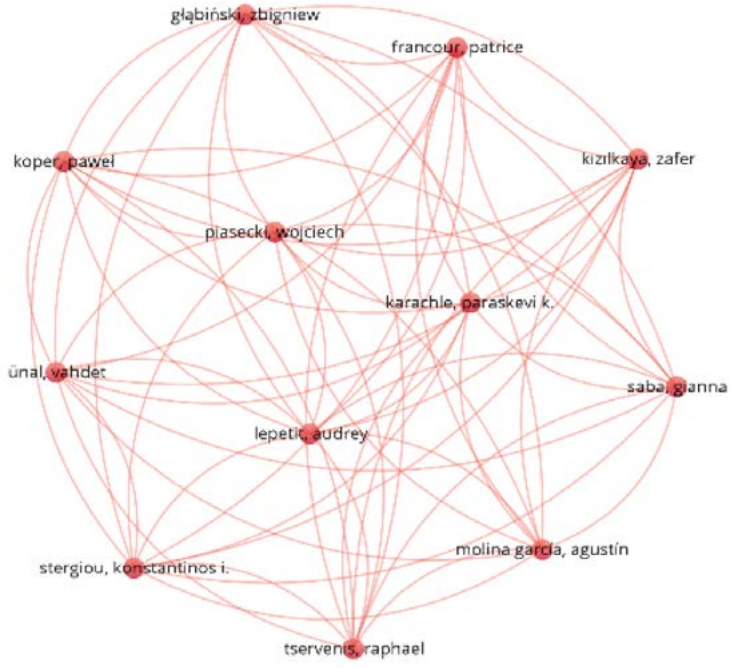

Figure 4. Roster of main authors globally.

Lakovidou O. and Kizos T. (1.83), revealing distinctive patterns of academic collaboration. This metric agrees with what was observed by Pérez-Galdós *et al.* (2022), who state that studies on agrotourism are evolving toward closer collaborative models, although selective, where researchers with high fractioned productivity tend to lead consolidated research groups with more extensive strategic collaborations. It is significant that Kizos T. appears both in fractioned productivity and in impact by citation, exemplifying what Moral-Cuadra and Orgaz-Agüera (2023) identify as "bridge researchers", capable of combining qualitative scientific production with strategic collaboration networks, thus becoming methodological references that transcend the regional borders of knowledge and consolidating their position as integral figures in the scientific advancement of the field.

Type of documents published

The main sources of publication are: IOP Conference Series: Earth and Environmental Science (34 articles); E3S Web of Conferences (17 articles); Journal of Environmental Management and Tourism (9 articles); AIP Conference Proceedings (8 articles); Springer Proceedings in Business and Economics (8 articles). This demonstrates the importance of conferences and acts, which suggests a dynamic field with many scientific encounters where advances are shared.

The most cited articles are: Sharpley R, 2002, Tour Manage (418 citations); Love DC, 2015, Aquaculture (302 citations); Bessière J, 2013, J Herit Tour (121 citations); Fachinello JC, 2011, Rev Bras Fruticultura (114 citations); and Kizos T, 2007, South Eur Soc Polit (82 citations). These studies represent the theoretical and empirical pillars of the field, and it is advisable to review them to understand the foundations of agrotourism.

It is evident that the implications for this study on agrotourism and alternative tourism in tropical agroecosystems have geographical opportunity in Indonesia, which leads scientific production (tropical country), but with low impact, suggesting that there is a niche for high-quality research in tropical environments. When it comes to the gap in knowledge, it is evident that there is a disconnect between production volume and scientific impact. There are countries with more publications, although they do not necessarily have the greatest impact, indicating an area of opportunity for innovative research.

On the other hand, the multidisciplinary and interdisciplinary approach, and the variety of sources and keywords indicate it is a field that connects agriculture, tourism, sustainability and rural development, suggesting the need for integrated approaches. With the collaboration networks, a low rate of international collaboration (9.92%) is perceived, which indicates an opportunity to establish multinational research networks, especially in tropical regions.

The recommendations for research encourage reviewing the most cited articles, especially those by Sharpley R. and Bessière J., which establish important conceptual frameworks. Deeply exploring the literature from Indonesia, since it represents a third of the global production and is probably focused on tropical agroecosystems that are similar to those of interest in other tropical regions. Identifying gaps in knowledge among tropical regions that are less studied could benefit from the research. Considering international collaborations to increase the potential impact of the publications. Focusing the research on

the intersection between agrotourism, rural tourism, sustainability, and rural development, which appear as central themes in the literature. Paying attention to recent contributions (2019-2025), since they represent nearly half of all the literature in the field and contain the most current advancements.

Studies on agrotourism and alternative tourism

Various modalities of alternative tourism such as rural tourism, ecotourism and agrotourism have stood out for their contribution to the sustainable development of rural communities (Gómez and Pérez, 2023), making this form of tourism an attractive option for the promotion of the activity in communities that seek to reach a harmonious and sustained development (Salas and Luz, 2021). To take advantage of these characteristics, tourism must be understood as a strategic opportunity for the economic development of countries, considering it from the local perspective, of development, to the socioeconomic and sociocultural reality where it is inserted. In this process, a coordinated and consensual action between public and private local actors, together with the local population, is required, so that they all plan together the best way of promoting the sector; under a strategy of participatory development that places tourism as an activity that generates employment, income and development.

Agricultural rural tourism promotes the development of sustainable productive systems by representing a feasible alternative for the generation of foods that favors food security, improves economic income of peasant families, eases access to activities in the primary sector, broadens the range of available products, and stimulates regional markets. Similarly, these initiatives constitute options that promote social progress and poverty reduction through the execution of productive initiatives destined to domestic consumption, which consolidate small-scale family businesses, using appropriate agricultural methods with ecological and economically accessible technologies. Consequently, it is fundamental to foster sustainable agricultural enterprises, where ecological tourism and specifically agrotourism act as catalysts for rural growth, at the same time as they contribute to reducing the impacts of global warming and strengthening local economies.

Agrotourism represents a modality of alternative tourism that is developed in rural spaces where farming constitutes the central axis of the experience. According to Flanigan et al. (2020), agrotourism is a recreational activity that takes place in active agrarian units, where visitors participate in rural life and productive practices as a fundamental part of their experience. This definition highlights the importance of interactivity with agricultural tasks, thus differing from other forms of rural tourism where the agrarian environment can be merely scenography.

The multidimensionality of the concept has been analyzed by Barbieri *et al.* (2022), who maintain that agrotourism constitutes a strategy of economic diversification for rural communities, combining traditional agricultural production with tourism services that allow the valuation of agrarian cultural heritage. This perspective is complemented by Phillip *et al.* (2021), who establish a classification based on the level of contact with agricultural activity, distinguishing between passive observation experiences and active direct participation experiences.

Within the context of sustainability, Sgroi and Donia (2023) argue that agrotourism represents a territorial development model that balances the preservation of agricultural traditions with innovation in tourism services, fostering environmental conservation and local socioeconomic wellbeing. This view is backed by Kizos and Losifides (2024), who have documented how successful agrotourism initiatives favor the permanence of the population in rural areas, the inter-generational transmission of traditional agricultural knowledge, and the creation of value chains that benefit multiple local actors, thus establishing a direct link between agrotourism and sustainable rural development.

CONCLUSIONS

The bibliometric analysis of alternative tourism and agrotourism reveals a research field in accelerated expansion, with an annual growth rate of 6.68% and concentration of 50% of scientific production in the 2019-2025 period. In Scopus, 363 publications were identified, demonstrating the growing academic interest toward tourism modalities that integrate sustainable development, agriculture, and environmental conservation.

Alternative tourism and agrotourism are consolidated as key elements for resilience and sustainable development of rural spaces, functioning as catalysts of economic diversification that strengthen food security, facilitate the inter-generational transmission of traditional agricultural knowledge, and generate local value chains. The evidence confirms their evolution toward a triple-impact model that balances economic profitability, environmental conservation and social justice. Critical research gaps were identified: the disconnect between scientific production volume and impact (Indonesia leads with 33.49% of publications, but with low impact by citation), limited international collaboration (9.92%), and underrepresentation of specific studies in tropical agroecosystems, particularly in Latin America. This gap represents a strategic opportunity to develop high-quality research in tropical contexts, taking advantage of its biodiversity, innovative potential, and needs for climatic adaptation.

The limitations of the analysis include the bias toward high-impact publications in English, the conceptual heterogeneity in definitions of agrotourism and rural tourism, and the quantitative approach that does not evaluate real social impact in the communities. To maximize the transforming potential of agrotourism, it is fundamental to develop specific normative frameworks, international collaboration programs, transversal integration of climate change into tourism policies, and participatory methodological approaches that actively involve local communities in contexts of tropical agroecosystems.

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Colegio de Postgraduados

Food fortification of pangola grass (*Digitaría eriantha* Steud) silage to increase its digestibility

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ABSTRACT

Objective: To evaluate the effect of different urea concentrations (2%, 3%, and 5%) on the nutritional composition and digestibility of pangola grass (*Digitaria eriantha* Steud) silage.

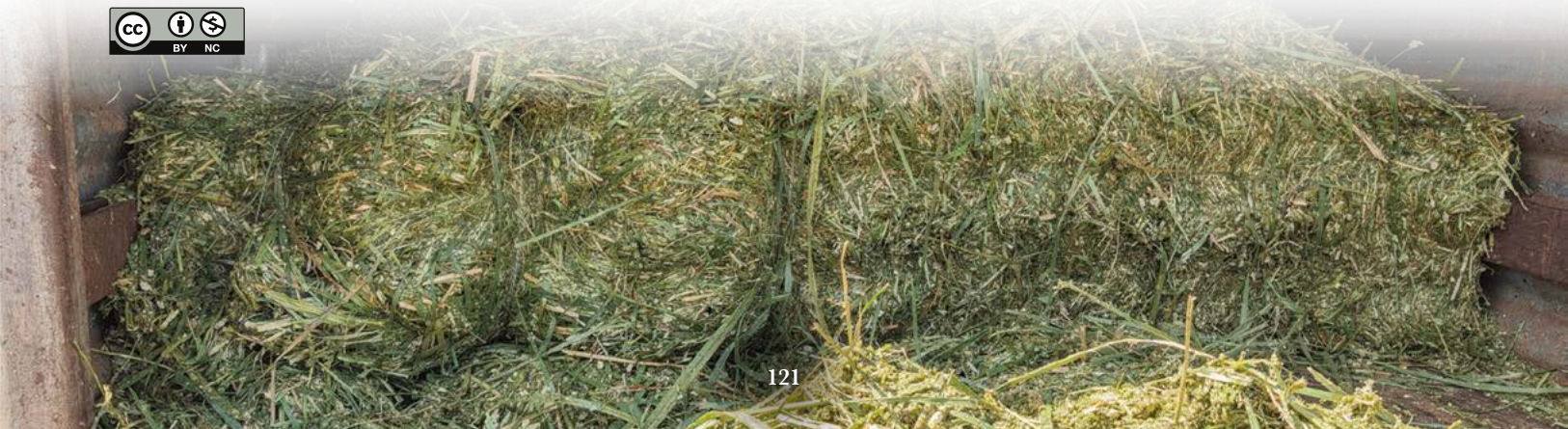
Design/Methodology/Approach: The study was conducted at the Instituto Tecnológico Superior, located in the municipality of Juan Rodríguez Clara, Veracruz. A completely randomized design with three repetitions was established. Pangola grass was harvested at 45 days of regrowth and three types of microsilos were prepared with 2%, 3%, and 5% urea, along with a control without treatment. After 30 days of fermentation, subsamples were taken from the microsilos to perform proximate and Van Soest analyses.

Results: The use of microsilos treated with 5% urea increased crude protein (CP) content, significantly improved protein content, and reached the highest concentration level. Additionally, it reduced acid detergent fiber (ADF) and neutral detergent fiber (NDF).

Study Limitations/Implications: The risk of urea intoxication in animals was the main limitation resulting from the urea-treated microsilo technique. This risk is directly related to incorrect dosages and the distribution of urea in the silage.

Findings/Conclusions: Adding urea to silage is a practical and cost-effective strategy to optimize livestock production in tropical regions, because it enhances the efficient use of forage resources and meets the nutritional needs of animals.

Keywords: Silage, grass, urea, nutritional quality.



INTRODUCTION

Silos are an alternative for forage preservation in livestock production systems. This method helps to preserve the nutritional quality of forage resources during drought periods (Patiño-Pardo *et al.*, 2022). Meanwhile, alternatives food sources for cattle in tropical regions include pangola grass (*Digitaria eriantha* Steud), whose adaptability to adverse conditions and high productivity (Murphy *et al.*, 2019) make it a strategic option to develop silage from tropical perennial grasses (Piltz *et al.*, 2022). The lack of quality forage is one of the most important challenges faced by producers during the dry season in the tropics. On the one hand, this situation does not only impact livestock health and productivity, but it also limits its sustainability. On the other hand, pangola grass can provide an advantage for the implementation of such strategies as silage production (Antonio-Medina *et al.*, 2024). Additionally, when it is fortified with urea, the nutritional value of pangola grass as crude protein (CP) records significant improvements (Wiyabot 2018; Almeida *et al.*, 2018).

Adding urea to silage is a feasible alternative to enhance the nutritional value of forages. Additionally, it acts as a fungicide and improves aerobic stability, minimizing matter losses once the silo has been opened and silage has been exposed to oxygen (Araujo *et al.*, 2023). Given its low cost, feeding ruminants with this type of grass is economically viable. Likewise, rumen microorganisms can metabolize silage, contributing to the synthesis of microbial proteins and consequently increasing nutrient availability (Wahyono *et al.*, 2022). The addition of urea to silages can increase their crude protein content and improve their digestibility (Calixto *et al.*, 2017); however, the dose and application conditions can prevent the risk of ammonia toxicity, guaranteeing a high-quality silage (González-Padilla and Merino-Zuñiga, 1984). In order to achieve a balance between safety and nutritional quality, adding urea to the silage technique applied to pangola grass must be optimized. Therefore, this research evaluated the effect of including urea on the nutritional characteristics of the silage during the preparation stage. The aim was to understand its performance according to the different doses. The ultimate purpose was to improve nutritional tropical forages and to provide practical solutions for livestock systems where pangola grass is a prevailing resource. This experiment resulted in an increase in crude protein (CP), dry matter (DM), ether extract (EE), net energy for lactation (NEl), net energy for maintenance (NEm), and net energy for gain (NEg), as well as a decrease in crude fiber (CF), acid detergent fiber (ADF), and neutral detergent fiber (NDF), increasing the digestibility and quality of pangola grass silage.

MATERIALS AND METHODS

Study Location

Pangola grass was sown at the Instituto Tecnológico Superior de Juan Rodríguez Clara, located in the municipality of Juan Rodríguez Clara, Veracruz (18° 00' 6.1" N and 95° 24' 1.7" W, 133 m.a.s.l.). According to the Köppen classification and the modifications thereof proposed by García (2004), the climate is warm subhumid (AW_0), with a mean temperature of 24.5° C, and a mean annual precipitation of 1,100 mm. The soil is dystric cambisol, with a sandy and crumbly texture and a highly acid pH (4.67) (Tosquy-Valle *et al.*, 2020).

Crop establishment

The collected material was established by hand on October 15, 2021, and the last general cutting was carried out on August 8, 2024. To prepare the microsilages, 45-cm tall mature grass was cut at 45 days after the last cutting.

Preparation of microsilages

At 45 days after the homogeneous cutting, microsilages were prepared in 20-L buckets. The grass was cut into 2-3 cm strips to make management and compacting easier. In addition to the amount of urea for the treatments (0, 2, 3, and 5%), the fresh weight of the grass was estimated for every dose (silos). Urea was uniformly, carefully, and homogeneously distributed. The resulting mix was placed in the silos and was then compacted. The air was expelled to create ideal anaerobic conditions. The silo was hermetically sealed to prevent the passage of air and to encourage lactic acid fermentation. After 35 days, the silos were opened to evaluate the quality of the silage (appearance, smell, and texture). Afterwards, the silage samples were collected, prepared, and sent to the Fogasa lab in Aguascalientes, where they were subjected to a proximate chemical analysis.

Nutritional composition

Dry matter (DM) and moisture (MOI)

Pangola grass was dried in an oven at 105 °C for 24 h to determine its DM and MOI. Both parameters were calculated based on the weight before and after the drying process (AOAC, 2005).

Crude protein (CP)

The CP content was analyzed through the Kjeldahl method. The sample was digested in sulfuric acid. Subsequently, it was neutralized and titrated to determine total nitrogen. The result was multiplied by a 6.25 factor to determine the amount of protein (Montegiove *et al.*, 2021).

Ether extract (EE)

The EE percentage was determined through the Soxhlet method. Hexane ether was used as solvent to extract lipids from the sample. Subsequently, the solvent was evaporated and the fat residue was weighted to estimate its ratio in the silage (AACC, 2009; Rybicka *et al.*, 2021).

Crude fiber (CF)

CF was determined through acid and alkaline digestion. Soluble components were eliminated and the fiber residue was measured (AOAC, 2019).

Digestibility

Neutral detergent fiber (NDF)

The method proposed by Van Soest *et al.* (1991) was used to measure the total fraction of the cell wall of the forage, including its cellulose, hemicellulose, and lignin. This

parameter was used to evaluate the amount of fiber which can be potentially digested by the rumen, because high levels can reduce the willing consumption of food.

Acid detergent fiber (ADF)

ADF was determined through digestion in an acid detergent solution. Hemicellulose was eliminated, leaving only cellulose and lignin. The efficiency with which ruminants digest silage was determined based on the less digestible fiber amount (Van Soest *et al.*, 1991).

Non-fiber carbohydrates (NFC)

NFCs are the energy fraction of soluble carbohydrates that is not available in food, excluding structural fiber. It was indirectly calculated based on the balance of dry matter components, using the following formula:

$$NFC = 100 - (CP + EE + NDF + Ashes)$$

Total digestible nutrients (TDN)

TDN was estimated based on the total energy contribution of the food (DM %). In the case of silage and forages, TDN was determined based on the ADF content, using the formula suggested by the NRC (2001).

Net energy for lactation (NEl) in Mcal/Kg

A predictive equation was applied to directly estimate NEl:

$$NEI = a + b \times CP + NDF$$

The coefficients (a, b) vary according to the type of feed and were determined for dairy cattle requirements (NRC, 2001).

Net Energy for Maintenance (NEm) in Mcal/Kg

The following equation was used to determine NEm:

$$NEm = a + b \times CP + d \times NDF$$

The coefficients (a, b, d) were adjusted by the type of feed, using the NRC requirement tables for dairy cattle (NRC, 2001).

Net energy for gain (NEg) in Mcal/Kg

The following equation was used to determine NEg:

$$NEg = a + b \times CP + d \times NDF$$

The coefficients (a, b, d) were adjusted by the type of feed, using the NRC cattle tables (NRC, 2001).

Statistical analysis

A completely randomized design (CRD) was used for the experiment, with four treatments (0%, 2%, 3%, and 5% urea) and three repetitions per treatment, resulting in 12 experimental units. The data were subjected to an analysis of variance (ANOVA) to evaluate the effect of the treatments on the nutritional and energy variables of silage. When significant differences ($p<0.05$) were detected, Tukey's honest significant difference test was applied to identify contrasts between treatments. Additionally, the Pearson correlation coefficient was estimated to explore relations between bromatological and energy variables. SAS v. 9.4 (SAS, 2014) was used for all statistical analysis and the figures were developed using GraphPad Prism v. 8 (2019).

RESULTS AND DISCUSSION

Nutrient composition

Figure 1a shows the DM and MOI behavior, reporting an increase in dry matter content with the 5% urea treatment. CP recorded the same behavior, unlike EE (Figure 1b). Finally, Figure 1c shows that the fiber values (CF, NDF, and ADF) significantly diminish as the urea level increases. This phenomenon could be related to a higher degradation of the cell wall under treatments with more urea.

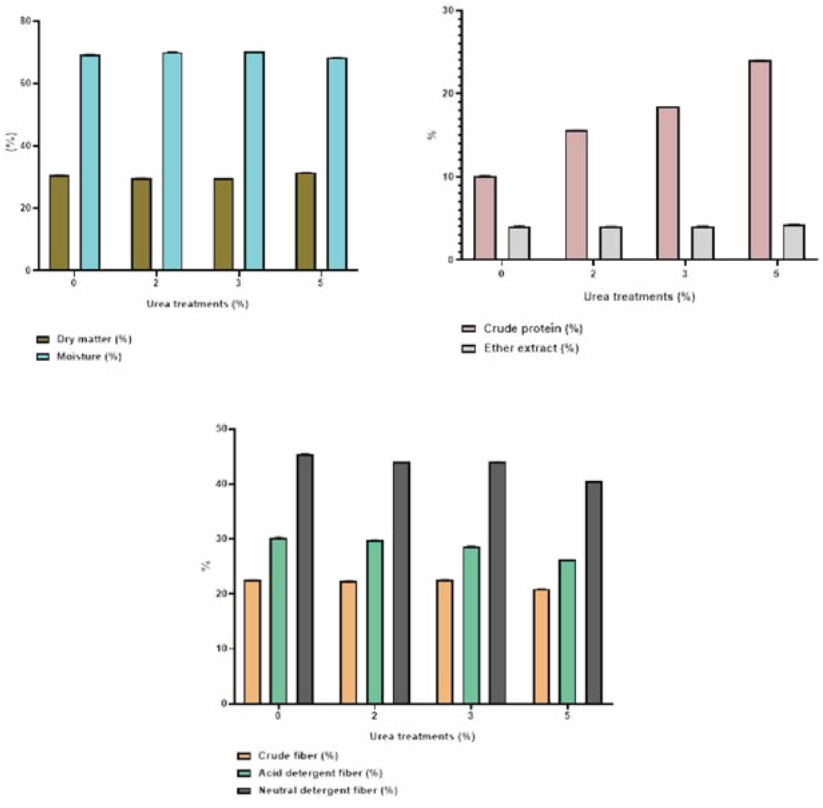


Figure 1. Behavior of the nutrient composition of pangola grass microsilos. a) Dry matter and moisture behavior (%). b) Crude protein and ether extract behavior (%). c) Crude fiber, neutral detergent fiber, and acid detergent fiber behavior (%), in pangola grass microsilages with the addition of various urea levels (0, 2, 3, and 5%).

The interpretation of the nutritional content of pangola grass microsilages treated with 2, 3, and 5% urea shows significant differences in all the evaluated variables ($p<0.05$ or $p<0.01$) (Table 1). In conclusion, the urea level impacts the nutritional parameters.

Figure 2 (NFC and TDN behavior) shows that the NFC content diminishes as the urea dose increases. Meanwhile, TDN had a slightly increasing trend, which reached its peak

Table 1. Analysis of the nutritional content of pangola grass microsilages, treated with 2, 3, and 5 % urea.

Variable	Control	Urea treatments			EEM
		2	3	5	
MS	30.7^{b}	29.8^{c}	29.5^{c}	31.6^{a}	0.02
HUM	69.3^{b}	70.1^{a}	70.4^{a}	68.4^{c}	0.02
PC	10.1^{d}	15.6^{c}	18.4^{b}	24^{a}	0.02
EE	4.1^{b}	4.0^{b}	4.0^{b}	4.3^{a}	0.01
FC	22.5^{ab}	22.4^{b}	22.6^{a}	20.9^{c}	0.01
FDA	30.2^{a}	29.8^{a}	28.7^{b}	26.2^{c}	0.05
FDN	45.5^{a}	44^{b}	44^{b}	40^{c}	0.01
CNF	32.3^{a}	28.3^{b}	25.4^{c}	23.0^{a}	0.05
TDN	85.05^{c}	85.2^{c}	86.0^{b}	87.6^{a}	0.03
ENl	1.9^{c}	1.9^{c}	1.9^{b}	2.0^{a}	0.00
ENm	2.1^{d}	2.1^{c}	2.1^{b}	2.2^{a}	0.00
ENg	0.8^{c}	0.9^{c}	1.01^{b}	1.19^{a}	0.00

DM (MS): dry matter (%). MOI (HUM): moisture (%). CP (PC): crude protein (%). EE: ether extract (%). CF (FC): crude fiber (%). ADF (DFA): acid detergent fiber (%). NDF (FDN): neutral detergent fiber (%). NFC (CNF): non-fiber carbohydrates. TDN: total digestible nutrients. NEl (ENl): net energy for lactation (Mcal Kg). NEm (ENm): net energy for maintenance (Mcal Kg). NEg (ENg): net energy for lactation (Mcal Kg). SEM (EEM): standard error of the mean. Tukey ($p=0.05$).

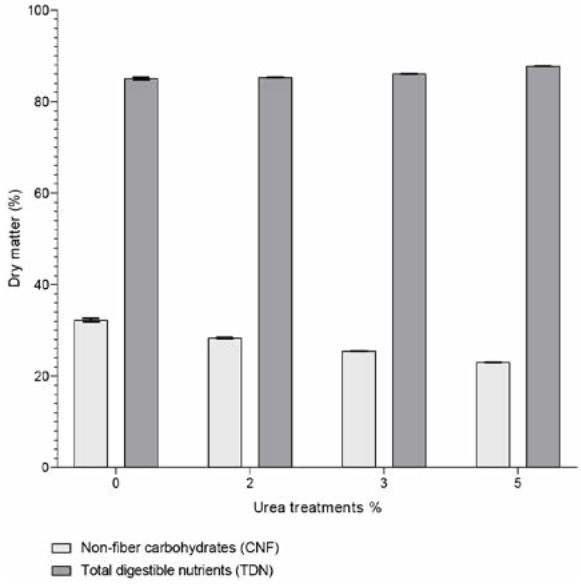


Figure 2. Behavior of non-fiber carbohydrate (NFC) and total non-digestible nutrients (TNDF) in pangola grass microsilages, with the addition of different urea levels (0, 2, 3, and 5%).

with the 5% urea treatment. Data suggest that urea modifies the energy composition of silage, reducing soluble sugars, while improving the total digestibility of forage.

Net energies (NEl, NEm, and NEg)

Figure 3 (net energy behavior) shows that NEl, NEm, and NEg consistently increase in microsilages as the urea concentration increases, reaching its peak with the 5% urea treatment. This behavior reflects an improvement on the energy value of microsilages using that treatment.

The correlation coefficients for the bromatological and energy variables of the microsilages (Table 2) show positive correlations between CP and the following energy fractions: NEl (r=0.97), NEm (r=0.97), and NEg (r=0.91). These results suggest that an increase in CP directly improves the energy available for the silage. Likewise, CP had negative correlations with ADF (r=−0.93), NDF (r=−0.94), and NFC (r=−0.99); therefore, adding urea reduces the fiber fraction and increases the CP quality of the forage. The fiber fractions (ADF) and energy (TDN) variables likewise had negative relationships (r=−1.00) —an inverse relationship that was to be expected. Correlations were statistically significant (*p<0.05 and **p<0.01).

Nutritional content

Table 1 shows that the nutritional content of microsilages treated with different urea levels (0, 2, 3, and 5%) (Table 1) point out significant changes in the evaluated variables with regard to the control. Unlike the 2% and 3% urea treatments, the 5% treatment caused a significant increase in DM. Given its high hygroscopic nature, urea tends to combine with the free water found in silages, consequently reducing moisture (Figure 1a) (Gutiérrez *et al.*, 2022).

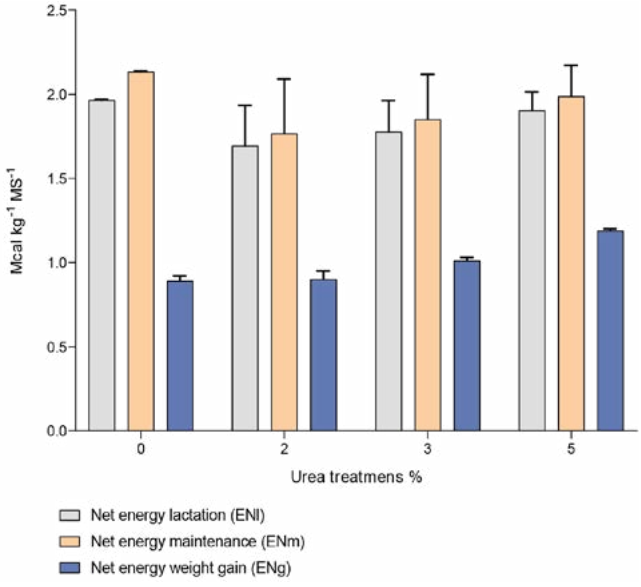


Figure 3. Behavior of net energy for lactation (NEl), net energy for maintenance (NEm), and net energy for weight gain (NEg), in pangola grass microsilages with the addition of different urea levels (0, 2, 3, and 5%).

Table 2. Correlation coefficients of the nutritional content of pangola grass microsilage, at the Instituto Tecnológico Superior de Juan Rodríguez Clara (spring-summer cycle 2023).

	HUM	PC	EE	FC	FDA	FDN	CNF	TDN	ENL	ENM	ENG
MS	−1.00	0.36	0.74	−0.85	−0.65	−0.63	−0.23	0.65	0.62	0.62	0.61
	<.0001	0.24	0.01	0.00	0.02	0.03	0.47	0.02	0.03	0.03	0.03
HUM		−0.36	−0.74	0.85	0.65	0.63	0.23	−0.65	−0.62	−0.62	−0.61
		0.24	0.01	0.00	0.02	0.03	0.47	0.02	0.03	0.03	0.03
PC			0.63	−0.79	−0.93	−0.94	−0.99	0.93	0.94	0.94	0.91
			0.03	0.00	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
EE				−0.80	−0.77	−0.73	−0.58	0.77	0.77	0.77	0.77
				0.00	0.00	0.01	0.05	0.00	0.00	0.00	0.00
FC					0.91	0.94	0.69	−0.91	−0.90	−0.90	−0.87
					<.0001	<.0001	0.01	<.0001	<.0001	<.0001	0.00
FDA						0.96	0.88	−1.00	−1.00	−1.00	−0.97
						<.0001	0.00	<.0001	<.0001	<.0001	<.0001
FDN							0.88	−0.96	−0.96	−0.96	−0.92
							0.00	<.0001	<.0001	<.0001	<.0001
CNF								−0.88	−0.90	−0.90	−0.87
								0.00	<.0001	<.0001	0.00
TDN									1.00	1.00	0.97
									<.0001	<.0001	<.0001
ENL										1.00	0.97
										<.0001	<.0001
ENM											0.97
											<.0001

DM (MS): dry matter (%). MOI (HUM): moisture (%). CP (PC): crude protein (%). EE: ether extract (%). CF (FC): crude fiber (CF%). ADF (FDA): acid detergent fiber (%). NDF (FDN): neutral detergent fiber (%). NFC (CNF): non-fiber carbohydrate (%). TDN: total digestible nutrients (%). NEl (ENl): net energy for lactation (Mcal Kg). NEm (ENm): net energy for maintenance (Mcal Kg). NEg (ENg): net energy for gain (Mcal Kg) ** Highly significant ($p<0.01$). * Significant ($p<0.05$).

The urea increase resulted in a significant CP increase, reaching the highest level with the 5% treatment (24%). In contrast, control recorded a 10.1% increase (Figure 1b). This increase is caused by the direct contribution of non-protein nitrogen (NPN) made by urea, which favors microbial growth and the conversion of NPN into protein (Wesseh and Ayantunde, 2021). According to Rodríguez-Romero (2004), lipids found in the soil are structural compounds which are not significantly influenced in any way by the addition of NPN. However, the slight advantage recorded by the 5% urea treatment is attributed to the indirect effect of an enhanced preservation of nutrients in the silage, resulting from a more efficient control of the fermentation process (Figure 1b). Likewise, a greater urea dose resulted in a reduction of fiber fractions (ADF and NDF), indicating an improvement in digestibility. For their part, Rashid *et al.* (2025) researched the impact of wheat straw treated with the urea of Azi-Kheli buffalos and recorded significant improvements in CF digestibility. This phenomenon is caused by the action of urea in the cell wall of plants. The decomposition of urea into ammonia causes a partial split of the lignocellulosic bonds,

reducing the structural fiber content of forage. This effect is more noticeable with higher urea levels (*e.g.*, 5%): a higher decrease in NDF and ADF enhances the digestibility of silage (Figure 1c) (Nascimento *et al.*, 2023). Net energies increased in proportion with the urea level, reaching the highest levels with the 5% urea treatment (Figure 3). Therefore, the energy availability of the feed improves as a result of a greater solubilization of the fiber components and the increase in CP —key factors for animal performance (NRC, 2001; Elizondo-Salazar 2020).

Figure 2 shows that the gradual increase of CP results in a decrease of non-fiber carbohydrates (NFC). These changes in the chemical composition are associated with a better digestibility of the silage and, therefore, a significant increase of the net energy value for lactation (NEl), maintenance (NEm), and gain (NEg). With 5% urea, NEl, NEm, and NEg reached 2.0, 2.2, and 1.19 Mcal/kg, respectively (Table 1). Villalba *et al.* (2021) pointed out that a lower NFC content, combined with a better balance of fiber and protein, optimizes ruminal fermentation and improves the efficient use of nitrogen in sustainable livestock systems. Meanwhile, López-Herrera *et al.* (2017) mentioned that grass and banana silages combined with nitrogen additives recorded a higher CP content and a lower fibrous fraction, favoring the fermentation and energy of the forage. For their part, Rodríguez-Chacón *et al.* (2014) reported that adding urea and treacle not just increased CP content, it also favored NFC and increased the total digestible nutrients (TDN), positively impacting the energy content of the silage (Table 2). Meanwhile, negative correlations between ADF, NDF, and NEl, NEm, and NEg indicate that the reduction of structural fiber significantly contributes to a better energy use in the forage and strengthens its nutritional value.

The results indicated that adding urea to the pangola grass silage significantly modified its chemical and energy composition (Table 2). Adding 5% urea increased CP and reduced the ADF and NDF levels, potentially improving the nutritional quality of the forage. Sánchez-Santillán *et al.* (2022) reported a CP increase and a NDF reduction in papaya waste and hay treated with urea and treacle, resulting in a higher silage digestibility.

Regarding the nutritional variables, a negative ratio was found between MOI and DM (Table 2), indicating that, when MOI decreases, DM increases. This factor is fundamental to preserve microsilages. Likewise, Krüger *et al.* (2020) pointed out that a higher DM content is associated with lower NDF and ADF content, which favors the nutritional quality of silage. Meanwhile, a positive and highly significant correlation between CP and energy parameters was recorded —*i.e.*, a higher CP content improves the efficiency of energy production, which is fundamental for lactating and fattening animals. These results match the findings of Calixto Junior *et al.* (2017) and Pineda-Cordero *et al.* (2016), who reported improvements in the nutritional and fermentation quality of grass silages combined with different treacle and urea additives.

These correlations strengthen the trend: CP had a negative correlation with ADF ($r=-0.9302$) and NDF ($r=-0.94246$). These results are similar to those reported by Krüger *et al.* (2020). Meanwhile, Juárez *et al.* (2009) recorded that a decrease in the fibrous fraction of tropical grasses had a positive association with the increase of energy and digestibility. In addition, CP and the energy fractions of the silage had a positive correlation: NEl 0.94%, NEm 0.94%, and NEg 0.91%. These results indicate an improvement of the protein content

and a better use of the energy available for the animals. Araújo *et al.* (2023) and Zamir *et al.* (2020) reported similar results: digestibility and energy significantly increased in the silages treated with urea and treacle. A positive ratio between CP and ADF (r=0.91005) and NDF (r=0.94434) indicates that these fractions are closely linked in the forage. These results match the findings of Pineda *et al.* (2016), who pointed out that a high CF content usually involves a higher ratio of structural fibers, which reduces the digestibility of the silage. Meanwhile, a negative correlation between NDF and the energy fractions (NEl, NEm, and NEg) suggests a lower fiber content, associated with a higher availability of energy in the feeding. These results also match the findings of Medina *et al.* (2008), who evaluated a supplementation for milking cows prepared with millet silage, urea, and other additives. Overall, adding of urea is a viable and economic strategy to improve the quality of pangola grass silage, particularly in tropical regions where forages have high levels of fiber and low levels of proteins.

CONCLUSIONS

Adding urea to pangola grass during silage is a highly effective strategy to improve the nutrient content of forages, because it increases crude protein and net energy content, optimizing food quality. Meanwhile, the positive impact of adding 5% urea on the silage nutritional profile requires further research. The said impact should be reflected on the performance of lactating and fattening animals. Livestock should be fed with a balanced and energetic diet, where urea plays a key role to reduce dependency on expensive protein supplements, during food shortage. In addition, urea could help producers in tropical areas to obtain a high-quality forage for their livestock.

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Foliar biofortification with iron, zinc and copper in maize under rainfed climatic conditions and its nutritional quality

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ABSTRACT

Objective: To evaluate nutrient absorption and forage quality in corn microsilos (*Zea mays* L.) cultivated under rainfed conditions and foliar biofortified with iron (Fe), zinc (Zn), and copper (Cu).

Design/Methodology/Approach: The study was carried out at the Juan Rodríguez Clara Higher Technological Institute, located in the municipality of Juan Rodríguez Clara, Veracruz. Foliar applications of Zn, Cu, and Fe were administered throughout the crop's growth cycle. Bromatological parameters and mineral content were analyzed through correlation and interpretation in relation to climatic variables, specifically temperature and precipitation.

Results: Moderate temperatures (22-23 °C) promoted Fe absorption (202 ppm); however, they also led to increases in neutral detergent fiber (NDF) (59%) and acid detergent fiber (ADF) (33%), enhancing plant structural rigidity. Conversely, cumulative rainfall of 200 mm negatively impacted Zn (29 ppm) and Cu (19 ppm) uptake due to leaching and water stress, which subsequently reduced the organic matter content (%OM) in the microsilos to 88%. A positive correlation was identified between Fe concentration and structural fiber content, while a negative correlation was observed between protein levels and hemicellulose, indicating a potential metabolic imbalance.

Study Limitations/Implications: Rainfall interfered with Zn and Cu absorption, underscoring the importance of aligning foliar fertilization strategies with prevailing weather conditions.

Findings/conclusions: Fe biofortification influences plant structure; water stress limits the absorption of Zn and Cu. Foliar applications must be adjusted according to the climate to optimize the nutritional quality of the forage.

Keywords: Maize, biofortification, microminerals, fibers.

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INTRODUCTION

Crop biofortification has emerged as a sustainable strategy to enhance the nutritional quality of food and ensure global food security (Sandhu *et al.*, 2023). Essential

micronutrients such as zinc (Zn), copper (Cu), and iron (Fe) play pivotal roles in plant metabolic processes, directly influencing their development, productivity, and nutritional quality (Niu *et al.*, 2020). However, the deficiency of these elements in agricultural soils remains a persistent challenge, limiting crop yields and compromising the quality of forage used in animal feed (Shukla & Behera, 2024; Rahman *et al.*, 2020). Among fertilization strategies, foliar application of micronutrients has been identified as an effective technique to enhance nutrient uptake, particularly in soils with low availability of essential elements or under water stress conditions (Fageria *et al.*, 2012; Fernández & Brown, 2013). This method enables nutrients to be directly absorbed through the leaves, thereby avoiding losses due to soil immobilization (Niu *et al.*, 2020). Nonetheless, various environmental factors such as temperature, precipitation, and accumulated heat units (growing degree days, GDD) significantly influence the efficiency of biofortification by affecting the absorption and distribution of these micronutrients within plant tissues (Havlin *et al.*, 2014; Ullah *et al.*, 2020). Scientific research has demonstrated that adverse climatic conditions, including drought stress and thermal variability, directly impact enzymatic activity in plants, altering fundamental physiological processes such as transpiration, root uptake, and nutrient mobilization (Shukla & Behera, 2024; Rao & Takahashi, 2022). These disruptions can lead to metabolic imbalances in which the plant prioritizes the development of robust structural components such as neutral detergent fiber (NDF) and acid detergent fiber (ADF) at the expense of nitrogenous compound accumulation, ultimately reducing forage digestibility and nutritional value (White & Broadley, 2009). In this context, the aim of this study is to evaluate nutrient absorption and forage quality in corn (*Zea mays* L.) microsilos developed under rainfed conditions and foliar biofortified with Fe, Zn, and Cu, while analyzing the influence of temperature and precipitation on the absorption of these minerals. Understanding these interactions will help optimize biofortification strategies, contributing to the development of more efficient and sustainable agricultural systems.

MATERIALS AND METHODS

Study site

The experiment was conducted at the Juan Rodríguez Clara Higher Technological Institute, located in the municipality of Juan Rodríguez Clara, Veracruz, Mexico (18° 00′6.1" N, 95° 24′1.7"′ W), at an altitude of 133 meters above sea level. According to the modified Köppen climate classification (García, 2004), the region presents a warm sub-humid climate (AW_0), with an average annual temperature of 24.5 °C and annual precipitation of approximately 1100 mm. The predominant soil type is a distric Cambisol (FAO-UNESCO, 1977), characterized by a sandy loam texture and strongly acidic pH (NOM-021-RECNAT-2000, 2000) (Tosquy-Valle *et al.*, 2020).

Crop establishment

The field trial was established on a 960 m^2 plot using a planting configuration of 80×0.15 cm with commercial hybrid maize seed. A randomized complete block design (RCBD) with three replications was implemented. Foliar applications of zinc (Zn), iron

(Fe), and copper (Cu) were carried out at a concentration of 1000 ppm. Applications were performed at three key growth stages: when the crop had three fully developed leaves, at stage V5-V6, and at stage V10-V12, prior to flowering (Crosby-Galván *et al.*, 2024).

Evaluated variables

Nutritional and mineral content of the corn microsilos were assessed. Silos were constructed using 8′ PVC tubes, filled with chopped maize plants (cut to <5 cm particle size) harvested at the milk-dough stage (45 days after sowing). Once filled, the containers were hermetically sealed and opened after 45 days for bromatological analysis. The following parameters were determined: dry matter (DM, %), ash (A, %), crude protein (CP, %), acid detergent fiber (ADF, %), neutral detergent fiber (NDF, %), hemicellulose (H, %), and lignin (L, %) following standard methods (AOAC, 1975; Van Soest *et al.*, 1994). Mineral content Fe, Cu, and Zn (ppm) was determined via atomic absorption spectrophotometry (AAS). Data were also collected to estimate growing degree days (GDD), based on maximum and minimum daily temperatures (°C) and the maize base temperature (10 °C) (Angel *et al.*, 2017).

Statistical analysis

To evaluate the effect of foliar treatments with Zn, Cu, and Fe (ppm) on the nutritional content (%), an analysis of variance (ANOVA) was performed under a randomized complete block design with three replications. Tukey's test ($p<0.05$) was applied to detect statistically significant differences among means. In the absence of significant differences, a Pearson correlation analysis was conducted between nutritional content variables (CP, NDF, ADF, H, and L) and mineral content (Fe, Cu, and Zn in ppm) to identify linear associations and interpret the physiological and structural effects of biofortification. Statistical analyses were performed using the SAS software package (SAS, 2009).

RESULTS AND DISCUSSION

The absence of statistically significant differences ($p>0.05$) among treatments in the evaluated variables (Table 1) may be attributed to the natural variability of climatic conditions under rainfed systems (Figure 1), which likely elicited a moderate physiological response of the crop to the foliar biofortification treatments.

Table 1. Effect of foliar biofortification with Zn, Cu, and Fe on the nutritional and mineral composition of maize (*Zea mays* L.) microsilos under rainfed climatic conditions.

Treatment	pH	Temp (°C)	DM (%)	Ash (%)	OM (%)	CP (%)	NDF (%)	ADF (%)	H (%)	L (%)
Control	5	23	97	5	95	7	54	25	29	4
Zn	4	22	97	4	96	8	58	31	27	5
Cu	4	23	98	12	88	8	58	30	27	5
Fe	4	22	97	4	96	8	59	33	26	5

Temp=Temperature; DM=Dry Matter; Ash=Ash Content; OM=Organic Matter; CP=Crude Protein; NDF=Neutral Detergent Fiber; ADF=Acid Detergent Fiber; H=Hemicellulose; L=Lignin.

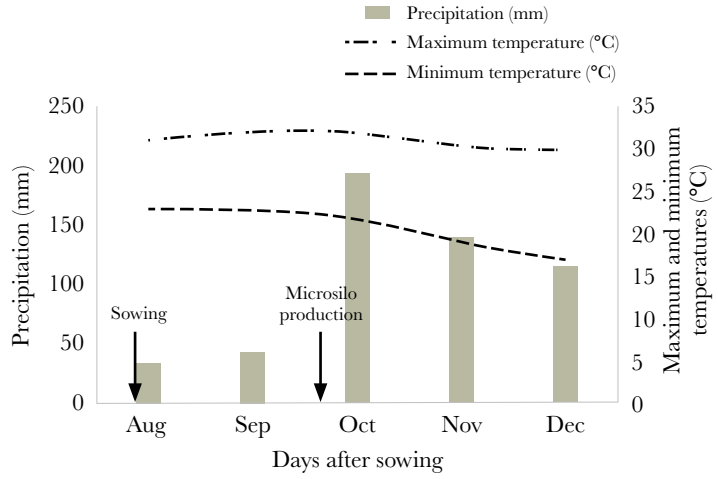

Figure 1. Precipitation and temperature behavior during the biofortification process of corn microsilos (*Zea mays* L.).

On the other hand, the lack of statistical significance does not preclude the presence of physiologically relevant effects (Tables 1 and 2). Therefore, the analysis was complemented with a Pearson correlation matrix between bromatological and mineral variables (Table 3), which revealed association patterns that better explain the structural and nutritional responses of maize. During the crop cycle, temperatures ranged between 22 °C and 23 °C an optimal range for nutrient absorption according to maize physiological requirements (El-Sappah *et al.*, 2022; Waqas *et al.*, 2021). Conversely, under water stress conditions, foliar transpiration is reduced, limiting nutrient translocation from the leaves to growing organs. This leads to an increase in structural fiber content, specifically neutral detergent fiber (NDF) and acid detergent fiber (ADF). In the case of foliar-applied Fe, absorption occurred, but its mobilization to cellular structures was hindered. Consequently, lignification of foliar tissues was observed as a physiological defense response to stress, with NDF and ADF levels reaching 59% and 33%, respectively (Table 1).

The Fe concentrations obtained induced a structural response in the plant, characterized by an increase in structural fibers. Stein *et al.* (2019) reported that iron application can promote lignification in roots, particularly in cultivars tolerant to Fe excess, such as rice, where lignin concentration in roots increased under high Fe treatments. In the present study, Fe absorption reached 202 ppm under moderate temperature conditions although lower than that observed in the control treatment (254 ppm) (Table 2) suggesting effective micronutrient mobilization via the root pathway into plant tissues.

Table 2. Comparative concentration of mineral elements in ensiled maize forage. Juan Rodríguez Clara Higher Technological Institute, Spring-Summer 2023 growing season.

Micronutrient	Control (ppm)	Biofortified (ppm)	Critical Value Range (ppm)[1]
Copper (Cu)	16	19	5-20
Zinc (Zn)	26	29	20-70
Iron (Fe)	254	202	50-250

[1]Critical values referenced from Mengel & Kirkby (1987) and Szerement *et al.* (2021).

This response can be attributed to the acidic nature of the soil (Tosquy-Valle *et al.*, 2017). Under acidic conditions, iron is predominantly present in its ferrous form (Fe^{2+}), which is more soluble and readily available for root uptake. In such environments, foliar absorption of Fe is generally less efficient than root uptake, due to structural barriers in the leaf and the dynamic behavior of the ion in solution. Additionally, physiological factors such as reduced foliar transpiration under water stress conditions limit the internal translocation of iron following foliar application. The effective absorption observed is likely due to moderate temperatures, under which Fe remains in its soluble (Fe^{2+}) form, facilitating its incorporation into plant tissues (Van Groeningen *et al.*, 2020). In contrast, elevated temperatures promote the oxidation of Fe into its insoluble ferric form (Fe^{3+}), reducing its bioavailability (Barker *et al.*, 2023). From a plant physiology perspective, thermal and water stress directly affect enzymatic activity, gene expression, and the function of ion channels involved in micronutrient uptake. When plants are exposed to excessive heat or water deficit, stomatal closure occurs as a survival mechanism, reducing foliar nutrient absorption and translocation. Although necessary for survival, this adaptive response compromises biofortification efficiency. Consequently, biofortified treatments with Cu and Zn, while showing slight increases, did not differ significantly from the control, suggesting inefficient biofortification (Table 2). This can be explained by the specific climatic and edaphic conditions during the study, characterized by acidic soil (pH 4.67) and low cation exchange capacity (3.9 $cmol^{+}\ kg^{-1}$) (Tosquy-Valle *et al.*, 2017). These conditions enhance micronutrient solubility but increase susceptibility to leaching and reduce nutrient retention. Therefore, even when foliar absorption is targeted, application immediately before heavy rainfall (200 mm accumulated) reduces effective nutrient uptake due to surface wash-off. Although Zn levels reached 29 ppm slightly higher than the control (26 ppm) this cannot be considered a significant improvement. Cu concentrations also increased marginally (19 ppm *vs.* 16 ppm in the control), suggesting limited phloem mobility and potential interference from Fe, which showed high concentration in the control (254 ppm). These findings align with reports by Ullah *et al.* (2020), Szerement *et al.* (2021), and Luo *et al.* (2024), who emphasize that the efficiency of foliar micronutrient application largely depends on timing, nutrient mobility, and post-application environmental conditions. Moreover, Zn and Cu absorption is also temperature-sensitive (Barker *et al.*, 2023). This may be due to ionic competition among micronutrients and the plant's transpiration rate, which regulates foliar uptake. Despite strategic timing, cumulative rainfall of 200 mm after microsilo establishment did not significantly affect nutrient absorption (Figure 1). Heavy rains washed away foliar-applied nutrients before absorption (López-Salazar *et al.*, 2019) and reduced mobility (Luo *et al.*, 2024). Lower concentrations of Zn and Cu were likely due to leaching or moisture saturation, which slows nutrient uptake. Additionally, water-saturated soils limit root oxygenation, thereby reducing soil nutrient uptake (Zhou *et al.*, 2024; Drechsel *et al.*, 2023). While foliar applications can serve as mitigation strategies under rainfed conditions, careful timing is essential (Wasaya *et al.*, 2017). Environmental conditions also influenced the nutritional composition of ensiled forage (Figure 2, Table 1). Biofortified treatments showed an increase in crude protein from 7% (control) to 8%, suggesting that thermal conditions favor nitrogen compound synthesis (Waqas *et al.*, 2021).

In contrast, the Fe treatment increased NDF to 59% and ADF to 33%, indicating greater development of structural components (lignification) (Table 2). Iron contributes to cell wall lignification, a process driven by enzymatic activity stimulated by moderate temperatures (Shoormij *et al.*, 2022). Antonio-Medina *et al.* (2021) also reported that Cu application in green wheat forage under controlled conditions increased ADF and NDF fractions, likely due to structural reorganization of the cell wall. This aligns with foliar Cu biofortification in maize, which also resulted in elevated NDF (58%) and ADF (30%) compared to the control (54% and 25%, respectively), though not statistically significant (Table 1). This suggests that Cu may exert a structural effect across plant species, even under uncontrolled edaphoclimatic conditions. Moreover, at moderate temperatures (22-23 °C, Figure 2), the accumulation of growing degree days (GDD) favored Fe absorption (202 ppm), but also increased NDF and ADF, leading to more fibrous plant tissues via lignification (Wu *et al.*, 2024). During the cropping cycle, planting occurred on August 2, 2023, coinciding with a steady rise in GDD, which promoted vegetative development until reaching the milk-dough stage (Figure 2). This phase, characterized by high metabolic activity, is critical for the accumulation of essential nutrients.

However, the occurrence of cumulative precipitation totaling 200 mm created a conducive environment for water stress, which adversely affected physiological processes and reduced the efficiency of micronutrient absorption (Figures 1 and 2). Physiologically, water and heat stress disrupt key processes such as transpiration, root absorption, and nutrient mobilization. These effects are evident in Table 1. Additionally, Table 3 presents the structural and nutritional components of biofortified maize (*Zea mays* L.) microsilos with zinc (Zn), copper (Cu), and iron (Fe), which were directly influenced by plant physiology and the absorption dynamics of these micronutrients. A strong positive correlation was observed between neutral detergent fiber (NDF) and acid detergent fiber (ADF) ($r=0.88$, $p=0.0002$), indicating that increased Fe absorption promotes the synthesis of structural components such as cellulose and lignin, thereby enhancing the mechanical strength of plant cell walls.

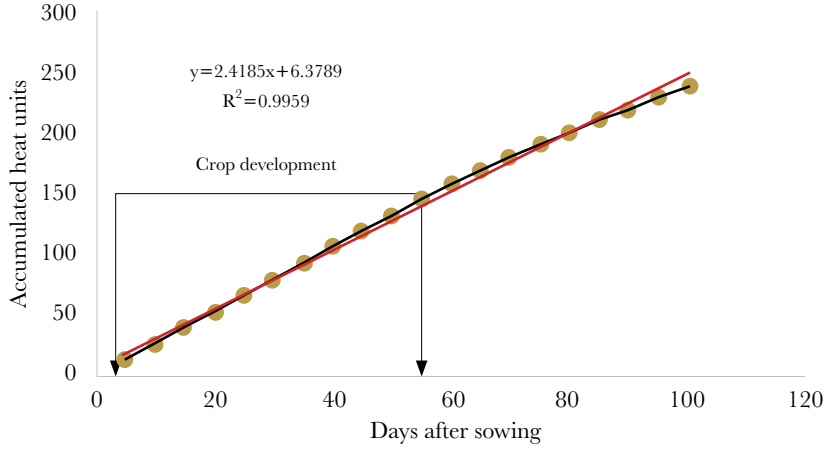

Figure 2. Relationship between days after sowing (DAS) and accumulated heat units with the maize (*Zea mays* L.) crop. Juan Rodriguez Clara Higher Technological Institute. 2023.

Table 3. Correlation between nutritional composition and mineral content in maize (*Zea mays* L.) microsilos biofortified with Fe, Cu, and Zn. Juan Rodríguez Clara Higher Technological Institute, Spring−Summer 2023 growing season.

Variables	NDF	ADF	H	L	Fe	Cu	Zn
CP	0.08 (p=0.798)	0.40 (p=0.204)	−0.69 (p=0.013)	0.49 (p=0.105)	0.03 (p=0.928)	0.03 (p=0.917)	−0.58 (p=0.048)
NDF		0.88 (p=0.0002)	−0.11 (p=0.741)	0.53 (p=0.074)	0.20 (p=0.526)	−0.31 (p=0.334)	0.10 (p=0.753)
ADF			−0.56 (p=0.056)	0.78 (p=0.003)	−0.03 (p=0.923)	−0.23 (p=0.463)	−0.04 (p=0.905)
H				−0.70 (p=0.011)	0.42 (p=0.176)	−0.04 (p=0.896)	0.27 (p=0.398)
L					−0.01 (p=0.978)	−0.10 (p=0.747)	−0.35 (p=0.268)
Fe						−0.19 (p=0.549)	−0.17 (p=0.597)
Cu							−0.24 (p=0.455)

PC=Proteína Cruda; FDN=Fibra Detergente Neutro; FDA=Fibra Detergente Ácida; H=Hemicelulosa; Li=Lignina Detergente Ácida; Fe=Hierro; Cu=Cobre; Zn=Zinc.

This physiological process occurs when the plant is subjected to stress conditions, prioritizing the formation of structural support tissues over other metabolic processes (Santiago & Malvar, 2010). A strong correlation was also observed between ADF and lignin (Li) ($r=0.77$, $p=0.003$), confirming that absorbed iron is closely linked to the lignification of plant tissues. This suggests that while the plant may tolerate adverse climatic conditions, it compromises forage digestibility by increasing cell wall rigidity, thereby negatively affecting forage quality once ensiled. Moreover, a significant negative correlation was found between crude protein content and hemicellulose (H) ($r=-0.69$, $p=0.0129$), indicating a metabolic shift in the plant toward the production of structural fibers at the expense of protein synthesis. This physiological response may result from climatic stress and biofortification with Fe and Cu elements known to stimulate the formation of protective tissues, but which can simultaneously reduce the nutritional value of the forage. These results demonstrate that the absorption of Fe, Zn, and Cu directly influences the structural composition of biofortified maize and that this effect does not improve upon silage conversion. The prioritization of structural tissue formation enhances plant resilience but compromises forage nutritional quality by decreasing protein availability and increasing fiber levels. Therefore, it is essential to achieve a balanced biofortification strategy that maximizes both micronutrient absorption and the nutritional value of the silage, thereby ensuring optimal nutrient supply for animal production systems.

Climatic conditions and micronutrient absorption

The climatic conditions recorded during the cropping cycle significantly influenced the absorption of zinc (Zn), copper (Cu), and iron (Fe) in biofortified maize (*Zea mays* L.), and no significant differences were observed once the crop was processed into silage.

Stable temperatures between 22 °C and 23 °C favored Fe absorption (202 ppm) in the biofortified treatment a result consistent with findings by Niu *et al.* (2020), who highlighted that moderate temperatures enhance the solubility and translocation of foliar-applied micronutrients. Under these thermal conditions, Fe remains in its soluble ferrous form (Fe^{2+}), facilitating incorporation into plant tissues and contributing to increased neutral detergent fiber (NDF) (59%) and acid detergent fiber (ADF) (33%) levels, which reinforce cellular structure through lignification (White & Broadley, 2009). However, the 200 mm of cumulative precipitation following foliar application negatively affected Zn (29 ppm) and Cu (19 ppm) absorption, likely due to leaching and water stress. According to Ullah *et al.* (2020), soil saturation reduces transpiration and limits foliar nutrient uptake. Rao and Takahashi (2022) noted that water stress alters enzymatic activity, stomatal conductance, and nutrient translocation. The behavior of Zn and Cu may also be explained by ionic competition, as the high Fe concentration could have interfered with their absorption. This interaction is especially relevant in moisture-saturated environments, where transpiration is limited (Szerement *et al.*, 2021). Water stress prior to rainfall, followed by excess moisture, hindered efficient nutrient uptake and limited the internal mobility of minerals such as Cu, which are critical to physiological processes related to plant growth (Antonio-Medina *et al.*, 2021).

Relationship between micronutrients and forage composition

The correlation matrix revealed key relationships between absorbed micronutrients and the structural composition of the forage. A strong positive correlation was observed between NDF and ADF ($r=0.88$, $p=0.0002$), indicating that Fe absorption promoted the synthesis of cellulose and lignin essential components for structural reinforcement in plant tissues (Liu, 2012). This finding is consistent with iron's role in cell wall lignification, enhancing mechanical resistance but potentially compromising forage digestibility (White & Broadley, 2009; Basnet & Khanal, 2022). Similarly, a positive correlation was found between ADF and lignin (Li) ($r=0.77$, $p=0.003$), supporting the notion that Fe absorption stimulates the formation of more rigid and lignified tissues, improving plant tolerance to environmental stress while potentially reducing the nutritional value of silage (Puren *et al.*, 2023). On the other hand, a significant negative correlation was identified between crude protein and hemicellulose (H) ($r=-0.69$, $p=0.0129$), suggesting a metabolic imbalance in which the plant shifts resources toward structural fiber production at the expense of nitrogen compound accumulation. This behavior may result from physiological responses to water stress and the presence of Fe and Cu, which stimulate the formation of defensive structures but compromise protein content (Szerement *et al.*, 2021; Rao & Takahashi, 2022). The findings highlight the need to synchronize foliar biofortification with climatic conditions to maximize micronutrient uptake and improve forage quality. Although pre-rainfall foliar applications helped prevent nutrient leaching, the proximity of heavy rainfall events affected the mobility and assimilation of Zn and Cu. This reinforces the importance of aligning agronomic management with climatic factors to optimize biofortification efficiency (Niu *et al.*, 2020; Xue *et al.*, 2023). The correlation between Fe absorption and increased NDF and ADF illustrates that while plant structural resistance improves, forage

digestibility may decline. Therefore, it is crucial to balance micronutrient application to enhance both absorption and silage nutritional value (Khulbe *et al.*, 2020).

CONCLUSIONS

Foliar application of iron, zinc, and copper in maize microsilos under rainfed conditions and acidic soils did not achieve effective biofortification. However, it did induce changes in structural and bromatological composition. Iron, in particular, influenced crude protein content but compromised nutritional quality by promoting forage lignification. Foliar biofortification is not solely dependent on dosage or nutrient type but also on timing, fertilizer formulation, and, most importantly, edaphoclimatic conditions. Therefore, it is recommended to adjust foliar application strategies according to soil characteristics and rainfall patterns to optimize crop response and forage quality. To ensure agronomic strategies such as biofortification are effective in animal production systems, a balanced approach must be implemented one that enhances nutrient uptake without compromising the nutritional value of silage.

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Effects of filter cake application on soil chemical properties in sugarcane agroecosystems

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ABSTRACT

Objective: To evaluate the effects of filter cake application on the chemical properties of a Fluvisol under a sugarcane agroecosystem.

Design/methodology/approach: A targeted soil survey was conducted at a surface depth of 0-30 cm in a Fluvisol cultivated with the sugarcane variety CP 72-2086, in order to identify areas with and without filter cake irregularly distributed at different depths and soil compartments. The soil chemical properties evaluated included pH, electrical conductivity (EC), organic carbon (OC), organic matter (OM), total nitrogen (TN), the C/N ratio, and available phosphorus (P).

Results: The incorporation of filter cake after eight years in a sugarcane-cultivated Fluvisol resulted in statistically significant differences in pH, organic carbon, and organic matter. In addition, filter cake application promoted low to medium levels of available phosphorus, mainly in the rhizosphere and in two filter cake thickness treatments.

Limitations on study/implications: The chemical composition of filter cake varies according to sugar mill processing conditions, clarification reagents, storage practices, and composting procedures, which may lead to variability in the results reported across studies.

Findings/conclusions: The results indicate that filter cake positively influences key soil chemical properties such as pH, organic matter, organic carbon, and available phosphorus, particularly in the rhizosphere, thereby enhancing the long-term sustainability of sugarcane soils.

Keywords: *Saccharum* spp., sustainability, soil amendment, soil fertility, agricultural residues

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INTRODUCTION

Mexico is a major sugarcane-producing country, operating 47 sugar mills during the 2024/2025 harvest season (CONADESUCA, 2025). In 2024, sugarcane cultivation covered a national area of 843,048.28 ha, of which 4.93 % (41,590 ha) was located in the state of Tabasco (SIAP, 2025). Fluvisols cultivated with sugarcane in this region have been subjected to intensive anthropogenic activities for more than 50 years (Ayuso, 2023). In

2024, the national area devoted to sugarcane cultivation increased by 1.11 times relative to the baseline year 2010 (SIAP, 2025). In parallel with this expansion of cultivated area, there has been a corresponding increase in the demand for and application of synthetic chemical fertilizers, leading to a decline in natural soil fertility, environmental contamination, and rising production costs.

These negative effects can be mitigated through the use of filter cake, an agroindustrial by-product which, when composted, functions as an organic fertilizer and soil amendment due to its high content of organic carbon, nitrogen, and other essential nutrients (Dotaniya *et al.*, 2016; López-González *et al.*, 2017; Salman *et al.*, 2023). Filter cake is a highly effective soil amendment that may contain varying proportions of waxes, plant fibers, sucrose, organic carbon, macronutrients, micronutrients, colloids, coagulants, and albuminoids, among other components (López, 1981; Salgado *et al.*, 2001; Kumar *et al.*, 2017). Interest in evaluating the effects of filter cake on agricultural soils has increased, as it represents a sustainable and cost-effective strategy to enhance soil fertility, reduce environmental pollution, and lower storage and disposal costs. The application of filter cake in agricultural systems has been shown to increase soil pH and improve soil reserves of organic carbon, nitrogen, phosphorus, and potassium (Arreola-Enríquez *et al.*, 2004; Basak *et al.*, 2021; Dotaniya *et al.*, 2025). Moreover, the impact of filter cake on the physical, chemical, and biological properties of the rhizosphere is of particular interest for understanding its role as an agricultural soil amendment (Solomon *et al.*, 2024). Accordingly, this study addresses the following research question: Has the incorporation of filter cake after eight years promoted improvements in the chemical properties of a Fluvisol cultivated with sugarcane? Answering this question contributes to addressing both national and local demands related to food security and agricultural sustainability.

MATERIALS AND METHODS

The study was conducted in a field plot located in the Ejido Rubén Jaramillo Lazo, municipality of H. Cárdenas, Tabasco, Mexico, at geographic coordinates 18° 09' 06" N latitude and 93° 37' 03" W longitude, and an elevation of 13 m above sea level. The climate of the study area is classified as warm humid, characterized by abundant rainfall during summer and autumn, with an estimated mean annual precipitation of 2,550 mm and temperatures ranging from 27 to 36 °C (INEGI, 2021).

For approximately 50 years, the agricultural soil has been managed under a conventional agronomic system involving the use of heavy agricultural machinery, synthetic chemical fertilizers, herbicides, and crop residue burning. However, in February 2015, the ejido farmers irregularly applied approximately 1 t ha^{-1} of dry filter cake on the soil surface, sourced from the Santa Rosalía sugar mill.

In February 2024, an *in situ* assessment of filter cake thickness (FCT) was carried out in the Fluvisol. The number of sugarcane rows was counted within a one-hectare area, and starting from the third row in an east-west direction, a targeted soil survey was conducted at 10 m intervals, considering a depth range of 0 to 30 cm. The objective was to identify sampling points containing sugarcane roots within the rhizosphere (RZ) and at least two

soil layers (C1 and C2) differing in color according to the Munsell Soil Color Charts (USDA, 2017), as well as in filter cake thickness (Figure 1A, B, and C).

The experimental design consisted of a 3×3 factorial arrangement with four replications (n=4), comprising three filter cake thicknesses in the soil and three soil compartments within the 0-30 cm depth interval. The FCT included three levels: FCT1 without filter cake, with a thickness of 0-11 cm (Figure 1A); FCT2 with filter cake, 0-17 cm thick (Figure 1B); and FCT3 with filter cake, 0-24 cm thick (Figure 1C). The soil compartment factor included three levels: RZ, non-root soil layer 1 (C1), and non-root soil layer 2 (C2). The combination of both factors resulted in a total of nine treatments, which are described in Table 1.

In total, 48 soil samples were collected, each weighing 100 g. The samples were placed in individually labeled polyethylene bags and transported to the Agricultural and Environmental Microbiology Laboratory at the Colegio de Postgraduados, Tabasco Campus, for processing and analysis.

For the analysis of chemical parameters, soil samples were air-dried at room temperature under shaded conditions for five days. Soil pH (hydrogen potential) and electrical

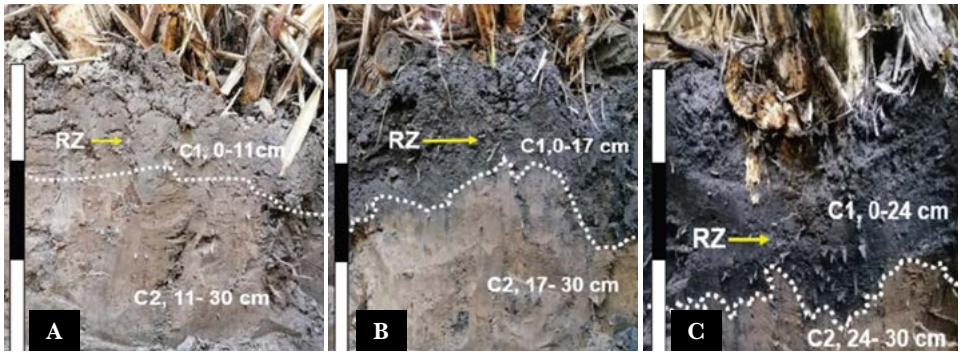

Figure 1. Fluvisol soil with filter cake thickness and surface soil compartments (0-30 cm) under sugarcane (*Saccharum* spp.) var. CP 72-2086. A) FCT1: soil layer without filter cake (0-11 cm, grayish brown); B) FCT2: soil layer with filter cake (0-17 cm, dark brown); and C) FCT3: soil layer with filter cake (0-24 cm, very dark brown). RZ: rhizosphere; C1: non-root soil layer 1; C2: non-root soil layer 2.

Table 1. *In situ* experimental treatments (with and without filter cake) and their corresponding soil compartments.

T	Code	Description
1	FCT1+RZ	Thickness 1 without filter cake (0-11 cm) + Rhizosphere
2	FCT1+C	Thickness 1 without filter cake (0-11 cm) + Non-root soil layer (0-11 cm)
3	FCT1+C2	Thickness 1 without filter cake (0-11 cm) + Non-root soil layer (11-30 cm)
4	FCT2+RZ	Thickness 2 with filter cake (0-17 cm) + Rhizosphere
5	FCT2+C1	Thickness 2 with filter cake (0-17 cm) + Non-root soil layer (0-17 cm)
6	FCT2+C	Thickness 2 with filter cake (0-17 cm) + Non-root soil layer (17-30 cm)
7	FCT3+RZ	Thickness 3 with filter cake (0-24 cm) + Rhizosphere
8	FCT3+C1	Thickness 3 with filter cake (0-24 cm) + Non-root soil layer (0-24 cm)
9	FCT3+C2	Thickness 3 with filter cake (0-24 cm) + Non-root soil layer (24-30 cm)

T: Treatment. FCT: Filter cake thickness. RZ: Rhizosphere. C1: Non-root soil layer 1. C2: Non-root soil layer 2.

conductivity (EC) were determined after shaking the samples in a soil-to-water suspension at a 1:2 ratio, following the methodology described by Jackson (1964), using a portable pH meter and electrical conductivity meter (HANNA Instruments, model HI 9811-5).

Soil organic matter (OM) and organic carbon (OC) content were quantified according to the procedure described by Nelson and Sommers (1982). Total nitrogen (TN) was determined using the micro-Kjeldahl method after digestion with sulfuric acid (H_2SO_4) (Bremner, 1965). Available phosphorus (P) was analyzed using the Olsen method (Olsen and Sommers, 1982), employing a 0.5 M sodium bicarbonate extracting solution adjusted to pH 8.5, and quantified by UV-visible spectrophotometry (GENESYS 10S UV-VIS spectrophotometer) at a wavelength of 882 nm.

Data obtained for each treatment were subjected to analysis of variance (ANOVA), and mean comparisons were performed using Duncan's multiple range test at a significance level of $p \leq 0.05$, using SAS statistical software (version 9.1.3; SAS Institute Inc., 2005).

RESULTS AND DISCUSSION

The results of this study indicate highly significant differences in soil pH (Duncan's test, $p \leq 0.05$; Table 2), particularly in treatments 8, 7, and 6, compared with treatments without filter cake (FCT1; treatments 1, 2, and 3). In addition, an increase in pH was observed in the C2 non-root soil layer with surface-applied filter cake (treatments 6 and 9) relative to the C2 layer without filter cake. This behavior can be attributed to an increase in hydroxyl ions in treatments with filter cake, resulting in a shift in soil reaction from acidic to moderately acidic conditions, according to the NOM-021-RECNAT-2000 classification (SEMARNAT, 2002).

These results are consistent with those reported by Bordin *et al.* (2024) in Brazil, who evaluated combinations of 10 t ha^{-1} of filter cake and 100 m^3 ha^{-1} of vinasse incorporated into soils cultivated with sunflower. In that study, soil pH increased from 5 to 6, which was considered beneficial due to the associated reduction in aluminum

Table 2. Changes in soil pH, electrical conductivity, and organic matter as affected by filter cake thickness and soil compartment.

T	Thickness / Compartment	pH	EC (dS m^{-1})	OM (%)
1	FCT1+RZ	4.7e	0.25b	6.1c
2	FCT1+C1	4.7d	0.23c	5.6cd
3	FCT1+C2	4.8d	0.15ef	4.2e
4	FCT2+RZ	5.8c	0.30a	8.2b
5	FCT2+C1	5.9c	0.18d	7.5b
6	FCT2+C2	6.1b	0.17de	5.0d
7	FCT3+RZ	6.1b	0.17de	9.4a
8	FCT3+C1	6.2a	0.14f	9.1a
9	FCT3+C2	6.0b	0.16ef	5.4 cd

T: Treatment. FCT1: No filter cake, soil thickness 0-11 cm. FCT2: With filter cake, soil thickness 0-17 cm. FCT3: With filter cake, soil thickness 0-24 cm. EC: Electrical conductivity. OM: Organic matter. Values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test ($p \leq 0.05$, $n=4$).

concentration in the soil. The observed pH increases in the C2 layer of treatments 6 and 9 may be explained by the role of this layer as a reservoir for leached materials originating from the C1 layer containing filter cake. The downward movement and removal of suspended materials across one or more soil layers have been previously documented by Weil and Brady (2017) and is influenced by physical, environmental, and anthropogenic factors.

The variability of this property has a substantial impact on crop performance, as a soil pH of 6.5 is considered optimal for sugarcane development (FAO, 2025). Although sugarcane is tolerant of low pH conditions, prolonged soil acidity may impose several limitations, including poor crop growth, nutrient deficiencies, reduced soil biological activity, and increased aluminum toxicity (Al^{3+}) due to its greater solubility at pH values below 5. Under such conditions, phosphate species ($H_2PO_4^-$, HPO_4^{2-}), sulfates (SO_4^{2-}), and molybdates (MoO_4^{2-}) are retained by aluminum oxides and hydroxides, thereby limiting their availability to plants (Weil and Brady, 2017; Barrow and Hartemink, 2023; Zhu *et al.*, 2025). Consequently, reversing or preventing excessively acidic pH conditions in soils cultivated with sugarcane is of considerable agronomic interest.

The highest EC values were observed in treatment 4, followed by treatments 1 and 2, which correspond to soils without filter cake application. In contrast, treatment 8 (FCT3+C1) exhibited the lowest EC value (0.14 dS m^{-1}) (Table 2). The EC values recorded across all nine treatments indicate that salinity was not a limiting or detrimental factor in this study (SEMARNAT, 2002). Therefore, although filter cake application as an organic amendment may contribute soluble salts and nutrients that gradually increase soil EC, this increase was not sufficient to classify the soil as "very saline."

Regarding soil organic matter (OM) and organic carbon (OC), treatments 8, 7, and 4 exhibited a positive effect of filter cake application, showing highly significant differences (Duncan's test, $p \leq 0.05$; Tables 2 and 3) compared with treatments without filter cake (1, 2, and 3). For agricultural soils, these values indicate a very high OC content according to the agronomic classification proposed by Rodríguez and Rodríguez (2015). This reflects an increase in the soil organic reservoir, which undergoes degradation and mineralization processes, thereby supplying essential elements such as nitrogen, phosphorus, and sulfur to the soil solution in accordance with plant root system demand. This effect is primarily associated with the properties of filter cake, as it is a by-product rich in organic matter (Zhou *et al.*, 2022; Pino-Ortega and Batista-Nieto, 2025).

Llanes-Hernández *et al.* (2025) reported that the combined application of filter cake, chemical fertilizers, and grazing in sugarcane-cultivated soils for more than 30 years, followed by crop rotations including vegetables, grains, and tubers, maintained high organic matter levels ranging from 3.23 to 3.78% when compared with uncultivated soils. Such soil enrichment supports and promotes overall improvements in physical and chemical soil properties, thereby contributing to enhanced agricultural ecosystem productivity. Similarly, in rice and wheat cropping systems in India, Basak *et al.* (2021) observed a 25% increase in organic carbon following filter cake incorporation, accompanied by higher crop yields.

The organic carbon results for treatments 7 and 4 reveal an interesting pattern under the influence of filter cake and soil compartment position, with increases of 54% and 34% in the rhizosphere, respectively, compared with treatment 1 without filter cake (FCT1+RZ). Accordingly, organic carbon content exhibited an inverse relationship with soil depth, decreasing as depth increased. This pattern can be explained by the greater activity of soil carbon sequestration and biogeochemical cycling processes in surface soil horizons (Trumbore, 2009). Consequently, the rhizosphere, as the shallowest compartment and the zone closest to plant roots, utilizes filter cake as a readily available organic input that enhances and maximizes soil functions through interactions with root exudates, biological activity, and other soil factors, thereby creating a dynamic and favorable environment for plant development.

Total nitrogen (TN) contents (Table 3) were agronomically classified as high across six treatments, with values ranging from 0.15% to 0.21%. This was especially notable in the rhizosphere (RZ), the C1 and C2 compartments of FCT1 and FCT2, and the RZ compartment of FCT3, all falling within the 0.15-0.25% range established by NOM-021-RECNAT-2000 (SEMARNAT, 2002). These values indicate a high TN content in the soil. However, in the C1 and C2 compartments of FCT1 and FCT3, TN levels decreased to a medium classification.

Filter cake incorporation did not have a significant effect on total nitrogen (TN) content in the FCT1, FCT2, and FCT3 treatments (Duncan's test, $p \leq 0.05$; Table 3). This behavior contrasts with the findings reported by Rivera-Cruz *et al.* (2010), who observed a 1.1-fold increase in TN following filter cake application in an acidic soil cultivated with sour orange. Similarly, Septyani (2019) reported that the addition of 17 t ha^{-1} of filter cake combined with 25% cattle manure in oil palm-cultivated soils increased TN content by 3.5 times compared with soils without filter cake.

The lack of a significant TN response observed in this study may be attributed to nitrogen immobilization processes driven by soil microbial activity stimulated in the rhizosphere and its interactions, as filter cake typically exhibits a high C/N ratio

Table 3. Organic carbon, total nitrogen, C/N ratio, and available phosphorus in soil compartments influenced by filter cake application.

T	Filter cake thickness / Compartment	OC (%)	TN (%)	C/N	P (mg kg^{-1})
1	FCT1+RZ	3.5c	0.19a	18.4cd	3.7d
2	FCT1+C1	3.2cd	0.18a	18.3cd	3.3d
3	FCT1+C2	2.4e	0.15b	17.3d	1.8e
4	FCT2+RZ	4.7b	0.21a	23.5bc	10.0a
5	FCT2+C1	4.4b	0.19a	23.6bc	9.0b
6	FCT2+C2	2.9d	0.18a	16.6d	2.5e
7	FCT3+RZ	5.4a	0.19a	29.1b	7.6c
8	FCT3+C1	5.2a	0.14b	38.8a	3.8d
9	FCT3+C2	3.1cd	0.13b	25.4b	0.6f

T: Treatment. FCT1: No filter cake, soil thickness 0-11 cm. FCT2: With filter cake, soil thickness 0-17 cm. FCT3: With filter cake, soil thickness 0-24 cm. OC: Organic carbon. TN: Total nitrogen. C/N: Carbon-to-nitrogen ratio. Values followed by the same letter within a column do not differ significantly according to Duncan's multiple range test ($p \leq 0.05$, $n=4$).

(Pérez-Méndez *et al.*, 2011). Over time, nitrogen associated with organic amendments undergoes mineralization and becomes available for plant uptake. However, in the present study, filter cake was incorporated eight years prior to sampling, with no subsequent applications. As a result, most of the nitrogen initially supplied by the filter cake may have already been mineralized or depleted, causing the soil to revert to conditions similar to those of soils without filter cake. This occurs despite the currently high TN values, which may be influenced by sugarcane management practices and natural nitrogen cycling processes. This interpretation is supported by the findings of Breza and Grandy (2025), who demonstrated that nitrogen mineralization and immobilization rates depend on the stoichiometry, quantity, and quality of the applied organic substrate.

The C/N ratio (Table 3) exhibited highly significant differences among treatments (Duncan's test, $p \leq 0.05$). The highest C/N ratio (38.8) was observed in FCT3+C1 (treatment 8), indicating that TN is largely stored within the organic matter of the filter cake and is either temporarily unavailable for plant uptake or immobilized due to microbial competition. This nitrogen pool is expected to be gradually mineralized, considering that the optimal C/N ratio for organic amendments to decompose without causing nitrogen immobilization ranges between 24:1 and 30:1 (Weil and Brady, 2017).

Treatments 4, 5, and 6 within FCT2 exhibited C/N ratios ranging from 16.6 to 23.5, while treatments 1, 2, and 3 within FCT1 (Table 3) showed lower C/N values ranging from 17.3 to 18.4, below the optimal range. From an agronomic perspective, these lower C/N ratios are favorable, as they indicate active release of plant-available nitrogen, reduce nutrient immobilization, and promote plant growth through enhanced nitrogen uptake.

With respect to soil phosphorus (P) (Table 3), highly significant differences among treatments were observed as a result of filter cake application (Duncan's test, $p \leq 0.05$). According to NOM-021-RECNAT-2000 (SEMARNAT, 2002), treatments 4, 5, and 7, corresponding to FCT2+RZ, FCT2+C1, and FCT3+RZ, respectively, exhibited P concentrations of 10.0, 9.0, and 7.6 mg kg^{-1}, which are classified as low to medium when compared with treatments 1, 2, and 3 representing soils without filter cake (Table 2). A decreasing trend in P concentration with increasing soil depth was also observed, with the rhizosphere showing the highest values.

These findings are consistent with those reported by Arruda *et al.* (2019), who demonstrated that filter cake application improved the uptake and availability of labile inorganic phosphorus in the rhizosphere and altered the structure of microbial and fungal communities, positioning filter cake as a practical and viable alternative to conventional chemical fertilizers. Furthermore, the decomposition of filter cake organic matter generates competition for phosphorus adsorption sites, reducing phosphorus fixation and increasing its availability to plants, rather than allowing it to remain immobilized in the soil (de Aquino *et al.*, 2021).

Treatments grouped under FCT3 (Figure 1C; Table 3) showed a slight decrease in soil P concentrations compared with FCT2 (Figure 1B; Table 3). This reduction may be associated with increased soil alkalinity in the layer where filter cake was applied (0-24

cm), as higher pH levels can negatively affect phosphorus concentration and availability. This effect occurs because increases in soil pH strongly influence phosphorus reaction mechanisms and fixation processes, depending on soil type (Penn and Camberato, 2019).

CONCLUSIONS

This study demonstrates that the incorporation of filter cake into Fluvisol soils, even eight years after its initial application, leads to significant and sustained improvements in several soil chemical properties under sugarcane (*Saccharum* spp.) cultivation. The key findings indicate that filter cake applied at two thickness levels effectively reverses soil acidity by increasing pH from acidic to moderately acidic conditions, while also enhancing organic matter and organic carbon contents, particularly in the rhizosphere. In addition, an increase in soil phosphorus availability was observed in response to filter cake application at a thickness of 0-17 cm (FCT2), with values ranging from low to medium.

The effect of filter cake was most pronounced in the rhizosphere, followed by the upper non-root soil layer (C1), where interactions between plant roots and biological activity maximize organic functions and nutrient retention.

In summary, filter cake acts as a valuable organic soil amendment that promotes soil health and sustainability in sugarcane agroecosystems by mitigating the negative effects of intensive management and chemical fertilization. These findings highlight key strategies for the repurposing of agroindustrial by-products to enhance agroecosystem sustainability from both economic and environmental perspectives. However, the application of such by-products should be evaluated on a site-specific basis to determine their potential to complement or partially replace chemical fertilizers within a sustainable agronomic management framework.

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Morphological characterization of native mexican tomato populations (*Solanum lycopersicum* (L.) Mill.)

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ABSTRACT

Objective: To characterize native populations of tomato (*Solanum lycopersicum* (L.) Mill.) collected from different regions of Mexico in order to determine their morphological diversity and potential use in breeding.

Design/methodology/approach: One hundred native tomato populations originating from 17 climatic regions in central and southern Mexico, along with two commercial hybrids, were cultivated in a completely randomized block design with two replications under greenhouse conditions. The crop was grown up to the tenth cluster, and morphological evaluation was carried out on the sixth cluster by measuring 28 variables. The statistical analysis included descriptive statistics, univariate analysis of variance, and mean comparison tests. Multivariate analyses included cluster analysis and principal component analysis.

Results: In the descriptive analysis, the variables with intermediate variability were average fruit weight and number of locules. Based on fruit shape, fruits were classified into the following types: kidney, squash, irregular shapes, pear, bell, round, flattened round, saladette, and cherry. In the principal component analysis, native populations were dispersed into eight groups according to their degree of domestication, earliness, and geographic origin. In the cluster analysis, the populations were distributed into 14 groups; Group 13 was shared by populations from Puebla, Guerrero, and the commercial hybrids, indicating that they share common traits.

Limitations of the study/Implications: Studies on culinary and nutritional quality in native tomato populations could be conducted to complement the information obtained.

Findings/Conclusions: Fruits from native tomato populations from Mexico exhibited considerable variability. This diversity is manifested in different fruit types, sizes, and number of locules. Understanding morphological variability opens the possibility for their inclusion in genetic improvement programs.

Keywords: tomato, native, diversity, variability, shape

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INTRODUCTION

The tomato (*Solanum lycopersicum* (L.) Mill) holds agricultural importance in Mexico due to the area cultivated (49,461 ha) and the production volume (3,636,927 t), which positions the country as the leading exporter worldwide (SIAP, 2024). Tomatoes grown

in Mexico originate from improved varieties, and the types (or shapes) marketed include: large round, flattened round, squash, guaje, pear, bell, grape, cocktail, cherry, saladette, and Raf (Maldonado-Peralta *et al.*, 2016). However, in regional markets of Campeche, Guerrero, Puebla, Oaxaca, Tlaxcala, Veracruz, and Yucatán, tomatoes from native and wild populations are sold and highly valued due to their superior organoleptic and nutraceutical quality (Figure 1), comparable to modern varieties (Urrieta-Velázquez *et al.*, 2012). This has been corroborated in collections from Campeche, Chiapas, State of Mexico, Guerrero, Hidalgo, Oaxaca, Puebla, Veracruz, and Yucatán (Bonilla-Barrientos *et al.*, 2014; Berrospe-Ochoa *et al.*, 2018; Figueroa-Cares, 2018; Leana-Acevedo *et al.*, 2022).

The center of origin of tomato is the Andean region in northern Peru and southern Ecuador (Blanca *et al.*, 2012; Peralta *et al.*, 2008). The center of domestication remains controversial; however, recent genomic studies indicate that tomato pre-domestication began in Ecuador and Peru approximately 80,000 years ago with the wild species *Solanum pimpinellifolium* L., giving rise to the wild species *Solanum lycopersicum* var. *cerasiforme*. This species was subsequently re-domesticated in Mexico, and after a process of 7,000 years, it gave rise to *S. lycopersicum* L. (Blanca *et al.*, 2012; Razifard *et al.*, 2020; Wang *et al.*, 2020). Therefore, the tinguaraque or cherry tomato (*S. lycopersicum* var. *cerasiforme*) is the direct ancestor of the tomatoes currently cultivated. In Mexico, the regions with the highest genetic diversity of native tomato are the Sierra of Veracruz and Puebla (Long, 1995), where *S. lycopersicum* L. and *S. lycopersicum* var. *cerasiforme* predominate (Nuez *et al.*, 1996).

The genetic diversity of a species is determined by the different populations that comprise it within a specific geographic area. These populations are dynamic, have a historical origin, and possess a distinctive identity.

In Mexico, tomato was cultivated by the Mexica people since pre-Hispanic times using the chinampa system (Estabrook, 2011; Long, 1995) and became integrated into local

Figure 1. Regional market in Tehuacán, Puebla, Mexico, where native tomato populations are sold to their high quality.

gastronomy (López-Terrada, 2015). The tomatoes used by the Mexica exhibited diverse shapes and a range of colors, including yellow, pink, and red (Sahagún, 1961). Following the conquest of Mexico, tomato was introduced to Spain and from there dispersed worldwide (Blanca *et al.*, 2012; Estabrook, 2011). Today, tomato and cherry tomato are widely cultivated and valued across countries on all continents (Prasanna *et al.*, 2023).

Wild populations are species that grow naturally in a specific region, having evolved and adapted to the local ecosystem without human intervention. These populations maintain a close genetic relationship with cultivated populations and are known as wild relatives (Majeed *et al.*, 2021). Native or local populations are materials that have been cultivated and selected by farmers over several generations, resulting in populations with specific traits according to the preferences of local consumers.

These native populations are adapted to the conditions of their environment and retain considerable genetic variation, which provides stability under environmental changes, making them of interest for genetic improvement. They also hold significant historical, cultural, and economic value for local communities, making their preservation and understanding essential (Ruíz *et al.*, 2016; FAO, 2020).

In Mexico, native tomato populations are diverse, locally adapted, and associated with traditional cultivation systems (Camacho *et al.*, 2005). Currently, the characterization of these native populations has advanced through collections from Campeche, Chiapas, State of Mexico, Guanajuato, Guerrero, Hidalgo, Jalisco, Michoacán, Morelos, Oaxaca, Puebla, San Luis Potosí, Tabasco, Veracruz, and Yucatán (Canul-Hu *et al.*, 2022; Juárez-López *et al.*, 2012; Maldonado-Peralta *et al.*, 2016; Marín-Montes *et al.*, 2016; Matos-Canul *et al.*, 2018).

Knowledge of the diversity and genetic structure of native tomato populations is still limited, making a systematic study —including their characterization— necessary to harness their genetic potential for inclusion in breeding programs. In this study, we conducted a morphological characterization of native Mexican tomato populations from different regions of Mexico to determine their morphological diversity and expand knowledge about these native populations.

MATERIALS AND METHODS

Genetic material

Information on the native populations and tomato controls is presented in Table 1. This research is part of the project "Comprehensive Assessment of the Diversity of Native Mexican Tomato" (CP-CONACYT), which focuses on native tomato populations. A total of 100 native populations from 17 climatic regions in central and southern Mexico, located across seven states, were selected. The controls were the commercial hybrids Reserva (H1) and Sun-7705 (H2). For characterization, the original seeds from the collections were used.

Experimental design

The experiment was established during the Spring-Summer cycle in a complete randomized block design (CRBD) with two replications under greenhouse conditions at the Colegio de Postgraduados, Montecillo Campus, Texcoco, Mexico. The experimental unit consisted of four pots.

Table 1. Origin of native tomato (*Solanum lycopersicum* (L.) Mill.) populations collected in seventeen climatic regions of Mexico.

Origin	Populations by climatic region
Puebla	Region 1: P1, P2, P4, P5, P6, P7, P8, P9, P10, P11, P12, P13, P14, P15, P16, P17, P18, P19, P20, P21, P22, P23, P25, P26, P27, P28, P29, P30, P31, P32, P33; Region 2: P3; Region 3: P24
Guerrero	Region 4: G1, G3, G4, G5, G6, G7, G8, G9, G11, G12, G13, G14; Region 5: G10; Region 6:G2
Oaxaca	Region 7: O5, O6, O8, O9, O10, O11, O12, O13, O14, O15, O16, O17, O18, O19, O20, O21, O22; Region 8: O1; Region 9: O3; Region 10: O2; Region 11: O4; Region 12: O7
Campeche	Region 13: C1, C2, C3, C4, C5, C6, C7, C8, C9, C10
Yucatán	Region 14: Y1, Y2, Y3, Y4, Y5, Y6, Y7, Y8, Y9, Y10
México	Region 15: M1, M2, M3, M4, M5, M6, M8; Region 16: M7
Veracruz	Region 17: V1, V2, V3
Hibrids	Region 18: H1; Region 19: H2

Experimental Management

The seeds received a pre-germination treatment (0.2% KNO_3) and were germinated in trays with peat-moss. Seedlings at 40 days of germination were transplanted in December 2011 into 10-L black polyethylene bags filled with red tezontle as substrate (particle diameter ≤ 12 mm). One tomato seedling was placed per pot.

Nutrition, Pest Control, and Cultural Practices

Plant nutrition was provided using Steiner solution adjusted to pH 5.5, osmotic pressure 0.072, and electrical conductivity 2.0-2.5 $dS \cdot m^{-1}$, supplemented with micronutrients. The crop was stimulated with Bioengorda®, Agromil Plus®, Boramin Ca®, natural extracts, plant hormones, vitamins, and diluents. Whitefly (*Bemisia tabaci*) and tomato russet mite (*Aculops lycopersici*) were controlled with the insecticides Nimicide®, Engeo®, and Agrimec®. Preventive control of gray mold (*Botrytis cinerea* Pers.), late blight (*Phytophthora infestans*), bacterial spot (*Pseudomonas syringae*), and powdery mildew (*Leveillula taurica*) was carried out using Mancozeb®, Cupravit®, Ridomil Gold®, Serenade®, Kasumin®, and Fungimycin®. Cultural practices included preparation of nutrient solutions, irrigation, foliar applications of biofertilizers, fungicides, and insecticides, staking, and pruning of lateral shoots and mature leaves.

Quantitative Variables

The crop was grown up to the tenth cluster, after which the plant was topped above the third leaf. Morphological evaluation was carried out on the sixth cluster. The variables were: PH1C=plant height to first cluster, SD6C=stem diameter at sixth cluster, NI=number of internodes, ID=internode distance, LC4=length of the fourth cluster, DC4=diameter of the fourth cluster, NS=number of fruit-bearing shoots, LL3C=leaf length before third cluster, NL6C=number of leaflets on the leaf before sixth cluster, LL6C=leaflet length on the leaf before sixth cluster, LW6C=leaflet width on the leaf before sixth cluster, LCR=leaf chroma, LHUE=leaf hue measured with Hunter Lab

D25-PC2 colorimeter, DF=days to flowering, DM=days to maturation, FS%=fruit set percentage at the third cluster, NS=number of sepals, PL=petal length, SCL=staminal column length, GL=gynoecium length, SE=stigma exertion, TNF=total number of fruits, AFW=average fruit weight measured with a granatary balance, FL=fruit length, FW=fruit width, FS=fruit shape, PT=pericarp thickness measured with a Truper vernier caliper, and NL=number of locules.

Fruit length was measured as the polar diameter, and fruit width as the equatorial diameter, both determined using a digital caliper. Fruit shape (FS) was calculated as the ratio of fruit length to fruit width (FL/FW); fruits with a ratio less than 1 were considered flattened, fruits with a ratio equal to 1 were considered round, and fruits with a ratio greater than 1 were considered elongated. The number of locules was determined by cutting the fruit in half and counting the cavities.

Statistical Analysis

Each variable was evaluated using descriptive statistics and univariate analysis of variance based on the Complete Randomized Block (CRB) model with two replications. Mean differences were determined using Tukey's test ($p \leq 0.05$). Similarity relationships among native populations were calculated using the Euclidean distance coefficient (data not shown). Multivariate analyses included cluster analysis and principal component analysis (PCA). Cluster analysis was performed using the unweighted pair-group method with arithmetic mean (UPGMA).

PCA was used to identify relationships among materials based on proximity and to determine the variables that contributed most to the variation (informative variables). Statistical analyses were performed using InfoStat statistical software (Di Rienzo *et al.*, 2008).

RESULTS AND DISCUSSION

Morphological Characterization

Table 2 presents the descriptive statistics of the variables and the analysis of variance. Dispersion statistics indicate the variation among the observed data; high values for range, variance, and standard deviation suggest dispersed data with greater variability, while low values indicate homogeneous data. Quantitative variables exhibiting a wide range were related to plant architecture (plant height to first cluster, length of the fourth cluster, leaflet length on the leaf before the sixth cluster, leaflet width on the leaf before the sixth cluster), phenology (days to flowering, days to maturation), yield (fruit set percentage at the third cluster, total number of fruits), and fruit quality (average fruit weight, fruit length, fruit width).

The coefficient of variation (CV) expresses the variability of a dataset relative to its mean and is used to compare the dispersion of two or more datasets. Variables with intermediate dispersion ($30\% < CV < 70\%$) were related to the fruit and included average fruit weight, number of locules, number of fruit-bearing shoots, and total number of fruits. Variables with low dispersion ($CV < 30\%$) were associated with plant architecture, flower characteristics, and phenology.

Analysis of variance detected significant differences for all variables, except for the diameter of the fourth cluster and the leaflet length on the leaf before the sixth cluster. In terms of phenology, populations C9, Y8, and P2 stood out, averaging 20 days to flowering. These populations were earlier than those from Guanajuato, Puebla, Guerrero, Hidalgo, Oaxaca, and Yucatán, which required 25 to 32 days (Vázquez-Ortiz *et al.*, 2010), and earlier than populations from Puebla, Michoacán, Oaxaca, and Chiapas, which required 55 days (Alvarado-Rodríguez *et al.*, 2022).

For days to maturation, populations P1, P2, and P3 stood out with 69 days, showing earlier maturity than populations from Puebla and Oaxaca, which required 100 days (Bonilla-Barrientos *et al.*, 2014). However, there are native populations from Guanajuato, Puebla, Guerrero, Hidalgo, Oaxaca, and Yucatán that are even earlier, reaching maturity in 46 days (Vázquez-Ortiz *et al.*, 2010). These results highlight the wide variation in earliness present in native Mexican tomato populations, providing useful information for the development of improved varieties.

In terms of number of locules, population Y4 stood out with 17 locules per fruit. This value is higher than that of populations from Oaxaca, which have 11 locules (Leana-Acevedo *et al.*, 2022), and the CAM25 population from Campeche, which has 13 locules (Maldonado-Peralta *et al.*, 2022). Regarding fruit weight, populations G4 and O5 stood out with 265 g and 219 g, respectively. These weights are much higher than those of populations LBCh231 and PUE10 from Puebla, with 145.27 g and 147.8 g, respectively; OAX100 from Oaxaca with 154.8 g; and the varieties Floradade and Cid F1 with 105.67 g and 112.6 g, respectively (Alvarado-Rodríguez *et al.*, 2022; Maldonado-Peralta *et al.*, 2022).

Regarding pericarp thickness, populations P13, P16, and P24 exhibited thickness similar to the hybrid H2, at 7 mm. These fruits had greater thickness than populations from Guerrero, Puebla, Oaxaca, Yucatán, Campeche, Veracruz, and the State of Mexico, which averaged 3.8 mm (Maldonado-Peralta *et al.*, 2016), but lower than population PUE100 from Puebla with 8.9 mm and the hybrid Cid F1 with 9.9 mm (Maldonado-Peralta *et al.*, 2022). In terms of petal length, populations P15, P30, P32, and Y2 stood out with 21 mm. These values are higher than those of populations collected in Morelos, Puebla, Oaxaca, Tabasco, and Veracruz, which averaged 16.75 mm (Canul-Ku *et al.*, 2022).

Regarding variables related to plant architecture, in breeding programs it is desirable for the position of the leaf before the third cluster to be semi-drooping, so that the plant is compact and receives light throughout the canopy. For the clusters, a larger peduncle diameter provides greater strength and prevents fruit drop, while cluster length is associated with more branching and a higher number of fruits.

Modern commercial varieties exhibit little variation (Miller and Tanksley, 1990), whereas native populations possess considerable morphological variability, as identified in this study, which allows for the exploitation of the outstanding traits of these native populations.

Figure 2 shows the fruit shapes or types identified in the native tomato populations. Fruit shape (FS) exhibited wide variability, allowing native populations to be classified into

Table 2. Descriptive statistics and analysis of variance for 28 quantitative variables evaluated in native tomato (*Solanum lycopersicum* (L.) Mill.) populations.

Variable	Range		AVG	SD	CV	Analysis of variance	
	Max	Min				SS (Region)	MS (Error)
PH1C	57.38	19.75	33.89	8.71	25.70	4425.81**	36.79
SD6C	15.77	10.24	13.00	1.24	9.53	88.55 **	0.75
NI	33.38	12.38	25.59	2.65	10.35	226.16**	5.49
ID	8.17	4.84	6.25	0.76	12.10	24.29**	0.38
LC4	60.50	16.75	32.18	8.63	26.83	3417.17**	46.73
DC4	11.65	4.67	7.89	0.96	12.20	12.36	0.92
NS	6.62	1.00	2.77	1.05	37.83	63.02**	0.54
LL3C	47.13	21.38	35.61	5.21	14.62	1825.79**	10.38
NL6C	8.00	5.00	6.89	0.50	7.28	7.92**	0.20
LL6C	161.78	84.17	124.51	13.90	11.16	3326.34	183.86
LW6C	81.19	40.14	59.33	8.04	13.56	1590.25**	56.18
LCR	17.77	9.61	12.30	2.12	17.23	341.13**	1.28
LHUE	−48.15	−53.89	−51.46	1.27	−2.47	53.77**	1.24
DF	45.38	19.25	32.15	6.68	20.79	2808.69**	19.35
DM	109.50	69.25	89.83	9.53	10.61	6289.49**	32.84
FS%	96.41	57.73	81.52	8.04	9.87	1401.02**	58.32
NS	9.38	5.25	7.11	0.93	13.03	55.11**	0.36
PL	21.67	12.43	17.00	2.26	13.28	299.73**	2.45
SCL	10.87	6.95	8.64	0.77	8.96	22.76**	0.43
GL	10.99	6.75	8.90	1.03	11.62	64.88**	0.49
SE	1.46	0.78	1.05	0.13	12.60	0.77**	0.01
TNF	123.13	25.50	63.64	23.07	36.25	33,240.31**	233.06
AFW	265.15	5.11	74.95	37.97	50.66	40,729.84**	1191.85
FL	106.25	16.13	44.08	10.91	24.76	4473.26**	85.89
FW	1.29	0.46	0.87	0.20	22.71	2.30**	0.02
FS	93.52	19.43	53.06	10.52	19.83	4847.78**	71.99
PT	8.02	2.09	5.22	1.22	23.36	100.03**	0.57
NL	17.13	2.25	6.45	2.79	43.21	486.48**	3.37

** Significant differences, Max: maximum, Min: minimum; AVG: averange, SD: standard deviation, CV: coefficient of variation, PH1C: plant height to first cluster, SD6C: stem diameter at sixth cluster, NI: number of internodes, ID: internode distance, LC4: length of the fourth cluster, DC4: diameter of the fourth cluster, NS: number of fruit-bearing shoots, LL3C: leaf length before third cluster, NL6C: number of leaflets on the leaf before sixth cluster, LL6C: leaflet length on the leaf before sixth cluster, LW6C: leaflet width on the leaf before sixth cluster, LCR: leaf chroma, LHUE: leaf hue, DF: days to flowering, DM: days to maturation, FS%: fruit set percentage at third cluster, NS: number of sepals, PL: petal length, SCL: staminal column length, GL: gynoecium length, SE: stigma exertion, TNF: total number of fruits, AFW: average fruit weight, FL: fruit length, FW: fruit width, FS: fruit shape, PT: pericarp thickness, NL: number of locules.

the following types: kidney, squash, irregular shapes (with a higher number of locules), pear, bell, round, flattened round, saladette, and cherry.

Flattened round, squash, ribbed bell, and kidney-type fruits had a flattened shape (FL/FW<1). Round fruits included the ball and cherry types (FL/FW=1). Saladette, pear, and bell-type fruits exhibited an elongated shape (FL/FW>1).

Figure 2. Fruit types of native Mexican tomato (*Solanum lycopersicum* (L.) Mill). Source: prepared by the autor with support from Ramiro Maldonado.

Kidney-shaped fruits had a higher number of locules than cherry, ball, bell, and saladette-type fruits. Locules are cavities that protect the seeds and derive from the carpels of the flower. The number of locules is associated with fruit shape and larger size, and this variability is genetically determined (Muños *et al.*, 2012).

These ribbed and flattened tomato variants are frequently observed in native populations and occur at low frequency in modern cultivars. These shapes are associated with multilocular ovaries and abnormal fusion of floral organs (Barrero and Tanksley, 2004).

Fruits with similar shapes have been reported in native populations from Campeche, State of Mexico, Guanajuato, Guerrero, Hidalgo, Oaxaca, Puebla, Veracruz, and Yucatán (Vásquez-Ortiz *et al.*, 2010; Maldonado-Peralta *et al.*, 2016; Bonilla-Barrientos *et al.*, 2014).

Table 3 presents the contribution of the variables to the Principal Component Analysis (PCA). The first two principal components explained 39% of the variation. PC1 accounted for 25% of the variation, with positive contributions from days to flowering, days to maturation, and pericarp thickness, and negative contributions from the number of locules and leaf length before the third cluster. PC2 accounted for 14% of the variation, with positive contributions from length of the fourth cluster, diameter of the fourth cluster, and number of internodes, and a negative contribution from average fruit weight.

Figure 3 shows the spatial distribution of the populations across two principal components. The 100 native populations and the tomato controls were grouped into eight clusters based on the degree of domestication, earliness, and geographic origin. Early-flowering native populations were grouped in quadrant IV, intermediate-flowering populations in quadrants I and IV, and late-flowering native populations with specific fruit shapes in quadrants I and II.

Table 3. Variable contributions to principal components.

Variable	CP1	CP2
Height to first cluster	0.20	0.19
Stem diameter at the sixth cluster	−0.06	0.21
Number of internodes	0.11	0.30
Internode distance	−0.05	0.18
Length of the fourth cluster	−0.13	0.36
Diameter of the fourth cluster	−0.03	0.34
Number of fruit-bearing shoots	−0.25	0.20
Leaf length before third cluster	−0.26	−0.01
Number of leaflets on the leaf before sixth cluster	0.11	0.17
Leaflet length on the leaf before sixth cluster	0.17	0.15
Leaflet width on the leaf before sixth cluster	0.20	0.22
Leaf chroma	−0.12	−0.01
Leaf hue	0.17	−0.09
Days to flowering	0.32	0.11
Days to maturation	0.30	0.03
Fruit set percentage at the third cluster	0.11	−0.08
Number of sepals	−0.25	−0.05
Petal length	0.29	0.16
Staminal column length	0.24	−0.03
Gynoecium length	0.13	0.22
Stigma exertion	−0.05	0.19
Total number of fruits	−0.22	0.26
Average fruit weight	0.12	−0.28
Fruit length	0.11	−0.23
Fruit width	0.09	−0.23
Fruit shape (Length/width ratio of the fruit)	0.02	−0.05
Pericarp thickness	0.26	−0.10
Number of locules	−0.28	−0.11
Explained variation (%)	25.00	14.00

Group A consisted of populations from Puebla (P10, P11, P12, P13, P14, P15, P17, P18, P19, P20, P21, P23, P24, P25, P26, P27, P28, P29, P30, P31, P32, P33) that produced ribbed bell-type fruits with four locules, thick pericarp, and intermediate fruit weight. The plants were low-growing with short leaves, long-petaled flowers, and late maturation.

Group B included populations from Guerrero (G3 and G4), Oaxaca (O4 and O5), Puebla (P16), and the controls H1 and H2. This group produced large fruits of the saladette and round types, with thick pericarp and high weight. The plants were low-growing with few internodes, short clusters, and late maturation. Within this group, population G4 stood out for producing round-type fruits with the highest weight.

Group C included populations from Campeche (C3, C4, C5, C7, C9), Yucatán (Y4, Y7, Y8), and Guerrero (G7) that produced round and flattened squash-type fruits with numerous locules (10 locules), thin pericarp, and high fruit weight. The plants had flowers with short petals and early maturation.

Group D consisted of populations from Puebla (P1, P2, P4, P6, P7, P8, P9), Veracruz (V1, V2, V3), and Oaxaca (O12) with characteristics similar to kidney-type native tomatoes. The fruits were squash- and kidney-shaped with numerous locules and thin pericarp. The plants had long leaves and early maturation. Kidney-type native tomatoes exhibit plants with longer internodes, small, flattened, and ribbed fruits (fruits with pronounced grooves). Group E included populations from Oaxaca (O1, O2, O3, O6, O8, O9, O10, O11, O14, O15, O16, O18, O19, O21, O22), Guerrero (G1, G5, G6, G8, G10, G14), and Campeche (C1, C8). These populations produced squash-, kidney-, and irregular-shaped fruits with high weight. The plants had long leaves, flowered at 30 days, and matured at 88 days.

Group F consisted of populations from Yucatán (Y1, Y2, Y3, Y5, Y6, Y10), Campeche (C2, C6), Guerrero (G2, G13), and Oaxaca (O20), producing squash-, pear-, and flattened round-type fruits with thick pericarp and intermediate weight (64.17 g). The plants had numerous internodes, long and wide clusters, and long-petaled flowers.

Group G included populations from the State of Mexico (M2, M3, M4, M5, M6, M7) and Guerrero (G11, G12) with pear- and squash-type fruits of intermediate weight (64.17 g) and thick pericarp. The plants exhibited late flowering and maturation.

Group H was represented by the population from the State of Mexico (M1), which produced cherry-type fruits with two or three locules, thin pericarp, and low weight. The plant had few internodes, long leaves, short-petaled flowers, early flowering at 23 days, and maturation at 80 days.

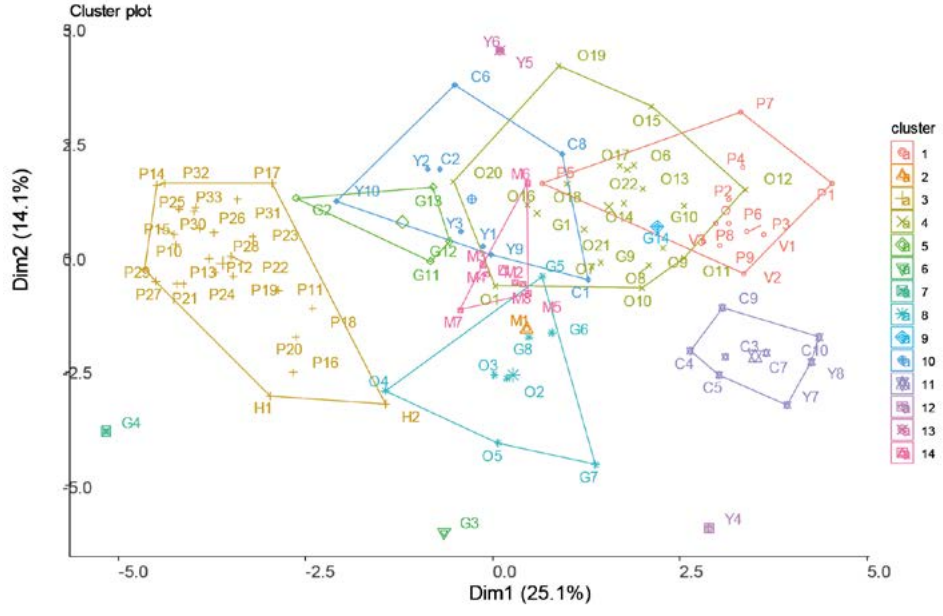

Figure 3. Principal Component Analysis diagram of 100 native tomato populations and two commercial hybrids (*Solanum lycopersicum* (L.) Mill.) based on quantitative morphological variables.

Hierarchical Cluster Analysis

Figure 4 shows the cluster analysis performed based on the 28 quantitative variables. A Euclidean distance cutoff of 1.19 defined 14 groups. Populations in groups 1 to 7 were separated individually according to their outstanding traits.

Group 1 included population Y2, which produced fruits with thick pericarp, long-petaled flowers, and long internodes. Group 2 included population Y4, which produced large round-type fruits with high weight and 17 locules; this population exhibited early flowering and flowers with short gynoecia and stigmas. Populations in groups 1 and 2 displayed traits of interest for breeding, as consumers prefer round and saladette-type tomatoes, which are large, have thick pericarp, and high fruit weight.

Group 3 included population C9, which exhibited early flowering and maturation, with flowers having short gynoecia. Group 4 included population G3, which produced fruits with few locules, short internodes, and flowers with long staminal columns; this trait may be associated with pollination efficiency. In tomato flowers, the stamens are fused by their anthers to form a staminal column (or cone) that releases pollen from the top through the buzzing of native bees, depositing it directly onto the pollinator's body (Vallejo-Marín *et al.*, 2022). A long staminal column ensures proper pollen deposition on pollinators but may limit self-pollination.

Group 5 included population G4, which produced round-type fruits with high weight, few fruits per plant, short-gynoecium flowers, and late flowering. Group 6 included population M5, which produced long fruits with thick pericarp, flowers with long gynoecia, and late flowering. Populations in groups 5 and 6 also exhibited large fruits with thick pericarp, traits of interest for genetic improvement.

Group 7 included population C5, which exhibited long leaflets and flowers with short gynoecium and staminal column. Group 8 included populations M1 and G14, which produced cherry-type fruits with few locules, high fruit set, and plants with long leaves, few internodes, and limited branching. This group displayed traits of interest for the development of cherry-type populations.

Group 9 included twenty-two populations from Oaxaca (O1 to O22) and seven from Guerrero (G1, G5, G6, G7, G8, G9, G10) with large kidney- or squash-type fruits, flowers with long gynoecia, and 7 to 9 sepals. These populations have traits of interest for breeding, as kidney-type tomatoes are appreciated by consumers for their characteristic acidic taste and are also tolerant to pests and drought (Matos-Canul *et al.*, 2018).

Group 10 included nine populations from Puebla (P1 to P9) and three from Veracruz (V1, V2, V3), producing a high number of small fruits with thin pericarp, early flowering and maturation, and flowers with short staminal columns and gynoecia. These native populations exhibit traits similar to Cherry tomatoes.

Group 11 included four populations from Campeche (C3, C4, C7, C10) and two populations from Yucatán (Y7, Y8) with intermediate-sized and weighted fruits, plants with long leaves, and flowers with short staminal columns and gynoecia.

Group 12 consisted of six populations from the State of Mexico (M2, M3, M4, M6, M7, M8) and two populations from Puebla (P12, P24) producing pear-type fruits, branched clusters, and flowers with long gynoecia. Pear-type fruits are of interest for the European

market, as fruits with these characteristics are used in preparing the cold soup gazpacho (Campra *et al.*, 2019).

Group 13 included twenty-four populations from Puebla (P10 to P33), four populations from Guerrero (G2, G11, G12, G13), two populations from Yucatán (Y5, Y6), and the two commercial hybrids (H1, H2). These populations produced long fruits with higher weight and thick pericarp, plants with late flowering, and flowers with long petals, staminal columns, and gynoecia. These populations exhibited traits similar to the commercial hybrids, making them favorable for genetic improvement.

Group 14 consisted of populations from Campeche (C1, C2, C6, C8) and Yucatán (Y1, Y3, Y9, Y10) with intermediate-weight fruits, plants with intermediate flowering, and flowers with short staminal columns and gynoecia. The staminal column forms a cone around the stigma, and its height affects pollination. In flowers with short pistils and styles (gynoecia), self-pollination and wind pollination are limited; however, entomophilous pollination can ensure fertilization. The buzzing of bees releases pollen through the pores and deposits it on the thorax, facilitating contact with the stigma. Buzz pollination increases fruit size and weight by enhancing the number of fertilized seeds (fruit set), thereby impacting yield (Bashir *et al.*, 2017).

In the descriptive analysis, the variables average fruit weight and number of locules showed intermediate variability. Variables related to plant architecture, flower, and phenology exhibited low to intermediate variation. Fruits were classified into the following types: kidney, squash, irregular, pear, bell, round, flattened round, saladette, and cherry. The variables contributing most to the variation were days to flowering, days to maturation, pericarp thickness, length of the fourth cluster, diameter of the fourth cluster, and number of internodes. In the PCA diagram, native and cultivated populations were

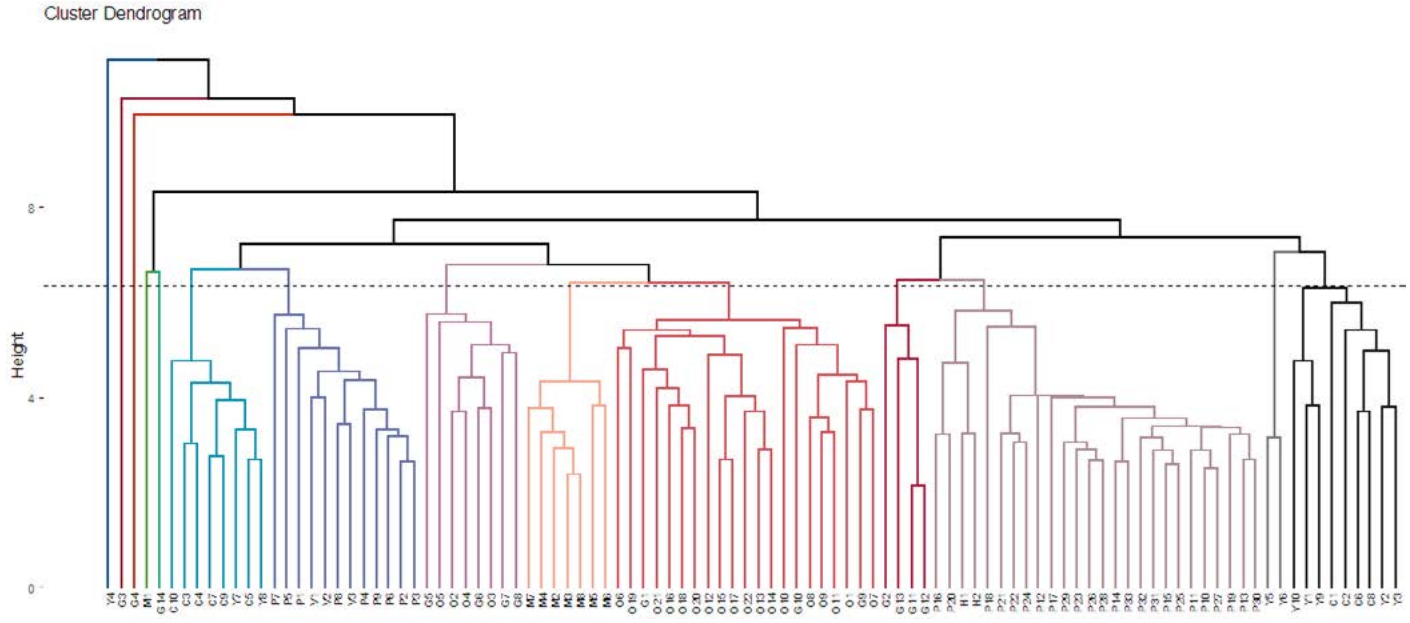

Figure 4. Hierarchical relationships among 100 native tomato (*Solanum lycopersicum* (L.) Mill.) populations using Euclidean distances from quantitative variables.

dispersed according to their degree of domestication, earliness, and geographic origin. In the cluster analysis, populations were distributed into fourteen groups, with group 13 shared by populations from Puebla, Guerrero, and the commercial hybrids, indicating they share common traits.

CONCLUSIONS

Native and local Mexican tomato populations exhibited high variability in the quantitative traits evaluated. The observed variability opens the possibility for their inclusion in breeding programs for its utilization and preservation.

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Antivenom properties of *Pentalinon* sp. (Apocynaceae) against the venom of *Bothrops asper*

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ABSTRACT

Objective: To evaluate the antivenin activity of organic extracts obtained from roots, stems and leaves of *Pentalinon* sp.

Design/methodology/approach: The phytochemical profile of the extracts was determined, and their inhibitory capacity was assessed against several effects induced by *Bothrops asper* venom, including lethality, proteolytic activity on azocasein, phospholipase A′ activity, and fibrinogenolytic activity.

Results: The extracts of *Pentalinon* sp. predominantly contained flavonoids and terpenoids. None of the tested extracts were able to neutralize the venom's lethality. However, the ethanolic leaf extract completely inhibited proteolytic activity on azocasein and partially reduced phospholipase A_2 activity.

Findings/conclusions: Root extracts partially inhibited proteolytic activity on azocasein and prevented the degradation of the alpha and beta chains of fibrinogen.

Keywords: Contrayerba, snakebite, traditional medicine, phytochemistry.

INTRODUCTION

Mexico is a country with great biodiversity, with around 4,000 species of medicinal plants (Ocegueda *et al.*, 2005) and 93 species of venomous snakes (Uetz *et al.*, 2024), which generally cause an average of 3,800 cases of bites (Neri-Castro *et al.*, 2020). The most affected population are children and agricultural workers living in rural areas, where medical services do not always have antivenom (Sevilla-Sánchez *et al.*, 2021). One of the snake species of medical importance that causes a high number of snakebite accidents in Mesoamerica and northern South America is *Bothrops asper*, known as the nauyaca, fournosed viper, or mahuaquite (Mora-Obando *et al.*, 2023). Its venom is mainly composed of three families of toxins: a) phospholipases A2 (PLA2) that are associated with effects such as edema, myotoxicity, cytotoxicity; b) snake venom metalloproteinases (SVMP) that act mainly in the microvasculature generating local and systemic hemorrhages, coagulopathies, myonecrosis, which affect hemostasis; and c) snake venom serine proteases (SVSP) that mainly contribute to defibrinogenation (Gutiérrez and Rucabado, 2000; Alape-Girón *et al.*, 2008; Gutiérrez *et al.*, 2009; Mora-Obando *et al.*, 2023).

Given the difficulties in obtaining antivenoms (Vásquez *et al.*, 2024), people often resort to using plant-based treatments to delay or counteract the effects of the venom; however, in most cases the plants used do not have scientific studies that validate their use (Bénard-Valle *et al.*, 2015), such is the case of contrayerba (*Pentalinon* sp.), used in traditional Mayan and Huastec medicine (Pulido and Serralta, 1993; Pérez-Bautista *et al.*, 2023).

In recent years, the search for inhibitors that complement antivenom therapy has gained importance (Gutiérrez *et al.*, 2021) and provide more time to reach the nearest medical service. In Mexico, there are few scientific studies evaluating the antivenom activity of plants that can help treat snakebites. Therefore, the objective was to evaluate the antivenom properties of *Pentalinon* sp. extracts against the venom of *Bothrops asper*.

MATERIALS AND METHODS

Location and collection of plant material

Roots, stems, and leaves of *Pentalinon* sp. were collected from wild populations in February and April of 2023 and 2024 in the municipality of San Felipe Orizatlán, Hidalgo, Mexico (21° 05' and 21° 24' N and 98° 27' and 98° 42' W), and in the municipality of San Martín Chalchicuautla, San Luis Potosí, Mexico (98°40'31.912" N and 21° 19' 28.950" W).

The experimental phase was carried out in the Phytochemistry Laboratory and the Laboratory of Biological Tests with Medicinal Plants at the Postgraduate College, Montecillo Campus, as well as in the Molecular Medicine and Bioprocesses Laboratory of the Institute of Biotechnology at the National Autonomous University of Mexico.

Taxonomic identification and extraction of *Pentalinon* sp.

The identification of *Pentalinon* sp. was carried out through molecular analysis at the Institute of Biology of the National Autonomous University of Mexico. Ethanolic extracts and extraction pools were obtained from dried roots, stems, and leaves (Table 1) by maceration with 70% ethanol, 96% ethanol, hexane, ethyl acetate, or methanol. Filtration

Table 1. Amount of plant material used to obtain organic extracts of *Pentalinon* sp.

Extract	Sample weight (g)	Abbreviation
Pentalinon sp. root ethanol	253.6	R
Pentalinon sp. stem ethanol	264.1	T
Pentalinon sp. leaf ethanol	87.7	H
Pentalinon sp. root pool	54	PR
Pentalinon sp. stem pool	150	PT
Pentalinon sp. leaf pool	128.1	PH

was performed every three days with solvent exchange until the absence of color was observed. The crude extract was obtained by removing the solvent with a rotary evaporator (IKA® RV10, automatic control/BUCHI R-114 Equipan S.A. de C.V., Switzerland). Extraction pools were formed from the mixture of the extracts obtained with hexane, ethyl acetate, and methanol. The extracts were kept refrigerated at 4 °C until use.

Obtaining the Phytochemical Profile of *Pentalinon* sp. extracts

The presence of phenols, flavonoids, tannins, saponins, and terpenes was evaluated using thin-layer chromatography (TLC) (Wagner and Bladt, 2009). 0.4 g of each extract were weighed and dissolved in 8 mL of methanol. Samples of each extract were placed on 10×10 cm aluminum plates (HPTLC Silica gel 60 F254 Merck). They were eluted using different elution systems according to the metabolite group. Finally, they were observed under ultraviolet light at 365 nm and developed with different developers (Table 2).

Bothrops asper venom

A pool of lyophilized *Bothrops asper* venom was used, obtained from 39 adult specimens. This venom came from the venom bank of the Biotechnology Institute of the National Autonomous University of Mexico (UNAM). The pool was stored at −20 °C until use.

Animal use

Mice (18-20 g) of the ICR strain, of mixed sex, were used. The mice were kept in acrylic boxes (30×15×15 cm) with a 12 h light/12 h dark cycle, with unlimited access to food and water (Laboratory Rodent Diet). Bioethical approvals were obtained from the Biotechnology Institute of UNAM, the project number is 495.

Table 2. Elution system and developer used for each group of metabolites analyzed in *Pentalinon* sp.

Metabolite	Elution system (v/v)	Developer
Phenols	Ethyl acetate:methanol (9:1)	Folin-Ciocalteu Reagent
Flavonoids	Ethyl acetate:methanol:water:formic acid (50:2:3:6)	NP-PEG
Tannins	Ethyl acetate:methanol (1:1)	
Saponins	Ethyl acetate:methanol (1:1)	Ferric Chloride
Terpenoids	Toluene:chloroform:ethanol (4:4:1)	Vanillin and 1% Sulfuric Acid in Ethanol
Alkaloids	Methanol-dichloromethane (1:9)	Anisaldehyde-Sulfuric Acid

Median lethal dose (LD_{50}) of *B. asper* venom and lethality neutralization with *Pentalinon* sp. extracts

The median lethal dose (LD_{50}) of the venom was obtained according to the methodology of Lorke (1983) with modifications. Three mice were inoculated intraperitoneally (IP) per dose of venom resuspended in phosphate buffer (PBS). The doses administered were 60, 100, 130, 150, and 170 μg mouse^{-1}. Deaths were recorded 24 h post-inoculation.

The evaluation of the lethality-neutralizing activity was determined using the methodology of Otero *et al.* (2000), with some modifications. Three mice were inoculated IP per treatment. The treatments were based on $3LD_{50}$ of the venom, which was pre-incubated for 30 min at 37 °C with different doses of extract. Controls consisted of $3LD_{50}$ of venom and some doses of extract (Table 3). Mice were monitored for 24 h, and mortality was recorded.

Inhibition of the proteolytic effect on azocasein

The methodology of Saravia-Otten *et al.* (2021) was followed with modifications. A dose of 1:48 venom:extract (w/w) was selected for evaluation; venom (20.5 μg) was used as a positive control, PBS as a blank, and PBS:DMSO:Tween 20% (1:0.2:0.2 v:v) as a negative control. Extracts with and without substrate were also included. All assays had a final volume of 20 μL and were pre-incubated at 37 °C for 30 min. 100 μL of an azocasein solution (Sigma A2765) of 10 mg mL^{-1} in an azocasein buffer was added, and the mixture was incubated for 30 min at 37 °C. Trichloroacetic acid (5%) was added to precipitate undigested proteins and stop the reaction. In 96-well plates, 150 μL of 0.5 M NaOH and 150 μL of supernatant were added. Absorbance was quantified at 450 nm using a BioTek Elx800 microplate reader with Gen5 Version 2.0 software. Experiments were performed

Table 3. Treatments evaluated for the neutralization of $3LD_{50}$ of *B. asper* venom with *Pentalinon* sp. extracts inoculated into mice (ICR).

Treatment	Venom	Extract (mg/mouse)
Venom*	3 LD_{50}	
Venom + ethanolic root extract	3 LD_{50}	5 20
Venom + ethanolic leaf extract	3 LD_{50}	20
Venom + ethanolic stem extract	3 LD_{50}	20
Venom + pool of leaf extracts	3 LD_{50}	20 50 80
Ethanolic root extract*	-	5 20
Ethanolic extract of stems*	-	20
Ethanolic extract of leaves*	-	20
Pool of leaf extracts*	-	20
Pool of root extracts*	-	20

*: control.

in triplicate. The absorbance of the extracts without substrate was subtracted from the activity of the extract incubated with the substrate to calculate the percentage of activity. Results were expressed as a percentage of proteolytic activity, considering 100% of the proteolytic activity generated by the venom.

Inhibition of phospholipase A2 (PLA2) activity

The evaluation was performed according to the methodology of Gutiérrez *et al.* (1988) with modifications. A selected dose of 1:623 venom:extract (w:w) was evaluated. As positive controls, *B. asper* venom (3 μg) and *Micrurus tener* venom were used; as negative controls, extracts, PBS, and PBS:DMSO:Tween 20% (1:0.2:0.2 v:v) were used. The venom:extract mixtures were pre-incubated for 30 min at 37 °C. The treatments were plated in triplicate on 0.1% agarose plates containing 2.0% egg yolk and rhodamine 6G. Subsequently, they were incubated for 20 h at 37 °C. The diameter of the halos was measured using GIMP 2.10.38. The average diameter of the halo generated by *B. asper* venom was taken as 100% phospholipase A2 activity.

Inhibition of fibrinogenolytic activity

The methodology described by Ware *et al.* (1942) was followed with some modifications. Mixtures of 50 μg of human fibrinogen with 1:221 and 1:442 venom:extract (w:w) doses were incubated for 30 min at 37 °C. Fibrinogen, venom (10 μg) with fibrinogen, and extracts with fibrinogen were used as controls. After incubation, a 6 μL aliquot of the sample was taken and mixed with 3 μL of 5x sample buffer containing $'\beta$-mercaptoethanol, bringing the final volume to 15 μL with distilled water. This mixture was boiled for 5 min and centrifuged at 13,000 rpm for 30 s. Perform electrophoresis under reducing conditions with 12.5% gels.

Statistical Analysis

The median lethal dose data were analyzed using GraphPad Prism Version 10.0 software, plotting the percentage of mortality as a function of the logarithm of the amount of venom using the sigmoid dose-response nonlinear regression method. The neutralization data were analyzed using GraphPad Prism Version 10.2.3 software. For the proteolytic inhibition and phospholipase A2 assays, a one-way ANOVA was performed to determine if there was statistical significance. Subsequently, a Dunnett's test was performed to determine if the treatments were significantly different from the control (venom). A p-value <0.05 was considered significant. The analyses and graphs were performed using GraphPad Prism Version 10.2.3 software.

RESULTS AND DISCUSSION

Taxonomic identification of *Pentalinon* sp.

According to the results of molecular analysis using the matK (maturase K) molecular marker for plant identification (Letsiou *et al.*, 2024), 100% pairwise identity and identical sites correspond to the genus *Pentalinon*. This genus has only two species, *Pentalinon andrieuxii* and *Pentalinon luteum*. In this case, it is suggested that the species used is *Pentalinon*

andrieuxii, based on evaluations of its morphological characteristics (results not shown) and the distribution of both species: *P. luteum* is reported mainly in Florida and the Caribbean islands, and *P. andrieuxii* in various states of Mexico and South America.

Obtaining extracts and phytochemical profile of *Pentalinon* sp.

Three ethanolic extracts and three extract pools were obtained; the yields are presented in Table 4. The pools were obtained by increasing the polarity of the extractions to achieve complete extraction of the metabolites.

Flavonoids, terpenoids, and saponins were found in greater quantities in the roots, stems, and leaves; phenols, tannins, and alkaloids were present in smaller quantities and were not found in the roots (Figure 1).

Some of the ethanolic extracts and extract pools showed variations in their compositions, which is related to the extraction method. Sterols, coumarins, and terpenes, including betulinic acid, urechitol A, and urechitol B, have been isolated from *P. andrieuxii* (Yampuc *et al.*, 2009; Domínguez-Carmona *et al.*, 2010; Pan *et al.*, 2012). A more in-depth phytochemical analysis of the phytochemical composition of *Pentalinon* sp. will be carried out in subsequent studies.

Table 4. Yields of the extracts obtained from *Pentalinon* sp.

Extract	Yield (%)
Pentalinon sp. root ethanol	14.45
Pentalinon sp. stem ethanol	12.54
Pentalinon sp. leaf ethanol	18.92
Pentalinon sp. root pool	n/d
Pentalinon sp. stem pool	n/d
Pentalinon sp. leaf pool	5.44

n/a: no data, the final extract was not weighed

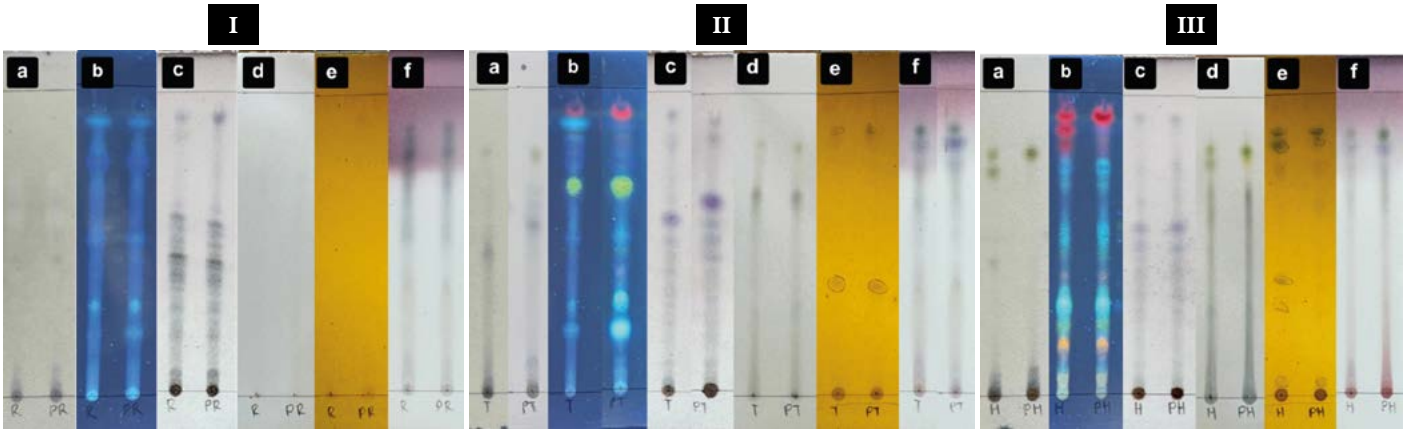

Figure 1. Chromatographic plates for the presence of a: phenols, b: flavonoids, c: terpenoids, d: tannins, e: alkaloids and f: saponins in extracts of *Pentalinon* sp. I: root, II: stems, III: leaves. R: ethanolic extract of root, T: ethanolic extract of stems, H: ethanolic extract of leaves, PR: pool of root extracts, PT: pool of stem extracts, PH: pool of leaf extracts.

Median lethal dose (LD_{50}) of *Bothrops asper* venom and neutralization of lethality with *Pentalinon* sp. extracts

The LD_{50} of the venom pool was 138.9 (131.5-145.9) μg/mouse. None of the evaluated extract doses neutralized the lethality of $3LD_{50}$ of *B. asper* venom (Figure 2). $3LD_{50}$ of venom was recorded as inducing death in mice between 15 and 18 min post-inoculation. The ethanolic leaf extract at a dose of 20 mg/$3LD_{50}$ delayed death for 3 h. The controls of the ethanolic extracts at a dose of 20 mg/mouse induced signs of toxicity (piloerection, loss of hind limb movement, decreased motor activity, and rapid respiration) for a few hours without causing death. The ethanolic root extract at a dose of 5 mg/mouse did not induce signs of toxicity. The root pool (20 mg mouse^{-1}) caused the death of the mice. Of the three doses of the leaf pool evaluated (20, 50, and 80 mg), none showed toxicity like the ethanolic extract. At 20 mg/$3LD_{50}$, the leaf extract pool delayed death by one hour. The 80 mg/$3LD_{50}$ dose did not prevent mortality 18 min post-inoculation.

People who report benefits from consuming this plant may be experiencing multiple factors that are difficult to monitor. These include the amount of venom injected by the snake, the possibility of dry bites, and the presence or concentration of metabolites that may interfere with the lethality of the venom. Furthermore, aspects such as the harvest time and the phenological stage of the plant could also influence the results observed when using *Pentalinon* sp. as an antivenom.

It has been documented that, during reproductive periods, plants tend to synthesize a greater quantity of bioactive compounds (Pires-Moreira *et al.*, 2024). However, none of the plants collected for this study were at that stage. On the other hand, the observed toxicity

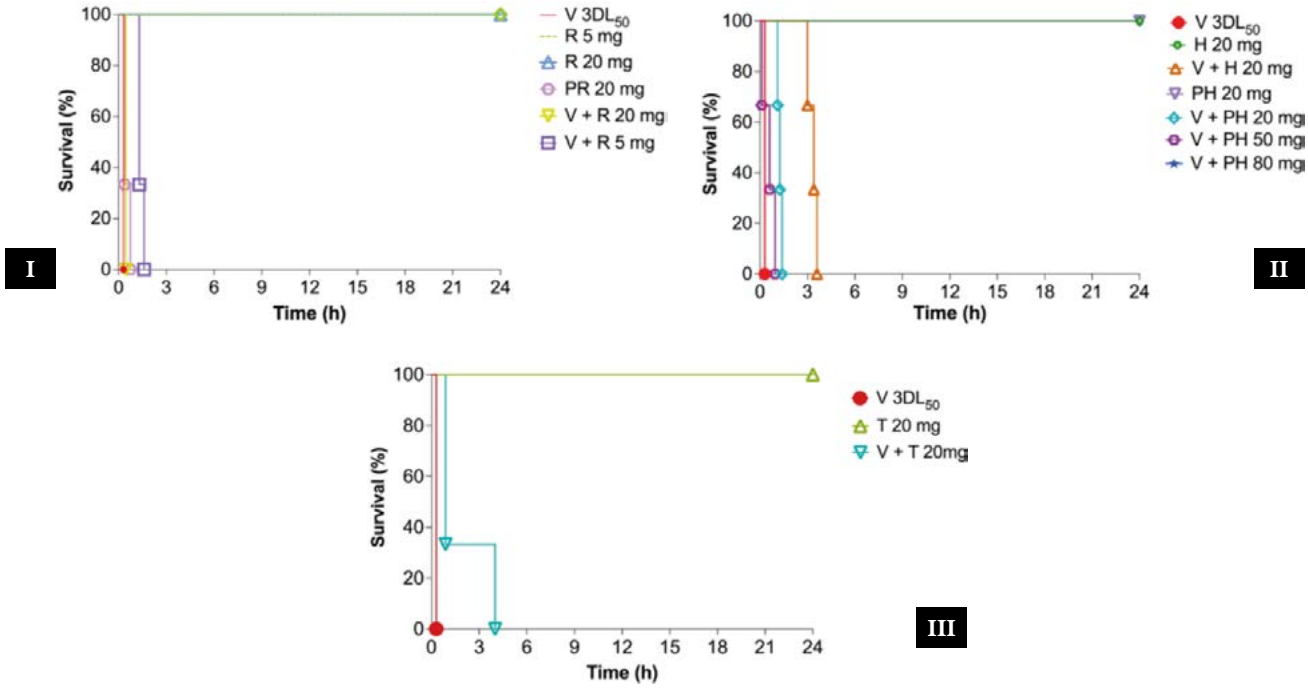

Figure 2. Percentage of survival of mice (ICR) injected with $3LD_{50}$ of *B. asper* venom and *Pentalinon* sp. extracts. I: root, II: leaves, III: stems. V: *B. asper* venom, R: ethanolic root extract, T: ethanolic stem extract, H: ethanolic leaf extract, PR: pool of root extracts, PT: pool of stem extracts, PH: pool of leaf extracts.

could be due to the presence of certain toxic terpenoids. According to Agus (2021), some monoterpenes have cytotoxic effects that vary depending on the dose and exposure time. This coincides with what was observed in the root extracts of *Pentalinon* sp., where 20 mg of the root pool did induce mortality, probably due to a higher concentration of these compounds resulting from the extraction method used. Similarly, it is possible that people who use this plant do not experience adverse effects because the dose used does not contain sufficiently high concentrations of these toxic compounds.

Inhibition of proteolytic activity on azocasein

The proteolytic activity on the venom's azocasein, associated with SVMPs, was significantly affected by the evaluated extracts at different percentages ($P<0.05$). The ethanolic leaf extract inhibited 100% of the proteolytic activity, while extracts PR (43.12±3.46%) and T (45.68±4.71%) had similar inhibition percentages, as did R (14.73±8.54%) and PT (10.16±2.7%). The leaf pool showed practically no inhibition (3.6±2.08%) (Figure 3). SVMPs are enzymes that depend on metal ions such as zinc to perform their catalytic functions (Adrião *et al.*, 2022).

Some flavonoids, such as pinostrobin, have the ability to chelate metal ions (Gómez-Betancur *et al.*, 2014; Kasprzak *et al.*, 2015) and have been shown to partially inhibit the proteolytic and hemorrhagic activity of *B. asper* venom in *in vitro* studies. The phytochemical profile of the roots, stems, and leaves of *Pentalinon* sp. revealed that all organs have a significant flavonoid content, which could be responsible for the inhibition of proteolytic activity. Differences in the phytochemical composition of ethanolic extracts and extract pools were also observed, which may be related to the varying percentages of inhibition among extracts from the same organ.

Inhibition of phospholipase A_2 (PLA2) activity

Of all the extracts evaluated, the ethanolic leaf extract was the only one to partially inhibit phospholipase A_2 activity. Root and stem extracts mixed with the venom generated

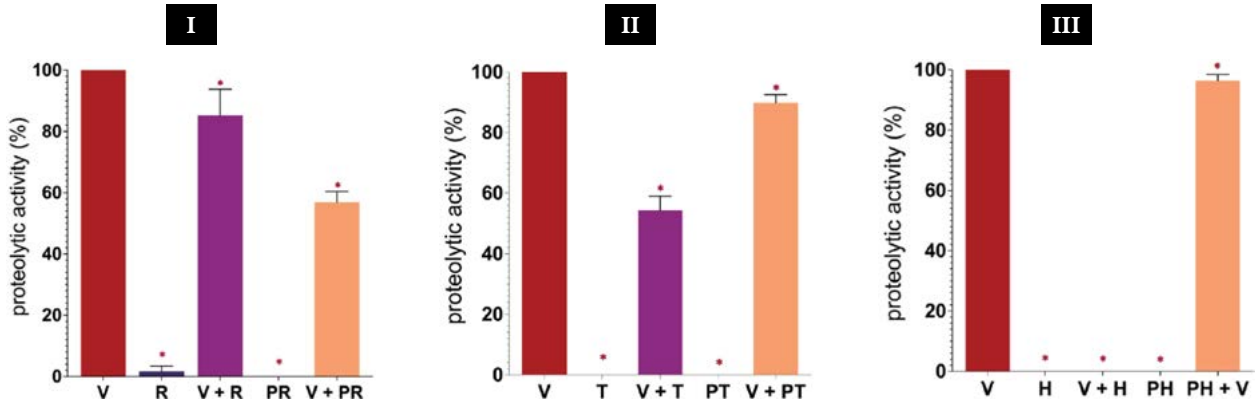

Figure 3. Inhibition of the proteolytic activity of azocasein in *B. asper* venom with extracts of *Pentalinon* sp. I: root, II: stems, and III: leaves. V: *B. asper* venom, R: ethanolic root extract, T: ethanolic stem extract, H: ethanolic leaf extract, PR: pool of root extracts, PT: pool of stem extracts, PH: pool of leaf extracts. Results expressed as a percentage of activity, considering the venom as 100%. Statistical significance ($p<0.05$) compared to the venom: *.

slightly larger halos than those generated by the venom alone (Table 5, Figure 4). The decrease in phospholipase A2 activity can be attributed to the interaction of phospholipase A_2 with components of the extract. Some phenolic compounds, flavonoids, coumestans, alkaloids, steroids, terpenoids, and tannins are among the main plant derived inhibitors of PLA2 (Carvalho *et al.*, 2013).

The phytochemical profile analysis shows that the leaves of *Pentalinon* sp. Some of these groups of compounds are present, which could act by preventing access to the hydrophobic channel for fatty acids, or by binding to functional sites on proteins, or by inducing protein oligomerization (Salvador *et al.*, 2019). In recent years, the search for molecules that inhibit specific venom toxins has gained increasing interest (Salvador *et al.*, 2019), and plants are an important reservoir of such molecules. Future research will need to identify the compounds present in the leaves of *Pentalinon* sp. that interact with phospholipases A_2 and how they interact.

Table 5. Phospholipase A_2 activity of *B. asper* venom and mixtures of venom with *Pentalinon* sp. extracts.

Treatment	Activity phospholipase A_2 (%)
Bothrops asper venom	100
Venom+Ethanolic root extract	108.8±6.505
Venom+Pool of root extracts	101.8±2.512
Venom+Ethanolic stem extract	110.5±8.23
Venom+Pool of stem extracts	104.2±4.149
Venom+Ethanolic leaf extract	60.78±6.618*
Venom+Pool of leaf extracts	95.44±2.702

Values corresponding to the percentage of PLA2 activity (± SD). Results of the average of three repetitions, corresponding to the 1:623 venom:extract (w:w) mixture. *: statistical significance of $p<0.05$ compared with the venom alone (100% activity).

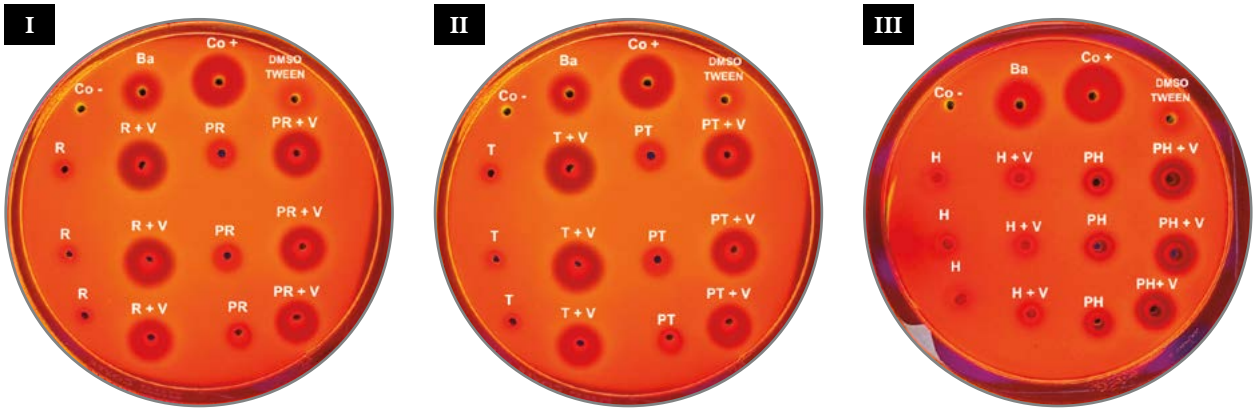

Figure 4. Inhibition of phospholipase A2 activity of *Bothrops asper* venom with extracts of *Pentalinon* sp. I: root, II: stems, III: leaves. Co-: PBS negative control, Co^+: Micrurus tener venom positive control, Ba: *B. asper* venom, V: *B. asper* venom, R: ethanolic root extract, T: ethanolic stem extract, H: ethanolic leaf extract, PR: pool of root extracts, PT: pool of stem extracts, PH: pool of leaf extracts.

Inhibition of fibrinogenolytic activity

The extracts of *Pentalinon* sp. acted in different ways against fibrinogen and venom. Figure 5A, corresponding to ethanolic extracts, shows how the root extract did not interfere with fibrinogen; all three chains remained intact. On the other hand, the stem and leaf extracts induced fibrinogen degradation, as the bands were faded. The ethanolic root extract partially inhibited the degradation of the fibrinogen β band caused by the venom; the ethanolic leaf extract plus venom induced the degradation of all three fibrinogen chains as well as venom components; no bands were observed; and the mixture of ethanolic stem extract with venom induced the same banding pattern as the venom alone (Figure 5A). The pools of stem and leaf extracts did not alter the α, β, and γ chains of fibrinogen. On the other hand, the pool of root extracts maintained all three chains, but they were diffuse (Figure 5B). Regarding the inhibition of fibrinogenolytic activity caused by the venom, although the pool of root extracts interacted with fibrinogen, it only partially inhibited fibrinogenolytic activity; the α and β bands were observed, but not entirely intact.

The stem pool prevented the degradation of the β chain, and the leaf pool showed no inhibition. To determine if the inhibition of chain degradation was better with higher doses, double the dose of root extracts (Figure C) and leaf extracts (Figure D) was evaluated. Root extracts partially inhibited fibrinogenolytic activity, as the α and β bands of the fibrinogen

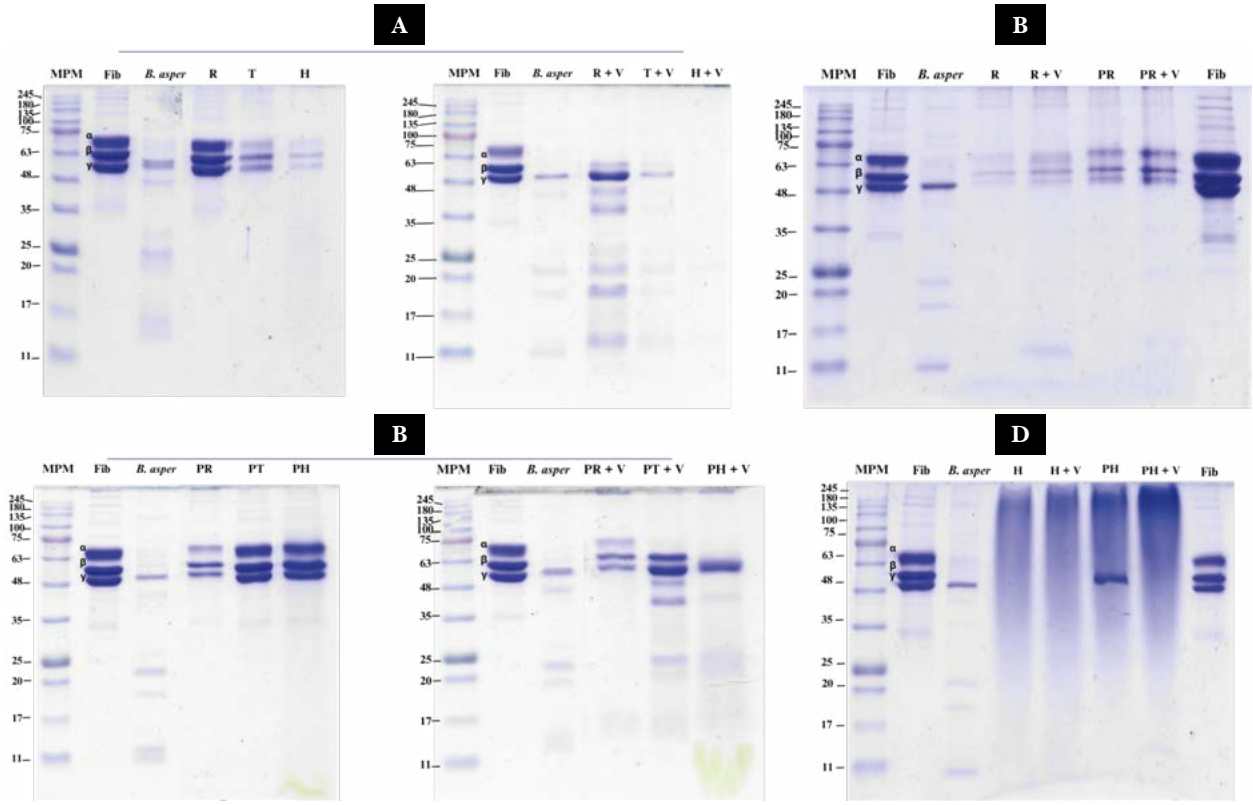

Figure 5. Inhibition of the fibrinogenolytic activity of *B. asper* venom with *Pentalinon* sp. extracts. A) ethanolic extracts, B) pool of extracts, C) root extracts, D) leaf extracts. MPM: molecular weight marker, Fib: fibrinogen as a negative control, *B. asper*: venom as a positive control, R: ethanolic root extract, T: ethanolic stem extract, H: ethanolic leaf extract, PR: pool of root extracts, PT: pool of stem extracts, PH: pool of leaf extracts.

chains, which the venom completely degrades, were faintly visible. With leaf extracts, the fibrinogen and venom bands were lost, showing runs along the gel. Based on these results, intermediate doses between those evaluated may show better inhibition. These results also reflect the different compositions of the extracts, which cause non-homogeneous inhibition. Roots have components that interact with venom enzymes, interfering with fibrinogenolytic activity. The search for inhibitors of venom serine proteinases is limited (Gutiérrez *et al.*, 2021). In future research it would be important to evaluate whether inhibition acts in the same way in coagulation assays, since the degradation of fibrinogen chains affects the transformation of fibrinogen to fibrin, which is the protein that forms the clot network (Park and Park, 2024).

This study represents an advance in the exploration of the antivenom properties of *Pentalinon* sp. Although the evaluated extracts did not inhibit the lethality of *Bothrops asper* venom, the presence of bioactive compounds, such as phenols, flavonoids, terpenoids, and saponins, was identified. These compounds interact *in vitro* with venom enzymes, achieving partial inhibition of proteolytic, phospholipase A_2, and fibrinogenolytic activities. Among the plant parts evaluated, the leaves stood out for exhibiting the greatest inhibitory capacity against proteolytic and phospholipase A_2 activities. Significant differences in phytochemical composition were detected between the ethanolic extracts and the extraction pools, which likely explains the variations in their neutralizing capacity. These results underscore the importance of conducting a comprehensive phytochemical analysis to identify the compounds responsible for these interactions and to further their structural and biological characterization.

It is important to emphasize that this study does not propose the use of *Pentalinon* sp. as a substitute for antivenoms, which remain the treatment of choice for snakebites. Instead, this work is positioned as a preliminary analysis aimed at identifying compounds that may complement current treatments, with the goal of addressing the limitations and challenges faced by patients affected by these envenomations. Although the extracts showed partial inhibition of certain enzymatic activities, this should not be interpreted as an indicator of in vivo efficacy. The results have demonstrated that these extracts do not neutralize the lethal activity of *Bothrops asper* venom. Furthermore, toxicity studies revealed that the pool of root extracts, administered at a dose of 20 mg, caused the death of mice, highlighting the need for more detailed toxicological analyses to evaluate their safety.

CONCLUSIONS

This study provides novel information on the potential interactions between extracts of *Pentalinon* sp. and *Bothrops asper* venom. To date, their efficacy and safety for use in treating snakebites have not been demonstrated. These findings open new lines of research, focused both on identifying specific compounds and on their preclinical evaluation, to determine their potential as complementary tools in the management of snake envenomation.

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Solid-state fermentation in cereal grains on chemical properties, gas and methane production *in vitro*

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ABSTRACT

Objective: to evaluate the effect of solid-state fermentation, *in vitro* with *Aspegillus oryzae*, on the chemical properties, gas production, and methane in maize, oats, barley, and sorghum grains.

Design/Methodology/Approach: maize and sorghum grains were fermented in solid for 5 days, and oats and barley for 7 days with *Aspergillus oryzae* and neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), flavonoids and *in vitro* production of gas at 3, 6, 9, 12, 24, 48, 72 and 96 h; and methane at 24 h.

Results: in general terms, solid-state fermentation (SSF) decreased neutral detergent fiber and increased flavonoid content in grains. Methane production in sorghum grains also decreased with five days of fermentation (5DF sorghum). Regarding gas production, SSF improved fermentation parameters by reducing lag time (A) and increasing gas production rate (k). There was, however, lower gas production due to partial consumption of soluble carbohydrates during solid-state fermentation.

Limitations/Implications of the study: results obtained in this study were *in vitro*, therefore, they are not yet applicable *in vivo*. They provided, however, a proper notion of compound degradation in the rumen, and on which of the fermented grains would help to reduce methane production.

Findings/Conclusions: solid-state fermentation improved grain structure, increased flavonoid content, and decreased methane production.

Keywords: cereal grains, solid-state fermentation, flavonoids, methane, *in vitro* determinations.

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INTRODUCTION

The issue of climate change has become very important, due to the devastating effects it has had on agriculture. Extreme variations in temperature have caused droughts, the spread of pests and diseases, changes in seasons and crop cycles, and low yields, among others (Cruz-González *et al.*, 2024). The lack of water and rain in some areas of Mexico

have caused the growth of cereal grains to be interrupted, which has affected the integral development of the grain.

In general, the cereal grain is made up of three parts: bran, endosperm and germ. The endosperm accounts for the majority of the cereal grain and its main component is starch. Grains whose endosperm is found with high degrees of hardness in their texture have lower rumen degradability (Ganesan & Rajauria, 2020). Cereals are a basic part of cattle feed, as they provide the most energy due to their carbohydrate content (Zhang *et al.*, 2022).

However, the lack of rainfall or water stress causes the development of β-type starch, which causes hardening in the endosperm and lower degradability (Yu *et al.*, 2016). For this reason, different techniques have been implemented that allow the components of the endosperm to be used and increase their degradability; grains with greater degradability have a higher nutritional value in animal feed (Ganesan & Rajauria, 2020).

Solid-state fermentation [SSF] is a biotechnological process in which microorganisms grow on solid substrates with low humidity levels, which allows for a degradation of certain compounds in the cell wall and a growth of single-cell protein from the microorganism (Liu *et al.*, 2022). During fermentation, microorganisms produce a variety of enzymes that degrade the cell wall and hydrolyze bonds between phenolic compounds and carbohydrates or proteins, increasing bio- accessibility and availability (Dulf *et al.*, 2016).

On the other hand, climate change has uncovered major risks associated with global warming caused by the accumulation of greenhouse gases (GHGs) in the atmosphere; GHGs are mainly composed of methane (CH_4), together with carbon dioxide (CO_2) and nitrous oxide (NO_2) (Ugbogu *et al.*, 2019). It is estimated that CH_4 methane contributes 14.5% of global anthropogenic emissions in the livestock sector (Molina-Benavides *et al.*, 2019). The emissions that ruminants produce are approximately 115 million tons of CH_4 per year (Cardoso-Gutiérrez *et al.*, 2021).

The methane gas CH_4 is produced as an effect of the degradation of carbohydrates in the rumen through the actions of the rumen microbiota and is associated with the loss of raw energy in the animal. However, it has been shown that through certain modifications in the diet and the presence of certain secondary metabolites in the feed, it is possible to reduce or affect rumen methanogenesis (Pámanes-Carrasco *et al.*, 2019; Romero *et al.*, 2023), which has motivated nutritionists and researchers around the world to find effective strategies to mitigate CH_4 emissions in the agricultural sector.

Thus, the objective of this study was to evaluate the effect of solid-state fermentation, *in vitro* with *Aspegillus oryzae*, on the chemical properties, gas production, and methane in maize, oats, barley, and sorghum grains.

MATERIALS AND METHODS

Cereal grains and fermentation microorganism

The grains of oat, barley and sorghum were purchased at a local market, while maize grains of the CAFIME variety were provided by Mexico's National Institute for Research in Forestry, Agriculture and Livestock - INIFAP in Durango. The fermentation fungus *Aspergillus oryzae*, from strain 2094 donated by Instituto Tecnologico de Durango, part of the Tecnologico Nacional de México, was kept on potato dextrose agar (PDA) at 30 °C.

Fermentation of grains and production of flours

Fermentation was done following the method of Bhanja Dey & Kuhad (2014). Cereal grains were cleaned, then weighed in lots per species (200 g of each) that were placed in glass jars. Afterwards, 200 mL of distilled water was added to each jar, then all jars were sterilized at 121 °C for 15 minutes. Inoculation was done with 20 mL of spore suspension (approximately 1×10^6 spores per mL). Then, jars were left to stand for several days of fermentation; five days for maize (5DF maize) and sorghum (5DF sorghum) grains, and seven days for oat (7DF oat) and barley (7DF barley) grains. All jars were incubated 30 °C. At the end of the days of fermentation, the grains were sterilized again; they were dried at 55 °C for 72 h, then ground to a particle size of 1 mm. These flours were stored in Ziploc™ bags at 5 °C until they were used.

Determination of neutral detergent fiber, acid detergent fiber and acid detergent lignin

The determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was performed in a fiber analyzer equipment, with the method described by the manufacturer (ANKOM Technology, 2020). Samples of 0.5 g of flour were stored into ANKOM F57 bags, sealed and deposited in the Fiber Analyzer 200 equipment (ANKOM Technology, USA). Then, sealed bags were washed with NDF solution (ANKOM Technology, USA) at 90 °C for 1 h. At the end of the procedure, bags were rinsed three times with hot distilled water for 5 minutes and then with acetone. The bags were then dried at room temperature and placed in an oven at 55 °C until they reached a constant weight. Upon completion of the NDF determination, the procedure for obtaining the ADF value was followed in accordance with the recommendations of the manufacturer (ANKOM Technology, 2020).

The determination of acid detergent lignin (ADL) was performed by the Van Soest *et al.* method. (1991), with those bags that came from the ADF analysis. For this ADL measurement, the bags were placed inside a beaker containing 72% H_2SO_4 for 3 h. At the end of the time, the bags were removed from the acid and rinsed three times with hot water and then with acetone, dried at room temperature and then taken to the oven at 55 °C until constant weight was obtained.

Determination of total flavonoids

The total flavonoid content was determined according to the method specified by Heimler *et al.* (2005). We added 75 μL of 5% sodium nitrite and 0.150 mL of 10% aluminum chloride solution (prepared on the day of analysis) and 0.5 mL of 1M sodium hydroxide to 0.25 mL of flour sample. The final volume was adjusted to 2.5 mL with deionized water. The mixture sample was left to stand for 5 minutes, and then its absorbance was measured at 510 nm against the control, which was prepared as the mixture, but without the flour sample. The mass of total flavonoids was expressed as μg EC g^{-1} (mg catechin equivalents per gram) of flour. The calibration curve was performed at 20-500 μg mL^{-1} ($R^2=0.9982$).

In vitro gas production

Samples of 0.5 g from each flour were weighed and deposited into calibrated 100 mL glass syringes (Fortuna®, Labortechnik, Germany), then incubated at 39 °C with a mixture of buffer solution and rumen fluid in a 2:1 ratio according to Theodorou *et al.* (1994). Gas production was recorded at times measured per hours; at the start (0), and after 3 hours, 6, 9, 12, 24, 48, 72 and 96 h. Gas production values were fitted to the Gompertz model (Murillo-Ortiz *et al.*, 2018):

$$GP = G\max * e^{-A*\exp^{(-k*t)}}$$

where, *GP*: gas production at time t (mL); *G*max: maximum gas production (mL g^{-1}); *k*: constant rate of gas production (mL h^{-1}); *A*: lag phase (h); *e*=Euler number or base of the natural logarithm (2.71828).

Methane production

Methane production was measured using glass modules (ANKOM Technology, USA) equipped with a wireless transducer to measure the pressure generated by the gas, according to the manufacturer specifications (ANKOM Technology, 2020). One gram of each flour was poured into glass flasks and incubated at 39 °C with buffer solution and rumen fluid in a 2:1 ratio, according to Theodorou *et al.* (1994) for 24 h. After that time, the valves were opened to release the pressure generated by the gas towards a portable analyzer (GEM^{TM}5000, LANDTEC, USA) to determine the proportion of methane produced, as specified by González-Arreola *et al.* (2019).

Statistical analysis

All determinations were made in triplicate and the data obtained were analyzed under a completely randomized design with the GLM procedure, as well as the estimation of gas production parameters. The comparison of means was performed with the Tukey's test, accepting less than 5% probability of error [$p \leq 0.05$] (SAS Institute Inc., 2009).

RESULTS AND DISCUSSION

Content of detergent fibers and acid detergent lignin

NDF neutral detergent fiber measures the main structural components of the plant (hemicellulose, cellulose, and lignin). NDF contents in raw and fermented grains showed significant variation ($p \leq 0.05$) (Table 1). In general, NDF content was higher in raw grains than in fermented grains, except raw maize grains which obtained the lowest value (30.49%). Raw sorghum grains obtained the highest value of NDF (89.97%). The high NDF values obtained in this study are due to incomplete starch degradation. This is because an enzymatic pretreatment was not applied, although the fermentation process did manage to reduce the amount of starch.

The increase in NDF in 5DF maize could be due to the formation of retrograded resistant starch during sterilization before fermentation. Retrograded resistant starch is a type of starch that, when gelatinized by cooking, reorganizes during cooling and forms

a dissolution-resistant crystal structure (Birt *et al.*, 2013). The ADF measures the less digestible components of the plant, such as cellulose and lignin. The results obtained with the SSF showed that the process increased ($p \leq 0.05$) the contents of neutral detergent fiber and acid detergent lignin (Table 1).

The lowest value of ADF was shown by raw barley grains (4.18%), while 5DF sorghum grains obtained the highest value (16.60%). Similarly, ADL values were lower ($p \leq 0.05$) in raw grains compared to fermented grains. The lowest percentage of ADL was presented by raw maize grains (0.71%), while the highest value was obtained by 7DF oat grains (4.20%). Increases in ADF and ADL in fermented grains can be explained as a reduction in cell content by the fungus during fermentation, with the consequent increase in the concentration of cell wall components (Jiménez-Alfaro *et al.*, 2020).

Kowieska *et al.* (2011) evaluated varieties of barley grains and reported lower NDF values (26.9 and 25.3%) than those in this study in raw barley grains; but similar ADF values (10.7 and 10.4%) in barley grains with seven days of fermentation. On the other hand, Yasar & Tosun (2018) observed that, when solid-state fermenting barley grains with whey for 48 h, NDF content decreased from 27.5% to 22.7% and ADF content from 7.81% to 3.90%. However, such effect was not found in ADF content in this study.

Total Flavonoid Content

Flavonoids are an important class of phenolic compounds in cereals, due to their antioxidant properties (Saharan *et al.*, 2017). Regarding flavonoid content, fermented grains showed higher values ($p \leq 0.05$) than raw grains (Table 1). The highest value ($p \leq 0.05$) was observed in 5DF sorghum grains (854.03 μg EC g^{-1} flour), unlike maize grains and barley grains that obtained the lowest values (169.76 and 184.97 μg EC g^{-1} flour).

The results shown here are similar to those reported by Cai *et al.* (2012), who increased the flavonoid content in oat subfractions, by fermenting them with different filamentous fungi. On the other hand, Sandhu *et al.* (2016) also observed the increase of flavonoids

Table 1. Chemical composition in raw grains and in solid-state fermented grains with *Aspergillus oryzae*.

Sample	NDF (%)	ADF (%)	Lignin (%)	Flavonoids (μg EC g^{-1})
Maize	30.49 ± 0.81^{f}	4.41 ± 0.13^{e}	0.71 ± 0.24^{e}	169.76 ± 9.73^{e}
5DF Maize	5.64 ± 1.18^{b}	10.77 ± 0.17^{b}	2.38 ± 0.07^{c}	227.36 ± 18.03^{de}
Oat	52.11 ± 3.07^{d}	4.53 ± 0.26^{e}	2.00 ± 0.23^{cd}	225.76 ± 20.05^{de}
7DF Oat	41.23 ± 2.18^{e}	9.30 ± 0.65^{cd}	4.20 ± 0.24^{a}	305.77 ± 16.00^{c}
Barley	78.27 ± 1.99^{b}	4.18 ± 0.40^{e}	1.41 ± 0.23^{de}	184.97 ± 8.80^{e}
7DF Barley	68.19 ± 2.27^{c}	10.12 ± 0.48^{bc}	3.42 ± 0.11^{b}	246.57 ± 11.20^{d}
Sorghum	89.97 ± 0.70^{a}	8.38 ± 0.18^{d}	3.89 ± 0.47^{ab}	743.09 ± 33.57^{b}
5DF Sorghum	66.96 ± 2.41^{c}	16.60 ± 0.84^{a}	3.23 ± 0.26^{b}	854.03 ± 33.04^{a}

NDF: neutral detergent fibre; ADF: acid detergent fiber; μg EC g^{-1} milligrams of catechin equivalent per gram of flour; Values expressed as means±standard deviation. Different letters in the same column indicate statistical differences ($p \leq 0.05$).

in six wheat varieties fermented with *Aspergillus awamori* Nakazawa for six days; those authors obtained the maximum values of flavonoids content at five days of fermentation, 324 to 426 μg EC g^{-1} of flour.

Similarly, Saharan *et al.* (2017) fermented rice, oats, maize, wheat and sorghum with *Aspergillus oryzae* for six days at 30 °C; those authors observed that all cereal grains showed an increase in flavonoids after fermentation; they recorded the highest values in wheat and rice (25.29 and 22.66 μg EC g−1). Likewise, Sandhu & Punia (2017) also reported the increase of flavonoids in six barley varieties fermented with *Aspergillus awamori* Nakazawa for six days at 30 °C. The highest values (3059 to 3686 μg EC g^{-1}) were recorded at five days of fermentation.

In vitro production of gas and methane

All *in vitro* gas production parameters showed significant differences ($p \leq 0.05$) (Table 2). The maximum volume of gas production (Gmax) was higher in raw grains than in fermented grains; Within raw grains, maize presented the highest value (181.67 mL g^{-1}). Grains of 7DF oat showed the lowest Gmax value (154.87 mL g^{-1}). In fact, these values are consistent with an increase in structural carbohydrates, such as lignin.

The increase in lignocellulolytic compounds also affects the process of degradability of food by the rumen microbiota. In this way, the process of gas generation by rumen fermentation tends to decrease (Murillo-Ortiz *et al.*, 2018). During gas production, microorganisms ferment soluble and complex carbohydrates, and within these, starch produces the largest volume of gas (González-García *et al.*, 2017). Regarding the A-parameter (lag phase), fermented grains had a shorter delay time in gas production than raw grains. The lowest value was presented by 7DF oat grains, 2.32 h, while the highest value was shown by raw barley grains (4.32 h). These results suggest a greater ability of rumen microorganisms to adapt to the substrate, which allows gas generation to start earlier. Conversely, the rate of gas production (k) increased in fermented grains compared to raw grains.

Table 2. *In vitro* production parameters of gas and methane from raw and solid-state fermented grains with *Aspergillus oryzae*.

Sample	*G*max	*A*	*K*	$CH_{4\ (24h)}$
Maize	181.67±1.75^{a}	3.78±0.12^{b}	0.15 ±0. 00^{e}	18.04±1.27^{a}
5DF Maize	157.53±1.21^{b}	3.28±0.25^{c}	0.17±0.00^{d}	16.09±1.19^{a}
Oat	178.23±0.75^{a}	3.98±0.07^{b}	0.21±0.01^{b}	20.07±0.33^{a}
7DF Oat	154.87±3.16^{b}	2.32±0.10^{d}	0.27±0.01^{a}	17.54±2.24^{a}
Barley	179.00±2.33^{a}	4.32±0.05^{a}	0.19±0.01^{c}	20.58±3.39^{a}
7DF Barley	158.20±8.96^{b}	2.36±0.08^{d}	0.23±0.00^{b}	18.20±0.44^{a}
Sorghum	173.50±0.89^{a}	3.92±0.09^{b}	0.12±0.01^{f}	16.20±1.53^{a}
5DF Sorghum	161.60±2.19^{b}	2.61±0.08^{d}	0.15±0.00^{e}	10.02±0.65^{b}

*G*max: maximum gas production volume at 96 h (mL g^{-1} sample), A: lag phase (h), k: gas production rate (mL h^{-1}). $CH_{4\ (24h)}$: methane production in 24 hours. Values expressed as means ± standard deviation. Different letters in the same column indicate statistical differences ($p \leq 0.05$).

This coincides with the results obtained in the maximum gas production (Gmax) in fermented grains, because there is less substrate available. That is, due to carbohydrates that were previously consumed in fungal fermentation, thus decreases the maximum gas production. Therefore, it took less time for microorganisms to produce gas. This means that, in a gas production kinetic curve, the exponential gas production phase would need less time to reach the asymptote.

No reports were found on the production of gas or methane in vitro from solid-state fermented grains, but there were reports from raw grains. For example, Cabral-Filho *et al.* (2005) measured the gas production *in vitro* at 96 h of eight varieties of sorghum grains, the values reported were higher (322 to 430 mL g^{-1}) than those of our study; also the lag phase varied from 3 to 5 h. On the other hand, González-García *et al.* (2017) reported lower Gmax values at 24 h, in maize and sorghum grains (89.02 and 94.94 mL g^{-1}), than those found in our study (158.49 and 139.18 mL g^{-1} unpublished data).

Methane production showed differences ($p \leq 0.05$) among grains (Table 2). All grains obtained similar methane values ($p > 0.05$), except 5DF sorghum, which obtained the lowest value ($p \leq 0.05$) in methane production (10.02 mL g^{-1}). In fact, sorghum is the fermented grain with the highest concentration of flavonoids per gram of flour (854.03 μg EC g^{-1}). It should be noted that Romero *et al.* (2023) published in a study that phenolic compounds, including flavonoids, are excellent hydrogen or free radical trappers. In this way, in a rumen fermentation process, where methane synthesis depends on the hydrogens released, it is reduced by competing against an efficient antioxidant.

However, this depends on the dose or concentration of flavonoids (Lasinskas *et al.*, 2023). On the other hand, Kim *et al.* (2013) determined methane production *in vitro* by different food ingredients, such as bran, vegetable proteins, and cereals. They observed that cereals (maize, barley and wheat) presented the highest methane production at 24 h, this due to their higher content of sugars, starches and hemicellulose. Their results for maize were slightly higher (21.33 mL g^{-1}) than those reported here, but for barley (8.17 mL g^{-1}) results were smaller (Table 2).

CONCLUSIONS

The results of the study showed that solid-state fermentation modified the structure of the maize kernel and thus facilitated microbial access and initial digestibility. It also increased the flavonoids content and decreased methane production. However, it also reduced the content of the total substrate.

The increase in flavonoids suggests that solid-state fermentation could be used as a technique to produce functional foods with potential to improve animal health, and reduce environmental impact by decreasing enteric fermentation.

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Colegio de Postgraduados

Changes in grassland structure in Sierra de Organos National Park associated with a 14-year grazing exclusion

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ABSTRACT

Objective. To evaluate the effect of 14-year grazing exclusion on land cover and on the dynamics of plant functional groups in the grassland of the Sierra de Organos National Park.

Design/Methodology/Approach. In 2008, at the beginning of the grazing exclusion, four transects were established to measure land cover and plant functional groups. The measurements were repeated in 2010, 2012, 2014, 2018 and 2022.

Results. During the first ten years of grazing exclusion, basal cover increased by 201%, as well as by 228.7% in litter accumulation and 219% in soil cover. In other words, bare soil was significantly reduced. However, in the following period of the evaluation, basal cover and soil cover decreased, which meant an increase in bare soil. In addition, during the first decade of grazing exclusion, grasses maintained a clear dominance in the plant community. However, since 2018 those decreased, which coincided with an increase in herbaceous plants and shrubs.

Limitations/Implications of the study. The study generated practical information applicable both in the study area and in livestock ranches in the Central-North Region of Mexico.

Findings/Conclusions. Grazing exclusion should be limited to a period of less than ten years, since during that time the vegetation cover increases and bare soil is reduced; however, as it continued, a degradation of the pasture and the substitution of grasses for other herbaceous and shrubs were observed.

Keywords: leaf litter, basal cover, plant functional groups.

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INTRODUCTION

Mexico's National Park "Sierra de Organos" (SONP) is an outstanding national ecotourism destination and an area of scientific interest due to its unique landscape and great biodiversity, which includes endemic and threatened species. However, the inadequate management of extensive livestock farming for decades led to overgrazing, which caused the deterioration of vegetation and soil degradation, affecting landscapes in the Park. To conserve its natural resources and encourage ecotourism, the SONP was declared a Protected Natural Area (ANP, in Mexico) in 2000. As part of management practices, during the second half of 2008 cattle grazing was excluded, with the aim of restoring vegetation, improving the soil and ensuring the ecological and tourist sustainability of the park in the long term.

Grazing exclusion (GE) has been promoted worldwide as an effective strategy for the restoration of grasslands degraded by overgrazing (Shu *et al.*, 2024). However, several studies have shown that its effects can vary significantly depending on the length of the exclusion period (Lian *et al.*, 2024). On the one hand, grazing exclusion in the short term (three to five years) tends to favour an increase in biomass and plant diversity (Zhan *et al.*, 2022; Yang *et al.*, 2023). On the other hand, prolonged periods of exclusion (eight years or more) can decrease vegetation cover and species richness, thereby reducing the expected benefits of restoration (Liu *et al.*, 2020; Zhan *et al.*, 2022; Yang *et al.*, 2023).

Prolonged grazing exclusion in grasslands favors the excessive accumulation of leaf litter on the soil surface. Studies have shown that this increase in organic material hinders the ecological restoration of degraded or poorly managed grasslands (Song *et al.*, 2025). One of the main negative effects of abundant leaf litter is that it forms a layer on the ground that acts as a physical barrier and reduces the amount of light available, which hinders the recruitment of plants from seed and limits the emergence and development of seedlings and shoots of perennial species (Facelli & Pickett, 1991; Jessen *et al.*, 2023). Another relevant impact is the increase in the number of native shrubs, which alters the original structure and composition of the plant community (Zhang *et al.*, 2024).

On the other hand, excessive litter accumulation increases the risk of fires, posing an additional threat to ecosystem stability and resilience (Vermeire *et al.*, 2018). Although leaf litter in moderate amounts benefits the ecosystem by attenuating the thermal oscillation of the soil, improving infiltration, decreasing evapotranspiration, reducing erosion and contributing organic matter to the in situ nutrient cycle (Pellant *et al.*, 2020).

During the first six years of grazing exclusion in the SONP (2008-2014), leaf litter cover increased from 17.8% to 39.9% (Valdez-Cepeda *et al.*, 2021), evidencing a considerable accumulation of surface organic material due to the absence of grazing. As a result, the authors recommended reviewing and adjusting the length of exclusion periods to avoid negative impacts associated with excess of litter and to achieve sustainable grassland management. However, as this strategy has not been modified to date, it is considered essential to re-evaluate grazing exclusion effectiveness to ensure the conservation and functionality of the ecosystem.

In order to address this problem and with the consideration of the environmental, ecotourism and socioeconomic importance of the SONP, this research was proposed to generate practical information that guides decision-making towards the sustainable management of this Mexico's natural protected area; and to provide elements with value for the conservation of grasslands in the regional livestock sector. Therefore, the objective was to evaluate the effect of 14-year grazing exclusion on soil cover and on the dynamics of plant functional groups in the grassland of the Sierra de Organos National Park.

MATERIALS AND METHODS

Study Area

The study was implemented in the Sierra de Organos National Park —SONP, part of Mexico's national system of protected natural areas (known as ANP), which is

located in the northwest of the municipality of Sombrerete, Zacatecas, Mexico; with extreme coordinates 23° 46' 54.31" N and 103° 46' 37" W, 23° 48' 06.39" N and 103° 49' 08.66" W, 23° 46' 07.71" N and 103° 47' 01.26" W and 23° 48' 28.80" N and 103° 48' 57.93" W (SEMARNAT, 2013). The National Park covers an area of 1124.65 hectares under Ejido's property. The climate is temperate sub-humid [C(w_0) (w) a (e)], with rainfall in summer; the annual averages of precipitation and temperature are 592.3 mm and 14.9 °C (INIFAP, 2025). The landscape is composite of a low mountain range of volcanic rock with escarpments, ridges and small valleys with flat-undulating topography and gentle slopes, where the altitude varies between 2120 and 2560 m (SEMARNAT, 2013). The vegetation present includes pine forest, oak forest, natural grassland, scrubland, riparian vegetation, as well as aquatic and subaquatic communities (SEMARNAT, 2013).

Rainfall

In the interannual variability of precipitation, from 2008 to 2022, four stages were identified in the area of influence of the SONP. A drought season (2008-2012) with values below the average (592.3 mm), with an extreme drought in 2011 (250 mm, 57.8% lower than the general average for the period). A sustained wet season (2013-2018) followed, with rainfall above the general average for the period and a maximum of 961 mm (in 2015, 62.2% higher than the general average for the period). Then, a brief return to dry conditions (2019-2020) and finally, a moderate wet season (2021-2022), the latter with records above the general average for the period (Figura 1). This information with annual records allowed us to explore the existence, or not, of associations between rainfall and the grassland variables analyzed in this study.

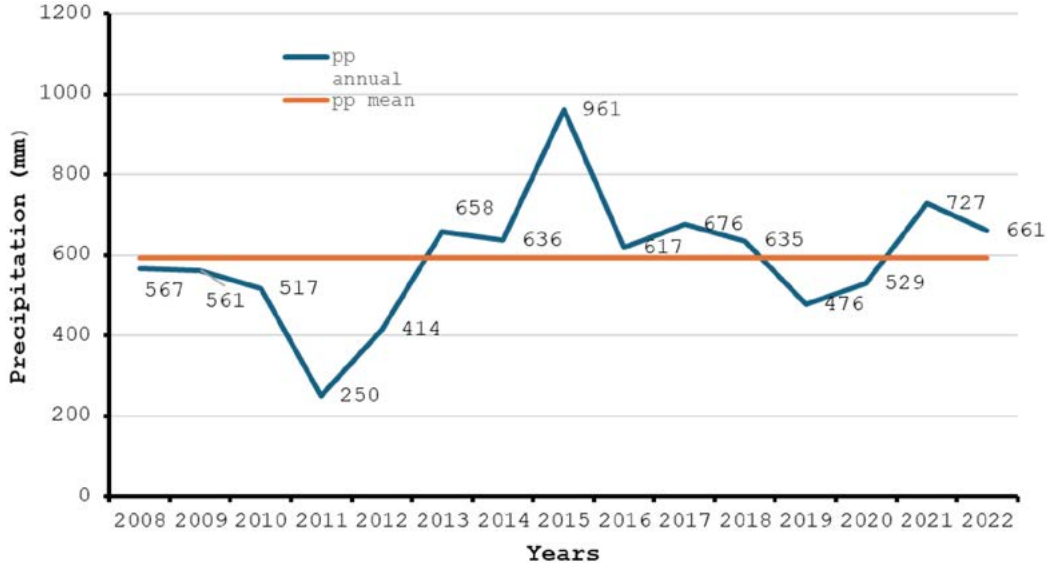

Figure 1. Annual rainfall (mm) recorded at the "Providencia" Meteorological Station during the period 2008-2022 (INIFAP, 2025). The orange horizontal line represents the general average for the period (592.3 mm), which represents the 23-year normalized climate value for rainfall in that Meteorological station (2000-2022).

Sampling procedure

This study was developed exclusively in the SONP grassland, which covers an area of 135 hectares (SEMARNAT, 2013). In this plant community, four transects were established for monitoring of variables, strategically distributed and identified as I, II, III and IV. In transects I, II and IV, 100 random measurement points were selected, while in transect III, due to physiographic conditions, 66 random points were established. The data collected at the measurement points allowed quantifying four variables of grassland soil cover, including the percentage of basal cover, leaf litter, soil cover and bare soil.

In addition, at each measurement point, the perennial plant species present were identified, which were grouped into four functional groups: trees, shrubs, grasses, and grasses. The first evaluation was done in 2008, to coincide with the beginning of the grazing exclusion. Subsequent evaluations were recorded in 2010, 2012, 2014, 2018 and 2022. Data were collected in the early fall of each of those years; the method 'Early Warning Biological Monitoring-Rangelands and Grasslands' of the Allan Savory Holistic Management Center was used (ASCHM, 1999).

Statistical analysis

The response variables used to evaluate the effect of grazing exclusion (GE), applied over 14 years in the grassland, included percentages of basal cover, leaf litter, soil cover, and bare soil, as well as the relative proportion of grasses, herbaceous plants, shrubs, and trees. To analyze these variables, an analysis of variance was performed for each; the number of years without grazing was considered as the source of variation. The statistical analysis, a one-way analysis of variance, was performed in Minitab® 2016 version. When significant differences were detected in any variable, a Tukey multiple mean comparison test was applied ($p \leq 0.05$).

RESULTS AND DISCUSSION

The length of the grazing exclusion (GE) period generated significant differences ($p \leq 0.001$) in the percentages of basal cover, leaf litter, soil cover and bare soil. As well as in the relative proportion of grasses, herbaceous plants and shrubs in the SONP grassland (Figure 2 and Figure 3); in contrast, the proportion of trees showed non-significant changes (Figure 3).

Basal cover

In the temporal dynamics of the percentage of basal cover of the grassland, three significant ($p \leq 0.001$) stages were distinguished (Figure 2). The first phase, called initial recovery (2008-2012), was associated with an 82.2% increase in the first two years, as basal cover rose from 9.0% to 16.4%; in 2012, value remained practically unchanged, which means that the GE favored the growth in diameter of the grass tillers during the first two years. The total length of this phase coincided with the dry period in the study area (reference in Figure 1).

The annual rainfall remained lower than the general average for the period, which generated a water deficit that reduced plant growth and prevented the grazing exclusion

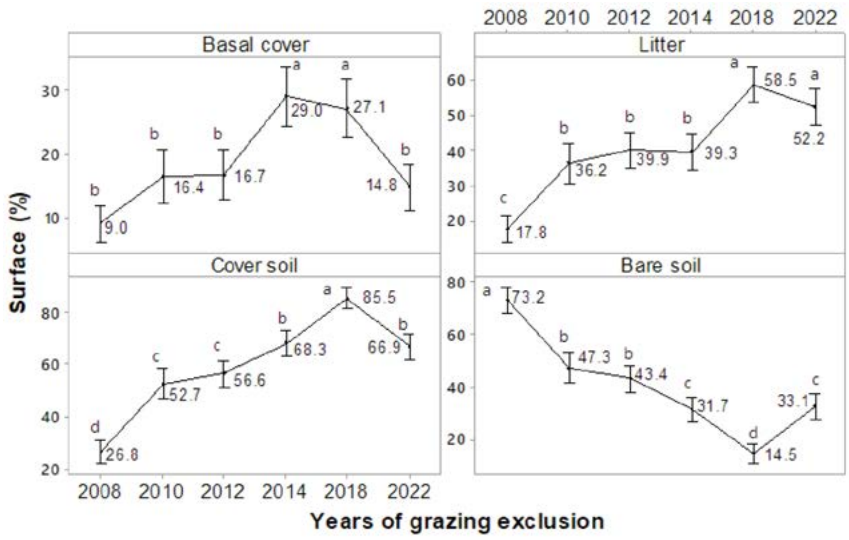

Figure 2. Percentages of area with basal cover, with leaf litter, with covered soil and in bare soil in the grassland of the Sierra de Organos National Park during 14-year grazing exclusion (2008-2022). Means with different letters indicate statistical difference (Tukey; $p \leq 0.05$). Lines represent the mean ± SE (1 unit of standard error).

to be reflected in only positive significant changes. During the second phase, we called peak and consolidation of basal cover (2013-2018), this variable reached its absolute maximum in 2014 (29%). This is more than triple its initial value, and decreased only to 27% in 2018, as an indication of a stabilization. This observed pattern indicates that, with six years of GE, the plant community reached its highest basal density and remained unchanged during the following four years. This stage coincided with a period of abundant annual rainfall, which favored both the recruitment of young plants and a greater development of grass tillers. As a result of this vegetative growth, a subsequent accumulation of leaf litter occurred.

Finally, during phase three, we defined as recent decline (2019-2022), basal cover showed a marked decrease up to 14.8%, this is a 45.4% decrease compared to that value in 2018. This decline is evidence that grazing exclusion alone does not ensure sustained increases for basal cover in the long-term. A moisture deficit was recorded during 2019-2020, while in 2021 and 2022, annual rainfall again exceeded the average. Despite the rebound in precipitation, basal cover did not recover; on the contrary, it decreased. This behaviour can be attributed, in part, to the lagging effects of the previous drought and, to a greater extent, to the grazing exclusion, since the prolonged absence of grazing limits grass regrowth and the removal of senescent biomass, which favors an excessive accumulation of leaf litter. This accumulation hinders both the development of plants already established, and the germination of seeds with establishment of new plants (Facelli & Pickett, 1991). According to our results, an optimal period of grazing exclusion is comprised between six and 10 years. After that time, the benefits began to reverse, as basal cover declined.

Overall, the results indicated that grazing exclusion acted as a trigger for the increase in basal cover, which peaked in 2014. However, the magnitude and duration of this response clearly depended on rainfall and litter accumulation. The sustained water volume between

2013 and 2018 boosted basal cover, while the increase in leaf litter reversed the growing trend. These facts show that the recovery of the grass stratum results from the interaction between the absence of grazing and the interannual availability of water. It is inferred that, after six years of grazing exclusion, it is advisable to suspend GE and to incorporate instead an adaptive pasture management system that integrates flexible grazing adjusted with rainfall variability. This means increasing grazing intensity in humid years and reducing animal load in dry seasons, in order to preserve both, productivity and vegetation cover (Derner *et al.*, 2022).

Leaf litter cover

The percentage of soil surface covered by leaf litter exhibited a pattern of four statistically different stages throughout the 14-year grazing exclusion ($p \leq 0.001$; Figure 2). During the first stage, we called initial accumulation (2008-2010), leaf litter cover was characterized by an increase from 17.8% to 36.2%, attributable to the absence of livestock grazing and trampling. In the second season, we identified as a transitional plateau (2011-2014), leaf litter cover showed almost constant values (39.9% in 2012 and 39.3% in 2014), suggesting a temporal balance between the fallen of leaf debris and their decomposition.

During the third stage, we defined as the maximum peak of accumulation (2015-2018), leaf litter cover reached its highest level in 2018, 58.5% which was three times the baseline. This value reflects the large basal cover of the period and the minimal biomass removal associated with the absence of grazing. Finally, during the fourth phase, we designated as the recent adjustment (2019-2022), leaf litter cover showed a slight decrease, up to 52.2% in 2022. The decrease in leaf litter observed in recent years is related to the parallel reduction of basal cover. Because less plant cover reduces leaf production and, consequently, the contribution of the material that compose the layer of leaf litter.

The results of our study are consistent with those of Song *et al.* (2025), who observed that, after four years of grazing exclusion, grasslands presented a notable accumulation of leaf litter. Similarly, Zhang *et al.* (2024) concluded that this accumulation in excluded grasslands weakened the biotic resistance of grasses, while favoring shrub growth and recruitment.

In general, the exclusion from grazing induced a sustained accumulation of leaf litter during the first decade, which was followed by a relative decrease during the following four years. In the initial stage, this increase implied a contribution of organic matter and a better retention of moisture in the soil (Pellant *et al.*, 2020). However, the accumulation of leaf litter also increased the fuel load and generated a physical barrier on the ground, especially when it reached its maximum value in 2018. That timing coincided with the onset of a marked decrease in basal cover.

This leaf litter barrier could negatively affect the regeneration processes and the dynamics of the vegetation in the grassland. According to Facelli & Pickett (1991), the reduction in light caused by leaf litter makes it difficult for seedlings to establish, and may even hinder the establishment of species with larger seeds. Chen *et al.* (2018) also observed that grass-leaves litter cover made it difficult to establish seedlings of invasive and native species coexisting in California grasslands. In addition, the layer of leaf litter represents a

physical obstacle for seeds, seedlings and shoots, as it can delay or prevent their arrival into the soil, also inhibiting their emergence and development.

Soil cover

The percentage of area with soil cover presented a four-phase course during the 14-year grazing exclusion ($p \leq 0.001$; Figure 2). In the first phase that we called initial accumulation (2008-2010), soil cover doubled from 26.8% to 52.7%, thus showing the early response of herbaceous vegetation and the rapid accumulation of leaf litter once GE began. During the second phase, we called the moderate-growth plateau (2011-2014), soil cover was characterized by a more attenuated increase (52.7% to 68.3%) indicating a phase of dynamic balance between the increase in basal cover and the processes of accumulation and decomposition of leaf litter.

In the third phase, we described as the maximum peak (2015-2018), soil cover reached its highest value (85.5%), more than three times the initial level. This maximum coincided with the highest proportion of surface covered with leaf litter, and with the second highest basal cover value. The physical protection of the soil was favored, which tipically reduces vulnerability to erosion and improves hydrological function (Pellant *et al.*, 2020). During the fourth phase, we called the recent adjustment (2019-2022), soil cover showed a small decrease, a value of 66.9% soil cover was recorded. Despite this reduction, this value remained higher than that one measured at the start of GE.

In general terms, grazing exclusion promoted a sustained increase in soil cover during the first decade, but showed a slight decrease in the last four years. Our results corroborate the findings of Song *et al.* (2020), who demonstrated that prolonged exclusion from grazing induces a statistically significant reduction in total coverage. Based on our results, it is recommended that grazing exclusion does not exceed 10 years; while the following step is the reintroduction of livestock through adaptive management strategies. This practice would make it possible to conserve the soil cover achieved, and to ensure the functionality and sustainability of the grassland in the long term.

Bare soil

During the 14 years excluded from grazing, the percentage of land area with bare soil showed an inverse trend to those observed in basal cover, leaf litter and covered soil. The percentage of surface area with bare soil decreased as those variables increased, then it increased as those decreased. This behavior was divided into four phases ($p \leq 0.001$; Figure 2). During the first one that we called the initial decline (2008-2010), bare soil was reduced from 73.2% to 47.4%, which means a decrease of 25.8 percentage points after the immediate elimination of grazing and cattle trampling.

In the second phase, we called gradual decline (2011-2014), the portion of bare soil continued to decrease to 31.7%, due to the expansion of basal cover and leaf litter cover. At the third phase, called absolute minimum (2015-2018), bare soil reached the lowest value (14.5%) in the evaluation; that is, a reduction of 80.2% compared to the initial value. This minimum coincided with the second highest value of vegetation cover and with the most extensive leaf litter cover, which decreased soil exposure. In the fourth

phase, called partial rebound (2019-2022), the area with bare soil showed an increase to 33.1%. That is, it remained at less than half of the baseline, but indicated the partial loss of the protection achieved. This uptick was related to the simultaneous decrease in basal cover and leaf litter cover.

When considering the entire period, grazing exclusion substantially reduced the area of bare soil during the first decade; however, in the following four years this trend was reversed and the exposed soil increased again. This represents an aggravating factor, since a higher proportion of bare soil increases the vulnerability of the grassland to erosion, both by water runoff and by wind (Pellant *et al.*, 2020).

Temporal dynamics of plant functional groups during the 14-year grazing exclusion

The temporal dynamics of the plant functional groups in the SONP grassland during the period excluded from grazing (2008-2022) was expressed as the relative frequency (%) of grasses, herbaceous plants, shrubs, and trees within the plant community (Figure 3).

Grasses, which constituted the dominant group at the beginning of GE, retained a proportion of more than 92% in the plant community during the first ten years. This confirms historical dominance as a stratum of grassland perennial plants. However, after 2018, the proportion of grasses decreased significantly, lowering to 83.1% in 2022, which coincided with a simultaneous increase in herbaceous plants and shrubs. This pattern could be due to the low incorporation of new plants and the progressive mortality of grass tillers. The latter is mainly induced by the natural aging of plants and the accumulation of leaf litter caused by prolonged grazing exclusion.

Herbaceous plants showed fluctuating dynamics in the period, with intermittent increases and decreases that reached their maximum value in 2022 (9.0%), with a

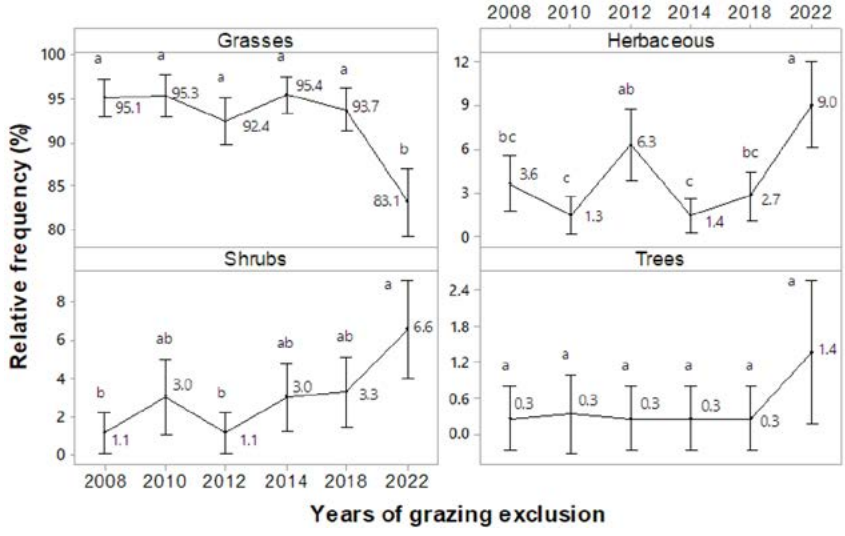

Figure 3. Dynamics of the relative frequency (%) of plant functional groups (grasses, herbaceous, shrubs, and trees) in the grassland vegetation of the Sierra de Órganos National Park during a 14-year grazing exclusion (2008-2022). Means with different letters indicate statistical difference (Tukey; $p \leq 0.01$). Lines represent means ± SE (1 unit of standard error).

statistically significant increase compared to most previous years. This trend indicates that these species have managed to establish themselves successfully, probably favored by prolonged grazing exclusion, and the consequent accumulation of leaf litter (Figure 3). Our results are consistent with those of Song *et al.* (2020), who observed that grazing exclusion in different periods favored the increase in the relative cover of herbaceous plants.

Shrubs showed a pattern of gradual increase, going from a relative frequency of 1.1% in 2008 to 6.6% in 2022. This statistically significant ($p \leq 0.01$) increase reflects a progressive colonization of the grassland by low-growing woody species, facilitated by the lack of grazing. Our results are consistent with those reported by Zhang *et al.* (2024), who also observed an increase in shrub abundance during a livestock exclusion period.

Similarly, Shi *et al.* (2023) documented this increase and noted that it is linked to both grazing exclusion and the competitive advantage that shrubs develop through interspecific interactions during that period. Those authors reported that the increase in shrubs would significantly decrease the quality of grasslands and suggested that livestock exclusion strategies should be implemented with caution, and with careful consideration of their possible long-term effects on the structure and functionality of ecosystems.

Therefore, one recommendation is to return grazing to the SONP through the adaptive grazing modality (Derner et al., 2022). Trees maintained a low and stable proportion (0.3%) during the first decade, but showed a significant increase in 2022 (1.4%). This increase may indicate the beginning of a transition to vegetation of greater height and structural complexity, which could be consolidated in later stages if grazing exclusion continues.

In general terms, the persistent dominance of grasses during the first ten years of GE confirms the initial success of this practice in conserving the grass layer. However, the significant changes observed in 2022 in the structure of plant functional groups, particularly the decline of grasses and the simultaneous increase of herbaceous plants and shrubs, suggest a turning point in the successional process. This is a shift towards more diverse plant communities, although potentially less productive from a grazing point of view.

The increase in shrubs and the expansion of herbaceous plants in the grassland highlighted the importance of reintroducing controlled disturbances, such as livestock grazing, through rational or adaptive systems that allow maintaining the dominance of grasses and counteracting the proliferation of other species. Porensky *et al.* (2020) supported this strategy, suggesting that maintaining light to moderate levels of grazing by large herbivores is key to preventing the invasion of undesirable species and preserving the ecological integrity of rangelands. In addition, the ecological feedback mechanisms generated by herbivores favor plant growth and regulate systemic ecological processes in grasslands, confirming the fundamental role of grazing in grassland dynamics and resilience (Frank *et al.*, 1998).

CONCLUSIONS

Grazing exclusion increased basal cover, soil cover, and leaf litter accumulation during the first decade, and reduced the proportion of bare soil. After that period, basal cover, leaf litter cover, and soil cover decreased, while bare soil increased. This is evidence that the

initial benefits of grazing exclusion were reversed. In the first ten years, grasses retained their dominance, but afterwards, herbaceous plants and shrubs increased towards a significant change in the structure and composition of the grassland.

Therefore, it is not recommended that grazing exclusion extends beyond six to ten years. But to reintroduce livestock through adaptive management schemes that adjust the animal load according to climate variability. This strategy helps to maintain basal cover, avoiding excessive accumulation of leaf litter and preventing the increase of bare soil. Also, it allows to conserve the levels of soil cover achieved during the first decade, maintaining the dominance of grasses, and limiting the expansion of herbaceous plants and shrubs. In the long term, it would be expected to favor both forage productivity and the ecological integrity of the SONP grassland.

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Characterization of the Production and Commercialization Process of Tilapia Aquaculturists from Laguna de Tres Palos, Acapulco, Guerrero

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ABSTRACT

Objective: To characterize the production process and commercialization channels of tilapia (*Oreochromis niloticus*) farmed in floating cages by producers from the Laguna de Tres Palos, Acapulco, Guerrero and to propose a marketing strategy.

Design/Methodology/Approach: The design consisted of a quantitative and qualitative approach and included a descriptive and cross-sectional scope. In addition, a non-probability convenience sampling and a semi-structured questionnaire with closed and open-ended questions were used. The questionnaire included overall producer and commercialization data. The SPSS software and the coding method were used to analyze the information.

Results: Producers have a low organization level which, consequently, favors buyers in the tilapia sale negotiation. Likewise, tilapia aquaculturists use a traditional commercialization approach, which the producers believe to be an acceptable income.

Study Limitations/Implications: The non-probabilistic convenience sampling design used means that the results of this study are exclusive to the analyzed sample. Consequently, the results of this study cannot be applied to all aquaculture producers in Laguna de Tres Palos. Probabilistic sampling should be used in future research to confirm these results at a population level.

Findings/Conclusions: A better integration of the different stages of the process could improve tilapia production and commercialization. In addition, including advertising and awareness-raising campaigns about tilapia consumption is recommended.

Keywords: Aquaculture, cages, cultivation, sustainable fishing.

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INTRODUCTION

The consumption of fish and aquaculture products provides essential proteins and nutrients to the most unprotected population. In 2023, the Mexican fish production

reached 1,947,601 t of landed catch. Out of total domestic production, 1,720,599 t (88.3%) came from the Pacific Ocean coastline, 217,090 t from the Gulf and Caribbean areas, and 9,912 t from areas without coastline (CONAPESCA, 2023). Meanwhile, the total production of tilapia (*Oreochromis* spp.) in Mexico recorded 75,573 t of landed catch —3.9% of the domestic fish production. Guerrero accounted for 1.9% (1,454 t) of the domestic tilapia production (CONAPESCA 2023). The contribution of aquaculture accounts for 78% (60,377 t out of a total of 77,687 t of live weight) of the domestic tilapia production. In this regard, tilapia aquaculture in Guerrero produces 95.5% (1,205 t) of live fish weight (CONAPESCA, 2023). Consequently, tilapia aquaculture is fundamental for fish production in Guerrero. In addition, the production of tilapia in floating cages ranks first in Guerrero, followed by catfish production. Fish production is carried out in La Villita dam, between Guerrero and Michoacán; however, this activity also takes place in Laguna de Tres Palos (SADREG, 2018). Consequently, tilapia is farmed more than other fresh water fish species in Guerrero. This state has 334 fattening tilapia, trout, catfish, and white shrimp farms, with different technification degrees and development of their production processes, in floating cages and concrete and mud ponds (Gobierno del estado de Guerrero, 2024). Out of this total, 227 production units farm tilapia. Forty-five of these production units are mainly located in Laguna de Tres Palos and use 1,320 floating cages.

Research about the characterization of the production process and commercialization of tilapia is scarce. Nevertheless, Lango-Reynoso *et al.* (2015) concluded that live tilapia commercialization is a strategy of market differentiation. In addition, the freshness of the product plays a key role, guiding the decision-making process of consumers. González *et al.* (2017) pointed out that producers use a traditional commercialization route —*i.e.*, producers sell directly to final consumers. However, both in the domestic and international markets, tilapia is sold in a wide array of presentations, including whole tilapia (fresh, frozen) and tilapia filets (fresh, frozen, and in affordable packages) (Lango-Reynoso *et al.*, 2015). These authors also mentioned that the commercialization channels are "producer-retailer-final consumer" and "producer-final consumer." Likewise, Ahmed *et al.* (2012) and Hsiao *et al.* (2025) indicated that the commercialization chains from the producers to the final consumers are "producer-final consumers" and "producer-wholesaler-retailer-final consumer". These commercialization chains are also a system, with different degrees of traditional commercialization. Consequently, the objective of this study was to characterize the production process and the commercialization channels of tilapia farmed in floating cages by producers from Laguna de Tres Palos, Acapulco, Guerrero. The ultimate aim was to propose sustainable commercialization strategies.

MATERIALS AND METHODS

Study Area

The research was conducted in the localities of San Pedro de las Playas, Plan de los Amates, El Arenal, and Laguna de Tres Palos, Acapulco, Guerrero, Mexico. Tilapia is mainly farmed in floating cages in these production areas (Figure 1).

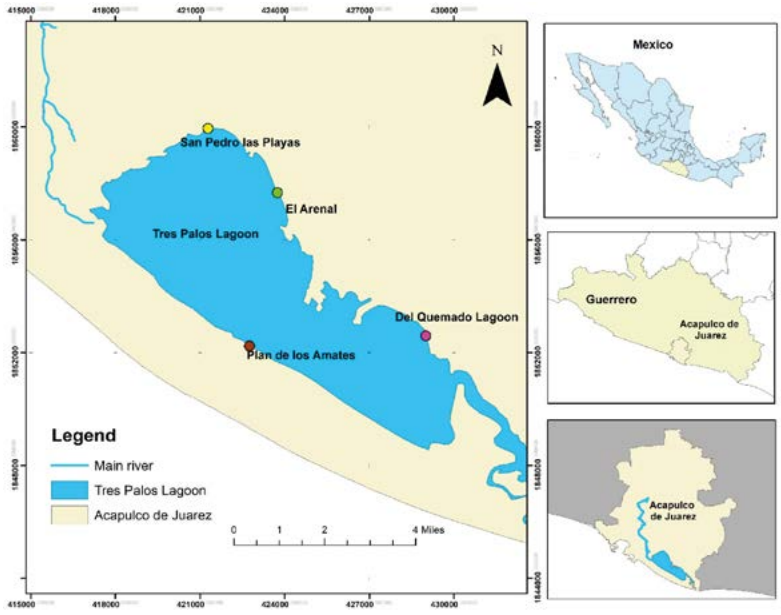

Figure 1. Location of Laguna de Tres Palos and the study areas, Acapulco, Guerrero.

Research Design

The design had a quantitative and qualitative approach, with a descriptive, cross-sectional, and non-experimental scope (Hernández-Sampieri and Mendoza, 2018; Müggenburg-Rodríguez and Pérez-Cabrera, 2018). A questionnaire was applied from November 2024 to April 2025 to determine the tilapia commercialization channels. The sampling population was made up of aquaculturists, wholesalers, retailers, and female sellers that buy fishes in the localities of San Pedro las Playas, Plan de los Amates, El Arenal, and Laguna del Quemado. In addition, a non-probability convenience sampling and a semi-structured questionnaire with closed and open-ended questions were used to gather overall data about the tilapia producers and the commercialization process. A previous pilot test with aquaculturists was conducted. Subsequently, the questions were adjusted to improve understanding and accuracy. In addition, the reliability degree of the open-ended questions of the tool was determined (Cronbach's Alpha=0.71). The appropriate reliability level validated the questionnaire (Bojórquez *et al.*, 2013).

Eighty-one individuals (50 producers and 31 buyers, divided into 4 wholesalers and 27 retailers) were interviewed. In addition to the interviews and direct monitoring, four work meetings were carried out with a focus group of producers that included leaders and chairpersons of fishing cooperatives. The aim was to find out their opinion about production processes and commercialization.

Data Analysis

The data obtained were analyzed using the SPSS v. 22.0 (Nel, 2014) software. Meanwhile, Excel was used to conduct the descriptive statistical analysis and to develop graphs. The Kruskal Wallis test was also used to differentiate the answers of the questionnaire by locality. The quantitative and qualitative data from the interviews

and meetings with producers were triangulated. Matching and different answers were compared in order to validate the findings. Subsequently, the categorization and codification process proposed by Rincón (2014) was used to analyze textual data. Only the traditional content analysis was used.

RESULTS AND DISCUSSION

Identification of Participants in the Tilapia Production Chain

Field information was used to identify the participants in the production, harvest, and commercialization processes of tilapia farming in Laguna de Tres Palos, Acapulco, Guerrero (Figure 2).

Aquaculturists, wholesalers and retailers, final consumers were identified as direct participants, while net, fingerling, and food providers were identified as indirect participants.

Production Link

The first link of the tilapia production chain in Laguna de Tres Palos includes the main social actors (aquaculturists) of the four localities showed in Figure 3. In this activity, men have a higher participation than women (Figure 3b). The average age of the producers is 50 (Figure 3c). Producers pointed out that they mainly started farming tilapia in floating cages due to the decrease of capture fishery (Figure 3d). In addition, they mentioned that they invested their own resources when they first engaged in this primary economic activity (Figure 3e).

The interviewees indicated that they did not have the permit granted by Comisión Nacional del Agua (CONAGUA). Nevertheless, they were enrolled in the National Fisheries Register and, when the questionnaire was applied, they were also using floating cages in their operations (Figure 3f).

Producers require net, food, and fingerling providers in order to farm tilapia in floating cages. The chairpersons of the cooperatives or leaders of the work groups interviewed were Argenie Morales Martínez, Benito Morales, Norberto Castillo Victorio, Jorge Nájera de la Paz, and Pedro Gómez Alonso. They mentioned that approximately 80% of the

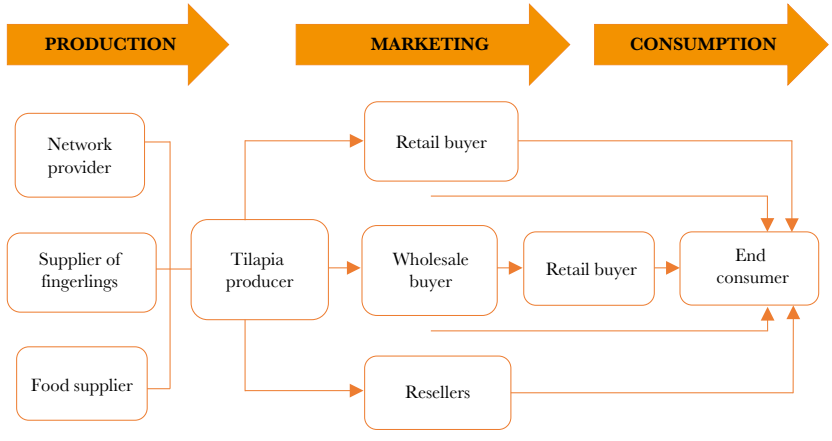

Figure 2. Value chain diagram of tilapia farmed in floating cages in Laguna de Tres Palos, Acapulco, Guerrero.

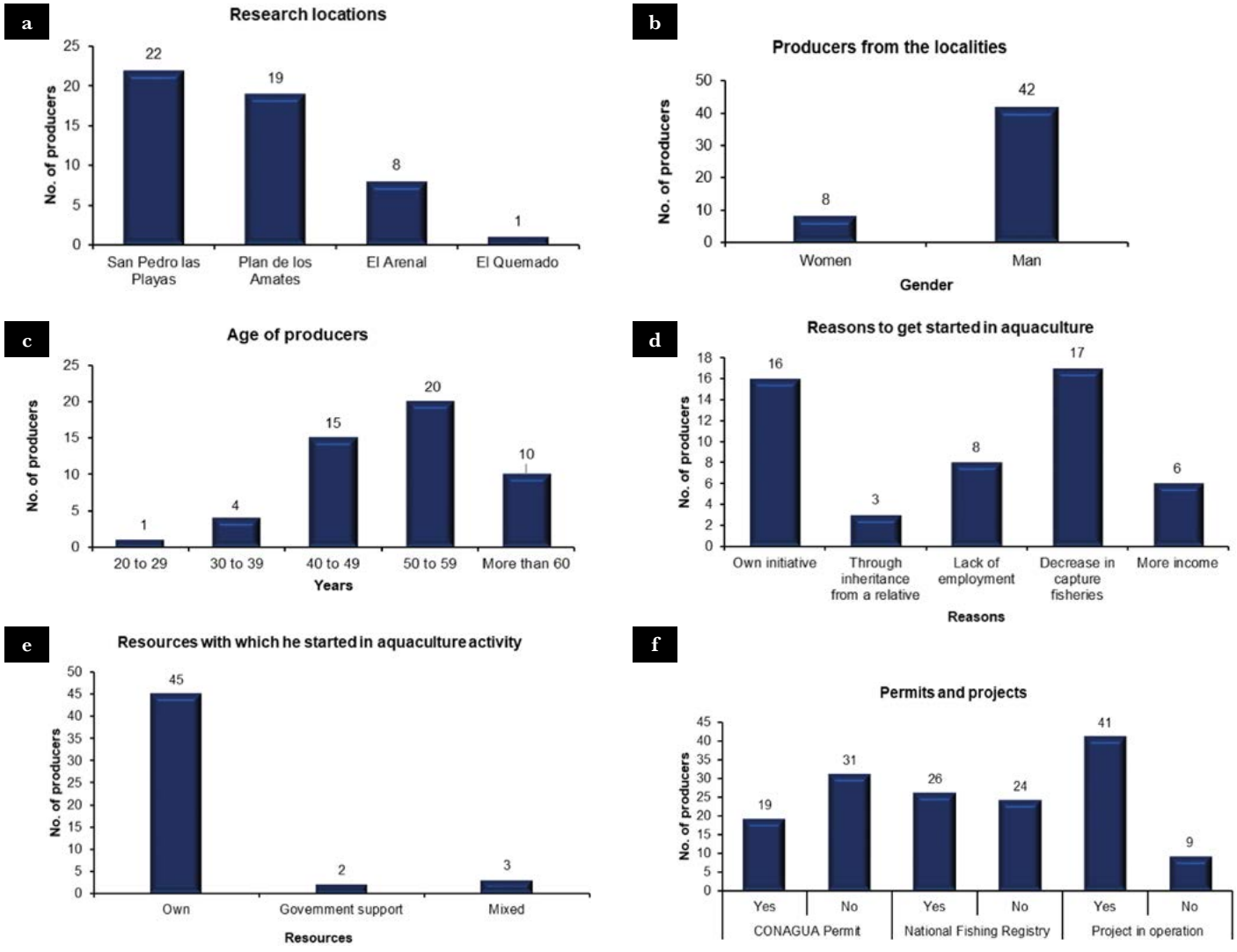

Figure 3. a) Localities. b) Gender. c) Age. d) Reasons to start aquaculture. e) Origin of the resources. f) Permits and operation projects.

aquaculturists purchase their main materials —including 9×1" tarred nylon fishing net, 16" nylon rope, #12 silk thread, and mosquito net —from providers in Guadalajara, while the remaining 20% buy their materials from local providers in Acapulco. In addition, 90% of aquaculturists purchase food for tilapias from a provider located in the Llano Largo locality, Municipality of Acapulco, Guerrero.

Construction of the Nurseries

A nursery is an area or infrastructure designed to handle and look after fingerlings (youngsters), providing them an optimal environment for their initial growth and development, before they can be moved to larger fattening cages. Nurseries are built with 10 m long × 5 m wide × 1.80 m high mosquito nets. Fingerlings are deposited in these nurseries for 30 days. The most common nurseries measured 5 m × 5 m × 1.50 m, 6 m × 6 m × 1.50 m, 3 m × 3 m × 1.50 m, and 4 m × 4 m × 1.50 m (Figure 4b).

Table 1. Characteristics of the Production Units (N=50).

Statistics/Item	Juvenile fish density per cycle	Fish density (fattening, cage)	Number of cycles per year	Production per cycle (t)	Weight (g) of harvested fish	Mortality reported per cycle	Number of cages at the time of interview
Average	22,020.00	2,520.00	1.48	1.98	496.00	144.00	3.18
Standard deviation	18,874.98	952.76	0.54	0.84	92.49	73.290	1.935
Minimum	1,000.00	1,000.00	1.00	1.00	400.00	100	0
Maximum	80,000.00	6,000.00	3.00	4.00	1,000.00	400	10

Where: t, tons; g, grams.

Construction of Fattening Cages

A 1-inch mesh size tarred nylon fishing net is used to build the fattening cages. Measures are taken with a tape measure and then the net is cut by hand. Afterwards, a plastic weaver and silk thread (No. 12) are used to sow the net to a nylon rope. Subsequently, the bottom and upper parts are sown together, creating 9 m long × 6 m wide × 1.50 m high rectangular cages. The cages are then anchored and nailed with mangrove branches by their four corners. In addition, a 0.30-m mosquito net is placed around the cage to prevent food spilling. According to the interviewees, square (58%) and rectangular cages (40%) are the most common shapes. The usual measures such as 5 m × 5 m × 1.50 m, 6 m × 6 m × 1.50 m, 3 m × 3 m × 1.50 m, and 4 m × 4 m × 1.50 m. In this regard, at the moment of the interviews, most of the producers had 1 to 3 floating cages (72%) (Figure 4a, b).

Production Process

In order to farm tilapia in floating cages, the leaders and chairpersons of aquaculture cooperatives pointed out in a work meeting that they stock an average of 80-100 fishes/m^3; however, the results of the questionnaire indicated that producers stock an average of 45-60 fishes/m^3 (Table1). Tilapia farming in floating cages requires various labors for the appropriate development of the fishes. The following labors are carried out during different stages of the process:

1. Stocking of tilapia fingerlings. Tilapia fingerlings (≈1 g) are introduced to nursery cages of different sizes (5 m × 5 m × 1.5 m, 6 m × 6 m × 1.5 m, 3 m × 3 m × 1.5 m, and 4 m × 4 m × 1.5 m). This process lasts approximately 30 days.
2. Moving tilapia to fattening cages. Fishes are moved from the nurseries to floating cages of different sizes built with 1-inch mesh size tarred nylon fishing nets.
3. Feeding. Depending on their development stage, fishes are fed with different rations of balanced feed. The portion should be 1% to 3% of the body mass of the fish per day, divided into two rations to prevent waste and to guarantee a good development.
4. Monitoring and management. This process includes measurements of water quality, daily check of fish health, net cleaning, and monitoring.
5. Harvest. Once the fishes have reached a 400-500 g body weight, they can be harvested (≈6 months). Aquaculturists harvest fishes in the small hours, between

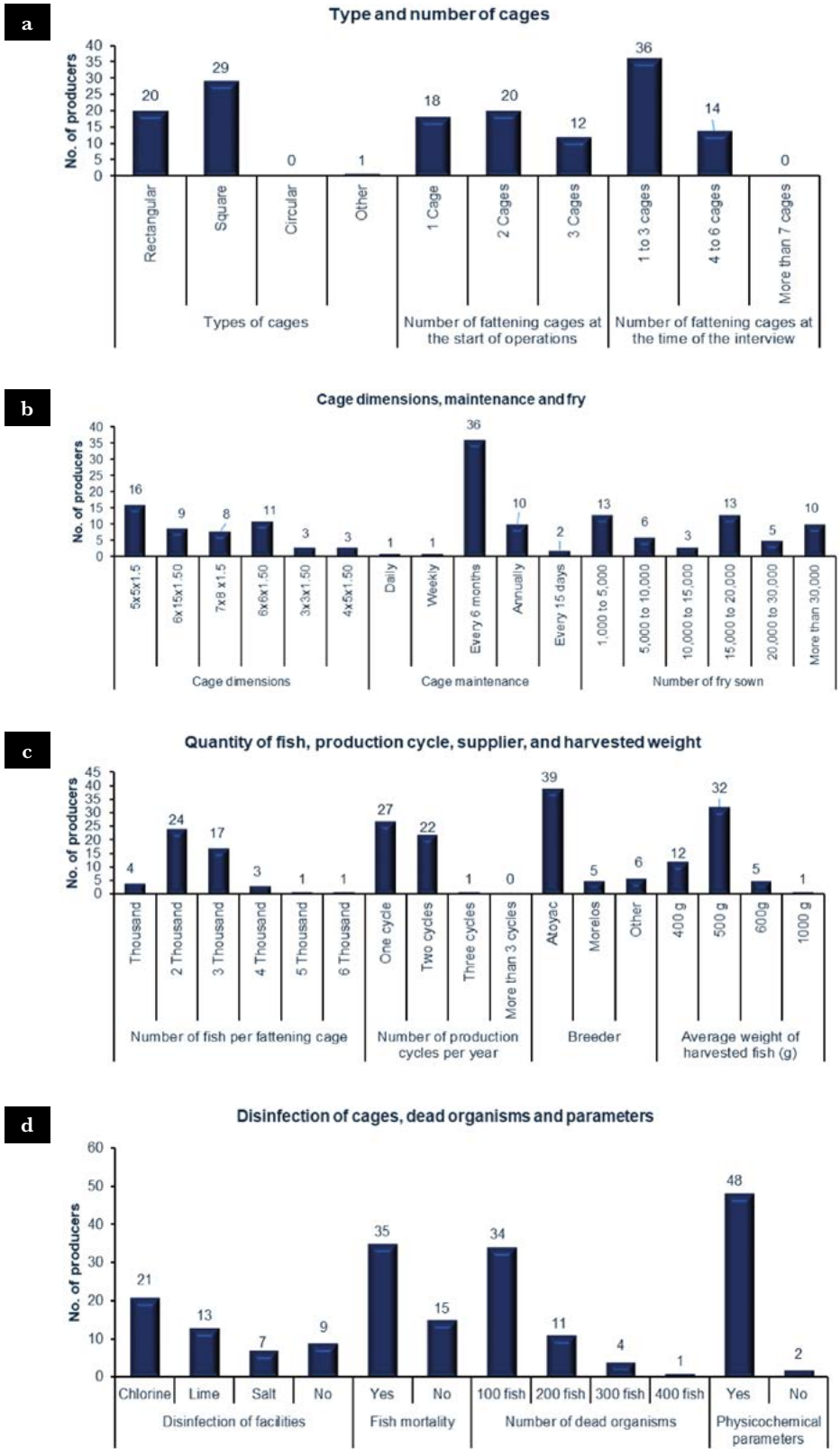

Figure 4. a) Types and number of cages. b) Measures and maintenance of the cages and number of stocked fingerlings. c) Number of stocked fishes, number of cycles, and providers. d) Disinfection, mortality, and physicochemical parameters.

3:00 am and 5:00 am. Afterwards, the catch is moved in rowboats and motorboats to the shore of the lagoon, where the producers sell it to wholesalers, retailers, or final consumers.

The results of this study indicated that 26, 26, 20, 12, 10, and 6% of the producers stocked their nurseries with 1,000-5,000, 15,000-20,000, >30,000, 5,000-10,000, 20,000-30,000, and 10,000-50,000 tilapia fingerlings per cycle, respectively (Figure 4b, Table 1). These tilapia fingerlings are purchased from the reproduction center located in Atoyac de Álvarez, Guerrero (Figure 4c). Meanwhile, during the moving stage, 48, 34, 8, 6, 2, and 2% of the producers moved 2,000, 3,000, 1,000, 4,000, 5,000, and 6,000 tilapias per fattening cage, respectively. Regarding the culture cycles, most of the producers (54%) carried out fattening in one cycle, while the rest (44%) implement a two-cycle fattening (Figure 4c, Table 1).

Although none of the aquaculturists monitor the physicochemical parameters of the water, 96% of them believe that the said measurements are crucial for a good tilapia production (Figure 4d). Nevertheless, as their main management practice, 82% of the producers disinfect their cages and materials with chlorine, lime, and/or salt, mainly at the end of the farming cycle. However, 70% of the producers also report fish mortality cases during the cycle. Most of the cases involve 100 to 200 deaths (Figure 4d, Table 1). Finally, most producers (72%) carry out maintenance of the cages every six months, while 20, 4, and 2% repair their cages every year, every fifteen days, and every week, respectively (Figure 4b).

Producers harvest 1 to 2 t per cycle, depending on the number of fingerlings and operating cages (Table 1). The average weight of six-month tilapia ranges from 400 to 500 g (88%). Regarding the farming cycles, most producers (54%) fatten fishes for only one cycle, while 44% practice a two-cycle fattening (Figure 4c, Table 1). Finally, 72% of the interviewees followed feeding protocols; however, 60, 24, and 16% feed the fishes twice, once, and thrice a day, respectively (Figure 5a). The amount of feed per ration mostly varies from 1-3 kg (66%) to 3-5 kg (26%), although some producers (8%) use ≥6 kg (Figure 5b). Throughout the production cycle, aquaculturists use 1 to 5 t of balanced feeds, divided as follows: 1 to 2 t (48%), 3 to 5 t (42%), and >5 t (10%) (Figure 5a). The most popular feed brands are Pedregal (34%), Grow Fish (32%), Malta Cleyton (32%), and Biofingerling (2%). Most producers scatter the feed in the whole cage (76%), although some (22%) pour it in the middle of the cage. Feeding from trays or baskets is uncommon (2%). Most producers (66%) use motorboats to feed the fishes, while 34% uses rowboats (Figure 5b).

Commercialization

The aquaculturists who farm tilapia in floating cages at Laguna de Tres Palos transport their fishes in any way possible to the wholesaler market. Subsequently, retailers and street vendors sell it to the final consumer. Therefore, aquaculturists, along with the fishermen and fisherwomen of the Laguna de Tres Palos, carry out three types of commercialization: 1) "producer-wholesalers/distribution center-retailers-final consumer"; 2) "producer-retailer-final consumer"; and 3) "producer-female sellers/housewives-final consumer," as

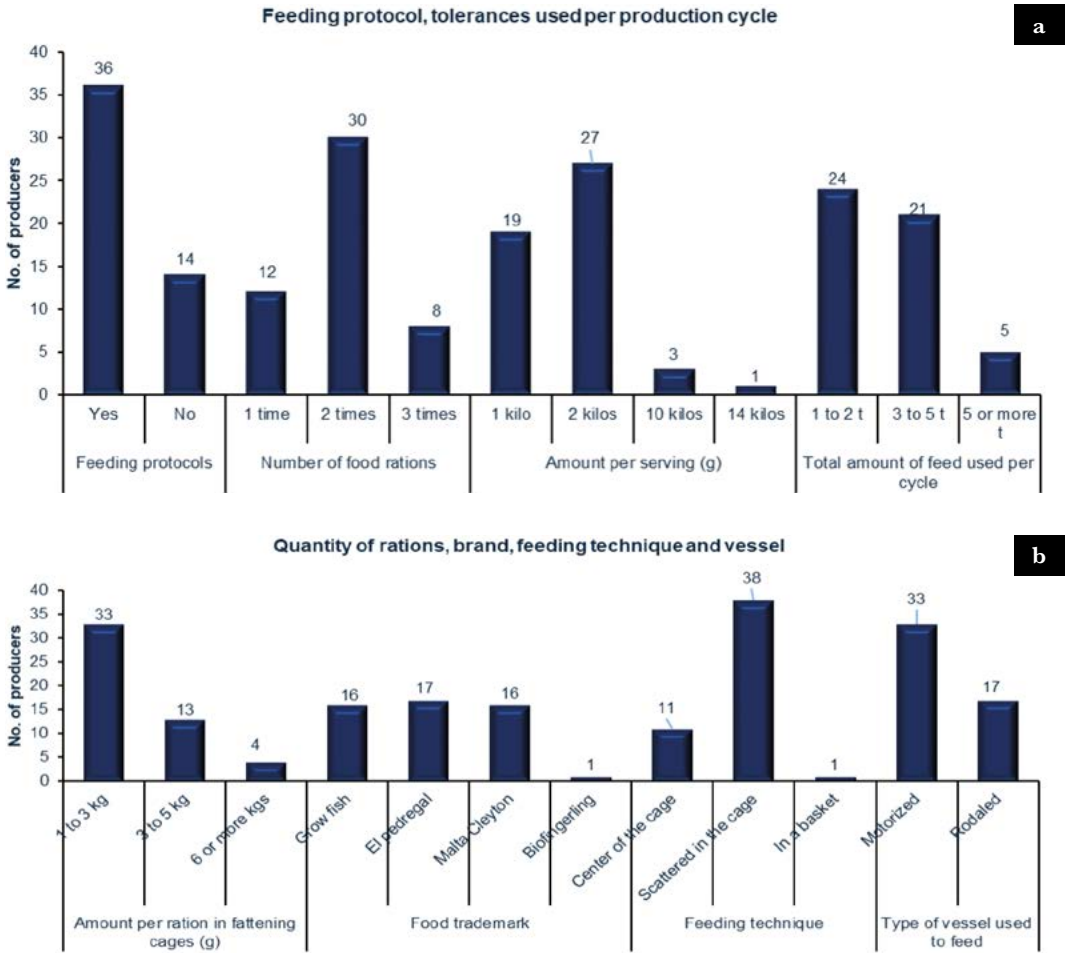

Figure 5. Feeding characteristics of tilapia farming in floating cages.

the most direct channel (Figure 1). Traditionally, the commercialization of the product, from the moment it leaves the farming cages, until it reaches the final consumer, takes place at the following levels: 1) local, from producers to wholesalers and retailers (74%); 2) regional, through wholesalers (20%); and 3) domestic, through wholesalers (6%). Producers sell their product either whole and fresh (94%) or whole and frozen (6%) (Figure 6b).

According to the information provided by the aquaculturists about the size of the tilapia commercialized, large fishes (500-1,000 g) account for most (56%) of the commercialized product and are sold for more than $60 Mexican pesos per kilogram. Medium-sized specimens (400-450 g) have a 34% participation and are sold from $35 to $50 Mexican pesos per kilogram. Finally, small tilapias (300-350 g) have a 10% participation and are sold from $25 to $30 Mexican pesos (Figure 6a). Additionally, 60% of the producers allocate ≈5% of their production to self-consumption. Producers frequently eat tilapia: 76% of them eat this type of fish on a weekly basis (Figure 6c, d). The commercialization channel is composed of four wholesalers and 27 retailers. Wholesalers sell large, medium, and small fishes at $100, $80, and $50 Mexican pesos, respectively. For their part, retailers sell tilapia at $100, $80, and $60 Mexican pesos per kilogram (Figure 6f).

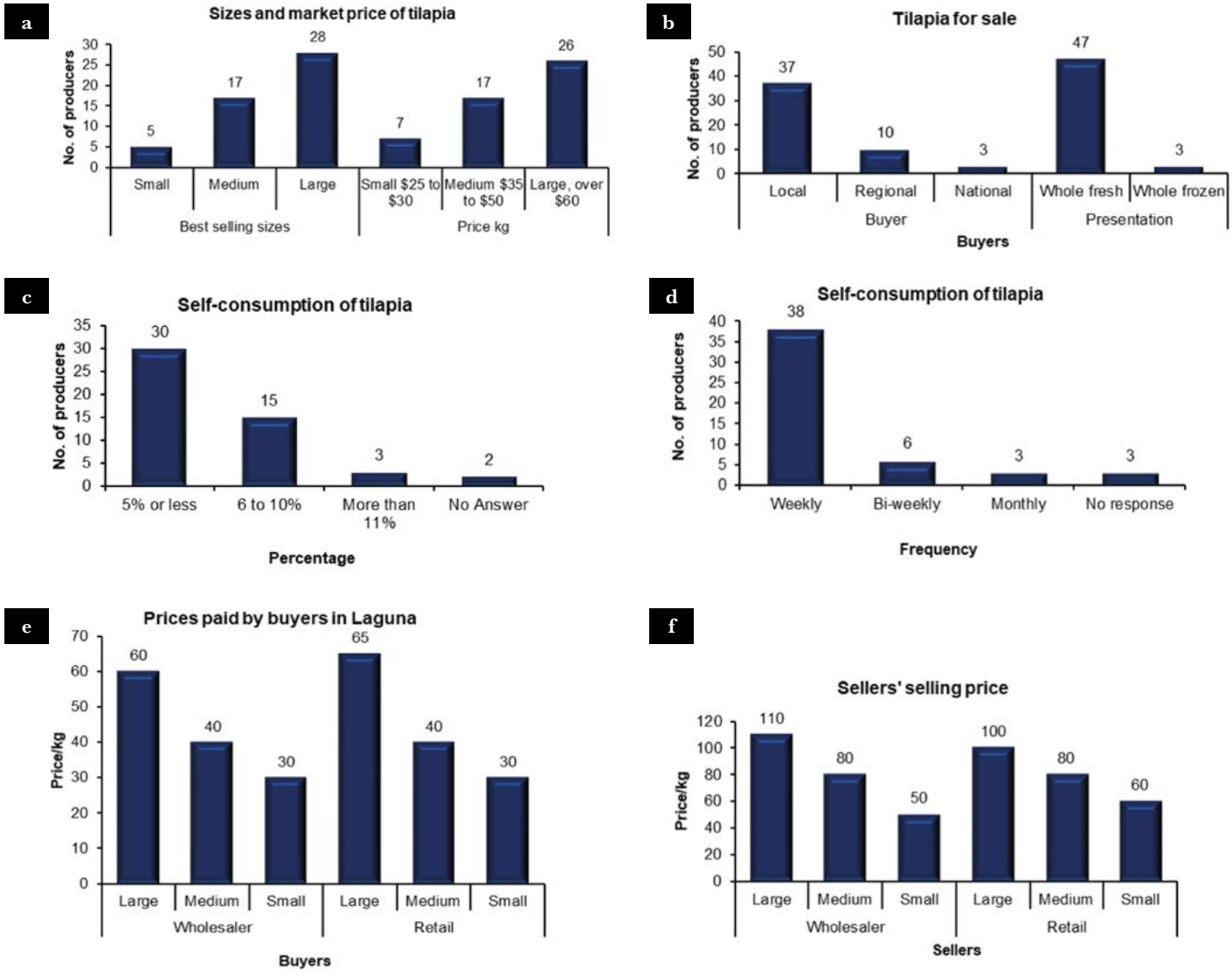

Figure 6. a) Best-selling size and price. b) Buyer type and tilapia presentations. c) Self-consumption percentage. d) Self-consumption frequency. e) Price per kilogram of tilapia paid by wholesaler and retailer buyers to the producer. f) Tilapia resale price charged by wholesaler and retailer buyers.

Meanwhile, according to the Kruskal Wallis test, only seven questions recorded significant differences ($p<0.05$) between the sites: 1) With what resources did you start this activity? (Plan de los Amates-El Quemado and San Pedro las Playas-El Quemado); 2) What is the size of your farming cages? (Plan de los Amates-El Arenal); 3) What is the size of your fattening cages? (Plan de los Amates-San Pedro las Playas); 4) Has your aquaculture unit recorded mortality cases? (Plan de los Amates-San Pedro las Playas); 5) Do you follow feeding protocols? (Plan de los Amates-San Pedro las Playas); 6) What is the ration you use in fattening cages? (Plan de los Amates-El Arenal); and 7) What type of boat do you use to feed the tilapias? (Plan de los Amates-San Pedro las Playas).

Likewise, based on the meetings with the producers, the research team was able to collect information about the production and commercialization cycles of tilapia farmed in floating cages and the factors that limit productivity.

Cycle of Tilapia Production in Floating Cages

Argenie Morales Martínez and Benito Morales (San Pedro las Playas), Norberto Castillo Victorio (Arenal), Jorge Nájera de la Paz (Laguna del Quemado), and Pedro Gómez Alonso (Plan de los Amates) are the leaders and/or chairpersons of the cooperatives. According to them, the production cycle usually starts in November or December with 1-2 g tilapia fingerlings mostly purchased from the reproduction center located in Atoyac de Álvarez, Guerrero. The size of the cage determines the number of fingerlings. Usually, each fattening cage holds 1,000 to 2,000 fingerlings, although that figure can sometimes reach up to 3,000. Tilapias are harvested at the end of March and in April; however, some producers harvest even in May. Producers estimate a 10-20% mortality rate, mainly caused by stress resulting from the lack of oxygen in the water body, as well as the pollution degree and even hurricanes.

Tilapia Commercialization

The San Pedro las Playas, Plan de los Amates, El Arenal, and Laguna del Quemado producers sell the tilapia they farm to wholesalers, retailers, and street vendors without any added value. Likewise, fish consumption reaches its peak during Easter week (March or April) and December; therefore, producers must plan ahead to have fresh product available for those festivities.

Finally, according to aquaculturists Argenie Morales Martínez, Benito Norberto Castillo Victorio, Jorge Nájera de la Paz, and Pedro Gómez Alonso, the main factors that impact tilapia productivity are the degree of water pollution, the reduction of oxygen, and the increase in water temperature. These phenomena usually take place in June or July of every year.

The production of tilapia in floating cages complies with the guidelines of the Instituto Mexicano de Investigación en Pesca y Acuacultura Sustentable (IMIPAS, 2018) and the Official Gazette of the Federation (DOF, 2012), as well as with the general production, building, and operation characteristics of floating cages established by Avilés and Lizawa (1993). For their part, Riveros *et al.* (2014) detailed the building of square cages with synthetic nets, using bamboo and polystyrene to keep them afloat. However, aquaculturists build square or rectangle cages with black net, anchored with mangrove branches from each corner.

The floating cages used by producers in this study match the square or rectangular cages reported by Estrada *et al.* (2023) and Pérez *et al.* (2021). Meanwhile, Estrada *et al.* (2023) found that 29, 33, and 23% of the producers started with 3-5, 6-10, and 11-20 cages, respectively. Regarding the size of the cages, 53% of the producers used 4 m × 4 m × 3 m cages, while 17, 15, and 3% used 3 m × 3 m × 2 m, 4 m × 4 m × 2 m, and 4 m × 4 m × 5 m cages, respectively. Some producers even use 3 m × 2 m × 1.5 m. For their part, Estrada *et al.* (2023) and Pérez *et al.* (2021) also reported square cages of the same size.

Various researches have reported variations in fish density per cubic meter. For example, Castillo-Capitan *et al.* (2014) determined that a 100 fishes/m^3 density favors greater growth, although Kunda *et al.* (2021) reported that 60 fishes/m^3 was an ideal density. Based on their years of experience, producers from Laguna de Tres Palos grow 80 to 100 fishes/

m^3. However, Costa *et al.* (2017) reported that 250 fishes/m^3 was the most adequate density to farm tilapia in floating cages. Given the discrepancies between the various studies, their specific factors must be taken into consideration, including their management system, good production practices, the size of the cages, and the environmental conditions, in order to determine the most appropriate culture density.

This research remedies the lack of published data about the tilapia commercialization chain in the State of Guerrero. The commercialization and productivity aquaculture practices in four key communities (San Pedro las Playas, Plan de los Amates, El Arenal, and Laguna del Quemado) are described, along with the prospects of the market participants (*e.g.*, wholesale and retail buyers, as well as local street vendors). In this sense, producers stick to traditional tilapia commercialization patterns: 1) "producer-wholesalers/distribution center-retailers-final consumer"; 2) "producer-retailer-final consumer"; and 3) "producer-female sellers/housewives-final consumer," as the most direct pattern. These results match the findings of Lango-Reynoso *et al.* (2015) who identified "producer-retailer market" and "producer-market-final consumer" patterns. Therefore, this research took into consideration local participants and described the specifics of the routes, reinforcing and deepening the understanding about the dynamics of the local or regional tilapia market.

For their part, Ahmed *et al.* (2012) and Hsiao *et al.* (2025) also identified the "producers to consumers" channel —including the intermediate "wholesaler to retailer" routes— as the traditional commercialization channel.

Eltholth *et al.* (2015) describe three different tilapia commercialization channels: "producer-wholesale," "producer-retail," and "producer-final consumer." According to them, these channels point out trends and help to achieve a basic understanding of the tilapia value chain. These results fully match the findings of this research.

Meanwhile, profitability is limited by several factors, including the high costs of inputs (fingerlings and feed), the low selling price of tilapia, and specific problems of local fish farms. In this last regard, the interviewees mentioned the pollution caused by the discharge of waters from point and non-point sources. A similar situation was reported by Antwi *et al.* (2017).

Likewise, Antwi *et al.* (2017) and Uddin *et al.* (2021) identified several limitations to the farming of tilapia in cages, including the lack of investment capital, fish robbery, the high cost of feed, education issues, and bad management. For their part, Aldama-Rojas *et al.* (2011) determined a wider list of obstacles, including the presence of predators, the lack of economic support and technical assistance, a defective oxygenation and changes in the color of water, strong winds, bad quality water, inadequate aquaculture practices, and water pollution resulting from inappropriate human practices. In contrast with production and environmental factors, Magcale-Macandog *et al.* (2014) highlighted producer experience as a determining factor for successful aquaculture. This human factor matches one of the key findings of this research. Likewise, the high cost of balanced feed and the lack of working capital force producers to diminish feeding rations. Given the weather conditions during the rainy season (*e.g.*, hurricanes) and the consequent loss of the whole production, producers prefer not to farm tilapia during that period.

In economic terms, on the one hand, Macfadyen *et al.* (2012) determined that tilapia commercialization does not achieve a satisfactory profit margin. This situation is attributed to the reduction of prices in real terms, the flotation of seasonal prices, and particularly buyer distrust caused by water pollution, just like in this research. On the other hand, the research of Villerías (2021) about commercialization channels concluded that, just like fishermen and fisherwomen, aquaculturists distribute their product from local buyers to final consumers. This arrangement matches the route identified in this research. Additionally, Villerias (2021) highlighted that producers obtain greater profits when they sell tilapia directly to the final consumer.

Regarding the different types of markets, Hernández-Arzaba *et al.* (2019) pointed out that tilapia commercialization is mainly concentrated in the local market (95.42%) and that it is almost non-existent in regional and domestic markets.

Acharjee *et al.* (2023) proved the existence of different routes for tilapia commercialization, particularly the "producer-middlemen (wholesaler)–middlemen–final consumer." Under this arrangement, producers sell 40% of their fish directly to middlemen. For their part, Reta-Mendiola *et al.* (2021) focused on the human factor and concluded that a large proportion of aquaculturists lack experience in both production and commercialization. This lack of experience results in a product with low productivity and competitiveness which consequently limits the capacity of producers to develop an industrialization plan. Therefore, their product can only be sold at the production unit, either to people or companies that collect the whole production or final consumers.

Hernández-Arzaba *et al.* (2023) divide the tilapia commercialization channels into direct routes —*i.e.*, "producer-local buyers-final consumer," based on their closeness and price— and routes where middlemen are involved — *i.e.*, "producer-middlemen (fisheries)-final consumer" and "producer-final consumer." In contrast to this commercialization channels, Jing *et al.* (2025) deploy a modernized approach, pointing out that the digital commercial channel for processed tilapia can be directly aimed to the final consumer. This sale method is attractive and provides a feasible opportunity for producers. Additionally, this digital channel justifies specific studies about the digital commercialization of tilapia in the region.

CONCLUSIONS

This research proved that aquaculturists have implemented inadequate production processes, mainly due to the prevailing lack of organization, resulting in favorable conditions for buyers during the tilapia sale negotiations. Meanwhile, the Laguna de Tres Palos aquaculturists have a brief participation in the commercialization chain of tilapias farmed in cages. The first tilapia commercialization chain in which producers are involved is the "producer-wholesalers/distribution center-retailers-final consumer" long form. The second route is the "producer-retailer-final consumer" chain. Finally, the most direct and slightly more profitable chain is composed of "producer-female sellers/housewives-final consumer." Despite the limited sale prices and the high costs of inputs, 88% of the participants reported that they were highly satisfied with their income; nevertheless, producers should sell their products through the direct commercialization channel.

All the tilapia produced by aquaculturists is commercialized whole and fresh. The main limitations to tilapia production are water quality and the cost of balanced feed. The limitations to tilapia commercialization are the low product prices and the point of origin of the fish. Interviewees considered that there are many potential pollution sources, including the discharge of polluted water from point-sources and non-point-sources in the vicinity of farming cages. However, they are acutely aware of the need to help in the preservation and restoration of the water body that they use as a source of income.

Participating aquaculturists should improve the commercialization chain through the enhanced integration of the various steps of production and distribution. An advertising and awareness campaign would promote the consumption of good quality tilapia farmed in floating cages at Laguna de Tres Palos. A project should be developed to improve the production and competitiveness of the tilapia value chain, taking into consideration the reality of the aquaculture producers.

In order to improve tilapia commercialization, two key actions have been proposed to achieve greater visibility: boosting trade through electronic tools (*i.e.*, digital marketing) and strengthening the distribution and supply chain. In addition, access to the product would increase consumer preference for tilapia.

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Agricultural structure and municipal resilience in the Northern region of Sinaloa

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ABSTRACT

Objective. To characterize the agricultural specialization of the municipalities that compose the northern region of Sinaloa, based on their patterns of specialization and diversification in the production of crops.

Design/Methodology/Approach. The agricultural structure of the five municipalities that compose the northern region of Sinaloa was evaluated based on the specialization indices of the planted area and the value of the production of the crops registered in 2023. With these indicators, four classes were constructed that combine patterns of concentration and diversification.

Results. The analysis showed three categories of agricultural structure, resilience-oriented, moderately resilience, and vulnerable or at risk. Each one reflects different capacities to face adverse situations and sustain productive stability over time.

Limitations/ Implications of the study. The study was limited to an agricultural cycle and did not incorporate external factors such as public policies or technological changes, which constrain the construction of future scenarios.

Findings/Conclusions. The geographical concentration of production of a small number of crops generates comparative advantages and greater market share but also implies structural risks. The identification of agricultural resilience categories provides a basis for the design of differentiated strategies that strengthen regional sustainability.

Keywords: specialization index, agricultural resilience, agricultural diversification.

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INTRODUCTION

In the current globalized and interconnected context, productive specialization is a frequent strategy for countries and regions to insert effectively into international markets (Johansson & Olaberría, 2014). Krugman (1991) explained that the geographical concentration of goods and services is the result of economies of scale, which allow companies to reduce costs and increase their efficiency.

In agriculture and agribusiness, specialization is understood as the significant production of a limited number of crops in a region. This model offers comparative advantages, since

quality improvements, as well as higher productivity and yields are achieved through technological innovation and efficient use of inputs. This allows producers to integrate into international markets (Zayas, 2018; Herment & Mignemi, 2021).

However, low agricultural diversification carries risks such as dependence on external markets and vulnerability to price fluctuations, trade policies, and tariff barriers. It also generates difficulties in adapting to changing conditions such as climate variability or reduced demand for certain products (Abson, 2019). Added to these factors are environmental impacts, including loss of biodiversity, soil degradation, pests spreading and intensive use of chemicals (Perfecto & Vandermeer, 2010; Cuadras *et al.*, 2021; Kaur *et al.*, 2024).

Studies reports also warn that excessive specialization and monocultures can lead to regional food insecurity and inequalities (FAO, 2018). Because they marginalize small producers, low-profitability crops, and other sectors, with negative effects on employment and opportunities (Campi *et al.*, 2021). Therefore, the need is posed to monitor levels of agricultural specialization in order to establish adequate limits of concentration, that at the same time promote diversified portfolios of products as a basic strategy for resilience (Klasen *et al.*, 2016).

In Mexico, agriculture is a pillar for economic development, food security, environmental conservation, and rural sustainability (Ayala *et al.*, 2012). The country ranks thirteenth in the world in food production and fifth in Latin America, but it is also the main food importer in the region (FAO & IFPRI, 2023), thus reflecting tensions between specialization and self-sufficiency.

The state of Sinaloa, in the northwest of the country, is established as one of the most productive agricultural areas, due to its level of contribution to national and international markets (SIAP, 2023). Sinaloa's leadership has been built around irrigated agriculture that has ensured high levels of productivity since the mid-20th century. This strategic role has led to this Mexican state being recognized as "the grain storehouse of Mexico" (Román & Carrillo, 2021).

Sinaloa's horticulture is characterized by its export orientation, mainly to the United States. Factors such as geographic location, climatic conditions, technological innovation, infrastructure, and trade agreements such as the North American Free Trade Agreement (NAFTA) have driven agricultural specialization (Fiscal *et al.*, 2017).

Despite this, studies on agricultural specialization in Mexico are limited and tend to focus on national or regional scales. Some analyze the production of a small group of fruits and vegetables (Cruz-Delgado *et al.*, 2013), while others focus on specific crops such as strawberries, asparagus, blueberries, green peppers or maize (Bustamante *et al.*, 2019, 2020; Portillo *et al.*, 2023; Pacheco *et al.*, 2024; Rodríguez *et al.*, 2025).

Although these studies made valuable contributions, there is still a lack of research focused on the productive structure of Sinaloa, and particularly on the northern region that is the most dynamic and productive in the state. In 2023, this area planted 72% of the 53 products grown in Sinaloa (SIAP, 2023), which confirms its strategic importance.

Hence, this analysis seeks to identify the productive profiles of Ahome, El Fuerte, Choix, Guasave and Sinaloa, the five municipalities that compose Sinaloa's northern region,

based on four classes that combine the degree of specialization, estimated as the planted area, and the value of production of crops as it was recorded in 2023. The objective of this study was to characterize the agricultural specialization of the municipalities that compose the northern region of Sinaloa, based on their patterns of specialization and diversification in the production of crops.

MATERIALS AND METHODS

For the analysis of the agricultural structure of the northern region of Sinaloa, the location index (or quotient) was used as a relative measure of the level of specialization shown by each municipality in regard to a specific crop (Castro & Fuentes, 2017). The index was calculated for both the planted area and the value of production in 2023, based on data obtained from Mexico's Agri-Food and Fisheries Information Service (SIAP, 2023).

The specialization index compares the share of crop *i* in municipality *j* with the share of the same crop in the entire state. It is obtained with the formula:

$$IEA_{ij} = \frac{C_{ij} / C_j}{C_{iE} / C_E}$$

IEA_{ij}: agricultural specialization index of *i*-crop in *j*-municipality; C_{ij}: area or value of production of the *i*-crop in the *j*-municipality; C_j: area or total value of production in the municipality. C_{iE}: area or value of production of the *i*-crop in the $_E$-state. C_E: area or total value of production in the state.

Values obtained as indicators are interpreted as follows: If IEA>1 it is understood that the municipality is overrepresented or specialized in a particular crop; If IEA<1 the municipality is underrepresented or not specialized in that crop; If IEA=1 the crop is considered as represented but the municipality is not specialized in it. The combination of the indices obtained by planted area (IEAs) and by production value (IEAv), allows us to classify the crops into four classes or types (Table 1).

Table 1. Classes of crops according to agricultural specialization indices.

Type	IEAs	IEAv	Description
I	>1	>1	Specialization in both cultivated area and production value
II	>1	<1	Specialization in cultivated area but not in production value (extensive, inefficient, or damaged crop)
III	<1	>1	Specialization in production value but not in cultivated area (intensive, efficient, or high-value crop)
IV	<1	<1	Not specialized in either aspect

IEAs: Agricultural specialization index by cultivated area. IEAv: Agricultural specialization index by production value.

RESULTS AND DISCUSSION

In 2023, the northern region of Sinaloa specialized in more than half of the crops planted (66% by planted area and 74% by value of production). In this way, it showed different patterns of agricultural activity in the five municipalities that compose the region (Table 2).

In Ahome, 87% of the crops in the region were planted (33/38). This municipality is characterized by its specialization in more than half of its crops, both in planted area (58%) and in production value (61%). In addition to presenting a diversified and specialized agricultural structure, Ahome has nine crops that exceed four or five times the state value, which indicates a high degree of agricultural specialization. However, it combines with this high specialization a diversified agricultural structure that provides the municipality with more secure economic conditions. Consequently, its agricultural structure, in addition to being competitive, implies a low level of risk when facing adverse situations such as abrupt changes in the market or other eventualities.

The opposite case to the previous one is Choix, where only 13% of the products of the northern region were planted (7/38). In addition, this municipality specialized in most of them, in terms of planted area (71%) and in more than half of crops in terms of production value (57%). Therefore, this municipality is characterized by little agricultural diversification and a very high specialization in more than half of its crops, which exceed the state value by more than 10 times. Both conditions together imply a risky situation in the face of market variations and other eventualities. This fact characterizes Choix as a vulnerable profile.

The rest of the municipalities in the region presented similar proportions in regard to the crops planted (El Fuerte, 43%; Guasave, 45% and Sinaloa, 42%). However, regarding specialization by planted area, or production value, Sinaloa (50% and 55%) and El Fuerte (52% and 48%) showed similar percentages in the two indicators. On the other hand, Guasave presented a notable difference between the two indicators, with 38% in planted area and 58% in production value, which indicates a more marked specialization in crops of high economic value.

An additional difference is that Sinaloa and El Fuerte have high and very high specialization in some crops, with values that exceed the state proportion by more than

Table 2. Agricultural specialization in municipalities according to crop number and type, planted area, and value of production in the northern region of Sinaloa (Mexico).

Municipality	Crops	Specialization		Type			
		Area	Value	I	II	III	IV
Ahome	33	19	20	19	0	1	13
Choix	7	5	4	4	1	0	2
El Fuerte	23	12	11	10	2	1	10
Guasave	24	9	14	10	0	1	13
Sinaloa	22	11	12	10	1	2	9
Northern region	38	25	28	22	2	7	7

five or 10 times. On the other hand, Guasave shows high specialization in a single crop. Therefore, although these municipalities can be considered semi-diversified and with a certain degree of specialization, they do not reach the same level of competitiveness as Ahome does.

As for the typology of municipal specialization, Ahome has more than half of the crops in type I (specialization both in area and in value), among which oats, chia, coriander, sunflower, vegetables, potato seed, wheat seed and marigolds are notable for their high specialization (Table 2). However, more than a third of the products sown are underrepresented (type IV) and sorghum is only overrepresented in its value of production (type III). This product can be intensive or have a certain efficiency in terms of the relationship between the area and the value of production shown in the different municipalities (Table 3).

The municipality of Choix has more than half of its crops in type I (sesame, peanut, watermelon, grassland and rangeland plants), although with very high specialization, since they exceed by more than 15 times the state proportion in planted area, and by more than 50 times the value of production, especially the cultivation of grassland and rangeland plants. These products can exhibit high efficiency depending on the surface-to-value ratio. The rest of the crops were classified as types II and IV, but two of those (maize and sorghum) presented total losses due unforseen contingencies. Choix's production was concentrated on efficient crops that gave this municipality a competitive advantage and a good position in the market. However, its poorly diversified agricultural structure exposes Choix to risks in the face of economic fluctuations or environmental contingencies. In addition to threatening the sustainability of the agricultural sector due to the agroecological deterioration that low diversification generates.

The municipality of Sinaloa has 45% of the products in type I: safflower, green peas, beans, chickpeas, potatoes, fodder sorghum, sorghum grain and wheat grain, chickpea seed and cauliflower. Among which the last two are outstanding for the high specialization in surface area, and very high in value of production. Due to the relationship between both variables, those latter could be considered efficient crops, along with fodder sorghum. The municipality has nine products in type IV (non-specialized), as well as peanuts and green tomatoes in type III. In addition to sesame seeds (type II) with a very low index in production value compared to that of the planted area, perhaps due to some eventuality, since this behavior is atypical in the region.

El Fuerte has 42% of its products in type I (sesame, peanut, pumpkin, green peas, green beans, maize ear —sweet corn—, beans, potatoes, sorghum grain, grassland and rangeland plants). Among these, green peas are outstanding for their very high specialization, both in surface area and in production value. Pumpkin and green beans also are outstanding, with an atypical relationship between the indicators (5.17 *vs.* 2.91 and 4.40 *vs.* 1.20, respectively). This suggests inefficient crops or the possible existence of losses; a situation similar to that observed in green pepper and zucchini, both classified as type II. The municipality has 46% of its crops in type IV (non-specialized), whereas safflower is classified in type III, as a crop with a certain degree of efficiency.

The municipality of Guasave concentrates more than half of the crops in type IV (non-specialized), in addition to 38% of its products classified as type I (green peas, cabbage,

Table 3. Agricultural specialization of municipalities in the northern region of Sinaloa (Mexico) based on the indices estimated per crop.

Crop	Ahome		Choix		El Fuerte		Guasave		Sinaloa	
	IEAs	IEAv	IEAs	IEAv	IEAs	IEAv	IEAs	IEAv	IEAs	IEAv
Sesame	1.94	1.96	15.86	57.20	1.57	1.70	0.00	0.00	2.14	0.24
Oat grain	4.58	5.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Eggplant	0.13	0.03	0.00	0.00	0.00	0.00	0.03	0.01	0.00	0.00
Peanut	0.28	0.16	20.74	242.21	1.52	1.87	0.00	0.00	0.78	1.09
Zucchini	0.92	0.91	0.00	0.00	0.16	0.06	0.95	0.85	0.37	0.43
Pumpkin	2.43	3.51	0.00	0.00	5.17	2.92	0.59	0.61	0.00	0.00
Safflower	0.35	0.45	0.00	0.00	0.61	1.09	0.34	0.52	2.49	3.78
Onion	0.22	0.16	0.00	0.00	0.80	0.16	0.51	0.64	0.60	0.43
Chia	4.58	5.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Green peas	0.00	0.00	0.00	0.00	14.03	15.76	1.94	2.29	1.15	1.66
Green pepper	0.29	0.60	0.00	0.00	1.22	0.73	0.28	0.40	0.48	0.42
Coriander	4.58	5.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cabbage	0.00	0.00	0.00	0.00	0.00	0.00	2.32	4.65	0.00	0.00
Cauliflower	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.36	12.84
Green bean	0.17	0.06	0.00	0.00	4.40	1.20	1.90	1.94	0.45	0.46
Maize ear (sweet corn)	3.36	4.05	0.00	0.00	3.70	3.42	0.39	0.35	0.20	0.23
Strawberry	4.14	2.99	0.00	0.00	0.00	0.00	0.51	2.43	0.00	0.00
Bean	1.50	1.66	0.07	0.14	1.48	1.75	1.83	1.98	1.40	1.85
Chickpea grain	0.14	0.19	0.00	0.00	0.04	0.06	1.40	1.71	2.53	3.14
Sunflower	4.58	5.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vegetables	4.58	5.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maize grain	0.80	0.94	0.04	0.00	0.84	0.99	1.04	1.18	0.71	0.97
Potato	2.42	2.64	0.00	0.00	1.83	2.26	1.27	1.47	1.54	2.12
Grassland and rangeland plants	0.00	0.00	50.83	236.35	2.79	2.12	0.00	0.00	0.00	0.00
Cucumber	0.38	0.13	0.00	0.00	0.14	0.04	0.31	0.30	0.21	0.15
Watermelon	1.55	1.38	21.52	52.54	0.47	0.40	0.28	0.34	0.08	0.09
Bean seed	1.35	1.03	0.00	0.00	0.00	0.00	3.69	4.65	0.00	0.00
Chickpea seed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.36	12.84
Maize seed	2.40	2.28	0.00	0.00	0.11	0.15	0.06	0.10	0.00	0.00
Potato seed	4.58	5.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Wheat seed	4.58	5.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Green fodder sorghum	0.08	0.14	0.00	0.00	0.30	0.51	0.00	0.00	3.89	6.23
Sorghum grain	0.84	1.44	1.61	0.00	1.88	2.25	0.96	1.54	1.41	1.43
Soy bean	2.73	2.97	0.00	0.00	0.00	0.00	0.31	0.30	0.00	0.00
Red tomato	0.07	0.04	0.00	0.00	2.55	0.94	0.51	0.37	0.73	0.38
Green tomato (tomatillo)	0.79	0.53	0.00	0.00	0.81	0.65	1.06	1.31	0.92	1.51
Wheat grain	3.16	3.57	0.00	0.00	0.62	0.54	0.45	0.51	1.11	1.45
MX marigold (cempasuchil)	4.58	5.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

IEAs: agricultural specialization index by cultivated area. IEAv: agricultural specialization index by production value. Mexican marigold is the native species *Tagetes erecta* or Cempohualxochitl (Nahuatl) that means "20-flowers flower"; It is called cempasuchil in Mexico.

green beans, beans, chickpeas, maize, potatoes, beans and green tomatoes). Of these, cabbage and beans are outstanding in the value of production (4.65). Cabbage also presents a relationship between the indicators that shows a certain efficiency in cultivation; similar to strawberries and sorghum, both classified in type III.

These latter three municipalities have few products that are efficient in terms of production value, and are highly specialized in a small number of products. For this reason, these municipalities do not reach the level of positioning presented by the other two profiles; however, their semi-diversified structure gives them relative security in the face of disruptive events. Therefore, these municipalities are defined by the pattern we called moderate resilience.

This analytical exercise corroborates the prominence of the northern region of the state of Sinaloa and characterizes their production profiles or patterns. This study not only allowed us to assess the current agricultural condition in the region, but it is also useful to establish strategies towards sustainability, conservation and food security.

CONCLUSIONS

The combination of the agricultural specialization indices, by planted area and value of production, allowed us the identification of three municipal patterns or profiles of production in Sinaloa that represent different opportunities and risks. The resilient pattern corresponds to Ahome, which is specialized in a wide variety of crops that provides security in the face of market changes. The vulnerable pattern is characteristic of Choix, with few types of crops and very high specialization in high-value production. The third profile, moderate resilience, corresponds to the municipalities of Sinaloa, El Fuerte and Guasave, which allocate a significant area to few crops, and also maintain a certain variety in types of crops and are specialized in a number of them.

The analysis of agricultural specialization corroborated the prominence of the northern region of Sinaloa, as well as the existence of different patterns or profiles of production within that region. This implies specific conditions of advantage or disadvantage per municipality in the face of possible contingencies or uncertain scenarios. This study not only allows us to know the current situation but is also useful to establish strategies aimed at strengthening the resilience and sustainability of the regional agricultural sector, towards the conservation of natural resources and food security in the long term.

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Colegio de Postgraduados

3-oxo-C12-HSL and C4-HSL promote root system development in *Solanum lycopersicum* L. *in vitro*

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ABSTRACT

Objective: To evaluate the effect of the synthetic C4-HSL and 3O-C12-HSL molecules produced by wild strains of *P. aeruginosa* as promoters of the root system of *Solanum lycopersicum* L *in vitro*.

Design/methodology/approach: Wild and control strains were studied, in which crude extraction in ethyl acetate and quantification of both molecules were performed. Different concentrations of synthetic 3O-C12-HSL and C4-HSL (10, 12, 24, 48, 96, and 192 μM) were used. The ability to elongate the primary root, as well as the length and density of root hairs under conditions were evaluated. Laboratory-controlled studies.

Results: The results obtained showed no beneficial effect on PAO1 in the crude extracts. However, a positive effect was observed when using the synthetic molecules 3O-C12-HSL and C4-HSL on primary root elongation at a concentration of 24 μM and on root hair density at a concentration of 96 μM, respectively, with a statistically significant difference ($p<0.05$) compared to the controls.

Findings/Conclusions: This study reports positive effects on primary root elongation and root hair density using the synthetic molecules C4-HSL and 3O-C12-HSL in *S. lycopersicum*.

Keywords: AHL, crude extract, 3O-C12-HSL, C4-HSL, *P. aeruginosa*, *S. lycopersicum*, *in vitro*.

INTRODUCTION

Currently, agriculture faces a high demand for the production of high-quality food; however, achieving this involves overcoming several challenges, including the loss of soil fertility, water scarcity, land-use change and climate change, the overuse of agrochemicals,

high costs, and low yields, which together make food production an unsustainable system. Microorganisms may represent an alternative to increase agricultural production in small spaces while producing organic food that benefits environmental and human health. Numerous studies have reported the use of Plant Growth-Promoting Bacteria (PGPB). *P. aeruginosa* is considered a PGPB, but it is also an opportunistic bacterium, which limits its use in agriculture. However, it has been reported that autoinducer molecules (AHLs) of the Quorum Sensing (QS) system modify root architecture and increase biomass in *Cicer arietinum* and *Triticum aestivum* (Gahoi *et al*., 2021; Ibal *et al*., 2021) when C8-, C10-, and C12-HSL are added, respectively. In *P. aeruginosa*, the QS system is well studied, in which two AHLs (autoinducers) have been described: N-3-oxo-dodecanoyl-L-homoserine lactone (3-oxo-C12-HSL) and N-butanoyl-L-homoserine lactone (C4-HSL). These molecules bind to their receptor proteins LasR and RhlR to activate the transcription of genes involved in virulence and persistence in diverse environments (Mukherjee *et al*., 2017). However, studies suggest that the agricultural use of these AHLs can promote germination, increase plant fresh and dry weight, and induce root elongation in *Arabidopsis thaliana* and *Lactuca sativa* L. (Von Rad and Ortiz Castro, 2008; Ortiz J. *et al*., 2024). It has also been reported that *P. aeruginosa* strains promote the growth of *Solanum lycopersicum* L. (Hariprasad *et al*., 2013; Adesemoye *et al*., 2008). This vegetable is important to study because it is part of the Mexican diet (Martínez-Rodríguez *et al*., 2017). Therefore, the objective of this study was to determine the effect of the synthetic C4-HSL and 3O-C12-HSL molecules and crude extracts produced by wild strains of *P. aeruginosa* on promoting root growth of *Solanum lycopersicum* L. under *in vitro* conditions, in order to evaluate their potential as a viable alternative for the development of tomato cultivation in current agriculture.

MATERIALS AND METHODS

Biological material: Wild strains of *P. aeruginosa* were isolated and identified using the automated VITEK system (bioMérieux®) from different environments in the state of Guerrero, and control strains were included (Table 1).

AHL extraction and elucidation: A pre-inoculum of the strains of interest was prepared in LB (Luria-Bertani) medium and grown overnight at 37 °C with shaking at 225 rpm. Subsequently, 30 mL of PPGAS broth (NH′Cl 0.02 M, 1.069 g/L; KCl 0.02 M, 1.49 g/L; Tris-HCl 0.12 M, 18.91 g/L; peptone 1%, 10 g/L; glucose 0.5%, 25 mL/L; MgSO′ 0.0016 M, 3.2 g/L) were inoculated with an appropriate volume of the pre-inoculum to reach an initial absorbance of 0.05 at 600 nm, and incubated at 37 °C for 24 h with shaking at 225 rpm. Cultures were centrifuged at 14,000 rpm for 10 min at 4 °C, and

Table 1. *P. aeruginosa* strains used in this study.

Origen	Cepa
Rizósfera de jitomate	MAZ 0105
Rizósfera de maíz	TIX 0303
Aislado clínico	MCD
Control	PAO1-UW
Mutante experimental	PAO1-Δ*rhlI*/Δ*lasI*

the supernatant was transferred to a clean 50 mL polypropylene tube. Then, 5 mL of supernatant were mixed with 5 mL of acidified ethyl acetate (1000 mL ethyl acetate + 100 μL acetic acid) in a 15 mL polypropylene tube and manually shaken vigorously for 15 min. Subsequently, the mixture was centrifuged at 3,500 rpm at 4 °C.

Once the phases were separated, the upper organic phase was collected, and 5 mL of acidified ethyl acetate were again added to the lower phase, repeating the procedure to obtain the organic extract. The upper-layer fractions were combined and evaporated in a fume hood until a volume of 1 mL was reached, transferred to a 1.5 mL Eppendorf tube, and allowed to evaporate completely in the hood. After this process, 50 μL of methanol were added to recover the extract, which was stored at −20 °C until use (Grosso-Becerra *et al.*, 2014). For C4-HSL confirmation, thin-layer chromatography (TLC) plates silica gel 60 F254 (MERCK® 105554) were used. Using a 1 μL micropipette, up to 5 μL of the sample were applied to the spotting point. Synthetic C4-HSL (SIGMA® 09945) and N-hexanoyl-L-homoserine lactone (SIGMA® 56395) were used as standards. Methanol-water (60:40) was used as the mobile phase. Detection was performed using the CV026 biosensor, incubated at 30 °C for 16 h (Grosso-Becerra *et al.*, 2014). Quantitative analysis of the TLC plates was carried out using ImageJ software (Phattanawasin *et al.*, 2016). For the detection of 3O-C12-HSL, a modification of the method reported by Morales *et al.* (2017) and Pearson *et al.* (1997) was used.

In vitro* culture conditions of *S. lycopersicum: The experiment was carried out in a plant growth chamber. A total of 700 *S. lycopersicum* seeds were obtained from the brand Vita®. The disinfection process was performed following the protocol of Rangel-Estrada *et al.* (2015). After the disinfection treatment, seeds were placed in groups of 10 in Petri dishes containing 1.5% agar and allowed to germinate in darkness at 25 °C for 72 h. Subsequently, only germinated seeds were selected and transplanted to Petri dishes containing 1.5% bacteriological agar, to which the treatment to be evaluated was added before gelation (50 °C) to reach the final AHL concentrations (Ortiz-Castro *et al.*, 2008).

The experiment was conducted in an *in vitro* plant culture incubation room under a photoperiod of 16 h of light at an intensity of 200 ± 50 μmol m^{-2} s^{-1}, temperature of 25 ± 2 °C, and relative humidity of 50-60%, followed by 8 h of darkness at 25 °C (Schwarz *et al.*, 2014).

Treatments used in the bioassay with *S. lycopersicum*: The treatments used in this study were as follows: negative control; solvent control (ethyl acetate); crude ethyl acetate extracts of AHLs from wild and control strains; and different final concentrations (10, 12, 24, 48, 96, and 192 μM) of the synthetic AHL molecules C4-HSL (SIGMA® 09945) and 3O-C12-HSL (SIGMA® 56395), respectively, for evaluation. Ten seeds were used per treatment, and three independent experiments were performed in triplicate.

Primary root growth and root hairs: The roots of *S. lycopersicum* were analyzed using an SMZ 10 stereoscopic microscope (NIKON®) after seven days of exposure. Root hairs were counted at 3x magnification, and micrographs were captured using a Motic Cam 5 5.0 MP camera (MOTIC®). The results were interpreted following the methodology described by Ortiz-Castro *et al.* (2008), and root hair density was calculated using the protocol of Ortiz *et al.* (2024).

Statistical analysis: The significance of the estimated concentrations of the main AHLs produced by *P. aeruginosa* strains was determined by one-way ANOVA with Tukey's *post hoc* test. The significance of the treatments in the *in vitro* bioassay with *S. lycopersicum* was determined by one-way ANOVA at a significance level of 0.05 ($p<0.05$), followed by Dunnett's *post hoc* test. The following software packages were used: SigmaPlot v16 for data tables, GraphPad Prism v8 for graph preparation, and ImageJ2 for quantitative measurement of TLC plates.

RESULTS AND DISCUSSION

Three environmental *P. aeruginosa* strains were isolated, selected, and identified, originating from the rhizosphere of maize and tomato, along with one clinical isolate. In addition, two control strains were included in this study, namely the PAO1 strain and the double mutant of the autoinducer synthase genes, as shown in Table 1. To strengthen the study, final concentrations of 10, 12, 24, 48, 96, and 192 μM of the synthetic AHL molecules C4-HSL and 3O-C12-HSL, respectively, were also included.

Regarding the concentration of AHL molecules in the extracts of each strain, PAO1 showed a concentration of 11.32 μM for 3-oxo-C12-HSL and 0.70 μM for C4-HSL, whereas no AHLs were detected in the mutant strain. In the wild strains, differential concentrations of both AHL molecules were detected, as shown in Figure 1.

Primary root growth and root hair density. Figure 2 shows the effect of crude ethyl acetate extracts from wild strains and the double mutant of *P. aeruginosa*, which caused a clear decrease in primary root length and root hair density, with statistical significance ($p<0.05$). In addition, strain MAZ 0105 inhibited primary root elongation and root hair density by 32%, with statistical significance ($p\leq0.05$).

In contrast, stimulation with synthetic AHLs at different concentrations produced an increase in primary root length at concentrations of 12, 24, and 48 μM for C4-HSL and 12 μM for 3O-C12-HSL, whereas a decrease was observed when roots were exposed to 3O-C12-HSL at a concentration of 192 μM (Figure 3).

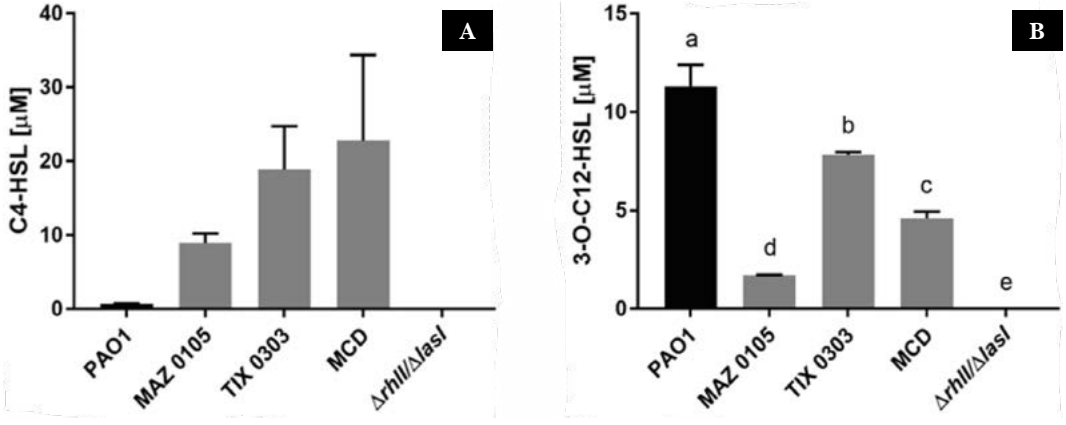

Figure 1. Estimation of the concentrations of the main AHLs produced by *P. aeruginosa* strains. (A) C4-HSL. Bars represent the mean ± SD (n=2). (B) 3O-C12-HSL. Bars represent the mean ± SD (n=3). Different letters indicate statistically significant differences ($p<0.05$).

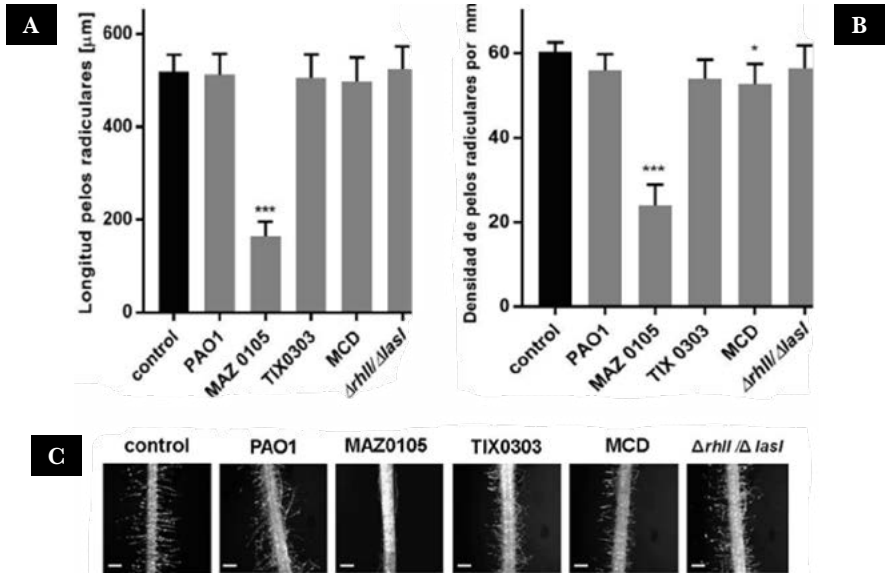

Figure 2. Primary root length and root hair density of *S. lycopersicum* exposed to crude ethyl acetate extracts from wild and control strains. (A) Root length. Bars represent the mean ± SD (n=30). (B) Root hair density per mm^2. Bars represent the mean ± SD (n=9), with statistically significant differences: ***=p≤0.05. (C) Micrographs at 3x magnification of seedlings from the strains; white bars correspond to 200 μm.

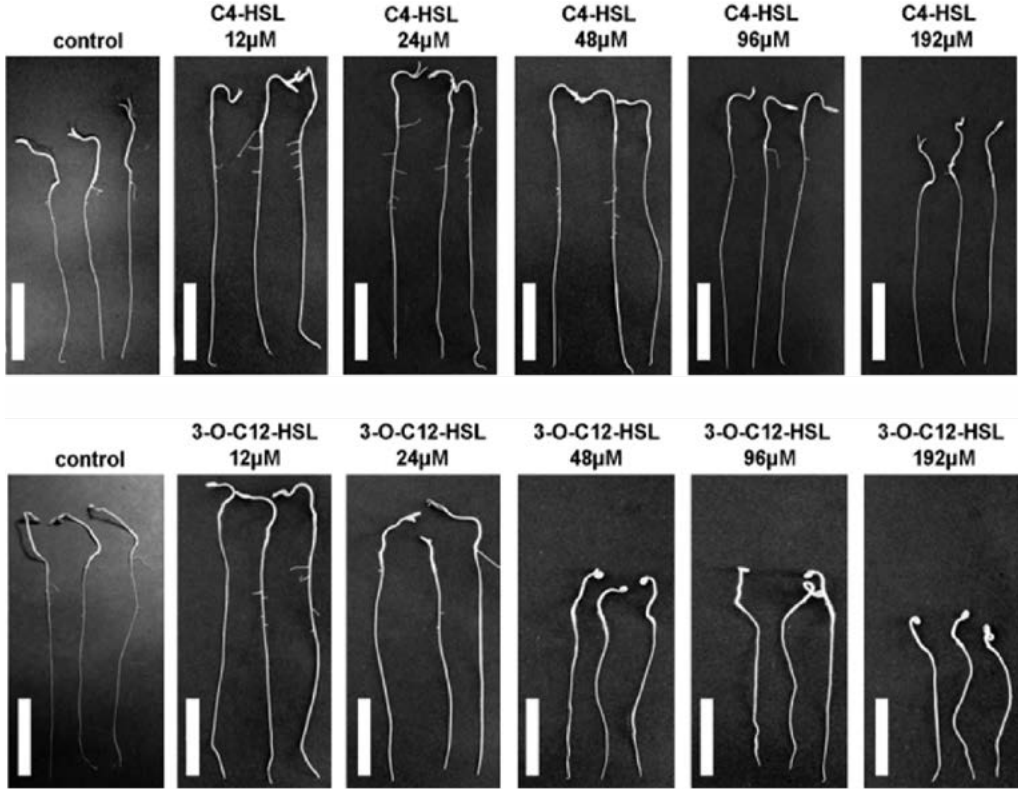

Figure 3. Images of seedlings exposed to different concentrations of synthetic AHLs. The upper panel shows the effects of C4-HSL and the lower panel shows those of 3O-C12-HSL. White bars correspond to 3 cm.

Plants treated with 3O-C12-HSL at a concentration of 24 μM showed an increase in root hair length, and at concentrations from 48 to 192 μM, both root hair length and density increased, with statistical significance ($p<0.05$). For C4-HSL, an increase in root hair length was observed only at 24 μM, whereas an increase in root hair density was observed at concentrations of 96 μM and 192 μM, with statistically significant differences compared to the control ($p<0.05$) (Figure 4).

The search for new strategies to promote plant growth and to produce food with higher nutritional value in a sustainable and low-cost manner has led us to investigate whether QS system molecules from *P. aeruginosa* can enhance primary root development and increase root hair density under *in vitro* conditions, thereby providing evidence for their potential use in agricultural production models. However, in this study, the results demonstrate that crude ethyl acetate extracts obtained from wild and control *P. aeruginosa* strains are not able to promote root elongation or root hair density under *in vitro* conditions in *S. lycopersicum* (Figure 2), suggesting that their concentrations may be too low to exert an effect. In contrast, strain MAZ0105 significantly inhibited root and root hair development, which further confirms that these molecules are present at very low concentrations and are insufficient to stimulate primary root and root hair development. Therefore, we confirmed a favorable inductive effect of both synthetic AHLs on root growth at a concentration of 24 μM and on root hair density at concentrations of 96 μM, respectively (Figure 4). In contrast, a concentration of 192 μM of 3O-C12-HSL reduced primary root growth but promoted root hair development in both length and density, in agreement with what has

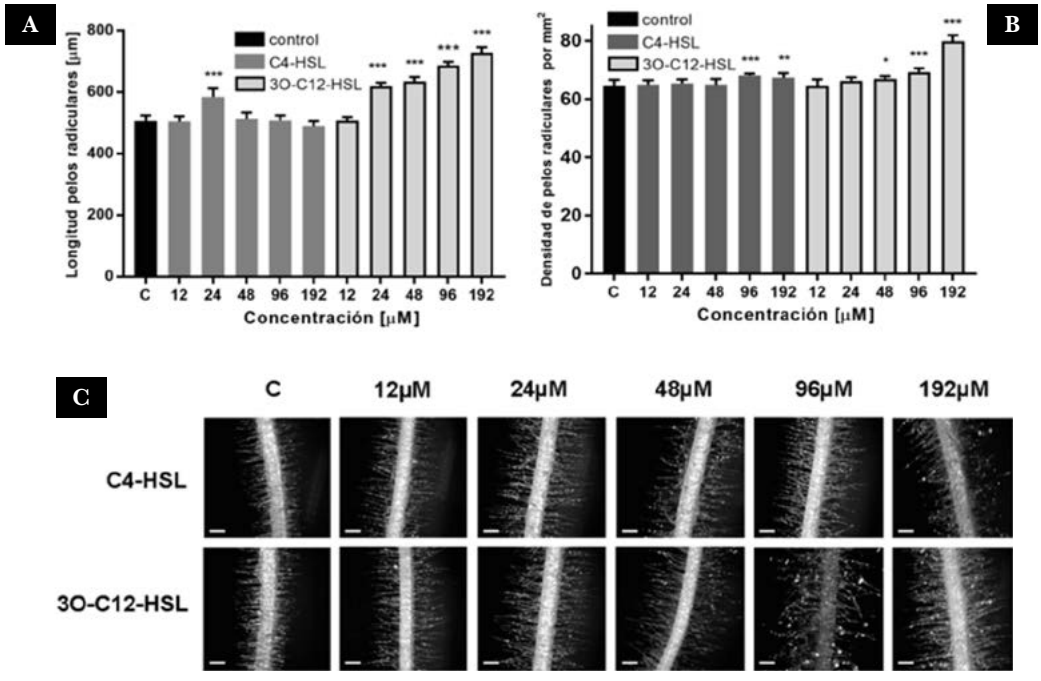

Figure 4. Determination of root hair length and density. (A) Root hair length and (B) root hair density of *S. lycopersicum* exposed to synthetic C4-HSL and 3O-C12-HSL, respectively. Bars represent the mean ± SD, with statistically significant differences: ***=$p\leq0.05$. (C) Micrographs at 3x magnification of plants grown with synthetic AHLs. White bars correspond to 200 μm.

been reported for *A. thaliana* (Von Rad *et al*., 2008; Ortiz-Castro *et al*., 2008). Several studies have demonstrated the influence of AHLs on the development of different plant species by modifying the root system and increasing biomass 30 days after application in *Cicer arietinum* and *Triticum aestivum*, as reported by Gahoi *et al*. (2021). In another study, Ibal *et al*. (2021) described the promotion of shoot and root growth when C8-, C10-, and C12-HSL were used, respectively. However, caution should be exercised in the use of these molecules, as they may alter the substrate microbiome and generate undesirable biological processes. Another study reported that the use of AHL-producing microorganisms is capable of promoting germination and increasing dry weight, shoot development, and root length in lettuce (*Lactuca sativa*) (Ortiz J. *et al*., 2024).

Although the use of AHL molecules to improve root development, plant height, and biomass production is very promising, it has been reported that treatments of *C. equisetifolia* with 3O-C10-HSL, 3O-C12-HSL, and 3O-C14-HSL significantly affect all developmental parameters under continuous monoculture, and that this effect may be due to the fact that these molecules promote the development of microbial communities in the soil and, in turn, through their enzymatic effects on soil nutrients, inhibit their availability, thereby negatively impacting plant development (Zhang, 2025). This indicates that we must be cautious in regulating AHL concentrations in agriculture, as they may enhance or ultimately impair biomass production.

The use of microbial molecules opens the possibility of promoting root and root hair development in various plant species, which may improve adaptation to environmental and soil conditions where they are cultivated (Tron *et al*., 2015). Our results in *S. lycopersicum* show that, although the AHLs studied share some effects at certain concentrations, C4-HSL tends to enhance longitudinal growth of the primary root, whereas 3O-C12-HSL more strongly promotes the growth and development of root hairs. Another advantage of using these AHLs as plant growth promoters in *S. lycopersicum* is that 3O-C12-HSL has been reported to induce systemic resistance against some pathogens (Hartmann *et al*., 2021). Nevertheless, further studies are still needed to demonstrate that these AHL molecules are beneficial and safe for agricultural applications.

CONCLUSIONS

The use of PGPB in agriculture represents an alternative to increase plant production; however, studying microorganisms that produce signaling molecules such as AHLs is a promising area for enhancing root development, biomass, and fruit production. In this study, we found that synthetic C4-HSL and 3O-C12-HSL in *S. lycopersicum* exhibited in vitro effects on primary root growth at a concentration of 24 μM and on root hair density at concentrations of 96 μM, respectively, whereas the concentrations obtained in crude ethyl acetate extracts were unable to generate a positive effect.

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Comparative quality analysis of habanero pepper (*Capsicum chinense* Jacq.) genotypes in Baja California

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ABSTRACT

Objective: To analyze the quality of genotypes habanero pepper (*Capsicum chinense* Jacq.) produced in Baja California.

Methodology: Experimental materials and a commercial control were evaluated in two locations. The results were analyzed using a completely randomized design with ten treatments and four replications, following the methodology described by Steel and Torrie (1980). Due to its high content of capsaicinoids accumulated in the fruit, the habanero pepper is a vegetable of interest to the pharmaceutical industry. It has been observed that capsaicin concentration may vary due to environmental effects, water and nutritional stress, low temperatures, lack of radiation, and low relative humidity.

Result: The habanero pepper represents a symbol and example of pungency. In this study, the concentration of capsaicin (Ccap), °Brix, and shelf life (SL) after harvest were determined and compared among habanero pepper (*Capsicum chinense* Jacq.) genotypes grown under greenhouse, open-field, and shade-net conditions in Baja California.

Conclusions: There are significant differences among the evaluated genotypes for each variable; however, these genotypes are promising candidates for further selection with commercial purposes.

Keywords: *Capsicum chinense*, Calidad, Invernadero, Genotipos.

INTRODUCTION

Before the discovery of the American continent, the inhabitants were already domesticating and improving chili plants. Its consumption is closely linked to the history of Mexico, as it is widely accepted that the first American settlers were the ones who domesticated this important crop. Archaeological evidence dating from 5200 to 3400

BC shows that Native Americans cultivated chili plants. Interest in chili cultivation is not only due to its economic importance but also because it has been demonstrated to be a significant source of natural colorants, vitamins (A, C, D, and E), and minerals, in addition to its characteristic pungency provided by capsaicin. Chiles have also been found to contain phytochemical compounds that exert beneficial effects on human health (Guzmán & Paredes, 1998).

Authors such as Borges *et al.* (2008) conducted a study to determine capsaicinoids in habanero pepper grown under different moisture and nutrient conditions. They found a significant response in capsaicin content with plant age, whereas dihydrocapsaicin did not show such a response. Fruit yield exhibited a significant response to increased nutrition and moisture, reaching an average of 1,391 g of fruit per plant at the highest nutrition and available moisture level. A significant relationship was observed between capsaicin content and third-grade fruit yield, as well as between dihydrocapsaicin content and the yield and number of second-grade fruits. Mexico is the country with the greatest genetic diversity of *Capsicum* in the world. Its genetic richness is largely due to the diversity of climates and soils, but also to traditional cultivation practices carried out by small-scale farmers using seeds from fruits selected from native plants (Latournerie *et al.*, 2002).

Among the great diversity of the *Capsicum* genus, the habanero pepper (*Capsicum chinense* Jacq.) has become a symbol and prime example of pungency due to its highest capsaicin content found in the fruit (Laborde & Pozo, 1984). The importance of capsaicinoids lies not only in providing the spicy flavor but also in their use by the pharmaceutical (Salazar-Olivo & Silva-Ortega, 2004), weapons, tobacco, cosmetic, and paint industries, among others, as an active ingredient in various products.

According to Harvell and Bosland (1979), the levels of pungency in chili are determined by two factors: the plant's genetics and those that interact with the environment. Studies have shown different responses of capsaicinoid content to water stress and mineral nutrition; for instance, in *Capsicum annuum* L. cv. Padrón, water stress had a strong effect on capsaicin production (Bernal *et al.*, 1995; Estrada *et al.*, 1999). In contrast, Velasco *et al.* (2001) reported that increasing the supply of N, P, and K in jalapeño pepper (*Capsicum annuum* L.) decreased capsaicin production.

To date, the effect of different moisture regimes and the application of N, P_2O5, and K_2O on capsaicinoid synthesis in habanero pepper remains unknown. Recently, there has been interest in quantifying certain antioxidant constituents of fruits and vegetables due to their potential functionality against various diseases, including diabetes, cancer, cardiovascular diseases, and neurodegenerative disorders such as Alzheimer's disease (Kaur & Kapoor, 2001).

Several studies have been conducted to identify the amounts of these compounds in species of the *Capsicum* genus. These studies have included different species as well as their various forms of consumption (fresh, dried, and processed); however, the results obtained are often inconsistent, as the amounts sometimes vary within the same species. Due to its high content of capsaicinoids accumulated in the fruit, the habanero pepper is a vegetable of interest to the pharmaceutical industry. It has been observed that the concentration of this substance can vary due to environmental conditions, water and nutritional stress, low

temperatures, lack of radiation, and low relative humidity. It has also been recognized that among the great diversity of the *Capsicum* genus, the habanero pepper represents a symbol and example of pungency. Given that capsaicinoids are compounds of interest, this study determined and compared the concentration of capsaicin (Ccap), °Brix, and shelf life (SL) after harvest in habanero pepper (*Capsicum chinense* Jacq.) genotypes grown under greenhouse, open-field, and shade-net conditions in Baja California.

MATERIALS AND METHODS

The study was conducted in the postharvest laboratory at the Agricultural Sciences Institute of the Autonomous University of Baja California, located in Ejido Nuevo León, Mexicali Valley, Baja California. The statistical model used was based on the methodology of Steel and Torrie (1980), for which a completely randomized design with ten treatments and four replications was applied, using an additive linear model.

$$y_{ij} = m + t_i + e_{ij}$$

The capsaicin content was determined using the method proposed by Davis *et al.* (2007). The procedure performed to determine the capsaicin content in habanero pepper (*Capsicum chinense* Jacq.) genotypes was as follows:

1. Weigh 1 g of fresh habanero pepper tissue,
2. macerate it in a glass tube,
3. add 10 mL of pure acetone,
4. blend the tissue to obtain a homogeneous sample,
5. heat the mixture in a water bath for 30 minutes at 60 °C,
6. allow the samples to cool to room temperature,
7. centrifuge the sample at 1000 rpm for 10 minutes,
8. let the sample cool and measure the absorbance using a spectrophotometer (Thermo Scientific Genesys 20) at a wavelength of 280 nm,
9. finally, record the readings and use the regression equation to adjust the data and determine the capsaicin content.

To evaluate the shelf life of the genotypes, ten fruits from each treatment and replication were selected. Data on polar and equatorial diameter, fruit weight, and total fruit weight were recorded. The fruits were placed in paper bags labeled with the corresponding genotype information and then transported to the laboratory, where they were stored in a refrigerator at 8 °C and 90% relative humidity. Measurements were taken weekly, and data were recorded throughout the entire conditioning period. For the shelf life data collection, the first measurement was conducted on September 30, 2017, and the last reading on November 30 of the same year, giving an average shelf life of 60 days for some genotypes. At the time of the final reading, the visual quality of the fruit was at 70%. To determine fruit quality, fruits without defects, damage, or deterioration were visually assessed, and the fruit color intensity was recorded. For °Brix measurements, a sample of fruits was juiced,

and a drop of the juice was placed on a digital refractometer to take the reading. The refractometer used was an ATAGO PAL-1 model. The results of the evaluated variables were subjected to analysis of variance (ANOVA) and multiple mean comparisons using Tukey's test ($p \leq 0.05$) in the R statistical software package.

RESULTS AND DISCUSSION

Tables 1, 2, and 3 show the results of the qualitative variables of habanero pepper genotypes grown under greenhouse, open-field, and shade-net conditions in the state of

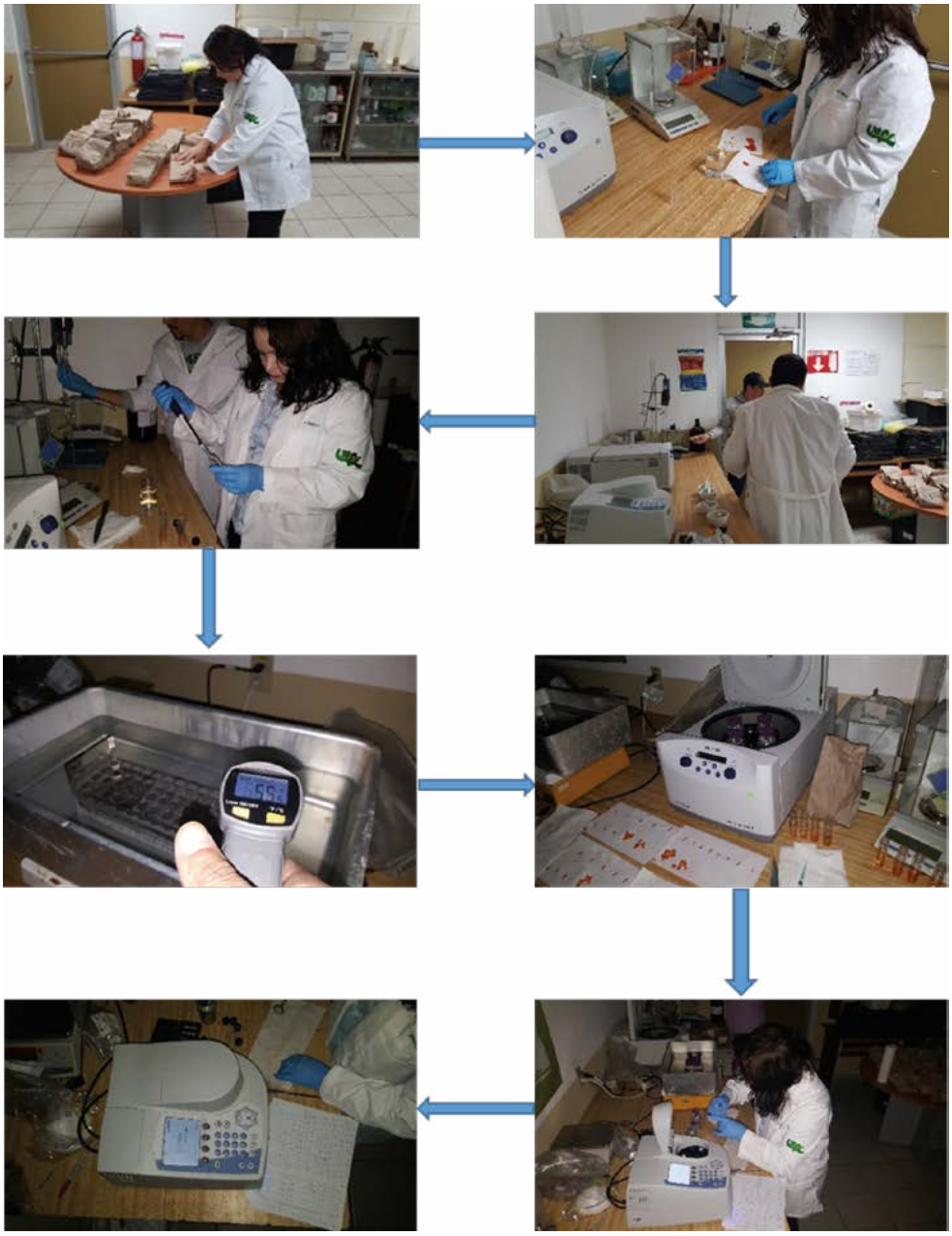

Figure 1. Flowchart for the determination of capsaicin content in habanero pepper (*Capsicum chinense* Jacq.) fruits at the Agricultural Sciences Institute laboratory. December, 2017.

Table 1. Results of the mean comparison for qualitative variables in 10 habanero pepper (*Capsicum chinense* Jacq.) genotypes grown under greenhouse conditions during the 2017 cycle. San Quintín, Ensenada, B.C.

Genotypes	°Brix	VA	Ccaps	UE
HRA 1-1	8.48 b	44.25 e	21.49 a	322,250 a
HRA 7-1	8.30 b	45.75 e	20.03 ab	300,000 ab
HAN 1-30	9.95 a	65.25 ab	19.86 ab	297,500 ab
HAN 25	9.75 a	64.25 abc	20.16 ab	301,750 ab
Jaguar INI	8.75 ab	58.25 d	19.49 b	291,750 b
HNY 201	9.70 a	61.25 abcd	19.86 ab	297,250 ab
HUX 15-1	9.52 a	65.50 ab	18.63 b	279,000 b
HQR 15-3	9.90 a	66.75 a	20.17 ab	302,000 ab
HAN 1-40	9.97 a	60.75 bcd	20.19 ab	302,500 ab
Jaguar Yuc	8.62 ab	59.00 cd	21.55 a	323,000 a

°BRIX=degrees brix, VA=shelf life in days, Ccaps=mM total capsaicin in g^{-1} fresh weight of fruit, UE=Scoville unit.

Baja California during the 2017 and 2018 cycles. Different heterogeneous groups were observed for each of the evaluated variables. The mean comparison using Tukey's test at $p \leq 0.05$ showed heterogeneity of variances and significant differences among the genotypes under study. The conditioning atmosphere for evaluating shelf life was maintained at a constant temperature of 8 °C and a relative humidity (RH) of 90%.

Shelf life

The shelf life of the genotypes grown under greenhouse conditions showed significant differences. The mean comparison test yielded five groups (a, b, c, d, e), indicating heterogeneity of variances and that the genotypes exhibit different shelf lives under the controlled-atmosphere conditions in which they were established. The red habaneros (HRA 1-1 and HRA 7-1) had a shelf life of 44 and 45 days, respectively, whereas lines HQR 15-3 and HAN 1-30 remained in postharvest for 66 and 65 days, respectively. The commercial variety Jaguar showed an average postharvest shelf life of 58 days, reaching up to 74 days. All evaluated genotypes maintained 70% of their quality characteristics until the end of their shelf life. In Table 2, the genotypes grown under open-field conditions are shown, which exhibited heterogeneity of variances with three heterogeneous groups (a, b, c), indicating significant differences among genotypes in terms of postharvest days. The red habanero lines showed shorter shelf life compared to the orange habaneros. HAN 1-30 had 63 postharvest days while maintaining 70% of its quality characteristics, whereas the HRA 1-1 line remained for 46 postharvest days. For the shelf life of genotypes harvested under shade-net conditions in the Mexicali Valley, the lines HAN 1-30 and HRA 1-1 were maintained under controlled atmosphere at 8 °C and 90% relative humidity for 51 and 43 days, respectively. The postharvest treatment received by fruits intended for consumption influences this quality parameter. Many types of chili peppers are dehydrated for consumption, such as pasilla, mulato, chiltepin, and bird's beak chili, among others.

Table 2. Results of the mean comparison for qualitative variables in 10 habanero pepper (*Capsicum chinense* Jacq.) genotypes grown under open-field conditions during the 2017 cycle. San Vicente, Ensenada, B.C.

Genotypes	°Brix	VA	Ccaps	UE
HRA 1-1	8.48 de	46 c	21.49 a	332,250 a
HRA 7-1	7.83 e	53 bc	20.28 abc	303,750 abc
HAN 1-30	9.50 ab	63 a	19.85 bc	297,500 bc
HAN 25	9.75 a	55 abc	20.15 abc	301,750 abc
Jaguar INI	8.38 de	48 bc	20.98 ab	314,250 ab
HNY 201	8.70 bcd	56 ab	19.86 bc	297,500 bc
HUX 15-1	9.53 ab	55 abc	19.38 c	290,250 c
HQR 15-3	9.40 abc	57 ab	20.17 abc	302,000 abc
HAN 1-40	9.48 ab	50 bc	20.44 abc	306,250 abc
Jaguar Yuc	8.62 cde	51 bc	21.55 a	323,000 a

°BRIX=degrees brix, VA=shelf life in days, Ccaps=mM total capsaicin in g^{-1} fresh weight of fruit, UE=Scoville unit.

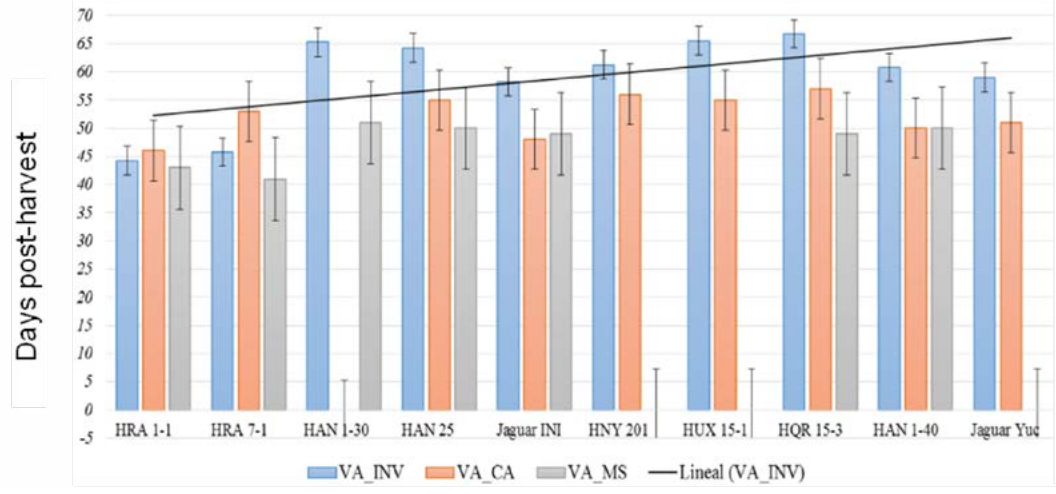

Figure 2. Shelf life of habanero pepper (*Capsicum chinense* Jacq.) genotypes evaluated under greenhouse, shade-net, and open-field conditions in Baja California, November 2017 and 2018.

In a study conducted by Vega *et al.* (2009), several drying temperatures (50-90 °C) were evaluated on red chili fruits (*Capsicum annuum* L.) for their effect on ascorbic acid concentration. The results showed more than 90% degradation of this chemical compound at all temperatures tested. Transpiration, dehydration, or water loss of fruits during postharvest constitutes the main factor that reduces their consumption quality. It has been observed that when fruits lose 6-7% of their weight, firmness and appearance decrease, consequently affecting quality and shelf life (Báez *et al.*, 2005).

°Brix

The sugar concentration in the habanero pepper genotypes showed significant differences across the evaluated environments. Tables 1, 2, and 3 present the mean values of the genotypes, showing heterogeneity of variances among each genotype, with

Table 3. Results of the mean comparison for qualitative variables in seven habanero pepper (*Capsicum chinense* Jacq.) genotypes grown under shade-net conditions during the 2018 cycle. Mexicali Valley, Baja California.

Genotypes	°BRIX	VA	Caps.	UE
HRA 1-1	8.57 b	43 bc	20.85 a	312,330 a
HRA 7-1	8.90 ab	41 c	21.63 a	324,000 a
HAN 1-30	9.47 a	51 a	20.96 a	314,000 a
HAN 25	8.87 ab	50 a	20.48 a	306,670 a
Jaguar INI	8.90 ab	49 ab	20.67 a	309,670 a
HQR 15-3	8.90 ab	49 ab	20.58 a	308,330 a
HAN 1-40	8.90 ab	50 a	20.20 a	302,670 a

°BRIX=degrees brix, VA=shelf life in days, Ccaps=mM total capsaicin in g^{-1} fresh weight of fruit, UE=Scoville unit.

five heterogeneous groups identified (a, b, c, d, e). The overall mean Brix value for the genotypes was 9.2%. In Figure 3, the mean comparison of sugar content across all genotypes established in the three evaluation environments is shown. No variation or significant differences were observed among the three environments for this variable.

Capsaicin Content (Capsaicin)

The capsaicin content for each genotype showed significant differences in the greenhouse and open-field environments, whereas the genotypes evaluated under shade-net conditions did not exhibit significant differences for this variable. In the greenhouse and open-field, three heterogeneous groups (a, b, c) were identified, indicating significant differences among the genotypes, and the Tukey test showed heterogeneity of variances (Tables 1 and 2). In Figure 4, the distribution of data regarding capsaicin content in all genotypes grown under greenhouse, shade-net, and open-field conditions is shown. The environment with the highest capsaicin content in its genotypes was the shade-net in the Mexicali Valley. These results are consistent with those reported by Morales *et al.* (2020), who conducted a study to determine capsaicinoids in habanero pepper genotypes

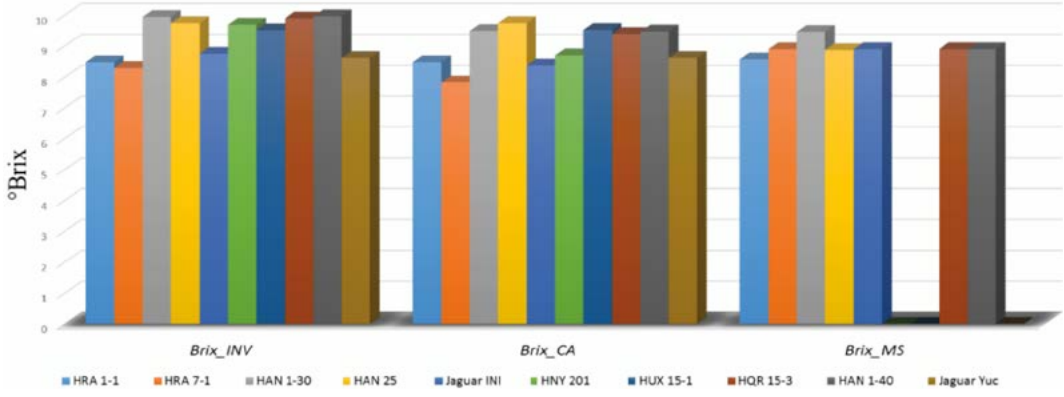

Figure 3. Sugar content of habanero pepper (*Capsicum chinense* Jacq.) genotypes evaluated under greenhouse, shade-net, and open-field conditions in Baja California, October 2017 and 2018.

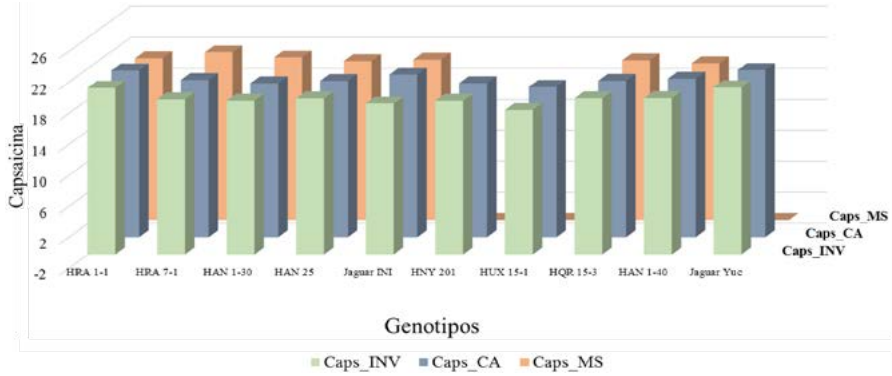

Figure 4. Capsaicin content per gram of fruit in habanero pepper (*Capsicum chinense* Jacq.) genotypes evaluated under greenhouse, shade-net, and open-field conditions in Baja California, December 2017 and 2018.

evaluated under greenhouse conditions. They reported that the highest total capsaicinoid content occurred at the commercial maturity stage of the fruits, with an average of 2.65 mg g^{-1} of capsaicin. On the other hand, Montoya *et al.* (2010) indicate that in some chili species, the highest concentrations of capsaicinoids are found in mature fruits. This reflects the differing behavior of the evaluated genotypes. The environment plays a decisive role in capsaicinoid concentration, as higher temperatures result in spicier peppers. Differences were observed between the results obtained under greenhouse and open-field conditions compared to those grown under shade-net in the Mexicali Valley, taking into account the extreme summer temperatures, which range between 40 and 50 °C. Borges *et al.* (2010), in their study on capsaicinoids in habanero pepper under different moisture and nutrient conditions, observed a significant response of capsaicin content to plant age. Velasco *et al.* (2001) reported that increasing the N, P, and K supply in jalapeño pepper reduced capsaicin production. To date, it remains unknown whether this effect is related to moisture and nutrition in the crop.

Scoville Units (US)

The pungency value for each genotype was calculated using the official AOAC method (1995), where 0.001 mg g^{-1} of capsaicinoids is equivalent to 15 Scoville units (US). Tables 1, 2, and 3 present the mean results for this variable in each habanero pepper genotype. The overall mean US was 311,000 units, with a coefficient of variation of 3.16% and a relative efficiency of 0.91, indicating that the design employed did not reduce the effect of experimental error. Capsaicin content is responsible for the pungency of habanero pepper fruits (González *et al.*, 2013), which showed significant variation among genotypes and maturity stages. Figure 5 shows the US values (pungency) of the genotypes under study. These results are consistent with those reported by SIAP (2016), which indicate that the US of habanero pepper range from 100,000 to 445,000.

In Figure 6, the characteristics of the six genotypes selected during the two evaluation cycles (2017-2018) for the agricultural regions of Baja California under greenhouse, shade-net, and open-field conditions are shown. These characteristics are based on phenotypic observations and characterizations of the genotypes, as reported by Ramírez *et al.* (2012),

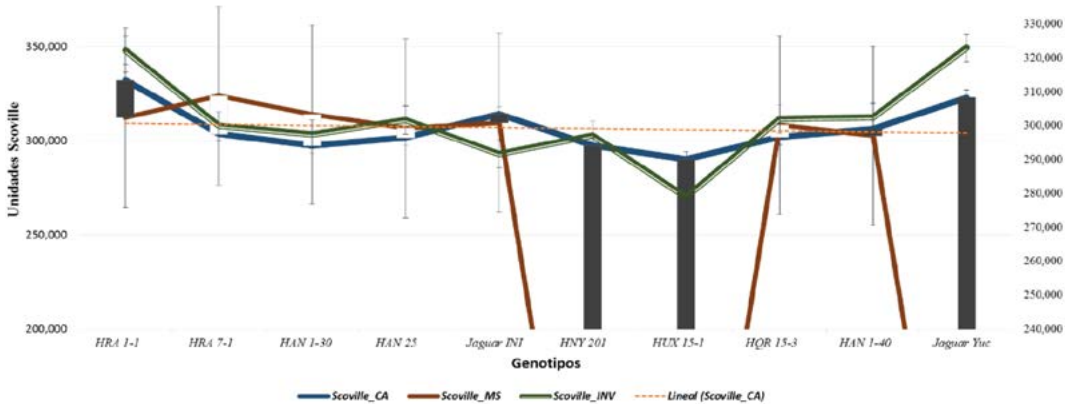

Figure 5. Scoville units (US) of habanero pepper (*Capsicum chinense* Jacq.) genotypes evaluated under greenhouse, shade-net, and open-field conditions in Baja California, December 2017 and 2018.

								
Color of unripe fruit		**Green**	**Pale Green**	**Green**	**Green**	**Pale Green**	**Green**	**Green**
Color of ripe fruit		Orange	Red	Orange	Orange	Red	Orange	Orange
Fruit weight in grams		9.3	10.8	10.2	10.2	10.4	9.4	9.7
Number of locules		3 to 4	3	3 to 4	3 to 4	3	3 to 4	3 to 4
Yield per plant in kilograms		1.9	2.2	2.1	2.2	2.5	1.8	2
mM total capsaicin in g^{-1} fresh weight of fruit		17.26	21.15	20.57	19.82	22.16	20.78	19.29
Shelf life		59	43	61	58	41	59	65
Degrees Brix		8.9	8.5	9.8	10.2	8.9	9.6	10.1
Yield in tons per hectare		58	55	58	68	53	53	55

Figure 6. Habanero pepper (*Capsicum chinense* Jacq.) genotypes selected over two evaluation cycles for continued use in the breeding program in Baja California. April, 2020.

who emphasized phenotypic characterization and varietal description of the experimental lines from the habanero pepper breeding program in the Huasteca region of Tamaulipas, Mexico. These efforts led to the development and validation of the commercial variety Jaguar.

CONCLUSIONS

Of the ten genotypes (Jaguar-INIFAP, HRA 7-1, HNY 201, HAN 1-30, HRA 1-1, HAN 25, HAN 1-40, HQR 15-3, HUX 15-1, and Jaguar Yucatán) grown under open-field and greenhouse conditions in the Ensenada Coastal Zone, seven were selected for further breeding based on phenotypic, qualitative, and quantitative characteristics of

the materials. Six experimental lines (HRA 7-1, HAN 1-30, HRA 1-1, HAN 25, HAN 1-40, HQR 15-3) were evaluated under shade-net conditions in the Mexicali Valley and compared with the commercial variety Jaguar as a control.

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Arbuscular fungi: key organisms for the symbiotic association in the genera *Agave* L. and *Opuntia* (L.) Mill

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ABSTRACT

Objective: Conduct a review of scientific literature with the aim of synthesizing current knowledge on the benefits of arbuscular mycorrhizal fungi (AMF) in *Opuntia* and *Agave* species to propose their use in the Complementary Agriculture (AgriCom) model, strengthening semi-desert production systems.

Design/methodology/approach: A systematic review (2013-2025) was conducted in databases (using keywords in English and Spanish) of studies that mentioned the role of AMF in *Opuntia* spp. and *Agave* spp.

Results: The review shows that symbiosis with AMF significantly improves biomass (increases of 35%-60% in *Opuntia*, 30%-55% in *Agave*), nutrient uptake (especially phosphorus, by 40%-70%), and drought tolerance in both genera. AMF increase water absorption in plant tissues, reduce oxidative stress, and promote more extensive root systems. Native AMF strains often showed superior benefits compared to commercial strains.

Limitations on study/implications: Most studies on Agavaceae were conducted under greenhouse conditions; therefore, further comparative field studies are required.

Findings/conclusions: Scientific evidence confirms that symbiosis with AMF is a key strategy for improving the productivity and resilience of *Opuntia* and *Agave* in adverse conditions. The use of AMF in the AgriCom model can increase crop growth.

Keywords: Arbuscular mycorrhizal fungi, *Opuntia*, *Agave*, water stress, AgriCom.

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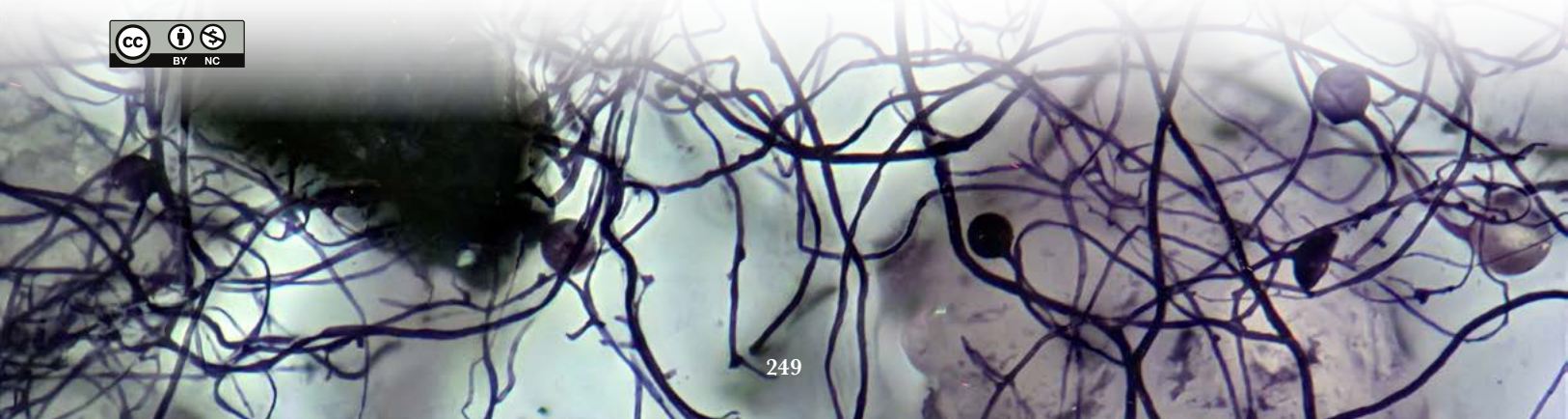
INTRODUCTION

In Mexico, the semidesert has extreme climatic conditions such as low rainfall, high temperatures, and degraded soils. These conditions cause erosion and biodiversity loss, which in turn limit soil fertility and the soil's water-retention capacity. These factors affect agricultural productivity and food security in rural communities (Cotler *et al.*, 2020). In the Potosino-Zacatecan semidesert, years with very little rainfall have been recorded (Díaz-Sánchez *et al.*, 2022), along with prolonged drought periods during which vegetation faces high water stress (Martínez & Pugnaire, 2009).



The conventional technologies used over the past few decades are no longer efficient, and conventional agriculture has generated serious social, economic, and environmental impacts. In this regard, the intensive use of agrochemicals has degraded soils, reduced biodiversity, and caused contamination of soils and water resources (Altieri & Nicholls, 2020). Furthermore, the use of monoculture systems has displaced local varieties, overlooking traditional knowledge and community identity (Sevilla Guzmán & Woodgate, 2013).

Although producers in these areas have experimented with new practices to adapt to and overcome climatic constraints, unfortunately this has not been enough (Nicholls & Altieri, 2019). Therefore, it is necessary to establish strategies to address the challenges faced by rural communities, which are highly susceptible to being affected. Agricultural diversification is a strategy to increase ecosystem resilience in order to ensure the sustainability of natural resources (Altieri & Nicholls, 2017).

Complementary Agriculture (AgriCom) is defined as small-scale agricultural and even livestock production modules whose planting, production, and harvesting times vary throughout the year, enabling rural residents to earn income at different intervals —in contrast to the seasonal harvest of a single crop— while benefiting from self-employment and avoiding or limiting migration (Díaz-Sánchez *et al.*, 2022). Currently, the AgriCom model includes 70 nopal genotypes in production and conservation modules (Díaz-Sánchez *et al.*, 2022). In the near future, the implementation of Agavaceae species is expected.

The implementation of productive systems with species such as nopal and agave can be a strategy for conserving the germplasm of these crops, benefiting food security and the economic income of rural communities. The consumption of nopalitos (young nopal pads) and their fruit (tuna) is ancestral (Reyes-Agüero *et al.*, 2005), but production has recently increased due to their nutraceutical properties. The nopal belongs to the Cactaceae family, which makes it ideal for growing in extreme temperatures and with little rainfall, as occurs in arid and semi-arid regions (Torres-Ponce *et al.*, 2015).

On the other hand, 75% of *Agave* species are found in Mexico (García-Mendoza, 2002), from which products such as mezcal, tequila, human food, and animal feed are obtained (Ramírez-Tobías *et al.*, 2014). These plants have the advantage of storing water in their tissues, allowing them to adapt to living in extreme conditions with prolonged periods of drought and high temperatures (García-Mendoza, 2007).

The inclusion of arbuscular mycorrhizal fungi (AMF) in the AgriCom model can help mitigate the effects of drought and erosion, as well as improve soil fertility and increase biodiversity in the Mexican semidesert. AMF, in the ecological context, can facilitate the recovery of soil microbial communities, thereby benefiting the restoration of disturbed areas by improving seedling survival and growth (Carrillo-Saucedo *et al.*, 2022), as well as crop productivity without reliance on chemical fertilizers, which, due to their indiscriminate use, have a negative impact on the environment (Herrera-Monroy *et al.*, 2022). These fungi have developed strategies to overcome the conditions found in these ecosystems, resulting in better use of available water and improved nutrient uptake (Martínez & Pugnaire, 2009).

Based on the above, the first objective of this study was to conduct a literature review to gain an understanding of the current knowledge regarding the various benefits of AMF in ecologically and economically important crops in the Mexican semidesert, such as *Opuntia*

spp. and members of the Agavaceae family (*Agave* spp.), and to suggest their incorporation into the agricultural practices of the Complementary Agriculture (AgriCom) model to strengthen productive systems in arid and semi-arid areas.

MATERIALS AND METHODS

Information search

The information search was conducted through a systematic review (2013-2025) of scientific articles in the Web of Science (WOS) database (Page *et al*., 2021). The search focused on studies addressing the function of AMF in *Opuntia* spp. and *Agave* spp. To narrow down the large number of publications, keyword combinations in English and Spanish were used, which were adjusted based on the results to retrieve articles (Page *et al*., 2021). The "keyword" method (Table 1) was applied to facilitate the process of searching for information on the research topic.

The literature selection was appropriate for the scope of this review, highlighting the benefits of AMF for *Opuntia* and *Agave* species and focusing on growth and drought tolerance.

RESULTS AND DISCUSSION

Studies of cacti

Arid and semi-arid zones cover more than half of Mexico, where the vegetation is diverse (Cervantes-Ramírez, 2005). Cacti, especially the genus *Opuntia*, have held cultural significance in Mexico since the beginning of its history. The morphological and physiological characteristics of the nopal cactus enable it to store water in its tissues in order to survive drought conditions (Reyes-Terrazas *et al*., 2020).

In rural semidesert communities, climatic conditions have intensified the drought, affecting crops (Díaz-Sánchez *et al*., 2022). Therefore, the use of AMF allows plants to increase their tolerance to water stress and nutrient uptake through a strengthened root system and hyphae that are widely distributed in the soil (Barredo-Pool *et al*., 1998). The symbiosis between *Opuntia* spp. and AMF favors successful establishment and productivity in arid and semi-arid zones. According to the scientific articles in this study, the genera *Glomus*, *Rhizophagus*, and *Funneliformis* are the most frequently associated with *Opuntia*, suggesting that they are preferred under stress conditions (Table 2).

Regarding nutrient uptake, all studies agree on the increase in phosphorus concentration in the plant's foliar tissues (cladodes) due to inoculation with AMF. Lahbouki *et al*. (2021)

Table 1. Keywords for information retrieval.

Keyword	*Opuntia* spp.	*Agave* spp.	AMF
Variants	Prickly pear cactus *Opuntia ficus indica* Nopal	*Agave salmiana* Otto ex Salm-Dyck *Agave americana* L. *Agave potatorum* Zucc.	Arbuscular mycorrhizal Micorriza AM
Synonyms and related words	*Nopalea* sp. *Opuntia* species	Maguey Agavaceae	Association Drought stress Growth

Table 2. Studies on cacti (*Opuntia*) and arbuscular mycorrhizal fungi.

Reference	*Opuntia* species/variety	AMF	Greenhouse/ Field	Benefits for *Opuntia* spp.
Pimienta-Barrios *et al.* (2003)	*Platyopuntia* sp.	Unspecified (native AMF)	Field (without inoculation, using benomyl to inhibit AMF)	30%-50% increase in CO_2 absorption Better root development (40%) Greater water retention under drought (25%) Reduction of oxidative stress
Neffar *et al.* (2015)	*O. ficus-indica*	*Glomus* spp. *Gigaspora* sp.	Field	Colonization ranges from 40% to 80% depending on the season. 45% increase in phosphorus uptake Greater drought resilience (30%) Maximum colonization in spring/ autumn
Bashan *et al.* (2000)	*Opuntia* spp. (plántulas)	*Glomus aggregatum* (*Rhizoglomus aggregatum* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl) (*Rhizophagus intraradices* N.C. Schenck & G.S. Sm) C. Walker & Schüßler)	Field Inoculation	50% greater growth under mesquite trees 90% survival in inoculated seedlings and 65% in non-inoculated plants Synergistic effect with nurse trees
Estrada-Luna & Davies (2001)	*Opuntia albicarpa* Scheinvar	*G. intraradices* (*R. intraradices*) *G. deserticola* (*Viscospora deserticola* (Trappe, Bloss & J.A-Mente) Tedersoo & Magurno, comb. nov.)	*In vitro*/*ex vitro* inoculation	40-60% increase in biomass Improved phosphorus absorption (70%) 25% greater water efficiency Post-transplant stress reduction
Lahbouki *et al.* (2021)	*O. ficus-indica*	*Rhizophagus irregularis* (Blaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler *Claroideoglomus etunicatum* (*Entrophospora etunicata* (W.N. Becker and Gerd.). Blaszk., Niezgoda, B.T. Goto, and Magurno, comb. nov.) *Glomus aggregatum* (*Rhizoglomus aggregatum* (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl)	Inoculation + vermicompost	Biomass increase (60% with vermicompost) Increase in phenolic compounds and antioxidants Higher relative water content in tissues (20%) 30% better water use efficiency (WUE)

Note: *Platyopuntia* is a historical taxonomic term for flat-cladode cacti now included within the genus *Opuntia*.

reported a 40% to 70% higher concentration of this nutrient in nopal. On the other hand, it has been shown that biomass increases by 35% to 60% even under water stress (Estrada-Luna & Davies, 2001; Pimienta-Barrientos *et al.*, 2003). Mention is also made of the use of AMF with amendments such as vermicompost, which, on its own, is presented as an alternative for soil recovery by improving nutrient availability (Flórez-Muriel, 2020).

AMF reduce the impact of drought through various mechanisms: a) maintaining higher water content (20%) in plant tissues (Pimienta-Barrios *et al.*, 2003); b) increasing the production of antioxidant metabolites (up to 65%), protecting nopal plants from cellular damage (Lahbouki *et al.*, 2021); and c) enhancing AMF colonization during periods of higher moisture content, thereby optimizing their activity (Neffar *et al.*, 2015). Based on the literature, the alliance between AMF and plants of the genus *Opuntia* can be confirmed, improving their growth, nutrition, and tolerance to water stress —fundamental factors in semiarid production systems with a limiting climate.

Studies of Agavaceae

Agave (also known as maguey) is found in many arid and semi-arid regions of Mexico, where its main appeal lies in the production of mezcal (Corona-Romero *et al.*, 2022). These types of ecosystems are characterized by low rainfall and prevailing high temperatures, to which the maguey must adapt (Mayagoitia-Toulet & Zamora-Gutierrez, 2024).

Agaves are important: a) ecologically, as they prevent erosion, provide refuge for various organisms, and supply resources for animals; and b) economically, since their extraction is aimed at producing food, beverages, and fibers (Mayagoitia-Toulet & Zamora-Gutiérrez, 2024). Furthermore, the popularity of their alcoholic beverages has increased interest in them, so sustainable harvesting schemes must be in place to prevent their irrational exploitation and the loss of genetic diversity (Mandujano-Bueno *et al.*, 2018).

The use of AMF can promote their conservation, diversity, and production (Trinidad-Cruz *et al.*, 2017), so a literature review on this symbiosis is essential to understand its benefits and how it can contribute to the conscious production of this vegetative resource. According to Table 3, AMF influence increased biomass, tolerance to water stress, and enhanced nutrient uptake. AMF have fundamental benefits for agave development, primarily: vegetative growth and tolerance to abiotic stress.

Table 3. Studies on agavaceous plants (*Agave*) and arbuscular mycorrhizal fungi.

Reference	Agave species/ variety	AMF	Greenhouse/Field	Benefits for Agave spp.
Carballar-Hernández *et al.* (2013)	*Agave potatorum* Zucc.	*Glomus aggregatum* (*R. aggregatum*) *G. etunicatum* (*E. etunicata*) *Acaulospora scrobiculata* Trappe +15 especies	Field	30% increase in root biomass Greater drought tolerance (80%)
Quiñones-Aguilar *et al.* (2023)	*A. tequilana* F.A.C. Weber	*R. intraradices* *Funneliformis mosseae* (T.H. Nicolson & GErd.) C. Walker & A. Schüßler	Inoculation Greenhouse	40% increase in biomass 35% increase in root length 90% drought tolerance
Quiñones-Aguilar *et al.* (2016)	*A. inaequidens* Koch	*R. intraradices* *F. mosseae* *Claroideoglomus claroideum* (*Entrophospora claroidea* (N.C. Schenk & G.S. Sm) Blaszk, NIezgoda, BT. Goto & Magurno)	Inoculation Greenhouse	50% increase in dry biomass Height increase (45%) 85% survival compared to 45% in non-inoculated plants.
Hernández-Cuevas *et al.* (2023)	*A. maximiliana* Baker	*R. aggregatus* (*R. aggregatum*) indigenous *R. intraradices* (allochthonous)	Inoculation/Comparison	38% increase in biomass. Native fungi were more efficient at promoting growth. 92% survival rate in native fungi
Moreno-Hernández *et al.* (2025)	*A. marmorata* Roezl	*R. irregularis* *F. mosseae*	Inoculation Greenhouse	55% increase in biomass 60% increase in root length 88% survival under drought conditions
Chávez-González *et al.* (2023)	*A. salmiana*	*R. irregularis* *F. mosseae* *Diversispora spurca* (C.M. Pfeiff., C. & Bloss) C. Walker & Schüßler)	Field	40% increase in biomass 75% survival rate during drought

In the case of water stress tolerance, plants inoculated with AMF exhibit more extensive and robust root systems, which increases their capacity to explore and absorb water from the soil under water-limited conditions (Quiñones-Aguilar *et al.*, 2023; Moreno-Hernández *et al.*, 2025).

Most studies conducted inoculation in a greenhouse; however, those carried out in the field demonstrate benefits from the AMF association in different *Agave* species. All studies report increased biomass and survival rates exceeding 75% in water-stressed environments (Carballar-Hernández *et al.*, 2013; Chávez-González *et al.*, 2023).

Chávez-González *et al.* (2023) mentioned that bacteria such as *Bacillus subtilis* increased mycorrhizal colonization by 60% and phosphorus solubilization by 35%. The authors conclude that the combined symbiosis of AMF and bacteria may be more effective than the independent inoculation of these microorganisms. Based on the literature, native AMF have advantages over introduced ones, such as greater adaptation to climatic conditions, and in plants they promote a more extensive and robust root system that demonstrates benefits for growth and water tolerance in *Agave*.

Implementation of arbuscular mycorrhizal fungi in the AgriCom model

The AgriCom model works by combining crops that are essential for maintaining soil fertility. Therefore, higher yields per unit area can be achieved, while also promoting biological balance and the production of diverse foods, thereby ensuring food and economic stability for rural families (Gutiérrez-Alzate, 2020). Local plant species or those adapted to the climatic conditions where the communities being served are located are used. In addition, local agrobiodiversity is preserved by including it in the planting modules, whether as a source of forage or human food (Acevedo-Osorio *et al.*, 2020).

In the AgriCom model, inoculation with AMF can be a strategy to enhance the capacity to improve the sustainability and resilience of production systems in arid zones. The scientific evidence analyzed shows that AMF increase biomass, nutrient uptake, and drought tolerance in cacti and agaves. AMF can not only promote the development of the model crops, but can also benefit soil fertility, improve biomass, and enhance tolerance to water stress.

CONCLUSIONS

The reviewed scientific evidence demonstrates that AM symbiosis benefits the productivity of *Opuntia* spp. and *Agave* spp., especially under water stress and in low-fertility soils, which are characteristic of arid and semi-arid regions. These benefits are fundamental to the semidesert's productive systems. Therefore, incorporating AMF into AgriCom can enhance crop growth and resilience in the model, as well as strengthen soil health. The implementation of these fungi can be a comprehensive solution for semidesert agri-food systems that also promotes food security and the conservation of plant resources.

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Colegio de Postgraduados

In Vitro Semi-Solid Fermentation of Two Prickly Pear (*Opuntia* sp.) Cultivars as Food Supplement for Ruminants

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ABSTRACT

Objective: To increase the protein content of two commercial prickly pear cultivars, fermenting and adding some components, in order to complement the diet of ruminants in areas whose conditions impose limitations upon agriculture.

Design/Methodology/Approach: A randomized complete bock design and a factorial treatment arrangement were used to test two prickly pears cultivars (Cristalino (*Opuntia albicarpa*) and Rojo Pelón (*Opuntia ficus-indica*)), two particle sizes (chopped and blended), and two non-protein nitrogen (NPN) sources and their combination (1% urea, 0.1% ammonium sulfate, and urea + ammonium sulfate). The substrates were fermented for 9 h. One percent yeast (*Saccharomyces cerevisiae*) and 0.25% *Saccharum* spp. treacle were added to the substrates.

Results: The levels of the tested factors recorded significant differences ($p<0.05$). The "Cristalino" cv, the "blended" particle size, and the "urea plus ammonium sulfate" NPN had the highest protein content (CP): 29.9%, 33.5%, and 37.7%, respectively. The treatment with the highest CP (46.1%) used the Cristalino cv, blended particles, and urea plus ammonium sulfate.

Study Limitations/Implications: The study faced no limitations.

Findings/Conclusions: Fermenting prickly pears is a nutritious option to feed ruminants.

Keywords: Prickly pear; fermentation; forage supplement; crude protein.

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INTRODUCTION

Prickly pears (*Opuntia* sp. (Caryophyllales: Cactaceae)) are native to North America (Griffith, 2004) and South America (Majure *et al.*, 2012). They are distributed in several arid, semi-arid, and temperate regions of Europe, Africa, Asia, Australia, and the Americas. Several species are grown in over 2.6 million ha for different purposes (Nefzaoui, 2018).

It is a major natural resource for these areas, as a result of its socioeconomic, agricultural, and environmental benefits, as well as its functional, nutraceutical, and biological properties

(Abbas *et al.*, 2022; Stavi, 2022). Since the climate change vulnerability of traditional crops has reduced their productivity and quality, prickly pear cultivation has intensified in the last years, given its morphological and physiological characteristics, its scalability, and its adaptability to scarce and erratic precipitation and soil degradation. Prickly pear survives as a consequence of its resilience, efficient water use, resistance to heat stress, low input requirement, high productivity, and other characteristics (Horibe, 2021; Jorge *et al.*, 2023; Sipango *et al.*, 2022; Thakuria *et al.*, 2020).

For a long time, prickly pear has been used as a complementary and emergency forage during droughts and winter, when forage and grain production becomes scarce (Fuentes-Rodríguez *et al.*, 2017). It has increasingly been used to feed animals in arid and semi-arid zones, because it is a promising forage option that provides energy, minerals, and water for several species, guaranteeing food safety and reducing hunger and poverty in those areas (Dubeux Jr. *et al.*, 2021). However, cladodes have a low fiber and crude protein content and must be complemented with other crops or otherwise enriched (Torres-Ponce *et al.*, 2015). This procedure makes up for the lack of such components and might also slow down its laxative effect (Gusha *et al.*, 2015). In this context, prickly pear could be a fundamentally strategic forage with high productive potential for various productive systems located in the semi-arid regions of the world (Rocha *et al.*, 2021).

Studies about the nutritional content of prickly pears show that they provide little protein; therefore, ingredients that add protein and energy are required to increase in their quality (Fuentes-Rodríguez *et al.*, 2017). Consequently, technologies must be implemented to increase their nutritional value through such biotechnological processes as silage (Veleta *et al.*, 2024). Silage is an anaerobic process that preserves wet crops through fermentation; under optimum silage conditions, bacteria mainly ferment soluble carbohydrates, preserving the nutrients of the forage (El Hajji *et al.*, 2022). Fermentation in containers (bioreactors) is another option that can improve the protein content of the substrate, increasing the unicellular protein in the cell wall of fermenting microorganisms (González *et al.*, 2019).

Solid state fermentation is a process that produces microbial protein degrading the glucose of the cladodes. This process is supported by the microorganisms that grow in the insoluble substrate, in the absence of added water. The said fermentation principle is based on the metabolization of glucose by yeast; therefore, an effective process requires the release of glucose (Torres-Ponce *et al.*, 2015).

The first studies about the enrichening of prickly pear were carried out in northeastern Brazil at the end of the past century, as a strategy to tackle the lack of quality forage, resulting from the low and erratic precipitation and the high costs of protein supplements. Oliveira (2001) recorded that crude protein increased by 12.8% when prickly pear was fermented with *Aspergillus niger*. Likewise, Araújo *et al.* (2005) reported a 26% increase in crude protein as a result of the use of *Saccharomyces cerevisiae*. In Mexico, Díaz-Placencia *et al.* (2012) increased protein content from 9.3 to 19.3% through *in vitro* fermentation of prickly pear, inoculated with a *Kluyveromyces lactis* yeast, obtained from waste apples. For their part, Flores-Hernández *et al.* (2021) reported that protein increased from 10.4 to 36.5%, when *S. cerevisiae* was applied to the *O. megacantha* cultivar. Likewise, Castro *et al.*

(2022) used the same yeast and increased protein from 3.9 to 27.5 %. Finally, Veleta *et al.* (2024) reported a similar increase in protein (6.6 to 32.7 %), 72 h after fermentation.

Considering that prickly pears have a high palatability, are easy to reproduce, grow fast, can recover after they are cut, have a high productivity per surface unit, and can be produced at a low cost, this species could withstand the challenges of global climate change. However, since several research teams have reported high variation in the crude protein content of different species and cultivars, the aim of this study was to determine the crude protein content of two prickly pear (*Opuntia* sp.) cultivars grown in the region, using two particle sizes and two sources of non-protein nitrogen, in a fermentation process, in order to increase the nutritional value of its forage. The purpose was to corroborate its potential as a forage alternative for livestock producers in central and northern Mexico.

MATERIALS AND METHODS

Study Site

The study was carried out under lab conditions, in the Water, Soil, and Plant Lab of the Colegio de Postgraduados - Campus San Luis Potosí, located in Salinas de Hidalgo, San Luis Potosí.

Preliminary Test

During the initial test, the effect of adding a 1% yeast or 1% yeast plus 2% fermented agave juice inoculum, to the prickly pear-based substrate on the crude protein content was evaluated. In addition, the substrate was fermented for 6, 8, 10, and 12 h to determine its effect on the crude protein content. In the absence of any significant differences, these factors were not included in the final test.

Final Test

This test used a factorial design to evaluate the effect of the cultivar, particle size, and non-protein nitrogen (NPN) on the crude protein content of fermented prickly pear. In the case of the cultivar (cv), mature cladodes from Rojo Pelón (*Opuntia ficus-indica*) without prickles and Cristalino (*O. albicarpa*) with prickles were used. The Rojo Pelón cv came from a germplasm collection located in Salinas de Hidalgo, San Luis Potosí (22° 37' 33.6" N, 101° 42' 39.4" W, at 2,075 m.a.s.l.); meanwhile, the Cristalino cv was collected at the community of La Victoria, Pinos, Zacatecas, México (22° 15' 27" N, 101° 37' 48" W, at 2,308 m.a.s.l.). The 10- to 12-month-old cladodes were collected in February 2024. Regarding particle size, the cladodes were prepared in one of two ways: 1) blended in a standard Oster® blender (BLSTBPST013), unwashed and without removing the prickles; and 2) cut with a knife into approximately 1.0 cm^2 squares. Finally, in terms of non-protein nitrogen (NPN), Flores *et al.* (2019) recommend adding 1% urea and 0.1% ammonium sulfate. Therefore, the effect of each recommended dose was evaluated separately and combined. Therefore, the following levels were added: 1% urea, 0.1% ammonium sulfate, and the same ratio of urea + ammonium sulfate. The factorial design resulted in 12 treatments (Table 1).

Table 1. Treatment and factors evaluated during the fermentation of two prickly pear (*Opuntia* spp.) cultivars.

Treatment	Cultivar (cv)	Particle size	Non-protein nitrogen source
CrLiUr	*Cristalino*	Blended	Urea
CrLiSa	*Cristalino*	Blended	Ammonium sulfate
CrLiUS	*Cristalino*	Blended	Urea + ammonium sulfate
CrPiUr	*Cristalino*	Chopped	Urea
CrPiSa	*Cristalino*	Chopped	Ammonium sulfate
CrPiUS	*Cristalino*	Chopped	Urea + ammonium sulfate
RpLiUr	*Rojo pelón*	Blended	Urea
RpLiSa	*Rojo pelón*	Blended	Ammonium sulfate
RpLiUS	*Rojo pelón*	Blended	Urea + ammonium sulfate
RpPiUr	*Rojo pelón*	Chopped	Urea
RpPiSa	*Rojo pelón*	Chopped	Ammonium sulfate
RpPiUS	*Rojo pelón*	Chopped	Urea + ammonium sulfate

Substrate Preparation

To prepare the substrates for fermentation, 250 g of each prickly pear cultivar and each size were initially put into 600-mL beakers. Subsequently, urea, ammonium sulfate, or both were added, depending on the treatment. In all cases, 1% yeast was added as inoculum, along with 0.25% molasses to provide energy and to facilitate fermentation.

Both the NPN and yeast sources were added in the order suggested by Flores *et al.* (2019): first, urea, followed by ammonium sulfate, yeast, and finally molasses. Additionally, 140 mL of deionized water were added to the cut pricky pear at 35 °C, to achieve an even distribution of yeast in the surface of the prickly pear.

Fermentation Process

The fermentation process was carried with a 45-kg Eberbach™ 5900 reciprocal shaker (Científica Senna, Mexico). The beakers with the substrates were placed in 18×4×12 cm box carriers and shaken at ≈60 oscillations min^{-1}. The substrates were continuously shaken during the 9-h fermentation. Additionally, the cut prickly pear substrate was subsequently shaken by hand for 1 min every hour, because yeast settled during the shaking, preventing its interaction with the whole substrate. The substrate temperature was measured with a mercury thermometer, ranging from 17 °C (at the start of the fermentation process, in the morning) to 26 °C (at the end of the fermentation process, in the evening).

To stop the fermentation of the substrate at the end of the process and to determine the crude protein content afterwards, samples were taken from each fermented substrate and placed in 50-mL Eppendorf centrifuge tubes®. Following the suggestions of Díaz-Plascencia *et al.* (2012), two drops of 86.5% phosphoric acid (orthophosphoric acid) were added. The samples were placed in a Thermo Scientific™ flask at −5 °C awaiting their analysis.

Protein Content

The samples were defrosted in water at room temperature and dehydrated in a FELISA® FE-292D oven (Monterrey and Mexico City), at 60 °C for 48 h, following the guidelines of Díaz-Plascencia *et al.* (2012). They were subsequently crushed in a mortar and kept in a 10-mL centrifuge tube. Finally, Parafilm was placed around the lid, awaiting analysis.

The Dumas method used to determine total nitrogen complies with the regulations of the Association of Official Analytical Chemists (AOAC). This method was employed to estimate total nitrogen, placing 0.1 g of the sample in a nitrogen-free tin foil cup. The cups were subsequently placed in each of the slots of the carousel of a Truspec N 4334 elemental determinator (LECO, USA). The Total Crude Protein (TCP) was determined based on the total nitrogen value and a 6.25 protein conversion factor. This determination process was also applied to the Cristalino and Rojo Pelón cultivar substrate controls (without any addition or fermentation).

Other Bromatological Analyses

In addition to their crude protein content, both the treatments and the controls were analyzed to determine their ash content, ether extract, humidity, total solids, and pH. Likewise, in terms of protein content, the Neutral Detergent Fiber of the best treatment in each cultivar and the controls was determined, following the official methods established by the AOAC (1975 and 1990). The ether extract, humidity, ash, and neutral detergent content were quantified using the AOAC method 945.39 (with a Soxhlet extractor), the AOAC method 966.02, the AOAC method 923.03, and the ANKOM method (1999), respectively. Total solids were quantified based on the humidity content. Finally, pH was measured with an Apera Instruments® PH700 pH meter.

Statistical Analysis

A completely randomized experimental design with a factorial arrangement was used to generate 12 treatments, with different numbers of repetitions. The data were subjected to an analysis of variance with a factorial design and means were compared with Tukey's test ($p<0.05$), using version 9.4 of the SAS statistical analysis system (SAS® 9.4). Finally, the 12 treatments with the factorial design plus two controls (prickly pear cultivars without treatments) were subject to an analysis of variance.

RESULTS AND DISCUSSION

Preliminary Test (Fermentation Time and Inoculum)

During the preliminary test, the fermentation time of the prickly pear substrates did not record significant differences in crude protein (CP) content ($p=0.57$). The highest CP content (30.0%) was recorded after a 10 h fermentation, while the lowest content (28.2%) was recorded after 8 h. Intermediate contents were reported at 6 h (29.6%) and 12 h (29.7%). Likewise, no significant differences ($p=0.34$) were found in CP content, when yeast or yeast plus fermented agave juice were added to the substrate. However, when yeast plus fermented agave juice was added, the CP content (29.8%) was higher than with yeast alone (29.0%).

Final Test

Table 2 shows the results of the factorial ANOVA for the crude protein (CP) content, especially in the three main factors (cultivar, particle size, and source of non-protein nitrogen). All interactions showed significant effects (cultivar*particle size) or highly significant effects (the rest).

Cultivar

ANOVA pointed out highly significant differences ($p<0.0001$) in CP percentage between both prickly pear cultivars (Rojo Pelón and Cristalino). Figure 1 shows that the Cristalino cv (29.9 %) had a higher CP content (Tukey $p<0.05$) than the Rojo Pelón cv (27.3 %).

Particle Size

The ANOVA showed highly significant differences ($p<0.0001$) in CP content, depending on the preparation of the substrates (cut or blended cladodes). Figure 2 shows that the CP value recorded after the fermentation of the blended cladodes (33.5%) was higher (Tukey $p<0.05$) than that of fermented cut cladodes (21.5%).

Source of Non-Protein Nitrogen (NPN)

The ANOVA also indicated highly significant differences ($p<0.0001$) in CP content, when different sources of nitrogen were added to the substrates for their fermentation.

Table 2. Effect of main factors and their interactions on the crude protein content of fermented prickly pear.

Source	F	p
Cultivar	25.63	<0.0001
Particle size	549.38	<0.0001
Nitrogen source	981.00	<0.0001
Cultivar*Particle size	3.90	0.0494
Cultivar*Nitrogen source	14.23	<0.0001
Particle size*Nitrogen source	115.60	<0.0001
Cultivar*Particle size*Nitrogen source	8.00	0.0004

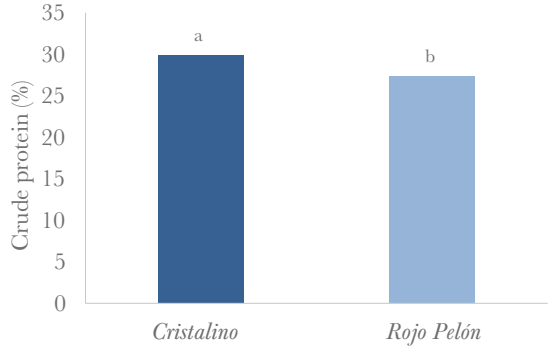

Figure 1. Effect of cultivar on the CP content (%) of fermented prickly pear substrates.

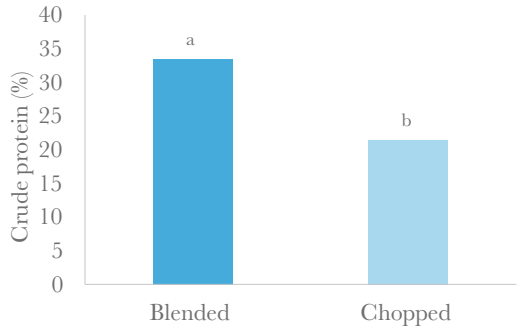

Figure 2. Effect of particle size on the CP content (%) of fermented prickly pear substrates.

Figure 3 shows statistical differences (Tukey $p<0.05$) between the three levels in this study: a higher PC content (37.7 %) was recorded with the addition of urea + ammonium sulfate. Meanwhile, the lowest CP value (13.2%) was reported when ammonium sulfate was added and an intermediate CP value (35.2%) was observed with the addition of urea.

CP Content per Treatment

ANOVA showed highly significant differences ($p<0.0001$) in CP content between the treatments evaluated. Figure 4 shows that the CrLiUS (*Cristalino*, blended, and urea + sulphate) treatment recorded the highest CP content (46.1%). However, it was not statistically different (Tukey $p<0.05$) from the RpLiUS (*Rojo Pelón*, blended, and urea + sulphate) and CrLiUr (*Cristalino*, blended, and urea) treatments, which recorded a 45.4 % and 43.7 % CP content, respectively. Nevertheless, its CP content was different from the other treatments. Finally, the controls (prickly pear cultivars without treatment) recorded the lowest CP values. Particularly, the *Rojo Pelón* cv had the lowest value (4.8 %) and was statistically different from the rest of the treatments, except for the *Cristalino* control.

Table 3 includes both the abovementioned CP results and the values of other determinations analyzed, based on the levels of the evaluated factors. The values of these variables are also shown for the controls (prickly pear without treatment).

Regarding the CP content, the highest Neutral Detergent Fibre (NDF) content (20.5%) in the substrate was recorded by the *Cristalino* cv (CrLiUS: *Cristalino*, blended, and urea + ammonium sulfate.). Meanwhile, the best treatment for the *Rojo Pelón* cv reached 18.0%

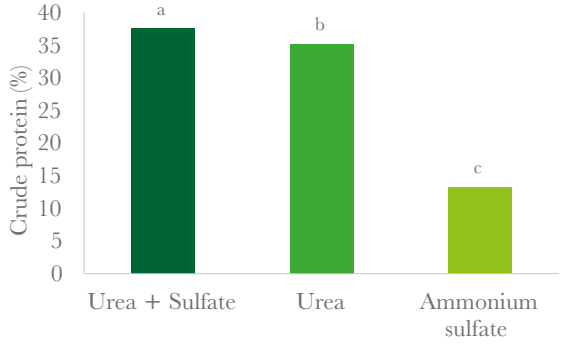

Figure 3. Effect of the nitrogen source on the CP content (%) of the fermented prickly pear substrates.

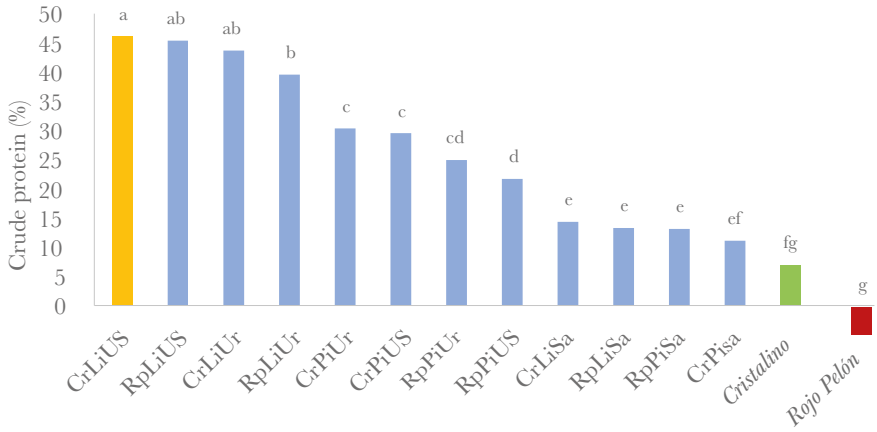

Figure 4. Crude protein content (%) per treatment. Cr=*Cristalino*, Rp=*Rojo Pelón*, Li=blended, Pi=cut, Ur=urea, Sa=ammonium sulfate, US=urea + ammonium sulfate.

Table 3. Bromatological characteristics and pH of the fermented prickly pear substrates and the controls.

Factor	Level	PC (%)	C (%)	EE (%)	H (%)	ST (%)	pH
Cultivar	Rojo pelón	27.3 b*	15.3 a	0.44 a	92.1 b	7.9 a	5.7 a
	Cristalino	29.9 a	15.0 b	0.36 a	92.3 a	7.7 b	5.2 b
Size	Blended	33.5 a	15.3 a	0.35 b	91.8 b	8.2 a	5.4 a
	Chopped	21.5 b	15.1 a	0.45 a	92.6 a	7.4 b	5.4 a
Source	Ammonium sulfate	13.2 c	16.4 a	0.44 a	92.5 a	7.6 c	4.8 c
	Urea	35.2 b	15.3 b	0.39 a	92.0 c	7.9 a	5.8 a
	Urea + Ammonium sulfate	37.7 a	13.9 c	0.37 a	92.2 ab	7.8 ab	5.6 b
Controls	Rojo pelón	4.8	9.6	0.16	91.2	8.8	4.7
	Cristalino	7.0	11.4	0.11	94.2	5.9	4.9

* Different letters between factor levels indicate significant differences (Tukey $p<0.05$). CP (PC)=crude protein, A(C)=ashes, EE=ether extract, H=humidity, TS (ST)=total solids.

(RpLiUS: *Rojo Pelón*, blended, and urea + ammonium sulfate). Finally, the NDF content of the controls (prickly pear without treatment) reached 17.2% and 19.0% in the *Rojo Pelón* and *Cristalino* cv, respectively.

The results of this study confirm that fermentation can improve the nutritional value of prickly pears (*Opuntia* spp.), making it a useful supplement for the diet of confined livestock, in association with other inputs. These findings support the results obtained in Brazil (Araújo *et al.*, 2005; Araújo *et al.*, 2008) and Mexico (Castro *et al.*, 2022; Díaz-Plascencia *et al.*, 2012; Flores-Hernández *et al.*, 2017; Flores *et al.*, 2019; Herrera-Torres *et al.*, 2014; Herrera *et al.*, 2017), the main countries in which this biotechnological process has been successfully tested. All those studies were conducted under various conditions, including different prickly pear cultivars, incubation methods, fermentation time, type of microorganism, NPN, and sugar sources. However, they all increased microbial protein from 200 to 400%, indicating their high potential as an alternative forage source for ruminants in the arid and semi-arid zones of the Mexican territory.

Few studies that explore the fortification of prickly pear in Mexico specify the species or cultivar used. Only a few point out that they are defenseless forage, including the AT-TV6 (Díaz-Plascencia *et al.*, 2012), AV6 (Herrera-Torres *et al.*, 2014), and one unspecified (Castro *et al.*, 2022) varieties. Only two researches involved wild varieties: *Opuntia leucotricha* (prickly) (Maldonado-Quiñones *et al.*, 2022) and *O. rastrera* (Fuentes-Rodríguez *et al.*, 2017). However, as far as this research team was able to determine, no work has used commercial cultivars to produce fruit. This study provides regional producers of prickly pear fruits an alternative for the exploitation of pruning waste and a source of high-quality forage supplement during summer and winter.

The CP content of the prickly pear cultivars used in this study showed statistical differences: the *Cristalino* cv (*O. albicarpa*) had a 29.9 % value, while the *Rojo Pelón* cv (*O. ficus-indica*) reached 27.3 %. Under similar *in vitro* conditions, Herrera-Torres *et al.* (2014) and Herrera *et al.* (2017) increased protein content from 14 to 16.31%, respectively. Under different fermentation and microorganism conditions, the values recorded in this study using a higher volume of prickly pear were higher than those obtained in Brazil by Araújo *et al.* (2005) and Araújo *et al.* (2008), who reported 26 and 10.4% values, respectively. Meanwhile, in Mexico, Díaz-Plascencia *et al.* (2012), Herrera-Torres *et al.* (2014), Herrera *et al.* (2017), and Maldonado-Quiñones *et al.* (2022) reported 19.4, 14, 16.3, and 22.7% values, respectively. Meanwhile, similar results were recorded by Flores-Hernández *et al.* (2017) (29.8%) and Castro *et al.* (2022) (27.5%), but Flores-Hernández *et al.* (2019) obtained better results (33.5%). This wide variability could be linked to the concentration levels of the substrate (Díaz-Plascencia *et al.*, 2012), because non-fibrous carbohydrates favor external fermentation (fortification) and internal fermentation (rumen fermentation) (Flores-Hernández *et al.*, 2017).

The two sizes tested had statistical differences. The blended prickly pear substrate recorded higher CP values (33.5%) than the cut prickly pears (21.5%). In most researches about the fortification of prickly pear the substrate has been cut (Castro *et al.*, 2022; Flores-Hernández *et al.*, 2017; Flores *et al.*, 2019), mainly due to its practicality (saving in labor and mechanization of the process) and technological issues (high humidity content), which hinder the reduction of the particle size. However, the results of this research indicate that the reduction in particle size can favor the development of the yeast microorganisms found in the substrate and the effect of the addition of non-protein nitrogen (NPN).

With regards to the NPN source, the tested levels had statistical differences. Adding both urea and ammonium sulfate increased the protein level to 37%, while separately adding each source caused a decreasing trend, both for urea (35.2%) and ammonium sulfate (13.2%). Herrera *et al.* (2017) recorded less protein when no nitrogen sources were added; they attributed the protein increase (from 5.12 to 16.31%) to the presence of native microbes in the substrate. According to Díaz-Plasencia *et al.* (2012) and Herrera-Torres *et al.* (2014), adding a combination of NPN and microorganisms to the substrate during the fermentation process facilitates the increase of protein levels, because they activate, accelerate, and make fermentation more efficient. For their part, Maldonado-Quiñones *et al.* (2022) mentioned that adding NPN to the prickly pear, through the urea and ammonium sulfate (which is not lost as ammonia), is a source of degradable protein for the rumen; however, it must

be synchronized with the addition of a source of rapidly degrading carbohydrates (grains and molasses), to avoid an increase in potentially toxic ammoniacal nitrogen. Therefore, detoxifying ammonia is particularly important.

Therefore, in terms of CP content, the best treatments were those to which urea and ammonium sulfate were added and in which cladodes were blended. The cultivar factor was less important, because the best treatments alternated between both cultivars. Therefore, the treatment with the highest CP content (46.1%) was based on blended *Cristalino* cv, to which urea plus ammonium sulfate (CrLiUS) was added, because its values were higher than those of cultivars without treatment: 4.8% for Rojo Pelón and 7.0% for *Cristalino*. The best treatment (CrLiUS) had a 46.1% CP content —higher than the reports of any of the works about the fortification of protein through prickly pear fermentation quoted in this study.

CONCLUSIONS

This study proved that fermentation increases the protein content of prickly pear and therefore, its quality as forage. The levels of the factors evaluated (prickly pear cultivar, particle size, and source of non-protein nitrogen) had significant differences ($p<0.05$). The highest CP values were recorded by *Cristalino* cv (Cr) on its own (29.9%), blended (Li, 33.5%), and with the addition of urea plus ammonium sulfate (US, 37.7%). The combination of these factors (CrLiUS) resulted in the treatment with the highest CP content (46.1%). The fermentation times used in this study (6, 8, 10, and 12 h) did not cause any significant differences, just like the inoculation with yeast only or with yeast plus fermented agave juice. Prickly pear fermentation is a nutritious and feasible option to feed ruminants. However, further research that resulted in the standardization and optimization of its processes would provide products with better dietary evenness, which could be used in livestock production at a low cost.

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