

AGRO PRODUCTIVIDAD

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Agradecimientos: Son opcionales y tendrán un máximo de tres renglones para expresar agradecimientos a personas e instituciones que hayan contribuido a la realización del trabajo.

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Cost of producing a replacement heifer in family dairy system

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ABSTRACT

Objective: To estimate the production cost of a replacement heifer in the family dairy system of the Los Altos Region of Jalisco, Mexico, providing economic evidence to support ranchers' decision-making in the region.

Design/methodology/approach: Information was obtained from three dairy farmers in the family farming system located in Tepatitlán, Jalisco, from May 2019 to May 2022, through the design and implementation of monthly tracking forms for technical and economic information. The unit of analysis was the heifer, with information obtained from 297 animals. These data were integrated into a database to analyze the activities and technologies implemented in the zootechnical areas and to estimate the unit cost of production of a heifer (CUPV). The CUPV was calculated by summing the quantity of each input used multiplied by its respective price in each production unit and dividing the result by the number of heifers produced.

Results: Eleven of the 17 activities and technologies analyzed were implemented by all three production units during the study period. None of the units had calving facilities or hired staff exclusively dedicated to calf care. The only activity that improved during the study period was the implementation of the progressive weaning method. The average CUPV was \$37,789, with feed representing 96%, labor 1.4%, reproduction-related costs 1.3%, and health-related costs 0.6%.

Limitations on study/implications: Estimating the CUPV is essential to support milk producers' decision-making in generating their own replacement heifers.

Findings/conclusions: Producing a replacement heifer costs on average \$37,789.00 from month 0 to first calving, with more than 90% of the total cost allocated to feed.

Keywords: Livestock economics, bovine milk, technology.

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INTRODUCTION

Bovine milk production in Mexico in 2024 was 13,553,069.380 thousand liters, with 2,713,534 cows in milk. Jalisco ranks first both in production and in number of breeding cows, with 2,845,110.294 thousand liters and 402,347 cows in milk, with 26,213 production units out of the 231,448 existing in Mexico (SIACON, 2024), which places it in second position nationwide in this category. It is estimated that milk production units in the state of Jalisco have an average of 15 breeding cows, with a production of 19.1 liters per cow per day (INEGI, 2022). These data show the importance of dairy activity in the state of Jalisco, but the number of breeding cows per production unit and the productivity per



cow indicate that the family or small-scale production system predominates. Within the state of Jalisco, the Los Altos region is one of the most important dairy regions, not only in the state but in the country; however, productivity levels and the use of technology are not optimal. A study conducted by Mariscal *et al.* (2017) mentions that the main problems faced by dairy producers in the region are related to high input costs, marketing problems, and other contextual threats, but it does not mention problems whose solution focuses on making the production process more efficient.

In studies reviewed on the problems of milk production in the Los Altos Region of Jalisco, the production of replacement heifers is not mentioned, which coincides with what was expressed by Barrón *et al.* (2025) regarding the limited research on heifer development and their productive and economic performance, which presents a dilemma in management and decision-making by ranchers, especially those in small-scale family systems where their priority is often to obtain an “immediate” income from milk sales and heifers are usually considered an extra economic burden (Barrón *et al.*, 2025). They also lack financial knowledge to decide what is more convenient, producing or buying their replacements, impacting their costs, as is done in other countries such as the United States, Canada, or the United Kingdom, where there are studies on the production costs of replacement heifers under different conditions, mentioning the advantages for dairy enterprises of buying or producing these replacements (Laflamme-Michaud *et al.*, 2025; Boulton *et al.*, 2017; Hawkins *et al.*, 2019). However, the lack of information in most production units also makes real cost calculation difficult, as it is not possible to separate the inputs used in raising replacement heifers from other livestock and agricultural activities.

Raising heifers from birth to first calving is a costly investment that requires at least two years to be integrated into milk production and begin to recover this investment (Hawkins *et al.*, 2019; Boulton *et al.*, 2017; and Laflamme-Michaud *et al.*, 2025). Heifer rearing is considered a significant cost, and it has been shown that the total cost of raising heifers ranks second in the annual operating cost of dairy production units (Heinrichs *et al.*, 2013; and Boulton *et al.*, 2015), accounting for up to 20% of production costs (Laflamme-Michaud *et al.*, 2025). However, milk producers in many important milk-producing countries, and dairy producers in Los Altos de Jalisco, Mexico, can undoubtedly be included, raise their replacement heifers aware that this is a costly activity, keeping (almost) all their newborn female calves with the aim of ensuring the availability of enough young females to replace cows that are eventually expected to be culled (Mohd *et al.*, 2015). Being aware that this is a high cost does not mean that producers know exactly “how costly” it is, as they often lack sufficient information to calculate it, since costs are rarely evaluated (Laflamme-Michaud *et al.*, 2025). Based on the above, the production costs of a replacement heifer in the family dairy system were estimated to provide economic elements to support ranchers’ decision-making.

MATERIALS AND METHODS

Selection of production units

The participation of three conventional producers, convinced of the importance of providing information through the implementation of specific technical and economic

records for heifer production, was obtained in the municipality of Tepatitlán de Morelos, Jalisco, Mexico. The municipality is among those with the highest milk production at the state level, with a predominance of family or small-scale and semi-technified production systems (Ríos *et al.*, 2015). It is located in the Altos Sur region of Jalisco (Figure 1), Mexico, 80 km from the state capital (Guadalajara) ($20^{\circ} 48' 54''$ N, $102^{\circ} 45' 31''$ W at 1880 m above sea level). It is part of the Bajío macro-region with a semi-warm semi-humid climate (type C(w1) according to Köppen-García), with average annual temperatures around 18-19 °C, rainy summers (June-August), and an average precipitation close to 870 mm (IIEG, 2021).

Integration of information

Monthly technical and economic monitoring formats were designed in the Excel[®] program and implemented from May 2019 to May 2022 in the three cooperating and representative production units of the family or small-scale dairy system. The technical monitoring formats included activities/technologies in the areas of feeding with six activities, four in management, three in pre-production, and three in health (Table 1). The record was made by marking the number 1 when the activity/technology was carried out and 0 when it was not.

The unit of analysis was the heifer, obtaining average information from 297 animals. The information was collected by a technician and subsequently compiled and analyzed.

Estimation of the cost of a replacement heifer

The estimation of the total cost was obtained by applying the following algebraic expression.



Source: Taken from IIEG, Statistical and Geographic Information Institute of the State of Jalisco, “General Map of Jalisco, 2012”, and INEGI, 2025.

Figure 1. Geographic location of the municipality of Tepatitlán de Morelos, Jalisco, Mexico.

Table 1. Activities/technologies related to heifer rearing carried out from 2019 to 2022.

Area	Activities/Technologies
Feeding	Provide colostrum within the first 24 h
	Receives colostrum from the dam
	Feed the calf individually during the milk-feeding period
	Provide starter feed or concentrate prior to weaning
	Provide milk replacer
	First forage offering, days
Management	Apply a weaning method
	Daily cleaning of the calf hutch or pen
	Daily cleaning of utensils
	Exclusive staff for calf care
	Protection against prevailing winds
Reproduction	Apply a gradual weaning method
	Separate the calf from the dam
	Has a calving pen
Health	Navel disinfection
	Deworming of replacement heifers
	Vaccination of replacement heifers

Source: Author's own elaboration.

$$\begin{aligned}
 TC = VC + FC = Pr_{i...n} * Q_{i...n} = & (Pr_{milk} * Q_{milk} + Pr_{milk\ replacer} * Q_{milk\ replacer} \\
 & + Pr_{forage} * Q_{forage} + Pr_{concentrate} * Q_{concentrate} + Pr_{vaccines, dewormers and medications} \\
 & * Q_{vaccines, dewormers and medications}) + \text{Reproduction cost}(18\text{ months} - \text{first calving}) + FC
 \end{aligned}$$

Where: TC =Total cost; VC =Variable cost; FC =Fixed cost; $Pr_{i...n} * Q_{i...n}$ =Price of input i to n multiplied by the quantity of input i to n .

The inputs considered in the variable feeding cost were the cost of milk, commercial milk replacer, forage (corn stover), and commercial concentrate; vaccines, dewormers, medications, and, in the 18 months-first calving stage, a reproduction cost. The labor cost was calculated based on the hours dedicated to heifers by permanent workers in relation to an eight-hour workday.

As a fixed cost in the short term, the proportional fraction of the depreciation of breeding cows was considered. Average production costs were obtained by growth stage, divided into: a) lactation from 0 to 60 days, b) post-lactation to 6 months, c) from 12 to 18 months, and d) from 18 months to first calving. The costs obtained were updated to 2024 using the annual inflation rate reported by INEGI (2025b). With the total costs obtained for each growth stage, unit costs were calculated by dividing the total cost per stage by the quantity produced.

Information analysis

The technical activity data and the economic data were validated with the technician; subsequently, labor calculations were performed, and average costs were estimated and updated. With the updated data, relative frequencies and averages were obtained.

RESULTS AND DISCUSSION

Activities and technologies for the production of replacement heifers

The three production units studied, located in the municipality of Tepatitlán, Jalisco, had a total of 743 confined Holstein cows, of which 402 were considered by the producers as high producers, 141 as medium producers, 105 as low producers, and 95 as dry cows. On average, the productive herd size was 248 animals, and the units had facilities for milk production such as roofed pens, drinkers and feeders, mechanized milking parlors, handling chutes, and areas assigned for calves and heifers, as well as land for grain and forage production and silos for silage production, all intended for milk production.

These producers are representative of the system, but also in their attitude toward carrying out specific activities/technologies to ensure proper development of the calves and heifers that will become part of production and of the producers' assets in the medium and long term. The activities/technologies were 16, grouped into feeding, management, reproduction, and animal health (Figure 2), of which 75%, 88%, and 63% were implemented in 2019 by Production Unit (PU) 1, PU2, and PU3, respectively.

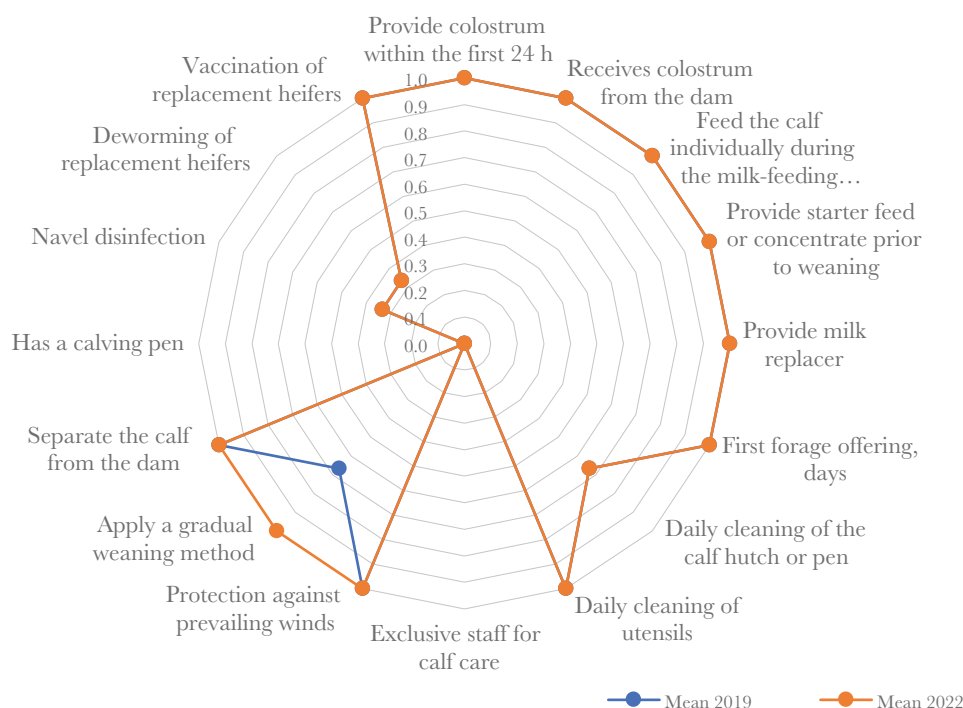


Figure 2. Average activities carried out for heifer production in the production units from 2019 to 2022. Source: Authors' own elaboration based on monitoring form data.

In 2022, the same number of activities/technologies was carried out, reflected in the same percentage obtained for PU1 and PU2; in the case of PU3, it increased to 69% with the implementation of the progressive weaning method (Figure 2). Within each area in the three production units analyzed, the six feeding-related activities were carried out at 100% both at the beginning in 2019 and at the end in 2022. Newborn females received colostrum from the mother within the first 24 hours after birth, approximately four hours after being born; they also received milk replacer to complement feeding since they were separated from their mothers. Calves were fed individually during the lactation stage. In the forage category, corn stover and silage were included, which were offered at approximately 60 days of age. In management, the cleaning of utensils and protection from prevailing winds stand out, activities carried out at 100% in the three units, unlike the daily cleaning of calf pens or corrals, which was only carried out in PU1 and PU2. In reproduction, the implementation of the progressive weaning method was the only activity not carried out in 2019 in PU3 and that had been incorporated by 2022. Umbilical disinfection and deworming of replacements were only carried out in PU2 (Figure 2).

Based on these data, more than 70% and up to 88% of the activities/technologies aimed at obtaining replacement heifers in good condition to be integrated into the productive herd were carried out in the production units. Considering that not all listed items are technologies and some remain only at the level of activities, this can still be explained as a high technological level, given that dairy producers in the state of Guanajuato under the same production system used technology within a range of 66% to 100% (Vélez *et al.*, 2013), and in dual-purpose small-scale production units the use of technology in general is higher than 50% (Espinosa *et al.*, 2018). However, these producers use more technology and carry out more activities in the areas of feeding and reproduction and genetics than reported by Vélez *et al.* (2013), with percentages of 64% and 69%, respectively, and they coincide in progress in animal health with more than 50%. On average, the producers had 99 animals for heifer replacement and a total of 297 (Table 2).

Cost of producing a replacement heifer in family or small-scale production units

The production cost of a replacement heifer in these units, considering the activities/technologies carried out during the production process by growth stage, averaged

Table 2. Animals intended for heifer replacement in 2022 by production unit.

Month and year	Calves in the milk-feeding period	Rearing from 3 to 6 months	Rearing from 6 to 12 months	Rearing from 12 to 18 months	Rearing from 18 months to first calving	Total
PU 1_May 2022	15	15	12	29	22	93
PU 2_May 2022	5	26	27	28	25	111
PU 3_May 2022	3	17	31	18	24	93
Total	23	58	70	75	71	297
Mean	8	19	23	25	24	99

Source: Authors' own elaboration based on monitoring form data.

\$2,198.00 during the lactation stage, and the cost for the animal to enter as a productive cow at first calving increased on average to \$35,592.00 Mexican pesos at approximately 24 months of age. The average cost of obtaining a replacement heifer at first calving, at 2024 prices, was \$37,789, and consistency is observed in the increase in costs according to the physiological growth of the animals over time (Table 3).

Table 3. Production costs of replacement heifers by growth stage in the state of Jalisco.

Stage	Production Unit			Mean
	1	2	3	
Lactation	2,303	2,300	1,990	2,198
Post-weaning to 6 months	5,006	2,844	5,012	4,287
6 a 12 months	7,418	6,955	8,345	7,573
12 a 18 months	11,074	7,617	9,766	9,486
18 months-first calving	17,985	11,357	13,396	14,246
Total	43,786	31,073	38,509	37,789

Source: Authors' own elaboration based on monitoring form data.

The average total cost of producing a replacement heifer coincided with that reported by Takeaways (2024), which reported an average value of \$38,797 (\$2,904 Canadian dollars, with an exchange rate of \$13.36 in 2024), based on data from 63 farms. Another study, with information from 1,972 farms located in Minnesota, United States, reported by Dairy Heifer Cost Variation from PennState Extension, Beck (2025), based on data from 2016 to 2021, mentions an average cost of a replacement heifer of \$35,684 (\$1,709 US dollars, at an exchange rate of \$20.88 in December 2024).

Although production systems differ from those existing in Mexico, and specifically from the family or small-scale dairy system, these studies serve as references given the scarcity of national information.

In the structure of variable production costs on average, feeding represented 96%, labor 1.4%, reproduction-related costs 1.3% considered in the 18 months-first calving stage, and health 0.6%, with recurrent problems of diarrhea and umbilical infections (Table 4). The percentage of feeding is double that reported by Hawkins *et al.* (2019), who analyzed the costs associated with pre-weaning management strategies for raising heifers on dairy farms in the United States, estimating that 46% of the total cost was allocated to feeding. A similar situation was reported by Boulton *et al.* (2017) in 101 farms in the United Kingdom, with 46.4% from birth to weaning and 36.8% from birth to calving, and by Laflamme-Michaud *et al.* (2025) in Canada, with 87 conventional Holstein dairy farms in Quebec reporting 46%, as well as 73% in the study reported by Heinrichs *et al.* (2013) based on 44 farms in Pennsylvania, United States. However, in a study conducted on the dairy operation of the Alfredo Volio Mata Experimental Station of the University of Costa Rica by Elizondo and Solís (2018), the percentage of feeding cost was 86.19% from 3 to 16 months and 77.56% from 16 months to the age at first

calving. The percentage of feeding cost was high compared to other studies, and it is possible that the feeding plan for the animals exceeds the recommended percentages for newborn calves, with milk quantities ranging from 8% to 10% of body weight according to González *et al.* (2022), as well as the amount of concentrate supplied, which increased the average feeding cost.

By production unit, the highest feeding cost was observed in PU3 and, therefore, the percentages in the remaining categories were lower. The forage cost was included from the 3-6 months stage to first calving and included corn stover and silage. The labor percentage was lower than 1.5%, which can be considered low when compared with the results obtained by Hawkins *et al.* (2019), who reported 33% of the total cost allocated to labor, and 22.3% reported by Boulton *et al.* (2017) based on information from 101 farms in the United Kingdom; in the case of a study conducted in Canada by Laflamme-Michaud *et al.* (2025), labor represented 16% in 80 farms and 23% in 44 farms in Pennsylvania, United States. What is confirmed is the limited time that permanent staff working in the production units dedicate to heifer care, which averaged 2 hours per day, with payment at the minimum wage and without personnel exclusively assigned to calf care.

Table 4. Structure of production costs of replacement heifers in percentage terms.

Item	Feeding	Health	Reproduction	Labor
UP1	96.3	0.5	1.7	1.4
UP2	94.8	0.8	1.5	1.4
UP3	97.4	0.4	0.6	1.3
Mean	96.2	0.6	1.3	1.4

Source: Authors' own elaboration based on monitoring form data.

Feeding cost in the production of replacement heifers in family or small-scale production units

During the lactation stage, the calves were fed with cow's milk (produced at the PUs), commercial milk replacer, and concentrate. In PU3, milk accounted for 55% of the total feeding cost during lactation stage, with a total cost of \$1,940, of which 11% was for milk replacer and 34% for concentrate. In PU1 and PU2, commercial concentrate represented 69% and 72%, respectively. During the following four stages, they were fed with forage and commercial concentrate. Forage represented 24%, 37%, and 32% of the total feeding cost in PU1, PU2, and PU3, respectively, while commercial concentrate accounted for 77%, 63%, and 68%. The total feeding cost was lowest in PU2, as its diet offered a smaller amount of concentrate and a larger amount of forage compared to the other two Pus, even though its lactation stage cost was higher than in PU3 and \$88.00 lower than in PU1 (Table 5).

Table 5. Average feeding cost of producing replacement heifers by growth stage in the state of Jalisco at 2024 values in Mexican pesos.

Stage	UP1	UP2	UP3	Average
Lactation	2,251	2,163	1,940	2,118
Post-weaning to 6 months	4,843	2,663	4,853	4,120
6 to 12 months	7,224	6,747	8,203	7,391
12 to 18 months	10,879	6,896	9,298	9,024
18 months-first calving	16,976	11,000	13,229	13,735
Total	42,172	29,469	37,521	36,387

Source: Authors’ own elaboration based on monitoring form data.

CONCLUSIONS

The use of technologies/activities by producers reflects the importance they place on using technologies and carrying out specific activities aimed at obtaining replacement heifers to be incorporated into the dairy herd, with more than 80% of activities/technologies implemented.

The production of heifers, whether for replacement or for sale to other producers, is an activity present in all milk production units and production systems, and it requires resources of all kinds for its development; therefore, estimating the cost of producing them becomes of utmost importance to support milk producers’ decision-making. In this study, the average cost was \$37,789.00 from month 0 to first calving, with more than 90% of the cost corresponding to feeding, which can serve as a reference to support decisions related to feeding programs, the acquisition of inputs at better prices, the review of labor activities and the number of hours dedicated to this activity, and the necessary and sufficient number of replacements to be generated and incorporated considering the average total and unit production costs.

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Agricultural productivity in Bahía de Banderas, Nayarit, Mexico: Analysis of corn production among medium- and large-scale farmers

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ABSTRACT

Objective: To determine the average productivity per hectare of commercial white corn among medium- and large-scale farming units in Bahía de Banderas, Nayarit, Mexico.

Design/methodology/approach: A quantitative approach was employed using a non-experimental, descriptive research design.

Results: The average productivity per hectare of commercial white corn during the 2022-2023 cycle was 1.291497419.

Limitations on study/implications: Access to farmers' plots for questionnaire data collection was challenging, as some were located far from the main access roads.

Findings/conclusions: Producers in Bahía de Banderas, Nayarit, Mexico, reported an average productivity of 1.29 tons per hectare of commercial white corn. However, some producers experienced negative returns during the study period due to high production costs and low corn prices. Additionally, there was considerable heterogeneity in production costs and income among the 81 farmers who participated in the study.

Keywords: productivity, farmers, corn.

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INTRODUCTION

In Mexico, the National Institute of Statistics and Geography (INEGI, 2022), classifies the primary sector into the following categories: agriculture; livestock breeding and production; forestry; fishing; and hunting. The Economic Commission for Latin America and the Caribbean (2022) estimated a 4.4% growth in Mexico's agricultural GDP for that year.

Reinforcing the above, Food and Agriculture Organization of the United Nations Mexico (2020) states that agriculture is more than a key productive sector for the country's economic development, particularly in rural areas, where 41.9% of the population depends on the primary sector as their main livelihood. In total, 24.6 million hectares in Mexico are allocated to primary sector activities.



Nayarit is one of the 31 states that, together with Mexico City (formerly the Federal District), constitute Mexico's 32 federal entities. Bahía de Banderas is one of the fastest-growing municipalities in the state. With respect to the agricultural sector, the grain crops with the highest planting demand in this municipality are beans, sorghum, sugarcane, mango, and grain corn (SIAP, 2023). Notably, corn was the leading export product of Bahía de Banderas in 2022, with sales valued at US\$1.34 million, primarily destined for France (Data México, 2023).

According to Caamal y Pat (2018), the primary sector faces problems related to low crop productivity; limited access to and availability of resources; unstable irrigation systems; and the absence of adequate social protection mechanisms for agricultural workers. Similarly, Hernández (2020) identifies low productivity as one of the most significant challenges in agriculture, attributing it to the limited adoption of technologies and innovations; the scarcity and obsolescence of agricultural machinery; reduced water availability; and the inefficient use of productive resources.

Based on the above, the objective of this study was to determine the average productivity per hectare of commercial white corn among medium- and large-scale farming units in Bahía de Banderas, Nayarit, Mexico.

MATERIALS AND METHODS

The study adopted a quantitative, non-experimental, descriptive research design. To determine productivity in agricultural units, the formula established by the Agri-Food and Fisheries Information Service (SIAP, 2022) in its Technical Standards for the Generation of Basic Agricultural Statistics (NTGEBa) was applied. According to these standards, productivity is measured as yield and expressed per hectare (unit of measurement per hectare; tons/ha).

For this study, the target population consisted of medium- and large-scale producers cultivating more than five hectares of corn, listed in the database of the Valle de Banderas Irrigation Unit Users Association, A.C., Bahía de Banderas delegation, for the 2022-2023 autumn-winter agricultural cycle. The total population comprised 81 producers. It is important to note that no sampling procedure was applied; instead, a census of all 81 observational units was conducted.

During the fieldwork phase, a survey was administered to collect data for subsequent analysis. The instrument was designed based on previously validated tools, such as the National Agricultural Survey (ENA, 2020).

Once the survey instrument was designed, construct validity was assessed through expert review. The field instrument consisted of a 33-item questionnaire. The validation process was conducted during in-person meetings held at the agricultural production units and at the offices of the irrigation association. After the instrument had been validated, it was administered to the agricultural units. In addition to the questionnaire, field observation techniques were employed. The data collected were processed using Microsoft Excel and IBM SPSS Statistics through descriptive statistical analysis, encompassing data collection, organization, presentation, analysis, and reporting of the observed results.

RESULTS AND DISCUSSION

In the primary sector, productivity is determined through the variable yield per unit of measurement, which in this study was expressed as tons per hectare (tons/ha). To obtain the results for this variable during fieldwork, the final output (tons of corn per harvested hectare) was considered.

In addition, the economic value of the resources used to achieve this level of production was calculated. It is important to emphasize that the findings reflect the average productivity per hectare of commercial white corn among the 81 farmers included in the study.

Figure 1 illustrates the method used to determine productivity, as well as the production factors involved, such as land, labor, materials and inputs, financial and miscellaneous costs, and machinery and equipment, applied to obtain the final output.

The average yield of farmers in Bahía de Banderas was 8.2901 tons of corn per hectare. An important factor is the sale price, which during the 2022-2023 production cycle was below market expectations; as a result, some producers were unable to recover their investment costs. The average price was \$6,343.20 Mexican pesos per ton.

Regarding land, the average rental price per hectare was \$5,680.25.

In terms of labor, an average of one permanent field worker (employed throughout the entire production cycle) and two temporary workers (hired for specific stages of the cycle) were contracted per farmer. With respect to gender composition, 95% of the labor force was male and 5% female.

The stages of the production process with the highest labor demand were fertilization and pest control, as well as harvesting, selection, and packaging.

The average daily wage paid to field workers was \$401.54 Mexican pesos. During the production cycle, permanent labor worked a total of 45.87 days, while temporary workers were employed for 43.09 days. After the harvest, temporary workers were dismissed, generally receiving only the legally mandated benefits.

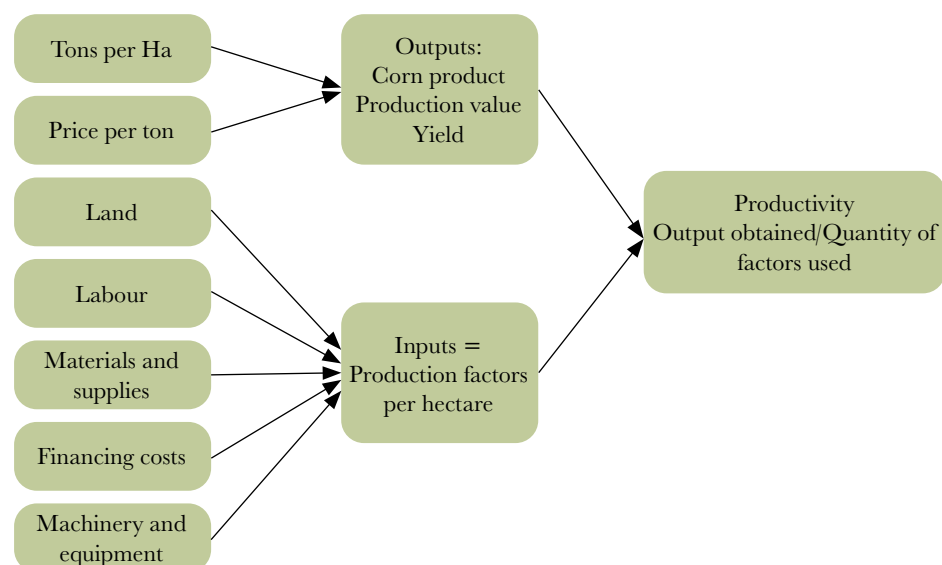


Figure 1. Elements to calculate productivity.

The producer actively participates in corn cultivation throughout the entire production process. Responsibilities include operational tasks, supervision, and administrative activities, which are performed based on accumulated experience and practical knowledge. On average, producers work 8 h and 40 min per day, with a maximum of up to 14 h daily, seven days a week, including holidays.

Most farmers do not receive a fixed salary; instead, their compensation depends on the net returns obtained at the end of the production cycle, after covering production costs and outstanding debts.

In addition, the inputs and materials used in the production of one hectare of commercial white corn included seeds, fertilizers, herbicides, insecticides, and pest control products, among others, with an average cost of \$22,163.27. Farmers in Bahía de Banderas purchase these inputs from local and regional suppliers.

Another production factor was financing cost. In this regard, 88% of the farmers did not obtain third-party loans for the production cycle, including credit from financial intermediaries or government programs, primarily because they considered that they did not meet the eligibility requirements and that interest rates were too high. Only 12% applied for and received a loan. The average financing cost was \$136.09 per hectare, while professional service fees averaged \$93.21.

Water use was one of the most critical resources in the production process. All surveyed farmers cultivated irrigated land. Irrigation services were provided by the Asociación de Usuarios de la Unidad de Riego Valle de Banderas AC. Water was delivered through canal systems and tanker trucks. For the 2022-2023 cycle, the irrigation cost was \$1,317.00 per hectare for all farmers.

Similarly, the cost of insurance, marketing, and technical assistance services averaged \$109.75 per hectare.

Machinery and equipment used by farmers represent a challenge to overcome, due to the high costs involved in acquiring, renting, and maintaining them. In the production cycle analyzed in this research, 53% of respondents reported renting machinery for this cycle, while 47% indicated that it was owned. Furthermore, this production factor was used for activities such as harrowing, subsoiling, plowing, sowing, spraying, cultivation, and threshing.

The total cost invested in the machinery and equipment production factor per hectare averaged \$7,179.47. Regarding the application of technology, drones were the only technology used by these farmers for the different irrigation stages of their land.

The procedure applied to obtain the average total production cost of one hectare of corn consisted of calculating the amount of production costs for each farmer separately (summing all the previously mentioned productive resources). Subsequently, the data from the 81 farmers who participated in the sample were added together and finally divided by the same number. The formula used is presented below.

Procedure for calculating the production cost for the 81 farmers

$$\text{Production cost} = r + L + ps + i + w + me + fc + mc$$

where: r =rent; L =labor; ps =profesional services; i =inputs and materials; w =water; me =machinery and equipment rental; fc =financing costs; mc =miscellaneous cost.

Finally, the average total production cost of one hectare of commercial white corn for the 2022-2023 cycle among the 81 medium- and large-scale farmers in the Bahía de Banderas region, Nayarit, was \$42,507.15.

Another important aspect is the average income per hectare of corn for the 2022-2023 cycle, which amounted to \$52,685.80, with an average profit of \$10,178.65.

Likewise, the study variable productivity per hectare was calculated according to the following procedure: first, yield per hectare was obtained using the formula tons/ha; it was then multiplied by the final sale price to obtain total income. Subsequently, this amount was divided by the total production cost of the same hectare in order to determine the productivity of each farmer.

$$Productivity = \frac{(ton / ha)(final price per ton)}{Total cost of the factors of production}$$

where: (ton / ha) =tons per hectare; $final price$ =Final market price per ton.

The average productivity per hectare of commercial white corn in the 2022-2023 cycle was 1.291497419. This result was obtained by dividing the productivity of all farmers by the same number of participants (Table 1).

Likewise, the previous table shows negative productivity for 14 (17%) farmers; that is, they incurred losses during this production cycle, as their input costs were higher than their income.

These results contrast with the technological package proposed by Trust Funds for Rural Development (FIRA, by its acronym in Spanish) (2023), which indicates that producing one hectare of white corn in Nayarit requires \$33,913.00 Mexican pesos. However, the findings of this study show an amount of \$42,507.15 for that same hectare in the same area, resulting in lower profit margins. Specifically, the results of this research estimate an average profit of \$10,178.65, compared to \$11,447 according to FIRA. Therefore, farmers receive lower income from their harvest.

In addition, Funds for Rural Development (FIRA) establishes a wage of 300 per workday for labor, whereas the results of this study show an average of \$401.54 per workday. In light of these data, the findings do not align with this institution, and farmers appear to be the least favored in terms of access to credit under the technological package developed by FIRA.

As noted by Sandoval (2023), Hernández (2021), Velásquez (2023), Gutiérrez (2023), National Agricultural Survey (ENA, 2020) y Ávila (2017), the main problem faced by agricultural production units is production costs. The results of this study confirm this situation and additionally highlight low sale prices, as pointed out by Gutiérrez (2023). These problems arise because farmers do not have guaranteed final prices for their products and must instead accept the prices offered by buyers.

Table 1. Productivity per hectare.

Number of farmers	Productivity	Number of farmers	Productivity	Number of farmers	Productivity
1	1.272322101	28	0.968532574	55	1.342786282
2	1.397750073	29	1.159012356	56	1.569867578
3	0.994425777	30	1.168687522	57	1.85437601
4	1.206109205	31	1.454394349	58	1.100110011
5	2.289377289	32	1.788672724	59	2.040446178
6	1.06379386	33	1.073701971	60	1.405337882
7	1.094376987	34	1.173468111	61	2.231376588
8	1.168017755	35	2.028992715	62	2.43405176
9	0.871444384	36	1.754550866	63	0.973885442
10	0.718670791	37	1.635343548	64	0.868827703
11	1.192543098	38	1.31755644	65	0.774316517
12	1.186805515	39	1.328211472	66	1.400239105
13	0.915720967	40	0.730394685	67	1.540770952
14	1.315648043	41	1.14093559	68	1.012526398
15	1.299625584	42	1.545479473	69	1.45075493
16	1.157814212	43	1.155046052	70	0.830361036
17	1.525179072	44	0.748732523	71	1.590544965
18	1.204197488	45	2.044692737	72	1.096304237
19	0.992171657	46	1.358261425	72	1.52025743
20	1.180873083	47	0.825140863	74	1.15166959
21	1.194479666	48	1.019619471	75	1.337579618
22	1.162018592	49	1.684800681	76	1.187267165
23	1.264498733	50	1.17172115	77	1.175529243
24	1.773039372	51	1.180615591	78	1.329014038
25	1.029090174	52	1.144574004	79	1.638334851
26	1.0074525	53	1.330613306	80	1.161574579
27	0.851072042	54	1.110489568	81	1.229799039
average productivity					1.291497419

Another factor influencing price formation is the lack of government support to regulate pricing policy, leaving producers to negotiate the final price of corn on their own. This situation reduces the value of production and directly affects the productivity of the economic units studied. Indeed, 17% of farmers reported low productivity, meaning that their costs exceeded their income. On the other hand, resources were not used efficiently or effectively.

Adding to the cost-related information, the findings also confirm what Ortiz and Castillo (2020) state: all agricultural processes generate both direct and implicit costs, which are important to estimate and analyze.

In the surveyed production units, those in charge do not keep formal records of their production costs, nor do they quantify them. Likewise, they do not consider the wages of

themselves or their family members who contribute to the work, which makes it difficult to calculate their actual profits accurately.

These results are similar to those established by Delgadillo and Leos (2016) who argue that financial costs are generally quantified for tax purposes and include all fixed and variable costs, but exclude those associated with business management and the wages of the producer or family members. Likewise, farmers keep informal accounting records, and few hire professional accounting services.

Another factor influencing the productivity of the surveyed producers is machinery and equipment used in operational processes. This production factor represents a challenge for farmers due to the high costs associated with acquisition, rental, and maintenance. In addition, a major problem lies in delays and the poor condition of rented machinery, as well as the lack of maintenance, adjustment, or repair, which causes interruptions in activities and results in additional costs. These are the same problems described by Hernández (2020).

Regarding technology adoption, its implementation is low; only a few producers use drones for irrigation in their production processes. On the other hand, the results of this research are consistent with Ramírez *et al.* (2022) and ENA (2020), in that lack of supervision, limited training, negligence, absence of a procurement and input planning strategy, slow and complex administrative procedures, and employee idleness contribute to problems in the overall operation of agricultural enterprises.

In the area where the surveyed farmers are located, the corn market is in constant fluctuation, influenced by macroeconomic variables such as inflation and climatic conditions, thereby generating difficulties for producers. In this regard, the findings are consistent with those of the FAO (2019), which explains that limited market access and the lack of organizational structure among farmers hinder the commercialization of their products.

With these results, productivity theory is confirmed, as it measures the efficiency of the resources used to obtain goods and services and their optimal management. In this sense, the findings are consistent with Muñoz (2012), who states that the fewer resources invested in producing the same quantity of goods, the greater the efficiency of the enterprise.

On the other hand, although farmers make extensive use of their available resources, they do not implement adequate controls to maximize their profits, leading to higher input costs, which directly affects low productivity.

CONCLUSIONS

In conclusion, producers reported an average productivity of 1.29 per hectare of commercial white corn. Some of them registered negative productivity during this period due to high production costs and low prices.

Another aspect to consider is the lack of homogeneity in production costs and income among the 81 farmers who participated in the study. Each of them applies their own methods for cost allocation and quantification, and in some cases, they do not maintain formal records, which makes the rapid and accurate collection of information difficult.

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Fruit quality characterization of wild and cultivated blackberries (*Rubus* sp.)

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ABSTRACT

Objective: To characterize wild and cultivated blackberries for fruit quality to identify those with outstanding traits for genetic improvement.

Design/methodology/approach: External and internal variables associated with fruit quality were evaluated in ripe blackberries. The variables evaluated were: fruit length, fruit width, fruit weight, fruit shape, seed area, pH, titratable acidity, and total soluble solids. Statistical analysis was performed for each variable. Cluster and multivariate analysis were assessed to determine variability and relationship between the germoplasm.

Results: The *Rubus glaucus* Benth. accession showed values similar to cultivated varieties in length, width, and fruit weight, number of drupelets per fruit, pH, and total soluble solids, while the *Rubus phillyrophyllus* Rydb. accession exhibited values similar to cultivated varieties in seed area, pH, titratable acidity, and total soluble solids. One classic variety and three contemporary varieties stood out with large, heavy fruits and more drupeoles, and one commercial variety had the highest total soluble solids.

Limitations on study/implications: Fruit quality characterization of wild and cultivated blackberries is an initial and essential step to identify germplasm with outstanding traits. Integrate this species into breeding programs will contribute to broadening the genetic base and incorporate their favorable alleles, actions that contribute to their utilization by humans and their conservation.

Findings/conclusions: The characterization allowed to identify the *R. glaucus* Benth. and *R. phillyrophyllus* Rydb. wild species with outstanding traits, which facilitates their appreciation and integration into breeding programs

Keywords: quality fruit, wild, cultivated varieties.

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INTRODUCTION

The blackberry is part of the berry group, whose consumption has increased internationally due to their visual and palatable appeal, including appearance, color, and taste. Additionally, their consumption provides various health benefits due to their high concentration of antioxidants, amino acids, organic acids, vitamins, and dietary



fiber (Bhat *et al.*, 2023; Schulz & Chim, 2019). These compounds give to blackberries antioxidant, neuroprotective, anti-inflammatory, antimicrobial, and antifungal properties (Mello *et al.*, 2023; González-Jiménez *et al.*, 2015; Risco *et al.*, 2019; Schulz & Chim, 2019; Villarruel-López *et al.*, 2025). High demand for blackberries has sparked farmers' interest in their cultivation for export, as it is a competitive and highly profitable activity (Zamora *et al.*, 2023). Michoacán is the region with the highest blackberry production due to land allocated to cultivation, climate, differentiated production methods, and cultural practices implemented (Rivas, 2016; Zamora *et al.*, 2023). This has made Mexico leading exporter of blackberries in America, with United States as primary commercial destination (SIACON, 2023). The most widely distributed and produced variety in Mexico is Tupy, developed by Embrapa in Brazil from cross between Comanche and Uruguay varieties and released in 1989 (Bassols *et al.*, 2022). Tupy variety replaced Brazos variety, released in 1959, because it produces large, firm fruits with a longer shelf life and no color reversion issues (Meza *et al.*, 2013).

Currently, blackberry production faces various field problems due to development of varieties from a reduced genetic base, leading to variability depletion. As a result, classic varieties developed in 1990s are being replaced. An alternative to overcome this barrier is crossing wild species with improved varieties, introducing new genes to broaden the genetic base and increase diversity.

Blackberries belong to genus *Rubus*, whose wild species are found on all continents and have adapted to diverse environmental conditions. China is considered Center of Origin for genus *Rubus*, while United States and China are Centers of Diversity due to their wide diversification of *Rubus* species (Meng *et al.*, 2022). However, in Mexico, several wild *Rubus* species have been described since arrival of Spanish conquistadors (BHL, 2006; UNAM, 2015). To date, 55 wild *Rubus* species have been reported, distributed in mountainous areas of Trans-Mexican Volcanic Belt, Sierra Madre del Sur, and Sierras of Chiapas and Guatemala (Rzedowski & Calderón, 2005; Reyes *et al.*, 2019; Rodríguez-Bautista *et al.*, 2021). These species grow in cold, temperate, and subtropical climates, at altitudes ranging from 1100 to 3600 meters above sea level, and are found in undergrowth, rural paths, ravines, and riverbanks.

Wild blackberry species are adapted to Mexico's diverse edaphoclimatic conditions, making them an important source of adaptation alleles for genetic improvement programs. It is important to note that several wild blackberry species in Mexico are threatened in their natural habitats due to invasive species, drastic climate changes, habitat destruction from human activities, urban growth (Hunter & Heywood, 2011; Rodríguez-Echeverry & Leiton, 2021), overharvesting of forest fruits, and societal unawareness of their conservation importance. Breeding programs would contribute to conservation of wild *Rubus* species by preserving live specimens in germplasm banks, botanical gardens, or botanical collections. Genetic improvement seeks to utilize wild species for human benefit and begins with understanding specific traits of each wild species that distinguish them from others through morphological, biochemical, physical, and quality evaluations. This evaluation allows selection of parental lines for crosses to develop new varieties. In Mexico, information on evaluation of wild blackberries is still limited. Evaluated species include *Rubus glaucus*

Benth., *Rubus adenotrichos* Schltdl., *Rubus sapidus* Schltdl., *Rubus cymosus* Rydb., *Rubus humistratus* Steud., and *Rubus coriifolius* Liebm., and *Rubus pringlei* Rydb. collected in Chiapas, Hidalgo, and Michoacán (Rodríguez-Bautista *et al.*, 2019; Rubio *et al.*, 2019; Rueda, 2017), as well as, *Rubus* spp. germplasm collected in Northeastern Sierra of Puebla and Sierra Centro of Veracruz (Ibañez, 2011). The objective of this research was to characterize fruit quality of wild and cultivated blackberries to identify those with outstanding traits for genetic improvement.

MATERIALS AND METHODS

Fruit collection

Blackberry fruits at commercial maturity were collected during 2021 and 2022 cycles. Germplasm identification and origin are presented in Table 1. Wild species accessions and classic varieties are part of *Rubus* collection of Colegio de Postgraduados Campus Montecillo, located in the State of Mexico (19° 27' 49.4" N and 98° 54' 19.5" W). The collection contains specimens of wild species from Chiapas, Hidalgo, Michoacán, and Puebla, and classic varieties from Michoacán. Name of classic varieties is due to time of their release between 1959 and 1990. Characterization included cultivars developed in recent years in Mexico (contemporary varieties) made up of public varieties of Autonomous University of Chapingo (UACH), identified with H, and commercial varieties provided by the company Splendor Produce, identified with V.

Determination of Quality Variables

The evaluation was carried out on 15 randomly selected ripe fruits without mechanical damage. External and internal variables associated with quality were: fruit length (FL),

Table 1. Blackberry genotype specifications.

Code	Wild species or variety	Class and type	Origin
ZS2	<i>Rubus</i> spp.	Accession	MEX
ZS7	<i>Rubus glaucus</i> Benth.	Accession	MEX
ZS10	<i>Rubus schiedeanus</i> Steud.	Accession	MEX
ZS11	<i>Rubus philyrophyllus</i> Rydb.	Accession	PUE
ZC1	Brazos	Classical, Public	MIC
ZC2	Tupy	Classical, Public	MEX
ZC3	Kiowa	Classical, Public	MEX
ZH1	Uruapan	Contemporary, Public	JAL
ZH2	América	Contemporary, Public	MIC
ZH3	Leonel	Contemporary, Public	MIC
ZH4	Cituni	Contemporary, Public	MIC
ZV3	Advanced selection 1	Contemporary, Commercial	JAL
ZV5	Advanced selection 2	Contemporary, Commercial	MIC
ZV6	Advanced selection 3	Contemporary, Commercial	MIC
ZV7	Advanced selection 4	Contemporary, Commercial	MIC

MEX: State of México, JAL: Jalisco, MIC: Michoacán.

fruit width (WF), fruit weight (WGTF), fruit shape (FS), hydrogen ion potential (pH), titratable acidity (TA), and total soluble solids (TSS). Additionally, number of drupeoles per fruit (NDF) and seed area (SA) were evaluated. Fruit length and width variables were considered to be polar and equatorial diameters; these attributes were measured with a digital Vernier caliper (OBI Model 215140). Fruit weight was obtained with a digital granataria scale. Fruit shape was calculated as length-to-width ratio; elongated fruits have $FFS > 1$, round fruits have $FS = 1$, and flattened fruits have $FS < 1$ (Magallanes-López *et al.*, 2020). Seed area was obtained with ImageJ program by determining ellipses in a digital image with 15 seeds (Schneider *et al.*, 2012; Ferreira & Rasband, 2012).

Blackberry juice or supernatant was used to evaluate internal variables: pH, TSS, and TA. Prior to this, fruits were disinfected in a diluted sodium hypochlorite solution, dried, ground with an electric mill, and centrifuged at 1000 rpm for 10 min. pH was measured in 3 g of juice diluted in 50 mL of distilled water using a potentiometer (Hanna). TSS were obtained with a refractometer (Atago) by placing two drops of juice and expressed in °Brix. These variables were measured 5 times. TA was determined according to AOAC 942.15 (2005). To do this, 3 g of juice were weighed, diluted in 50 mL of distilled water, and titrated with a 0.1 M NaOH standard solution (J. T. Baker) to pH 8.1 using a potentiometer (Hanna). Titratable acidity was assessed in triplicate. Percentage of citric acid per 100 g of sample was determined by the Equation 1.

$$TA = (V_{NaOH} \times N_{NaOH} \times meq \text{ citric acid} / W) \times 100 \quad (1)$$

where V_{NaOH} =volume of sodium hydroxide spent; N_{NaOH} =normality of 0.1 M NaOH, 1 meq citric acid=0.064 g of citric acid; W =weight of sample.

Statistical Analysis

Descriptive statistics were calculated for each variable and subjected to Kolmogorov-Smirnov normality test ($p > 0.05$) and Levene's homogeneity of variance test ($p > 0.05$). Kruskal-Wallis test was used to determine difference between variances, and DCG test was used to compare means (Di Rienzo *et al.*, 2002). Cluster analysis was performed using Ward's coefficient and squared Euclidean distance. Principal component analysis (PCA) was used to identify correlations between variables and with germplasm, and to determine similarity between genotypes by proximity. InfoStat Statistical Software (2008) was used to analyse the data.

RESULTS AND DISCUSSION

Table 2 presents average values for external fruit quality variables. Fruits of contemporary varieties were similar in width and length, except for varieties ZV6 and ZH1 (Uruapan), which stood out for their greater length, width, and weight, with a significant difference ($p < 0.05$). Fruits of accession ZS7 were similar in size to contemporary varieties, suggesting that *R. glaucus* Benth. has potential for genetic improvement. Accessions ZS2, ZS10, and ZS11 had smaller fruit width, length, and weight, with a significant difference ($p < 0.05$). Similar values for length, width, and weight of wild fruits have been reported for *R. pringlei*,

R. glaucus, and *R. adenotrichos* collected in Michoacán (Rubio *et al.*, 2019) and for fruits of *Rubus* spp. collected in Sierra de Puebla (Ibañez, 2011).

Regarding fruit shape, ZS2, ZS10, ZH3 (Leonel) and ZV6 presented flattened fruits, ZC1 and ZC2 presented round fruits and ZS7, ZH4 (Cituni), ZV3 and ZV7 presented elongated fruits, reiterating outstanding features of species *R. glaucus* Benth. In general, wild species of blackberry have rounded fruits, while improved varieties have an elongated shape. Shape of fruit is determined by the receptacle; therefore, complementary studies must be carried out to determine their relationship. Wild fruits of *Rubus* spp. with similar shapes were collected in Sierra de Puebla (Ibañez, 2011).

Number of drupeoles per fruit was similar in contemporary varieties. Commercial variety ZV6 stood out with a higher number of drupeoles, with a significant difference ($p < 0.05$). The fruits of varieties ZH1 (Uruapan), ZH3 (Leonel), and ZH4 (Cituni) from UACH had a similar number of drupeoles to commercial varieties. Accession ZS7 stood out with the highest number of drupeoles per fruit, with a significant difference ($p < 0.05$), verifying potential of *R. glaucus* Benth species for improvement. The fruits of accessions ZS2 and ZS10 had a lower number of drupeoles, with a significant difference ($p < 0.05$). These values are higher than those reported by Rodríguez-Bautista *et al.* (2019) in fruits of *R. adenotrichos*, *R. cymosus*, *R. humistratus*, *R. palmeri*, *R. pringlei* and *R. sapidus* collected in Chiapas, Hidalgo and Michoacán with 3 to 14 polydrupes per fruit. However, they are lower than number of drupeoles per fruit reported by Rubio *et al.* (2019) in *R. glaucus* and *R. pringlei* with 70 and 89 drupeoles, respectively, and by Ibañez (2011) in fruits of *Rubus* spp. with 30 to 89 drupeoles.

In this regard, it is important to note that fruit size is affected by anatomical, environmental, and genetic factors. Anatomically, position and number of stigmas in flower limits complete pollination, which affects number of drupeoles. Regarding environmental factors, fruit size depends on attraction of pollinators, absence of pests (vectors of diseases and/or viruses), nutrition, and environmental conditions. Genetically, fruit size depends on polyploidy. Blackberries are diploid (2x) or polyploid plants that can have from 2 to 18 pairs of chromosomes (Hummer *et al.*, 2016). Increase in size of fruits of improved varieties with respect to wild species is due to increase in ploidy (number of DNA copies), which generates plants with larger fruits due to greater number of drupeoles. In nature, this generates benefits such as more robust plants that are better adapted to climate changes (Alix *et al.*, 2017; Sattler *et al.*, 2016).

In general, seed area of blackberry fruits was lower in accessions of wild species. Accessions ZS2, ZS7, ZS10 and varieties ZV7 and ZH1 (Uruapan) from UACH stood out with the lowest seed area, with a significant difference ($p < 0.05$). Varieties with the largest seed area were ZV5 and ZC3, with a significant difference ($p < 0.05$). These values are similar to Indonesian *Rubus* species reported by Surya *et al.* (2021): *R. chrysophyllus*, *R. lineatus*, *R. niveus* and *R. glomeratus* with 3.39, 4.35, 6.73 and 6.99 mm², respectively. However, the genus *Rubus* presents wide diversity in this characteristic, as indicated by Surya *et al.* (2021), the species *R. pyrifolius*, *R. acuminatissimus* and *R. alpestris* have a larger seed area with 22.30, 19.56 and 14.7 mm², respectively, and the species *R. fraxinifolius*, *R. ellipticus* and *R. rosifolius* have a reduced seed area with 1.86, 2.03 and

2.66 mm², respectively. Seed area is related to surface area and size of seed, this variable is important because consumers prefer fruits with small or seedless seeds due to their ease of consumption, which has generated development of various seedless fruits such as: strawberry (*Fragaria* × *ananassa* Duchense ex Weston), grape (*Vitis vinifera* L.), papaya (*Carica papaya* L.), banana (*Musa paradisiaca* L.), etc.

Table 3 shows average internal fruit quality variables. pH was very similar among accessions and cultivated blackberries grown with pH between 3 and 4. Accessions ZS7 and ZS10 stood out with pH less than 3, with a significant difference ($p < 0.05$), indicating that these fruits are acidic. Varieties ZV5 and ZC1 stood out with pH greater than 4, with a significant difference ($p < 0.05$). pH of fruits is consistent with values obtained for species *R. adenotrichos*, *R. glaucus*, *R. pringlei* and Tupy variety from Michoacán with 3.1, 3.2, 3.3 and 3.3, respectively (Rubio *et al.*, 2019), as well as with wild materials collected in Sierra of Puebla with pH ranging from 2.8 to 3.2 (Ibañez, 2011) and with Tupy variety with pH 3.4 (Alonso, 2017). These results indicate that pH also varies depending on genetics of germplasm. It should be noted that pH is related to water activity and metabolites present in fruit, so detailed studies on the chemical composition of fruits are necessary to detect the cause of this variation.

Organic acids in fruit are determined by titratable acidity; acid present in blackberries is citric acid. Titratable acidity was very similar among accessions and cultivated blackberries, with average values close to 1.50. Accessions ZS7 and ZS10 stood out with higher titratable acidity, with a significant difference ($p < 0.05$), indicating that they have a higher citric acid concentration. This trait could be of interest in breeding to improve

Table 2. External fruit quality variables of wild and cultivated blackberries from México.

Code	FL (mm)	WF (mm)	WGTF (g)	FS	NDF	SA (mm ²)
ZS2	14.00±0.45d	15.20±0.86d	1.56±0.16d	0.93±0.05b	17.40±1.44e	3.10±0.22c
ZS7	27.40±1.12c	20.40±0.24c	7.08±0.24c	1.35±0.07a	139.40±5.72a	3.56±0.21c
ZS10	15.20±1.16d	16.00±1.52d	2.36±0.24d	0.96±0.06b	14.00±1.10e	3.67±0.26c
ZS11	17.00±0.45d	13.80±0.49d	1.66±0.10d	1.24±0.07a	33.60±1.72e	4.25±0.25b
ZC1	18.20±0.49c	17.80±0.49c	3.26±0.12c	1.03±0.05b	50.60±4.15d	4.30±0.26b
ZC2	24.40±0.40c	22.40±0.75c	5.86±0.15c	1.09±0.04b	78.80±8.38c	3.44±0.23c
ZC3	29.20±0.49c	24.20±1.24c	10.54±1.18b	1.22±0.07a	88.80±8.73c	6.22±0.62a
ZH1	51.32±7.87a	42.44±4.25a	11.40±0.25b	1.19±0.14a	80.50±1.50c	3.25±0.33c
ZH2	24.80±1.50c	20.60±0.93c	5.02±0.35c	1.21±0.08a	53.80±6.76d	4.96±0.41b
ZH3	19.40±0.40c	21.60±1.57c	4.40±0.15	0.92±0.07b	81.20±7.54	4.89±0.32b
ZH4	28.60±0.98c	19.40±0.60c	6.58±0.57c	1.48±0.05a	85.60±6.82c	4.36±0.44b
ZV3	28.80±1.11c	19.80±0.97c	10.30±0.87b	1.47±0.11a	86.00±3.65c	4.21±0.31b
ZV5	25.40±1.60c	19.80±0.66c	5.10±0.29c	1.29±0.09a	80.00±5.59c	6.27±0.51a
ZV6	28.00±4.15b	31.40±2.38b	13.18±0.83a	0.95±0.21b	110.40±12.09b	5.45±0.57b
ZV7	26.00±1.79c	19.40±0.24c	10.92±2.05b	1.34±0.10a	73.80±9.36c	3.20±0.10c

FL: fruit length, WF: fruit width, WGTF: fruit weight, FS: fruit shape, NDF: number of drupeoles per fruit, SA: seed area. Means with different letters indicate significant differences ($p < 0.05$) ± standard error.

Table 3. Internal fruit quality variables of wild and cultivated blackberries from México.

Code	pH	TA (%)	SST (°Brix)
ZS2	3.28±0.19b	1.88±0.09b	7.07±0.07c
ZS7	2.83±0.01e	3.64±0.06a	11.82±0.46b
ZS10	2.85±0.01e	3.32±0.04a	6.70±0.60c
ZS11	3.69±0.16b	1.05±0.14c	9.36±0.97c
ZC1	4.59±0.04a	0.47±0.01d	15.20±0.86b
ZC2	3.13±0.12d	1.73±0.11b	9.88±0.71c
ZC3	3.05±0.02d	2.15±0.23b	7.20±0.37c
ZH1	3.35±0.06b	1.72±0.30b	8.85±1.09c
ZH2	3.47±0.01b	0.94±0.08c	11.88±0.95b
ZH3	3.54±0.02b	0.79±0.01c	10.30±0.63c
ZH4	3.35±0.01b	1.14±0.05c	13.67±0.33b
ZV3	3.26±0.04b	1.24±0.07c	14.44±0.57b
ZV5	4.67±0.02a	0.39±0.14d	17.40±1.44a
ZV6	3.37±0.01b	1.05±0.06c	12.54±0.64b
ZV7	3.48±0.02b	0.92±0.01c	13.30±1.48b

pH: hydrogen ion potential; TA: titratable acidity; TSS: total soluble solids. Means with different letters indicate significant differences ($p < 0.05$) $\pm$ standard error.

nutraceutical quality of fruits. Varieties ZC1 and ZV5 presented lower titratable acidity, with a significant difference ($p < 0.05$). Titratable acidity was similar to those obtained in wild materials collected in Sierra of Puebla with 0.7 to 2.0 % citric acid/100 g of sample (Ibañez, 2011) and in *R. adenotrichos*, *R. glaucus*, *R. pringlei* and Tupy with 0.8, 0.9, 1.05 and 1.10% citric acid/100 g of sample, respectively (Rubio *et al.*, 2019).

It is important to mention that blackberry ripening process involves consumption of citric acid through metabolism, which decreases its concentration and increases concentration of amino acids and sugars, which is why presence of citric acid in ripe blackberries is low and total soluble solids increase. Ripening also modifies color of fruits, which is associated with secondary metabolites: anthocyanins and anthocyanidins, whose stability depends on pH and presence of glycosides (sugars). Variations in any of these conditions modify chemical conformation of anthocyanins and anthocyanidins, resulting in changes in color of blackberries (Reyes-Landa *et al.*, 2023; He and Giusti, 2020). Fruit ripening and quality are also related to longer shelf life (Clark *et al.*, 2007).

Total soluble solids determine the amount of sucrose present in fruits, which is associated with sweetness or sweetening power present in fruits. In general, contemporary varieties presented a higher concentration of total soluble solids than wild accessions, except for accession ZS7, with a significant difference ($p < 0.05$). Fruits of contemporary varieties are sweeter than wild fruits due to consumer preference; this preference has guided plant breeders towards selection of fruits with a higher concentration of total soluble solids, seeking greater market acceptance. In this regard, Clark & Finn (2011)

pointed out that sweet taste of fruits is detected by palate when they contain at least 10% of total soluble solids.

Variety ZV5 stood out with a higher concentration of total soluble solids, with a significant difference ($p < 0.05$), that is, it presented sweetest fruits. Accessions ZS10, ZS2, ZS11 and varieties ZC2, ZC3, ZH1 (Uruapan) and ZH3 (Leonel) presented fruits with a lower concentration of total soluble solids, with significant difference ($p < 0.05$). Fruits with similar concentrations of total soluble solids were obtained from *R. adenotrichos*, *R. glaucus* and *R. pringlei* collected in Michoacán with 12.4, 7.6 and 10.7 ° Brix, respectively (Rubio *et al.*, 2019). As well as in wild materials collected in Sierra of Puebla with 8.58 to 12.0 °Brix (Ibañez, 2011) and in Tupy variety with 9.0 and 9.8 °Brix (Alonso, 2017; Rubio *et al.*, 2019).

Accessions ZS2 and ZS11 presented pH and titratable acidity similar to cultivated varieties, accession ZS7 presented a quantity of soluble solids similar to cultivated varieties, so these accessions present traits of interest for genetic improvement.

The contrasts in fruit quality variables between wild accessions, classic varieties, and contemporary varieties indicate path followed by plant breeders in traditional genetic improvement of this species. Selected variables are related to fruit size, fruit shape, and total soluble solids. According to Clark & Finn (2011), another important point to consider in blackberry breeding is maintaining an adequate balance between titratable acidity and total soluble solids, as this balance imparts an intense flavor to fruit.

Clustering analysis is shown in Figure 1. Plant materials formed 3 groups: the first group was divided into two branches, one branch was made up of accession ZS7 and varieties ZV7, ZV3 and ZH4 (Cituni), which presented fruits with a similar number of drupeoles,

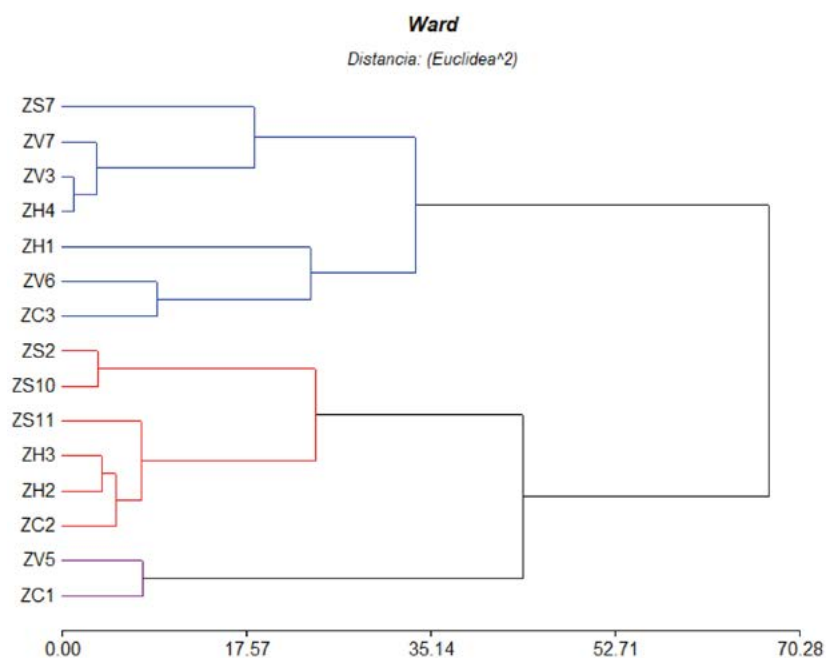


Figure 1. Clustering generated from Ward similarity index and squared Euclidean distance using quality variables from wild and cultivated blackberries.

this corroborates that species *R. glaucus* Benth. has outstanding characteristics. The other branch was made up of varieties ZH1 (Uruapan), ZV6 and ZC3 that share in common large fruits with a larger seed area. The second group was made up of two branches, one branch with accessions ZS2 and ZS10 that had in common small fruits with a lower number of drupeoles and a lower concentration of total soluble solids. The other branch was shared by accession ZS11, classic variety ZC2 and contemporary varieties ZH2 (America) and ZH3 (Leonel), these materials present similarity in fruit length and width, seed area, pH, titratable acidity and total soluble solids, so species *R. phillyrophyllus* Rydb. has novel traits. The third group consisted of varieties ZV5 and ZC1, which share a similar fruit width. Their fruits also had higher pH and soluble solids levels, and lower titratable acidity. It is important to reiterate that accessions ZS7 and ZS11 shared a group with several cultivated blackberries. Therefore, *R. glaucus* Benth. and *R. phillyrophyllus* Rydb. exhibit traits of interest for genetic improvement and should be considered for integration into genetic improvement programs. Further studies on ploidy of these materials will be necessary to determine feasibility of crosses between wild species and cultivated blackberries.

Table 4 and Figure 2 show Principal Component Analysis. Components PC1 and PC2 explain 65.9% of the variability. First component explained 36.4% of variation, with the largest contributions from variables: fruit weight, fruit length, number of drupeoles per fruit, and fruit width. Second component explained 29.5% through positive contributions of variables: pH and total soluble solids, and negative contribution of titratable acidity. Regarding correlation circle, external variables: number of drupeoles per fruit, length, width, and fruit weight were highly correlated. Meanwhile, the internal variables: total soluble solids and pH were negatively correlated with titratable acidity. Furthermore, there was a correlation between external and internal variables: total soluble solids and seed area. This indicates that fruits with larger seed areas will have a higher concentration of total soluble solids, meaning they will be sweeter.

Variety ZH1 (Uruapan) was characterized by high values of number of drupeoles per fruit and in length, width and weight of fruit, contrary to accession ZS11 and variety ZC1 located in the opposite quadrant, in the opposite direction to these mentioned

Table 4. Contribution of fruit quality variables to variability of Principal Components.

Variable	CP1	CP2
Fruit lenght	0.43	−0.16
Fruit width	0.49	−0.12
Fruit weight	0.50	−0.08
Fruit shape	0.27	0.12
Number of drupeoles per fruit	0.45	−1.8E-03
Seed area	0.12	0.33
pH	−0.04	0.56
Titrrable acidity	−0.05	−0.52
Total soluble solids	0.19	0.50

CP: Main component

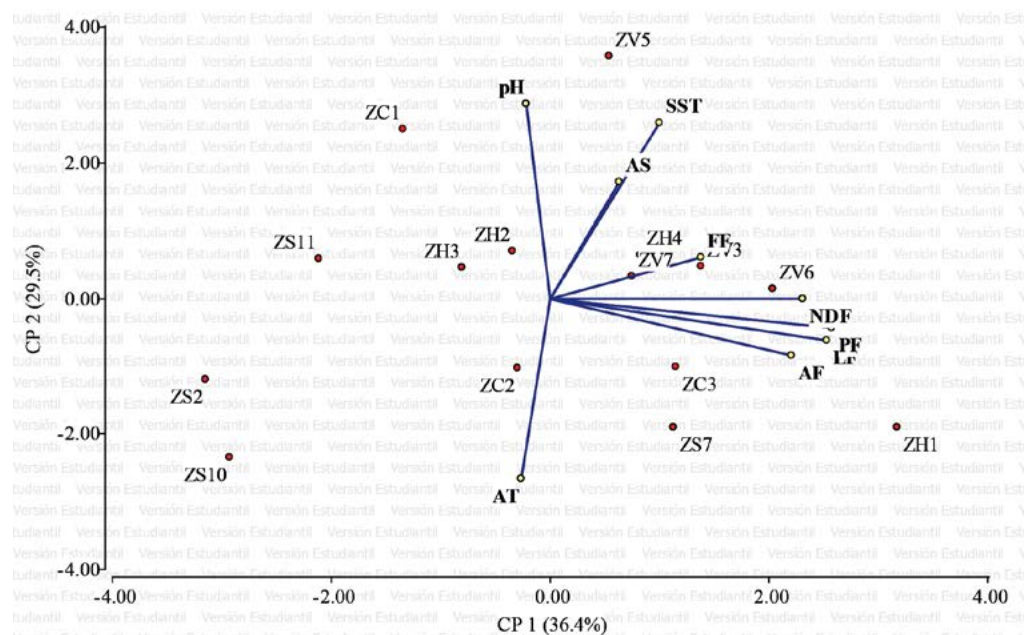


Figure 2. Principal Components Analysis obtained from Ward similarity index using external and internal variables of fruit quality determined in wild species and cultivated blackberries.

vectors (variables). Variety ZV5 was characterized by higher values in pH and total soluble solids. In PCA diagram, three groups were formed whose materials share quality variables between them. The first group was made up of accession ZS7 and varieties ZC3, ZH1 (Uruapan), ZV3, ZV4, ZV6 and ZV7, the second group was made up of accession ZS11 and varieties ZC1, ZC2, ZH2 (América) and ZH3 (Leonel) and the third group were accessions ZS2 and ZS10.

CONCLUSIONS

Characterization of blackberry fruit quality variables is essential for identifying wild species with outstanding traits, enabling their assessment and integration into genetic improvement programs. The results allowed to identify that *R. glaucus* Benth. and *R. phillyrophyllus* Rydb. wild species had outstanding traits, which facilitates their appreciation and integration into breeding programs. These actions contribute to their utilization by humans and their conservation.

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Economic and Productive Impact of Porcine Epidemic Diarrhea during Three Post-Infection Reproductive Cycles

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ABSTRACT

Objective: To evaluate the long-term productive and reproductive performance of a naïve group of sows infected with the Porcine Epidemic Diarrhea virus (PEDv) and to calculate the cost per weaned piglet.

Design/Methodology/Approach: A repeated-measurement design was used to compare productive performance over a farrowing horizon. The analysis included five reproductive cycles: a) farrowing prior to the PEDv outbreak; b) farrowing during the PEDv; c) first post-PEDv farrowing; d) second post-PEDv farrowing; and e) third post-PEDv farrowing. A general cost formula was used to estimate the cost per weaned piglet.

Results: Significant differences were recorded between the number of weaned piglets and the non-productive days during the outbreak ($P < 0.05$). Prior to the PEDv, sows weaned an average of 10.55 piglets. This figure dropped to 3.49 during the outbreak. Over the next three post-PEDv farrowings, the average remained below 9.60 piglets. The first post-outbreak farrowing recorded a 0.62 increase in stillborn piglets. The cost per piglet before the outbreak reached USD \$22.03; however, during the three subsequent farrowings the price per piglet after the outbreak was USD \$23.82, USD \$23.64, and USD \$23.66, respectively.

Study Limitations/Implications: Understanding the production costs of PEDv enables the development of strategies to lessen its financial impact on farms. It also provides an effective guide for sow management that reduces the impact of this disease on productivity.

Findings/Conclusions: The analysis of the productivity and economic impact—which included a review of sow productivity—showed that the cost per weaned piglet remained high for at least three subsequent farrowings after the outbreak was controlled. As a result of the emergence of highly virulent strains in Asia and North America, PEDv remains a significant pathogen in pork production.

Keywords: Swine coronavirus; pig diseases; productivity; weaned piglets; long-term disease.

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INTRODUCTION

Currently, the Porcine Epidemic Diarrhea virus (PEDv) is one of the four main emerging coronaviruses that impact swine production (Wang *et al.*, 2019). In China (2010) and in the USA (2013), large-scale outbreaks of highly virulent strains caused significant economic losses and forced farmers to design or strengthen biosafety protocols (Russell *et*



et al., 2019; Jarvis *et al.*, 2016). During this period, PEDv spread at a fast pace in North and Central America. In addition, the concomitant circulation of the Porcine Deltacoronavirus (PDCoV) was confirmed (Perez *et al.*, 2019; Niederwerder and Hesse, 2018; Trujillo *et al.*, 2016).

Porcine Epidemic Diarrhea (PED) is particularly hard on neonatal piglets of sows that were not previously exposed to the virus. Mortality rates range from 50 to 100%, as a consequence of diarrhea, vomit, and dehydration. The third effect, along with the increase of the production costs—due to the control measures adopted by producers—are the main cause of the high economic impact of this disease (Ji *et al.*, 2020).

In 2017, the USA reported 2,754 positive pig farms. In countries such as Colombia, PEDv still impacts farms (Qi *et al.*, 2020; Ajayi *et al.*, 2019). The emergence of new strains and the potential synergy with other infectious agents—such as porcine circovirus type 2 and 3 (PCV2/PCV3) and PDCoV—could aggravate the infection (Zhang *et al.*, 2021; Guo *et al.*, 2020; Jung *et al.*, 2020). In Mexico, García-González *et al.* (2023) analyzed 1,054 serums collected in eight states of the country during 2019–2021 and detected 61.66% of PEDv positive cases. According to these authors, Jalisco was the state with the highest percentage of positive samples (256; 21.44%). In addition, the circulation of emerging PEDv strains with amino acid insertions in protein S has been recorded. These insertions can produce new antigenic variants (Revels-Félix *et al.*, 2020).

The United States Department of Agriculture (USDA) stopped monitoring the disease in 2018, when the number and intensity of the cases diminished. Nevertheless, some authors pointed out that the emergency of less virulent PEDv strains—able to persist in young animals—could result in an endemic disease (Lin *et al.*, 2019; Chen *et al.*, 2016; Crawford *et al.*, 2016; Langel *et al.*, 2016). There are several inactivated vaccines available in the market; however, herd immunity through a controlled exposition to the virus in the field (feedback) is the most common method to stimulate the production of the secretory immunoglobulin A (S-IgA) (Hsueh *et al.*, 2020; Chang *et al.*, 2018). The feedback method stimulates the passive immunity of the sows against circulating strains; however, the lack of a standardized inoculum preparation method can modify the results or even worsen the health situation of the farms. Although research is currently focused on designing first-generation vaccines for the different strains (Yu *et al.*, 2020), several studies agree that the PED impact on the (re)productive performance of the herd depends on the physiological stage in which sows are exposed to the virus, but that it, at least, includes the subsequent farrowing (Furutani *et al.*, 2018; Sasaki *et al.*, 2017). Olanratmanee *et al.* (2010) reported that this effect is more severe on gilts and sows during early gestation stages.

Langel *et al.* (2020) emphasized that the gestation stage in which immunity protocols are executed is significant for the improvement of the protection of the herd against PEDv, ultimately leading to an optimization of the control measures. Several strategies have been simulated to evaluate the PED impact on the future productivity of the farms; however, post-outbreak data are still limited.

As a consequence of the persistent importance of this disease for pork production in Mexico, the objectives of this study were: (1) to evaluate the long-term effects of PEDv on

the (re)productive performance of infected sows; and (2) to estimate the cost per weaned piglet in the three subsequent farrowings once the initial outbreak had been controlled.

MATERIALS AND METHODS

Pig farm and PEDv outbreak description

The study was carried out in a full cycle pig farm (from farrow-to-finish), located at 1,670 m.a.s.l. in the plateau of the State of Mexico. The average stock included 170 Landrace-Yorkshire hybrid sows, and a reproductive cycle target of 116 gestation days, 21 lactation days, and 7 days until the first post-weaning. Productive and financial data were directly obtained from the database of the pig farm, from October 2013 to June 2015.

Before the outbreak, the farm recorded an average of 2.36 farrowings per sow per year and an 84% fertility average. The initial outbreak was confirmed during the last week of March 2014. A polymerase chain reaction (PCR) was applied to the feces, diarrhea, and small intestines (jejunum and ileum) during the necropsies of recent diseased piglets. The results of the tests were positive and helped to rule out the presence of the transmissible gastroenteritis virus (TGEV) and rotavirus (Hernández *et al.*, 2018).

In order to determine the PEDv long-term effect, 61 sows that initially contracted the disease were evaluated and monitored during three subsequent farrowings. A week after the symptoms appeared, a herd immunity process was conducted using the exposition method (feedback). The intestines of infected piglets were collected, crushed, and mixed to prepare an approximately 10-ml oral solution that was administered to pregnant, lactating, and weaned sows, as well as replacement gilts once a day for 13 days.

Statistical analysis

In order to determine the long-term (re)productive performance of the group of sows infected with PEDv, the following variables were analyzed: total number of born piglets, total number of liveborn piglets, total number of stillbirths, total number of mummified piglets, total number of weaned piglets, lactation duration, non-productive days, and interval between farrowings.

A repeated measure analysis (Park *et al.*, 2009) used time as a classification variable and (re)productive performance as a dependable variable. The best covariance structure (heterogeneous-autoregressive (HAR) model) was determined. In addition, an adjusted Tukey's test was conducted to establish significance (Kramer, 1956). Five periods were established: 1) before the PEDv outbreak; 2) during the PEDv outbreak; 3) first post-PEDv farrowing; 4) second post-PEDv farrowing; and 5) third post-PEDv farrowing. The analyses were carried out using SAS OnDemand for Academics (SAS Institute, Cary, NC, USA; License: u58666318).

Cost analysis per weaned piglet

The total cost formulas proposed by Muñoz and Rouco (1995) and Amador *et al.* (2022) were used to analyze the costs, focusing on the cost per weaned piglet (Table 1). The costs were calculated using the USD \$1=MXN \$14.7414 exchange rate published by the Banco de México in August 2014.

Table 1. Formulas used to calculate the cost per weaned piglet.

Formula		
General	$C = F + V$	C =Cost per weaned piglet. F =Fixed cost. V =Variable cost.
Fixed cost	$F = L + S + Co + R + A + Fi + COf + Ot$	L =Labour costs. S =Inputs costs. Co =Energy and fuel costs. R =Cost of repairs and maintenance. A =Amortization of fixed assets. COf =Fixed opportunity costs. Ot =other costs.
Variable cost	$V = ((SA + SF + AI + PF + M + T + COv) / (TOOSOW \times W)) \times z$	SA =Sow amortization SF =Sow feeding cost. AI =Artificial insemination. PF =Piglet-feeding cost. M =Medicines. T =Transport. COv =Variable opportunity cost. $TOOSOW$ =Total number of sows. W =Piglet-weighting factor. z =Number of weaned piglets.
Sow amortization	$AR = (PS - (PC - (1 - MORR))) / (AFPS / AFSY) - REP$	PS =price per sow. CS =price per culled sow. $MORR$ =% of sow mortality. $AFPS$ =total average number of farrowings per sow. $AFSY$ =average number of farrowings per sow per year. REP =sow replacements.
Number of farrowings (Regardless of sows' productive stage).	$PARM = \sum (CER \times n) / TOTSOW$	CER =Number of sows. n =Number of farrowings. $TOTSOW$ =Total number of sows.
Number of farrowings per sow per year.	$PAR = 365 / (116 + LAC + INT) \times (1 - NAB + VAC / CUB)$	LAC =Lenght of lactation. INT =Weaned-to-fertile service interval. NAB =Number of abortions. VAC =Non-productive shows. CUB =Number of reproductive services.
Piglet weighting factor	$w = PAR \times VIV \times (1 - MOR)$	VIV =Number of live-born piglets per farrow. MOR =Piglet mortality

The cost per weaned piglet was estimated for the periods previously established in the repeated measure design. The following combined costs were calculated to determine the long-term economic impact of PEDv: a) a weaned piglet, including the cost during the outbreak and the first post-PED farrowing (Goede and Morrison, 2016); b) a weaned piglet, including the cost during the outbreak and the first and second post-PED farrowings ; and c) a weaned piglet, including the cost during the outbreak and the first, second, and third post-PED farrowings.

RESULTS AND DISCUSSION

Productive and reproductive performance

PEDv did not have an impact on the total number of born piglets, liveborn piglets, mummified piglets, or the lactation duration (Table 2). Nevertheless, a 0.62 increase of stillbirths during the first farrowing post-outbreak was recorded ($P<0.05$). The number of weaned piglets and the non-productive days were different during the outbreak ($P<0.05$).

Before the PED outbreak, the group of sows weaned an average of 10.55 piglets, but during the outbreak they only weaned 3.49 piglets. During the subsequent three post-PEDv cycles, the average did not exceed 9.60 piglets. The non-productive days variable had an erratic behavior; however, a significant effect on farrowings ($P<0.05$) was clearly identified during rather than before the outbreak (16.16 ± 2.07 days). No differences were recorded when the farrowing cycles during the outbreak were compared with the two subsequent post-PEDv farrowing cycles.

Analysis of the cost per weaned piglet

The lower cost per weaned piglet was recorded before the PEDv outbreak (USD \$22.30). The highest cost was registered during the outbreak farrowing (USD \$32.50), followed by the cost of the third post-PED farrowing (USD \$23.98) (Table 3). Combining the costs before and during the outbreak farrowing cycles, the estimated cost per weaned piglet was USD \$24.43. The following formula was used to determine the combined cost per farrowing cycle: before the outbreak + during the outbreak + first post-PEDv farrowing + second post-PEDv farrowing + third post-PEDv farrowing. Likewise, the combined costs per farrowing cycle were higher than the costs recorded before the outbreak (Table 3).

Regarding the variable cost structure, the total feed for sows (FSTOT) heading did not show a significant variation between reproduction cycles. The feed during lactation (FL) and feed for weaned piglets (FWP) headings diminished during the outbreak, while the feed after the weaning (FW) increased (Table 3). The FL and FWP costs were lower during the two subsequent farrowings. The FW cost was higher during the first subsequent farrowing than before the outbreak.

Table 2. Productive and reproductive performance of the farrowings of a group of sows infected with PEDv during the last gestation or lactation stages: before the outbreak, during the outbreak, and first, second, and third post-PED farrowings.

Variable	Before	After	1st post-PED	2nd post-PED	3rd post-PED
n	52	61	51	46	32
Total piglets born	12.18 $\pm$ 0.47	12.44 $\pm$ 0.43	12.90 $\pm$ 0.50	12.16 $\pm$ 0.50	11.92 $\pm$ 0.66
Live-born piglets	11.29 $\pm$ 0.43	11.48 $\pm$ 0.39	10.93 $\pm$ 0.44	11.22 $\pm$ 0.45	10.80 $\pm$ 0.61
Stillborn piglets	0.43 $\pm$ 0.14 ^a	0.61 $\pm$ 0.13 ^a	1.23 $\pm$ 0.14 ^b	0.56 $\pm$ 0.15 ^a	0.48 $\pm$ 0.20 ^a
Mummified piglets	0.45 $\pm$ 0.12	0.36 $\pm$ 0.11	0.73 $\pm$ 0.13	0.38 $\pm$ 0.13	0.64 $\pm$ 0.17
Weaned piglets	10.55 $\pm$ 0.44 ^a	3.49 $\pm$ 0.40 ^b	9.60 $\pm$ 0.45 ^a	9.20 $\pm$ 0.46 ^a	9.48 $\pm$ 0.62 ^a
Length of lactation	18.90 $\pm$ 1.09	15.03 $\pm$ 0.03	17.40 $\pm$ 1.13	19.35 $\pm$ 1.16	21.08 $\pm$ 1.56
Number of unproductive days	7.59 $\pm$ 2.11 ^a	16.16 $\pm$ 2.07 ^b	11.00 $\pm$ 2.13 ^{ab}	8.00 $\pm$ 2.40 ^{ab}	5.37 $\pm$ 3.02 ^a
Farrowing interval	144.81 $\pm$ 4.40	144.06 $\pm$ 3.17	148.49 $\pm$ 3.20	146.28 $\pm$ 3.37	148.88 $\pm$ 4.04

^{a,b} Values with different letters in the same column are statistically different ($P<0.05$).

Table 3. Structure of the cost of the farrowings for the group of sows infected during the last gestation or lactation stages: before the outbreak, during the outbreak, during the subsequent first, second, and third post-PEDv farrowings.

Item	Before	During	+ 1	+ 2	+ 3
Fixed cost					
Total fixed cost	25,689.87	25,689.87	25,689.87	25,689.87	25,689.87
Variable cost					
SA (Sow amortization)	1,425.84	1,425.84	1,425.84	1,425.84	1,425.84
FG (Feed for gestation stage)	33,694.80	33,870.22	32,859.75	31,584.63	32,773.67
FL (Feed for lactation stage)	20,766.25	16,600.09	18,644.33	19,906.50	22,528.34
FW (Feed for sow at weaning stage)	2,117.96	4,532.86	2,993.43	2,090.18	1,457.51
FSTOT (Sow total feed)	56,579.01	55,003.17	54,517.51	53,581.31	56,759.52
FB (Feed for boar)	2,123.19	2,123.19	2,123.19	2,123.19	2,123.19
FWP (Feed for weaned piglets)	1,003.54	333.71	890.55	819.39	877.11
AI (Artificial insemination)	2,028.30	2,028.30	2,028.30	2,028.30	2,028.30
M (Medicines)	6,375.91	6,492.38	6,064.82	5,941.22	5,993.80
VO (Variable opportunity)	3,665.65	3,550.44	3,527.68	3,462.37	3,645.72
Total variable cost	73,201.44	71,007.03	70,577.89	69,381.62	72,853.48
Total variable cost*	73,897.91	23,285.64	66,843.92	61,247.85	69,038.25
Total cost	99,587.78	48,975.51	92,533.79	86,937.72	94,728.12
Cost per weaned piglet	22.03	32.58	23.07	23.55	23.98

*Including: / (TOTSOW * W) *z. + 1 = 1st post-PED farrowing, + 2 = 2nd post-PED farrowing, + 3 = 3rd post-PED farrowing.

Based on the analysis of the combined cost structure of the farrowing cycles —before and during the outbreak and during the three subsequent farrowings—, the cost of feed during lactation (FL) and feed for piglets (FP) was lower than the costs recorded before the outbreak (Table 4). Meanwhile, the cost of feed for weaned sows (FW) increased when farrowings during the outbreak and the three post-PED farrowings were included in the analysis. The inclusion of the productive performance of the PEDv infected sows in the estimated cost per weaned piglet resulted in a higher value than the farrowing cost before the outbreak (Table 4).

Productive and reproductive performance

PEDv does not change the total number of born piglets, liveborn piglets, mummified piglets, or lactation duration (Furutani *et al.*, 2018; Sasaki *et al.*, 2017); however, it can increase non-productive days and the number of stillbirths during the first post-outbreak farrowing (Olanratmanee *et al.*, 2010), as a consequence of the premature loss of litters (Lin *et al.*, 2016). In the evaluated pig farm, the number of stillbirths increased during the first post-PED farrowing, possibly as a result of the reduction of daily feed consumption by sows and the inefficient use of nutrients, which led to a delayed estrus and a lower milk production.

Tsukahara *et al.* (2018) and Gao *et al.* (2021) reported that the PED infections can take place even after the application of immunity protocols. For their part, Murai *et al.* (2018) concluded that the mortality increase in complete cycle pig farms could be the consequence

Table 4. Structure of the combined cost of farrowing cycles for the group of sows infected during the last gestation or lactation stages: before and during the outbreak, and during the first, second, and third post-PEDv farrowings.

Item	Before	Before + During	Before + During + 1	Before + During + 1 + 2	Before + During + 1 + 2 + 3
Fixed cost					
Total fixed cost	25,689.87	25,689.87	25,689.87	25,689.87	25,689.87
Variable cost					
SA (Sow amortization)	1,425.84	1,425.84	1,425.84	1,425.84	1,425.84
FG (Feed for gestation stage)	33,694.80	33,782.28	33,469.07	33,440.78	33,305.19
FL (Feed for lactation stage)	20,766.25	18,688.58	18,673.56	19,268.43	19,930.99
FW (Feed for weaning stage)	2,117.96	3,322.27	3,210.63	2,959.82	2,654.49
FSTOT (Sow total feed)	56,579.01	55,003.17	55,353.26	55,669.03	55,890.67
FB (Feed for boar)	2,123.19	2,123.19	2,123.19	2,123.19	2,123.19
FWP (Feed for weaned piglets)	1,003.54	669.50	744.55	775.07	795.81
AI (Artificial insemination)	2,028.30	2,028.30	2,028.30	2,028.30	2,028.30
M (Medicines)	6,375.91	6,433.99	6,308.65	6,293.22	6,293.26
VO (Variable opportunity)	3,665.65	3,608.20	3,580.86	3,598.76	3,608.68
Total variable cost	73,201.44	71,292.19	71,564.65	71,913.41	72,165.55
Total variable cost*	73,897.91	47,997.28	54,190.98	56,860.76	59,129.60
Total cost	99,587.78	73,687.15	79,880.85	82,550.63	84,819.47
Cost per weaned piglet (USD)	22.03	24.43	23.82	23.64	23.66

*Including: / (TOTSOW * W) *z. 1=1st post-PED farrowing, 2=2nd post-PED farrowing, 3=3rd post-PED farrowing.

of the long persistence of the virus during the finish line, where swines can be infected and spread the virus without showing any symptom. Meanwhile, García-González *et al.* (2023) highlighted that PEDv is still present in key swine regions of Mexico and that its impact on the domestic production could be higher than expected, suggesting a long persistence of the virus. The serums analyzed by these authors belonged to 6-month-old piglets from slaughterhouses and backyard farms. Reveles-Félix *et al.* (2019) recorded the circulation of non-Indel-S emerging strains with insertions of protein S in 15 Mexican states (2016-2018), suggesting that the virus still circulated and persisted in the population. According to Saif *et al.* (2019), the virus can be found in circulation for more than 200 days.

The sows of the impacted farm weaned less piglets during the outbreak and the three subsequent farrowing cycles than they did before the outbreak. Furutani *et al.* (2020) reported that the PED-impacted farms that applied herd immunity processes right after the first symptoms appeared recorded an increase in the number of piglets that died during lactation (5.9 per litter during the first month) and a reduction (2.5 per litter during the third month post-outbreak). This study shows that neither the number of stillbirths nor the number of weaned piglets returned to the pre-outbreak levels. This situation could be the consequence of the short duration of the protection granted by the immunity process (Langel *et al.*, 2020), the diversity of the strains with different virulence levels found in the field, the synergy with other infectious agents (Zhang *et al.*, 2021); the use of non-

standardized oral inoculums for the feedback process; or the lack of biosafety measures in the handling of the feed.

Jung *et al.* (2020) argue that immunity achieves better results if applied during the middle of the gestation period. Meanwhile, the number of stillbirths per litter increase and the sows have less neutralizing antibody titers if the treatment is applied during early or later stages. Regarding the long-term effects of PED, Perri *et al.* (2019) reported that eliminating the virus from an infected pig farm takes approximately 43 weeks, while Jang *et al.* (2021) pointed out that its full eradication may take up to 24 months. García-Cano *et al.* (2023) mentioned that the seroconversion changes throughout time, depending on the inoculum amount and mean.

The remedial strategies to tackle PEDv in the field must be coordinated to strengthen biosafety in room and during transportation —through standardized cleaning and disinfection, a drying processes, and the application of heat, whenever possible— (Baker *et al.*, 2017) and feed biosafety —through origin and storage control and the application of pelletized heat treatments (Trudeau *et al.*, 2017; Cochrane *et al.*, 2017). In addition, prioritizing lactogenic immunity with mother immunization and gilt handling must be aligned to the gestation stage to maximize S-IgA content in colostrum and milk (Wei *et al.*, 2024).

Analysis of costs per weaned piglet

The estimation of the economic impact of PEDv can be inaccurate due to the variability in the outbreak severity and duration (Schulz and Tonsor, 2015). Goede and Morrison (2016) found significant productivity variations resulting from the effects of PED, as well as a 7-64-week recovery time (29.5 weeks in average). The authors agree that the main financial impact of PED lays in the reduction of the number of weaned piglets per litter, highlighting the importance of the analysis of the cost per weaned piglet.

This study focused on the average number of weaned piglets: 10.55 before the outbreak, 3.49 during the outbreak, and 9.60, 9.20, and 9.48 in the first, second and third subsequent farrowings, respectively. These fluctuations had important economic impacts.

Although no productive statistical difference was recorded between the number of weaned piglets before the outbreak and during the first, second, and third subsequent farrowings, the cost per weaned piglet increased by USD \$1.04, USD \$1.52, and USD \$1.95, respectively. Amador *et al.* (2022) reported a production cost per weaned piglet of USD \$114.50 and USD \$133.94 during the first and second week of a PED outbreak in Mexican farms. They agreed that the production costs remained high for at least eight weeks after the initial outbreak.

This study determined that PED had a clear impact on the group of infected sows, particularly during the first post-outbreak farrowing, and that the cost per weaned piglet returned to its previous levels after at least three subsequent reproductive cycles. Jang *et al.* (2021) highlighted the importance of an appropriate handling of gilts in endemic farms, because they can be infected during the acclimatization process, without showing symptoms, consequently, spreading the virus in the farrowing units. Mai *et al.* (2020) pointed out that the persistent presence of the virus in slurries and in the fattening-finishing line increases

the possibility of exposure and infection among gilts. Weng *et al.* (2016) proposed different intervention strategies, including the handling of gilts, which can significantly reduce the impact of PED.

The closure of the pig farm can impact production as a consequence of the lack of herd restocking, while adding new gilts after the outbreak could cause re-infections due to the introduction of susceptible non-immunized animals. Implementing strict biosafety protocols (feedback immunity) is fundamental, because this process involves a contamination risk. Nevertheless, Brown *et al.* (2019) and Won *et al.* (2020) agree that the exposition to field strains creates higher antibody titers. The reduction of losses associated with PED justifies the cost of applying this type of control measures (Weng *et al.*, 2016).

The few available reports about the economic impact of PED do not include its impact on consumption and feed conversion. Although the objective of this study was not to evaluate feed performance, the feed cost varied depending on the productive stage: the cost of feed for weaned sows increased and the cost of lactating and gestating sows decreased. Feed for piglets also recorded a lower cost during at least the three first post-outbreak farrowings, as a result of a lower number of weaned piglets.

Álvarez *et al.* (2016) reported higher mortality rates and lower weight gain among weaned piglets after a PED outbreak, as well as the persistence of the virus in the fattening line; consequently, taking into account the low post-PEDv yield of piglets is fundamental.

In financial terms, the review of the performance of sows showed variations in the cost per weaned piglets in the farrowings during the outbreak and the first, second, and third post-PED farrowings, recording USD \$2.40, USD \$1.79, USD \$1.69, and USD \$1.63 increases, respectively. Therefore, the herd produced an average increase of USD \$2,044.24 and USD \$1,539.43 of the return on investment during the lactation and the weaning stage, respectively. The increase of the total variable cost reached USD \$429. At least three subsequent farrowings were required after the outbreak to recover the total losses associated with the disease. These losses were estimated for a herd of 170 sows, which resulted in 487 piglets, almost twice the 261 and 287 piglets reported by Amador *et al.* (2022) and Goede and Morrison (2016), respectively. The current study is one of the first scientific approaches that takes into account both long-term productive and economic indicators of sows impacted by PED.

CONCLUSIONS

The PEDv outbreak had a significant negative impact on the number of non-productive days and the weaned piglets during its duration. The number of stillbirths increased during the first post-outbreak farrowing. The evaluated group of sows only recovered its previous production levels after, at least, three subsequent reproductive cycles. Likewise, taking into account the productivity of the sows, the cost per weaned piglet was higher than the cost before the outbreak for at least three subsequent farrowings. Since the emergence of highly virulent strains in Asia and North America, PEDv remains a major pathogen for swine production.

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Identification of sources of resistance to *Meloidogyne* spp. nematodes in striped Jalapeño pepper (*Capsicum annuum* L.)

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ABSTRACT

Objective. To identify striped Jalapeño pepper plants (*Capsicum annuum* L.) as a source of genotypes resistant to *Meloidogyne enterolobii* and *Meloidogyne incognita* that enable genetic diversification in the development of improved crops within genetic improvement programs.

Design/Methodology/Approach. The resistance of 15 accessions of striped Jalapeño peppers native to northern Hidalgo (Mexico) to *Meloidogyne enterolobii* (Me), and of 12 accessions to *Meloidogyne incognita* (Mi) were evaluated. The experimental unit was one plant per replicate, with four replicates per accession. Each plant was inoculated with 1000 J2 of Me and Mi obtained through monoxenic selection. The California Wonder (CW) variety was used as a susceptible control against both *Meloidogyne* species, and the CM-334 line was used as a resistant control against Mi. Resistance was evaluated using the variables number of galls, number of egg masses, number of eggs per gram of root, and reproduction index. Evaluations were performed 34 days-post inoculation (dpi) for Me and 42 dpi for Mi. A randomized complete block design was used in R Studio[®].

Results: Accessions CP922, CP933, CP937, CP938, CP939, and CP946 showed resistance to both species. Whereas accessions CP932, CP903, and CP942 showed specific resistance to Mi, and accessions CP941 and CP935 showed resistance to Me. In particular, the striped Jalapeño accessions CP922, CP933, CP937, CP938, CP939, and CP946 showed evidence of a response range from moderately resistant to highly resistant to both *Meloidogyne* species.

Limitations/Implications of the study. This research is an important starting point for identifying intraspecific variation leading to selection for resistance to root-knot nematodes in Jalapeño peppers. At the same time, it constitutes an initial exploratory phase, in which the characterization of this variation does not yet allow for the identification of the physiological, biochemical, or molecular mechanisms that explain the observed resistance. Further research is therefore suggested.

Findings/Conclusions. The identified accessions represent a promising alternative for use in genetic improvement programs for *Capsicum* spp., toward resistance to the root-knot nematodes *Meloidogyne enterolobii* and *Meloidogyne incognita*. However, for the development of appropriate integrated pest management strategies, it is recommended to incorporate molecular biology, transcriptomics, or metabolomics tools to validate the strategic use of the identified variation.

Keywords: reproduction index, *Meloidogyne enterolobii*, *Meloidogyne incognita*, genetic improvement.

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INTRODUCTION

Nematodes of the genus *Meloidogyne*—present in various agricultural areas of Mexico—affect vegetables, fruit trees, ornamentals, and staple crops. Although more than 100 species of this genus have been described (Moreira *et al.*, 2018), studies have focused primarily on *Meloidogyne incognita*, *M. hapla*, *M. javanica*, and *M. arenaria*, due to their polyphagous nature and the serious damage they cause worldwide. However, in recent years, *M. enterolobii* has gained greater relevance due to its increasing geographic distribution, wide host range, and high aggressiveness and virulence (Sikandar *et al.*, 2021). Losses caused by these nematodes in agricultural production have driven the search for new control alternatives. Among those, one of the most promising strategies is the identification of resistant genotypes for the development of genetic improvement programs aimed at obtaining crops tolerant or resistant to these pathogens (Devran *et al.*, 2023).

The first source of genetic resistance to *Meloidogyne* spp. was identified in wild forms of tomato [*Lycopersicon peruvianum* L.] (Arias *et al.*, 2009). However, it is recognized that native crops constitute a fundamental reservoir of genes associated with resistance to diseases and adverse environmental conditions (Després *et al.*, 2007). Mexico represents a center of genetic diversity for *Capsicum annuum* L., distributed throughout virtually the entire territory. Plants with genetic resistance to *M. incognita* have been identified in Serrano and Huacle chili pepper lines (Gómez-Rodríguez *et al.*, 2017), as well as in native peppers from different Mexican states with resistance to *M. enterolobii* (Carrillo *et al.*, 2020).

However, to date, there are not commercially available chili pepper varieties with proven resistance to *M. enterolobii*. This highlights the need to continue exploring materials with potential resistance to Me. In this regard, researchers from the Genetics Program at Colegio de Postgraduados (Mexico) have collected various chili pepper varieties from different states in Mexico, including striped Jalapeño pepper accessions from the state of Hidalgo, which have not been evaluated for their resistance to plant parasitic nematodes.

Therefore, the objective of this study was to identify striped Jalapeño pepper plants (*Capsicum annuum* L.) as a source of genotypes resistant to *Meloidogyne enterolobii* and *Meloidogyne incognita* that enable genetic diversification in the development of improved crops within genetic improvement programs

MATERIALS AND METHODS

We evaluated the resistance of 15 accessions of striped Jalapeño pepper native from the north of the state of Hidalgo (CP903, CP907, CP908, CP922, CP932, CP933, CP934, CP935, CP937, CP938, CP939, CP941, CP942, CP945 and CP946) against *Meloidogyne enterolobii* (Me), and of 12 accessions (CP903, CP907, CP922, CP932, CP933, CP935, CP937, CP938, CP939, CP941, CP942 and CP946) against *Meloidogyne incognita* (Mi). California Wonder (CW) chili pepper was used as a susceptible control against both nematode species, whereas the CM-334 line was used as a resistant control against *M. incognita*.

Seeds of the accessions were provided by the Genetics Program of Colegio de Postgraduados (CP, colpos.mx) and preconditioned for *in vitro* germination (De la Rosa

et al., 2012). They were then placed in plastic containers with absorbent paper moistened with sterile distilled water and kept in a controlled growth chamber (APT.line™ KBWF, E5.1 model) at 28 °C, with cycles of 23 °C per 6 h for 5 days and subsequently at 28 °C for 15 days.

Once germinated, the seedlings were individually transplanted into 25 cm³ beakers filled with sterile fine sand. Plants were maintained in a controlled growth chamber of the Botany Program CP under conditions of 26±2 °C, a photoperiod of 14 h light and 10 h dark, 80% relative humidity, and daily watering. Sixty plants were evaluated per nematode species. When the seedlings reached three pairs of true leaves, they were inoculated with 1000 second-stage juveniles (J2) per plant apiece of both *Meloidogyne* species (Filialuna *et al.*, 2022). The classification of resistance to *Meloidogyne* was based on the reproduction index (RI%) used by Hadisoeganda and Sasser (1982) and defined by;

$$RI = (Nh_{accession} / Nh_{control}) \times 100$$

where: *RI* = reproduction index (%); *Nh accession* = number of eggs per g of root of each accession evaluated; *Nh control* = number of eggs per g of root of the susceptible control × 100 (Table 1).

The experimental unit consisted of one plant per replicate, with four replicates per accession. The trial was established using a randomized complete block design. Variables evaluated were number of egg masses, number of galls, and number of eggs per gram of root; at 34 days-post inoculation (dpi) for *Meloidogyne enterolobii* (Me) and at 42 dpi for *M. incognita* (Mi) (Castagnone-Sereno, 2012). The data obtained for each variable were subjected to an analysis of variance at a 95% confidence level. Statistical differences between means were determined using Tukey’s test ($p \leq 0.05$), and the open-source statistical software R Studio[®] was used. Before analyses, the data were transformed to logarithmic form using the log10 (x+1) function to normalize their distribution.

RESULTS AND DISCUSSION

The 15 striped Jalapeño pepper accessions evaluated showed significant differences ($p \leq 0.05$) in the response variables to infection by *Meloidogyne enterolobii* compared to the susceptible control California Wonder (CW) (Table 2).

Table 1. Classification of the resistance response to *Meloidogyne* spp. (Hadisoeganda and Sasser, 1982).

Category	Reproduction index (RI%)
Highly resistant	RI < 1%
Very resistant	1% ≤ RI < 10%
Intermediate resistant	10% ≤ RI < 25%
Moderately resistant	25% ≤ RI < 50%
Susceptible	RI ≥ 50%

Table 2. Response of 15 striped Jalapeño pepper accessions to infection by *Meloidogyne enterolobii*, and resistance classification based on the reproduction index (Hadisoeganda and Sasser, 1982).

Accession	Number of galls	Number of masses	Eggs per gram of root	Resistance
CP934	3.59±1.66 b	6.40±1.40 ab	162.83±14.44 ab	SC
CP942	5.12±1.81 ab	9.19±4.07 ab	187.01±31.16 ab	SC
CP933	4.19±1.38 ab	4.11±3.23 b	70.70±32.70 d	IR
CP932	5.99±1.56 ab	10.05±1.81 ab	172.01±21.99 ab	SC
CP935	5.95±0.63 ab	10.24±2.04 ab	143.55±15.82 abc	MR
CP937	5.26±1.03 ab	7.76±1.99 ab	128.62±8.52 bc	MR
CP938	4.90±1.81 ab	8.71±4.55 ab	141.89±22.55 abc	MR
CP903	4.92±1.39 ab	8.90±2.70 ab	175.74±39.65 ab	SC
CP939	3.71±1.61 b	6.00±2.69 ab	78.36±27.21 d	IR
CP946	4.03±1.51 ab	4.77±2.92 b	84.65±48.47 d	IR
CP922	4.63±1.02 ab	7.67±0.63 ab	137.80±18.59 abc	MR
CP945	4.40±0.63 ab	8.23±0.58 ab	173.71±21.83 ab	SC
CP941	3.92±1.76 b	6.82±2.06 ab	122.29±13.38 bc	MR
CP908	4.51±0.71 ab	8.88±1.02 ab	189.57±47.84 ab	SC
CP907	5.07±1.17 ab	8.05±2.27 ab	192.72±50.34 ab	SC
CM-334	5.32±0.73 ab	8.97±0.39 ab	114.77±20.41 cd	MR
CW	7.65±1.97 a	11.46±1.96 a	207.14±25.06 a	SC

SC: Susceptible, MR: Moderately resistant, IR: Intermediate resistant, VR: Very resistant, HR: Highly resistant. Means with different letters in the same column indicate a statistically significant difference (Tukey, $p \leq 0.05$).

Among them, accessions CP934, CP939, and CP941 had the fewest galls, while CP933 and CP946 had the fewest egg masses. Similarly, accessions CP933, CP939, and CP946 had the fewest eggs. Although CP934 had the fewest galls, its reproduction index (RI) reached 61.48%, thus indicating a susceptible response.

This result suggests that gall formation is not an essential requirement for nematode development, as noted by Cook and Starr (2006). In the case of tomato, Navarro *et al.* (2009) reported a similar phenomenon when analyzing the behavior of the FA 572-Katerine and LT-M12 genotypes against *M. incognita*. Additionally, Roberts *et al.* (2008) demonstrated in *Phaseolus lunatus* L. that nematode reproduction and root galling can be controlled by independent genes (*Mir-1*, *Mig-1*, and *Mjg-1*) in species of the genus *Meloidogyne*.

According to the resistance classification used in this study, accessions CP933, CP939, and CP946 were categorized as intermediate resistant, while CP935, CP937, CP938, CP922, and CP941 were moderately resistant, as did the CM-334 pepper line against *M. enterolobii*. In contrast, accessions CP334, CP942, CP332, CP903, CP945, CP908, and CP907 showed a behavior similar to the susceptible control CW.

Among the accessions qualified as intermediate resistant, CP933 and CP946 had significantly ($p \leq 0.05$) the lowest number of eggs per gram of root and egg masses, while

CP939 recorded in turn the lowest number of eggs and galls, reaching a RI between 10 and 25%.

In general, of the more than 100 chili pepper varieties evaluated worldwide against *M. enterolobii*, only a few are reported as resistant (Gonçalves *et al.*, 2014; Carrillo *et al.*, 2020). In this study, accessions CP933, CP939, and CP946 were classified as intermediate resistant ($10\% \leq \text{RI} < 25\%$). On the other hand, accessions CP935, CP937, CP938, CP922 and CP941, along with CM-334, showed a moderately resistant response ($25\% \leq \text{RI} < 50\%$). Although, since the latter is reported as susceptible (Villar-Luna *et al.*, 2016), these accessions can be considered borderline resistant.

In the evaluations of the accessions against *Meloidogyne incognita*, significant differences ($p \leq 0.05$) were detected in all the variables analyzed (Table 3). Accessions CP946, CP939, CP938, CP903, and CP942 showed very resistant response; CP932, CP922, and CM-334 were classified as highly resistant; CP933 presented moderate resistance, and CP937 intermediate resistance. Among these, accessions CP903, CP922, and CM-334 recorded the lowest number of galls, egg masses, and eggs, while CP946, CP939, CP938, CP932, and CP942 presented a low number of galls and egg masses. In particular, accession CP903 showed as Very resistant ($1\% \leq \text{RI} < 10\%$), in contrast to CM-334 line, which exhibited High resistance ($\text{RI} < 1\%$). However, no significant differences were observed between the two in the number of galls, egg masses, and eggs (Table 3).

This response could be associated with what was described by Pegard *et al.* (2005) in CM-334, who pointed out the existence of two resistance mechanisms against *Meloidogyne incognita*, *M. arenaria* and *M. javanica*: i) a preventive resistance based on physical barriers

Table 3. Response of 12 striped Jalapeño pepper accessions to infection by *Meloidogyne incognita* and resistance classification based on the reproduction index (Hadisoeganda and Sasser, 1982).

Accession	Number of galls	Number of masses	Eggs per gram of root	Resistance
CP922	1±0 c	1.65±1.13 e	1.13±0.23 b	HR
CP932	1±0 c	4.22±0.45 e	2.69±0.27 ab	HR
CP938	1±0 c	11.78±1.58 e	2.20±0.45 ab	VR
CP939	1±0 c	15.65±6.69 e	2.20±0.45 ab	VR
CP942	1±0.23 c	19.15±4.61 e	2.92±0.82 ab	VR
CP946	1±0 c	25.53±0.85 de	4.19±0.24 ab	VR
CP903	1.48±0.83 bc	12.22±10.48 e	1.13±0.23 b	VR
CP907	5.11±0.46 a	194.42±35.53 a	7.94±2.29 ab	SC
CP941	4.67±0.45 ab	112.07±10.31 b	8.87±1.12 a	SC
CP933	3.87±2.63 abc	59.64±13.33 cd	6.82±5.67 ab	MR
CP935	2.46±1.53 abc	91.73±2.24 bc	5.62±5.39 ab	SC
CP937	2.65±1.56 abc	37.91±21.37 de	4.15±1.46 ab	IR
CM-334	5.11±0.46 bc	7.29±0.78 e	1.52±0.18 b	HR
CW	4.91±0.84 a	105.09±8.38 b	6.89±2.02 ab	SC

SC: Susceptible, MR: Moderately resistant, IR: Intermediate resistant, VR: Very resistant, HR: Highly resistant. Means with different letters in the same column indicate a statistically significant difference (Tukey, $p \leq 0.05$).

and exudation of nematostatic compounds that limit the penetration of J2 juveniles, and ii) an induced resistance mediated by a hypersensitivity reaction (HR) and the accumulation of chlorogenic acid, which inhibit the formation of galls and egg masses by blocking the migration, development and reproduction of the nematode.

According to Roberts *et al.* (2008), nematodes of the genus *Meloidogyne* often induce gall formation in compatible interactions, since the main resistance genes suppress both gall formation and nematode reproduction, primarily affecting pathogen multiplication in the later stages of infection. In addition, Villar-Luna *et al.* (2015) noted that in the incompatible interaction *Mi-CM-334* an overexpression of genes (*EAS*, *HMG2*, *WRKY-a*, *PR-1*, and *POX*) associated with plant defense is observed. As well as the accumulation of the phytoalexin capsidiol, a compound linked to restricting nematode establishment and reproduction.

The results obtained in accessions CP903 and CP922, classified as very resistant and highly resistant, coincide with those reported by Gómez-Rodríguez *et al.* (2017). Those authors described in Serrano and Huacle pepper lines, a low number of galls and eggs of *M. incognita*. In particular, they highlighted the Serrano pepper lines 41-1 and 41-2, in which no galls or eggs were recorded, a behavior similar to that observed in the Morelos native chili pepper line CM-334.

Results of this research show that plants of native chili pepper responded differentially to *Meloidogyne incognita* and *M. enterolobii*. This variation supports the findings of Després *et al.* (2007), who indicated that defense levels can vary within the same species depending on the genotype and the environment. Also, that native populations constitute a valuable reservoir of genes associated with resistance. These genetic resources are key to identify new sources of resistance, and to support breeding programs that prioritize genetic resistance through the evaluation, selection, and validation of promising materials under contrasting conditions, with their eventual incorporation as resistant genotypes.

CONCLUSIONS

In this study, striped Jalapeño peppers from northern Hidalgo demonstrated differential resistance to *Meloidogyne enterolobii* and *M. incognita*. This characteristic placed these accessions evaluated as a potential alternative for inclusion in genetic improvement strategies for chili pepper.

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Variation among populations and effect of gibberellic acid in the germination of wild pepper seeds (*Capsicum annuum* var. *glabriusculum*) from Northwestern Mexico

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ABSTRACT

Objective: to assess the effects of doses of gibberellic acid (GA) on the germination of seeds of wild chili pepper (*Capsicum annuum* var. *glabriusculum*) to estimate the variation among populations, correlating it to the main climate factors of the seed collection sites in northwestern Mexico.

Design/Methodology/Approach: ripe wild pepper fruits were collected at five sites in the states of Sinaloa and Sonora in northwestern Mexico. After collection, the seeds were immersed in concentrations of 0, 125, 250 and 500 ppm of gibberellic acid (GA). The treated seeds were sown in 60-cavity seedling trays, with Peat moss™ as the substrate.

Results: the application of GA significantly increased germination in all the populations studied. Higher GA doses were more effective in promoting higher germination percentages. Greater weight of seeds was correlated with higher germination percentages. Correlation values were not clearly determinant, however, annual rainfall in the collecting sites of seeds was correlated with the germination percentages of the populations.

Limitations/Implications of the study: wild chili pepper is a species threatened by human activities; by changes in land use, particularly. Also, low germination percentages in this species are limitations for production and conservation.

Findings/Conclusions: the application of GA significantly increased germination in all the populations evaluated, GA doses 250 ppm and 500 ppm were the most effective in promoting germination. Regardless the treatments, variation among populations was high for the germination percentages.

Keywords: germination capacity, gibberellic acid, variation among populations, seed weight to germination capacity correlation.

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INTRODUCTION

Seed germination is a fundamental process in the establishment and survival of plants under natural conditions. Natural selection is considered to have favored mechanisms that allow seeds to respond to environmental conditions that favor plant development after germination. It is well known that there is variation in germination between populations, among and within individual plants.



The variation in the mechanisms that regulate germination between and within plant species is interpreted as adaptation to the conditions of their habitats at local and regional scales. In addition, the maternal environment (seed origin) is considered to explain the observed differences in germination capacity between populations of the same species.

Under experimental conditions, the application of gibberellic acid (GA) breaks seed dormancy mechanisms, accelerates germination, and frequently replaces the need for environmental stimuli, such as light and temperature in several plant species (Morales-Pizarro *et al.*, 2023), including *Capsicum* (Hernández-Verdugo, 2018; Beltrán-Burboa *et al.*, 2020).

Several studies have reported that seed weight or seed mass vary widely between populations, also among individual plants and within each plant originate physiological changes that are associated with a variety of traits related to adaptation, such as survival, growth, and reproduction (Mendoza-Pedroza *et al.*, 2023). Some researchers reported a positive relationship between seed weight and germination percentage (Gelviz-Gelvez *et al.*, 2020; Mendoza-Pedroza *et al.*, 2023).

Wild relatives of cultivated plants are an important genetic resource that constitutes a primary gene pool, which can help solve problems of current or future agriculture, such as tolerance or resistance to pests and diseases, and increase the quality and quantity of production (Retes-Manjarrez *et al.*, 2016, 2017, 2018; Hernández-Verdugo, 2018; Osuna-Rodríguez *et al.*, 2023). In addition, wild chili pepper fruits are widely consumed in the regions of Mexico where they grow naturally. Previous quantitative studies (Ornelas-Ramírez *et al.*, 2021; Romero-Higareda *et al.*, 2022, 2023) have indicated that wild chili pepper populations in northwestern Mexico maintain high levels of genetic variation between and within their populations.

These populations have also shown high levels of variation between and within their populations in the germination capacity of their seeds. The objective of this study was to assess the effects of doses of gibberellic acid (GA) on the germination of seeds of wild chili pepper (*Capsicum annuum* var. *glabriusculum*) to estimate the variation among populations, correlating it to the main climate factors of the seed collection sites in northwestern Mexico.

MATERIALS AND METHODS

Ripe fruits of wild chili pepper (*Capsicum annuum* var. *glabriusculum*), which afterward were dried in the shade at room temperature for 1 month, the location of collected populations and their geographic and climatic data are shown (Table 1). The populations where we collected seeds were Presa el Oviachic and Mazocahui in the state of Sonora, Yecorato, Lo de Vega and Buyubampo, in the state of Sinaloa (Figure 1).

Germination experiments

The seeds were immersed for 24 h in concentrations of 0, 125, 250 and 500 parts per million (ppm) of gibberellic acid (GA) dissolved in distilled water. The seeds were then put to dry on paper for 24 hours. The treated seeds were sown in 60-cavities seedling trays, using Peat mossTM as substrate. Sixty replicates of seven seeds from each population were sown in each GA treatment.

Table 1. Geographic and climatic data of the collection sites of the populations of wild chili pepper (*Capsicum annuum* var. *glabriusculum*) evaluated.

Population (seed collection site)	Latitude (North)	Longitude (West)	Altitude (m)	Average annual rainfall (mm)	Average annual temperature (°C)
1. Mazocahui	29° 35'	110° 00'	326	586	21.1
2. Presa Oviachic	27° 49'	109° 54'	582	389	25.5
3. Yecorato	26° 26'	108° 12'	405	855	24.1
4. Buyubampo	26° 38'	108° 39'	225	632	25.1
5. Lo de Vega	26° 12'	108° 36'	116	670	23.4

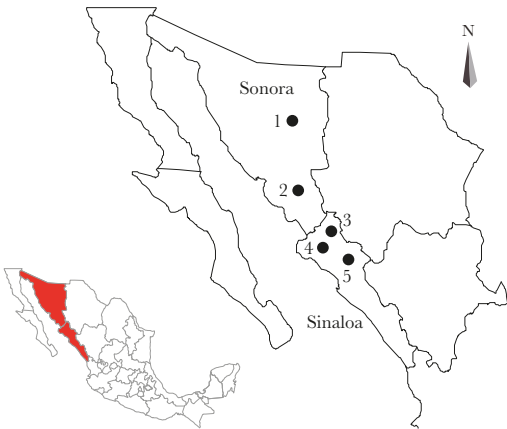


Figure 1. Collection sites of wild chili pepper populations (*Capsicum annuum* var. *glabriusculum*) in the states of Sonora and Sinaloa in northwestern Mexico (names of sites and features in Table 1).

The seeds of each replicate were weighed on a Sartorius™ analytical balance with an accuracy of 0.1 mg. As a germination response variable, the final percentage of germination was recorded at 30 days after planting.

Statistical analysis

In all treatments, the analyzed variable was the germination percentage accumulated at the end of the experiment. The number of seeds germinated daily during the first week was counted, then counted every third day. Count stopped one week after the absence of germination was observed. The germination percentage values were transformed into their arcsine values before the analyses, but the results are reported in percentages. Analyses of variance were performed in two instances. First, a two-ways analysis of variance was performed with all populations with GA treatments as the random factors, to detect general patterns of differences in germination. Univariate (one-way) analyses were then performed between populations for each treatment.

When the differences were significant ($p \leq 0.05$), a multiple mean test (Tukey-Kramer) was performed to determine the differences among the populations studied. Variation in seed weight between populations was estimated using an analysis of variance. Correlation analyses were also performed for germination percentages with the main climate factors of

the collection sites of the populations, and for germination percentages with average seed weight. All analyses were performed using JMP in SAS[®] version 11.

RESULTS AND DISCUSSION

Gibberellic acid treatments on germination

Overall, germination percentages were very low in all populations non treated with GA, which showed a mean 4.4% of germination within the interval 0.5-6.1% (Table 2, Figure 2A).

In contrast, the application of GA doses 125 ppm, 250 ppm and 500 ppm significantly increased the germination rate in all populations. In those treatments, 250 ppm and 500 ppm doses of GA were the most effective. The average germination percentage of all populations at 250 ppm GA was 31.4%, within the range 2.4-54.2%. The average germination percentage at 500 ppm GA was 30.2% within the 3.8-51.4% range. In the 125

Table 2. Germination percentages (mean $\pm$ 1 SE) of the studied wild chili pepper populations (*Capsicum annuum* var. *glabriusculum*) at different concentrations of gibberellic acid (GA).

Population	Treatments (GA, ppm)				Mean
	0	125	250	500	
1. Mazocahui	6.0	13.3	21.9	12.2	13.4 $\pm$ 1.1 c
2. Presa Oviachic	2.3	5.2	2.4	3.8	3.4 $\pm$ 0.6 d
3. Yecorato	6.1	39.8	49.1	51.4	36.6 $\pm$ 2.0 ab
4. Buyubampo	0.5	56.4	54.2	49.1	40.1 $\pm$ 3.3 a
5. Lo de Vega	5.1	27.9	37.39	41.0	27.8 $\pm$ 1.7 b
Mean	4.4 $\pm$ 0.5 c	26.4 $\pm$ 1.7 b	31.4 $\pm$ 1.8 a	30.2 $\pm$ 1.8 a	

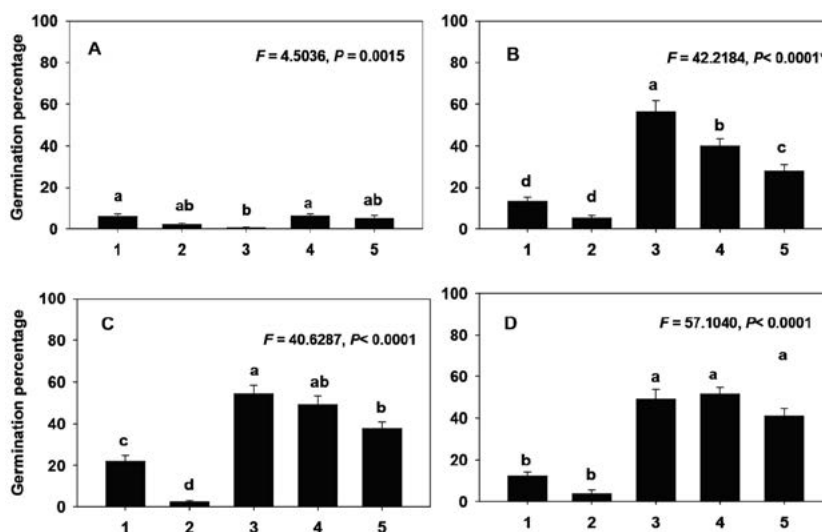


Figure 2. Average final germination percentage ($\pm$ 1 standard error), with A: 0 ppm, B: 125 ppm, C: 250 ppm and D: 500 ppm GA, of the wild chili pepper populations (*Capsicum annuum* var. *glabriusculum*) from Mazocahui, Presa Oviachic, Buyubampo, Yecorato and Lo de Vega (Numbers in X-axis correspond to the populations in Table 1).

ppm GA treatment, the average germination percentage was 26.4% within the rage 5.2-56.4% (Table 3, Figure 3).

The results of this study showed that populations of wild chili pepper (*Capsicum annuum* var. *glabriusculum*) had very low germination capacity in the treatment with no GA (0 ppm), which indicates that these seeds have physiological dormancy that can be broken with the exogenous application of GA (Hernández-Verdugo, 2018).

Of the three concentrations applied, 250 ppm and 500 ppm were the most effective in promoting higher percentages of germination in the seeds of the populations studied. These results are consistent with other studies that have shown that endogenous application of GA is an effective method to increase germination capacity in seeds of other plant species (Morales-Pizarro *et al.*, 2023), including the genus *Capsicum* (Hernández-Verdugo 2018; Beltrán-Burboa *et al.*, 2020).

Table 3. Average seed weight (mean ± 1 SE) of the populations of wild chili pepper (*Capsicum annuum* var. *glabriusculum*) studied.

Population	Average seed weight (mg)
Yecorato	3.47 ± 0.04
Mazocahui	2.79 ± 0.03
Presa Oviachic	1.81 ± 0.03
Buyubampo	2.94 ± 0.05
Lo de Vega	4.09 ± 0.04

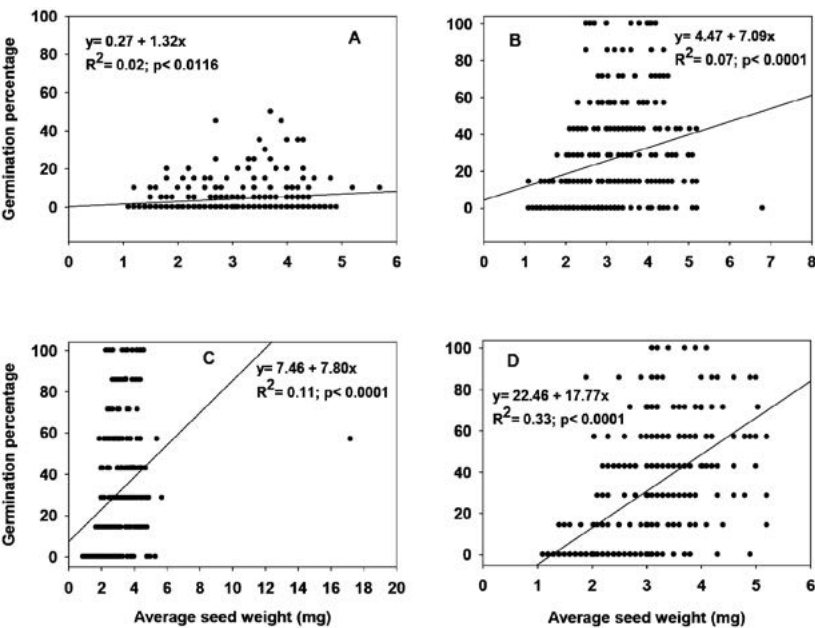


Figure 3. Germination percentage as a function of the average seed weight in treatments with A: 0 ppm, B: 125 ppm, C: 250 ppm and D: 500 ppm GA, of the wild chili pepper populations (*Capsicum annuum* var. *glabriusculum*) from Mazocahui and Presa Oviachic (Sonora), Yecorato, Buyubampo and Lo de Vega (Sinaloa).

Differences between populations in germination

The studied populations of wild *C. annuum* showed significant differences in their germination capacity in all treatments (Table 2, Figure 2).

Considering all treatments, the populations Buyubampo (population 4) and Yecorato (population 3) had the highest germination percentages, with 40.1% and 36.6% as average germination rates (Table 2, Figure 2). The Lo de Vega (population 5) and Mazocahui (population 2) populations showed intermediate germination percentages, while Presa Oviachic population (population 1) presented the lowest average germination percentage of all populations (Table 2, Figure 2).

Variation between populations in seed weight

There were statistically significant differences between populations in average seed weight ($F=545.73$; $p\leq 0.0001$). The average seed weight ranged from 1.81 mg in the Presa Oviachic population to 4.09 mg in the Lo de Vega population (Table 3).

Germination variability and climate factors

In regard to germination capacity, all populations differed significantly in all treatments (Table 2, Figure 2). The variation in germination capacity, between populations within the same species, has been interpreted as an adaptation related to habitat characteristics, particularly local climate factors. However, because we observed very few correlations in germination capacity with the main climate factors of the collection sites, we cannot interpret such differences as adaptations to local ecological conditions.

Only the mean annual rainfall showed a positive and marginally significant correlation in the treatments with 250 ppm GA ($R^2=0.686$, $p=0.0845$) and 500 ppm GA ($R^2=0.0710$, $p=0.0731$). Whereas the rest of the climate and geographical variables of the collection sites considered in this study showed no influence on the germination capacity of the wild chili pepper populations studied.

Based on the fact that most rainfall in Northwest Mexico occurs during the germination, growth, flowering and fruiting periods of the studied populations of wild chili pepper, it is likely water availability to be one of the main factors regulating the germination of these populations.

Correlations of seed weight to germination capacity

Four correlation analyses were run between the average seed weight of the five populations studied and the germination percentages. Average seed weight was positively and significantly correlated with germination percentage in each of the four GA treatments (Figure 3), which indicates that heavier seeds are more likely to germinate. These results suggest that the environmental conditions where wild chili-pepper plants grow may influence the weight and germination capacity of their seeds. Other researchers have reported similar results Gelviz-Gelvez *et al.* (2020) and Mendoza-Pedroza *et al.* (2023) showed that larger seeds of Pigeon pea (*Cajanus cajan* (L.) Mill) had higher germination percentages and germination speed rate than smaller seeds.

Seedlings from large seeds had higher dry matter content in roots, stems, and leaves than seedlings from small seeds. Thus, the more vigorous seedlings obtained from large seeds can be more successful during the growth process, which facilitates the absorption of water and nutrients. Gelviz-Gelvez *et al.* (2020) also reported that the heaviest seeds of seven wild plant species growing in semi-arid environments in central Mexico showed significantly higher germination percentage than the smaller seeds ($p \leq 0.05$), which may be due to heavier seeds have a greater capacity to retain water in arid or semi-arid environments.

The seeds of the populations used in this study come from a semi-arid environment, with low rainfall (Table 1) in the states of Sinaloa and Sonora. The high variation observed in seed germination of populations of wild chili pepper in Northwestern Mexico, coincides with the high levels of variation in morphology, genetics, or susceptibility to pathogens and salinity, found in the plants of those populations (Hernández-Verdugo, 2018).

The observed differences in germination patterns among the evaluated populations of wild chili pepper (*Capsicum annuum* var. *glabriusculum*) may be part of a mechanism that allows this species to colonize different habitats. Wild chili pepper populations are distributed from tropical forest sites to highly disturbed localities, such as the vegetation remaining on the roadsides of the low deciduous forest. In addition, this high variation in germination capacity of wild chili pepper may have contributed for reaching the wide geographical distribution this plant currently exhibits in the entire continent of America; this means, from the south of the United States to the south of Peru and north of Argentina (Hernández-Verdugo, 2018).

CONCLUSIONS

Germination of wild pepper seeds from the studied populations was promoted by the endogenous application of gibberellic acid (GA). Of the three concentrations of GA assessed, 500 and 250 ppm were the most effective in promoting higher germination percentages of these seeds. The populations studied differed significantly in their germination percentages in all the treatments tested. Seed germination percentages of the studied populations showed positive but marginally significant correlations with the mean annual rainfall of the collection sites, especially with the 250 ppm and 500 ppm GA treatments. It is possible that the availability of water during the growth and reproduction of wild chili-pepper plants is an important environmental factor for the germination of these seeds. Seed weight was positively correlated with germination percentages in the four GA treatments, indicating that seeds with higher weights showed higher germination percentages than seeds with lower weight.

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The birdlife in the Botanical Garden of Benemérita Universidad Autónoma de Puebla, a sustainable alternative

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ABSTRACT

Objective: to contribute to the knowledge of the birdlife of the Botanical Garden of the Benemerita Universidad Autonoma de Puebla (JB-BUAP) through a sustainable birdwatching project.

Design/Methodology/Approach: a survey was administered to 94 randomly selected visitors to the JB-BUAP to assess their interest and perceptions. Weekly monitoring was conducted over a one-year period, with 47 field trips to identify and count the number of bird species visiting the study site.

Results: with the trips conducted, 101 bird species were identified, distributed taxonomically among 12 orders and 36 families. Of these species, 10 are endemic, 6 are semi-endemic, and 2 are quasi-endemic. Regarding their conservation status, one species was identified as threatened, the Mexican duck (*Anas diazi*). Three species are subject to special protection, the Least grebe (*Tachybaptus dominicus*), the Peregrine falcon (*Falco peregrinus*), and the MacGillivray's warbler (*Geothlypis tolmiei*).

Limitations/implications of the study: the visitors interviewed were mostly young people with a high school diploma; others were workers, that highly valued the biodiversity present in the JB-BUAP. Therefore, this study could be extended to a more general and varied population that has not yet visited the JB-BUAP.

Findings/Conclusions: there is a general interest in wildlife within urban environments, and in this particular case towards birds in a space intended for the public. It is advisable to promote the promotion of habitats to maintain the populations of these birds; that in this and other places, such as agroecosystems, could function as a natural pest control.

Keywords: endemic, semi-endemic, quasi-endemic, birds, conservation biology, integrated management.

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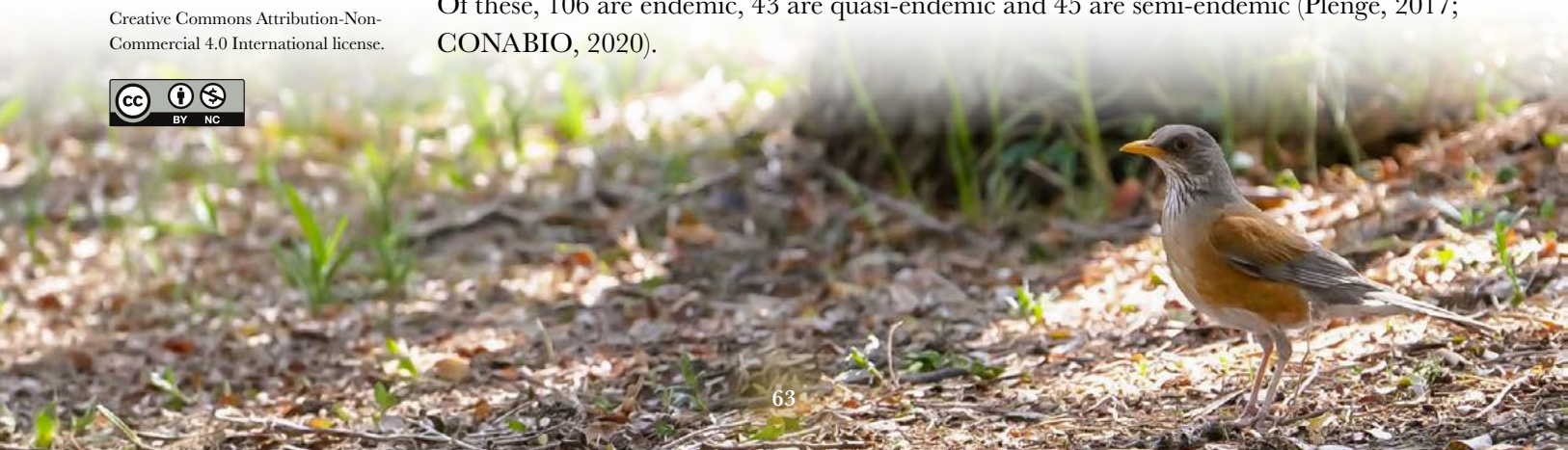
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INTRODUCTION

There are about 10 500 species of birds in the world. Colombia ranks first among the ten countries with the highest diversity of birds with 1815 species. Then come Peru with 1781; Brazil, 1712; Indonesia, 1604; Ecuador, 1559; Bolivia, 1406; and Mexico ranks eleventh with 1136 species, of which 194 are characterized by some level of endemism. Of these, 106 are endemic, 43 are quasi-endemic and 45 are semi-endemic (Plenge, 2017; CONABIO, 2020).



In order to practice birdwatching, binoculars, monocular, telescopes and cameras are usually used, depending on the intended level of detail. Many birders write down the species they can identify in a field notebook (Mackinnon, 2005). Then, to achieve bird identification, they rely on illustrated bird guides or mobile applications such as Merlin Bird, eBird, iNaturalist, Audubon Bird Guide, BirdNET. In Mexico, one of the most widely used illustrated bird guides is that of National Geographic (Dunn & Alfer, 2017).

Birdwatching is free and inclusive, it is a field practice linked to outdoor spaces and at the local level, it develops a sense of belonging to a specific community (Gelder, 2007). In Mexico there are several species of birds of great interest for their conservation, management and, therefore, for their observation. One of the places where you can find a wide variety of birds is the Botanical Garden under the Meritorious Autonomous University of Puebla [Benemerita Universidad Autonoma de Puebla], from this point on (JB-BUAP).

The BUAP botanical garden acts as a refuge for various species of birds that include migratory, endemic, resident and cosmopolitan birds. This birdwatching study was approached from an agroecological point of view as the scientific approach, also has a public character, due to its location in an urban area accessible to anyone interested. Both boarding students and students from other institutions, workers and the public can participate in enjoying birdwatching and learning about the local birdlife. This study, then, aimed to contribute to the knowledge of the birdlife of the Botanical Garden of the Benemerita Universidad Autonoma de Puebla (JB-BUAP) through a sustainable birdwatching project.

MATERIALS AND METHODS

Study area

The research was conducted in the botanical garden of the BUAP (JB-BUAP), a space that has been part of the Mexican Association of Botanical Gardens since 1998. This garden has several accreditations, among which the certification as an Environmental Education Center is notable, with the Level III Quality Certificate granted by the Mexico's Secretariat of Environment and Natural Resources (Secretaría de Medio Ambiente y Recursos Naturales-SEMARNAT), valid until 2015 and in the process of revalidating that accreditation. It is the first botanical garden in Mexico accredited by the Botanic Gardens Conservation International (BGCI) since 2000, and by the Arboreta Network-ArbNet at Level IV since 2019 (Jardín Botánico BUAP, 2025).

The site is inscribed in a temperate subhumid climate with summer rainfall, has a rainfall of 800-1000 mm per year and an average annual temperature of 14-16 °C (INEGI, 2020). Since 1977, the JB-BUAP is identified as an agroecosystem, due to its management and conservation and its human activities. This management has favored a remarkable diversity of plants in that site (Hernández, 1977). It is located at coordinates 19° 00' 00.24" N and 98° 11' 49.61" W, at an altitude of 2128 m; and is part of the University City (CU) of the BUAP, at Av. San Claudio s/n, Col. San Manuel, C.P. 72570, Puebla, Puebla (Mexico). The botanical garden covers a total area of 10.8 ha (about 26.8 acres), with a central lagoon of 3.3 ha (about 8.15 acres) that includes an islet of 0.472 ha (1.16 acres) in its center (Figure 1).

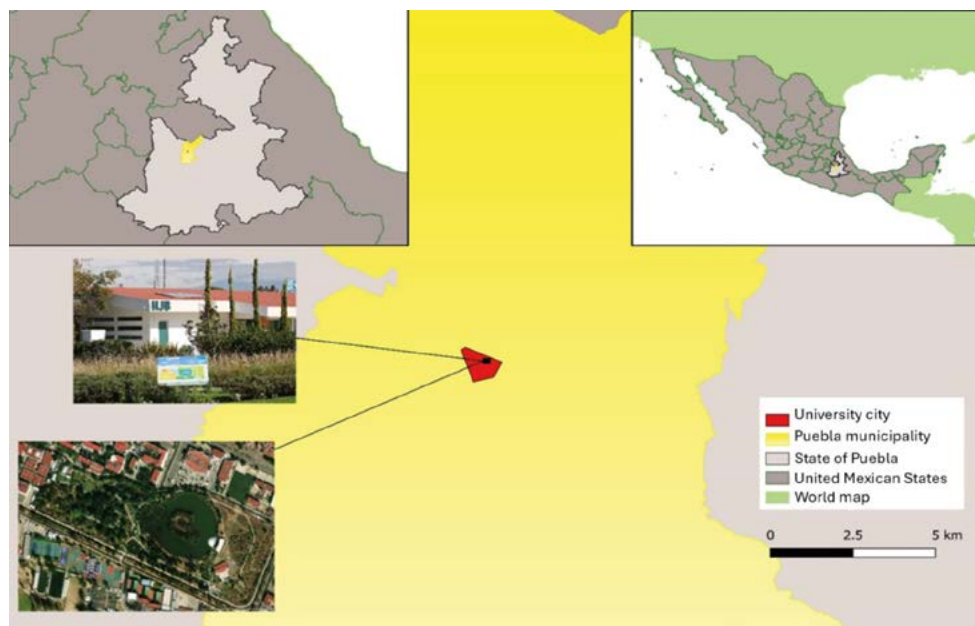


Figure 1. Map of the Botanical Garden of the Benemerita Universidad Autonoma de Puebla (JB-BUAP).

Survey application

The average number of visitors to the JB-BUAP in a year was considered as population size, that is, 3200 people. The sample size calculation was made for a finite population according to the formula of Almaguer *et al.* (2022);

$$n = (p)(q)(N)(Z^2) / [E^2(N-1) + Z^2(p)(q)]$$

where: n =was the necessary sample size (94); N =size of the study population (3200); Z =Z-score value (1.96) corresponding to the 95% confidence level; p =expected proportion of the total population with the visitor characteristic, usually (0.5) to obtain the maximum sample size; E =desirable margin of error (0.1).

The interview was applied completely randomized to measure the level of knowledge that the community has about birdlife and to evaluate the acceptance of birdwatching. In the design of the project activities, the degree of knowledge of the participants was adjusted on a scale (Very low, Low, Medium, High, Very high). The language used to communicate information was adapted to this level of knowledge. The questionnaires designed were applied to visitors when they left the JB-BUAP, to take advantage of their recent experiences in the place. Likewise, the application was distributed throughout the day, to avoid interviewing both the first few and the last few people to leave, to ensure the randomness of the opinions (Hernández *et al.*, 2010). This procedure was followed for 33 consecutive weeks, from June 2024 to February 2025; three questionnaires were applied per week during the interview period.

Birdwatching

The study is defined as observational, prospective, longitudinal and descriptive (Méndez, 1996). The monitoring, to follow visits and birdwatching, was implemented for one year at the JB-BUAP. Because in addition to identifying the different species of birds present and quantifying them, we sought to determine which are the most abundant in each season of the year evaluated. Birdwatching was done by the itinerary method (González, 2019) with tours along the roads that are already formed within the JB-BUAP (1162 m). The tours started from different points on each date. During the monitoring, several species of birds were identified by direct and indirect methods. The direct method includes observation with binoculars, taking photographs and visual identification of the birds. Meanwhile, the indirect methods were the collection of feathers and footprints, the identification of songs, and the observation of nests to determine to which species the evidence found belonged.

The tours were made from 7:30 am to 10:30 am, with two field observers (October 7, 2023 to October 26, 2024). A field notebook, a Canon® ECOS Rebel T7 camera with a Canon® EF 70-300 mm f/4-5.6 telephoto lens and a Sony® Alpha 7rv camera, a Sony® G 200-600 telephoto lens, were used. Tasco® binoculars, model Futura SE, 10×50 mm and other Osprey® Waterproof binoculars, 10×40 mm, were also used. For communication between the observers, portable radio communication equipment (Motorola® Waterproof Talkabout) was used. The data were concentrated weekly in an Excel® records spreadsheet organized by species as they were found. In addition to their taxonomical classification, order, species, common name, scientific name, seasonality of sightings, status of endemism and, where appropriate, protection status.

Statistical methods

The data obtained were processed with descriptive statistics in the IBM statistical program. SPSS v. 27.0.0, to represent them in tables and graphs with numerical and percentage values, according to the objective.

RESULTS AND DISCUSSION

General data and perceptions of the interviewees

Of the 94 people interviewed, 51.06% (n=48) belong to the male sex, 45.74% (n=43) to the female sex, and 3.19% (n=3) do not perceive themselves as having these gender identities. Age ranged from 19 to 63 years old, with an average of 26 years, and mode of 23. The figure is slightly lower than the average municipal age (27) according to data from the population and housing census in the municipality of Puebla (INEGI, 2021).

Regarding the level of schooling, 75.53% (n=71) of the total number of people interviewed have completed high school (Figure 2A), which contrasts with the state data (14.6%) of the Secretariat of Public Education of Puebla in the year (SEP, 2022). Regarding employment status, 84.04% (n=82) are students (Figure 2B).

With a qualitative consideration to measure criteria or perceptions in the surveys, it was found that 70.21% (n=66) of the people interviewed do like to practice birdwatching (Figure 2C). And that 6.38% (n=6) of the people interviewed do not want to have a guide on their visit to explain the birdlife of the JB-BUAP, while 93.62% (n=88) do want a guide

to obtain information. Opinion questions were also included to evaluate what value they give to the biodiversity present in the JB-BUAP, through a scale (Very low, Low, Medium, High, Very high). According to the answers obtained, 61.70% (n=58) of the interviewees rated the value of bird biodiversity present in the JB-BUAP as very high (Figure 2D).

These results point to the importance declared by visitors about bird biodiversity in the JB-BUAP, 97.87% of the people interviewed (92/94) rated bird biodiversity with a High and Very high value, and no opinions were found with Low or Very low values.

Another important piece of information that the survey yielded was the season (spring, summer, autumn, winter) when visitors have seen the greatest variety of bird species. A 45.74% (n=43) of the people have seen more birds in spring. However, in the monitoring tours that were made, the season in which the most bird species were identified was autumn (n=79), in that season 84.04% of the total species identified in the year was represented (Figure 3).

Data obtained in birdwatching sightings

A total of 101 species of birds were identified in the 10.8 ha of the botanical garden with the 47 tours. These species are taxonomically distributed in 12 orders and 36 families. Our data are higher than the 50 species reported by Universidad Autonoma Chapingo (UACH), in Texcoco de Mora, State of Mexico (Valencia-Trejo *et al.*, 2014). They also exceed the 67 species reported in Jardin Valladolid in Yucatán, Mexico (Guzmán-Canul & Sánchez-Soto, 2024). However, this species richness was lower than that reported for the entire area of Ciudad Universitaria of the BUAP, that is, 112 species (González, 2019). As well

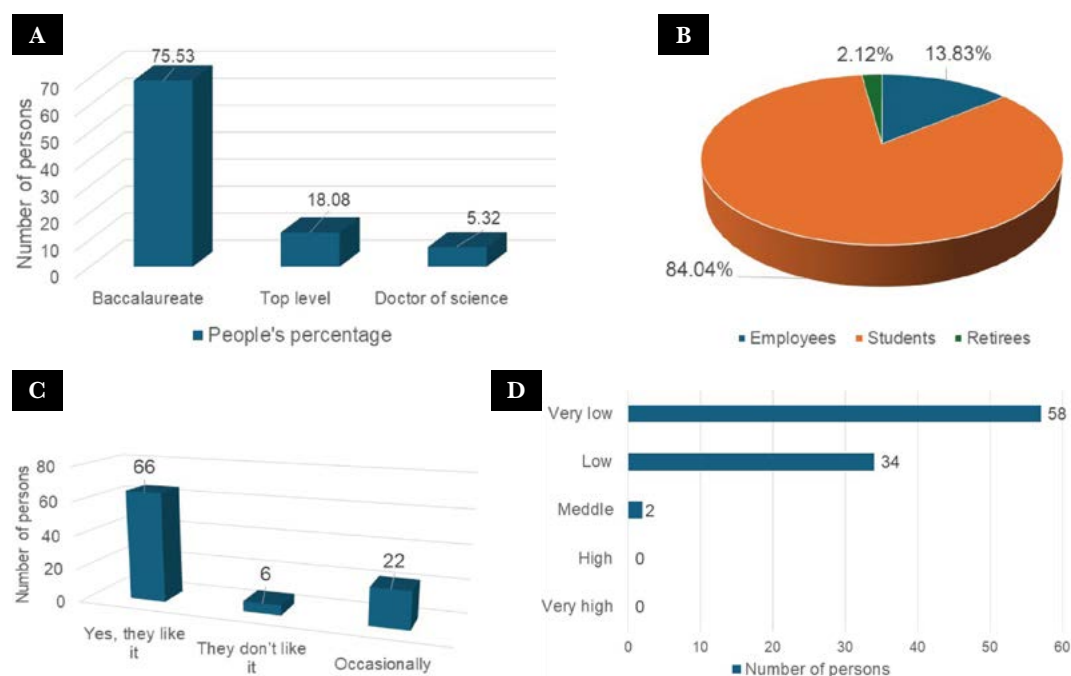


Figure 2. General characteristics of the interviewees A: level of schooling (%); B: employment status (%); C: like or dislike for birdwatching; and D: value given to bird biodiversity, in the botanical garden of the Benemerita Universidad Autonoma de Puebla (JB-BUAP).

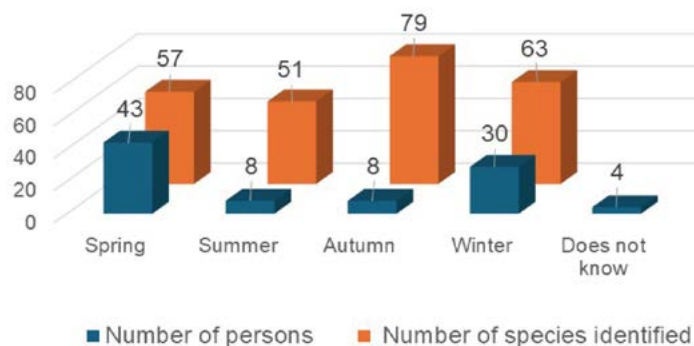


Figure 3. Number of people who observed more bird species in the different seasons of the year *vs.* species data records obtained by monitoring in the Botanical Garden of the Benemerita Universidad Autonoma de Puebla (JB-BUAP).

as, fewer than the 210 species reported for the entire municipality of Puebla (Camacho *et al.*, 2012) or than the total number of bird species reported (595) for the state of Puebla, according to a historical record of sightings (Mendoza *et al.*, 2012).

On the other hand, that value (101) is lower than that in the State Reserve of Palmar, Yucatán, which reported 104 species of birds in 47 931 ha (Chablé-Santos *et al.*, 2008). Another case is the Biosphere Reserve Tehuacan & Cuicatlan, between the states of Puebla and Oaxaca, with 113 species reported in 490 186 ha (Vázquez *et al.*, 2009). It is also lower compared to what was recorded in the Sierra de Cuatro Venados, Oaxaca, where 160 species were observed in 221 000 ha (Lavariega *et al.*, 2016).

On average, 23 species were recorded per tour and of the total species sighted, 18 are in categories of endemism (Table 1). In terms of seasonality, 58 species are resident, 37 are migratory and 6 are transitory. In addition, in regard to their status of endemism, 10 endemic species (E) were present, among which the Mexican duck (*Anas diazi*) (Figure 4A); the rufous-backed thrush (*Turdus rufopalliatus*) (Figure 4B); the dusky hummingbird (*Cyanthus sordidus*) (Figure 4C); the Boucard's wren (*Campylorhynchus jocosus*) (Figure 4D); and the Mexican chickadee (*Poecile sclateri*). As well as, 6 species of semi-endemic (SE) birds, among which are the Broad-billed hummingbird (*Cyanthus latirostris*) and the Cassin's kingbird (*Tyrannus vociferans*). It was also possible to identify 2 species of quasi-endemic birds (QE), these two species were the Russet-crowned motmot (*Momotus mexicanus*) (Figure 4E) and the Grey silky-flycatcher (*Ptiliogonys cinereus*) (Figure 4F).

These results differ from the data reported in the High Mountains region of Veracruz, Mexico by Alcántara-Salinas *et al.* (2020), as they reported 22 endemic species, 12 quasi-endemic, and 17 semi-endemic bird species. On the other hand, in terms of its protection status, it was observed that there is one threatened species that is the Mexican duck (*Anas diazi*) (Figure 4A) and three species that are subject to special protection, the Least grebe (*Tachybaptus dominicus*), the Peregrine falcon (*Falco peregrinus*) and the MacGillivray's warbler (*Geothlypis tolmiei*) (Mendoza *et al.*, 2012). This comparison is also inferior to what was reported for the High Mountains of Veracruz by those authors, who identified 57 species subject to special protection and 29 are threatened (Alcántara-Salinas *et al.*, 2020).

Table 1. Endemic (E), semi-endemic (SE), and quasi-endemic (QE) bird species of Mexico.

Taxonomic Order	Taxonomic Family	Common name	Scientific name	Endemism (status)*
Anseriformes	Anatidae	Mexican duck	<i>Anas diazi</i>	E
Apodiformes	Trochilidae	Dusky hummingbird	<i>Cynanthus sordidus</i>	SE
		Broad-billed hummingbird	<i>Cynanthus latirostris</i>	SE
		Violet-crowned hummingbird	<i>Amazilia violiceps</i>	SE
Coraciiformes	Momotidae	Russet-crowned motmot	<i>Momotus mexicanus</i>	QE
Passeriformes	Tyrannidae	Brown-crested flycatcher	<i>Myiarchus tyrannulus</i>	E
		Cassin's kingbird	<i>Tyrannus vociferans</i>	SE
	Turdidae	Blue-hooded euphonia	<i>Chlorophonia elegantissima</i>	E
		Rufous-backed thrush	<i>Turdus rufopalliatius</i>	E
	Parulidae	Black-throated gray warbler	<i>Setophaga nigrescens</i>	SE
		Golden-crowned warbler	<i>Basileuterus culicivorus</i>	E
	Paridae	Mexican chickadee	<i>Poecile sclateri</i>	E
	Troglodytidae	Stotz warbler	<i>Troglodytes solstitialis</i>	E
		Rufous-naped wren	<i>Campylorhynchus rufinucha</i>	E
		Boucard's wren	<i>Campylorhynchus jocosus</i>	E
	Ptiliognatidae	Grey silky-flycatcher	<i>Ptiliogonys cinereus</i>	QE
	Passerellidae	Clay-colored sparrow	<i>Spizella pallida</i>	SE
	Icteridae	Scott's oriole	<i>Icterus parisorum</i>	se

*Endemism status of the species of Mexico: E: endemic; SE: semi-endemic; QE: quasi-endemic.



Figure 4. A) Mexican duck (*Anas diazi*). Photographer: Miguel Antonio Silveira Caminero. B) Rufous-backed thrush (*Turdus rufopalliatius*). Photographer: Oscar Agustín Villarreal Espino Barros. C) Dusky hummingbird (*Cynanthus sordidus*). Photographer: Miguel Antonio Silveira Caminero. D) Boucard's wren (*Campylorhynchus jocosus*). Photographer: Miguel Antonio Silveira Caminero. E) Russet-crowned motmot (*Momotus mexicanus*). Photographer: Miguel Antonio Silveira Caminero. F) Grey silky-flycatcher (*Ptiliogonys cinereus*). Photographer: Miguel Antonio Silveira Caminero.

As for the different seasons of the year, in spring-summer 74.19% (n=69) of the bird species visualized during a year were identified, which taxonomically belong to 12 orders and 31 families. On the other hand, in autumn-winter, 95.70% (n=89) of the total bird species identified in the year of travels were sighted, belonging to 12 orders and 33 families. This difference in the number of bird species is possibly due to migration in this seasons (autumn-winter).

Between 194 and 212 endemic bird species have been recorded in Mexico, representing 18-20% of the total species of our national territory (Navarro-Sigüenza *et al.*, 2014). This study at JB-BUAP reports 101 species of birds, which include 10 of the 17 endemic species identified in the municipality of Puebla (Mendoza *et al.*, 2012). The 101 species represent 58.8% of the endemic species observed in the region. This finding highlights the importance of the area for the conservation of the endemic birdlife of Mexico. The presence of these species is evidence of the great biological diversity of the region, and its relevance for the protection of these ecosystems. These results underscore the need to continue conservation efforts to protect both the endemic species and their natural habitat within the JB-BUAP.

Species and relative abundance

The bird species that are most abundant in the JB-BUAP are in the first place the Barn swallow (*Hirundo rustica*), the most observed in one day (94 individuals on September 14, 2024). Next one is the House finch or Mexican sparrow (*Haemorrhous mexicanus*), with 72 individuals observed in one day (November 25, 2023). The Clay-colored sparrow (*Spizella pallida*) was represented by 58 individuals (20 April 2024); in fourth place is the Cinnamon-rumped seedeater (*Sporophila torqueola*) with 46 individuals on the same date (April 20, 2024). They are followed by the Mexican duck (*Anas diazi*), with a national label of 'threatened species', 40 individuals in one day (November 11, 2023) and the last most identified species was the Scarlet flycatcher or Austral vermillion flycatcher (*Pyrocephalus rubinus*) with 22 individuals sighted in one day (June 22, 2024). These data are essential for the temporality of the presence of birds in the JB-BUAP, which can contribute to conservation strategies and proper management of this important habitat for local birdlife.

Among the diversity of birds that have been meticulously identified, 6 outstanding species are characterized by their ability to adapt and colonize a wide range of habitats in different parts of the world. These cosmopolitan birds belong to 4 different orders and 6 families, embodying the ability of birds to conquer diverse and often very distant territories (Table 2).

These 6 cosmopolitan species exemplify the ability of birds to thrive in a variety of environments, from cities to rural areas and from temperate to tropical regions. This illustrates the amazing adaptability and biological diversity that characterizes birds around the world.

In terms of areas with specific status as Protected Natural Areas (in Mexico, ANP's), in the State Reserve of Palmar, Yucatán (47,931 ha); the Biosphere Reserve Tehuacan & Cuicatlan, Puebla-Oaxaca (490,186 ha), and in the Sierra de Cuatro Venados, Oaxaca (221,000 ha), there are of course more bird species due to their geographical surfaces. Although the JB-BUAP only has 10.8 ha (about 26.8 acres), 101 identified species were

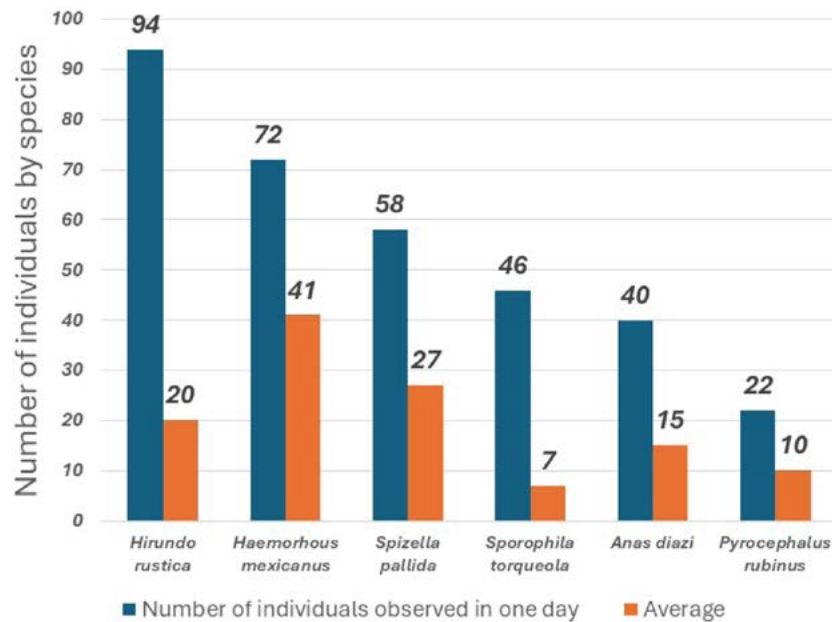


Figure 5. Bird species with the most individuals observed in a day *vs.* the average number of individuals observed by each species during a year, in the botanical garden of the Benemerita Universidad Autonoma de Puebla (JB-BUAP).

Table 2. Cosmopolitan bird species identified in the Botanical Garden of the Benemerita Universidad Autonoma de Puebla (JB-BUAP).

Taxonomic Order	Taxonomic Family	Common name	Scientific name
Columbiformes	Columbidae	Rock dove	<i>Columba livia</i>
Pelecaniformes	Ardeidae	Cattle egret	<i>Bubulcus ibis</i>
Accipitriformes	Pandionidae	Osprey or Fish hawk	<i>Pandion haliaetus</i>
Passeriformes	Sturnidae	Common starling	<i>Sturnus vulgaris</i>
	Passeridae	House sparrow	<i>Passer domesticus</i>
	Hirundinidae	Barn swallow	<i>Hirundo rustica</i>

found, of which 10 are endemic, 6 semi-endemic and 2 quasi-endemic. In addition to the successful sighting of a species classified as threatened according to NOM-059-SEMARNAT-2010 (SEMARNAT, 2010), the Mexican duck (*Anas diazi*). The urban character of this facility (JB-BUAP) highlights the importance of an area immersed in a metropolis, which preserves great diversity and endemism in visiting birds. This may be due to the botanical management of the site, as it created mini-habitats, which are preferred by visiting birds.

From an ecological perspective, the study promotes the conservation of species and the botanical garden functions as an environmental education space that, being located in an urban area, helps to conserve species from different regions of the state of Puebla and encourages knowledge and recreation related to birds in the metropolitan area of Puebla.

The study also seeks to consolidate the management of JB-BUAP as an agroecosystem in an urban environment that provides natural habitat for birds and strengthens their

conservation. As well as to declare this site as a formal tool for environmental education. An additional benefit in terms of administration management is the possibility of increasing the public interest in visiting the botanical garden. This would benefit the facilities and the employees, 20 people, including the director, a curator of scientific collections, as well as researchers, technicians specialized in areas such as conservation and *in vitro* plant production, and gardeners.

CONCLUSIONS

The visitors interviewed were mostly young people, with a high school diploma, as well as workers who highly value the biodiversity present in the JB-BUAP. Among the 101 species identified, 10 are endemic, 6 semi-endemic and 2 quasi-endemic; in addition to the successful sighting of a species classified as threatened, the Mexican duck (*Anas diazi*). This highlights the need for conservation efforts to protect endemic species and their habitat of choice in the botanical garden of the BUAP.

In autumn and winter, more species of birds were identified; this is a migratory seasonality and greater variety was found in sightings during this period. This is indicative of significant biological diversity and the relevance of the site studied. More than 90% of the people interviewed want a guide to explain about the birdlife of the JB-BUAP. This shows the relevance of trained guides, and informs about the interest of most visitors in obtaining professional information during their visit. The Botanical Garden of the Benemerita Universidad Autonoma de Puebla can be a formal site for conservation, and sustainable management and use for birdwatching and amateur photography.

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The effectiveness of ozone as a therapeutic alternative for treating bovine mastitis

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ABSTRACT

Objective: This narrative review aimed to evaluate the effectiveness of ozone as a therapeutic alternative for bovine mastitis, considering its antimicrobial and anti-inflammatory properties in comparison with conventional antibiotic treatments.

Design/methodology/approach: A review of the literature published between 2000 and 2025 was conducted, encompassing experimental assays, field studies, theses, reviews, and scientific articles. The purpose of this review was to analyze the doses used, the routes of administration, and the clinical and bacteriological outcomes observed in both clinical and subclinical mastitis.

Limitations on study/implications: The principal limitation identified was the inconsistency among the protocols employed, particularly with regard to doses, concentrations, frequency of application, and routes of administration.

Findings/conclusions: The local or intramammary use of ozonotherapy has been shown in several studies to be as effective as antibiotics. Its main advantages include the absence of milk residues, low cost, and a reduction in the use of antimicrobial agents. Nevertheless, further controlled studies are required to establish standardized protocols that can be effectively implemented in veterinary practice.

Keywords: Bovine mastitis, ozonotherapy, veterinary medicine.

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INTRODUCTION

Bovine mastitis is one of the most prevalent and economically significant diseases in the dairy industry worldwide (Ruegg, 2017). It is defined as an inflammation of the mammary gland, generally caused by bacterial infections, which may occur in either clinical or subclinical form, thereby affecting both the quality and quantity of the milk produced (Bradley, 2002). In addition to compromising animal welfare, mastitis causes substantial economic losses due to decreased milk production, treatment costs, disposal of contaminated milk, and the premature culling of animals from the herd (Rainard *et al.*, 2018).



Traditionally, the treatment of this condition has relied on the use of intramammary and systemic antibiotics. However, the prolonged and, in some cases, inappropriate use of these drugs has led to problems such as the development of bacterial resistance, the presence of residues in milk, and the environmental impact derived from the use of pharmacological products (Kalmus *et al.*, 2011). In light of these limitations, it is necessary to explore more sustainable, effective, and safe therapeutic alternatives for both the animal and the consumer (Pereira *et al.*, 2016). Moreover, the intramammary application of ozone leaves no residues (Prabhakaran *et al.*, 2025), eliminating the need for withdrawal periods that result in economic losses, while also offering low cost and demonstrated effectiveness in the treatment of both clinical and subclinical mastitis (Sciorsci *et al.*, 2020).

In this context, the use of ozone has emerged as a promising option. Ozone, a gas with a high oxidative capacity, possesses antimicrobial, anti-inflammatory, and regenerative properties that have been exploited in human medicine and, more recently, in veterinary medicine (Filippi *et al.*, 2012). Several studies have shown that ozonotherapy applied topically, intramammarily, or in ozonated water may reduce bacterial load, accelerate tissue recovery, and decrease the need for antibiotics in the treatment of mastitis (Ogata and Nagahata, 2000), in both its clinical and subclinical forms (Fuentes *et al.*, 2023; Machado *et al.*, 2023).

The use of ozone constitutes a viable therapeutic alternative for the treatment of bovine mastitis, owing to its potential to improve clinical outcomes, reduce the adverse effects associated with conventional treatments, and contribute to safer and more sustainable dairy production.

CURRENT CHALLENGES

Bovine mastitis has been extensively studied because of its high prevalence in dairy herds and its complex interaction with environmental, microbiological, and physiological factors. It is a multifactorial disease that, in most cases, results from a bacterial infection, although it may also be caused by yeasts, mycoplasmas, viruses, and even trauma (Ruegg, 2017). Its development is closely associated with the ability of microorganisms to colonize the mammary parenchyma and trigger a local inflammatory response characterized by leukocyte infiltration, release of proinflammatory cytokines, tissue damage, and alterations in milk composition, which may progress clinically or subclinically depending on the pathogen involved and the animal's immunological status (Bradley, 2002).

The most common etiologic agents include contagious bacteria such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma* spp., as well as environmental bacteria such as *Escherichia coli*, *Klebsiella* spp., and *Streptococcus uberis* (Rainard *et al.*, 2018). The usual route of entry is the teat canal, through which microorganisms penetrate and colonize the mammary tissue. The ability of these pathogens to evade the immune system and adhere to the mammary epithelium is crucial for establishing infection, especially in chronic cases such as those caused by *S. aureus* (Kalmus *et al.*, 2011).

From a pathophysiological perspective, the inflammatory process begins when bacteria or their products are recognized by pattern recognition receptors (PRRs), mainly Toll-like

receptors (TLRs), present in macrophages and mammary epithelial cells. This interaction activates an inflammatory cascade mediated by cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF- α), which induce fever, pain, and leukocyte recruitment to the site of infection (Rainard *et al.*, 2018). Local inflammation manifests as edema, hyperemia, and pain in the mammary gland, accompanied by alterations in the color, texture, and homogeneity of the milk.

The immune system responds through the rapid migration of neutrophils to the infected tissue, where they phagocytose bacteria and release enzymes and free radicals. Although this response is essential for controlling infection, it may also damage secretory epithelial cells, leading to reduced milk production and, in prolonged cases, to fibrosis and mammary atrophy (Ruegg, 2017).

Clinically, mastitis is classified into two main forms: clinical and subclinical. Clinical mastitis is characterized by visible signs in the milk (clots, blood, pus) and in the udder (edema, redness, heat, and pain). Systemic signs such as fever, anorexia, and decreased productive performance may also be observed. Clinical mastitis may be mild, moderate, or severe, depending on the intensity of the inflammation and the type of etiological agent involved (Bradley, 2002). In contrast, subclinical mastitis presents no evident clinical signs, but involves persistent inflammation that causes alterations in milk composition, including an increase in somatic cell count (SCC), a decrease in lactose and protein content, and the presence of pathogens. It is more difficult to detect, yet it has a significant economic impact because of its silent and persistent effect on dairy production (Ruegg, 2017).

For diagnosis, several tools are available to identify the presence and type of mastitis. Among the tests most commonly used in the field is the California Mastitis Test (CMT), which detects an increase in somatic cell count (SCC) through the formation of a viscous gel. SCC values above 200,000 cells/mL generally indicate subclinical inflammation (Ruegg, 2017). In addition, microbiological culture of milk is essential to identify the etiological agent and determine its antimicrobial susceptibility profile. At present, molecular techniques such as polymerase chain reaction (PCR) have been incorporated as more sensitive and specific methods, particularly for detecting chronic infections caused by *Mycoplasma* spp. or strains of *S. aureus* (Rainard *et al.*, 2018).

The pathophysiology of bovine mastitis represents a major challenge for dairy herd health. Subclinical forms, owing to their silent nature, may go unnoticed and generate sustained production losses, whereas clinical forms may severely compromise animal health. Understanding these processes is fundamental for the development of more effective therapeutic strategies, including alternatives to conventional treatment such as ozonotherapy.

JUSTIFICATION

Ozone (O₃) is a molecule composed of three oxygen atoms, characterized by its high reactivity and oxidative capacity. Its therapeutic use is based on the controlled application of specific concentrations that induce moderate oxidative stress, capable of activating antioxidant defense mechanisms without causing cellular damage (Bocci, 2011). This

property makes it a therapeutic alternative of growing interest, especially in infectious and inflammatory contexts in which conventional treatments, such as antibiotics, have lost efficacy or promoted microbial resistance (Franzini *et al.*, 2016). Ozone exhibits a potent broad-spectrum antimicrobial action, effective against Gram-positive and Gram-negative bacteria, fungi, and viruses. Its mechanism of action is based on the oxidation of membrane lipids, proteins, and nucleic acids of microorganisms, leading to loss of cellular integrity and bacterial death (Sechi *et al.*, 2001; Zielinska *et al.*, 2020). This property is particularly relevant against persistent pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli*, as well as against mycotic agents such as *Candida* spp. Recent studies have demonstrated that the intramammary application of ozone is effective in cases of mycotic mastitis, achieving clinical and microbiological resolution without the use of conventional antifungals, thereby broadening its therapeutic spectrum beyond bacterial infections (Köker, 2024). Furthermore, ozone promotes modulation of the cellular antioxidant system. Upon reacting with unsaturated fatty acids and other biological compounds, ozone generates reactive oxygen species (ROS) and lipid oxidation products (LOPs), including hydrogen peroxide (H_2O_2). These molecules act as second messengers that activate intracellular pathways such as the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which stimulates the production of endogenous antioxidant systems, including superoxide dismutase, catalase, and glutathione peroxidase, thereby strengthening the organism's capacity to limit tissue damage, preserve mammary epithelial cells, and favor recovery of milk production (Bocci, 2011; Filippi *et al.*, 2012). Another important aspect of ozone's mechanism of action is its ability to modulate the immune response. At the cellular level, ozone has been shown to stimulate the activation of macrophages, T lymphocytes, and natural killer (NK) cells, promoting the production of T helper type 1 (Th1) cytokines such as interferon gamma ($IFN-\gamma$) and tumor necrosis factor alpha ($TNF-\alpha$), while reducing immunosuppressive cytokines such as interleukin-10 (IL-10) and transforming growth factor beta ($TGF-\beta$). This modulation contributes to a more effective immune response against infections and to the regulation of chronic inflammatory processes. In addition, an increase in phagocytosis and the destructive capacity of neutrophils following ozone exposure has been documented (Zielinska *et al.*, 2020). In veterinary medicine, ozone may be administered in various forms, including ozonated gas, ozonated water, carrier oils such as ozonated sunflower oil, or by means of ozonated autohemotherapy. These routes allow for the effective treatment of infectious diseases such as mastitis, as well as inflammatory conditions, wounds, dermal infections, and metritis (Fuentes *et al.*, 2023). In the specific case of bovine mastitis, recent studies suggest that ozone not only reduces bacterial load, but also promotes recovery of mammary tissue by stimulating angiogenesis, improving microcirculation, and reducing local inflammation (Franzini *et al.*, 2016). The use of ozone represents a safe, effective, and environmentally friendly alternative in the treatment of infectious diseases, particularly in the context of increasing antimicrobial resistance. Unlike antibiotics, it does not generate residues in milk or meat, and its controlled application has been shown to be well tolerated by animals, with no relevant adverse effects when established protocols are followed (Filippi *et al.*, 2012).

The earliest studies in this field include the work of Ogata and Nagahata (2000), who administered intramammary gaseous ozone in cases of acute clinical mastitis using concentrations of 20-30 $\mu\text{g/mL}$ (10 mL per mammary quarter for three consecutive days). They obtained a recovery rate of 60% in quarters treated exclusively with ozone and detected no residues in milk. Complementarily, Enginler (2015) evaluated different intramammary doses and concluded that moderate concentrations (20-30 $\mu\text{g/mL}$) achieved a more favorable balance between therapeutic efficacy and tissue tolerance. In the field of subclinical mastitis, Fuentes *et al.* (2023) demonstrated that the application of ozone (20-40 $\mu\text{g/mL}$, 10 mL/quarter for three days) significantly reduced somatic cell count (SCC) and improved milk quality parameters, although its efficacy was lower than that of conventional antibiotic treatments. Likewise, Argudo and Soria (2017) compared the efficacy of gaseous ozone (50 mL at 35 $\mu\text{g/mL}$), ozonized saline solution (50 mL at 35 $\mu\text{g/mL}$), and intramuscular ceftiofur (1.6 mg/kg) in mild and moderate clinical mastitis, obtaining recovery rates of 77.8%, 33.3%, and 83.3%, respectively. The authors concluded that gaseous ozone showed efficacy comparable to that of the antibiotic, whereas ozonized saline solution was less effective.

Other studies, such as that of Wakade *et al.* (2025), evaluated the combined use of gaseous ozone (25 $\mu\text{g/mL}$, 10 mL/quarter) and antibiotics, reporting cure rates of 60% with ozone alone, 70% with antibiotics, and 100% with the combination, thus suggesting a potential synergistic effect. Sertkol *et al.* (2018) reported the clinical recovery of cows treated with intramammary ozone in Turkey, further supporting its utility under production conditions. Research has also explored alternative formulations. Raimundo *et al.* (2024) used intramammary ozonated oil (10 mL, $\sim 1,200 \text{ mg/L}$ of O_3) in cows with mild clinical mastitis, achieving clinical improvement in four of six animals and negative cultures in half of the cases. *In vitro* studies by Sechi *et al.* (2001) and Orlandin *et al.* (2021) demonstrated the potent bactericidal activity of ozonated oils against mammary pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae*. In field studies, Torrico *et al.* (2018) applied intramammary gaseous ozone (30 $\mu\text{g/mL}$, 10 mL/quarter for three days) in 165 affected quarters, observing a 39% reduction in microbial load and absence of clinical signs in 23% of cases. Kwon *et al.* (2005) also reported positive results in the treatment of bovine mastitis with gaseous ozone, reinforcing its therapeutic action against this disease. Taken together, the literature indicates that ozonotherapy has broad potential as an alternative or adjunct in the treatment of bovine mastitis (Table 1). Its benefits include reduction of bacterial load, improvement of clinical and productive parameters, and absence of residues in milk, thereby contributing to a reduction in antibiotic use (Travagli *et al.*, 2020). However, the heterogeneity of protocols, concentrations, and routes of administration, together with the limited sample size in some trials, underscores the need to establish standardized guidelines and to conduct larger controlled clinical trials capable of confirming its efficacy and safety (Sciorsci *et al.*, 2020). Moreover, although several studies mention lower costs associated with the use of ozone instead of antibiotics for the treatment of mastitis, no studies were found in which a direct cost comparison between both treatments was performed.

Table 1.

Author / Year	Design / Sample	Treatment and dose	Main results	Clinical observations
Ogata & Nagahata (2000)	19 cows with acute clinical mastitis	O ₃ gas, 10 mL/quarter, 20-30 µg/mL for 3 days (intramammary administration)	60% recovery; no milk residues detected	Better outcomes in cases caused by <i>Streptococcus</i>
Sechi <i>et al.</i> (2001)	In vitro study with mastitis-associated pathogens	Ozonized oil (Oleozone), dose not specified	High <i>in vitro</i> bactericidal activity	Supports the use of topical formulations
Kwon <i>et al.</i> (2005)	Clinical trial	O ₃ gas, dose not specified (intramammary administration)	Clinical recovery observed	Protocol not described in detail
Enginler (2015)	Dose-comparison study	O ₃ gas, 20-70 µg/mL; optimal range: 20-30 µg/mL (intramammary administration)	Moderate doses showed the best balance between efficacy and safety	High doses may irritate mammary tissue
Ablondi & Pogliacomì (2004)	1,323 cases of various conditions	Autohemotherapy + O ₃ + local treatment	Good response in mastitis caused by <i>S. aureus</i> , <i>S. uberis</i> , among others	Not effective against coliforms or <i>S. agalactiae</i>
Maldonado <i>et al.</i> (2017)	Cows with subclinical endometritis	Ozonized water, 45 µg/mL, via intrauterine administration	Reduced inflammation; increased conception rate	Potentially extrapolable application
Argudo & Soria (2017)	54 cows with mild mastitis	O ₃ gas, 35 µg/mL (50 mL) for 3 days <i>vs.</i> saline solution <i>vs.</i> antibiotic (intramammary administration)	O ₃ : 77.8%; antibiotic: 83.3%; saline: 33.3%	Gaseous ozone showed efficacy comparable to that of the antibiotic
Sertkol <i>et al.</i> (2018)	Cows with clinical mastitis	O ₃ gas, 20 mL, 60 µg/mL for 3 days (intramammary administration)	85% clinical recovery; 70% bacteriological recovery	No local irritation observed
Torrico <i>et al.</i> (2018)	165 quarters from 73 cows	O ₃ gas, 10 mL/quarter, 30 µg/mL for 3 days (intramammary administration)	39% reduction in bacterial load; 23% of cases showed no clinical signs	Evaluated under real production conditions
Soto <i>et al.</i> (2022)	52 animals with mastitis and endometritis	Ozone <i>vs.</i> antibiotic, dose not specified; administered according to the pathology, either intramammarily or intrauterinely	Mastitis: 53.8% with ozone <i>vs.</i> 23.1% with antibiotic	O ₃ was more effective and was also associated with a better reproductive response
Fuentes <i>et al.</i> (2023)	12 cows with subclinical mastitis (randomized study)	O ₃ gas, 20-40 µg/mL, 10 mL/quarter for 3 days (intramammary administration)	Reduced somatic cell count (SCC); improved milk parameters; lower efficacy than antibiotics	A viable adjunct therapy that may reduce antimicrobial use
Machado <i>et al.</i> (2023)	5 cows with clinical and subclinical mastitis	Ozonized saline solution, 500 mL/quarter, plus intramammary O ₃ at 40 µg/mL for 5 min; repeated every 24 h	Improvement within 24 h; reduced inflammation; clinical mastitis shifted to subclinical status, and subclinical cases became negative	No pain, edema, or erythema; reduction in clots
Raimundo <i>et al.</i> (2024)	6 cows (pilot study)	Ozonized oil (~1,200 mg/L) for 5 days (intramammary administration)	Clinical improvement in 4/6 cases; negative cultures in 3/6	No control group
Wakade <i>et al.</i> (2025)	30 cows with clinical mastitis	O ₃ gas, 25 µg/mL <i>vs.</i> antibiotic <i>vs.</i> combination therapy (intramammary administration)	60% with ozone; 70% with antibiotic; 100% with the combination	Potential therapeutic synergy

CONCLUSIONS

Bovine mastitis remains a disease of considerable economic and sanitary importance. Although antibiotics have proven effective, their use is constrained by important limitations, particularly bacterial resistance and the presence of residues in milk. The literature reviewed indicates that ozonotherapy, in its various forms, including gaseous ozone, ozonized solutions, and ozonized oils, represents a promising therapeutic alternative owing to its antimicrobial and anti-inflammatory properties, its lack of residue generation, and its lower cost. Although some findings remain variable, recent reviews consistently suggest that ozone may reduce the use of antimicrobials in cattle and contribute to food safety. Accordingly, ozonotherapy may be regarded as a viable therapeutic alternative for the treatment of both clinical and subclinical bovine mastitis. Nevertheless, greater standardization of doses, routes of administration, and treatment protocols is still required in order to consolidate its application in veterinary practice.

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Genetic effects on resistance to *Phytophthora capsici* in chile of agua (*Capsicum annuum* L.)

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ABSTRACT

Objective: Genotypes of chile de agua (*Capsicum annuum* L.) were evaluated under greenhouse conditions to identify resistance to *Phytophthora capsici*.

Design/methodology: The experiment was arranged as a randomized complete block design (RCBD) with two replications and two treatments (inoculated and non-inoculated). Twenty-one single-cross F₁ hybrids were generated using Griffing's diallel method II (parents and F₁s). With the seven parents and 21 F₁ crosses, a total of 28 genotypes were assessed.

Disease severity was rated, and the Disease Index (DI) and the Area Under the Disease Progress Curve (AUDPC) were computed.

Results: Analysis of variance (ANOVA) revealed significant differences among genotypes. Crosses 1×5, 4×6, and 6×7 exhibited the lowest DI and AUDPC values. Diallel analysis indicated significant general combining ability (GCA) and specific combining ability (SCA) effects. Parents 3, 5, and 8, together with the crosses, are promising sources for breeding programs targeting resistance to *P. capsici*.

Findings/conclusions: The study was conducted in Saltillo, Coahuila, Mexico, in 2024.

Keywords: genetic resistance, general combining ability, heterosis, *Phytophthora capsici*.

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INTRODUCTION

The cultivation of chili pepper (*Capsicum annuum* L.) holds substantial importance in Mexico, a country recognized for its extensive genetic diversity, attributable to its status as the center of origin and domestication of this species (Arroyo-Vargas *et al.*, 2013; Luna Ruiz *et al.*, 2018). Within this species, the chile de agua landrace, endemic to the Central Valleys of Oaxaca, stands out due to its pronounced economic and cultural relevance in the region (Montaño-Lugo *et al.*, 2014; SIAP, 2023). *Phytophthora capsici* L. is an oomycete first reported infecting *Capsicum annuum* in 1922 in New Mexico, USA, and subsequently in Mexico by Galindo (1960) in crops from Chapingo. This pathogen



induces root, crown, and fruit rot, leading to sudden wilting and, under favorable environmental conditions, yield losses that may reach 80-100% (Mendoza, 1999; Pérez *et al.*, 2003). It is regarded as one of the principal soilborne pathogens affecting chili pepper, alongside *Fusarium oxysporum* f. sp. *capsici*, *Rhizoctonia solani*, and *Ralstonia solanacearum*, all of which are associated with the wilting syndrome (Vásquez-López *et al.*, 2009). In Mexico, its impact is particularly severe in irrigated production areas such as Chihuahua, Zacatecas, and Sinaloa, where recurrent incidence compromises yield stability and crop sustainability. Conventional management strategies, including crop rotation and fungicide application, have demonstrated limited effectiveness due to the prolonged survival of oospores and the pronounced genetic variability of the pathogen (Barchenger *et al.*, 2018). Moreover, the intensive use of chemical inputs entails environmental risks and a progressive decline in efficacy. Consequently, genetic resistance constitutes the most sustainable alternative for disease management. Notably, the Criollo de Morelos 334 (CM334) variety has been identified as a prominent source of resistance, along with diverse native and wild accessions that have exhibited partial resistance under field conditions (Oelke *et al.*, 2003; Sy *et al.*, 2008; Gómez-Rodríguez *et al.*, 2017). Nevertheless, the complex inheritance of resistance encompassing recessive and dominant genes, as well as polygenic control with epistatic interactions has hindered its effective incorporation into breeding programs (Chunthawodtiporn *et al.*, 2019; Siddique *et al.*, 2019). Diallel analysis constitutes a fundamental approach for partitioning genetic variation into general combining ability (GCA) and specific combining ability (SCA). General combining ability (GCA) reflects the average performance of a parent across multiple crosses and is primarily associated with additive genetic effects. In contrast, specific combining ability (SCA) quantifies the particular deviation of a given cross from the value expected based on the GCA of its parents and is related to dominance and/or epistatic effects (Sprague & Tatum, 1942; Griffing, 1956). In Griffing's Method II, Model I, which includes parents and all direct crosses under a fixed-effects framework, inferences are restricted to the evaluated set; conversely, a random-effects approach is appropriate when parents represent a sample from a broader population (Griffing, 1956). For resistance-related traits (*e.g.*, disease index or area under the disease progress curve [AUDPC]), negative GCA values indicate parental donors of alleles that reduce disease severity, whereas negative SCA values in a cross suggest heterosis and favorable non-additive effects. In *Capsicum*, empirical evidence supports the utility of the GCA/SCA framework for complex traits. Martínez *et al.* (2005) reported significant GCA in parental lines and significant SCA for yield, highlighting opportunities for hybrid development and trait-specific selection. Pech May *et al.* (2010) identified a predominance of additive effects for yield, modulated by environmental conditions, recommending hybridization to enhance productivity and inbreeding combined with selection for plant and fruit traits. In guajillo chili, Hernández-Mendoza *et al.* (2021) found highly significant GCA and SCA effects for fruit number and yield, identifying outstanding heterotic crosses. Collectively, these findings substantiate the estimation of GCA and SCA for resistance to *Phytophthora capsici* and inform strategic decisions: line selection when GCA predominates and hybrid

development when SCA exerts a greater influence (Martínez *et al.*, 2005; Pech May *et al.*, 2010; Hernández-Mendoza *et al.*, 2021). The aim of this research was to elucidate the genetic effects of seven creole populations of chile de agua (*Capsicum annuum* L.) and their F₁ crosses under controlled artificial inoculation with *Phytophthora capsici* in greenhouse conditions. This information provides a robust foundation for initiating a breeding program for this landrace, aimed at developing cultivars specifically adapted to the agroecological and productive conditions of the Central Valleys of Oaxaca.

MATERIALS AND METHODS

The experiment was conducted during the fall of 2024 in Greenhouse Seven at the Universidad Autónoma Agraria Antonio Narro (UAAAN), located in Saltillo, Coahuila, Mexico. Seven parental genotypes (creole populations) of chile de agua (*Capsicum annuum* L.) were used, all collected from villages in the Central Valleys of Oaxaca. Each accession was designated according to its community of origin. Seeds were obtained from fruits harvested directly under field conditions.

Sowing was carried out in 200-cell trays containing a commercial substrate. Two sowing dates, spaced one week apart, were implemented to ensure an adequate number of plants. Twenty seeds were sown per genotype (28 genotypes in total: 7 parents + 21 crosses), resulting in eight trays overall. Thirty-five days after germination, when seedlings exhibited 5-6 true leaves, they were transplanted into 12 oz expanded polystyrene cups containing the same substrate. Prior to transplantation, both the working surfaces and containers were thoroughly washed and disinfected with a household chlorine solution to ensure sanitary conditions. The greenhouse operated under temperatures ranging from 26 to 32 °C. Irrigation was applied by flooding. As the study focused exclusively on the seedling stage, fertilization was provided only at the tray stage (starter fertilization), with no subsequent applications after transplantation. No fungicides were applied that could interfere with the experimental infection process. A total of 28 genotypes were evaluated: 7 parental lines and 21 simple F₁ crosses. The number of crosses corresponded to $n(n-1)/2 = 7 \times 6 / 2 = 21$, in accordance with Griffing's diallel design, Method II, Model I (fixed effects, without reciprocals).

Preparation of the culture medium and inoculum

The *Phytophthora capsici* isolate was provided by the Centro de Investigación en Alimentación y Desarrollo (CIAD), Culiacán, Sinaloa, Mexico, by Dr. Raymundo Saúl García Estrada and his research team. A V8 agar culture medium was used. To prepare 500 mL, 100 mL of V8 juice were filtered, transferred to a 1 L Erlenmeyer flask, and mixed with 400 mL of distilled water, 10 g of bacteriological agar, and 1.5 g of calcium carbonate (CaCO₃). The mixture was homogenized on a shaker and sterilized in an autoclave at 15 psi for 15-20 min. Subsequently, the medium was cooled and poured into Petri dishes under sterile conditions. Isolate 20 of *P. capsici* was reactivated by placing a mycelial plug in the center of Petri dishes and incubating them at 27 °C for eight days. To increase inoculum production, the methodology described by Fernández-Pavía *et al.* (2004) was followed with modifications. The absence of contaminants was confirmed, and 20 mL

of sterile distilled water were added to each plate, which were then incubated for 72 h to promote sporangial formation. Plates were subsequently subjected to a thermal shock by alternating 4 °C and 27 °C for 30 min at each temperature to induce zoospore release. The resulting suspension was collected in a sterile flask, and zoospore concentration was quantified using a Neubauer hemocytometer (Neubauer-imp[®], Marienfeld, Germany), adjusting the final concentration to 10^5 zoospores mL⁻¹.

Greenhouse conditions and inoculation

The experiment was established using a randomized complete block design (RCBD) in a split-plot arrangement, with two blocks, two replicates, and two treatments (T1=non-inoculated; T2=inoculated). Inoculation was performed using a 20 mL syringe, applying 1 mL per plant of the suspension at 1×10^5 zoospores mL⁻¹. After transplantation, plants were maintained under adequate moisture conditions to favor infection.

Disease assessment

Evaluation of the response to *P. capsici* began six days after inoculation and was repeated every three days, for a total of eight assessments. Healthy, diseased, and dead plants were recorded using the severity scale proposed by Glosier *et al.* (2008), where 0=healthy plant, 1=leaf yellowing without stem necrosis, 2=slight stem necrosis, 3=moderate necrosis and slight wilting, 4=severe necrosis with marked wilting, and 5=dead plant. For genetic and severity analyses, only T2 (inoculated) was considered, as T1 did not develop symptoms and served as the negative control.

Calculation of disease index (DI) and area under the disease progress curve (AUDPC)

Using the severity-scale data, the disease index (DI) was calculated as:

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

where: X_i =disease severity in the i -th seedling; n =number of plants evaluated.

Using these indices, the area under the disease progress curve (AUDPC) was calculated according to Shaner and Finney (1977):

$$AUDPC = \sum_{i=1}^n \left[\frac{X_{(i+1)} + X_i}{2} \right] (T_{(i+1)} - T_i)$$

where: X_i =proportion of disease at the i -th observation; $T_{(i+1)} - T_i$ =time interval between two consecutive assessments; n =number of evaluations.

Statistical models

ANOVA (split-plot design)

Data were analyzed using analysis of variance (ANOVA) to evaluate the effects of sowing dates, treatments, genotypes, and their interactions on disease severity. Missing data were imputed using a general linear model (GLM) with fixed effects for the parental lines:

$$Y_{\{tfg\}} = \mu + F_f + R(F)_{\{tf\}} + G_g + (GF)_{\{gf\}} + \varepsilon_{\{tfg\}}$$

where: F_f : Sowing dates (fixed; main plot); $R(F)_{\{tf\}}$: Replication within sowing dates; G_g : Genotypes (fixed; subplot); $(GF)_{\{gf\}}$: Fixed interaction (genotype $\times$ sowing date); $\varepsilon_{\{tfg\}}$: Residual error.

Diallel analysis (Griffing, Method II, Model I)

A general combining ability (GCA) and specific combining ability (SCA) analysis was performed based on Method II proposed by Griffing (1956), which includes both parents and F_1 hybrids.

Additive linear model:

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + y_k + \varepsilon_{ijk}$$

where: Y_{ijk} =observed phenotypic value of the cross; μ =overall mean of all observations; g_i, g_j =general combining ability (GCA) effects of parents i and j ; s_{ij} =specific combining ability (SCA) effect of the cross ($i \times j$); y_k =effect of the k -th replication; ε_{ijk} =experimental error associated with observation i_{jk} .

Software and packages

Analyses were performed in R (R Core Team, 2025), using RStudio and the tidyverse, agricolae, and DiallelAnalysisR packages.

RESULTS AND DISCUSSION

Table 1 presents the mean squares from the analysis of variance, in which significant differences were observed among genotypes in their response to inoculation with *Phytophthora capsici*.

Significant effects ($p < 0.01$) were detected for both evaluation dates and genotypes, indicating substantial variation in disease progression (Table 1). The genotype $\times$ date interaction was not significant, suggesting that disease progression was consistent across the evaluated genotypes. The observed differences can be attributed to the genetic variability of the creole materials collected from different communities in the Central Valleys of Oaxaca. The delayed onset of symptoms (after day six) and the slower progression observed in some genotypes suggest the possible presence of major genes and/or mechanisms of partial resistance.

According to Van der Plank (1984), delayed disease onset is associated with vertical resistance (*i.e.*, major genes), whereas slow disease development is related to a reduced rate

Table 1. Mean squares from the analysis of variance for the reaction of parental genotypes and F₁ hybrids to inoculation with *Phytophthora capsici*.

Source of variation	Degrees of freedom	Middle squares
Evaluation dates	7	8924**
Replications/Dates	7	262
Genotypes	27	6060**
Genotype × Dates	189	186
Error	216	849
Total	447	
CV (%)		82.9

Highly significant at 0.01.

of disease increase. Previous studies have reported similar patterns. Martínez (2011) found no significant differences among chile mirador collections, although symptom progression was more rapid. Hernández (2009) evaluated 128 materials and reported that only one accession (118) maintained resistance for 10 weeks.

Retes-Manjarrez *et al.* (2020) identified six highly resistant local races when evaluating 32 *Capsicum* accessions under controlled conditions, with clear differences in the phenotypic expression of the disease. Table 2 presents the results for the disease index (DI) and the area under the disease progress curve (AUDPC), which enable the identification of genotypes exhibiting distinct levels of resistance to *P. capsici*.

According to Ristaino (1990), only materials with a disease index (DI) between 0 and 20 can be considered resistant. Under this criterion, only the parent San Andrés (DI=19.38) and the cross San Andrés × San Pablo (DI=5.31) were classified as resistant, highlighting their value for genetic improvement. The lowest area under the disease progress curve (AUDPC) value also corresponded to this cross (1282.5%·days), followed by La Raya × Ocotlán and San Andrés × Santa Cruz, both with 116.25. Although the values below 100% reported by Martínez (2011) as indicative of high resistance were not achieved, these combinations exhibited moderately resistant performance. According to Van der Plank (1984), this partial resistance is attributable to horizontal resistance of a polygenic genetic nature, which does not prevent disease development but rather slows its progression. This was evident in crosses such as San Andrés × Ocotlán (DI=11.17; AUDPC=455.29) and San Pablo × Zimatlán (DI=27.31; AUDPC=525). In contrast, Zimatlán × Vigallo and Ocotlán × Vigallo showed the highest DI (60 and 58.28) and AUDPC (1275 and 1154.88) values, indicating high susceptibility. Among the parents, Zimatlán and La Raya were the most susceptible, whereas San Andrés, San Pablo, and Santa Cruz exhibited lower disease progression, suggesting the presence of horizontal-resistance genes. Although this resistance is complex to fix, it is stable and durable (Van der Plank, 1984; Shaner & Finney, 1977). Overall, these results are consistent with Cíntora Portuguese (2016), who emphasized the utility of AUDPC as a key criterion for identifying resistant genotypes.

Overall, disease progress exhibited a steady increase throughout the evaluation period, as shown in Figure 1. Parental lines displayed higher AUDPC values than their corresponding F₁ crosses, indicating greater severity and more rapid infection development.

Table 2. Means of the disease index (DI) and area under the disease progress curve (AUDPC) in parental lines and F₁ crosses of chile de agua inoculated with *Phytophthora capsici*.

Genotype	Type	Disease Index (%)	Distribution of the area under the disease progress curve (%·days)
San Andrés	Parent	19.38	427.5
San Pablo	Parent	29.38	611.25
La Raya	Parent	30.94	615
Santa Cruz	Parent	41.41	926.25
Ocotlán	Parent	49.38	1106.25
Zimatlán	Parent	76.56	1635
Vigallo	Parent	78.13	1687.5
San Andrés × Santa Cruz	Cross F ₁	9.58	116.25
La Raya × Ocotlán	Cross F ₁	36.41	116.25
Ocotlán × Zimatlán	Cross F ₁	52.5	207
San Pablo × Vigallo	Cross F ₁	32.03	234.55
Santa Cruz × Vigallo	Cross F ₁	51.31	288.75
Santa Cruz × Zimatlán	Cross F ₁	48.75	318.75
San Andrés × Ocotlán	Cross F ₁	11.17	455.29
San Pablo × Zimatlán	Cross F ₁	27.31	525
San Pablo × La Raya	Cross F ₁	21.68	571.875
San Andrés × La Raya	Cross F ₁	7.97	573.45
La Raya × Zimatlán	Cross F ₁	38.75	712.5
San Pablo × Ocotlán	Cross F ₁	26.88	735
La Raya × Vigallo	Cross F ₁	39.38	765
San Andrés × Zimatlán	Cross F ₁	15.16	776.25
San Pablo × Santa Cruz	Cross F ₁	23.28	791.25
La Raya × Santa Cruz	Cross F ₁	32.81	986.25
Santa Cruz × Ocotlán	Cross F ₁	44.69	1095
San Andrés × Vigallo	Cross F ₁	15.78	1098.75
Ocotlán × Vigallo	Cross F ₁	58.28	1154.88
Zimatlán × Vigallo	Cross F ₁	60	1275
San Andrés × San Pablo	Cross F ₁	5.31	1282.5

Nevertheless, parents 3 and 5 stood out, as their AUDPC values were comparable to—or lower than—those observed in some crosses, suggesting relatively higher resistance. These findings confirm the presence of genetic variability in resistance to *Phytophthora capsici* among the evaluated materials and support the selection of parental lines with favorable additive effects for genetic improvement. Table 3 presents the effects of general combining ability (GCA) and specific combining ability (SCA) on the disease index (DI), enabling the identification of parents with favorable additive effects and cross combinations exhibiting significant non-additive interactions.

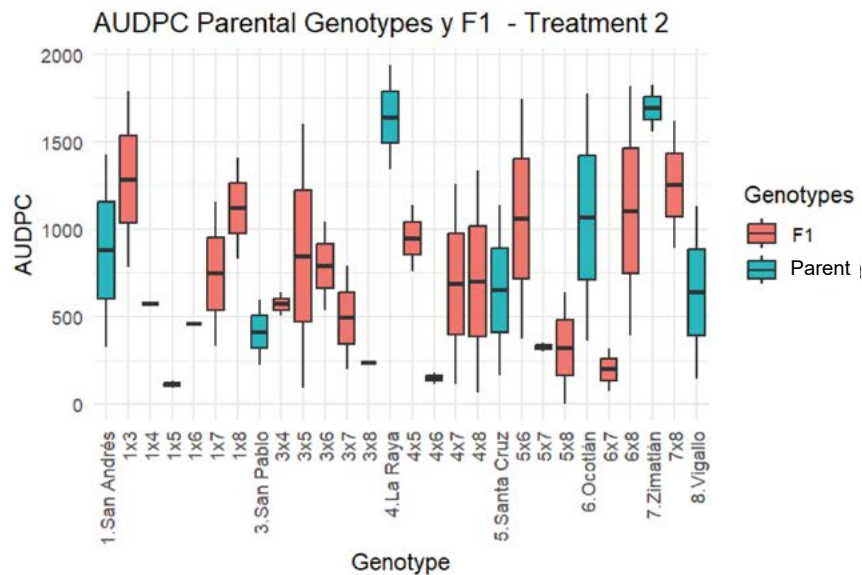


Figure 1. Distribution of the area under the disease progress curve (AUDPC) for parental lines and F₁ crosses of chile de agua inoculated with *Phytophthora capsici*.

Table 3. General combining ability (GCA) and specific combining ability (SCA) effects on the disease index (DI) in 21 F₁ crosses of chile de agua inoculated with *Phytophthora capsici*.

F ₁ Crosses	Disease index (%)	General combinatorial ability	General combinatorial ability	Specific combining ability
1×3	60	0.54	−4.78	29.09 **
1×4	27.31	0.54	4.85	−13.22 *
1×5	5.31	0.54	−5.58	−24.79 **
1×6	21.68	0.54	−0.49	−13.52 *
1×7	36.4	0.54	5.53	−4.81
1×8	52.5	0.54	−0.07	16.88 **
3×4	26.87	−4.78	4.85	−8.34
3×5	39.37	−4.78	−5.58	14.59 *
3×6	38.75	−4.78	−0.49	8.87
3×7	23.28	−4.78	5.53	−12.62
3×8	11.16	−4.78	−0.07	−19.14 **
4×5	44.68	4.85	−5.58	10.28
4×6	7.96	4.85	−0.49	−31.54 **
4×7	32.81	4.85	5.53	−13.50 *
4×8	30.93	4.85	−0.07	−7.12
5×6	48.75	−5.58	−0.49	19.67 **
5×7	15.15	−5.58	5.53	−19.94 **
5×8	15.78	−5.58	−0.07	−13.72 *
6×7	9.58	−0.49	5.53	−30.61 **
6×8	51.31	−0.49	−0.07	16.72 *
7×8	58.28	5.53	−0.07	17.66 **

**, *, very significant 0.01, significant 0.05, resp.

In the GCA and SCA analysis of the 21 simple F_1 crosses of chile de agua, the negative GCA values of parents 3 (−4.78) and 5 (−5.58) highlight their additive potential to reduce the disease index, making them promising candidates for parental-line selection. However, strongly negative SCA effects revealed the most heterotic combinations. Crosses such as 1×5 (SCA=−24.79; DI=5.31%), 4×6 (SCA=−31.54; DI=7.96%), and 6×7 (SCA=−30.61; DI=9.58%) exhibited reductions in severity far greater than expected based on the GCA of their parents, suggesting favorable gene interactions (dominance and/or epistasis) that could be exploited to develop highly resistant hybrids. In contrast, crosses such as 1×3, 7×8, and 1×8 showed positive SCA effects and DI means close to 60%, indicating particularly susceptible combinations. Overall, these results suggest that an effective breeding program should integrate the selection of parents with favorable GCA and the identification of specific crosses with strongly negative SCA to maximize resistance to *P. capsici*.

CONCLUSIONS

The evaluation of 27 chile de agua crosses revealed significant genetic variability in response to *Phytophthora capsici*. Crosses 1×5, 4×6, and 6×7 exhibited the lowest disease index (DI) and AUDPC values, indicating moderately resistant performance. Parents 3 and 5 showed favorable additive effects (negative GCA), whereas specific combinations with highly negative SCA suggest useful non-additive gene interactions for the development of resistant hybrids. These results support the use of parents with strong GCA in combination with high-performing specific crosses, as an effective strategy for breeding chile de agua resistant to *P. capsici*.

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Characterization of Maize Producers in the Paso de Ovejas Micro-Watershed, Veracruz, Mexico

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ABSTRACT

Objective: The objective of this study was to determine the producer profile and the management of productive activities along the Xocotitla-Angostillo transect in Veracruz, Mexico.

Design/methodology/approach: A questionnaire consisting of n=49 items was administered, including socioeconomic and technical questions to assess the situation of producers in the study area.

Results: Rainfed agriculture was identified as the main production system, with yields of 3.4 t ha⁻¹ and 3.1 t ha⁻¹ in Xocotitla and Angostillo, respectively. Likewise, producers reported receiving no government support, low maize (*Zea mays*) prices, and limited commercialization opportunities. In addition, livestock production was used as a secondary activity.

Findings/conclusions: It is recommended to update knowledge on maize management by exploring new alternatives that reduce ecological impact, adopting new technologies for irrigation and grain storage, and promoting access to technical assistance and fair market prices for producers.

Keywords: Water, agriculture, backyard livestock farming, agronomic management, soil.

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INTRODUCTION

Land use and land occupation for agricultural development have been key aspects of socioeconomic development, transforming rural landscapes (Liu, 2018). These changes increase risk and vulnerability to natural and technological disasters in a context where poverty, although lower than in the twentieth century, remains significant and a large proportion of the population still lacks the minimum resources required for a dignified existence (Gligo *et al.*, 2020). Landscape change is driven by several complex drivers that involve feedbacks between human-ecosystem relationships (Fischer *et al.*, 2015).

Global production of food, fiber, and industrial crops continues to grow significantly in line with increasing demand (UNESCO, 2020). Furthermore, the need to reach urban markets exerts greater pressure on rural regions and drives rapid landscape changes. The extent of human transformation of the Earth's surface and ecosystem functions has led to the crossing of multiple planetary boundaries, particularly climate change, biodiversity loss, and nitrogen cycle alteration.



Human influence on ecosystem transformation is irreversible. Perhaps the most important driver of these changes is the conversion of ecosystems into agricultural systems dedicated to the production of food, fiber, and fuels necessary for human well-being (Andersen *et al.*, 2015).

These landscape changes affect community members, who must adapt the ways in which they use, manage, conserve, and interact with the agroecosystems and natural resources that belong to them.

In the state of Veracruz, Mexico, various productive activities modify the landscape, particularly agriculture and livestock production. According to data reported by INEGI (2022), there are 5.8 million hectares with agricultural or livestock use potential, of which 2.6 million hectares are used for agriculture, making Veracruz a state with significant landscape modification driven by productive activities.

Specifically, in the municipality of Paso de Ovejas, where a micro-watershed is formed, there is a highly marginalized area consisting of a transect that includes the communities of Angostillo and Xocotitla. Although productive activities are carried out in this area, the management practices and modifications implemented by farmers to develop these activities remain poorly documented. Over time, these activities change constantly due to seasonal variations, natural resource modifications, and social factors.

Due to the region's topographic diversity, access to water varies depending on location, creating different environmental conditions for the development of productive activities. Therefore, it is important to understand how producers manage the natural resources required for production, particularly water and soil. Accordingly, this study identified the profile of rural producers in the Xocotitla-Angostillo transect, Veracruz, in relation to productive activities and the management of natural resources (water and soil) they employ.

MATERIALS AND METHODS

The research was conducted from October to December 2022 in the central region of the state of Veracruz, where the Paso de Ovejas micro-watershed is located, specifically in the communities of Angostillo and Xocotitla along the transect situated near the Tecolutla River (Figure 1).

A survey was administered to $n=180$ producers from both communities using a structured questionnaire consisting of 49 items. Five aspects were considered: producer profile, crop management and productivity, production costs, commercialization, and livestock component.

The sampling method used to establish the study population was snowball sampling (linear sampling), which consists of interviewing one producer who then refers other producers known to them for subsequent interviews, and so on. Key contact agents were the presidents of the ejido commissariats in each community, who facilitated access to producers and the administration of the surveys (Mendieta-Izquierdo, 2015).

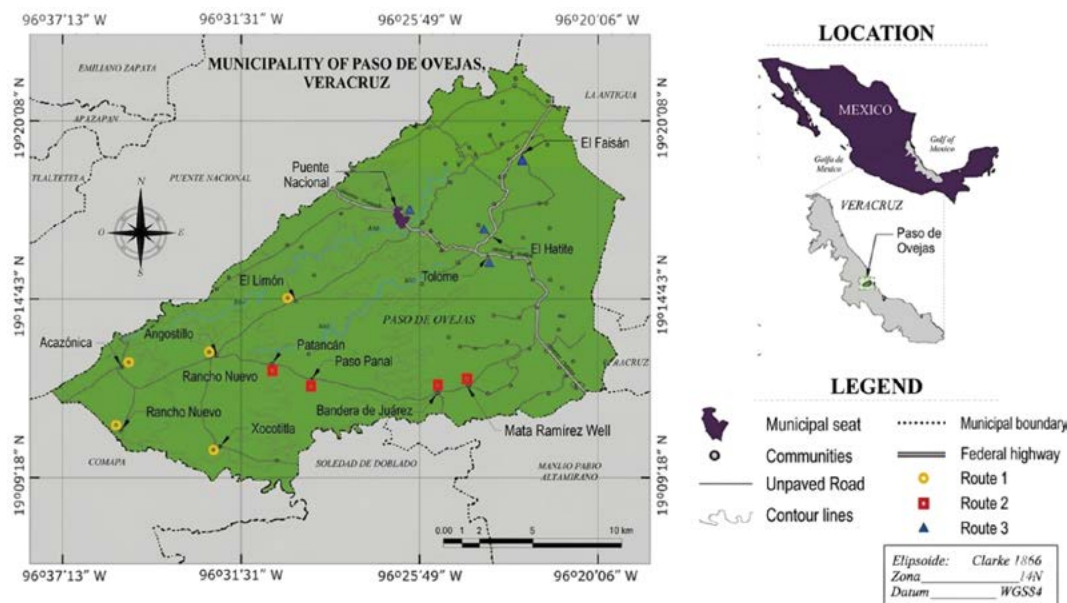


Figure 1. Geographic location map of the study site. Source: Cruz-Bautista *et al.* (2021).

RESULTS AND DISCUSSION

Producer profile

According to the data obtained, the average age of producers was 59.74 and 63 years in Angostillo and Xocotitla, respectively, showing a difference of 3.26 years between the two communities. This indicates that the majority of the population belongs to older age groups. This pattern is explained by the limited involvement of younger generations (adults under 30 years old) in these activities, as most of them tend to migrate.

Producers have an average schooling of 5.2 years, indicating that most of them only reached elementary primary education, and the majority did not complete this level.

Producers are evenly distributed between both communities. Most of them practice agriculture in areas where tomato and papaya were previously cultivated under irrigation; however, these crops were abandoned due to high production costs. Currently, producers focus on rainfed maize cultivation, as it involves lower production costs and requires less investment. Due to economic constraints and the need for a stable income, nearly half of the producers engage in livestock production or combine this activity with agriculture (40%).

100% of the producers reported operating as individual producers, and land tenure is ejidal. However, they own their plots through inheritance from their ancestors, where productive activities are carried out, and they do not pay land rent. Their properties have legal registration, which provides land tenure security and allows them greater autonomy in managing maize cultivation.

Regarding cultivated area, producers in Xocotitla and Angostillo cultivate an average of 5.8 and 5.9 ha, respectively (with some producers planting between 1 and 30 ha). This indicates that they are considered small-scale producers, as their land area is smaller than 13.5 ha (INEGI, 2017), and production is mainly oriented toward commercialization,

while only a small portion (10-20%) is used for self-consumption. Producers also reported that they previously cultivated other crops such as tomato (*Solanum lycopersicum*) and squash (*Cucurbita* sp.); however, due to changing climatic conditions, such as prolonged droughts, they have shifted to maize cultivation, as its production requirements are lower than those of the aforementioned crops.

According to the findings from the producer profile, educational level is a key factor influencing human development and poverty reduction, as recognized by UNESCO (2020). According to INEGI (2022), the average years of schooling in Mexico is 9.84 years, while in the state of Veracruz it is 8.75 years. In contrast, the schooling level in the study area is below both the national and state averages, revealing significant educational gaps. Evidence has shown that education has gained increasing relevance in a world undergoing profound transformations driven by the rapid advancement of science and its applications (de la Rosa *et al.*, 2021). Completing at least 12 years of schooling (primary to upper secondary education) is considered by the population to be the minimum level required to obtain conventional employment that does not require a university degree or engineering training.

School dropout occurs across all educational levels (Hernández and Montes, 2020). The factors associated with school dropout in upper secondary education are diverse and vary in nature, including economic, academic, family-related, individual, and broader external factors (Vizcarra-Muro, 2024).

It is worth noting that in 2020 at least one million students at all educational levels dropped out of school nationwide (SEP, 2020). However, the level of education among producers allows them to understand processes that do not require specialized training and to make decisions regarding crop management, such as land preparation and input application for crop development. It should be emphasized that the educational level observed among producers is minimal (primary education). Furthermore, producers no longer pass on agricultural activities to their children, as previously mentioned, because younger generations migrate to pursue education and, after attaining higher levels of schooling, remain in urban areas to work, where they perceive higher wages compared to agricultural or livestock activities. Nevertheless, improving the educational level of the population or encouraging younger generations to return to work in rural communities could enhance overall development and well-being for children, youth, and adults by fostering new ways of living (Miranda-López, 2018).

Crop management and productivity

The maize cultivated in the communities is predominantly hybrid (DK-370) in most cases (65.6%), while the remaining portion corresponds to locally sourced maize obtained from a regional supplier, and a small minority of producers use seeds saved from the previous harvest. Rainfed agriculture is practiced, and therefore irrigation infrastructure is lacking; the sowing season varies from May to July, which consequently affects the harvest period.

Regarding agronomic crop management, activities include soil preparation, sowing, cultural practices, and the application of fertilizers and pesticides (Ávila *et al.*, 2024). In

this regard, 100% of producers prepare the land mechanically, using a tractor to carry out harrowing. Likewise, all producers in both communities use tractors to establish planting furrows with a spacing of 0.8 m between rows.

All producers perform sowing and cultural practices manually and apply fertilizers at least once (before flowering) and pesticides (during the early growth stage) to control pests affecting the crop.

For seed preparation prior to sowing, 100% of producers apply a pre-treatment with thiodicarb to prevent pest attacks. The grain is sown in the field after the first three rains of the season to ensure adequate soil moisture. Three days after sowing, and once weeds reach approximately 20 cm in height, herbicide is applied to control weeds that could affect maize development.

During this phenological stage, producers apply glyphosate (50%) or a paraquat + diuron mixture (50%). Although glyphosate is currently banned, producers continue to apply it, stating that it is more effective than the alternative. For narrow-leaf weeds, nicosulfuron is applied, while for broadleaf weeds, Fito Amina is used. Approximately eight weeks after sowing, the flowering stage begins, at which point 100% of producers apply urea at a rate of 300 kg ha⁻¹. Regarding pests affecting the crop, the most common are fall armyworm and stem borer (*Diatraea* sp.). To control these pests, producers apply DENIM at 0.05% (4'-epi-methylamino-4'-deoxyavermectin B1 benzoate), using 200 L ha⁻¹ (100 mL diluted in 200 L of water).

Regarding maize production parameters, the average yield was 3.1 t ha⁻¹ and 3.4 t ha⁻¹ in Angostillo and Xocotitla, respectively, while planting density was approximately 41,625 plants ha⁻¹.

These yields are slightly higher than those reported for communities in the Tierra Blanca region of Veracruz, where rainfed maize production shows yields of 1.26 t ha⁻¹ (Jaramillo *et al.*, 2018).

In contrast, in areas where irrigation technology is used, yields are higher, reaching values of 8.13 t ha⁻¹ and 9.77 t ha⁻¹ in Sonora and Sinaloa, respectively (SAGARPA-SIAP, 2021). This variation in production yield may be due to different factors such as constant access to water, since nutrient distribution and uptake in crops improves in the presence of water, thereby optimizing plant development, whereas in the study site water is only available when rainfall occurs.

Another factor that affects production yields is plant density, as it has been reported that for improved varieties with short and intermediate plant height, around 62,500 plants ha⁻¹ should be used under irrigation and fertilization conditions (Saltos, 2018). In other studies, such as that conducted by García-López and Hernández (2023), it is mentioned that plant populations of up to 90,000 plants ha⁻¹ can be achieved.

In contrast, in studies where irrigation is not used (Medina-Méndez *et al.*, 2018), 70,000 plants ha⁻¹ have been sown, which is higher compared to what was found in the study sites (41,625 plants ha⁻¹), with a difference of more than 20,000 plants. Planting density in maize has a strong influence on grain yield and agronomic characteristics, since grain yield increases with population density until reaching a maximum point and decreases when density increases beyond this point. León-Aguilar *et al.* (2018) mention

that population density is one of the most important controllable factors in maize production available to producers to obtain higher grain yields. However, if population densities higher than the optimum are used, competition for light, water, and nutrients increases, causing a reduction in root volume, number of ears, grain quantity and quality per plant, and increasing the frequency of root and stem rot, which leads to lodging, whereas low population densities promote weed problems and underutilization of the soil (Moya-Cisneros, 2016).

Another factor to consider is ear size and weight. According to data obtained from producers, ear weight ranged from 150 to 220 g, while ear length was 18 cm and width was 5 cm. These values are higher than those reported by Sánchez-Hernández *et al.* (2019), where ears from Loma Bonita, Oaxaca, Mexico, had a length of 14 cm. Ramírez *et al.* (2020) found that ears from the same area had an average weight of 139.73 g, being slightly higher in the region of the present study. In addition, other factors such as climate, temperature, soil type, and crop management practices may affect yield, since high temperatures may prevent the plant from reaching the required cold hours for adequate production, and similarly, soil mineral deficiencies may lead to inadequate ear development.

Production costs

Production costs do not show considerable variations among producers. In the case of both communities, 100% of producers obtain their agricultural inputs from regional suppliers, since there are no stores in the communities that sell these products. In both communities, the purchased inputs are used to improve yields, grain quality, and based on recommendations among producers themselves. Production costs range from \$12,000.00 Mexican pesos (USD \$670.00) to \$14,500.00 (USD \$805.00) per hectare, with these differences due to the location where the inputs are obtained.

In addition to the required inputs, external labor used during the sowing and harvesting seasons must be included. During this period, 100% of producers hire external labor. Each producer hires an average of 5 to 10 laborers, with each laborer receiving an average payment of MN \$300.00 per day (USD \$17.00), which is higher than the current minimum wage (USD \$11.50). These laborers work for the duration of the harvest period, which can last up to approximately five days, since ear harvesting is not difficult and therefore does not require much time (Morales-Ibarra, 2021). Production costs vary according to the amount of inputs used, such as the quantity of fertilizers and pesticides applied depending on the number of applications, the type of machinery used for land preparation, the number of laborers employed, among others. According to the data collected, production costs are higher compared to those reported by Medina-Méndez *et al.* (2018), who mention that maize production cost USD \$590.00 per hectare, where 27% of the total amount is used for fertilization, 28% for seed and sowing, 19% for soil preparation, 10% for herbicides, 7% for pesticides, and the remaining 9% for harvesting. In contrast, in the present study approximately 40% of the total amount is used for pesticides and herbicides. The complete dependence on rainfed conditions in maize cultivation results in high risk, which limits resource investment, and under this scenario

maize becomes a highly soil-extractive agroecosystem, as some producers, in order to save as much as possible, use inadequate fertilization formulas and some even reach the point of not applying fertilizers or pesticides.

Comercialization

After harvesting, the ears are dried in the sun and then shelled. The grain obtained is stored in sacks for commercialization. Although maize could also be stored in metal drums or storage facilities, producers do not have this type of technology (SADER, 2024).

The grain can be used as livestock feed or consumed by humans in the form of fresh maize (elote), or once nixtamalized, as tortillas and a wide variety of products (Sánchez-Rodríguez, 2016). Because several maize-based products are part of the basic food basket, most of the production is commercialized mainly to industries. In the case of producers, a small portion of production is kept for self-consumption (20%), while most is commercialized or sold (Figure 3).

In both localities, producers mentioned that the price at which the collector purchases the grain is low, receiving a payment of USD $\$0.40 \text{ kg}^{-1}$ and USD $\$0.36 \text{ kg}^{-1}$ in Xocotitla and Angostillo, respectively, while the collector sells this same maize at a price of approximately USD $\$0.54 \text{ kg}^{-1}$. The difference between producer and trader, translated into tons of production, represents a considerable amount. However, between 70-80% of producers consider the crop to be profitable, as it generates some benefit.

In addition, it is important to mention that producers do not export the maize they produce nor sell it directly to companies, as the individual production volume is small and they do not have a producers' organization through which they could coordinate to gather the necessary production volume to meet the demand of a specific company, which could also provide them with higher profits by eliminating intermediaries in commercialization.

They also indicate that the government should support maize production in the region, as they do not receive any type of support, lack infrastructure (transport, packing facilities, storage containers), and their commercialization is limited due to being a marginalized area. In both localities, maize cultivation presents constraints such as low production yields, soil erosion, lack of irrigation technology or access to water, pests, lack of technical assistance, and commercialization limitations (IICA, 2018).

In the case of the study localities, maize production (approximately 2,737.80 tons) is sold or commercialized to an intermediary (collector), who sells the production to companies that require large volumes of maize, or in some cases to buyers who export the grain to other countries where there is demand.

Although it is mentioned that the payment they receive is not fair, mainly because production costs are high and therefore profits are reduced, producers consider that commercialization prices could be improved if a guaranteed price were established so that producers could obtain profits, as well as by establishing a collection center near the region to avoid selling to intermediaries. Likewise, the establishment of a producers' association could allow them to set their own guaranteed price or make agreements directly with companies and consequently obtain greater benefits from grain sales.

The reduced payment received by producers causes economic difficulties, motivating them, directly or indirectly, to seek alternatives to obtain income, and one of these is through livestock raising.

Livestock component

Animal husbandry in rural areas is used as an alternative subsistence activity (20-30% of income), which is related to rural activities and together contribute to the food sovereignty of producers. According to the surveys conducted, it was found that each maize producer had on average five head of cattle, in addition to some chickens and pigs.

In this sense, 100% of the producers raise chickens under backyard conditions. It was also found that producers had on average five cows, mainly raised in pastureland, and to a lesser extent an average of one pig per producer raised in backyard conditions.

Maize production together with backyard livestock farming constitutes a peasant production system in which agricultural and livestock activities converge with the purpose of providing families with basic foods such as meat, eggs, seeds, among others (Cuca *et al.*, 2015). The importance of livestock in rural families lies mainly in the fact that the products obtained are destined for self-consumption, as they represent family savings (Montero and Martínez, 2015). In addition, in case of emergency or under precarious conditions, these animals can be sold to generate income (García-Navarro *et al.*, 2022), which ensures family stability. Pig raising (*Sus scrofa* ssp.) generates income through live-animal sales, and most pigs are fed with waste or leftovers from the kitchen or harvest. Likewise, chickens (*Gallus gallus*) are part of the producers' diet and have great importance in the communities, being essential for the subsistence of many low-income farmers, as they are often the only asset they possess. They are also used as food during celebrations and gatherings, helping to build networks and relationships with other peasants in the community, as they are part of their uses and customs (Morales-Jiménez *et al.*, 2024).

Based on the aforementioned, it was determined that animal husbandry in the localities of Xocotitla and Angostillo constitutes a strategy that provides food for self-consumption and also becomes a means to meet certain needs, as these animals can be sold in case of financial emergencies and can therefore be considered a form of savings.

In addition, producers stated that when maize production is good, it helps sustain animal feeding, providing capital availability to improve animal husbandry and better control their diseases, which leads to better development and consequently greater profit for the producer.

The characterization of producers made it possible to determine that the main productive activity was agriculture, with rainfed maize as the principal crop, with an average of 5.8 ha cultivated and yields of 3.1 t ha⁻¹ and 3.4 t ha⁻¹ in Angostillo and Xocotitla, respectively. Producers in both localities could benefit from the implementation of new technologies or products to improve production yields and thus obtain greater benefits. Backyard animal husbandry, such as cows, pigs, and chickens, helps support family subsistence. The limiting factors affecting crop production are water availability, pests, lack of government support for infrastructure (machinery and storage), and technical assistance, and consequently poor crop management.

CONCLUSIONS

It is necessary to update producers on crop management and to consider more effective and economical alternatives to those currently used in the study localities in order to reduce ecological damage; likewise, it is essential to adopt irrigation technologies for crop production and grain storage. Similarly, it is important for the government to support producers in Xocotitla and Angostillo through governmental programs that provide technical assistance and infrastructure, in addition to guaranteeing a fair selling price for producers. It would also be important for the government to conduct periodic censuses in this type of locality in order to develop databases that would facilitate future research.

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Synergies between organizational resilience and knowledge absorption capacity: An empirical study of aquaculture PYMES in Northwest Mexico

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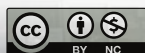
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ABSTRACT

Business resilience and knowledge absorption capacity are decisive for the long-term success of small and medium-sized enterprises, enabling them to adapt and remain competitive and operational in times of uncertainty.

Objective. To identify key factors that affect resilience and knowledge absorption capacity in aquaculture organizations in the municipality of Ahome, Sinaloa.

Methodological design. Using a quantitative approach, an analysis was conducted using a Likert scale to assess the dimensions of resilience and knowledge absorption. The results show that organizations have a strong capacity for foresight and knowledge acquisition, although they face challenges in adaptation, transformation, and exploitation. The main limitation of the study is that the sample is concentrated in a single region or sector, which restricts the generalization of the results. The main finding indicates that organizations are strong in foresight and knowledge acquisition, but have significant weaknesses in adaptation, transformation, and exploitation of knowledge, which limits their ability to generate innovation and competitive advantage.

Keywords: resilience, knowledge absorption, competitiveness, Ahome, aquaculture.

INTRODUCTION

The dynamics of a globalized market force organizations to operate in increasingly uncertain and volatile environments, where competitiveness and sustainability depend on the strategic use of knowledge and the development of solid organizational capacities. This challenge is particularly critical in productive sectors such as aquaculture, characterized by a high exposure to sanitary, climate and market risks, which demand flexible and adaptive organizational responses (Garrido-Moreno *et al.*, 2024).



From the perspective of knowledge management, the literature has pointed out that internal sources are insufficient to generate value in contexts of accelerated technological and competitive transformation (Nonaka and Takeuchi, 2023). Under this scenario, the knowledge absorption capacity (KAC) of organizations is consolidated as a strategic capability, by allowing them to acquire, assimilate, transform, and exploit external knowledge to strengthen their innovation and adaptation processes (Cohen and Levinthal, 1990; Zahra and George, 2002). The development of this capacity contributes to strengthening the basis of organizational knowledge and to optimizing the use of available resources (Flatten *et al.*, 2011).

In addition, organizations can be conceived as dynamic systems that require constantly adapting to disruptive contexts. In this sense, organizational resilience capability (RC) refers to the ability to anticipate, respond, and recover in the presence of external disturbances, articulating strategy, processes, technology, and human capital (Sanchis Gisbert and Poler Escoto, 2014; Arocena *et al.*, 2020). The importance of this capability was widely evidenced during the COVID-19 pandemic, when its role was confirmed in the operative continuity and organizational stability (Vidal and Vidal, 2020; Do *et al.*, 2022).

Although the KAC and the RC have been traditionally addressed as differentiated constructs, recent studies suggest that both can interact synergically, strengthening processes of organizational learning and adaptation (Hillmann and Guenther, 2021). However, there is still an empirical void regarding how this relationship is manifested in organizations of the agriculture and livestock sector, particularly in the scope of shrimp aquaculture in land ponds.

Facing this void, the objective of this study is to analyze jointly the knowledge absorption capacity and the organizational resilience capability in aquaculture organizations in the municipality of Ahome, Sinaloa, Mexico, with the aim of identifying their main strengths and limitations. From a quantitative empirical approach, the hypothesis set out is that higher levels of knowledge absorption capacity are associated with higher levels of organizational resilience. The study's contribution lies in providing sectorial empirical evidence on the interaction between both capabilities in a productive context that is scarcely explored by the literature, generating relevant analytical inputs for the organizational management and sustainability of the aquaculture sector.

Organizational resilience in PYMES

Organizational resilience has been widely recognized as a key capability for the sustainability and continuity of organizations, especially in contexts characterized by high vulnerability and uncertainty. In general terms, the literature conceptualizes resilience as the ability to anticipate, absorb and recover from external disturbances without compromising the organizational design.

From an empirical perspective, Dahles and Susilowati (2015) analyze business resilience in small tourism enterprises from Indonesia through a longitudinal study of ethnographic nature. Their findings show that resilience is constructed from adaptative subsistence strategies in the presence of recurrent crises. However, the qualitative nature of the study

and its sectorial approach limit the generalization of results to other productive contexts, such as agriculture and livestock production.

Saad *et al.* (2021), based on a systematic review of 118 studies on resilience in PYMES, identify important conceptual and methodological inconsistencies, as well as scarce empirical production in developing countries. The authors underline the need to strengthen analytical frameworks and contextualized evidence, which is particularly relevant for primary sectors exposed to frequent external shocks.

In the Latin American context, Contreras, López and Zalthen (2023) develop and validate a scale to measure the business Resilience Capability (RC) in Mexican PYMES, based on the dimensions of foresight, adaptation and recovery. Applied to 346 businesses in Campeche, the instrument shows adequate levels of reliability and validity. However, the study is centered on impacts derived from COVID-19 and does not delve into the relationship between resilience and other organizational capabilities, such as knowledge management, which opens a space for complementary studies.

Knowledge absorption capacity (KAC)

Knowledge absorption capacity (KAC) has been consolidated as a central construct in the literature about innovation and competitiveness. Since its initial formulation by Cohen and Levinthal (1990), KAC is defined as the ability to acquire, assimilate, transform, and exploit external knowledge with productive purposes.

Mariano and Walter (2015), through content and text analysis of 186 articles published between 1990 and 2013, show significant conceptual evolution of the construct, but also high fragmentation in its empirical operationalization. This finding suggests the need for applied studies that connect the KAC with specific productive contexts.

In Latin America, Sánchez and Rivera (2021) carry out a systematic and bibliometric review of KAC, organizational learning, and information technologies, highlighting the complementarity between these variables. However, their analysis remains at the macro level and does not address specific productive sectors.

In the Mexican context, Solís *et al.* (2017) empirically analyze the influence of KAC on innovation in the petrochemical sector of Tamaulipas, and they find a positive and significant relationship. Although this study contributes relevant empirical evidence, its approach in a specific industrial sector limits the direct transference of their findings to primary activities such as aquaculture, where productive dynamics and risks differ substantially.

The interaction between organizational resilience capability (RC) and knowledge absorption capacity (KAC)

In recent years, this interaction has sparked growing interest in specialized literature. From a theoretical perspective, resilience allows maintaining organizational stability in the presence of disturbances, while KAC eases the incorporation of external knowledge for innovation and adaptation.

Studies such as those by Castañeda and Cuellar (2020) and García-Valenzuela *et al.* (2023) suggest that a developed KAC strengthens organizational resilience by favoring

continuous learning processes. In turn, resilient organizations create favorable conditions for the integration and exploitation of knowledge.

This approach converges with the Theory of Dynamic Capabilities, which explains how organizations reshape resources and competences to respond to changing environments. However, most of these studies are kept in conceptual plans or in industrial and service sectors, leaving an empirical void in agriculture and livestock sectors and, particularly, in Mexican aquaculture.

MATERIALS AND METHODS

This study adopted a quantitative, exploratory approach, with non-experimental and transversal design. It was conducted in the northern zone of the state of Sinaloa, municipality of Ahome. This research was limited to a universe of N=98 aquaculture units that breed and fatten shrimp of the species *Litopenaeus vannamei* registered at the Committee for Aquaculture Health of Sinaloa and the Local Sanity Board. Based on field diagnosis, a sample framework of 88 operatively active units was identified, and convenience non-probabilistic sampling was used, achieving the effective participation of n=70 organizations. This represents a response rate of 79.5%, level which exceeds the standards suggested for organizational studies. This sample size is representative of the sector in the region of Ahome, considering that the inclusion criterion was that the organization was operating and available during the sampling period. The instrument was a structured questionnaire, based on scales validated by Contreras, López and Zalthen (2023) for RC, and Carrazco Escalante and León Balderrama (2017) for KAC.

The validation of the instrument was carried out in two phases. In the first, an expert panel in aquaculture management and methodology evaluated the pertinence, clarity and coherence of the items. In the second, the reliability of the scales was determined through Cronbach’s alpha coefficient, with values that range from 0.678 to 0.905. These results confirm an acceptable internal consistency, guaranteeing the homogeneity of the items, and their contribution to the measurement of the global construct (Frías-Navarro, 2022).

Table 1. Synthesis of prior studies and research voids.

Authors	Main variable	Context	Methodology	Main contributions	Limitations/Gaps
Dahles & Susilowati (2015)	Resilience	Tourism - Indonesia	Qualitative	Resilience as an adaptive strategy	Low sectoral generalization
Saad <i>et al.</i> (2021)	Resilience	Multisectoral	Systematic review	Identifies conceptual gaps	Lack of empirical evidence
Contreras <i>et al.</i> (2023)	Resilience	SMEs - Mexico	Quantitative	Validated scale (RC)	Does not integrate KAC
Mariano & Walter (2015)	KAC	Multisectoral	Content review	Conceptual evolution of KAC	Empirical fragmentation
Solís <i>et al.</i> (2017)	KAC	Industry - Mexico	Quantitative	KAC drives innovation	No primary sector
This study	RC - KAC	Aquaculture - México	Quantitative	Integrated RC - KAC evidence	-

Therefore, it is confirmed that the instrument has the necessary stability for the analysis of the data collected in the aquaculture sector. Another validation of the instrument was through the Exploratory Factorial Analysis; the results confirmed the validity of the construct with KMO index of 0.705 and significant Bartlett sphericity test with value $p \leq 0.000$. The factorial structure obtained was consistent with the theoretical dimensions of the original scales (Contreras *et al.*, 2023; Carrazco Escalante and León Balderrama, 2017), explaining 60% of the total variance. These tests guarantee that the instrument has as much internal stability as structural, coherent and adequate validity for the scale (Table 2).

The information was obtained through interviews with “aquaculture” owners, general managers, and biologists as operation chiefs, contributing reliability to the data. About the sociodemographic profile: a) Most have studies at the high school or university level; a minority have truncated studies; b) Undergraduate studies in Biology, Marine Biology, and Oceanology are the most common; and c) The population active in shrimp breeding is concentrated at 19 to 64 years, with minimal participation between 65 and 85 years old.

The greatest generational representation in the sector corresponds to the first and second generation of aquaculture producers, who express uncertainty about continuing with the activity started by their parents. However, recognizing the value of this labor, which with effort and dedication has contributed to the development of vulnerable communities on the northern coast of Sinaloa.

The measurement instrument was made up of 38 items distributed in two dimensions: 1) RC (foresight, adaptation and recovery) and b) KAC (acquisition, assimilation, transformation and exploitation). The questionnaire was applied to a sample of 70 shrimp farms settled on the northern coast of Ahome. A 7-point Likert type scale was used, with the following response options: (1) Totally disagree; (2) Moderately disagree; (3) Disagree; (4) Undecided; (5) Agree; (6) Moderately agree; (7) Totally agree.

Analysis of the scale reliability

The internal consistency of the instrument was evaluated through Cronbach's Alpha coefficient, using it to estimate the reliability of the measurements. According to Frías-Navarro (2022), adequate Cronbach's Alpha value is between 0.70 and 0.95; higher numbers can indicate redundancy between items.

Table 2 shows Cronbach's Alpha values for the RC and KAC variables, calculated by subdimension. The results are within the acceptable range, which evidences a high reliability of the instrument, guaranteeing the homogeneity of the items and their contribution to the measurement of the global construct.

However, at the global level, a reliability coefficient between 0.838 and 0.905 was obtained, which confirms that the instrument fulfills the requirements of internal consistency. These results support the close relationship between the organizational RC and the KAC. The analysis of quantitative data was carried out with SPSS software version 2.3 (Table 3).

Table 2. Variables and their measurement (RC-KAC).

Variable	Items	Authors
RC: a) prevention, b) adaptation, c) recovery.	We allocate a budget item to the implementation of actions that improve business resilience.	Contreras, López y Zalthen (2023).
	We allocate a budget item to support the implementation of actions that improve business resilience.	
	We have a strategic financial plan to deal with any contingency.	
	We have a strategic marketing plan to deal with any contingency.	
	We examine the possible outcomes of a project or process, comparing the potential benefits against the potential risks of the investment.	
	We have a strategic administrative plan in place to deal with any contingencies.	
	We encourage cooperation among suppliers to achieve the desired level of stability.	
	We encourage cooperation among workers to achieve the desired level of stability.	
	We easily adapt to new requirements imposed by the government, such as the implementation of safety and hygiene measures.	
	We adapt to situations arising from external disruptive events that directly affect the normal operation and stability of the company.	
	We can respond more quickly to changing conditions than our competitors.	
	We document the knowledge gained from experience with historical disruptive events.	
	We record the actions taken in response to a disruptive event in the action protocol, in case a similar event occurs again.	
	We have recorded the strategies that proved obsolete when facing a disruptive event.	
	We implement knowledge recording activities based on our experience with disruptive events that we have faced.	
	We act in accordance with the established protocol in the event of a disruptive event.	
	We have recorded the strategies that proved effective in dealing with a disruptive event.	
	We respond to external disruptive events that threaten the survival of this company.	
	We make the necessary changes to face and overcome obstacles based on our objectives.	
	We have measures in place to enable recovery from critical situations that disrupt normal operations and stability.	
KAC: a) Acquisition, b) Assimilation, c) Transformation, d) Exploitation	We gather information on developments in the sector through discussions with business partners in the aquaculture industry.	Carrasco Escalante y León Balderrama (2017).
	Our company participates at least twice a year in industry seminars and conferences organized to update and enrich our experience and knowledge.	
	We allocate considerable time to establishing contacts with agents who can provide us with knowledge and information on innovations in the sector.	
	I believe we have sufficient skills to establish contacts with agents who can provide us with knowledge and information about innovations in the sector.	
	Our company is always among the first to recognize changes in technical possibilities.	
	Our company is always among the first to recognize changes in health regulations.	
	Our company is always among the first to recognize changes in market competition.	
	Our company is very skilled at identifying new opportunities to serve new customers.	
	Our company allocates a lot of time to consulting with advisors to recognize changes in the market in advance.	
	Our aquaculture farm has sufficient skills to consult with advisors on how changes in the market can be used to make changes in the operation of our plant.	
	We record and store newly acquired knowledge for future reference.	
	Our company quickly recognizes the usefulness of new external knowledge for our existing knowledge.	
	We discuss at least monthly with external advisors how market trends could be used to improve our business.	
	We allocate a considerable amount of time to translating external information into adaptations for our business.	
	I believe we have sufficient skills to translate external information into adaptations for our business.	
	We translate external information directly into new business applications.	
	We apply external information to our business to contribute to our productivity.	
	I believe we have sufficient skills to convert external information into productive results.	

Table 3. Reliability scale Resilience and Knowledge Absorption.

Resilience Capacity		Knowledge absorption capacity	
Sub-Dimensions	Cronbach's Alpha	Sub-Dimensions	Cronbach's Alpha
Ability to anticipate	0.678	Acquisition Capacity	0.776
Ability to adaptation	0.716	Assimilation Capacity	0.780
Ability to recover	0.880	Transformation Capacity	0.600
Total	0.838	Total	0.905

RESULTS AND DISCUSSION

This study offers a detailed analysis of the organizational capabilities in aquaculture farms, evaluating both resilience and knowledge absorption. Through a quantitative approach and through a 7-point Likert scale, four subdimensions of the KAC and three of the RC were analyzed.

The objective is to provide a comprehensive framework that allows understanding how aquaculture organizations —shrimp aquaculture— manage knowledge and strengthen their capabilities of adaptation and recovery in face of disruptive events. The quantitative analysis revealed significant relationships between the allotment of budget for business resilience and various organizational capabilities in aquaculture PYMES. Next, the main findings of the data obtained are detailed:

- 1. Strategic plans for contingencies:** A positive and significant relationship between PYMES that channel budget to business resilience and those that have a strategic plan for contingencies was observed (contingency coefficient=0.747, $p=0.000$). This indicates that they invest in resilience, increasing the probability of designing formal plans to act in the presence of critical situations.
- 2. Adaptation to new regulations and government measures:** A significant relationship is reported (coef.=0.558, $p=0.011$) between the budget for resilience and the capacity for normative adaptation. PYMES with proactive approach for resilience show greater agility to fulfill government requirements.
- 3. Adaptation to disruptive external events:** The coefficient of contingency of 0.710 ($p=0.000$) reflects a strong relationship between investment in resilience and the capability for adaptation in the presence of disruptive events. This indicates that resilient organizations are better prepared to face unexpected scenarios.
- 4. Speed of response to changes in the environment:** With a coefficient of .737 ($p=0.000$), it is evidenced that PYMES which devote resources to resilience respond faster to changes in the environment, exceeding in agility those that do not invest in these capabilities.
- 5. Documentation and learning from past disruptive events:** There is a strong correlation (coef.=0.636, $p=0.001$) between the budget for resilience and the practice of documenting experiences. This demonstrates that resilient organizations value learning as a mechanism for continuous improvement.

6. Registry of obsolete strategies in face of disruptive events: A high coefficient of contingency of 0.770 ($p=0.000$) indicates that resilient organizations tend to record and identify inefficient strategies in the presence of disruptive events, which helps to avoid errors and improve their preparation.

7. Responsiveness to external disruptive events: With a coefficient of 0.693 ($p=0.000$), it is confirmed that PYMES which allot financing to resilience, have greater responsiveness to threats that risk their stability or operative continuity.

Aquaculture organizations that proactively invest in resilience develop skills to cope with contingencies, adapt to regulatory changes, and capitalize on the knowledge acquired. This strategic capability provides them with a competitive advantage in contrast with those that do not destine specific resources to strengthen their resilience (Table 4).

The aquaculture organizations that invest in resilience show greater capacity to adapt, learn and respond to threats, strengthening their competitiveness. Through quantitative analysis with a 7-point Likert scale, the dimensions of KAC (acquisition, assimilation, transformation and exploitation) and RC (foresight, adaptation and recovery) were evaluated. The results evidence differentiated performances in both capabilities, confirming the direct relationship between knowledge management, resilience and competitive sustainability in the organizations analyzed. Next, Table 5 is presented, adapted to reflect the results obtained according to that scale, illustrating the performance of the organizations in each dimension:

Table 4. Findings.

Capacity or Strategy	Contingency Coefficient	Significance Value (p)	Interpretation
Strategic contingency plan	0.747	0.000	Strong relationship. SMEs with a budget for resilience have contingency plans.
Adaptation to new government regulations	0.558	0.011	Positive relationship. Business resilience makes it easier to adapt to government regulations.
Adaptation to external disruptive events	0.710	0.000	Strong relationship. SMEs with business resilience respond better to disruptive events.
Rapid response to changes in the environment	0.737	0.000	Significant relationship. Resilient SMEs respond quickly to changes in the environment.
Documentation and learning from past disruptive events	0.636	0.001	Significant relationship. Resilient SMEs gather past experiences to drive future improvements.
Record of obsolete strategies in response to disruptive events	0.770	0.000	Strong relationship. SMEs with a budget for resilience record outdated strategies for improvement.
Response to external disruptive events that threaten survival	0.693	0.000	Significant relationship. Resilient SMEs respond effectively to external threats that jeopardize their survival.

Table 5. Results of the Absorption Capacity and Resilience Capability.

Dimension	Item	Result (Average)	Percentage (%)	Standard Deviation	Interpretation
Cap-Acquisition (KAC)	SMEs regularly access valuable information from outside sources.	5.6	76.6%	0.77	The organization has a high level of acquisition.
Cap-Assimilation (KAC)	SMEs quickly process the knowledge they acquire and apply it.	5.3	71.66%	0.72	The organization assimilates effectively.
Cap- Transformation (KAC)	Acquired knowledge is converted into practical actions and operational processes.	5.2	70%	0.64	The transformation process is effective but could be optimized.
Cap-Exploitation (KAC)	SMEs effectively apply transformed knowledge.	5.8	80%	0.801	Knowledge exploitation is effective.
Cap-Prevention (RC)	SMEs can anticipate regulatory and technological changes.	5.7	78.33%	0.778	The organization could anticipate change.
Cap-Adaptation (RC)	SMEs quickly adjust to changes in the environment.	5.4	73.33%	0.75	High adaptability, although there is room for improvement.
Cap-Recovery (RC)	SMEs quickly overcome operational problems.	5.5	75.5%	0.76	Good resilience to adverse events.

The shrimp farming organizations evaluated report a positive performance in knowledge management. In the Acquisition Capacity (76.67%), they show solid abilities to obtain valuable information, although they could improve the systematization. In Assimilation Capacity (71.66%), they absorb external knowledge adequately, although there is a margin to improve their integration and exploitation. The Transformation Capacity (70%) reflects strategic management of knowledge, although without reaching excellence. Regarding the Exploitation Capacity (80%), it is the most outstanding dimension, where they optimize operation and reduce costs efficiently, evidencing a practical use of knowledge that strengthens their operative performance (Table 6). In terms of RC, the aquaculture organizations maintain a strong capability of anticipation to regulatory and technological changes (78.33%). Adaptation (73.33%) to the environment is firm, together with 75.5% having the capability of recovering in face of operative events; together, this type of productive unit evidence agility and resilience, although they can strengthen specific categories.

Table 6. Quantitative evaluation (KAC).

1. Acquisition Capacity (Percentage: 76.7%): Aquaculture organizations rank between “Agree” (5) and “Somewhat agree” (6), indicating a good capacity to acquire relevant external information, although there are still opportunities to improve the systematization of this information.
2. Assimilation Capacity (Percentage 71.66%): Organizations adequately absorb knowledge, although their full integration can still be optimized.
3. Transformation Capacity (Percentage 70%): Organizations demonstrate an ability to convert knowledge into strategic actions. The average indicates sustainable, but not exceptional, practices, with room for strengthening innovation.
4. Exploitation Capacity (Percentage: 80%): • Best performing dimension. Organizations effectively leverage acquired knowledge, optimizing operations and minimizing costs, positioning themselves between the levels “Agree” (5) and “Somewhat agree” (6).

The results in this study show that aquaculture organizations from Ahome, Sinaloa, present a moderately high knowledge absorption capacity and resilience. The organizations declare to be “Moderately in agreement” (6) regarding the acquisition and exploitation of knowledge, although the dimensions of assimilation and transformation could strengthen their internal processes, which would offer a potential opportunity to improve their performance. In addition, the capabilities of foresight, acceptance and recovery show adequate development, contributing in a relevant way to their competitiveness and sustainability in the market (Table 7).

This analysis is crucial to understanding the key capabilities that allow organizations to adapt and be successful in such a dynamic environment. When specific improvement areas are detected, the research provides a solid basis to optimize the operative efficiency and to foster greater resilience in the long term, thus contributing to the strengthening of the aquaculture sector of the region in a profitable manner.

The Exploitation Capacity (5.8) exceeds the RC (5.5), indicating that organizations are more efficient in the use of knowledge than in adaptation to changes or recovery after crises. This finding suggests a more reactive approach; once knowledge is acquired, the organizations exploit it efficiently, although their capabilities of resilience could benefit from a more proactive approach, especially in the anticipation to changes.

Organizational resilience

The average of 5.07 in the dimension of resilience demonstrates a generalized capability to adapt, as well as to manage and exceed business challenges, although with internal variability in the perceptions. Organizational resilience manifests as the ability of an economic entity to absorb tensions and transform them into opportunities for improvement. Approximately 20% of the PYMES manifested high confidence in their resilience mechanisms, while 5% expressed disagreement, suggesting asymmetry in the implementation of strategies for risk management. This finding agrees with Coutu (2002), who maintains that resilience is not a uniform attribute, but rather a practice that is developed differentially according to organizational culture.

The results suggest that reactive resilience directed at responding to immediate crises is more consolidated than proactive resilience, linked to anticipation and innovation (Duchek, 2020). The “moderately agree” responses (44%) reflect a stage of partial formalization, where resilient practices exist, although they still lack full integration into strategic processes, in line with the idea that resilience requires progressive institutionalization and continuous adaptive learning (Linnenluecke, 2017).

Table 7. Exploitation Capacity vs. Resilience Capability.

Dimension	Average	%	Interpretation
Explotation of capacity	5.8	80	They demonstrate a high capacity to exploit transformed knowledge, reflecting efficiency in its application.
Resilience Capabilities	5.5	75	Performance in foresight and recovery is good, although slightly lower than knowledge utilization.

Results in Chile and Colombia reflect an operative resilience, although low anticipatory capability due to financial and political limitations of fragmented innovation. In Chilean salmon production, the responses have been reactive in face of environmental crises (Bustos-Gallardo and Irrázaval, 2021; Billi, 2022), while in Colombia, aquaculture faces financial restrictions, overregulation and weak institutionality in CTI, which limit its innovation and adaptation, requiring a territorial and multiscale approach (Rodríguez Giraldo and Franco Borrero, 2024).

Knowledge absorption capacity (KAC)

The average of 5.13 points reflects a favorable valuation of the KAC, showing that organizations have competences to acquire, assimilate and apply external knowledge, which are key for innovation (Zahra and George, 2002). However, 54% of the responses “moderately agree” suggest that this capability is still not fully institutionalized. According to Cohen and Levinthal (1990), KAC depends not only on the acquisition of information, but rather on the internal ability to transform it and exploit it productively.

Although 21% of the survey respondents were totally in agreement with the efficacy of this process, the heterogeneity between teams could be explained by differences in leadership, technical formation or technological integration (Flatten *et al.*, 2011). This variability frames the need to systematize the mechanisms of organizational transference and learning, avoiding the concentration of knowledge in key actors.

This agrees with Latin American studies that highlight the role of territorial innovation networks in knowledge absorption, such as those reported in fishing clusters of the Mexican Pacific Ocean and Ecuador’s coast (Beltrán-Lugo *et al.*, 2023). These experiences show that inter-organizational collaboration and associativity are determinant to transform external knowledge into productive and sustainable innovation.

Comparative: resilience and knowledge absorption

The comparison between organizational resilience (5.07) and the knowledge absorption capacity (KAC) (5.13) demonstrates an intermediate level of institutional maturity. In coherence with the theory of dynamic capabilities (Teece, Pisano and Shuen, 1997), both dimensions are mutually strengthened: resilience favors flexibility to incorporate emerging strategies, while a high KAC eases anticipation, learning and adaptation in face of changing environments. Recent studies confirm this reciprocal relationship, showing that knowledge absorption strengthens resilience by promoting learning after crises, and that resilient organizations create conditions for innovation (Saad *et al.*, 2021).

The balance observed suggests the need to consolidate an organizational culture of continuous learning, especially in sectors exposed to market variations or climate change, such as aquaculture in northwestern Mexico (Orsato, 2017). In Latin America, the evidence shows that PYMES which integrate resilience and organizational learning attain greater competitiveness and risk reduction, consolidating this synergy as a basis of territorial sustainability. In shrimp production clusters in Mexico, interorganizational cooperation and KAC strengthen their adaptability in the presence of crises (Carrasco Escalante and León Balderrama, 2017), while in Ecuador, collective innovation improves

productive stability (González-Illescas and Zaragoza-Sáez, 2023). Likewise, Chilean agro-industrial clusters show that institutional networks and shared learning drive regional competitive development, confirming that sustainability depends on resilient organizations directed at knowledge.

The organizational resilience and knowledge absorption capacity constructs are closely interconnected with the cultural, institutional and economic contexts of each organizational entity. A culture that boosts learning accelerates for the tacit resource to be acquired, disseminated and transformed into practical benefits, while institutionality favors proactive resilience regarding disruptive events and crises, through policies for technological progress and collaborative networks (Máñez-Guaderrama *et al.*, 2012; Pacheco-Mangas, Palma-García, and Hombrados-Mendieta, 2020; Giraldo and Borrero, 2024).

Similarly, having access to adequate financial resources and presence in clusters for organizational progress contributes to transforming knowledge into productive solutions that are sustainable in time (Beltrán-Lugo *et al.*, 2023; Carrasco Escalante and León Balderrama, 2017; DiBella *et al.*, 2023). These elements allow understanding why some organizations implement and develop better resilience than KAC, or reciprocally, evidencing the need to promote systematic learning and interorganizational cooperation to consolidate both dynamic capabilities (González-Illescas and Zaragoza-Sáez, 2023; Velázquez Ríos, Contreras Montellano, and Olea Miranda, 2024).

Implications and recommendations

Results confirm that the synergy between organizational resilience and KAC is decisive for territorial sustainability and regional competitiveness of aquaculture PYMES. The organizations that institutionalize collective learning achieve greater stability in the presence of environmental and market volatility (González-Illescas and Zaragoza-Sáez, 2023). It is recommended to advance towards proactive resilience, based on risk anticipation, technological integration, and inter-business cooperation, strengthening knowledge networks and practice communities that articulate innovation, adaptation and sustainable territorial development.

This study offers empirical evidence about the synergy between organizational resilience (RC) and KAC in shrimp producing PYMES of Ahome, Sinaloa. The results reveal strengths in the acquisition and exploitation of knowledge, as well as in recovery in the presence of crises. However, areas for improvement in processes of knowledge assimilation and transformation are identified, which are crucial to maintain innovation and respond proactively and strategically to changing productive environments (Zahra and George, 2002).

From this context, the findings point to the idea that investing sensibly in resilience and organizational learning is not only a defensive measure, but also an intelligent strategy to consolidate competitiveness of the aquaculture sector. For aquaculture producers, this involves heading towards institutionality of practices such as documentation of past experiences, critical review of failed tactics, and allotment of specific resources to planning for contingencies. In this sense, the results report that those responsible for public policy

can perform a relevant role in promoting mechanisms that incentivize cooperation between aquaculture PYMES, strengthening knowledge networks, and investing in organizational capabilities aimed at character resilience based on prevention and anticipation.

The findings from this study converge with what was set out by Do *et al.* (2022) and Hillmann and Guenther (2021), by validating that a firm organizational resilience allows not only facing crises but also learning from them and reconfiguring the resources available. Similarly, this is reinforced by what was proposed by Mariano and Walter (2015), by evidencing that processes of well-structured organizational learning contribute to articulating business innovation, adaptation and sustainability. In the Latin American context, the findings are in consonance with what was reported by Lindemann *et al.* (2024), who state that cooperation networks, together with mutual learning, help transform scenarios of vulnerability into opportunities for sustainable development.

CONCLUSIONS

Although this study presents certain limitations associated with its transversal design, such as the use of perceptual data, its results offer a solid base for future research. In particular, the recommendation is to develop comparative studies between different farm shrimp producing regions, as well as longitudinal studies that allow observing the evolution of organizational resilience and knowledge absorption capacity throughout time. Similarly, the adoption of mixed methodological approaches could deepen the understanding of cultural, institutional and financial factors that affect the consolidation of these capabilities in the agricultural production systems of Latin America.

Ethical declaration

The authors declare that the information obtained was collected with informed consent from the participants, respecting data confidentiality. There are no conflicts of interest, and the research was developed according to institutional and national ethical principles.

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Effects of additives on silage African oil palm forage waste and its acceptability in sheep

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ABSTRACT

Objective: The objective was to evaluate the effects of different levels of microbial inoculum (MI; 0, 3 and 6%), molasses (0, 3 and 6%), and ground corn (GC; 0 and 3%) on the fermentation and chemical composition of silage made from African oil palm forage waste (APFW), as well as its acceptability in sheep.

Design/methodology/approach: A total of 54 micro-silos were used in a completely randomized design with a 3×3×2 factorial arrangement and three replications. Additionally, 0.1% urea was added to all treatments. The variables evaluated were pH, lactic acid (LA), dry matter (DM), crude protein (CP), ash, neutral detergent fiber (NDF), and acid detergent fiber (ADF). Significant interactions were analyzed using SLICE in SAS. Acceptability was evaluated using a cafeteria test with four Pelibuey sheep.

Results: Treatments with MI ≥3% and molasses ≥3% reduced pH (from 4.9 to 3.7; $p \leq 0.05$) and increased LA concentration, indicating stable fermentation. MI at 6% decreased NDF and ADF ($p \leq 0.05$), whereas molasses increased DM ($p \leq 0.05$). GC (3%) increased ($p \leq 0.05$) pH and LA concentration when combined with molasses at 3-6%, while maintaining indicators consistent with adequate fermentation. Sheep showed greater preference ($p \leq 0.05$) for the silage, coinciding with higher DM and CP, and lower NDF and ADF.

Limitations/Implications: The study was conducted in laboratory-scale micro-silos, with a limited animal sample size ($n=4$) and without evaluation of productive parameters. Therefore, it is recommended to validate these results under field conditions, including the assessment of aerobic stability, performance, and cost-benefit.

Findings/conclusions: The combination of MI (3-6%) with molasses (3-6%) improved the fermentation of APFW, reduced NDF and ADF, and increased its acceptability in sheep, supporting its potential as a feed resources for small ruminants in tropical regions.

Keywords: Silage, agroindustrial waste, microbial inoculum, African oil palm, acceptability.

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INTRODUCTION

African oil palm (*Elaeis guineensis* Jacq.) is an agroindustrial crop of great global importance due to its high oil (Pasicznik, 2022). In Mexico, its cultivation is concentrated in the states of Chiapas, Veracruz, Campeche, and Tabasco, where large amounts of residual biomass —particularly leaves— are generated during harvesting and agronomic



management (SIAP, 2024). Despite their potential as a resource for ruminant feeding, these leaves are often underutilized and remain in the field as agricultural waste.

African oil palm leaves can be incorporated into ruminant diets as a non-conventional forage (Ebrahimi *et al.*, 2013; Syamsuddin *et al.*, 2024). However, their use in fresh form is limited by their short shelf life, attributed to rapid deterioration and oxidation processes when the material remains exposed to the environment (Ang, 2017). From a nutritional perspective, agricultural residues can contribute to ruminant diets; nevertheless, their adoption is often low due to logistical and preservation constraints, as well as variability in their quality, which limits their effective use in production systems (Alkhtib *et al.*, 2017). In the particular case of African oil palm forage waste (APFW), these materials are characterized by a neutral detergent fiber (NDF) fraction ranging from 69.5 to 78.05% and an acid detergent fiber (ADF) content of 50.9 to 56.9% (Wilson *et al.*, 2020). This composition may restrict ruminal degradation and, consequently, their intake and utilization potential if conservation and technological improvement strategies are not implemented.

Ensiling is a viable alternative for valorizing PFW, as it allows the material to be preserved for prolonged periods under anaerobic conditions, reducing losses and minimizing deterioration associated with storage or exposure to environmental conditions (Da Silva *et al.*, 2019). The production of high-quality silage depends on the occurrence of predominantly lactic fermentation, characterized by a rapid decline in pH. In this process, water-soluble carbohydrates (WSC) are essential because they serve as fermentable substrates for lactic acid bacteria (LAB), which are responsible for lactic acid production and the acidification that inhibits undesirable microorganisms (Oladosu *et al.*, 2016; Aguirre *et al.*, 2024). When the material to be ensiled is low in WSC or presents characteristics that hinder acidification, fermentation may be slow and unstable, increasing the risk of proteolysis and undesirable fermentations. Therefore, the use of additives is considered a key technological strategy to direct the fermentation process.

The incorporation of additives during ensiling can improve the fermentative quality and nutritional value of the material, promoting its acceptance by animals and their productive response (Diogénes *et al.*, 2023). In particular, the use of microbial inoculum aims to ensure a sufficient population of lactic acid bacteria (LAB) with high acidification capacity, while energy sources such as molasses provide rapidly fermentable water-soluble carbohydrates (WSC), and ground corn can contribute to the dry matter content and energy density of the silage, with potential effects on its fermentation profile and acceptability. Therefore, the objective of this study was to evaluate the effects of different levels of microbial inoculum, molasses, and ground corn on the fermentative quality, chemical composition, and acceptability of African oil palm forage residue silage in Pelibuey sheep.

The hypothesis was that the combination of microbial inoculum, molasses, and ground corn at increasing levels would improve the fermentation process and nutritional value of silage made from African oil palm forage waste, thereby increasing its acceptability in sheep.

MATERIALS AND METHODS

Collection of African oil palm forage waste

APFW were collected at Rancho La Esperanza, located in Colonia El Arenal (15° 10' 19" N, 92° 42' 00" W), in the municipality of Acapetahua, Chiapas, Mexico. The leaflets were manually separated from the rachis, and the material was chopped to a particle size of 2-3 cm using a 13 HP Honda® hammer mill. The material was transported to the Animal Science Laboratory (Colegio de Postgraduados, Campus Tabasco; Cárdenas, Tabasco) and processed on the same day for the preparation of micro-silos.

Experimental design and treatments

For the fermentative and chemical variables, a completely randomized design with a 3×3×2 factorial arrangement was used: MI (0, 3, and 6%), molasses (0, 3, and 6%), and GC (0 and 3%), expressed on a fresh basis (w/w). A total of 18 treatments (Table 1) with three replications were evaluated (n=54 micro-silos). Each micro-silo consisted of a 2.5-L hermetic polycarbonate jar with a screw cap. In all treatments, 0.1% urea was added on a fresh basis (1 g kg⁻¹ of fresh mixture) to ensure a minimum of 10% CP in the mixture.

Statistical Analysis

An analysis of variance was performed for each measured variable. The model included the fixed effects of MI, molasses, and GC, as well as their interactions. Mean comparisons

Table 1. Percentage inclusion of ingredients in the experimental treatments on a fresh basis.

Treat	APFW (%)	MI (%)	Molasses (%)	GC (%)	Urea (%)
T1	99.9	0	0	0	0.1
T2	96.9	0	0	3	0.1
T3	96.9	0	3	0	0.1
T4	93.9	0	3	3	0.1
T5	93.9	0	6	0	0.1
T6	90.9	0	6	3	0.1
T7	96.9	3	0	0	0.1
T8	93.9	3	0	3	0.1
T9	93.9	3	3	0	0.1
T10	90.9	3	3	3	0.1
T11	90.9	3	6	0	0.1
T12	87.9	3	6	3	0.1
T13	93.9	6	0	0	0.1
T14	90.9	6	0	3	0.1
T15	90.9	6	3	0	0.1
T16	87.9	6	3	3	0.1
T17	87.9	6	6	0	0.1
T18	84.9	6	6	3	0.1

Treat: treatment; APFW: African oil palm forage waste; MI: microbial inoculation; GC: ground corn.

were conducted using Tukey's multiple comparison test. When significant interactions were detected, simple effects were evaluated using the SLICE option in PROC MIXED (SAS, 2013), following the approach described by Maxwell and Delaney (1990). Specifically, the effect of factor A was evaluated within each combination of factors B and C, the effect of B within each combination of A and C, and the effect of C within each combination of A and B.

The linear model including all main effects and interactions was:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + (AB)_{ij} + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + \varepsilon_{ijkl}$$

where: μ is the overall mean; A_i , B_j , and C_k are the main effects; $(AB)_{ij}$, $(AC)_{ik}$ and $(BC)_{jk}$ are the two-way interaction effects; $(ABC)_{ijk}$ represents the three-way interaction effect; and ε_{ijkl} is the experimental error term. The assumptions of normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) were verified using the model residuals. When any of the assumptions were not met, the data were transformed using the Box-Cox procedure, and subsequent analyses were performed on the transformed scale.

Preparation of micro-silos

Ground APFW was mixed with MI, molasses, and GC according to the treatments (Table 1), and a sample (200 g) was taken to determine the initial moisture content. The mixture was placed in polycarbonate jars (2.5 L), compacted in a standardized manner (recording the net filling weight and minimizing headspace) to promote anaerobic conditions, and then hermetically sealed. The micro-silos were incubated for 30 days at ambient temperature in a cool area, without direct exposure to light.

Preparation of the microbial inoculum

The microbial inoculum, also known as Vitafert[®] (Iraola *et al.*, 2019), was produced by submerged liquid fermentation following the method described by Ramos *et al.* (2021). The following ingredients were mixed in a 20-L plastic bucket: 4% soybean meal, a source of proteins and essential amino acids for bacterial growth (Zhu *et al.*, 2022); 4% rice bran, which provides carbohydrates and fiber as an energy substrate (Lovegrove *et al.*, 2020); 15% molasses, rich in readily fermentable sugars that promote microbial multiplication (Zentou *et al.*, 2017); 0.5% mineral salts, necessary to maintain basic metabolic functions (Elliott & Sharp, 2020); 0.5% urea, used as an additional nitrogen source (Dos *et al.*, 2016); 0.3% magnesium sulfate, which provides magnesium as an enzymatic cofactor (Li *et al.*, 2025); 5% natural yogurt (Yoplait[®]), used as a starter inoculum of lactic acid bacteria (Kandola, 2018); and 7.7 L of water, required for the dissolution of the components and cellular activity. The mixture was allowed to ferment for three days and was stirred six times per day for three minutes on each occasion. At the end of fermentation, the inoculum had a pH of 4, 81.1% moisture, 24.9% crude protein, and concentrations of 8.5 and 4.7 log₁₀ CFU mL⁻¹ of lactic acid bacteria and yeasts, respectively.

Fermentative variables

At the end of the fermentation period, a 10 g sample was taken from the center of the micro-silo and placed in a 125 mL Erlenmeyer flask, to which 90 mL of distilled water was added. The mixture was placed on an orbital shaker (VWR[®], model DS-500E) at 180 rpm for 30 minutes. Subsequently, the samples were filtered through sterile gauze. The pH of the filtrate was immediately measured using a potentiometer (HANNA HI 2210) previously calibrated (pH 4.0 and 7.0). Lactic acid (LA) concentration in the filtrate was determined according to the method described in the *Diario Oficial de la Federación* (2010).

Chemical variables

A 400 g sample was taken from the central portion of each micro-silo and placed in paper bags for drying in a forced-air oven at 60 °C (Tecsa[®], model HDT-28) in the Animal Science Laboratory at Colegio de Postgraduados, Campus Tabasco. When the samples reached constant weight, the percentages of moisture, ash, and CP were determined following the methodology described by AOAC (2012). Dry matter was calculated as 100 minus the moisture percentage. NDF and ADF were determined according to the method described by Van Soest *et al.* (1991).

Acceptability test

A cafeteria test with sheep was conducted at the Experimental Field of Colegio de Postgraduados, Campus Tabasco, to evaluate feeding preference, following the methodology described by Wamatu *et al.* (2017). Silage from treatment 16 (6% MI, 3% molasses, 3% GC; Table 1) was used. The trial lasted 15 days (10 days for adaptation and 5 days for measurement). Four male Pelibuey sheep (average BW 29.25 kg; similar body condition) were used and housed individually.

The experimental unit consisted of 12 feeders arranged in four pens (blocks), with one sheep per pen. The data were analyzed using a mixed model (PROC MIXED, SAS, 2013) under a randomized complete block design with repeated measures (days). Treatments were considered fixed effects, animal was included as a random effect, and day was treated as the repeated factor. Means were compared using Tukey's test when appropriate ($p \leq 0.05$).

Animals confined in their pens were simultaneously offered, *ad libitum*, three treatments (T): T1, fresh giant grass forage (*Pennisetum purpureum* Schumach); T2, fresh APFW; and T3, APFW silage. The position of the feeders was randomized daily to avoid habit bias associated with feed location.

Animals were managed in accordance with the institutional regulations for the use and care of animals for research at Colegio de Postgraduados. Prior to the beginning of the trial, the sheep were supplemented with vitamins and dewormed according to their body weight. Each animal was housed in an individual pen (2 m²) with *ad libitum* access to drinking water. The amount of each feed offered and refused was recorded daily to calculate intake (fresh basis), and DM was determined to express intake on a dry matter basis. The feeds offered were analyzed for moisture, CP, and ash content following the methodology previously described.

RESULTS AND DISCUSSION

A significant three-way interaction (MI×molasses×GC) was found for pH, LA, CP, and ash, indicating that the effect of the microbial inoculum depends on the availability of soluble carbohydrates provided by molasses and on the inclusion of ground corn. For this reason, the effects of MI were interpreted using the SLICE procedure in SAS within each combination of molasses and GC.

Regarding pH, with 0% MI and without molasses or GC, the highest pH value (4.97) was recorded. The inclusion of molasses at 3 and 6% significantly reduced pH ($p \leq 0.05$) to values of 3.76 and 3.77, respectively. A similar pattern was observed with 3% MI, where pH was 3.80 without molasses or GC and decreased to 3.71 and 3.67 with 3 and 6% molasses, respectively, with no statistical differences between these levels. With 6% MI, pH was 3.79 without molasses or GC and remained stable (3.70-3.73) when molasses was added at 3 and 6%, with no significant differences (Table 2).

In the absence of GC, the inclusion of molasses at all MI levels maintained the pH below 4.0 (Table 2), indicating adequate silage fermentation and consistent with the findings reported by Wang *et al.* (2021) for silages with high availability of soluble carbohydrates.

The inclusion of 3% GC tended to increase silage pH. With 0% MI, pH increased significantly ($p \leq 0.05$) in all combinations with molasses. In treatments with 3 and 6% MI,

Table 2. Effect of microbial inoculum, molasses, and ground corn levels on pH, lactic acid, crude protein, and ash in African oil palm forage waste silages.

MI (%)	M (%)	GC (%)	pH	AL (%)	CP (%)	Ash (%)
0	0	0	4.97 ^b	6.54 ^b	10.71 ^c	13.56 ^c
		3	5.56 ^a	6.96 ^a	11.73 ^b	15.09 ^a
	3	0	3.76 ^d	6.90 ^a	12.29 ^b	14.90 ^a
		3	4.01 ^c	6.90 ^a	11.88 ^b	14.10 ^{bc}
	6	0	3.77 ^d	6.90 ^a	12.07 ^b	14.71 ^{ab}
		3	3.98 ^c	6.96 ^a	12.89 ^a	13.89 ^c
3	0	0	3.8 ^{bc}	6.72 ^a	12.26 ^b	13.32 ^{bc}
		3	4.0 ^a	6.84 ^a	13.30 ^a	13.36 ^{bc}
	3	0	3.71 ^c	6.84 ^a	10.80 ^c	12.85 ^c
		3	3.90 ^{ab}	6.96 ^a	10.53 ^c	14.59 ^a
	6	0	3.67 ^c	6.78 ^a	11.76 ^b	13.98 ^{ab}
		3	3.79 ^{bc}	6.90 ^a	10.82 ^c	13.54 ^{bc}
6	0	0	3.79 ^{bc}	6.78 ^b	12.14 ^a	14.43 ^a
		3	4.07 ^a	6.96 ^{ab}	11.58 ^a	13.46 ^{bc}
	3	0	3.70 ^c	6.78 ^b	10.56 ^b	13.39 ^c
		3	3.92 ^{ab}	7.20 ^a	10.75 ^b	14.15 ^{ab}
	6	0	3.73 ^c	6.96 ^{ab}	10.72 ^b	13.62 ^{bc}
		3	3.85 ^{bc}	7.20 ^a	10.61 ^b	13.15 ^c
SE±			0.06	0.10	0.19	0.23

^{abc} Means with different superscript letters within the same column, for each Molasses×GC combination, indicate statistical differences among MI levels ($P \leq 0.05$). SE: standard error; MI: microbial inoculation; M: molasses; GC: ground corn; LA: lactic acid; CP: crude protein.

pH increased with 0 and 3% molasses; however, with 6% molasses no significant changes were observed (Table 2).

Overall, MI contributed to reducing pH under all scenarios, with more consistent effects when molasses was included at 3-6% and in the absence of GC. This behavior suggests that the soluble carbohydrates provided by molasses promote rapid and sustained acidification of the silage. Final pH values ≤ 3.9 observed in treatments with MI are consistent with adequate fermentations for high-quality silages (Ferreira *et al.*, 2015). Previous studies have documented that the incorporation of microbial additives and sources of simple sugars favors pH reduction and improves silage stability (Ling *et al.*, 2022). The slight increase in pH observed with the inclusion of GC could be attributed to the fact that molasses constitutes the main source of rapidly fermentable carbohydrates, whereas corn starch undergoes slower fermentation during the initial stages of the process (Araiza *et al.*, 2015).

Regarding lactic acid (LA) content, the treatment without MI, molasses, or GC showed the lowest value (6.54%), with significant differences ($p \leq 0.05$) compared with the rest of the evaluated combinations (Table 2). The inclusion of molasses, regardless of the level, increased LA content compared with the treatment without additives.

In treatments with 3% MI, the addition of molasses and GC did not significantly modify LA content, with similar values observed among all evaluated combinations. In contrast, with 6% MI, the inclusion of GC showed a tendency to increase LA, particularly when combined with 3 and 6% molasses, where the highest LA values (7.20%) were recorded, although without consistent differences among all treatments (Table 2).

These results are consistent with those reported by Da Silva *et al.* (2019), who indicated that the addition of microbial inoculants and sources of fermentable carbohydrates promotes lactic fermentation in high-fiber forages.

The higher LA production observed in treatments with MI and molasses is desirable, as it promotes rapid and stable silage fermentation. Inoculation provides lactic acid bacteria (LAB), which promote the conversion of soluble sugars into lactate and accelerate the decline in pH, reflecting the widely documented inverse relationship between pH and LA (Ghimire and Raya, 2022). Similarly, Kim *et al.* (2021) reported that the addition of LAB is a key factor in stabilizing pH and improving the preservation of ensiled forage. Alhaag *et al.* (2019) highlighted that species such as *Lactobacillus plantarum* and *Pediococcus acidilactici* efficiently convert soluble carbohydrates into LA, accelerating silage acidification. Likewise, Bohórquez and Mena (2024) noted that molasses, as a source of fermentable substrate, enables efficient lactic fermentation in materials with low soluble carbohydrate content.

Regarding crude protein (CP), the treatment without MI, molasses, or GC showed the lowest value (10.71%; Table 2). In treatments with 0% MI, the inclusion of molasses increased CP compared with the control, with the highest value observed at 6% molasses and 3% GC (12.89%; $p \leq 0.05$), while the remaining combinations showed intermediate values. When 3% MI was included, CP reached its highest value when molasses was not added and 3% GC was included (13.30%; $p \leq 0.05$). At this MI level, the inclusion of molasses (3 or 6%) was associated with lower CP concentrations, particularly when combined with GC, where the lowest values were recorded (10.53-10.82%; Table 2).

With 6% MI, CP was higher in the absence of molasses (12.14 and 11.58% for 0 and 3% GC, respectively; $p \leq 0.05$), with no differences between both GC levels. In the presence of molasses (3 and 6%), CP remained around 10.56–10.75% and 10.61–10.72%, respectively (Table 2), suggesting a modulatory effect of molasses on the final CP percentage.

The variations observed in CP are expected, since the addition of energy sources (molasses and/or corn) may modify the relative percentage of CP due to dilution effects and changes in fermentative dynamics and nitrogen conservation during the ensiling process. Overall, the final CP values obtained are considered compatible with ruminant diets, in which moderate CP concentrations favor nitrogen supply for microbial protein synthesis in the rumen (Pashayan and Vunsh, 2024).

Regarding ash content, significant differences were observed among treatments, determined by the combination of MI, molasses, and GC (Table 2). In the absence of MI, molasses, and GC (0-0-0), the silage presented an intermediate ash content (13.56%). At this same MI level (0%), the inclusion of 3% GC without molasses significantly increased ash content to the highest value observed (15.09%; $p \leq 0.05$). Likewise, with 0% MI and the addition of molasses (3 or 6%) without GC, ash remained high (14.90 and 14.71%, respectively); in contrast, when molasses was combined with 3% GC, lower values were recorded (14.10 and 13.89%).

When 3% MI was included, treatments without molasses showed the lowest ash contents (13.32–13.36%). The combination of 3% molasses and 3% GM significantly increased ash content (14.59%; $p \leq 0.05$), whereas with 6% molasses the values remained within an intermediate range (13.98 and 13.54% for 0 and 3% GM, respectively).

With 6% MI, ash content was higher in the absence of molasses (14.43% with 0% GM) and decreased when 3% GM was included (13.46%). In the presence of 3% molasses, ash values were low (13.39 and 14.15% with 0 and 3% GM, respectively), whereas with 6% molasses intermediate values were observed (13.62 and 13.15% with 0 and 3% GM, respectively).

Ash content in forages represents the total mineral fraction and constitutes an indicator of the mineral contribution of the material for ruminant feeding (Block, 2022). This component is essential for maintaining animal health, productive efficiency, and metabolic balance (Fawaiq, 2022), and may vary depending on the supplements or additives incorporated during the ensiling process (Dilaga, 2024).

Regarding final dry matter (FDM), a significant interaction between microbial inoculum (MI) and ground maize (GM) levels was observed ($p \leq 0.05$); therefore, the effects of this combination were interpreted using the SLICE procedure (Table 3).

With 0% MI, the addition of GM significantly reduced the FDM content, with values of 57.21 and 55.51% for 0 and 3% GM, respectively ($p \leq 0.05$). In contrast, with 3% MI, the inclusion of GM significantly increased FDM, rising from 54.67% without GM to 56.59% with 3% GM ($p \leq 0.05$). When 6% MI was used, no significant differences in FDM were observed between the evaluated GM levels, with values of 56.25 and 56.01% for 0 and 3% GM, respectively (Table 3).

These results agree with those reported by Kung *et al.* (2018), who indicate that the objective of ensiling is to preserve the original nutrients of the forage while maintaining

Table 3. Effect of microbial inoculum and ground corn levels on the final dry matter content of African oil palm forage waste silages.

Microbial inoculation (%)	Ground corn (%)	Final dry matter (%)
0	0	57.21 ^a
	3	55.51 ^b
3	0	54.67 ^b
	3	56.59 ^a
6	0	56.25 ^a
	3	56.01 ^a
SE±		0.38

^{ab} Means with different superscript letters in the same column indicate differences between levels of the GC factor ($P \leq 0.05$) for each MI level. SE: Standard error; FDM: Final dry matter.

adequate dry matter that allows the quality of the material to be conserved and ensures its availability during periods of forage scarcity.

With respect to initial dry matter (IDM), neutral detergent fiber (NDF), and acid detergent fiber (ADF), no significant interactions were detected among the evaluated factors (MI×molasses×GM; Table 4); therefore, the main effects were analyzed.

For IDM, the levels of MI and GM did not show significant differences ($P \geq 0.05$). However, the level of molasses did influence this variable, with a significant increase ($P \leq 0.05$) observed at 3% molasses compared with 0% (53.93 and 51.10%, respectively), whereas the 6% level showed intermediate values (Table 4).

Table 4. Effect of microbial inoculum, molasses, and ground corn levels on initial dry matter, neutral detergent fiber, and acid detergent fiber in African oil palm forage waste silages.

Factor	Variables		
	IDM (%)	NDF (%)	ADF (%)
MI (%)			
0	51.45 ^a	76.81 ^a	67.02 ^a
3	52.80 ^a	74.90 ^a	64.65 ^b
6	52.57 ^a	69.64 ^b	61.82 ^c
EE±	0.42	2.14	1.50
Molasses (%)			
0	51.10 ^b	73.51 ^a	64.70 ^a
3	53.93 ^a	73.90 ^a	65.03 ^a
6	51.78 ^{ab}	73.94 ^a	63.75 ^a
EE±	0.85	0.14	0.38
Ground corn (%)			
0	51.49 ^a	74.27 ^a	64.70 ^a
3	53.05 ^a	73.30 ^a	64.29 ^a
SE±	0.78	0.49	0.21

^{ab} Means with different superscripts in the same column within each factor indicate significant differences ($P \leq 0.05$). SE: Standard error; MI: Microbial inoculation; IDM: Initial dry matter; NDF: Neutral detergent fiber; ADF: Acid detergent fiber.

Regarding NDF, no significant differences were observed between 0 and 3% MI; however, the inclusion of 6% MI significantly reduced ($P \leq 0.05$) the NDF content, with values of 76.81, 74.90, and 69.64% for 0, 3, and 6% MI, respectively. The levels of molasses and GM did not have a significant effect on this variable (Table 4).

Similarly, ADF showed a progressive reduction as the level of MI increased, with a significant decrease ($P \leq 0.05$) from 67.02 to 61.82% when MI increased from 0 to 6%. In contrast, the levels of molasses and GM did not significantly modify the ADF content (Table 4).

The reduction in NDF and ADF associated with increasing MI suggests a possible partial solubilization and/or structural disruption of the cell wall, as a consequence of more efficient lactic fermentation. This reduction in the fibrous fraction is relevant from a productive standpoint, since it is associated with greater potential digestibility and, therefore, with a higher expected intake of the silage by ruminants (Alves *et al.*, 2016).

Overall, the results indicate that the inclusion of microbial inoculum (MI) at levels of 3 to 6%, in combination with molasses between 3 and 6%, favors the establishment of a stable acidic environment, characterized by low pH values and higher concentrations of lactic acid, in addition to preserving crude protein in relative terms. In particular, the addition of 6% MI reduced neutral detergent fiber by 7.17 percentage units (from 76.81 to 69.64%) and acid detergent fiber by 5.20 percentage units (from 67.02 to 61.82%), results consistent with silage of African oil palm forage waste of adequate quality. The inclusion of 3% ground maize caused a slight increase in pH; however, this effect did not alter the overall fermentative profile when combined with MI and molasses.

Our results are consistent with the findings of Syamsuddin *et al.* (2024), who reported fermentative improvements when applying inoculants and sources of WSC to palm residues. However, other authors report variable effects of MM depending on its proportion and the type of forage (Alkhtib *et al.*, 2017), so its inclusion should be evaluated according to the production system.

Regarding the feeds offered during the cafeteria test, significant differences in chemical composition were observed among treatments ($P \leq 0.05$; Table 5). Green forage (T1) presented the lowest values of DM and CP, whereas APFW silage (T3) showed higher concentrations of DM, CP, and ash, as well as lower contents of NDF and ADF compared with fresh APFW (T2) and grass (Table 5).

For the variables intake index (II) and total intake (TI), statistical differences were observed only for the treatment factor ($P \geq 0.05$). The sheep showed a marked preference for the silage (T3), with higher II and TI on a dry matter basis compared with fresh APFW (T2) and grass (T1) ($P \leq 0.05$; Table 6). This response may be associated with the nutritional profile of the silage, characterized by lower fiber concentration (NDF and ADF) and greater DM and CP density, in addition to the effect of fermentation metabolites (*e.g.*, lactate and organic acids) that provide aroma and acidity and may improve palatability (Sighny and Irfandy, 2024).

Due to the small sample size (4 sheep), these results should be interpreted with caution and require validation at a larger scale, incorporating productive variables and an economic

Table 5. Chemical composition of the diets offered to Pelibuey sheep during the cafeteria test.

Trat	DM (%)	Ash (%)	PC (%)	NDF (%)	ADF (%)
T1	18.63 ^c	11.47 ^b	7.10 ^c	77.89 ^a	55.95 ^a
T2	43.92 ^a	8.52 ^c	8.16 ^b	73.65 ^b	51.27 ^b
T3	44.31 ^a	13.65 ^a	10.50 ^a	62.72 ^c	45.14 ^c
EE±	8.11	1.50	1.01	4.52	5.43

^{abc} Means with different letters in the same column are significantly different ($P \leq 0.05$). Trat: Treatment; T1: fresh giant grass forage; T2: fresh African oil palm forage waste; T3: African oil palm forage waste silage. DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber.

Table 6. Total intake and intake index on a dry matter basis in sheep during the cafeteria test.

Treatment	Intake index	Total intake (%)
T1	0.19 ^b	11.90 ^c
T2	0.13 ^b	12.70 ^b
T3	2.11 ^a	75.40 ^a
EE±	0.03	0.09

^{abc} Means with different superscripts in the same column are different ($P \leq 0.05$). T1: Giant grass fresh forage; T2: Fresh African oil palm forage waste; T3: African oil palm forage waste silage.

feasibility analysis to quantify the zootechnical impact of using APFW silage in tropical production systems.

Overall, the data suggest that APFW silage is an alternative with high acceptability and a more favorable nutritional profile than fresh material and green forage, with potential to help sustain intake during periods of forage scarcity in African oil palm producing regions.

CONCLUSIONS

The combined application of microbial inoculum (IM) between 3 and 6% and molasses between 3 and 6% significantly improved the fermentative quality of African oil palm forage waste (APFW) silage, as evidenced by a reduction in pH, an increase in lactic acid, higher dry matter content, and reductions in NDF and ADF. The use of ground corn at 3% showed minimal impact on the evaluated variables. Treatments with IM and molasses also increased the acceptability of the silage in Pelibuey sheep, which preferentially consumed this material compared with fresh APFW and grass. However, these results should be interpreted with caution due to the limited animal sample size ($n=4$) and the absence of productive performance metrics. APFW silage treated with IM and molasses represents a viable alternative for feeding small ruminants in tropical regions. Nevertheless, validation under field conditions is required, considering aerobic stability, productive response, and economic analysis to estimate its technical and financial feasibility.

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Effect of Dietary Energy Level on Milk Production in New Zealand White and Californian Does

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ABSTRACT

Objective: To evaluate milk yield in New Zealand White and Californian rabbit does fed high-energy diets.

Methodology: Milk production was measured in 72 New Zealand White and Californian does from kindling to 35 days postpartum. Four high-energy diets were formulated using the same ingredients, maintaining 17% crude protein (CP) and 14% crude fiber (CF), with progressively higher digestible energy (DE; kcal kg⁻¹) achieved by adding soybean oil. Milk yield was estimated under a controlled suckling scheme across six consecutive 7-day periods. A 2×4 factorial arrangement (breed×diet) was implemented in a completely randomized design. Data were analyzed using the MIXED procedure in SAS (v. 9.4). Wood's lactation model was fitted to describe milk-production dynamics, estimate the day of peak yield, and assess lactation persistence.

Results: No differences in milk production were detected among treatments. The lactation curve showed an early peak (day 17) and moderate persistence (0.5). Average feed intake was similar across treatments, with no significant differences ($p > 0.05$).

Study limitations: The effects of high-energy diets were assessed in purebred animals; however, it would be pertinent to include both direct and reciprocal crosses in future studies.

Conclusions: Diets containing at least 17% crude protein and 2700 kcal DE·kg⁻¹ are recommended to sustain adequate milk production in New Zealand White and Californian rabbit does.

Keywords: Lactating doe, milk yield, digestible energy, Wood's model.

INTRODUCTION

In Mexico, small-scale rabbit production in family farms is based primarily on New Zealand White (NZW), Californian (CAL), and Chinchilla breeds, as well as



their crosses. The NZW and CAL breeds exhibit high prolificacy, with litter sizes of 8 to 10 live-born kits (Ponce *et al.*, 2003), dressing percentages ranging from 56 to 58% at the end of the fattening period (Pla *et al.*, 1998), and high growth rates that allow them to reach market weight at 10 to 12 weeks of age (Lounaouci *et al.*, 2008), with daily weight gains of 35-42 g/day and slaughter weights of 2.0-2.2 kg (Blumetto, 2007). Rabbit meat production is considered an alternative to meet future food needs in rural and peri-urban production units, where this species can provide substantial amounts of meat for household consumption with relatively low investment (Bronio & Guzmán, 2003; Murillo, 2016). One of the key biological components to monitor within the family rabbit production system (FRPS) is kit output and milk production (MP), as the latter plays a pivotal role in kit survival and optimal growth (Gómez, 2006; Gómez *et al.*, 2011). Ludwiczak *et al.* (2020) estimated milk production in rabbit does and reported total solids contents of 27.6-29.0%, protein at 8.4-11.6%, fat at 11.6-13.8%, lactose at 1.4-2.6%, and ash at 1.7-2.1%. In addition, gross energy content has been reported at 2,220 kcal kg⁻¹ (Rommers *et al.*, 1999). Maertens *et al.* (2006) noted that rabbit milk contains higher protein and fat concentrations than that of other species. This distinctive nutritional profile may help explain the rapid growth of kits from birth to weaning, as the elevated levels of these nutrients contribute to accelerated offspring development (Rommers *et al.*, 1999). Reproductive does frequently experience a negative energy balance due to the high nutrient demand associated with milk synthesis. These requirements are often not fully met by voluntary feed intake, leading to the mobilization of body reserves to compensate for the energetic deficit (Xiccato *et al.*, 1999). A common strategy to mitigate excessive mobilization of body reserves in does is to increase the dietary energy density during lactation through the inclusion of fat or highly digestible carbohydrates, such as starch (Cervera *et al.*, 1993; Fortun-Lamothe, 1997). Prasad and Karim (1998) compared three energy and protein levels in the performance of lactating-pregnant does, obtaining average MP values of 105.7 g day⁻¹. Similarly, Pascual *et al.* (1999) evaluated the effects of three dietary energy levels on milk yield and composition in NZW and CAL does, reporting increased milk volume with high-fat diets and improved litter survival. The working hypothesis was that consumption of diets with a high concentration of digestible energy would increase milk production in does without altering feed intake. Therefore, the aim of this study was to compare milk production and feed intake in New Zealand White and Californian rabbit does fed high-energy diets during the lactation period.

MATERIALS AND METHODS

All animal-handling procedures and analyses were conducted in accordance with the Animal Welfare Committee regulations of the Colegio de Postgraduados (COBIAN/025/25; COLPOS, 2016).

Study location

This study was carried out at the rabbit unit of the Colegio de Postgraduados, Montecillo Campus, located at 19° 27' 35" N and 98° 54' 16" W, at an altitude of 2,220 m above

sea level, with a mean annual temperature of 15.9 °C and mean annual precipitation of 686 mm (INEGI, 2025). A total of 36 New Zealand White (NZW) and 36 Californian (CAL) does were used. Animals were housed in individual Extrona cages (90×50×40 cm), each equipped with an automatic drinker and a feeder (20×20×20 cm). The study was conducted from June to August; during this period, the mean recorded temperature was 22.5 ± 2.1 °C. The experimental period comprised 35 days of evaluation.

Reproductive management

The does used in this study were second-parity females. Artificial insemination (AI) was performed in all breeding does. Semen collection and evaluation procedures followed Luis *et al.* (2020). Semen was obtained from adult males of the same breed, 18 months of age, previously trained for semen collection. Semen was diluted with 0.9% sodium chloride solution (PISA) to achieve a final concentration of 20×10^6 spermatozoa. New Zealand White and Californian does were inseminated with a 0.5 mL dose deposited into the reproductive tract using a QHWJ (China) insemination gun; immediately thereafter, 0.2 mL of the gonadotropin-releasing hormone (GnRH) analog buserelin (SANFER) was administered intramuscularly (Dimitrova *et al.*, 2009). Pregnancy diagnosis was performed 14 days post-insemination. On day 28 of gestation, a nest box containing wood shavings was placed in each doe's cage and completed with fur pulled by the doe. Within the first hours after kindling, litter size and litter weight were recorded. Subsequently, litters were standardized to eight kits per doe in both breeds to promote maximal milk production (Singh, 1996).

Treatments and experimental diets

All diets were formulated according to the nutritional requirements recommended by De Blas and Wiseman (2020) for lactating does, varying the energy content across treatments. The calculated composition and the ingredients used in diet preparation are presented in Table 1.

Four isoproteic diets (T1, T2, T3, T4) were formulated with increasing digestible energy (DE) in 100-unit increments: 2600, 2700, 2800, and 2900 kcal kg⁻¹, respectively. The increase in energy was achieved by raising the inclusion level of soybean oil, while maintaining protein (17%), neutral detergent fiber (NDF; >30%), acid detergent fiber (ADF; 20%), and other nutrients as recommended by De Blas and Wiseman (2020). Diets were then mixed and pelleted to a 3-mm diameter. Recently kindled does (36 NZW and 36 CAL) were randomly allocated to the four treatments, with nine replicates per breed.

Feed intake

A total of 1.00 kg of feed per doe per day was offered in the morning (08:00 h). Feed intake was determined by weighing feed refusals and calculated as the difference between the amount offered and the amount refused. Measurements were recorded across five 7-day periods until the end of lactation (35 days).

Table 1. Composition of high-energy diets for lactating rabbit does.

Ingredient	Diet 1 (%)	Diet 2 (%)	Diet 3 (%)	Diet 4 (%)
Ground alfalfa	40.93	42.02	43.18	44.44
Corn	20.00	20.00	20.00	20.00
Dried distillers grains	15.00	15.00	15.00	11.42
Wheat bran	9.40	5.82	1.98	0.00
Canola meal	7.00	7.00	7.00	7.00
Soybean oil	4.16	5.82	7.50	9.36
Soybean meal	1.88	2.71	3.60	5.89
Common salt (NaCl)	0.30	0.30	0.30	0.33
L-lysine HCl	0.26	0.25	0.24	0.21
Mineral premix	0.25	0.25	0.25	0.25
Yeast	0.20	0.20	0.20	0.20
DL-methionine	0.19	0.20	0.21	0.25
Orthophosphate	0.14	0.23	0.33	0.45
L-threonine	0.11	0.11	0.10	0.11
Coccidiostat (Robenidine)	0.10	0.10	0.10	0.10
Calcium carbonate	0.08	—	—	—
Calculated nutrient composition				
Nutrient	Diet 1 (%)	Diet 2 (%)	Diet 3 (%)	Diet 4 (%)
Digestible energy (DE; kcal kg ⁻¹)	2600	2700	2800	2900
Crude protein (CP; %)	17.00	17.00	17.00	17.00
Crude fiber (CF; %)	14.00	14.00	14.00	14.00
Dry matter (DM; %)	90.21	90.43	90.66	90.89
Ether extract (EE; %)	7.33	8.91	10.51	12.09
Ash (%)	7.16	7.18	7.29	7.48
Neutral detergent fiber (NDF; %)	33.26	32.48	31.64	30.32
Acid detergent fiber (ADF; %)	20.12	20.13	20.14	20.06
Lysine (Lys; %)	0.90	0.90	0.90	0.90
Methionine + cysteine (Met+Cys; %)	0.69	0.69	0.69	0.69
Threonine (Thr; %)	0.74	0.74	0.74	0.74
Tryptophan (Trp; %)	0.20	0.20	0.20	0.21
Calcium (Ca; %)	0.80	0.80	0.83	0.88
Phosphorus (P; %)	0.45	0.45	0.45	0.45

Abbreviations: DE, digestible energy; CP, crude protein; CF, crude fiber; DM, dry matter; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; Lys, lysine; Met+Cys, methionine + cysteine; Thr, threonine; Trp, tryptophan; Ca, calcium; P, phosphorus.

Milk production

Milk production (MP) was estimated throughout lactation using the controlled-lactation method described by Gómez *et al.* (2008), which consisted of recording kit body weight immediately before and after nursing. The resulting weight difference was attributed to the amount of milk produced by the doe (McNitt & Lukefahr, 1990; Gómez *et al.*, 2008).

Statistical analysis

The Shapiro-Wilk test was used to assess data normality. To compare the effects of treatment and period, as well as their interaction, a joint adjustment was performed by simultaneously incorporating the effects of breed and energy level, grouping treatments in a 2×4 factorial arrangement (two breeds and four energy levels). Milk-production data were analyzed using a model that included the fixed effects of treatment and period, as well as their interaction (treatment $\times$ period), with doe included as a random effect. The general model was as follows:

$$Y_{ijkl} = \mu + T_i + d_{ij} + P_k + (TP)_{ijk} + \varepsilon_{ijkl}$$

where: Y_{ijkl} : Daily milk production of the doe receiving the i -th treatment in the j -th period; μ : Overall mean; T_i : Effect of the i -th treatment ($i=1-4$); d_{ij} : Error term associated with the effect of the i -th treatment; P_k : Effect of the k -th period ($k=1-6$); $(TP)_{ijk}$: Effect of the interaction between the i -th treatment and the j -th period; ε_{ijkl} : Random error.

To characterize the dynamics of the milk-production curve and feed intake across periods, a nonlinear exponential regression was performed by fitting Wood's model (1967), which describes an incomplete gamma function, as follows:

$$Y_n = a n^b e^{-cn}$$

where: Y_n : Mean milk production of the doe across different time periods; a : Milk yield at the onset of lactation ($g \text{ day}(b+1)$); b : Rate of increase up to peak lactation; c : Rate of decline after peak lactation (days); n : Time period at which milk production is estimated; e : Base of the natural logarithms (2.71828).

Wood's equation (1967) describes the milk-yield curve, allowing estimation of the rate of increase in milk production from kindling until the doe reaches peak yield, followed by a gradual decline. This model enables the estimation of maximum yield, time to peak production, and lactation persistence.

To estimate the day at peak production, maximum milk yield, and persistence, the following equations were used: day at peak production: $n = b/c$; maximum yield $MY = a(b/c)^b e^{-b}$; persistence: $S = c - (b+1)$.

RESULTS AND DISCUSSION

Milk production

Milk production in rabbit does is a critical factor for achieving heavier kit weights at weaning (35 days). Milk-production (MP) results for New Zealand White (NZW) and Californian (CAL) does fed high-energy diets are presented in Table 2. No differences in MP were observed ($p > 0.05$) among the different energy levels (2600, 2700, 2800, and 2900 kcal DE kg^{-1}). In NZW does, the highest MP was recorded in period 4 (day 21) for treatments (T) 2, 4, and 3, with values of 229.78, 209.11, and 197.78 g day^{-1} , respectively;

Table 2. Mean milk production and feed intake of New Zealand White and Californian rabbit does by period.

Period (days)	New Zealand White				California			
	MP/ Treatment (g day ⁻¹)				MP/ Treatment (g day ⁻¹)			
	1	2	3	4	1	2	3	4
1	88.44 ^a	81.11 ^a	70.67 ^a	88.00 ^a	62.44 ^a	72.00 ^a	67.11 ^a	71.33 ^a
7	146.00 ^b	172.22 ^a	118.67 ^c	143.56 ^b	134.89 ^a	136.00 ^a	118.89 ^b	112.44 ^b
14	195.78 ^b	215.78 ^a	176.44 ^c	191.33 ^b	189.33 ^a	194.00 ^a	173.56 ^b	177.56 ^a
21	184.44 ^c	229.78 ^a	197.78 ^b	209.11 ^b	198.44 ^a	199.43 ^a	156.89 ^b	151.67 ^b
28	136.83 ^a	148.44 ^a	140.67 ^a	151.11 ^a	148.22 ^b	192.29 ^a	157.41 ^b	140.22 ^b
35	77.78 ^c	126.89 ^a	91.11 ^b	104.44 ^b	97.33 ^b	132.00 ^a	120.22 ^a	74.44 ^c
SEM	10.53	10.50	10.50	10.50	10.50	11.90	10.57	10.50
** Feed intake (g day ⁻¹)								
	318.80	335.50	295.00	294.00	338.69	335.45	299.69	291.47

Abbreviations: T, treatment; P, period; T×P, treatment×period; MP, milk production (g day⁻¹); feed intake, mean feed intake (g day⁻¹).
Notes: ^{a, b, c} Means with different superscripts within periods differ (p>0.05).

in T1, the highest value occurred on day 14 (195.78 g day⁻¹). In CAL does, the greatest MP was observed in period 4 (day 21) for T1 and T2, with values of 198.44 and 199.43 g day⁻¹, respectively, whereas in T3 and T4 the highest MP occurred on day 14 (173.56 and 177.56 g day⁻¹, respectively). These findings are consistent with Pascual *et al.* (1999), who evaluated lactating NZW×CAL does fed three diets differing in energy density (2600, 2915, and 2965 kcal DE kg⁻¹) and reported that increasing dietary energy did not translate into significant increases (p>0.05) in MP.

Food consumption

In the New Zealand White (NZW) breed, the highest feed intake was observed in T2 (335.45 g day⁻¹), followed by T1, T3, and T4, with intakes of 318.80, 295.00, and 294.00 g day⁻¹, respectively. As shown in Figure 1, feed intake increased across the four treatments; however, no significant differences were detected (p>0.05).

In Californian (CAL) does, the highest feed intake was observed with diet 1 (338.69 g day⁻¹), followed by T2, T3, and T4, with values of 335.45, 299.69, and 291.61 g day⁻¹,

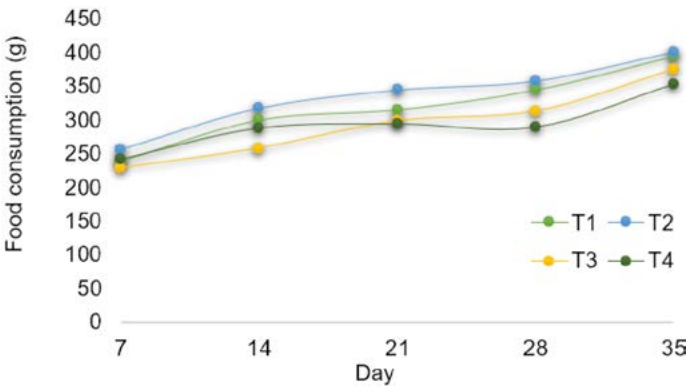


Figure 1. Feed intake of 36 New Zealand White (NZW) does across five observation periods.

respectively. As shown in Figure 2, feed intake increased across the four treatments, similarly to what was observed in NZW; however, no significant differences were detected among treatments ($p>0.05$).

The present results are consistent with those reported by Xiccato *et al.* (1995), who found feed intakes of 307, 315, and 302 g day⁻¹ in NZW and CAL does fed diets containing 2600, 2800, and 2900 kcal DE kg⁻¹, respectively. In contrast, Pascual *et al.* (1999) reported lower feed intake during lactation than that observed in the present study, with a mean intake of 150 g day⁻¹ across three diets. Likewise, Prasad and Karim (1998) reported feed intakes of 113.07 g day⁻¹ when varying dietary energy and protein levels.

Feed intake was similar across breeds and treatments, suggesting that increasing dietary energy through soybean oil inclusion did not adversely affect diet palatability. Nevertheless, a tendency toward lower intake was observed as dietary energy concentration increased. This pattern may reflect intake regulation driven by dietary energy density. These findings align with Xiccato (1996), who indicated that, in rabbits, feed intake is primarily regulated by a chemostatic mechanism aimed at maintaining a stable daily energy intake as part of energy homeostasis. This regulation is more pronounced in lactating animals than in growing rabbits, reflecting a physiological adaptation to meet the high nutrient demands of milk production.

Wood's model

Lactation is a highly complex biological process influenced by both genetic and environmental factors (Quintero *et al.*, 2007). Accordingly, a model capable of predicting maximum milk yield, lactation persistence, and the day on which peak production occurs is required. For this purpose, the incomplete gamma function fitted by Wood's model has been applied in rabbit does, allowing analytical estimation of maximum yield and time to reach peak production (Gómez *et al.*, 2023).

Milk production under the different diets, as a function of days in lactation, follows an exponential equation described as an incomplete gamma function. Figure 3 presents the mean milk production across the six periods for each treatment in NZW does, along with the MP trend line.

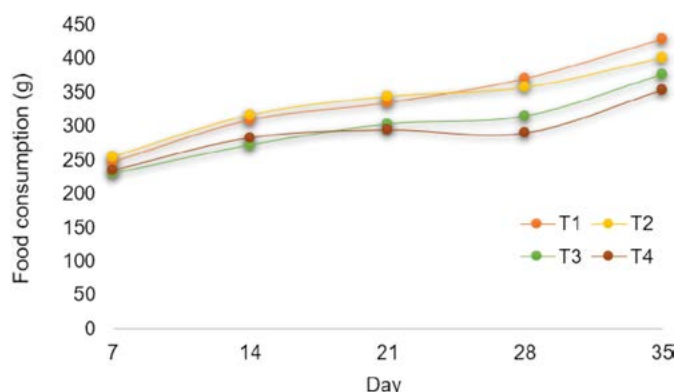


Figure 2. Feed intake of 36 Californian (CAL) does across five observation periods.

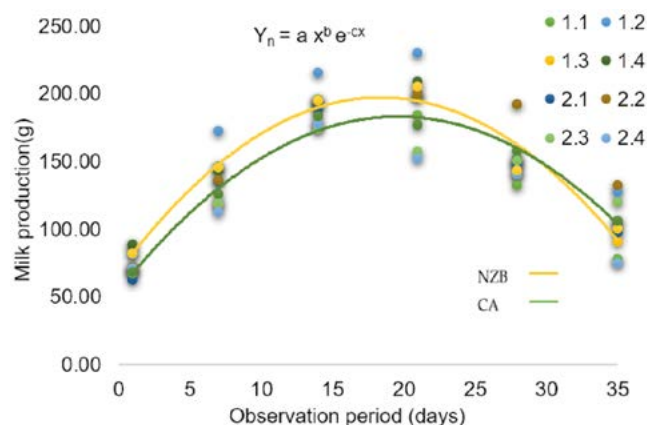


Figure 3. Milk-production curves for 36 New Zealand White (NZW; 1.1-1.4) and 36 Californian (CAL; 2.1-2.4) does, and their mean milk yield throughout a 35-day lactation.

The relationship between days in lactation and milk production (MP; Figure 3) indicates that, on average, does reached their highest MP between days 17 and 19, and MP began to decline from day 21 onward.

For CAL does, mean MP across the six periods for each treatment, together with the MP trend line, is also shown in Figure 3. The day-in-lactation-MP relationship similarly indicates that, on average, does achieved peak MP between days 18 and 19, after which MP began to decline from day 21 onward. In both breeds, peak production occurred at approximately day 19, closely matching the findings of Partridge and Allan (1982), who evaluated two diets with DE levels of 2800 and 2900 kcal and reported a production peak at 19 days. Xiccato *et al.* (1995) reported the highest production under high-energy diets (2600, 2800, and 2900 kcal DE kg⁻¹) between days 10 and 20 of lactation, with means of 233, 236, and 255 g day⁻¹, respectively. Other authors have reported peak production at 17 days (Gómez *et al.*, 2023) and as late as day 21 (Singh, 1996).

After fitting the incomplete gamma function using Wood's model solution procedure (Table 3), no significant differences were detected. In NZW does, the maximum yield (MY) was 215.33 g day⁻¹ for T2 on day 13 of lactation, followed by T4, T1, and T3, with values of 188.79, 187.95, and 173.75 g day⁻¹, respectively.

For CAL does, the maximum yield (MY) was observed in T1 (187.36 g day⁻¹), with peak production occurring on day 14 of lactation, followed by T2, T3, and T4, with values of 166.97, 162.91, and 159.15 g day⁻¹, respectively. Persistence (S) was similar in both breeds (NZW: 0.48-0.59; CAL: 0.45-0.67), suggesting a relatively pronounced post-peak decline from the third week onward, consistent with the increased energetic cost of lactation and the eventual mobilization of body reserves by the doe. This aligns with Gómez *et al.* (2023), who reported S values of 0.44.

An early lactation peak, occurring between days 18 and 21, represents the period of greatest nutrient demand, underscoring the need for adequate nutritional support during this phase to maximize MP and promote lactation-curve persistence (Maertens *et al.*, 2006). Mean MP values were slightly higher than those reported by McNitt and Lukefahr (1990), who observed a maximum MP of 185 g day⁻¹ at 20 days of lactation in NZW, CAL,

Table 3. Mean milk production and incomplete gamma-coefficient values for New Zealand White and California rabbits.

Estimators	New Zealand White			
	Treatments			
	1	2	3	4
MP	137.40±19.72	162.37±22.76	132.55±19.98	147.92±19.26
A	75.39±30.72	49.95±30.05	44.05±21.77	58.96±18.76
b	0.61±0.26	0.75±0.36	0.87±0.29	0.55±0.19
c	0.05±0.02	0.05±0.02	0.06±0.02	0.03±0.01
S	0.50	0.56	0.59	0.48
b/c	12.06	13.19	14.30	13.13
MY	187.95	215.34	173.75	188.79
Estimators	California			
	Treatments			
	1	2	3	4
MO	138.44±21.41	154.28±20.54	131.15±15.48	121.27±17.52
A	71.21±24.43	74.89±28.47	50.60±21.62	55.66±27.99
b	0.70±0.21	0.58±0.24	0.62±0.25	0.67±0.31
c	0.05±0.01	0.04±0.02	0.03±0.02	0.05±0.02
S	0.66	0.50	0.45	0.54
b/c	14.31	18.30	17.10	13.05
MY	187.36	166.98	162.91	159.15

MP: Mean milk production throughout lactation (g day^{-1}); a: Milk yield at the onset of lactation (g day^{-1}); b: Rate of increase in milk production up to the maximum; c: Rate of decline after the maximum; S: Persistence; b/c: Day at peak production; MY: Maximum yield (g day^{-1}).

CAL×NZW, and NZW×CAL does. Similarly, Xiccato *et al.* (1995) reported production values of 171, 180, and 191 g day^{-1} in NZW and CAL does. Pascual *et al.* (1999) reported a mean MP of 173.02 g day^{-1} , very close to that observed in the present study, and found that the highest MP occurred in the two high-energy diets (2915 and 2965 kcal). In another study, Pascual *et al.* (1999) reported mean milk production in NZW and CAL does of 164.0, 155.2, and 149.8 g day^{-1} for low (2350), medium (2650), and high (2950) DE diets, respectively. Gómez *et al.* (2008) reported peak production at 19 days (160 g day^{-1}) and a mean production of 122 g day^{-1} . In the study by Ludwiczak *et al.* (2020), which aimed to analyze milk quality, the highest values were reported (216.88 g day^{-1}) when the lactation period was shortened to 21 days, which may overestimate peak production and underestimate the decline phase when considering a 35-day lactation period.

CONCLUSIONS

Increasing digestible energy from 2600 to 2900 kcal kg^{-1} in diets for lactating rabbit does did not produce significant differences in milk production over the 35-day lactation period. Feed intake was similar across treatments and breeds, with a tendency to decrease as dietary energy increased, suggesting physiological regulation of intake as a function of energy supply. The incomplete gamma function adequately described the lactation curve in rabbit does. The lactation curve exhibited an average peak at day

17 postpartum, followed by a moderate decline from day 21 onward. Persistence was similar between breeds. Overall, the results suggest that, within the evaluated energy range, diets containing 17% crude protein and 2700 kcal DE kg⁻¹ are recommended to maintain MP in NZW and CAL does.

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Sembrando Vida Program: Analysis of Food Security and Social Organization in Communities of the Central Veracruz Agroecosystem

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ABSTRACT

Objective: To compare the Sembrando Vida Program with respect to food self-sufficiency, adoption of the Milpa Intercalada en Árboles Frutales (MIAF; Intercropped Milpa with Fruit Trees), and social organization across three communities situated along a contrasting altitudinal gradient within the central Veracruz agroecosystem.

Design: The methodology relied on a survey approach using a structured questionnaire. We interviewed 90 registered participants (“sowers”) from peasant learning communities in three localities in the state of Veracruz, complemented by semi-structured interviews with 15 sowers.

Results: The Sembrando Vida Program showed 100% recognition and acceptance among formal members. All participants (100%) reported that the program transformed and made more visible gender relations both within the broader community and within the peasant learning community. Participants also reported improvements in household and community-wide economic conditions and food security.

Limitations of the study/Implications: The significance of this study is limited to participants in the Sembrando Vida Program. Nonetheless, it offers a grounded account of current realities and of the program’s impacts on the lives of those involved.

Findings/conclusions: The evidence presented supports the program’s positive contributions to the transformation and development of rural communities and their principal actors, particularly women. The Sembrando Vida Program is associated with increased women’s participation across activities, advances in gender equity, enhanced food security, biodiversity conservation within agroecosystems, improvements in household economies, and strengthened social cohesion, alongside more structured planning and allocation of tasks in both agricultural and domestic spheres.

Keywords: Poverty, agroecosystems, social well-being, reforestation

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INTRODUCTION

Agriculture is a strategic sector for society at large, as it underpins the global food supply and contributes to the development of rural communities. Nevertheless, agriculture faces multiple local and global challenges that constrain rural community development.



These challenges are increasingly complex and include climate change, degradation of natural resources, rural outmigration, and the persistent impoverishment of rural communities. In Mexico, the National Population Council (CONAPO, 2006) estimates that by 2050 the population will increase by 17% relative to the current figure of 129.7 million inhabitants (INEGI, 2024). In response to these dynamics and to pervasive poverty in Mexico, the federal government implemented the Sembrando Vida Program (Programa Sembrando Vida; PSV) in 2019, with the aim of reversing negative trends attributed to neoliberal policies. PSV seeks to promote the social well-being of rights-holders, mitigate environmental degradation, reduce rural poverty, foster and strengthen a sense of belonging to rural life and land, and reinforce food sovereignty. In addition, the program is designed to reduce social vulnerability through food self-sufficiency, employment generation, and the equitable participation of women and men (Secretaría de Bienestar, 2020a). The main pillars of PSV are the Peasant Learning Communities (Comunidades de Aprendizaje Campesino; CAC), Agroforestry Systems (Sistemas Agroforestales; SAF), the Milpa Intercalada en Árboles Frutales (MIAF; Intercropped Milpa with Fruit Trees), agroecological practices, biofactories (biofábricas), and the living pharmacy (farmacia viviente) (DOF, 2020b). In recent years, multiple studies have assessed the impacts of PSV, yielding mixed perspectives within both the scientific and journalistic spheres. The scientific literature identified to date comprises 24 publications one indexed in Scopus and the remainder in SciELO. Overall, these studies report moderate yet encouraging assessments across several indicators, including agroecology, food production, training, and the production and use of bioproducts. The knowledge gap addressed by this study is to examine PSV performance from the perspective of the sowers themselves, using a comparative and territorial approach (altitudinal gradient), and to assess whether program implementation has been able to meet objectives related to food sovereignty, social fabric regeneration, and deforestation. Accordingly, the research question guiding this study was: What is the level of effectiveness of the Sembrando Vida Program in reducing rural poverty, environmental degradation, and in reactivating the local economy, strengthening food sovereignty, and regenerating the social fabric in rural communities along an altitudinal gradient in the central region of Veracruz State, Mexico? Therefore, the aim was to compare the impact of the Sembrando Vida Program (PSV) in terms of food self-sufficiency, adoption of Agroforestry Systems (SAF), and social organization, with particular emphasis on gender inclusion and empowerment, across communities in the central Veracruz agroecosystem situated along an altitudinal gradient.

MATERIALS AND METHODS

This study employed a survey-based approach grounded in a structured questionnaire technique.

Study area. The research was conducted in three localities in the central region of the state of Veracruz, Mexico, situated along an altitudinal gradient: Tetla, in the municipality of Chocamán (1,600 m a.s.l.); Zacamilola and Ameyala, in Zongolica (>2,300 m a.s.l.); and Pachuquilla and Remudadero, in Puente Nacional (400 m a.s.l.). Each municipality was represented by two communities, corresponding to two Peasant Learning

Communities (Comunidades de Aprendizaje Campesino; CAC). These three zones differ in their edaphoclimatic characteristics particularly climate, vegetation, and soils (Figure 1). The Zongolica region is characterized by coniferous vegetation, a cold climate, and

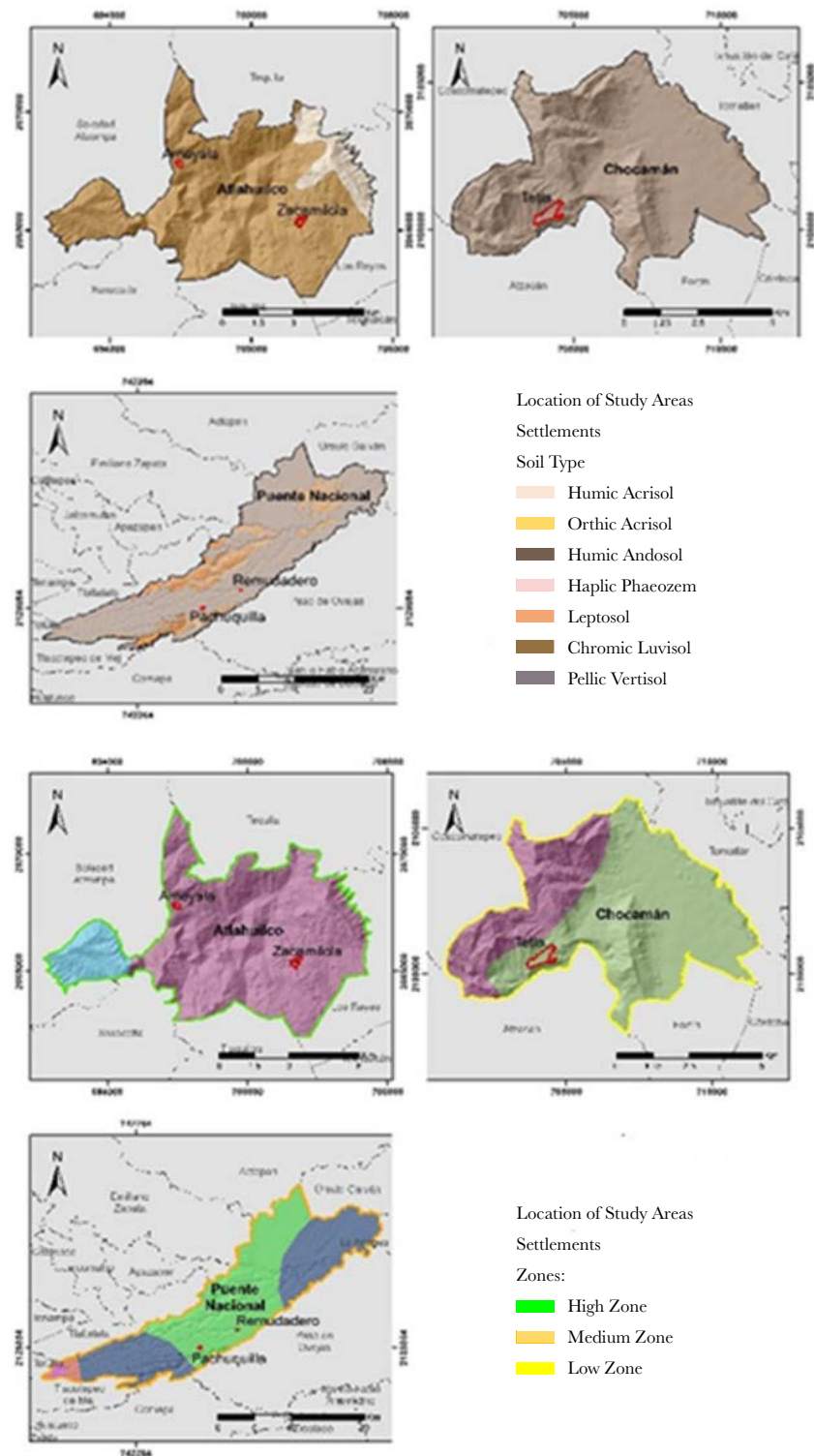


Figure 1. Location of the study areas. Authors' own elaboration based on CONABIO 2024.

Chromic Luvisol soils; Chocamán by a temperate climate, cloud forest vegetation, and Humic Andosol soils; and Puente Nacional by tropical dry forest (*selva baja caducifolia*), a subhumid tropical climate, and alluvial soils.

Communities were selected purposively, using the criterion of contrasting the program along an altitudinal gradient in order to capture the cultural diversity and the range of productive systems in the central region of Veracruz. This design enabled the assessment of program management under differing biophysical constraints, ensuring that the data reflected disparities in PSV implementation across contrasting contexts.

Type of study. This was a comparative study across three communities, conducted from January to August 2023. Data collection followed a mixed-methods approach that integrated: (1) administration of a structured survey to 90 program participants (“sowers”) belonging to the selected communities; and (2) semi-structured interviews with an additional 30 beneficiaries, selecting five key informants from each Peasant Learning Community (Comunidades de Aprendizaje Campesino; CAC). This methodological design enabled triangulation of information and ensured direct interaction with 100% of registered rights-holders in the study communities, thereby achieving full representativeness of the target population. The structured questionnaire was expected to standardize responses from the 90 sowers and provide a robust statistical basis for identifying general trends.

Semi-structured interviews were used to explore the underlying “why” of the observed patterns, allowing the 30 interviewees to articulate the particularities of their local contexts. By integrating both data sources, internal validation was achieved by contrasting and corroborating the survey’s quantitative evidence with qualitative insights.

The questionnaire underwent a content-validation process through expert judgment by specialists in rural development and agroecology, who assessed the relevance and clarity of each item in relation to the study objectives. Subsequently, a pilot study was conducted with 10% of the sample in a community with characteristics analogous to the study area. This procedure enabled refinement of technical terminology and ensured comprehension of the different items. Finally, instrument reliability was assessed using Cronbach’s alpha coefficient, yielding a value above 0.80, which indicates strong internal consistency and suitability for data collection. The unit of analysis consisted of PSV beneficiary sowers, selected according to inclusion criteria requiring a minimum of three years of participation in the program and active implementation of the MIAF and SAF systems. The data collection instrument was a structured questionnaire comprising seven components: (1) sociodemographic profile, (2) farming system, (3) agricultural practices, (4) local economy, (5) environmental degradation, (6) food security, and (7) social fabric.

After data entry in Microsoft Excel, descriptive statistics were applied along with the chi-square (χ^2) test of independence. The latter was justified by the categorical nature of the data and was used to determine whether there was a statistically significant association between the altitudinal gradient and respondents’ perceptions regarding the program’s effectiveness in reducing poverty, strengthening food security, promoting gender empowerment, and regenerating the social fabric.

RESULTS AND DISCUSSION

Rights-holders in Zongolica belong to Indigenous Nahuatl-speaking peoples and originate from rural communities with a predominantly agricultural vocation and high crop-species diversity. This setting differs from the other two study communities in the majority participation of women, the prevalence of Nahuatl as the primary language, and distinct economic, environmental, social, and cultural conditions (including practices, values, and beliefs). This pattern is consistent with the Economic Commission for Latin America and the Caribbean (ECLAC/CEPAL, 2021), which reports that more than 58 million people in Latin America and the Caribbean identify as Indigenous, and that a substantial share of this population experiences exclusion due to political, economic, social, environmental, health, and sanitary inequalities (ECLAC/CEPAL, 2021). In contrast, Spanish is the only language spoken in the other two communities (Table 1).

Characterization and perceptions of rights-holders

The operational structure of the Sembrando Vida Program (Programa Sembrando Vida; PSV) in central Veracruz is anchored in the Peasant Learning Communities (Comunidades de Aprendizaje Campesino; CAC) across three contrasting regions: Zacamilola and Ameyala (Sierra de Zongolica), Tetla 1 and Tetla 2 (Chocamán), and Remudadero and Pachuquilla (Puente Nacional). The demographic analysis revealed a population with extensive experiential knowledge, with mean ages ranging from 51.4 (SD=6.9) to 59.4 (SD=4.8) years. This maturity is perceived as a programmatic strength, as it facilitates the consolidation of agroecological practices grounded in traditional knowledge. In this regard, accumulated experience among older producers has been documented as a key determinant of agroecosystem resilience and the conservation of agrobiodiversity (Altieri & Nicholls, 2017). These means fall within the economically active age range (Vásquez, 2012).

Regarding educational background, primary schooling predominates (63%-70%, depending on the region), a trend that remains consistent along the altitudinal gradient. Despite this educational profile, perceptions of technical outcomes in nurseries and field

Table 1. Characterization of the study areas.

Study area	Peasant Learning Community (CAC)	Indigenous-language speakers	Main forest species	Main fruit species
Sierra de Zongolica	Zacamilola and Ameyala	100% Nahuatl	Pine*, oak, walnut, ilite, ocote (pine resin)	Avocado*, guava, peach, apple, lime, coffee, Hass avocado
Chocamán	Tetla 1 and Tetla 2	1% Nahuatl	Rosewood cedar, ilite, casuarina, ocote (pine resin), poplar, oak	Macadamia, lime, coffee, orange, banana, chayote
Puente Nacional	Pachuquilla and Remudadero	0.72% Indigenous language	Cedar, oak, mahogany	Guava, lime, fig, pineapple, orange, nanche, sapodilla, soursop, passion fruit

CAC (Peasant Learning Community).

plots are strongly positive, suggesting that the CAC training model has effectively overcome barriers associated with formal education. In addition, sowers report high satisfaction with family integration, perceiving PSV not only as economic support but also as a catalyst for household improvement opportunities.

Finally, perceptions of women's empowerment differ markedly by land tenure. In the Sierra de Zongolica, 86% of female participants are landowners, in contrast to 17% in Chocamán and 35% in Puente Nacional. Across all regions, women's participation substantially exceeds the national average of 15% (SADER, 2019), indicating that program implementation in Veracruz is achieving gender inclusion above federal benchmarks, particularly in higher-altitude areas.

The implementation of the Sembrando Vida Program (Programa Sembrando Vida; PSV) in central Veracruz exhibits a high degree of consolidation and continuity. In the Sierra de Zongolica, all rights-holders (100%) joined during the foundational cycle (2019), whereas early enrollment reached 93% in Chocamán and 97% in Puente Nacional. From beneficiaries' perspective, this early incorporation translates into greater perceived technical mastery of Agroforestry Systems (Sistemas Agroforestales; SAF) and the Milpa Intercalada en Árboles Frutales (MIAF; Intercropped Milpa with Fruit Trees), given that most sowers have completed the five-year learning cycle.

This, in turn, enables a more robust agroecological transition compared with those who joined in later stages (2020 and 2021). The association between length of participation and technical mastery aligns with diffusion of innovations theory, whereby accumulated experience reduces uncertainty and facilitates the internalization of complex practices (Rogers, 2003). Likewise, the literature notes that agroforestry systems require extended maturation periods before tangible benefits become evident, reinforcing the perception of success among foundational cohorts (Pinho *et al.*, 2012). With respect to social structure, family stability predominates, with most sowers being married or in common-law unions (94% in Zongolica, 83% in Puente Nacional, and 77% in Chocamán).

These results suggest that rights-holders view PSV as a family life project rather than a purely individual endeavor. This sociodemographic configuration is pivotal for regenerating the social fabric, as conjugal cohesion facilitates task allocation on plots and participation in collective CAC activities. In this context, "bonding" social capital (internal ties) is fundamental in traditional agroecosystems, where the family unit functions as the primary labor force and as emotional support in the face of external risks (Reimer *et al.*,

Table 2. Profile of PSV rights-holders.

Municipality/ Locality	Gender (M/F)	Average schooling (%)	Cultivate coffee, maize, and beans	Mean age (years)
Sierra de Zongolica	30/20	Primary (P)* 70, Secondary (S) 24, Upper secondary (SE) 20	81%	52.6 (SE)
Chocamán	43/5	Primary (P) 63, Secondary (S) 17, Upper secondary (SE) 20	91%	59.4 (SE)
Puente Nacional	47/15	Primary (P) 68, Secondary (S) 20, Upper secondary (SE) 18	86%	51.4 (SE)

Primary education (P), secondary education (S), no formal schooling (SE).

2008). Nevertheless, the diversity of marital status observed in areas such as Chocamán (13% single and 7% widowed) indicates that the program also operates as a social safety net for female and male heads of household who farm independently, adapting to the distinct demographic realities along the altitudinal gradient. This protective function is critical because, in contexts of rural vulnerability, integrating single-parent households into collective learning networks (CAC) mitigates exclusion risks and strengthens community resilience (Mancini, 2013).

Farming systems and agricultural practices

PSV implementation in central Veracruz unfolds across contrasting biophysical settings that shape beneficiaries' technical perceptions. In the Sierra de Zongolica (>2,300 m a.s.l.), sowers perceive steep terrain and limited access not as constraints, but as opportunities for diversification, achieving an exceptional agrobiodiversity of 145 species.

Particularly noteworthy is the strategic perspective reported among women in this region, who have incorporated ornamental plants into their productive systems, linking biodiversity to market niches associated with local festivities thereby constituting a distinctive model of economic management. This mountain-zone diversification is consistent with the argument advanced by Toledo and Barrera-Bassols (2008), who posit that biological complexity in Indigenous agroecosystems is an adaptive response that minimizes climatic and economic risks through the use of multiple ecological niches. At the level of practices, there is unanimous recognition (100%) of the importance of agrobiodiversity for community sustainability.

An attitudinal shift toward agroecology is evident: all rights-holders have adopted the use of bioproducts and biofactories, perceiving these inputs as effective substitutes for chemical agroinputs (used by only 6.6% of the sample). This technological appropriation is reflected in universal recognition of MIAF and SAF as pillars of food sovereignty and the generation of economic surpluses. The reported low dependence on agrochemicals suggests a process of input “de-substitution,” whereby technical empowerment enables producers to regain control over the productive process, aligning with the food sovereignty principles articulated by Gliessman (2015).

Chi-square (χ^2) test results confirm that the altitudinal gradient significantly influences the assessment of the program's environmental impacts (Figure 2). Soil conservation and deforestation: In the Sierra de Zongolica, perceptions are strongly positive (91% and 100% “strongly agree,” respectively), suggesting that under steep-slope conditions producers assign greater value to PSV conservation techniques as critical tools for ecosystem persistence ($\chi^2=13.54$, $p=0.0011$). Water management and fauna: Puente Nacional (lowland zone) reports the highest satisfaction regarding fauna and water conservation, with 93% expressing strong agreement. In contrast, Chocamán (mid-elevation zone) exhibits more conservative and dispersed perceptions (50%-63% strong agreement), indicating variability in water-related management that may be associated with pronounced soil differences identified in that region ($\chi^2=17.89$, $p=0.0013$).

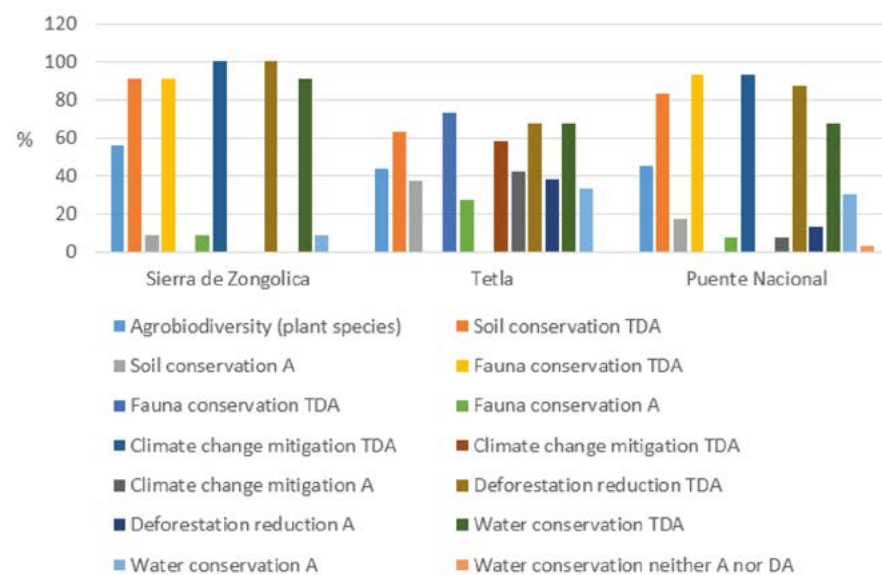
These regional differences underscore the relevance of a “biocultural landscape” approach, wherein perceptions of environmental success depend not only on the

technique applied, but also on the specific vulnerability of each thermal belt (Boege, 2008). Nevertheless, critical authors caution that positive perceptions may be influenced by the program's economic subsidy, which warrants validation through field-based biophysical indicators to confirm whether perceived conservation corresponds to genuine ecological improvement (Giraldo, 2018). Overall, rights-holders' perceptions indicate that PSV has fostered a paradigmatic shift toward the protection of natural resources. However, the statistical variability across CAC demonstrates that the success of agroecological management is not uniform; rather, it is mediated by territorial geography and by the adaptive capacity of productive systems to respond to the specific challenges of each agroecosystem.

Food security

Perceptions of food security under PSV are not homogeneous; rather, they adjust to the logistical and productive realities of each thermal belt. The χ^2 test revealed statistically significant differences across all evaluated dimensions, indicating heterogeneous program management and impacts:

Availability and access: In Puente Nacional (lowland zone), perceptions of increased food availability predominate (57% “strongly agree”; $\chi^2=15.28$, $p=0.0005$), possibly favored by flatter terrain that facilitates the flow of inputs. In contrast, in the Sierra de Zongolica, although the consensus regarding improvement is high (100%), only 47% reported the highest level of agreement, suggesting that difficult physical access continues to filter perceptions of availability. This regional disparity is consistent with Bacon *et al.* (2014), who note that in mountainous areas “hungry months” may persist despite support programs due to precarious transport infrastructure and distance to markets.



TDA (Strongly Agree). A (Agree). ni A ni DA (Neither Agree nor Disagree)

Figure 2. Perceptions of improvements in agricultural practices across PSV agroecosystems.

Utilization and stability: The Sierra de Zongolica stands out in biological utilization of foods (100% agreement), whereas Tetla and Zongolica report greater stability (40% “strongly agree”) relative to the lowland zone ($\chi^2=6.14$, $p=0.05$). Taken together, these findings indicate that, at the local level, PSV addresses the FAO (2016) pillars of food security and aligns with Mexico’s reported improvement in the Global Food Security Index (GFSI), which reached 69.1% in 2022. The high valuation of biological utilization in Indigenous settings suggests that productive diversification within agroforestry systems contributes directly to more balanced, higher-quality diets (Altieri & Nicholls, 2017).

Local economy: diversification and interdependence

In terms of economic management, PSV functions as a catalyst for rural investment. All rights-holders (100%) perceive the support not merely as a subsidy, but as investment capital for their plots, housing improvements, and the hiring of day labor, thereby energizing intra-community employment. This economic dynamic is reinforced by a multi-income strategy:

Complementarity: Beneficiaries combine PSV support with other social programs (*e.g.*, 60 y Más, Bienestar para Niños), particularly in Tetla (67%).

Pluriactivity: All sowers (100%) supplement their income through off-farm activities (construction, handicrafts, petty trade), strengthening perceived economic resilience in the face of rural poverty. As Ellis (2000) argues, pluriactivity should not be interpreted as a lack of specialization, but as a rational livelihood strategy that reduces rural household vulnerability to climatic or market shocks.

Regeneration of the social fabric and empowerment

The program has contributed to a revalorization of identity and social capital, albeit with significant regional nuances ($\chi^2=13.14$, $p=0.001$). In the Sierra de Zongolica, 100% of participants perceive strengthening of cultural and linguistic identity, whereas in Puente Nacional this effect is reported with lower intensity (87%). This suggests that, in higher-altitude and predominantly Indigenous areas, PSV operates as a mechanism of cultural resistance and collective pride. However, authors such as Fox (2016) caution that the effectiveness of such programs depends on organizational structures not being imposed externally, but rather on achieving genuine dialogue with local governance forms:

Perceptions of decision-making also vary: while Zongolica and Puente Nacional report total inclusion (100%), Tetla declines to 87%, indicating opportunities to strengthen participatory management within this CAC. Notably, there is unanimous agreement (100%) on critical indicators that did not require χ^2 analysis due to complete success.

Legitimacy and gender: The program is perceived as an effective tool for narrowing gender gaps, positioning rural women as leaders with tangible access to resources. This form of perceived empowerment is crucial; however, its sustainability depends on accompanying structural shifts in the domestic workload burden (Vásquez, 2012).

Worldview and dialogue: Participants report full respect for ancestral knowledge and intergenerational dialogue, consistent with Secretaría de Bienestar (2020) guidelines on knowledge exchange.

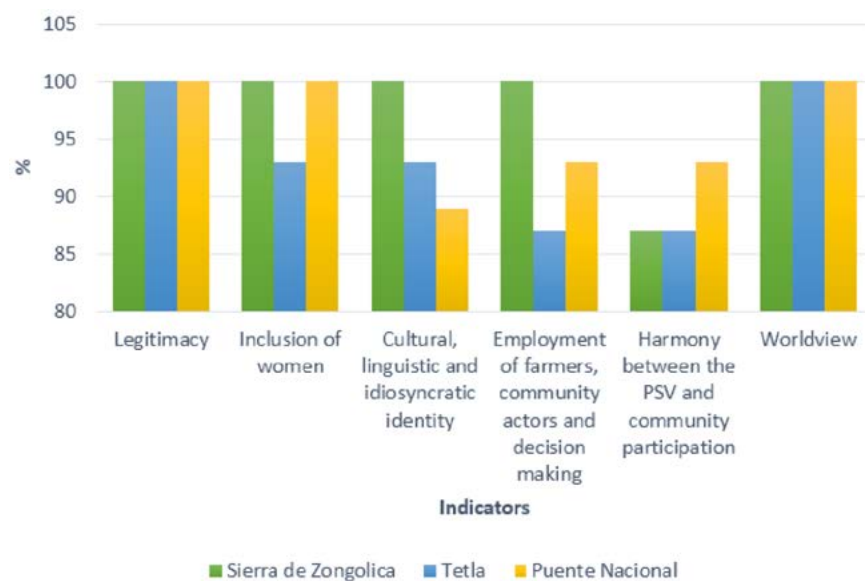
Life planning and empowerment: a unanimous consensus

Perceived structural impacts of the program on individual well-being show absolute consensus (100%) across the three regions. Rights-holders report that PSV has systematized their planning and organizational capacities, translating into tangible improvements in working conditions, housing, and control over productive processes. Given this unanimity, the chi-square (χ^2) test was not applicable for contrasting CACs, underscoring a generalized success in the social and economic recognition of sowers irrespective of geographic location. This “positive homogenization” of perceptions suggests that the program operates as a mechanism for standardizing a minimum threshold of well-being, whereby the security of a fixed income and an organizational structure reduce the uncertainty inherent to peasant livelihoods (Barkin, 2021).

Social capital and cohesion

Sowers perceive CAC management as materializing essential pillars of social cohesion:

Participation and trust: All beneficiaries (100%) recognize full alignment between program organization and community participation. Trust is expressed through unanimous perceptions of women’s empowerment: women’s active participation across all activities and collective learning within CACs are viewed as key drivers strengthening cooperation and trust across genders. Nonetheless, critical literature cautions that perceived empowerment should be examined alongside care burdens and the “double workday” that women may



PSV (Sembrando Vida Program). CAC (Peasant Learning Community)

Figure 3. Perceptions of improvements in the PSV social fabric.

assume when participating in intensive rural development programs (Herrera Flores, Miguel, 2022).

Identity and decision-making

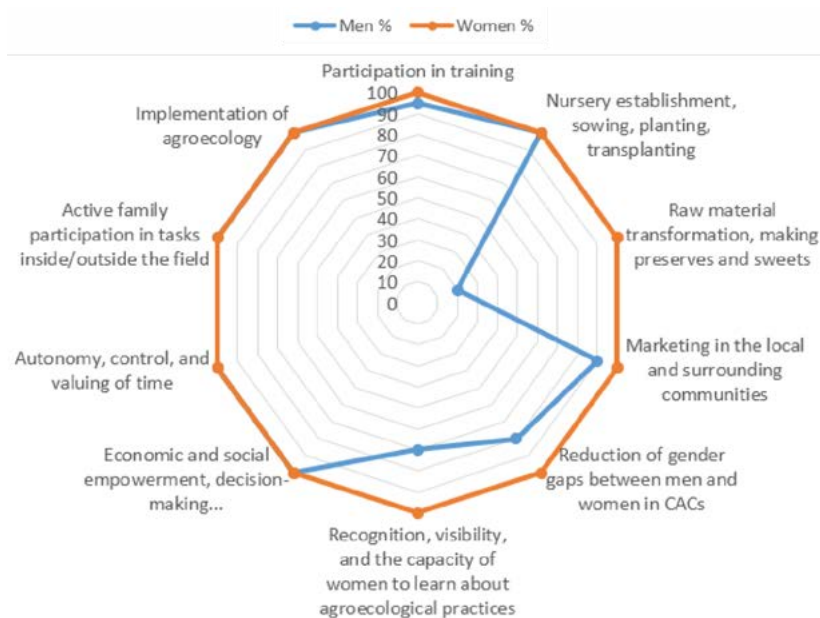
Perceptions of success in community promotion display statistically significant regional nuances ($\chi^2=13.14$, $p=0.001$). In the Sierra de Zongolica, 100% perceive that the program reflects and strengthens cultural identity and local idiosyncrasy, whereas in Puente Nacional this perception decreases to 87%. Similarly, the inclusion of local actors in decision-making is perceived with lower intensity in Tetla (87%) relative to full consensus in the other regions, suggesting that the expression of social capital varies by local context. This variation confirms that social capital is not static; rather, it depends on pre-existing networks and the degree of internal cohesion within each community (Woolcock & Narayan, 2000).

Indigenous inclusion and cultural diversity

The chi-square test revealed an extreme cultural asymmetry in linguistic identity ($\chi^2=285.88$, $p<0.0001$). In the Sierra de Zongolica, 100% of rights-holders are Nahuatl speakers, rigorously meeting the program's Indigenous inclusion criteria. In contrast, mother-tongue speakers are nearly testimonial in Tetla (1%) and Puente Nacional (0.72%). This substantial difference indicates that PSV has adapted to markedly divergent contexts: in the highland zone it functions as a refuge for Nahuatl worldview and language, whereas in mid- and lowland zones it focuses more directly on addressing poverty and inequality through organization and economic empowerment. Across both scenarios, sowers ultimately perceive PSV as a program that facilitates intergenerational dialogue and revalorizes ancestral knowledge, consistent with Secretaría de Bienestar (2020) guidelines, while also contributing to job creation and territorial rootedness. Recognition of linguistic and cultural diversity is a critical component of food sovereignty, since language is the primary vehicle for transmitting agroecological knowledge and conserving native seeds (Guzmán Luna *et al.*, 2022) (Figure 4).

This analysis underscores the importance of considering cultural and linguistic diversity when evaluating program implementation and impacts across regions. Whereas the Sierra de Zongolica is characterized by a high concentration of Nahuatl-speaking Indigenous populations within its Peasant Learning Communities (Comunidades de Aprendizaje Campesino; CAC), Tetla and Puente Nacional exhibit linguistically distinct demographic compositions among participating groups. This heterogeneity may have implications for how program strategies are implemented, the cultural relevance of training processes, and the ways in which the revalorization of ancestral knowledge is addressed.

The comparative assessment of the Sembrando Vida Program (PSV) in central Veracruz indicates that the altitudinal gradient functions as an axis of productive and cultural differentiation, although it does not appear to influence overall perceptions of well-being. In terms of perceptual findings, an absolute consensus emerged regarding improved living conditions and women's empowerment. Nevertheless, perceptions of food security and environmental conservation displayed statistically significant variation: while lowland areas prioritize food availability, highland areas place greater value on soil protection



CACs (Peasant Learning Communities).

Figure 4. Beneficiaries' perceptions of the Sembrando Vida Program (PSV).

and ecosystem stability. This pattern suggests that beneficiaries interpret the program's utility in relation to the most pressing needs of their immediate biophysical environment. Contextual implications are substantial with respect to social capital and identity.

CONCLUSIONS

The study shows that PSV operates under two territorial logics: in the Sierra de Zongolica it consolidates as a space of Nahuatl cultural and linguistic resistance, whereas at lower elevations it manifests primarily as an organizational model fostering economic inclusion and pluriactivity. The adoption of SAF and MIAF systems, together with the transition toward bioinputs, reflects an operational plasticity that enables the convergence of ancestral and technical knowledge, facilitating intra-community job creation and legitimizing rural women in leadership roles. Regarding methodological limitations, the results are confined to the opinions of rights-holders within the selected CACs, providing an internal perspective on program management. Because purposive sampling was employed, extrapolation of findings to the regional level should be approached with caution, particularly given that uncontrolled variables such as fluctuations in local markets may shape long-term food security stability. In addition, while the chi-square test allowed identification of significant associations between altitude and perceptions, the homogeneity of responses for social-fabric indicators constrained the scope for statistical variance analysis in those specific domains.

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Determinants of Rice Production, a Food-Dependent Crop in Mexico (*Oryza sativa*)

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ABSTRACT

Objective: To identify the economic factors that impact national rice production from 2003 to 2022, and to assess the production stage of each factor.

Design/methodology/approach: The methodology involves calculating growth rates for the relevant variables and estimating a multiple linear econometric model. The determinants of the production model are the planted area, the economically active population in the agricultural sector, and the total use of nitrogen fertilizers.

Results: The estimators indicate a positive relationship between production and planted area ($\hat{\beta}_1 = 0.825$); a positive relationship between the economically active population in the agricultural sector ($\hat{\beta}_2 = 1.276$); and a positive relationship with nitrogen ($\hat{\beta}_3 = 0.458$). The findings support the theoretical expectations. The results indicate increasing marginal returns for the economically active population agriculture, which has the greatest impact on production, while planted area and nitrogen indicate decreasing marginal returns.

Findings/conclusions: In general, the sum of the elasticities indicates increasing returns to scale (2.559). This finding helps in designing strategies focused on the main production factors recognizing real limitations.

Keywords: *Oryza sativa*, econometric model, production function, production factor, elasticity.

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INTRODUCTION

In Mexico, self-sufficiency in rice production was achieved in 1988. In subsequent years, however, the rice sector underwent a structural transformation characterized by limited production incentives (Economics Commission, 2022, p. 6). Trade liberalization and a process of accelerated modernization profoundly reshaped Mexico's agricultural production and commercial activities. The trade agreement facilitated the reallocation of cultivated land and improved yields across several crops (González, 2001). Rice, wheat, yellow maize, and beans experienced a decline in production due to insufficient international competitiveness. Policy priorities shifted toward the cultivation of forage crops, legumes, fruits, and vegetables (Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food - SAGARPA, 2017, p. 2). Consequently, staple grains progressively lost strategic prominence, ultimately positioning the country as a net importer. According to Article 179 of the Sustainable Rural Development Law (SRDL), rice is classified as a strategic crop

and fulfills the objective of planning and organizing agricultural production to guarantee food security (Sustainable Rural Development Law, 2021, p. 53). According to Reyes *et al.* (2024), *per capita* rice consumption amounts to 8.5 kilograms. The magnitude of domestic insufficiency is evident: in 2016, national rice production satisfied only 21.62% of total domestic consumption. Consequently, 929,482 tons were imported to meet internal demand, primarily from the United States, Brazil, and Uruguay (SAGARPA, 2017, p. 2). By 2021, rice imports had reached 1,018,000 tons, accounting for 90% of domestic demand (Economics Commission, 2022, p. 6), thereby exacerbating dependence on the external sector. Enhancing domestic rice production would reduce vulnerability to international market volatility and mitigate adverse effects arising from price surges or food crises that could disrupt the regular flow of imports (Puyana & Romero, 2004, p. 108). In Mexico, imports originate predominantly from the United States, a country that holds a geographical advantage in transportation costs relative to other exporters. At the national level, the principal rice-producing states are Campeche, Nayarit, Tamaulipas, Michoacán, Veracruz, Colima, and Morelos. The cultivated area in Mexico totaled 40,841 hectares, generating a production volume of 257,000 tons (Agrifood and Fisheries Information Service - SIAP, 2022).

The literature on rice studies encompasses diverse market analyses, particularly those estimating supply, demand, and local competitiveness dynamics. However, empirical analyses of national crop production and its relationship with production factors remain underdeveloped and relatively scarce. This issue can be addressed through the Cobb-Douglas model, which enables a parsimonious yet robust representation of the relationship between inputs and output under supporting assumptions, including exogenous total factor productivity and factor substitutability. By employing a generalized abstraction of rice production through an econometric model, it is possible to estimate the elasticity of each production factor. The study posits a direct relationship between cultivated area and rice output, assuming technical efficiency and abstracting from climatic considerations. Likewise, a positive relationship between labor and rice production is hypothesized, based on the inferred existence of labor surplus derived from latent population within the primary sector capable of expanding production, as documented by Pérez *et al.* (2019). Rice is a crop with high demand for urea (nitrogen-based fertilizer); therefore, a direct relationship with rice production is anticipated, consistent with studies on fertilizer optimization. Within a context of pronounced trade dependence and limited domestic output, identifying the relationship between national rice production and production inputs will enable the quantification of both the direction and magnitude of their effects. The aim of this study is to estimate the elasticities of productive inputs with respect to national rice output. Such estimates may inform the design of strategic policies aligned with the efficient use of production factors, thereby fostering increased output and mitigating external dependence, while recognizing production factors as essential instruments of agricultural development.

MATERIALS AND METHODS

This research adopts a quantitative, empirical, and econometric approach. The estimation of a growth rate constitutes a fundamental indicator for assessing the evolution

of a variable over a specified period. The growth rate for the period is expressed as follows:

$$Tc p = \left(\left(\frac{VF}{VI} \right) - 1 \right) \times 100 \quad (1) \text{ and the average annual growth rate is; } Tc p a = n \sqrt[n]{\left(\left(\frac{VF}{VI} \right) - 1 \right)}$$

(2). The calculation is performed to identify the temporal dynamics of an economic variable.

To analyze production dynamics, the methodology employed by Pérez *et al.* (2019) is adopted, specifically the estimation of a Cobb-Douglas production function, which is widely applicable at both the microeconomic and macroeconomic levels and enables the assessment of the impact of production inputs land, labor, and capital (Mejía *et al.*, 2023). At the microeconomic level, the Cobb-Douglas function approximates the technological behavior of productive units, whereas at the macroeconomic level it serves as an aggregated representation of the production process. This abstraction provides analytical simplicity and facilitates the direct interpretation of parameters. Its application rests on restrictive assumptions, including perfect competition, well-defined factor markets, and stable (exogenous) technology over time. Moreover, it assumes a constant elasticity of substitution equal to one among factors, attributing changes in output exclusively to variations in productive inputs rather than to shifts in the production function itself (Gujarati, 2010). In the present analysis, the model is justified as an empirical approximation under the principle of parsimony, explicitly acknowledging its theoretical and econometric limitations. Its economic interpretation, statistical properties, and theoretical coherence link production theory through a Cobb-Douglas-type production function. Through an adaptation of the methodology applied by Pérez *et al.* (2018) and Guzmán *et al.* (2019), a log-log econometric model is proposed and estimated using the Ordinary Least Squares (OLS) method. The estimated parameters of the variables influencing rice production represent the output elasticities of the production factors.

The model was estimated using the RStudio statistical package. The selected variables are theoretically relevant and recurrently employed in agricultural literature as proxies for land, labor, and capital inputs. The availability of official statistical information disaggregated by production factor and crop at an aggregated level is limited; therefore, proxy variables are employed to operationally approximate these theoretical constructs. Consequently, the estimated coefficients should be interpreted as approximate associations rather than exact structural effects, a limitation explicitly acknowledged in the analysis. Data on rice production and harvested area were collected from the Agrifood and Fisheries Information and Consultation System (SIACON). The economically active population in the agricultural sector was obtained from the National Institute of Statistics and Geography (INEGI), and total nitrogen fertilizer use was sourced from the Food and Agriculture Organization of the United Nations Statistical Database (FAOSTAT). The study covers the period 2003-2022 with annual frequency.

Y: Rice production, measuring the total quantity of output obtained during the calendar year. Unit of measurement: metric tons (t).

SSe: Harvested rice area, defined as the land area sown with rice during a calendar year. Unit of measurement: hectares (ha).

PEAa: Economically active population in the agricultural sector, defined as the number of individuals engaged in the sector during the specified year. Unit of measurement: number of persons. This variable serves as a proxy for labor input in the study. The approximation is based on the assumption that, at an aggregated level, fluctuations in the agricultural economically active population reflect changes in the relative availability of labor within the sector's productive activities. The limitations associated with this factor are acknowledged; results should be interpreted as an aggregate approximation rather than as a direct measure of the specific labor input used in rice production.

N: Nitrogen use or fertilizer use by nutrient, measured as nitrogen fertilizer applied per unit of cultivated land. Unit of measurement: kilograms per hectare (kg/ha). Given the difficulty of quantifying crop-specific local fertilizer use to construct a consistent time series, total national nitrogen use is employed as a proxy variable, under the assumption that its temporal evolution reflects changes in the average fertilization intensity of the agricultural sector. Results associated with this variable should therefore be interpreted as a general approximation rather than as a direct measure of effective fertilizer use in rice cultivation.

Rice production is modeled as a function of harvested rice area, the economically active population in the agricultural sector as a proxy for labor potential, and total nitrogen fertilizer use within the sector:

$$\ln(\hat{Y}_i) = \hat{\beta}_0 + \hat{\beta}_1 \ln(SSe_i) + \hat{\beta}_2 \ln(PEAa_i) + \hat{\beta}_3 \ln(N_i) + \hat{\varepsilon}_i \quad (3)$$

where: $\hat{Y}_i$ represents rice production; $\hat{\beta}_i$ denotes the estimated parameters; SSe_i is harvested rice area; $PEAa_i$ is the economically active population in the agricultural sector; N_i is nitrogen fertilizer use; and $\hat{\varepsilon}_i$ is the stochastic error term.

The corresponding Cobb-Douglas production function is specified as:

$$Y_i = \beta_0 SSe_i^{\beta_1} PEAa_i^{\beta_2} N_i^{\beta_3} \quad (4)$$

According to agricultural production theory, the following relationships are expected:

- Rice production exhibits a direct relationship with harvested rice area.
- Rice production exhibits a direct relationship with the dynamics of the economically active population in the agricultural sector.
- Rice production exhibits a direct relationship with nitrogen fertilizer use.

Potential endogeneity may arise between Y and SSe , as changes in output can influence decisions regarding cultivated area through expectations of yields, prices, climatic conditions, or direct production support policies. The inclusion of harvested area as an explanatory variable may also introduce a conceptual multicollinearity concern. Therefore, results must be interpreted cautiously, recognizing that the estimated relationship reflects structural dependence rather than an identifiable causal effect.

Given that the analysis is based on a time series comprising 20 observations, the identification of long-run patterns is subject to statistical limitations. Visual inspection is useful as an exploratory tool, yet insufficient to formally establish the stochastic properties of the series. Consequently, unit root tests are applied to assess stationarity in small samples. Due to sample size constraints, the Generalized Least Squares Dickey-Fuller (DF-GLS) test is employed, as it has been proposed as a more efficient alternative to the conventional Augmented Dickey-Fuller (ADF) test in small samples. Table 1 presents the values obtained for each variable from the set of unit root tests. The results provide mixed evidence, indicating stationarity in at least one of the applied tests for each series. For the DF-GLS test, a specification including a constant and no trend was adopted, since the variables are expressed in logarithms and, according to graphical inspection, fluctuate around a constant mean without displaying a clear deterministic trend. It is acknowledged that unit root tests exhibit low statistical power in small samples; therefore, the results should be interpreted with caution and in conjunction with other elements of the econometric analysis.

Given the limited sample size, the results must be interpreted with caution, as residual-based cointegration tests may exhibit low statistical power in small samples. Dynamic multicollinearity may increase the variance of individual estimators and affect their statistical significance; however, it does not introduce bias into the estimated coefficients nor invalidate the interpretation of short- and long-run relationships when evidence of cointegration exists. Its potential presence is therefore acknowledged as an inherent limitation of the adopted dynamic framework. Upon applying the Bounds Test within the Autoregressive Distributed Lag (ARDL) approach, considering the four variables included in the model, an F-statistic of 5.8245 (p -value=0.006783) is obtained, allowing for the rejection of the null hypothesis of no cointegration. The computed statistic exceeds the upper critical bound $I(1)$ values reported by Pesaran *et al.* (2001), thereby supporting the existence of a long-run equilibrium relationship among the analyzed variables. The associated p -value (0.006783) further reinforces this evidence. Nevertheless, given the limited sample length, the Bounds Test results must be interpreted prudently. The analysis is confined to identifying the presence of such a relationship and does not explicitly address

Table 1. Auxiliary Estimation of Unit Root Tests.

Variable	ADF (p-value)	KPSS (p-value)	ADF-GLS F-Statistic	ADF-GLS (p-value)	Test Statistic
Production (lnY)	0.725	0.100	1.359	0.317	-2.440 <CV
Harvested Area (lnSSe)	0.750	0.044	0.679	0.650	-1.687 >CV
Labor (lnMO)	0.173	0.100	1.945	0.173	-2.794 <CV
Nitrogen (lnNitro)	0.707	0.028	0.468	0.792	-1.091 >CV

short-run dynamics through an Error Correction Model (ECM). The estimation of the ECM is beyond the scope of the present study. In light of the cointegration evidence under the ARDL framework, the empirical estimation proceeds through a log-linear Ordinary Least Squares (OLS) model to obtain average long-run elasticities. This approach does not aim to capture the full short-run dynamics or the speed of adjustment toward equilibrium; rather, it seeks to provide a parsimonious and structurally coherent approximation of the long-run relationship among the variables.

RESULTS AND DISCUSSION

The analysis based on growth rates enables the evaluation of short-run dynamics among the variables. Agricultural production theory establishes a causal direction from harvested area, labor, and nitrogen use toward output determination. Growth rates facilitate the examination of short-term changes; thus, causal relationships among variables can be inferred in a temporal and partial manner, while a fully structural approach remains beyond the scope of this section. The calculation of the period growth rate and the average annual growth rate is presented in Table 2. A negative growth rate of -40.67% is observed for harvested area, with an average annual growth rate of -2.03% . Similarly, rice production exhibits a negative growth rate of -9.86% and an average annual growth rate of -0.49% . This negative coevolution is consistent with a short-run causal mechanism in which the contraction of the land factor precedes and partially explains the decline in output. The economically active population in the primary sector displays a positive growth rate of 0.57% and an average annual growth rate of 0.03% , reflecting short-run rigidity in the labor factor. Given the pronounced decline in production, these results suggest that labor is unlikely to constitute the principal causal factor underlying output variation during the analyzed period. Nitrogen fertilizer use presents a positive growth rate of 8.05% and an average annual growth rate of 0.40% , despite the simultaneous decline in production. This pattern indicates that the increase in input use was insufficient to offset the contraction in harvested area, thereby pointing to an indirect and conditional causal effect of fertilizer application.

When interpreted through growth rates, the identified causality must be regarded as indicative and confined to the short run, without implying a strict structural causal relationship. Harvested area emerges as the principal causal factor behind the decline in production; labor assumes a largely passive role during the period, and nitrogen use, although increasing, proves incapable of reversing the downward trajectory of output. Overall, the evidence suggests a causality predominantly driven by extensive changes (land area) rather than intensive factors. Nevertheless, this interpretation warrants caution, as it derives from descriptive growth rates rather than from an explicitly identified structural

Table 2. Period Growth Rate (PGR) and Average Annual Growth Rate (AAGR).

Variable/Rate	Production (Y)	Harvested Area (SSe)	Population (PEAa)	Nitrogen (N)
PGR	-9.86%	-40.57%	0.57%	8.05%
AAGR	-0.49%	-2.03%	0.03%	0.40%

model. The magnitude of the negative growth rates observed particularly in harvested area suggests the presence of a structural break during the 2011-2013 period, which altered the underlying trend behavior of the productive variables. This break may be interpreted as an exogenous or institutional shock, potentially associated with changes in production incentives, adverse climatic conditions, or land-use reallocation affecting the land factor. From a causal perspective, the decline in production would therefore not reflect a gradual adjustment of inputs, but rather a discrete shift in the production regime, where adjustment occurred primarily through the extensive margin. The nearly inertial behavior of the agricultural economically active population reinforces this interpretation by discounting labor as the source of the break, while the simultaneous increase in nitrogen use suggests a compensatory mechanism that ultimately failed to counteract the negative impact of the structural disruption on output. In this context, the structural break constitutes a key causal determinant that conditions the observed growth rates and constrains the validity of interpretations based on a stable input-output relationship throughout the entire period.

Based on the econometric evaluation, the variables included in the log-log Cobb-Douglas specification are statistically significant according to the Student's t-test at a 95% confidence level. The coefficient of determination (R^2) is 0.796, indicating that the model explains approximately 79.64% of the variation in rice production. The results are presented in Table 3. Furthermore, the estimated parameters exhibit the theoretically expected signs for the explanatory variables, thereby confirming consistency with agricultural production theory.

The estimated regression model is:

$$\hat{Y}_I = 1.15 \times 10^{-8} + 0.825 Sse_i + 1.276 PEAa_i + 0.4586 N_i \quad (5)$$

And the estimated Cobb-Douglas production function is:

$$\hat{Y}_I = 1.15 \times 10^{-8} \left(SSe^{0.825} PEA_i^{1.276} N_i^{0.4586} \right) \quad (6)$$

Because the regression is specified in logarithms, the estimated parameters can be interpreted directly as elasticities. The following ceteris paribus effects are observed:

- A 1% increase in harvested rice area is associated with a 0.825% increase in rice production. Thus, rice output exhibits diminishing marginal returns with respect to the land factor.

Table 3. Model parameters and statistical significance.

Variable	Estimated Parameter	Standard Error	t-Value	Prob> t
Intercept	-18.2837	8.715519	-2.10	0.052
lnSSe	0.825909	0.107363	7.69	0.000
lnNitro	0.458698	0.144882	3.17	0.006
lnMO	1.276668	0.536724	2.38	0.030
R^2	0.7964			

- A 1% increase in the economically active population in the primary sector is associated with a 1.276% increase in rice production, indicating increasing marginal returns with respect to labor.
- A 1% increase in total nitrogen fertilizer use is associated with a 0.4586% increase in rice production. Therefore, rice output exhibits diminishing marginal returns with respect to the capital proxy.

The sum of rice production elasticities is given by $0.825 + 1.276 + 0.4586 = 2.5596$, which exceeds unity and suggests increasing returns to scale within the estimated econometric model.

Since the sum of the estimated elasticities is greater than one (2.5596), increasing returns to scale are inferred. However, this relationship reflects aggregated econometric behavior and does not allow for a conclusive inference regarding a specific stage of the production process, as the estimation does not explicitly identify technological or structural conditions of the production system. Table 4 presents the results of the statistical evaluation, including the corresponding diagnostic tests.

The diagnostic tests indicate normally distributed residuals according to the Jarque-Bera and Shapiro-Wilk tests. To assess residual autocorrelation, the Breusch-Godfrey test suggests no evidence of serial correlation. Although the Durbin-Watson statistic indicates autocorrelation, the LM test corroborates the absence of first-order autocorrelation. Homoskedasticity is supported by the Breusch-Pagan test. In addition, the Variance

Table 4. Results of the rice production model tests.

Test	Statistical	Diagnostic
Jarque Bera	JB=0.50089	Normality in errors
	p-value=0.7785	
Shapiro-Wilk	W=0.96406	Normality in errors
	p-value=0.62774	
Breusch-Godfrey	p-value=0.3936	No autocorrelation
Durbin Watson	DW=1.101	Autocorrelation
	p-value=0.002898	
LM-test	LM test=2.2654	No autocorrelation at one lag
	p-value=0.1323	
Breusch-Pagan test	BP=4.1268	Homoscedasticity
	p-value=0.2481	
VIF	INSse=1.85; INPEAa=1.81; INNitro=1.21	Multicollinearity Values <10
Ramsey	RESET=4.1409	Model specification
	Prob>F=0.0599	

InSse=logarithms harvested rice area; InNitro=logarithms nitrogen; InMo=logaritms population; JB=Jarque Bera; W=Shapiro-Wilk statistic; DW=Durbin Watson; LM=Lagrange Multiplier test; BP=Breusch-Pagan test; VIF=Variance Inflation Factor, and RESET: Ramsey RESET test.

Inflation Factor (VIF) results indicate no evidence of severe multicollinearity among the independent variables.

The Ramsey RESET test points to a potential model misspecification; although the null hypothesis is not rejected at the 5% level, the result lies near the threshold, suggesting that the model may be omitting nonlinearities, interaction terms, or other relevant variables. Overall, the model is deemed efficient within the limits of an abstract and parsimonious specification. The study confirms a decline in both rice production and harvested area, consistent with Reyes *et al.* (2024), who analyze the rice market in Mexico and document a structural relationship associated with the contraction of cultivated area. The findings are also aligned with Ireta *et al.* (2016), who report a negative effect of area on rice production (-7.7), suggesting land-use-related production constraints. In contrast, these results differ from Caicedo *et al.* (2019) and Rivera *et al.* (2015), who argue that production expansion does not depend primarily on cultivated area but rather on yield improvements. A key limitation is that rice is the second highest-yielding cereal nationally; moreover, the gap between sown and harvested area, together with sensitivity to climatic variability, introduces additional uncertainty into the area-production relationship an important constraint acknowledged but not explicitly modeled in this study. Harvested area is identified as the second-most influential variable in rice production, with an estimated elasticity of 0.825. This positive effect is conditioned by the heterogeneity of production systems, which imposes additional constraints on crop expansion. In Mexico, rice-growing regions operate under distinct technological regimes, including flood irrigation in central and southern regions, direct seeding under irrigation in northern and western regions, and rainfed cultivation in the southeast (Favilla & Herrera, 2023).

These technological differences substantially affect productivity; however, they are not incorporated explicitly in the empirical analysis in accordance with the principle of econometric parsimony, favoring a simple specification estimable with a short sample. Consequently, the estimated coefficients should be interpreted as average elasticities conditional on an implicit technological level. From the econometric evaluation, the variable with the largest positive impact is the economically active population in the agricultural sector (1.276), implying a more-than-proportional association with rice output. Notably, agricultural labor participation has exhibited a downward trend in employment generation, consistent with García and Omaña (2001), who identify a negative composition effect: structural change in the productive system reduced employment levels at a rate of -0.2% annually, reflecting limited absorption capacity in agricultural employment. The result for the economically active population differs from Pérez *et al.* (2019), who find a negative relationship with production (-2.274%), attributing it to an oversupply of workers in the agricultural sector.

It also contrasts with evidence reported by Zhu *et al.* (2024), in which the rural economically active population plays a critical role in reducing rice cultivation intensity. In the present study, the computed growth rate of the agricultural economically active population (0.5%) may be linked to rural-urban migration and the persistence of labor informality phenomena that reduce labor retention in the primary sector and constrain short-run expansion, as documented for the Mexican context (Lara *et al.*, 2020). Regarding

nitrogen dynamics, the estimated impact on rice production is comparatively smaller, reflecting less than one percentage point in output response to a 1% increase in nitrogen use. Although fertilizer is a productive input with a positive effect, Mexico is characterized by substantial dependence on imports, reaching 67.1% (Gaucín & Torres, 2011). Tosquy *et al.* (2008) report that the use of nitrogen fertilizers can increase rice production costs by up to 27%. In the literature, studies linking rice and fertilizer often emphasize price elasticities; for example, Reyes *et al.* (2024) estimate -0.2 , and Guzmán *et al.* (2019) report -0.32 for bean cultivation. It is also estimated that the expansion of high-yield programs promoted excessive use of chemical nitrogen fertilizers (González, 2017, p. 1743). Given the selection of proxy variables particularly the economically active population and nitrogen use the analysis faces constraints derived from limited empirical documentation, which restricts the depth with which the results can be contrasted and contextualized.

CONCLUSIONS

The study demonstrates a sustained contraction in both rice production and harvested area, alongside a positive output response to production inputs. The estimates reflect associations and should not be interpreted as strict causal relationships. The estimated relevance of labor, harvested area, and nitrogen use is conditioned by the use of proxy variables, the omission of technological factors, and the aggregated nature of available information. Therefore, while the findings are consistent with economic theory, they remain subject to limitations stemming from sample size, data availability, and the scarcity of crop-specific empirical evidence.

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Biodegradable films derived from devil fish skin collagen (*Pterygoplichthys pardalis*)

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ABSTRACT

Objective: This study aimed to evaluate the feasibility of using collagen extracted from *Pterygoplichthys pardalis* skin to develop biodegradable films.

Design/methodology/approach: Collagen was extracted by assessing the effects of the acid-to-skin ratio and extraction time using a 2² factorial experimental design. The recovered collagen was characterized by SDS-PAGE, Fourier-transform infrared spectroscopy (FTIR), and ultraviolet-visible (UV-Vis) spectroscopy. Subsequently, films were formulated using a 2² factorial design with collagen concentrations (1 and 2%) and glycerol levels (10 and 20 mL), incorporating arabic gum. Mechanical performance including tensile strength, elongation at break, and Young's modulus was determined, alongside thermal properties (glass transition temperature, Tg; denaturation temperature, Tm) and biodegradability.

Results: The maximum collagen extraction yield reached 54%. The resulting films exhibited an average tensile strength of 1.26 ± 0.17 MPa, elongation at break of $15.99 \pm 0.07\%$, and Young's modulus of 22.09 ± 0.078 MPa. Tg ranged from -13 °C to -17 °C, while Tm varied between 140 °C and 158 °C. The biodegradability index attained $77.74 \pm 6.76\%$.

Limitations on study/implications: The work was performed at laboratory scale; therefore, additional studies are required to evaluate process scalability and the prospective industrial applicability of the films.

Findings/conclusions: Collagen derived from *P. pardalis* skin represents a viable alternative feedstock for producing biodegradable biomaterials with promising mechanical and thermal characteristics, supporting fish-waste valorization and the development of more sustainable materials.

Keywords: Fish waste; biodegradability; texture; spectroscopy; SDS-PAGE.

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INTRODUCTION

For decades, polymers such as polyvinyl chloride, polyethylene, polypropylene, and polystyrene have been extensively used in the manufacture of plastic packaging owing to their low weight, water resistance, and adequate mechanical performance (Jones *et al.*, 2020). Nevertheless, their non-biodegradable nature and improper disposal have driven the excessive accumulation of plastics across multiple ecosystems, resulting in



substantial and long-lasting environmental impacts (Esterhuizen & Kim, 2022). In response, packaging-material research has increasingly focused on developing films and materials derived from biomolecules including proteins, polysaccharides, and lipids as sustainable alternatives to petroleum-based materials (Amin *et al.*, 2021). In particular, agro-industrial and fishery residues have gained prominence as renewable sources of structural compounds most notably cellulose, chitosan, and collagen due to their availability, low cost, and strong valorization potential (Teixeira-Costa & Andrade, 2022). Within this context, collagen has become one of the most widely utilized proteins for biomaterial fabrication because of its bioactivity, biocompatibility, and biodegradability, as well as its ability to form polymeric networks with tunable mechanical and functional properties (Gallo *et al.*, 2022; Yamaura *et al.*, 2022). Structurally, collagen consists of polypeptide chains characterized by the repeating amino-acid motif [Gly-X-Y]_n, which gives rise to its distinctive triple helix. This architecture directly governs its thermal stability, intermolecular interactions, and bioactivity (Zeugolis & Raghunath, 2011). Fish represent a relevant source of collagen, predominantly type I, which is highly demanded by the pharmaceutical, biomedical, and cosmetic industries (García-Sifuentes *et al.*, 2019; Atay *et al.*, 2022; Irastorza *et al.*, 2021; Boland *et al.*, 2018; Tacias-Pascacio *et al.*, 2020; Valbuena *et al.*, 2020). Moreover, more than 70% of the biomass generated by the fishing industry is considered waste, which has encouraged the use of skins, scales, and skeletons as feedstocks for collagen recovery, often achieving yields comparable to or higher than those obtained from traditional mammalian sources (Erasmus *et al.*, 2021; Venugopal, 2021; Wijaya & Juanito, 2021). This approach not only reduces the environmental burden associated with fishery by-products, but also mitigates risks related to transmissible diseases and circumvents religious restrictions commonly associated with terrestrial collagen sources (Rodríguez-Salvador & Dopico, 2020). Most studies to date have focused on the extraction and characterization of collagen from high-commercial-value species, such as tilapia (*Oreochromis niloticus*), salmon, and cod (Hoyer *et al.*, 2012; Raftery *et al.*, 2016; Lovett *et al.*, 2020). In contrast, a knowledge gap persists regarding the utilization of collagen from invasive or non-commercial species, particularly with respect to its application in forming biodegradable films and packaging materials. *Pterygoplichthys pardalis* is a widely distributed invasive species in diverse aquatic ecosystems, whose proliferation generates negative ecological and economic impacts. The skin of this species constitutes a promising collagen source with a relevant hydroxyproline content an amino acid associated with triple-helix stability and enhanced intermolecular interactions which may translate into materials with improved thermal and mechanical performance (Nurubhasha *et al.*, 2019; Sun *et al.*, 2021). However, studies exploring collagen from invasive species for the development of films or functional biomaterials remain limited.

Against this backdrop, the present work aims to produce and characterize a biodegradable material based on collagen extracted from *P. pardalis* skins via an acid-based method, in order to assess its technological potential as a film for packaging applications. Implicitly, we hypothesize that the hydroxyproline content of this collagen promotes the formation of polymeric networks with greater thermal stability and superior

mechanical properties, thereby supporting the development of a sustainable alternative to conventional plastics and enabling the valorization of invasive biomass.

MATERIALS AND METHODS

Sample collection and skin pretreatment

Fish were obtained from the Peñitas Reservoir, in the municipality of Ostuacán, Chiapas, Mexico (latitude 17.843, longitude -93.487). A total of 68 adult *P. pardalis* individuals (mean weight: 389.96±99.61 g) were collected and transported in coolers under refrigerated conditions. Approximately 2381.2±129.45 g of fresh skin were excised, washed, and stored under refrigeration. The skin was dried at 60 °C for 72 h; these conditions do not compromise type I collagen integrity, as type I collagen from fish skin has been reported to exhibit a denaturation temperature of approximately 97.8 °C (Majeed *et al.*, 2025). Subsequently, the dried skin was ground and sieved to a particle size of 0.6 mm to maximize the available surface area.

Pretreatment of raw skins was carried out according to the methodology proposed by Sun *et al.* (2021), with minor modifications. Briefly, 100 g of dry raw material (skin) were mixed with 0.1 M NaOH at a 1:10 (w/v) ratio for 6 h at 4 °C, replacing the NaOH solution every 3 h to remove non-collagenous proteins and skin pigments. The skin was then washed with distilled water until neutral pH (7.0-7.5) was reached. Next, the skin was defatted using 10% butanol at a 1:10 (w/v) ratio for 24 h and subsequently washed three times with distilled water.

Acid-soluble collagen extraction

Collagen extraction was performed following the methodology described by Atef *et al.* (2020). A completely randomized 2² factorial design was applied with two factors: acid-to-skin ratio (10:1 and 20:1, v/w) and extraction time (24 and 72 h). Each treatment was conducted in triplicate (Table 1).

The samples were mixed with 0.5 M acetic acid as the extraction solvent, given its reported effectiveness for mild collagen extraction. The mixture was maintained at 4 °C, filtered, and neutralized with 0.1 M NaOH to pH 7.0 (monitored using a potentiometer). Subsequently, the suspension was centrifuged at 4000 rpm (Eppendorf model 5810R, Hamburg, Germany) for 20 min at 4 °C to separate soluble collagen in the supernatant from the precipitated fraction. The supernatant was then lyophilized at 0.520 mBar for 48 h (Labconco, Kansas, USA) and stored at 4 °C until further use.

Table 1. Experimental design for acid-soluble collagen extraction.

Treatment	Acid:skin ratio (v:w)	Extraction time (h)
I	10:1	24
II	20:1	24
III	10:1	72
IV	20:1	72

v:w=volume:weight ratio.

Characterization of the extracted collagen

Extraction yield

Extraction yield was determined as the ratio between the mass of the lyophilized product and the mass of the initial material (dry skin weight), as described in Equation 1:

$$Yield(\%) = \frac{Collegen(g)}{Dry\ skin\ weight(g)} \times 100$$

UV-Vis spectroscopy

Lyophilized collagen was dissolved in 0.5 M acetic acid at a 1:100 (w/v) ratio. The solution was transferred to a 1 cm quartz cuvette and scanned from 190 to 1100 nm to identify the wavelength of maximum absorbance using a DR 5000 spectrophotometer (HACH, Loveland, USA). The baseline was established using 0.5 M acetic acid.

Protein quantification

Protein concentration was determined using the Lowry method, employing a bovine serum albumin (BSA) calibration curve. Briefly, 2 mL of collagen sample solutions (1 g/mL in 0.5 M acetic acid) were mixed with 5 mL of Lowry reagent, and UV absorbance was measured at 500 nm (DR 5000, HACH, Loveland, USA) (Song *et al.*, 2021).

Fourier-transform infrared spectroscopy (FTIR)

Functional groups present in *P. pardalis* collagen were identified following the procedure reported by Zhang *et al.* (2020). FTIR spectra were recorded using a Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific Inc., Madison, USA). Lyophilized collagen was placed on an attenuated total reflectance (ATR) cell at ambient temperature. Signals were collected over 64 scans in the 4000-650 cm^{-1} range at a resolution of 2 cm^{-1} and compared with a background spectrum acquired from a clean, empty cell at room temperature (25 °C).

Molecular-weight profile of the extracted collagen

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970), with minor modifications. The resolving gel was prepared at 8% and the stacking gel at 5%. Gels were stained with 1% Coomassie Brilliant Blue. Electrophoresis was conducted at 80 V for 20 min followed by 120 V for 50 min, using 1x running buffer (25 mM Tris-Cl/250 mM glycine/0.1% SDS). Type I collagen extracted from rat tail was used as the standard (Sigma-Aldrich, USA). Electrophoresis was carried out in a Mini-PROTEAN system (Bio-Rad, Hercules, USA).

Preparation of collagen films

Films were formulated using a dispersion of collagen, gum arabic, and glycerol. First, gum arabic (1.5%) was hydrated for 24 h using triple-distilled water. Glycerol was then added, followed by the gradual addition of collagen. Formulations were prepared according

to a 2^2 experimental design with two central points and two axial points, using collagen concentration (1 and 2%) and glycerol (10 and 20%) as factors.

Dispersions were held for 24 h at room temperature, heated to 70 °C, and stirred, then cast into 5 cm-diameter Teflon-coated aluminum molds and dried at 23 °C for 24 h, as described by Andonegi *et al.* (2020).

Film analysis

Mechanical properties

Prior to mechanical testing, films were equilibrated at 25 °C and 75% relative humidity for 48 h. Mechanical properties tensile strength (TS), elongation at break (EAB), and Young's modulus (E) were determined according to ASTM D882 (American Society for Testing and Materials, 1996) using a TA.XT Plus texture analyzer (Stable Micro Systems, London, UK), with a 20 mm initial grip separation, a crosshead speed of 5 mm/min, and 80% deformation. Films were cut into 50×10 mm strips, and all measurements were performed in triplicate.

EAB was calculated as the ratio between the change in film length at rupture (L) and the initial gauge length (L'), as described in Equation 2:

$$EAB = L \frac{L}{L_0} \quad (2)$$

Cutting force (F) was obtained using Exponent software v6.1.16.0 (Stable Micro Systems, London, UK) at the moment of film rupture. Tensile strength (TS) was estimated using Equation 3, where F denotes the cutting force and A corresponds to the film cross-sectional area.

$$TS = \frac{F}{A} \quad (3)$$

Young's modulus (E) was calculated as the ratio between the increase in applied stress (TS) and the corresponding increase in elongation (EAB) of the tested material, using Equation 4:

$$E = \frac{TS}{EAB} \quad (4)$$

Differential scanning calorimetry (DSC)

The glass transition temperature (T_g) and denaturation temperature (T_m) of the films were determined in triplicate (n=3) using a Q-2000 differential scanning calorimeter (TA Instruments, New Castle, USA), following the methodology of Valencia *et al.* (2019). Approximately 10 mg ± 0.002 mg of sample were placed in aluminum pans under a dry nitrogen purge (50 mL/min). Films were cooled to 0 °C and subsequently heated from −80 to 200 °C at a heating rate of 20 °C/min. This analysis provided

indirect information regarding the thermally stable structural integrity of collagen within the films.

Biodegradability index

The biodegradability index (BI) was determined according to the oxygen-consumption methodology described in OECD 301 (Organization for Economic Cooperation and Development, 1992), which is based on the ratio between chemical oxygen demand (COD) and biochemical oxygen demand (BOD'), as expressed in Equation 5. All analyses were performed in triplicate.

$$BI = \frac{DBO_5}{DQO} \times 100 \quad (5)$$

Chemical oxygen demand (COD) was estimated using the micro-reflux method described in the HACH manual for chemical reactors. An aqueous solution of skin collagen (1:10,000, w/v) was prepared and digested at 150 °C for 2 h. Subsequently, the sample was transferred to a quartz cuvette, and absorbance was measured at 620 nm using a UV-Vis spectrophotometer (DR-5000, HACH, Loveland, USA).

Biochemical oxygen demand was determined as the difference between the initial dissolved oxygen and the dissolved oxygen remaining after five days of incubation at 20 °C. Incubation was conducted using a BOD Trak I respirometric analyzer (HACH, Loveland, USA), with PolySeed® as the inoculum. Although this method is not specific to solid polymers, it provides a preliminary indicator of the intrinsic biodegradability of collagen as an exploratory stage of the study.

Statistical analysis

All experiments were performed in triplicate. One-way ANOVA was used to assess the effects of the factors and their interactions on extraction yield at a 95% confidence level. Mean differences were evaluated using confidence intervals with Tukey's test. Statistical analyses were conducted using Statgraphics software (version XV).

RESULTS AND DISCUSSION

Fish skin is a valuable collagen source, as reported by Virtanen *et al.* (2017). Collagen recovery yields from *P. pardalis* skin are presented in Table 2, where a statistically significant difference ($p < 0.05$) was observed among all treatments. In this experiment, yields ranged from 37.4% to 57.05% and were significantly higher than those reported for eel skin (Govindharaj *et al.*, 2019), elasmobranch by-products (Seixas *et al.*, 2020), and swim bladders from Atlantic cod (*Gadus morhua*) (Sousa *et al.*, 2020). The pronounced variability in collagen yields is attributed to differences in collagen architecture among the distinct by-products and species evaluated (Wu *et al.*, 2016). The highest yield was achieved using an acid-to-skin ratio of 1:20 (v/w) and an extraction time of 24 h.

Analysis of variance showed that the acid-to-skin ratio (v/w) exerted a statistically significant positive effect ($p < 0.05$) on extraction yield, whereas the interaction between

Table 2. Extraction yields of acid-soluble collagen from *P. pardalis* skin.

Ratio (mL g ⁻¹)	Time (h)	Yield (%)
10:1	24	37.40d
20:1	24	57.05a
10:1	72	41.16c
20:1	72	51.88b
LSD		3.68

LSD=Least significant difference. Different letters within the same column indicate statistically significant differences among treatments ($p<0.05$).

both factors had a statistically significant negative effect on the response variable (Figure 1). The positive influence of the acid-to-skin ratio has been previously described for collagen extraction processes (Dhakal *et al.*, 2018) and is attributed to an enhanced hydrolysis-driven solubilization mechanism without inducing collagen degradation (Arumugam *et al.*, 2018).

Regarding the statistically significant negative effect of the interaction between both factors, this outcome may be explained by the fact that increasing both solvent concentration and extraction time can promote collagen denaturation, thereby reducing the amount of collagen recovered (Surasani *et al.*, 2019). In this respect, it is crucial to establish whether a critical solvent concentration exists beyond which further increases no longer enhance protein extraction. Otherwise, prolonged extraction times may intensify solvent-collagen interactions, potentially leading to deprotonation of functional groups that are not exclusively associated with cross-links between collagen α -helices, ultimately destabilizing and denaturing the protein (Tan & Chang, 2018).

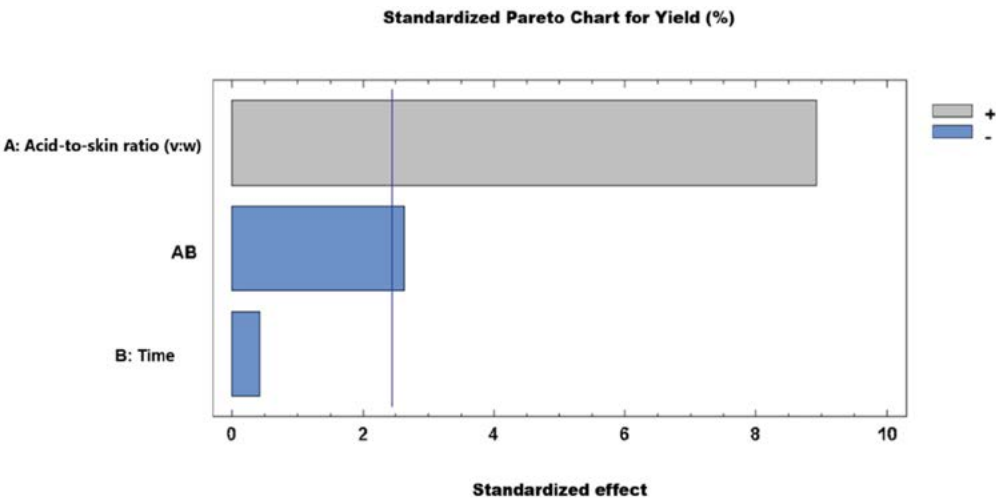


Figure 1. Standardized Pareto chart for collagen extraction yield obtained via acid extraction from *P. pardalis* skin. A represents the raw-material-to-extraction-solution ratio (w/v), and B represents extraction time (h).

Spectroscopic characterization of collagen

Fourier-transform infrared spectroscopy (FTIR)

The IR spectrum of *P. pardalis* skin collagen exhibited absorption bands at 2930, 1640, 1550, 1415, and 1019 cm^{-1} . The band at 2930 cm^{-1} corresponds to the asymmetric stretching of the $\text{Csp}^3\text{-H}$ bond (secondary amine) (Moula Ali *et al.*, 2017). Bands in the 1600-1700 cm^{-1} region have been associated with C=O stretching vibrations and hydrogen bonding coupled with COO^- , corresponding to amide I (S. S. Wang *et al.*, 2019). The absorption at 1550 cm^{-1} arises from N-H bending coupled with C-N stretching of amide II. Finally, amide III was represented by the absorption band at $\sim 1410\text{-}1415\text{ cm}^{-1}$, attributed to $\text{Csp}^3\text{-H}$ vibrations (Bi *et al.*, 2019). This band is related to the degree of molecular order in collagen and participates in the formation of the triple-helix structure (Chen *et al.*, 2016). In addition, a band near 1000 cm^{-1} corresponding to the $\text{Csp}^3\text{-O}$ bond characteristic of hydroxyproline was observed. Overall, the IR signals were consistent with those reported for collagen extracted from *P. pardalis* (Nurubhasha *et al.*, 2019) and from other fish species (El-Rashidy *et al.*, 2015; Sionkowska *et al.*, 2015).

UV-Vis spectroscopy

To obtain the UV-Vis spectrum of the protein extract, a spectral scan from 190 to 1100 nm was performed, revealing a maximum absorbance at 220 nm. Because fish collagen contains low levels of tryptophan, tyrosine, and phenylalanine and due to the presence of C=O , -COOH , and CONH_2 groups the maximum wavelength of acid-soluble collagen (ASC) is shorter ($\lambda_{\text{max}}=220\text{ nm}$) than that of many other proteins (Veeruraj *et al.*, 2015), whose absorption maxima are typically near 280 nm (Wang *et al.*, 2018). Comparable results have been reported for collagen extracted from Pacific cod (*Gadus macrocephalus*) skin (Sun *et al.*, 2017), catla (*Catla catla*), and rohu (*Labeo rohita*) (Pal & Suresh, 2016).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

To identify the collagen type extracted from *P. pardalis*, SDS-PAGE was used to separate protein fragments. Bands with molecular weights of 243 kDa, 122 kDa, and 110 kDa were detected (Figure 2). Although SDS-PAGE does not allow unequivocal assignment of the characteristic α - and β -chains of collagen, the observed banding pattern is consistent with the type I collagen profile previously reported for chemically extracted acid-soluble collagen from Nile tilapia skin (Hu *et al.*, 2017), as well as from tilapia and grey mullet (Shalaby *et al.*, 2020). Moreover, SDS-PAGE confirmed the effectiveness of the extraction method, as no bands attributable to non-collagenous proteins were observed.

Tensile strength, elongation at break, and Young's modulus of the films

Tensile strength values ranged from 0.23 to 2.92 MPa across all treatments, and were significantly lower than those reported by Erciyes & Ocaik (2019) for collagen-hydrolysate films. According to conventional benchmarks, the tensile strength of the films developed in this study falls below recommended values (Hosseini *et al.*, 2016). Figure 3 illustrates the effects of collagen concentration and glycerol content, as well as their combined influence,

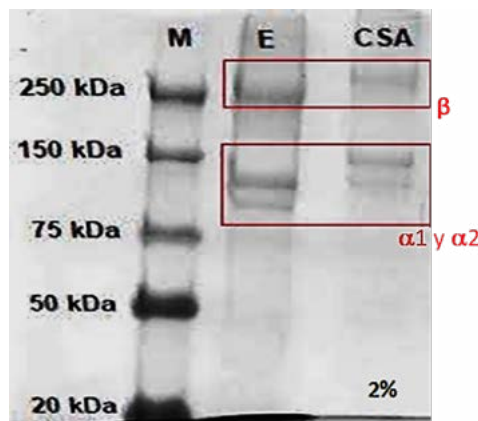


Figure 2. Electrophoretic profile of collagen extracted from *Pterygoplichthys pardalis* skin. M=molecular-weight marker; E=type I collagen standard; ASC=acid-soluble collagen.

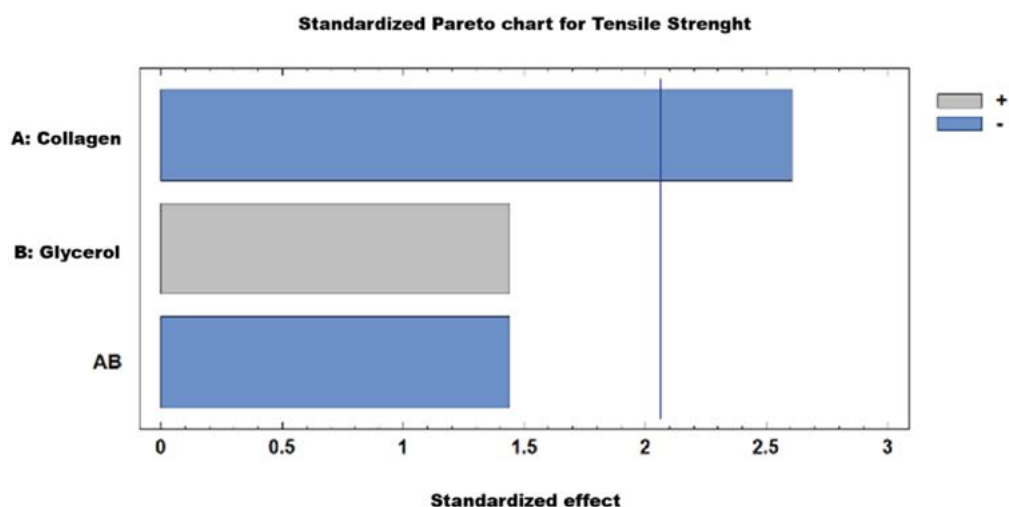


Figure 3. Effect of collagen (%) and glycerol (%) concentrations on the tensile strength of collagen films obtained from *P. pardalis* skin.

on the mechanical properties of the films (tensile strength, elongation at break, and Young's modulus).

Figure 4 shows a statistically significant negative effect of collagen concentration on tensile strength. This indicates that, as collagen concentration increased, film tensile strength decreased. This behavior may be explained by the fact that, rather than forming an effectively cross-linked network with gum arabic and/or glycerol, collagen molecules may slide relative to one another (Gómez-Guillén *et al.*, 2011; Tian *et al.*, 2021), potentially due to partial loss of mechanical functionality during the extraction process (Vergne *et al.*, 2018). In contrast, no statistically significant effect of the factors on film elongation at break was detected ($p > 0.05$). Nevertheless, a slight trend toward higher elongation at break with increasing collagen concentration was observed, a phenomenon commonly attributed to the hydrophilic character of collagen, which promotes film hydration (Slimane & Sadok, 2018).

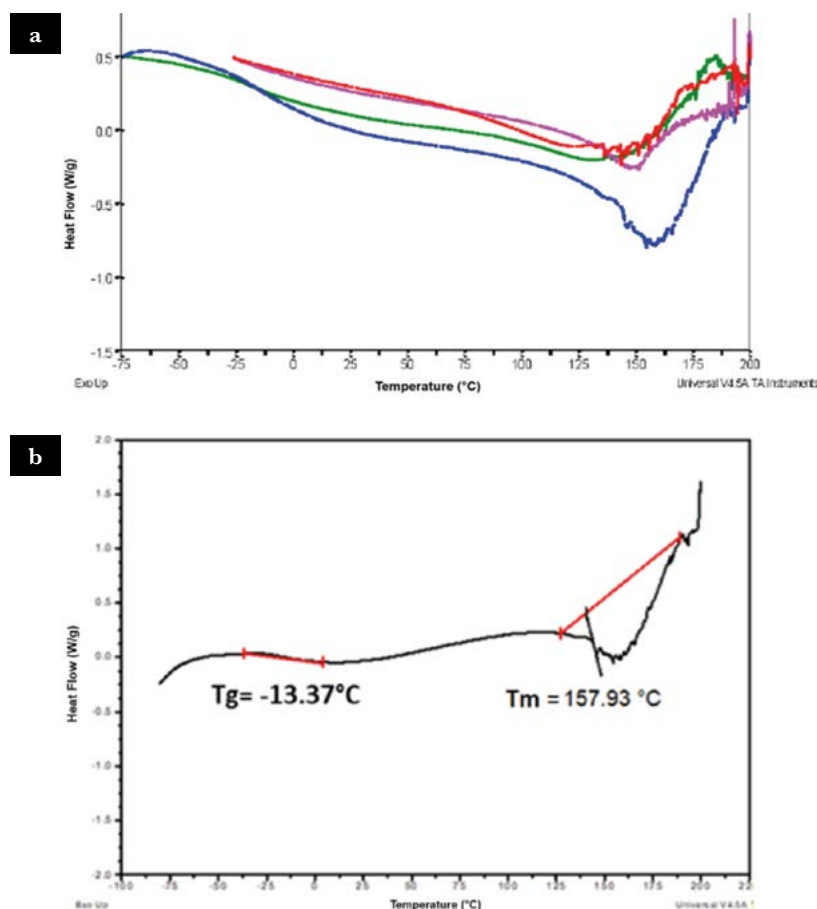


Figure 4. [a] Glass transition (Tg) and [b] denaturation (Tm) temperatures in thermograms of collagen films extracted from *P. pardalis* skin.

Ahmad *et al.* (2016) reported that the glass transition temperature (Tg) is governed by the structural features of polymeric materials, including molecular weight, chain branching, and the degree of cross-linking among the formulation components. Consistent with the tensile-strength trends, the absence of pronounced cross-linking between collagen and gum arabic and/or glycerol may explain why the Tg values of the films evaluated here were higher than those reported by Valencia *et al.* (2019), who obtained Tg values below -50°C . The endothermic peak around 150°C was associated with the film denaturation temperature (Chakrapani *et al.*, 2012), as previously described for collagen-based films (Andonegi *et al.*, 2020). The higher denaturation temperatures observed for *P. pardalis* collagen may be attributed to the stability conferred by its elevated hydroxyproline content (Sun *et al.*, 2021), an amino acid that can promote a higher degree of intermolecular cross-linking due to the presence of hydroxyl groups (Tatli *et al.*, 2018).

Biodegradability index

The biodegradability index (BI) is defined by the relationship between chemical oxygen demand (COD) and biochemical oxygen demand (BOD₅). This parameter was calculated after five days of incubating the samples at 20°C . The films developed in this

study presented COD values of $1.203 \times 10^6 \pm 3.682 \times 10^4$ mg O₂/L and BOD₅ values of $9.354 \times 10^5 \pm 5.310 \times 10^4$ mg O₂/L, yielding a BI of $77.74 \pm 6.76\%$. This indicates that 77.74% of the organic matter present in the sample is biodegradable within five days at 20 °C. Accordingly, the films can be classified as biodegradable materials under the criterion reported by Arias *et al.* (2012). The biodegradability index exhibited by collagen films produced from *P. pardalis* skin supports the inference that these films could serve as an alternative to materials such as nylon, given that they displayed a BI higher than that reported by Tachibana *et al.* (2013) for four types of nylon films. The COD, BOD₅, and BI values obtained in this work are particularly relevant, as biodegradability assessments for this type of film have not been previously reported. Atarés & Chiralt (2016) attributed film biodegradability to the organic and biological nature of the components used in their formulation, although this has not been conclusively demonstrated. Based on the present results, it can be stated that films fabricated from acid-extracted *P. pardalis* skin collagen, glycerol, and gum arabic are biodegradable, since most of the organic and inorganic matter present can be oxidized biochemically.

CONCLUSIONS

Collagen extraction from *P. pardalis* skin represents a promising route for the commercial valorization of this invasive species. Under controlled conditions (acid-to-skin ratio 1:20, v/w; extraction time 24 h), an extraction yield close to 60% was achieved. Using this collagen, films with high elongation capacity and a high biodegradability index (>70%) were produced, features that may position this collagen as a valuable feedstock for the biopolymer industry. However, further studies should evaluate alternative extraction strategies such as enzymatic methods which may better preserve the molecular structure and, consequently, the mechanical performance of collagen.

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Sanitary analysis of compost derived from canine origin: pilot study

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ABSTRACT

Objective: To evaluate the sanitary quality of compost derived from canine origin through parasitological, bacteriological, biochemical, and molecular analyses, focusing on the detection of enteropathogens relevant to public health.

Design/methodology/approach: Samples of compost derived from canine origin underwent parasitological, bacteriological, biochemical, and molecular analyses to detect enteropathogens such as *Escherichia coli* (ETEC) and *Salmonella* spp. Additionally, 16S rRNA gene sequencing was performed for species-level identification.

Results: No parasite structures were found, and molecular tests for *E. coli* and *Salmonella* spp. were negative. However, colonies suggestive of *Salmonella arizonae* were isolated by the research team. Subsequent 16S rRNA sequencing analysis revealed homology with *Citrobacter arsenatis*, a recently described bacterium.

Limitations on study/implications: The study was limited to a single compost source and focused on a few bacterial pathogens reported in canines (*Escherichia coli* y *Salmonella* spp.); broader microbiome analysis and pathogenicity assessments are recommended. The findings underline the need for rigorous characterization before the agricultural application of animal-origin compost.

Findings/conclusions: Compost derived from canine origin does not appear to pose a significant public health risk due to the presence of enteropathogens such as *Escherichia coli* (ETEC) and *Salmonella*, according to our analyses, although the pathogenicity of *Citrobacter arsenatis* is unknown. However, as it is compost derived from dog carcasses, it may contain opportunistic or pathogenic microorganisms of sanitary importance. Thus, its use is recommended for ornamental plants, lawn, trees.

Keywords: Carcasses, Composting, Public Health, Zoonosis.

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INTRODUCTION

Composting is a biological process that takes place under aerobic conditions, in which microorganisms carry out the biotransformation of organic matter through the biogeochemical cycles of carbon and nitrogen. The process includes three stages of temperature that result from microbial metabolism: a) Mesophilic I, b) Thermophilic (up to 60 °C), and c) Cooling or Mesophilic II, in addition to a stage of maturation of variable duration (Román, 2013). As a result, a biological product whose microbiological



complexity is related to biofertilizing activities is obtained. Composting constitutes one of the best alternatives for treatment and valuation of organic urban solid residues (Pérez, 2019).

Animal residues in Mexico have scarce specific regulations in relation to compost elaboration (Rodríguez and Córdova, 2006). In livestock production, mortality composting is an alternative for the management of animal residues, following a locally adapted methodology, with an emphasis on cost savings, reduction of environmental risks, and generation of potential biofertilizers (Bass and Lupis, 2010). Regarding the remains of companion animals, there has been an increase in private incineration services or those offered by public institutions (PROFECO, 2022), although there are scenarios such as shelters or animal control centers where there is a lack of equipment or elevated costs, generating a problem for the safe elimination of biological residues, for which composting could be an alternative.

Although aerobic composts harbor high microbiological diversity, including microorganisms with effects that promote plant growth and biological control of plant diseases (Martínez-Yáñez *et al.*, 2023), in the case of compost of animal origin, the presence of microorganisms with potential risks for public health is likely. Therefore, it is necessary to determine the sanitary quality of animal-based composts (Bass and Lupis, 2010). In this context, thermo-tolerant coliforms are a group of Gram-negative bacteria that belong to enterobacteria with pathogenic potential. According to the United States Environmental Protection Agency, a high level of thermotolerant coliforms is an indicator of the likely presence of pathogenic bacteria such as enterotoxigenic *Salmonella*, *Shigella*, *Leptospira*, *Listeria* and *E. coli*, which cause public health problems (Román, 2013).

Escherichia coli, Gram-negative facultative anaerobic bacillus, is a microorganism that is part of the normal microbiota of canines, and can become an important nosocomial pathogen (Alberca, 2017).

Salmonella, Gram-negative facultative anaerobic bacillus, non-sporulated, generally mobile by peritrichous flagella, is a microorganism found in asymptomatic carrier dogs of both antimicrobial-resistant and potentially pathogenic *Salmonella* strains. Dogs can harbor a large bacterial load in their intestines and mesenteric lymphatic ganglia, which can be eliminated through feces, with possibility of transmission to humans (Usmael *et al.*, 2022).

Another opportunistic nosocomial pathogen is *Citrobacter* spp., Gram-negative facultative anaerobic bacteria, which does not form spores and is rod-forming, reported in intestinal tracts of animals and humans; it is commonly associated with infections of various organs in adults and neonates (Jabeen *et al.*, 2023).

In this study, the hypothesis is set out that compost elaborated from canine carcasses will contain enteropathogenic microorganisms with zoonotic potential. With this purpose, the objectives were to identify parasites and enterobacteria of sanitary importance through isolation methods, biochemical characterization, and molecular confirmation. This analysis is relevant given the absence of specific regulations for the handling of animal carcasses such as dogs and cats, and the need for environmentally safe alternatives and socially viable for their disposal.

MATERIALS AND METHODS

Compost

Humus from compost elaborated with the technique based on the model by Rodríguez and Córdova (2006) was received, where a 3-month-old canine carcass, sawdust (product from sterilized wood, 11 kg), plant carbon ash (5 kg), zeolite of volcanic origin (1 kg), plant residues (2.5 kg), and water (8 L), were used in a 56 cm by 36 cm drum-type plastic recipient, with aeration holes and temperature monitoring. No clinical analysis or previous treatments were carried out with the live animal. The compost was monitored for 35 days, performing the first turning at 15 days and then once per week. During the thermophilic stage, 60 °C and 60% relative humidity were recorded. Personal protection gear was used both for the elaboration and while turning the compost.

Molecular analysis: DNA extraction from composts and molecular detection of thermotolerant enterobacteria

For the DNA extraction of the compost sample by triplicate, the DNeasy Powersoil Kit (QIAGEN Cat. No. 47014) was used, following the instructions from the manufacturer. For the molecular detection of thermotolerant enterobacteria, *Escherichia coli* pathotype ETEC (López-Saucedo, 2003) and *Salmonella* sp. (Liu, 2002), the pairs of oligonucleotides and PCR conditions described in Table 1 were used.

The cycling program for PCR was 5 min at 94 °C initial denaturalization, followed by 35 cycles for 45 s at 94 °C denaturalization, 45 s at the alignment temperature for each fragment and 90 s at 72 °C for polymerization.

The amplification of PCR fragments was verified through electrophoresis in agarose gel at 1% with TAE 1X buffer (Tris Base 4.84 g/L; Acetic acid 11.4 ml/L; EDTA 7.44 g/L). The gels were stained with ethidium bromide (1 µg/1ml) and visualized with UV light with a photo-documenter device (Labnet).

Table 1. Oligonucleotides used for PCR for the molecular detection of *E. coli* pathotype ETEC and *Salmonella* sp.

Enterobacteria	Gene	Oligonucleotide sequence	Tm	Expected product (bp)	Reference
<i>E. coli</i> ETEC H10407	lt	F: GGCGACAGATTATACCGTGC R: CGGTCTCTATATTCCCTGTT	55 °C	450	López-Saucedo, 2003
	st	F: ATTTTCTTTCTGTATTGTCTT R: CACCCGGTACAAGCAGGATT	48 °C	190	
<i>Salmonella</i> spp.	invA	invA1: CTGTTGAACAACCCATTTGT invA2: CGGATCTCATTAATCAACAAT	57.4 °C		Liu, 2002
Universal	16S rRNA	F27: AGAGTTTGGATCMTGGCTCAG R1492: TACGGYTACCTTGTTCGACTT	55 °C	1500	Heuer <i>et al.</i> , 1997

Bacterial culture

Isolation and identification of *Salmonella*

For the isolation and identification of *Salmonella*, the protocols established by norm NOM-210-SSA1-2014 “Microbiological testing methods: determination of indicator microorganisms and determination of pathogenic microorganisms” were used. Briefly, a dilution of 1:10 of the compost sample in peptone water was made, and then it was passed to Rappaport Vassiliadis Soya (RVS) broth and incubated at 37 °C. Then, it was plated in Salmonella-Shigella and brilliant green agars and incubated at 37 °C. Bacterial colonies with characteristic growth suggestive of *Salmonella* spp. were subjected to biochemistry analysis (short form), using the TSI tests, urea, citrate and SIM tests.

Molecular identification of colonies with characteristics suggestive of thermotolerant enterobacteria

DNA extraction from isolated colonies suggestive of *Salmonella* spp. was carried out with a lysis buffer based on 50 mM Tris base, 50 mM EDTA and 3% sodium dodecyl sulfate (SDS). It was done by preparing a bacterial culture in 3 ml of King's B liquid medium incubated 24 hours at 30 °C; the cells were harvested by centrifugation for 3 minutes at 10,000 rpm and the supernatant was discarded. It was suspended in 500 µl of lysis buffer (Tris base 50 mM, EDTA 50 mM, 3% SDS) and incubated in a water bath for 40 minutes at 65 °C. Then, 500 µl of phenol chloroform isoamyl alcohol was added and mixed by inversion. It was centrifuged at 10,000 rpm for 10 minutes, the aqueous (superior) phase was separated, and it was transferred to another tube. Then, 100 µl of sodium acetate 3M was added and mixed by inversion; 1 ml of absolute ethanol was added and incubated in ice for 25 minutes. It was centrifuged for 20 minutes at 10,000 rpm, the ethanol was eliminated, and the pellet was washed with 400 µl ethanol at 70%. It was centrifuged for 5 minutes at 10,000, the ethanol was eliminated, and it was left to dry at 37 °C. It was suspended in 40 µl of TE water and stored at 20 °C. To amplify the fragment of 1500 pb of the 16S rRNA of the isolated colonies, the oligonucleotides F27 and R1492 were used with the PCR software mentioned before (Table 1). The PCR products of the expected size were purified with the commercial kit DNA Clean & Concentrator (Zymo Research), according to the manufacturer's indications and were sent for sequencing at the National Laboratory of Agricultural, Medical and Environmental Biotechnology (Laboratorio Nacional de Biotecnología Agrícola Medica y Ambiental, LANBAMA, IPICYT). The sequences obtained were analyzed taxonomically through BLASTn (Basic Local Alignment Search Tool) (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Parasitological analysis

To conduct the parasitological study, a modified sedimentation/floating technique was used with sodium nitrate solution (NaNO₃) with 1.25 spg. (Raissi *et al.*, 2020). The samples were analyzed by duplicate, with 10 grams of sieved humus (fine fraction) (Raissi *et al.*, 2020). As positive control, substrate and concentrated canine feces positive to parasites such as *Toxocara* sp., *Dipylidium* sp., *Ancylostoma* sp. and *Trichuris* sp., were used. After performing the technique, the samples were observed with the microscope to carry out the analysis.

RESULTS AND DISCUSSION

Molecular analysis

Detection of *E. coli* ETEC and *Salmonella* sp., through amplification by PCR of gene fragments *it* and *invA*, respectively, had negative results. In this regard, it is likely that populations of the microorganisms mentioned are reduced and, therefore, detection by conventional PCR in a complex matrix such as composts cannot be achieved (Chen *et al.*, 2023; Prosser *et al.*, 2007).

Bacteriological analysis

Given the likely reduced population density of *E. coli* ETEC and *Salmonella* sp., the isolation *Salmonella* spp. was carried out, following specific protocols. This was done because composts have biofertilizer applications in agricultural crops and vegetables that could be susceptible to contamination by these pathogens and transmitted to humans through consumption, thus risking the safety of those foods (Sharma *et al.*, 2016).

After incubation of the enriched and diluted compost sample, it was observed that in the Salmonella-Shigella agar, 2 colonies grew with morphology suggestive of *Salmonella*.

Biochemistry analysis (short form) was performed on those colonies, which showed the following results: TSI reaction 4, negative citrate, negative urea, negative SIM to indole and positive to motility and production of H₂S, which, when compared with identification tables, matched with *Salmonella arizonae* although the citrate was negative (Table 2).

However, bacteria from genus *Citrobacter* present high genotypical similarities with *Salmonella* which is why it is frequent for them to be identified incorrectly when differential biochemical tests are used (Pławińska-Czarnak *et al.*, 2021).

Bioinformatic analysis of 16S rRNA sequences from isolated strains

Knowing that biochemistry (short form) presents limitations for identification (Pławińska-Czarnak *et al.*, 2021), the 1500 pb fragment of 16S RNAr gene from the colonies identified as *S. arizonae* was amplified by PCR. Sequencing of both 16S RNAr fragments revealed an identity of 74.73 and 93.3% with *Citrobacter arsenatis* (Table 3).

Taxonomically, the *Citrobacter* genus is closely related to *Salmonella* spp. and *Escherichia coli* (Katzenellenbogen *et al.*, 2017; Schoch *et al.*, 2020). Based on antigen O of the liposaccharide (LPS), a total of 43 serogroups O of *Citrobacter* are recognized, and a total of 20 chemical groups have also been classified according to the sugar composition of their lipopolysaccharides (LPS) and 19 genomospecies of *Citrobacter* based on the DNA relationship (Jabeen *et al.*, 2023). This is probably why similarity was found at

Table 2. Biochemical tests used.

Bacteria	TSI	Gas in glucose	H ₂ S	Indole/motility	Citrate	Urea
<i>Salmonella arizonae</i>	A/A	d	+	-/+	+	-
<i>Citrobacter</i> spp.	A/A	d	+	-/+	+	+
Isolated bacteria	A/A	+	+	-/+	-	-

TSI: triple sugar iron, A/A: acid/acid, d: different reactions (Barrow, 1993).

Table 3. Sequencing results of the 16S rRNA region.

Sample	BLASTn	Evalue	Cover	Identity	Accession
Grey strain grown in McConkey cultivation medium	<i>Citrobacter arsenatis</i> strain LY-1 16S ribosomal RNA, partial sequence.	5e-85	81%	74.73%	NR_180353.1
Black strain grown in Salmonella-Shigella cultivation medium	<i>Citrobacter arsenatis</i> strain LY-1 16S ribosomal RNA, partial sequence.	1e-157	93%	93.33%	NR_180353.1

the level of culture and and biochemistry (short form) with *Salmonella*, although with negative results in the PCR.

Parasitological identification

No structures suggesting canine parasites were found in the compost sample, although mites, mite eggs, and free-living nematode eggs were found. The free-living mites, although not taxonomically identified, were very similar to *Klinckowstroemia bifurcata*, which are commonly found associated to environments in or under decomposing logs and soil (Villegas-Guzmán *et al.*, 2012), considering that materials such as sawdust and plant residues were used which could transport them.

There are reports that indicate that to inactivate helminth eggs, it is recommended to raise the temperature to $>40^{\circ}\text{C}$, decrease moisture $<5\%$ or keep both for a period of time (Escobar *et al.*, 2014); and from temperatures of 60°C for 30 to 60 minutes, eggs of parasites such as *Trichuris* sp., *Strongyloides* spp., *Trichostrongylus* sp. and *Haemonchus* sp. are inactivated, among others (Mahmud *et al.*, 2016). This suggests that, through the thermal and biological composting process, potentially zoonotic pathogens in different animal species are eliminated (canines, Nemiroff and Patterson, 2007; bovine and human, Mahmud *et al.*, 2016; human and birds, Manga *et al.*, 2016).

However, no parasitological analyses were conducted of the individual used in the composting process, so the presence or absence of common parasites in puppies cannot be assured.

The composting process applied to dead animals is an alternative whose popularity is increasing and it is a viable alternative; it has been shown that there is low risk of environmental contamination when using pig carcasses. In addition, the composting technique reduces the risk of transmission of external animal diseases to production (Pepin *et al.*, 2021). Because of this, the compost elaborated based on dog carcasses could be an alternative to use to eliminate these organic residues; however, in our country there is a lack of guidelines and regulations concerning the risks and use that must be considered and avoided in composting of carcasses. Likewise, the carcasses used for compost can imply a risk of contamination of soil and water, because of their organic composition and the potentially pathogenic agents that present what would represent an impact on the environment (Vithanage, 2021).

In this study, bacterial colonies that showed high similarity at the level of 16S RNAr sequence with *Citrobacter arsenate* were isolated from the sample of compost from dog carcasses. This bacterium is a microorganism of recent isolation in freshwater sediments

in China, which presents the ability to reduce arsenate to arsenite (Wang *et al.*, 2021). Likewise, the *Citrobacter* genus has been described as a bacteria susceptible of acquiring resistance genes through horizontal transference, in equines in Europe and birds in Asia and Nigeria (Anyanwu *et al.*, 2022; Anyanwu *et al.*, 2023). Because it is a species of recent identification, there are no reports of association with pathologies in humans. However, the *Citrobacter* genus is associated to pathologies in humans as opportunist and is frequently found in various environments including the soil (Wang *et al.*, 2021), and because of this we cannot dismiss the likely pathogenicity of this new species.

Compost elaborated from carcasses of different animals has been used as a safe environmental method to eliminate the carcasses (Pepin *et al.*, 2021; Guan *et al.*, 2010), and it has even been observed that there is a low risk of contamination of air or underground water by important viruses in the pork industry (Pepin *et al.*, 2021). Although no important parasites or bacteria for public health were identified in this study in the compost from dog carcass, it should be highlighted that future metagenomic and metabolome studies are required, to assess the behavior of the microbial communities in the different states of the compost. Something to underline is that various metagenomic studies in composts made from pig, ruminant and bird manure have suggested the presence of bacterial genes involved in resistance to antibiotics, and therefore, inherent risks of transmission and proliferation of those phenotypes in the ecosystem (Zhang *et al.*, 2023; Fan *et al.*, 2023; Zhao *et al.*, 2024). In this regard, the thermophilic step during the composting process significantly reduces the risks of antibiotic resistance transfer, as a result of the low population density of the communities that harbor them and the dynamic interactions with other microbial communities (Werner, 2022; Eckstrom and Barlow, 2019).

In addition to researching the taxonomic and metabolomic profiles of the microbial communities that develop after the application of compost to the soil (Meneghini, 2017) to assess whether it can be used to grow vegetables, the proposed approaches aim to ensure the lowest possible risk for public, livestock and environmental health.

CONCLUSIONS

Bacterial and molecular analyses indicate that compost derived from canine origin presents low sanitary risk regarding enteropathogens such as *E. coli* ETEC and *Salmonella*. However, the detection of *Citrobacter arsenati*, whose pathogenic relevance is still not well-established, points to the need for additional studies that include more specific methods and a greater number of samples.

Evidence suggests that this compost could be viable for agricultural uses not destined for human consumption, although it is necessary to evaluate its safety in various contexts of application. Finally, these results highlight the convenience of advancing towards specific normative criteria for composts derived from companion animals, to ensure their safe handling and use.

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Effect of hydrocooling with calcium chloride and silicon dioxide on post-harvest quality of Saladette tomatoes ‘Macizo’ and ‘Moctezuma’

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ABSTRACT

Objective: Saladette tomato (*Solanum lycopersicum* L.) is valued for its nutritional properties; however, the effects of postharvest applications of Ca²⁺, Si, and hydrocooling remain insufficiently characterized.

Design/methodology/approach: This study evaluated calcium chloride (CaCl₂; 1% w/v), silicon dioxide (SiO₂; 1% w/v), and their combination. Treatments were applied at 0 °C (hydrocooling) for 5 min to ‘Macizo’ and ‘Moctezuma’ cultivars. Fruits were stored at 23 ± 1 °C (ambient temperature) and 24 ± 1% relative humidity for 21 days. Quality attributes were measured during storage. Measurements included weight loss, firmness, color, soluble solids content, and titratable acidity.

Results: Both cultivars lost weight and firmness. Hydrocooling, alone or with calcium chloride, delayed this loss. Ripening was characterized by an increase in a*, a decrease in b*, and an initial decline followed by partial recovery in L*. Titratable acidity decreased, with the most pronounced decline observed in the presence of silicon dioxide. Soluble solids content fluctuated. The maturity index increased, indicating advanced ripening.

Findings/conclusions: These findings highlight the potential of hydrocooling alone or combined with calcium chloride as an effective postharvest strategy to preserve Saladette tomato quality during storage.

Keywords: Firmness, quality, *Solanum lycopersicum*, ripening, climacteric fruit.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.; Solanales: Solanaceae) is a globally important commercial crop (Flores-López *et al.*, 2024). Mexico is its major exporter (Montaño-Méndez *et al.*, 2021). In 2024, 50,086.64 hectares were allocated to its cultivation,



resulting in the production of over 3.7 million tons (AEPA, 2025). Despite this progress, Mexico faces considerable post-harvest losses at the retail level, threatening its commercial competitiveness. Reducing these losses is crucial for maintaining Mexico's market share in the global tomato industry. The value of tomatoes extends beyond economic output and export volume, including their fresh and processed uses, as well as the nutritional benefits of carotenoids and phenols (Wu *et al.*, 2022; Bertin *et al.*, 2025). Maintaining the quality of tomato fruits, such as firmness, size, color, flavor, and nutritional value, until they reach consumers is difficult due to factors like pests, diseases, or handling during marketing (Bapary *et al.*, 2024; Flores-López *et al.*, 2024; Wang *et al.*, 2025). Cultivar, maturity and production methods influence tomato quality, but post-harvest processes, handling, storage, and transportation most substantially affect the final fruit characteristics (Ayuso-Yuste *et al.*, 2022; Bapary *et al.*, 2024). Importantly, because tomatoes are climacteric fruits, their natural ripening increases respiration and ethylene production, thereby accelerating quality loss and shortening shelf life (Salas-Méndez *et al.*, 2019). Elevated ethylene, in turn, influences phenolic content and fruit texture, which reduces consumer acceptance (Khalil *et al.*, 2020; Al-Dairi *et al.*, 2021; Baninaiem & Dastjerdi, 2023). Notably, in Latin America, consumers associate firmness with freshness, therefore any decrease in firmness caused by postharvest changes directly reduces retail prices.

Reducing postharvest quality loss depends on effective storage. Cold storage, high humidity, and controlled atmospheres help preserve tomato quality and extend shelf life (Flores-López *et al.*, 2024), but they can also cause chilling injury (Park *et al.*, 2018). Retail environments often lack these ideal conditions and typically experience higher temperatures and lower humidity (Sumonsiri *et al.*, 2022). These conditions increase respiration and moisture loss, causing faster deterioration in quality and economic losses (Baninaiem & Dastjerdi, 2023; Tigist *et al.*, 2013; Nkolisa *et al.*, 2018). Addressing these storage challenges is essential to minimizing postharvest decay.

Since most retail environments lack temperature and humidity control, we need to implement additional postharvest strategies. Physical methods such as hydrocooling and exogenous Ca^{2+} or silicon (Si) treatments delay firmness loss and extend shelf life in tomatoes and other fruits, especially at safe doses (Wang *et al.*, 2014; Shehata *et al.*, 2021; Wu *et al.*, 2025). Wang *et al.* (2014) found that dipping cherries for 5 min in cold water (0 °C) with CaCl_2 significantly increased the calcium content in fruit tissue. The increase in Ca and its effects on fruit and pedicel quality was dose-dependent: dipping in 0.2-0.5% CaCl_2 for 5 min increased tissue Ca and preserved both fruit and pedicel quality, whereas higher concentrations (1.0-2.0%) further increased tissue Ca but caused pedicel damage. CaCl_2 increases firmness by acting on cell wall pectin (Ornelas-Paz *et al.*, 2018), while sodium silicate $\text{Na}_2\text{O}_3\text{Si}$, creates a protective surface, enhancing resistance and reducing water loss (Rombolà *et al.*, 2023). Applying treatments (CaCl_2) and (SiO_2) during hydrocooling shows potential for maintaining tomato quality during storage and retail (Maya-Meraz *et al.*, 2024). Previous reports indicate that Ca^{2+} and Si improve fruit quality. We hypothesize that postharvest hydrocooling and treatments with Ca^{2+} , Si, or both will extend tomato shelf life by strengthening cell walls, reducing postharvest stress, slowing ripening, and preserving overall quality during storage.

Especially in the ‘Moctezuma F1’ and ‘Macizo’ cultivars, currently cultivated under greenhouse conditions in Cuauhtémoc, Chihuahua. ‘Moctezuma’ is a hybrid tomato; it is a vigorous, early-maturing plant with excellent firmness, long shelf life, extra-large fruit, and high yield potential. ‘Macizo’, on the other hand, is an improved genotype emphasizing quality and resilience. Both cultivars share key attributes such as firmness, shelf life, and stress tolerance. We aimed to demonstrate that hydrocooling with calcium chloride, silicon dioxide, or both can extend shelf life and maintain freshness in ‘Macizo’ and ‘Moctezuma’ tomatoes during storage.

MATERIALS AND METHODS

Reagents and plant material

Food-grade CaCl_2 and SiO_2 were purchased from Food Technologies Trading S.A. de C.V., Mexico. Saladette tomatoes from the ‘Macizo’ and ‘Moctezuma’ cultivars were selected for uniform size, color, and the absence of lesions or infections. Tomatoes were harvested manually at the red stage of physiological and commercial maturity from a commercial greenhouse in Cuauhtémoc, Chihuahua, Mexico. A total of 224 fruits (112 per cultivar) were immediately disinfected by immersion in a 1% (v/v) chlorine bleach solution for 3 min. Fruits were then washed twice with sterile distilled water and dried at room temperature in commercial plastic bags. Twenty-eight tomatoes were used per treatment. Post-harvest evaluations were performed on days 0, 7, 14, and 21 after treatment.

Post-harvest treatments of tomatoes

Four hydrocooling solutions were prepared and maintained at 0 °C using distilled water and ice: T1 (hydrocooling control; distilled water), T2 (1% CaCl_2), T3 (1% SiO_2), and T4 (0.5% CaCl_2 + 0.5% SiO_2) (Wang *et al.*, 2014). Tomatoes were immersed in one of the four solutions for 5 min, drained, dried on brown paper at room temperature, and placed on plastic racks. The fruits were stored in a controlled chamber at 23 °C and 24% relative humidity for 0, 7, 14, or 21 days (Nkolisa *et al.*, 2018; Vigneault *et al.*, 2000). At each storage interval, fruits were evaluated for weight, firmness, color, soluble solids content (SSC; °Brix), and titratable acidity (TA).

Measurement of physicochemical attributes in tomatoes

Physicochemical changes were evaluated by measuring weight, firmness, color, SSC, and TA at 0, 7, 14, and 21 days after treatment. For each measurement, 28 fruits per treatment were randomly divided into four groups. Seven biological replicates were destructively analyzed at each time point, ensuring that independent fruits were assessed. Fruit weight (g) was determined using a Velab™ electronic balance (Model VE-5000, Texas, USA).

Tomatoes’ firmness was measured by puncture tests using a Universal Texture Analyzer (Ta-XT2i Texture Technologies Corp., USA). A 6 mm diameter metal probe was used to measure the maximum force (N) at a penetration speed of 5 mm s⁻¹ and a depth of 15 mm (Ruiz-Cisneros *et al.*, 2018).

Skin color was measured at two opposite anatomical locations using a CR-300 colorimeter (Minolta Co. Ltd., Osaka, Japan) on the CIE-L*a*b* scale, as described by Ruiz-Cisneros *et al.* (2018). In this scale, L* indicates lightness (0=black, 100=white), a* represents the green-red axis, and b* the blue-yellow axis. The colorimeter was calibrated using a white standard prior to each measurement.

Soluble solids content (SSC, °Brix) and titratable acidity (TA, expressed as % malic acid according to NMX-F-102-S-1978) were measured in fruit juice. SSC was measured using a digital refractometer (PAL-1 pocket, Atago Co. Ltd., Tokyo, Japan). TA was determined by potentiometric titration of 50 mL of tomato juice, diluted 25-fold using 0.1 N NaOH as the titrant (Nkolisa *et al.*, 2018), and 0.5% phenolphthalein as the indicator (Ruiz-Cisneros *et al.*, 2018). Each sample was analyzed in quadruplicate. The maturity index was calculated as the SSC/TA ratio.

Experimental design and statistical analysis

Data were analyzed using a completely randomized 4×4×2 factorial design, with treatment (4 levels), storage time (4 levels), and cultivar (2 levels) as factors. Each experimental unit consisted of seven replicates (fruits) and was assigned a unique combination of factor levels to ensure independence.

The assumptions of normality, homogeneity of variances, and error independence were evaluated using the Shapiro-Wilk test ($W=0.988$; $p=0.070$), Levene's test ($F=1.2978$; $p=0.148$), and the Durbin-Watson statistic ($DW=1.8952$; autocorrelation=0.04546), respectively. Since these assumptions were satisfied, an analysis of variance (ANOVA) was performed to identify significant differences, followed by Tukey's test ($p\leq 0.05$) for mean comparisons. All analyses were conducted using SAS software version 9.0 (SAS Institute Inc., Cary, NC, USA, 2002).

RESULTS AND DISCUSSION

The main finding was that hydrocooling and CaCl_2 or SiO_2 treatments, whether applied alone or together, influenced the physicochemical properties of tomatoes depending on the cultivar ('Macizo' and 'Moctezuma') and the storage time (0, 7, 14, and 21 days). As expected for climacteric fruits, both treated and untreated tomatoes exhibited physicochemical changes related to senescence, with a typical shelf life of 2-3 weeks (Thole *et al.*, 2021). Previous studies have indicated that the effects of postharvest treatments, such as Ca^{2+} application, harvest timing, and storage temperature, differ by cultivar and influence fruit quality and shelf life (Correia *et al.*, 2019; Al-Dairi *et al.*, 2021). During storage, physiological processes such as oxidation, transpiration, ethylene production, and enzymatic activity occur. Cooler temperatures (around 12 °C) are typically used to slow these processes and extend tomato shelf life (Park *et al.*, 2018; Sumonsiri *et al.*, 2022). In the present study, rapid changes in tomatoes may be attributed to storage under higher temperature (23 °C $\pm$ 1 °C) and lower humidity (24%), supporting the notion that elevated temperatures negatively affect tomato quality (Thole *et al.*, 2021).

Effect of calcium chloride and silicon dioxide on tomatoes' weight

Weight loss is a key factor influencing the quality and shelf life of horticultural crops, such as tomatoes. In this study, weight loss increased progressively in most treatments over time (Figure 1), consistent with previous findings (Chandra-Dhami *et al.*, 2023). Conversely, tomatoes of 'Macizo' cultivar in the hydrocooling control group gained weight by the end of storage, possibly due to moisture absorption during hydrocooling. Weight loss during storage results from metabolic, transpiration, and respiration processes (Al-Dairi *et al.*, 2021), which release water, as well as from vapor pressure differences that drive moisture transfer. Godana *et al.* (2015) observed greater weight loss in tomatoes stored at room temperature, probably because of higher vapor pressure deficit and water loss. Kenghe *et al.* (2015) noted that 80%-90% relative humidity (RH) is ideal, as higher RH reduces water loss. However, low RH ($\sim 24\%$) and high temperature ($23 \pm 1^\circ\text{C}$) likely accelerated evaporation, so hydrocooling or CaCl_2 treatment did not sufficiently reduce weight loss (Figure 1). Low RH during storage has been attributed to water loss in tomatoes, resulting in weight reduction (Peralta-Ruiz *et al.*, 2020), highlighting the importance of environmental conditions. Hydrocooling and CaCl_2 -based treatments delayed weight loss during storage in both tomato cultivars (Figure 1), with the effect being most pronounced in 'Macizo' compared to the other cultivar. Hydrocooling treatments retain a large amount of water in tissues, resulting in slow weight loss (Vigneault *et al.*, 2000; Zainal *et al.*, 2019).

Previous research indicates that CaCl_2 treatment delays weight loss, depending on the cultivar and Ca^{2+} concentration (Shehata *et al.*, 2021; Chandra-Dhami *et al.*, 2023; Correia *et al.*, 2019). In this study, the hydrocooling alone group and CaCl_2 in both cultivars showed the most significant delay in weight loss (Figure 1) and maintained firmness (Figure 2). Continuous exposure to CaCl_2 or hydrocooling is known to increase tissue Ca levels (Vigneault *et al.*, 2000). This could be caused by increased Ca^{2+} absorption or easier Ca^{2+} penetration through the exocarp/cuticle and stem scar/pedicel. Fruits with larger cell size may also accumulate Ca differently (Wu *et al.*, 2025). Increased Ca^{2+} in fruit tissue likely inhibits ripening and softening enzymes, delaying weight loss and preserving firmness during storage (Chandra-Dhami *et al.*, 2023). CaCl_2 may also help prevent rapid water loss and slow metabolic changes, thereby extending shelf life (Breda *et al.*, 2017; Shehata *et al.*, 2021). It may form a semipermeable film on the fruit surface, reducing transpiration (Moradinezhad *et al.*, 2019), or act as a calcium pectate hydrogel, retaining water and delaying dehydration (Turmanidze *et al.*, 2017). Additionally, it has been suggested that Ca^{2+} may block stomata and reduce respiration, further slowing weight loss.

SiO_2 and $\text{CaCl}_2 + \text{SiO}_2$ treatments increased weight loss in both cultivars. This effect was most evident in 'Macizo' tomatoes at the end of storage (Figure 1). SiO_2 forms a thin layer on the fruit surface, helping retain water (Rombolà *et al.*, 2023). We anticipated that combining CaCl_2 and SiO_2 would slow weight loss and keep tomatoes firmer than either treatment alone. However, the results did not support this: the combined treatment was no more effective than the individual treatments in reducing weight loss or enhancing firmness. Therefore, no synergy between CaCl_2 and SiO_2 was observed under the tested conditions.

In ‘Moctezuma’ tomatoes, those treated with $\text{CaCl}_2 + \text{SiO}_2$ and SiO_2 lost weight and firmness more quickly than those treated with hydrocooling control and CaCl_2 by day 21 (Figures 1, 2).

Effect of calcium chloride and silicon dioxide on tomato firmness

Firmness is an important quality attribute in tomatoes. It depends on structural components, including parenchymal cells, fibers, water content, pectin, and hemicellulose, which together influence texture and help maintain fruit integrity (Wang & Seymour, 2022). During storage, firmness drastically decreased in both cultivars, from approximately 75.42–88.75 to 29.35–37.86 N in ‘Macizo’ and from 69.22–79.66 to 13.25–34.69 N in

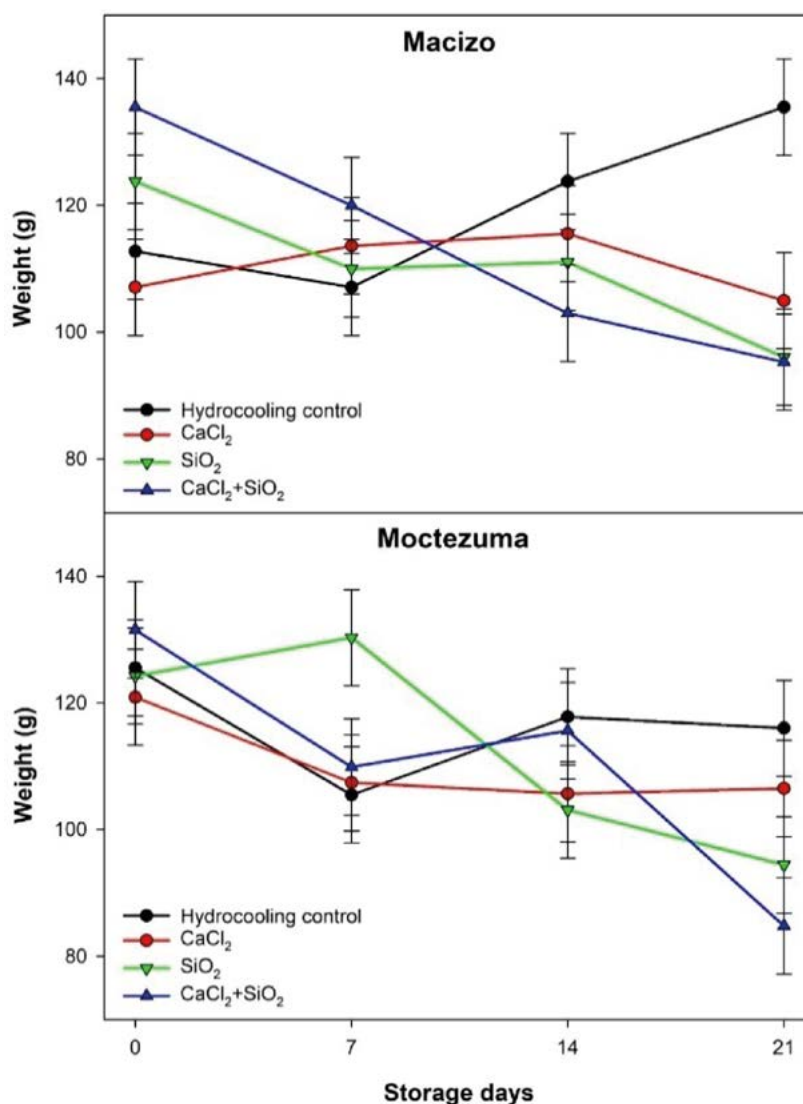


Figure 1. Effect of hydrocooling, CaCl_2 , and SiO_2 (alone or combined in hydrocooling) on the weight of Saladette-type tomato cultivars ‘Macizo’ (upper) and ‘Moctezuma’ (lower) during storage. Values represent means $\pm$ standard error ($n=7$). Significant effects were observed for storage time ($F_{3,192}=7.96$, $p<0.001$) and the treatment $\times$ time interaction ($F_{9,192}=3.96$, $p<0.001$).

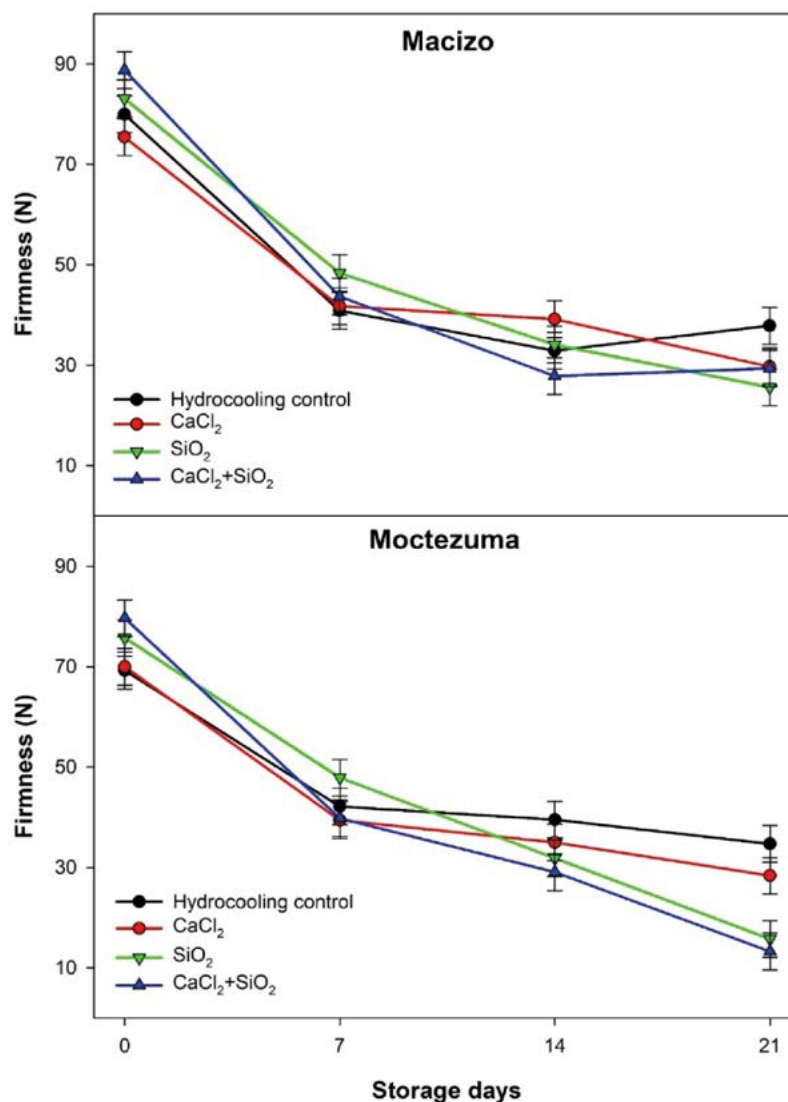


Figure 2. Effect of hydrocooling, CaCl₂ and SiO₂ (alone or combined in hydrocooling) on tomato firmness in Saladette-type cultivars ‘Macizo’ (upper) and ‘Moctezuma’ (lower) during ambient storage. Values represent means $\pm$ standard error (n=7). Significant effects were observed for storage time ($F_{3,192}=306.58$, $p<0.001$), cultivar ($F_{1,192}=10.59$, $p=0.001$), treatment $\times$ time interaction ($F_{9,192}=5.03$, $p<0.001$), and time $\times$ cultivar interaction ($F_{3,192}=2.80$, $p=0.042$).

‘Moctezuma’ (Figure 2). By day 7, firmness had decreased approximately 49.22% to 58.17% in ‘Macizo’ and about 49.91% to 63.21% in ‘Moctezuma’. Notably, on day zero, initial firmness was higher in both ‘Macizo’ (>75 N) and ‘Moctezuma’ (>70 N) compared to values reported for ‘Merlice’ tomatoes (20.9 to 51.6 N; Ruiz-Cisneros *et al.*, 2018). At the end of storage, ‘Moctezuma’ tomatoes showed greater firmness than ‘Macizo’, especially in the hydrocooling control and CaCl₂ treatments, indicating cultivar-dependent differences in texture. This confirms previous findings that genotype significantly influences tomato firmness (Gong *et al.*, 2022; Nie *et al.*, 2024).

The lowest firmness (below 40 N) was observed on day 21 in both the treated and hydrocooling control groups, indicating that chemical and natural changes, such as cell

damage or death, contributed to the fruits' softening during storage. Fruit softening occurs due to increased respiration, water loss, and pectin solubilization as the cell wall matrix progressively disassembles (Peng *et al.*, 2022). Cell wall-modifying enzymes degrade structural polysaccharides, including pectin, hemicellulose, and cellulose, thereby influencing the ripening process (Wang *et al.*, 2018). The loss of monosaccharides such as galacturonic acid, galactose, and arabinose from the cell wall further decreases firmness (Roohanitaziani *et al.*, 2022).

CaCl₂ treatment delayed firmness loss in 'Macizo' fruits, unlike the other treatments tested. This aligns with previous research, as delays caused by Ca²⁺ applications are well documented in fruits such as sweet cherries (*Prunus avium* L.; Correia *et al.*, 2019; Rombolà *et al.*, 2023), jujube (*Ziziphus jujuba* Mill; Moradinezhad *et al.*, 2019), blueberries (*Vaccinium corymbosum*; Angeletti *et al.*, 2010), Chilean strawberries (*Fragaria chiloensis*; Figueroa *et al.*, 2012), tomatoes (Shehata *et al.*, 2021), and others (Wang *et al.*, 2014).

CaCl₂ interacts with pectin in the cell wall (Ornelas-Paz *et al.*, 2018), thus delaying enzymatic degradation and lowering respiration and transpiration rates. By reducing water loss, CaCl₂ helps preserve cell wall structure and the middle lamella, which ultimately maintains tomato firmness (Shehata *et al.*, 2021). Consequently, increasing Ca²⁺ concentrations in the tested solutions and extending hydrocooling exposure times may further delay fruit softening. SiO₂ and CaCl₂ + SiO₂ treatments did not maintain fruit firmness after 21 days in either cultivar (Figure 2). In 'Moctezuma', firmness decreased by day 14 under SiO₂ treatment and by day 7 under CaCl₂ + SiO₂. This loss may be attributed to increased activity of cell wall-degrading enzymes, such as polygalacturonase and pectin methyl esterase, which degrade pectin in the cell wall and middle lamella, thereby causing tissue softening (Wang & Seymour, 2022). As fruit nears senescence, the production of essential compounds decreases, speeding up the degradation of proteins, chlorophyll, lipids, and nucleic acids (Gapper *et al.*, 2013). Ethylene also helps regulate phenolic metabolism and accelerates firmness loss (Khalil *et al.*, 2020).

Loss of firmness is often associated with changes in fruit color (Ayuso-Yuste *et al.*, 2022). For example, hydrocooling control 'Moctezuma' tomatoes exhibited greater softening and higher L* values, indicating chlorophyll degradation and lycopene accumulation (Choi & Park, 2023; Antala *et al.*, 2025).

Effect of calcium chloride and silicon dioxide on color in tomatoes

Color is a key visual quality attribute that strongly influences consumer preference for tomatoes (Baninaiem & Dastjerdi, 2023). During storage, peel color in both 'Macizo' and 'Moctezuma' cultivars changed as chlorophyll degraded and lycopene accumulated (Kapoor *et al.*, 2022; Zhu *et al.*, 2022). Starting on day 7, the L*, a*, and b* values changed in both cultivars. Specifically, a* increased by day 7 across all treatments, whereas b* decreased progressively until day 21, regardless of treatment.

L* initially decreased in all treatments and then increased from day 14 to 21, except in fruits treated with CaCl₂ + SiO₂, where a decline was observed only at the end of storage (Figure 3), consistent with previous reports (Breda *et al.*, 2017; Shehata *et al.*, 2021). This suggests that the CaCl₂ + SiO₂ treatment delayed the loss of lightness until day 21,

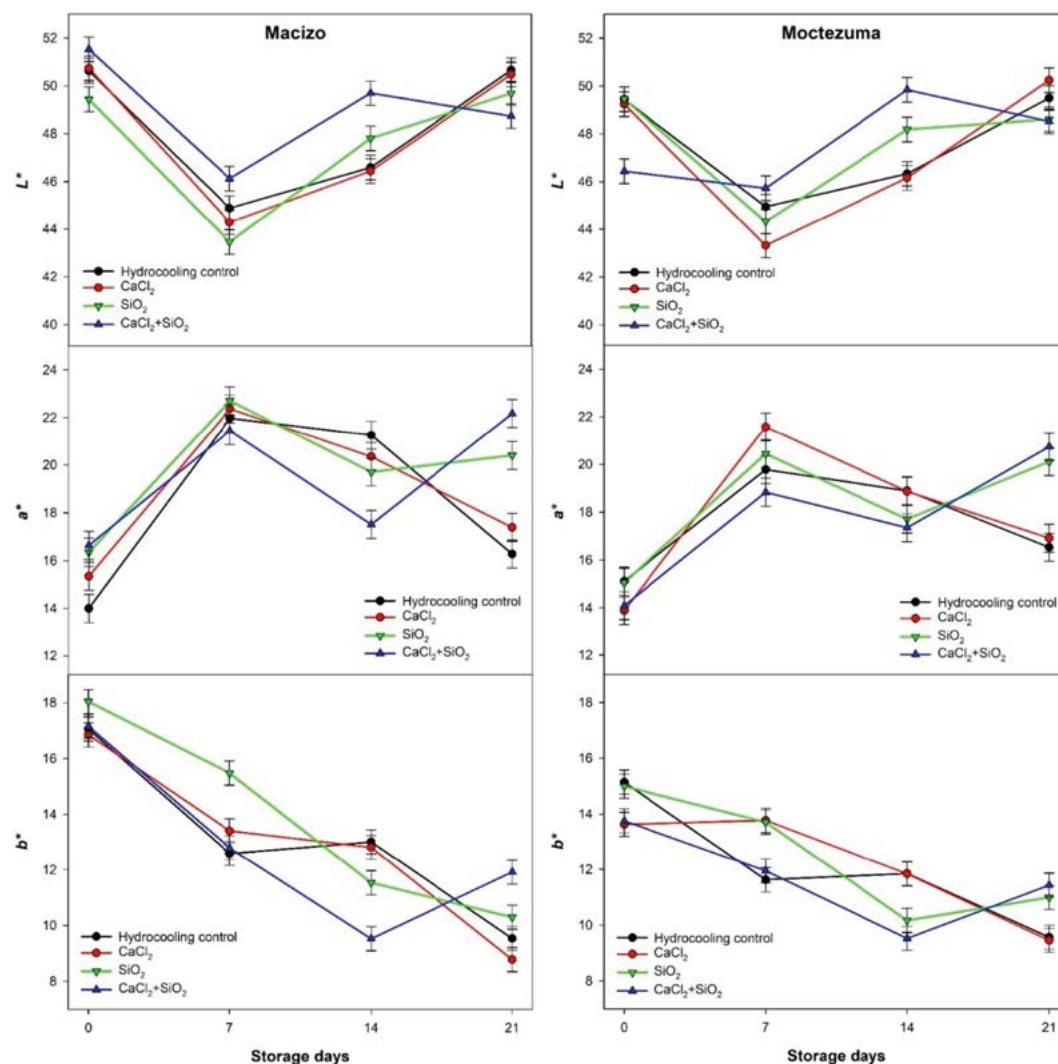


Figure 3. Effect of hydrocooling, CaCl_2 , and SiO_2 (alone or combined in hydrocooling) on the color parameters L^* , a^* , and b^* of Saladette-type tomato cultivars 'Macizo' and 'Moctezuma' during ambient storage. Values represent means $\pm$ standard error ($n=7$). Multivariate analysis indicated significant effects of treatment (Wilks' $\lambda=0.75$, $p<0.001$), storage time (Wilks' $\lambda=0.015$, $p<0.001$), cultivar (Wilks' $\lambda=0.76$, $p<0.001$), and the treatment $\times$ time $\times$ cultivar interaction (Wilks' $\lambda=0.75$, $p=0.001$). Univariate ANOVA showed significant treatment $\times$ time interactions for L^* , a^* , and b^* ($p<0.001$).

possibly due to slower ripening or reduced dehydration (Lopez-Camelo & Gomez, 2004). By day 21, hydrocooling and CaCl_2 treatments maintained higher L^* values than SiO_2 and $\text{CaCl}_2 + \text{SiO}_2$ treatments. Higher L^* values have been associated with softer fruit, reflecting greater chlorophyll degradation and lycopene accumulation (Ayuso-Yuste *et al.*, 2022; Shehata *et al.*, 2021).

Consistent with this, loss of firmness influenced changes in tomato color, with effects varying among treatments. In hydrocooling controls, lightness (L^*) initially increased and then decreased. In contrast, redness (a^*) and yellowness (b^*) gradually decreased in both cultivars, likely indicating pigment changes during ripening.

By day 21, SiO_2 and $\text{CaCl}_2 + \text{SiO}_2$ treatments showed higher a^* values than hydrocooling controls, indicating a more intense red color (Figure 3). In contrast, the hydrocooling control and CaCl_2 treatments showed lower b^* values on day 21. Fruits with b^* values below 20 are generally considered suboptimal in red coloration, which might negatively affect perceived ripeness (Shehata *et al.*, 2021).

Effect of calcium chloride and silicon dioxide on TA and SSC in tomatoes

Titrateable acidity (TA) indicates the organic acid content in fruits (Baninaiem & Dastjerdi, 2023). Similarly, soluble solids content (SSC) includes sugars, organic acids, and other soluble compounds that act as substrates for respiration (Shehata *et al.*, 2021; Wang *et al.*, 2023; Xu *et al.*, 2025). As expected, the TA and SSC values varied in tomatoes of both cultivars. TA decreased progressively with increasing storage time across all treatments (Figure 4). This trend is consistent with studies on tomatoes stored under ambient (25 °C; Hatami *et al.*, 2013) and cold conditions, where TA also decreased over time (Baninaiem & Dastjerdi, 2023; Chandra-Dhami *et al.*, 2023). In contrast, Shehata *et al.* (2021) reported an initial increase in TA up to 21 days of storage, followed by a subsequent decline. The gradual reduction in TA observed during storage is generally attributed to the use of organic acids as respiratory substrates and their depletion or oxidation during metabolic processes (Al-Dairi *et al.*, 2021; Wang & Seymour, 2022; Chandra-Dhami *et al.*, 2023; Zheng *et al.*, 2023).

Notably, SiO_2 -treated ‘Moctezuma’ fruits showed higher TA values on day 21. This indicates that SiO_2 may have temporarily preserved acidity during mid- to late-stage storage. Although differences in fruit size have been associated with variations in acidity, this factor is unlikely to explain the observed behavior, since fruit size stayed consistent across treatments (Tigist *et al.*, 2013). Instead, the response may indicate different metabolic regulation or delayed acid use in SiO_2 -treated ‘Moctezuma’.

Regarding SSC, both cultivars showed temporal fluctuations consistent with typical ripening patterns (Figure 4). In ‘Macizo’, lower SSC values at day 7 under CaCl_2 and $\text{CaCl}_2 + \text{SiO}_2$ treatments, compared to the hydrocooling control, suggest a delay in sugar accumulation or a temporary suppression of carbohydrate metabolism.

Notably, these effects were temporary, as SSC levels in SiO_2 and hydrocooling control treatments were similar to those of the CaCl_2 and $\text{CaCl}_2 + \text{SiO}_2$ by days 14-21. By the end of storage, only SiO_2 -treated fruits showed higher SSC values, indicating a late-stage enhancement in sugar accumulation specific to SiO_2 .

In ‘Moctezuma’, SSC values were generally lower in CaCl_2 and hydrocooling-treated fruits compared to the SiO_2 and $\text{CaCl}_2 + \text{SiO}_2$ treatments at the end of storage. This reduction was most significant in hydrocooling control-treated tomatoes, which stayed at lower levels until day 21. These findings suggest that $\text{CaCl}_2 + \text{SiO}_2$, compared to the hydrocooling control, may reduce metabolic rate (Wang *et al.*, 2023; Kaygısız *et al.*, 2025) by limiting respiration and slowing the conversion of reserve carbohydrates into soluble sugars (Turmanidze *et al.*, 2017; Shehata *et al.*, 2021). Therefore, the lower SSC values observed with CaCl_2 treatment in both tomato cultivars probably indicate delayed ripening rather than a decrease in total sugar content (Shehata *et al.*, 2021; Chandra-Dhami *et al.*, 2023).

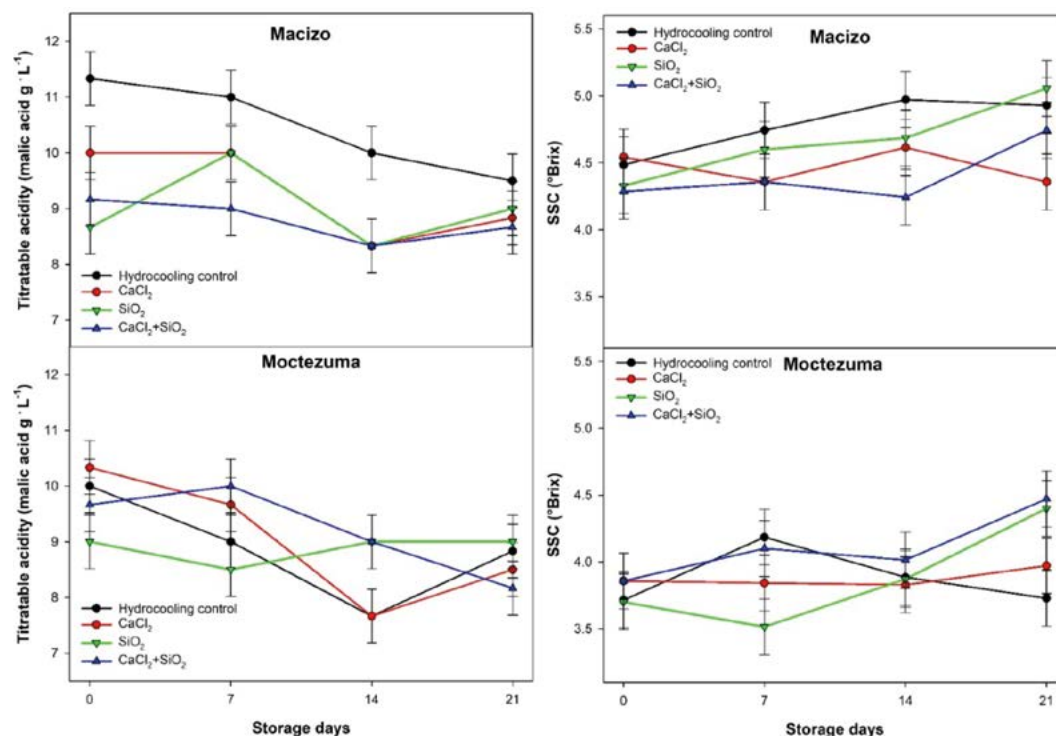


Figure 4. Effect of hydrocooling, CaCl_2 , and SiO_2 (alone or combined in hydrocooling) on titratable acidity (TA; left) and soluble solids content (SSC; right) in Saladette-type tomato cultivars 'Macizo' and 'Moctezuma' during ambient storage. Values represent means $\pm$ standard error ($n=7$). For TA, significant effects were observed for treatment ($F_{3,64}=3.77$, $p=0.015$), storage time ($F_{3,64}=12.75$, $p<0.001$), cultivar ($F_{1,64}=5.13$, $p=0.027$), and the interaction between treatment and cultivar ($F_{3,64}=6.23$, $p<0.001$). For SSC, significant effects were found for storage time ($F_{3,192}=4.15$, $p=0.007$), cultivar ($F_{1,192}=76.91$, $p<0.001$), and the interaction between treatment and cultivar ($F_{3,192}=3.27$, $p=0.022$).

At the end of storage, higher SSC values were observed in 'Macizo' tomatoes treated with SiO_2 or hydrocooling control, and in 'Moctezuma' fruits under $\text{CaCl}_2+\text{SiO}_2$ or SiO_2 , compared to other treatments for each cultivar. This increase is consistent with advanced ripening stages (Tigist *et al.*, 2013; Shehata *et al.*, 2021), during which the degradation of structural carbohydrates and polysaccharides results in the accumulation of soluble sugars (Baninaiem & Dastjerdi, 2023; Chandra-Dhami *et al.*, 2023). Additionally, water loss during storage can increase the concentration of soluble solids, which further increasing SSC (Tigist *et al.*, 2013; Al-Dairi *et al.*, 2021; Shehata *et al.*, 2021). Taken together, these factors indicate that although all treatments influenced SSC dynamics, their effects were largely mediated by cultivar-specific physiological responses and the ripening process during storage.

Effect of Calcium chloride and silicon dioxide on the maturity index in tomatoes

The SSC to TA ratio is a key indicator in tomatoes because both components significantly influence fruit maturity and postharvest quality (Flores-López *et al.*, 2024). The maturity index (SSC/TA) of both cultivars varied, sometimes even showing opposite

trends. In ‘Macizo’, hydrocooling control treatments resulted in a higher maturity index from days 07 to 21 of storage (Figure 5), indicating that these treatments accelerated ripening in this cultivar. In contrast, in ‘Moctezuma’, SiO_2 treatments generally resulted in lower maturity indices from day 14 until the end of storage. The flavor of tomatoes depends on the composition and interaction of sugars and acids (Kaur *et al.*, 2023). An increase in the SSC/TA ratio occurs when SSC is high and TA is low (Al-Dairi *et al.*, 2021). Fruit maturation mainly influences SSC, TA, and the SSC/TA ratio, and changes in the tomato SSC/TA ratio are associated with respiratory activity during storage (Flores-López *et al.*, 2024).

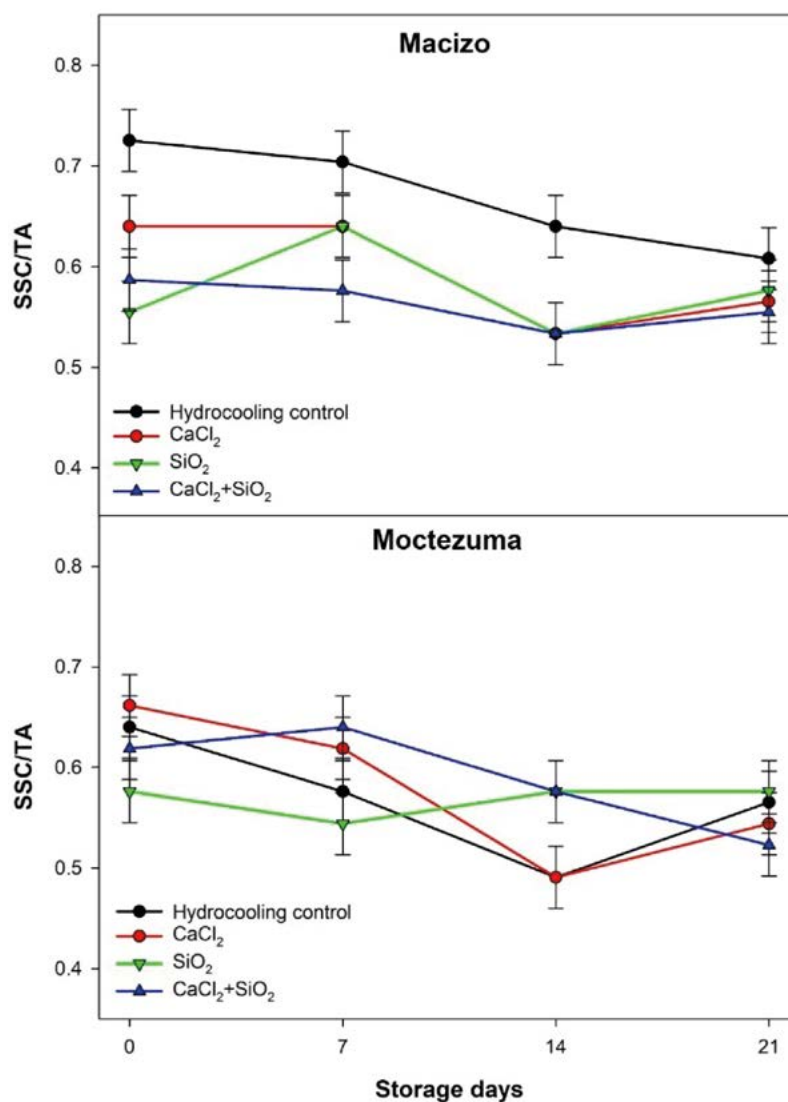


Figure 5. Effect of hydro-cooling, CaCl_2 , and SiO_2 (alone or combined in hydro-cooling) on the maturity index (SSC/TA) of Saladette-type tomato cultivars ‘Macizo’ (upper) and ‘Moctezuma’ (lower) during storage at ambient conditions. Values represent means $\pm$ standard error ($n=7$). Significant effects were observed for treatment ($F_{3,64}=3.77$, $p=0.015$), storage time ($F_{3,64}=12.75$, $p<0.001$), cultivar ($F_{3,64}=5.13$, $p=0.027$), and treatment $\times$ cultivar interaction ($F_{3,64}=6.23$, $p<0.001$).

CONCLUSIONS

Regardless of cultivar, storage conditions strongly influence postharvest fruit quality. Among the treatments evaluated, hydrocooling alone or combined with CaCl_2 was effective in reducing weight and firmness loss, particularly in the ‘Macizo’ cultivar. Although lightness (L^*) decreased by day 7 across treatments, it gradually recovered by days 14 and 21. Hydrocooling treatments also contributed to maintaining firmness until the end of storage. In contrast, SiO_2 treatment reduced titratable acidity in ‘Moctezuma’ and soluble solid content in ‘Macizo’. Additionally, b^* values decreased progressively during storage in both cultivars. These findings emphasize the potential of hydrocooling as an effective postharvest method to maintain tomato quality during storage.

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Optimal wheat distribution in Mexico

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ABSTRACT

Wheat is one of the most widely consumed cereals worldwide, and its availability is pivotal to food security in Mexico. This study employed linear programming, using the simplex method, to optimize wheat distribution routes throughout the country. Two scenarios were examined: a closed economy scenario, which demonstrated that domestic production is insufficient to satisfy internal demand, and an open economy scenario, which incorporated imports to offset the deficit. The model identified the optimal routes under state-level supply and demand constraints, highlighting Sonora as the principal domestic supplier, accounting for 31% of demand, and Veracruz as a strategic entry point for imported wheat. These findings underscore that the logistical optimization of both domestic production and imports is essential to safeguarding national food security.

Keywords: simplex method, wheat in open economy, wheat in closed economy.

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INTRODUCTION

In Mexico, wheat was introduced by the Spanish in 1520 and currently constitutes, together with maize and rice, one of the most important and widely demanded crops among the population (Ministry of Agriculture and Rural Development [SADER], 2021). Its relevance lies not only in its direct consumption, but also in its strategic role within the national agri-food system and the country's food security.

Within the national territory, wheat is classified into five groups: strong (Group I), medium-strong (Group II), soft (Group III), short and tenacious (Group IV), and durum (Group V). This classification is based on gluten properties (Agri-Food and Fisheries Information Service [SIAP], 2017). Four of these groups are mainly intended for the production of bread, flour, and other soft food products, whereas durum wheat is used predominantly in pasta production.

With respect to national production, durum wheat is concentrated primarily in Sonora, followed by Sinaloa, Michoacán, Guanajuato, Baja California, Jalisco, and Chihuahua (SIAP, 2023). This type of wheat exhibits a production surplus; therefore, part of its output is even allocated to livestock feed. In contrast, bread wheat or soft wheat, which is primarily

destined for flour production, recorded an output of 1.5 million tons in that year, cultivated across 21 states, with Sonora, Guanajuato, and Michoacán standing out as the principal producers (SADER, 2022).

In the sphere of foreign trade, Mexico exports wheat mainly to countries such as Algeria, the United States, Guatemala, Türkiye, and Venezuela. However, the country remains highly dependent on imports, with the United States serving as the main supplier, followed by Canada, Russia, Ukraine, and Argentina (FAOSTAT, 2024). This trade structure clearly reveals a relative insufficiency of domestic supply to meet internal demand, particularly in the case of bread wheat.

Moreover, it is important to acknowledge external constraints that limit national production, such as diseases and pests. In the irrigated regions of Northwestern Mexico, Northern Mexico, and El Bajío, the principal diseases affecting wheat are rusts, notably leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis* f. sp. *tritici*), the former being the most common and widespread in the country (Huerta *et al.*, 2011).

Added to this are international factors, such as the armed conflict between Russia and Ukraine, which has partially disrupted the global wheat supply, generating price increases and risks of shortage (Stanziani, 2023). In Mexico's case, this situation is further exacerbated by its commercial dependence on the United States to satisfy domestic demand (Espinosa, 2022), thereby exposing the country to supply vulnerabilities.

Although these elements reveal a structural problem surrounding wheat production and trade, there remains a knowledge gap regarding the efficiency of the grain's domestic distribution. In this context, logistics optimization emerges as a key factor in ensuring the timely and sufficient availability of wheat at the various points of consumption, even under scenarios of supply restriction.

Accordingly, the objective of the present research is to determine an optimal wheat distribution scheme in Mexico by identifying efficient routes that minimize resource use and make it possible to satisfy national demand. The study examines this issue under both closed- and open-economy scenarios in order to demonstrate the insufficiency of domestic supply and the strategic importance of optimal logistical allocation for strengthening the country's food security.

MATERIALS AND METHODS

In order to ensure the reproducibility of the results, this study employed a linear programming model solved using the simplex method (Ariza & Llinás, 2019). Linear programming makes it possible to optimize an objective function subject to linear constraints, under the assumptions of proportionality, additivity, divisibility, and certainty in the model parameters. The analysis was conducted under two scenarios:

- Closed economy, in which no foreign trade exists.
- Open economy, in which imports are incorporated through land and maritime customs entry points.

The model was implemented in LINGO 18 to verify the consistency of the results.

Case 1. Closed economy:

In the absence of foreign trade, the objective was to minimize the total transportation cost between producing states ($i=1, 2, \dots, m$) and consuming states ($j=1, 2, \dots, n$). The objective function is expressed as (Equation 1):

$$\text{Min } Z_{EC} = \sum_{i=1}^m \sum_{j=1}^n C_{ij} X_{ij}$$

where: Z_{EC} : linear objective function in a closed economy; C_{ij} : distance between origin i and destination j ; X_{ij} : quantity of product transported, to be determined, originating in i and destined for j .

Distances were obtained using the Point-to-Point Routes tool of the Ministry of Infrastructure, Communications and Transportation (SICT, 2024).

The 31 states and Mexico City were considered:

- If state production exceeded consumption, the state was classified as a supplier.
- If production was lower than consumption, it was classified as a demander.

Constraints: constraints refer to all variables that condition the objective function and indicate the available quantity that each supplier can provide to demanders (Alvarado, 2009). Within the system of constraints, both supply and demand restrictions were established.

Supply: to estimate total supply by state (i), total state production minus total state consumption was used, based on agricultural year-end data from the Agri-Food and Fisheries Information Service (SIAP, 2023). Therefore, (Equation 2):

$$E_i = PROD_i - CON_i \quad \forall i = 1, 2, \dots, m$$

where: E_i : wheat supply by state of origin i ; $PROD_i$: total wheat production at origin i ; CON_i : total wheat consumption at origin i .

In turn, total wheat consumption at origin (CON_i) was estimated using (Equation 3):

$$CON_i = \frac{CNA_{wheat}}{POB} \times POB_i$$

where: CNA_{wheat} : apparent national wheat consumption; POB : national population; POB_i : total population of state of origin i .

To calculate apparent national wheat consumption, total wheat imports were added to total national wheat production, and total wheat exports were subtracted (Equation 5):

$$CNA_{wheat} = PROD_{wheat} + IMP_{wheat} + EXP_{wheat}$$

where: CNA_{wheat} : apparent national wheat consumption; $PROD_{wheat}$: total wheat production; IMP_{wheat} : total wheat imports; EXP_{wheat} : total wheat exports.

Regarding imported and exported wheat quantities, data from the Statistics Division of the Food and Agriculture Organization of the United Nations (FAOSTAT, 2023) were used.

Thus, the constraint on the total quantity supplied by each origin (E_i), from each i to the m -th origin, is expressed as (Equation 5):

$$\begin{aligned} \sum_{j=1}^n X_{1j} &= E_1 \\ \sum_{j=1}^n X_{2j} &= E_2 \\ &\vdots \\ \sum_{j=1}^n X_{mj} &= E_m \end{aligned}$$

Demand: to determine total demand by destination (j), production at destination (j) was subtracted from consumption at destination (j). For these estimations, Equations 3 and 4 were adapted to the destination states j . Production data were obtained from the agricultural year-end records of the Agri-Food and Fisheries Information Service (SIAP, 2023), while national and state population data were drawn from the population census of the National Population Council (CONAPO, 2025). Finally, imported and exported wheat quantities were obtained from the Statistics Division of the Food and Agriculture Organization of the United Nations (FAOSTAT, 2023). Thus, demand was estimated as follows (Equation 6):

$$D_j = CON_j - PROD_j \quad \forall j = 1, 2, \dots, n$$

where: D_j : wheat demand by destination state j ; $PROD_j$: total wheat production at destination j ; CON_j : total wheat consumption at destination j .

The demand constraints for each destination (D_j), from each j to the n -th destination, are expressed as (Equation 7):

$$\begin{aligned} \sum_{i=1}^m X_{i1} &\leq D_1 \\ \sum_{i=1}^m X_{i2} &\leq D_2 \\ &\vdots \\ \sum_{i=1}^m X_{in} &\leq D_n \end{aligned}$$

Finally, the problem for the closed-economy case was formulated as follows (Equation 8):

$$\text{Min } Z_{EC} = C_{11}X_{11} + C_{12}X_{12} + \dots + C_{mn}X_{mn}$$

Subject to $(m+n)$ restrictions and non-negativity condition:

$$\begin{array}{cccccc} X_{11} & +X_{12} & \dots & +X_{1n} & = & E_1 \\ X_{21} & +X_{22} & \dots & +X_{2n} & = & E_2 \\ \vdots & \vdots & \ddots & \vdots & & \vdots \\ X_{m1} & +X_{m2} & \dots & +X_{mn} & = & E_m \\ \\ X_{11} & +X_{21} & \dots & +X_{m1} & \leq & D_1 \\ X_{12} & +X_{22} & \dots & +X_{m2} & \leq & D_2 \\ \vdots & \vdots & \ddots & \vdots & & \vdots \\ X_{1n} & +X_{2n} & \dots & +X_{mn} & \leq & D_n \\ \\ X_{ij} \geq 0 \quad \forall i=1,2,\dots,m \quad \wedge \quad \forall j=1,2,\dots,n \end{array}$$

Case 2. Open economy:

In this scenario, w customs entry points were incorporated (19 along the northern border, 2 along the southern border, and 17 maritime entry points), excluding airport customs due to their higher costs. The objective function was defined as follows (Equation 9):

$$\text{Min } Z_{EA} = \sum_{i=1}^m \sum_{j=1}^n C_{ij}X_{ij} + \sum_{k=1}^w \sum_{j=1}^n B_{kj}M_{kj}$$

where: Z_{EA} : linear objective function in an open economy; C_{ij} : distance between origin i and destination j ; B_{kj} : distance between origin k and destination j ; X_{ij} : quantity of product transported, to be determined, originating in i and destined for j ; M_{kj} : quantity of imported product transported, to be determined, originating in k and destined for j .

For all domestic origins (i)=1,2,..., m ; customs entry points of origin (k)=1,2,..., w ; and destination regions (j)=1,2,..., n . To estimate the distance from origin (i) and customs entry points (k) to destination (j), the Point-to-Point Routes tool of the Ministry of Communications and Transportation of Mexico (SCT, 2024) was used.

Supply: in addition to the constraints established in Equation 3, imported supply at customs entry points was incorporated (Equation 10):

$$\begin{aligned}
\sum_{j=1}^n M_{1j} &\geq 0 \\
\sum_{j=1}^n M_{2j} &\geq 0 \\
&\vdots \\
\sum_{j=1}^n M_{wj} &\geq 0
\end{aligned}$$

Demand: quantity demanded by each destination (D_j), from each j to the n -th destination (Equation 11):

$$\begin{aligned}
\sum_{i=1}^m X_{i1} + \sum_{k=1}^w M_{k1} &\leq D_1 \\
\sum_{i=1}^m X_{i2} + \sum_{k=1}^w M_{k2} &\leq D_2 \\
&\vdots \\
\sum_{i=1}^m X_{in} + \sum_{k=1}^w M_{kn} &\leq D_n
\end{aligned}$$

In another format, the problem in an open economy was posed as the following expressions (equation 12):

$$Min Z_{EA} = C_{11}X_{11} + C_{12}X_{12} + \dots + C_{mn}X_{mn} + B_{11}M_{11} + B_{12}M_{12} + \dots + B_{wn}M_{wn}$$

Subject to $(m+n+w)$ restrictions and non-negativity condition:

$$\begin{aligned}
X_{11} + X_{12} + \dots + X_{1n} &= E_1 \\
X_{21} + X_{22} + \dots + X_{2n} &= E_2 \\
\vdots &\vdots \\
X_{m1} + X_{m2} + \dots + X_{mn} &= E_m \\
M_{11} + M_{12} + \dots + M_{1n} &\geq 0 \\
M_{21} + M_{22} + \dots + M_{2n} &\geq 0 \\
\vdots &\vdots \\
M_{w1} + M_{w2} + \dots + M_{wn} &\geq 0
\end{aligned}$$

$$\begin{aligned}
X_{11} + X_{21} + \dots + X_{m1} + M_{11} + M_{21} + \dots + M_{w1} &\leq D_1 \\
X_{12} + X_{22} + \dots + X_{m2} + M_{12} + M_{22} + \dots + M_{w2} &\leq D_2 \\
\vdots &\vdots \\
X_{1n} + X_{2n} + \dots + X_{mn} + M_{1n} + M_{2n} + \dots + M_{wn} &\leq D_n
\end{aligned}$$

$$X_{ij}, M_{kj} \geq 0 \quad \forall i=1,2,\dots,n \quad \wedge \quad \forall j=1,2,\dots,m \quad \wedge \quad \forall k=1,2,\dots,w$$

In the case of the open economy, in addition to the 31 states and Mexico City, 38 customs offices (19 on the northern border, 2 on the southern border, and 17 maritime) were considered as a complement to the supply; to reduce costs, airport customs offices were excluded.

To ensure the robustness of the results, it was validated that all constraints were met in both scenarios.

RESULTS AND DISCUSSION

According to the information collected, by the end of 2023 Mexico had recorded a total wheat production of 3,476,026.80 tons (SIAP, 2023). In that same year, imports amounted to 4,022,527.23 tons, whereas exports totaled 126,158.51 tons (FAOSTAT, 2023). Based on these figures, apparent national wheat consumption was estimated at 7,372,395.52 tons, of which 47.15% originated from domestic production and 52.85% from foreign sources. The total population was 131,135,337 inhabitants; therefore, annual *per capita* consumption was estimated at 0.056 tons per person.

Case 1. Closed economy

In this first scenario, only three states were identified as self-sufficient in wheat production: Baja California, Sinaloa, and Sonora. The model considered the surplus of each of these states as origin supply.

The results showed that wheat allocations followed a geographically coherent pattern consistent with distance minimization. Baja California supplied Baja California Sur and Nuevo León; Sinaloa supplied exclusively Jalisco; whereas Sonora, the largest producer with more than 1.8 million tons, covered a broad range of destinations.

The optimal distribution made it possible to supply only 15 of the 29 states with demand, as shown in Table 1.

Sonora had the capacity to cover 31% of demand, Sinaloa 2%, and Baja California 0.6%, which together represented only 33.6% of national demand. The State of Mexico

Table 1. Optimal distribution of wheat in a closed economy in Mexico.

Origin (y): national supply (tons)	Destination (j): national demand (tons)	
Baja California: 36,088.52	Baja California Sur: 25,985.59 Nuevo León: 10,102.93	
Sinaloa: 117,345.46	Jalisco: 117,345.46	
Sonora: 1,821,319.50	Aguascalientes: 84,931.39	México: 163,989.81
	Chihuahua: 129,382.50	Michoacán: 7,899.22
	Coahuila: 169,984.70	Nayarit: 72,755.32
	Colima: 42,581.12	Nuevo León: 310,624.34
	Durango: 97,957.33	Querétaro: 142,413.38
	Guanajuato: 98,175.05	San Luis Potosí: 164,764.00
	Jalisco: 246,660.80	Zacatecas: 89,200.54
Total supply=1,974,753.48	Total demand=1,974,753.48	

Own source.

constituted the most critical case, recording a deficit of 809,962.94 tons, since its demand amounted to 973,952.76 tons and it received only 163,989.81 tons. An additional 14 states received no supply whatsoever, accumulating a further deficit of 3,086,405.71 tons. Overall, the national deficit under the closed-economy scenario reached 3,896,368.65 tons. This indicates that Mexico was able to satisfy only 1 out of every 3 tons demanded.

The geographical distribution of supply reveals a clear bias toward the northern region of the country, which is consistent with the location of the producing states and the cost-minimization objective function. Figure 1 illustrates this pattern.

These results are consistent with previous studies that have highlighted the high regional concentration of wheat production in Mexico, particularly in Sonora and the northwestern region of the country (Garza & Taddei, 2016; Brambila *et al.*, 2017). As these studies have warned, such concentration entails a structural vulnerability of the system under scenarios involving logistical or sanitary constraints.

Likewise, the risk associated with the Ug99 fungus, as documented in the specialized literature (Reyes, 2019), further reinforces the importance of distributing production across more regions and avoiding excessive dependence on a single producing state.

The analysis also confirms what was reported by Brambila *et al.* (2017): in order to achieve equilibrium between supply and demand, wheat yield would need to increase by at least 30%, a figure consistent with the gap observed between national demand and supply in this scenario.

In summary, the closed-economy scenario is not viable, even under optimistic assumptions of efficient distribution. Its inability to cover two-thirds of demand demonstrates that Mexico is structurally dependent on foreign trade to guarantee its food security.

Case 2. Open economy

In this scenario, the supplying states were once again Baja California, Sinaloa, and Sonora. However, due to the national deficit, imported supply from 11 optimal customs

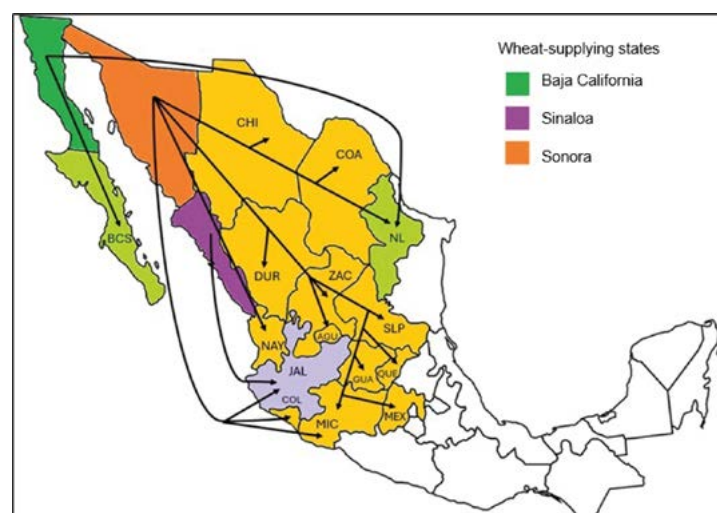


Figure 1. Wheat distribution network in Mexico under a closed-economy scenario. Source: Prepared by the authors based on the output results presented in Table 1.

entry points (1 land border crossing and 10 maritime entry points) was incorporated. The national distribution is presented in Tables 2, 3, and 4.

Here, a different pattern was observed compared with the closed-economy case: Baja California ceased supplying Nuevo León and instead supplied Coahuila. Sinaloa expanded its coverage to include Aguascalientes. Sonora increased the volume allocated to Jalisco and the State of Mexico.

Demand could not be met through domestic supply alone and was therefore complemented by imports, whose optimal pattern is shown in the corresponding tables.

The distribution presented in Table 2 contrasts with the results shown in Table 1. Baja California no longer satisfies the demand of Nuevo León, but instead supplies Coahuila; similarly, Sinaloa, in addition to covering the demand of Jalisco, also extends its supply to Aguascalientes. Nevertheless, demand is not fully satisfied by domestic supply alone; therefore, the deficit was complemented with imports. The optimal import quantities are presented in Tables 3 and 4, which highlight 11 customs entry points: one along the northern border and 10 maritime entry points. Accordingly, the open-economy scenario constitutes the optimal alternative for covering the remaining demand, which had previously been estimated at 66.4%.

The results identified only one land border customs entry point, namely Ciudad Miguel Alemán in Tamaulipas. Given that it is located along the border with the United States of America, this finding also suggests that this customs point would receive wheat from that country and potentially from Canada as well. With regard to maritime customs entry points, several of them converged on the same state, namely Veracruz, which emerged as a

Table 2. Optimal distribution of wheat in an open economy in Mexico.

Origin (i): national supply (tons)	Destination (j): national demand (tons)	
Baja California: 36,088.52	Baja California Sur: 25,985.59	
	Coahuila: 10,102.93	
Sinaloa: 117,345.46	Aguascalientes: 84,931.39	
	Jalisco: 32,414.07	
Sonora: 1,821,319.50	Chihuahua: 129,382.50	Michoacán: 7,899.22
	Coahuila: 159,881.77	Nayarit: 72,755.32
	Durango: 97,957.33	Querétaro: 142,413.38
	Guanajuato: 98,175.05	San Luis Potosí: 164,764.00
	Jalisco: 331,592.19	Zacatecas: 89,200.54
	México: 527,298.19	
Total national supply: 1,974,753.48	Total national demand: 1,974,753.48	

Prepared by the author using the output of results.

Table 3. Optimal distribution of imported wheat supply in Mexico at border customs offices.

Customs office of origin (k): imported supply (tons) Destination (j): domestic demand (tons)	Customs office of origin (k): imported supply (tons) Destination (j): domestic demand (tons)
Northern border: Ciudad Miguel Alemán, Tamaulipas: 320,727.28 Nuevo León: 320,727.28	Northern border: Ciudad Miguel Alemán, Tamaulipas: 320,727.28 Nuevo León: 320,727.28

Prepared by the authors using the output data.

strategic gateway for wheat distribution. Specifically, the optimal quantities are presented in Table 4.

Within Table 4, it was observed that the customs entry point of Tuxpan distributed the largest volume of wheat within Mexican territory, amounting to 1,553,905.54 tons, which represents 40% of the total estimated imports. Moreover, this customs point is located in the state of Veracruz; therefore, when the volumes from the Coatzacoalcos and Veracruz customs entry points are added, they total 2,344,388.73 tons, representing 61% of imported wheat supply. In other words, the state of Veracruz emerges as a fundamental pillar for the entry of imported wheat into Mexico.

Subsequently, the second state with the highest allocation for imported wheat entry was Tamaulipas, through the customs entry points of Altamira and Ciudad Miguel Alemán, which together accounted for 519,264.14 tons, representing 13% of the total imported quantity. In third place was the state of Guerrero, through the customs entry point of Acapulco, with a total of 316,308.93 tons, corresponding to 8% of imports. In the case of the remaining states, their combined share of total imports accounted for 18%.

By adding domestic supply to imported supply, national demand was fully satisfied. Specifically, total national wheat demand in Mexico was estimated at 5,871,122.15 tons, of which domestic supply, with 1,974,753.48 tons, covered 33.6%, whereas imported supply, with 3,896,368.68 tons, covered 66.4% of national demand. Thus, 2 out of every 3 tons demanded in Mexico must be supplied through imports, making foreign trade essential for meeting the needs of the Mexican wheat industry.

Table 4. Optimal distribution of imported wheat supply in Mexico at maritime customs.

Customs of origin (k): imported supply (tons)	Destination (j): national demand (tons)
Marítima: Manzanillo, Colima: 42,581.12	Colima: 42,581.12
Marítima: Acapulco, Guerrero: 316,308.93	Guerrero: 202,628.06
	Morelos: 113,680.87
Marítima: Salina Cruz, Oaxaca: 230,979.31	Oaxaca: 230,979.31
Marítima: Subteniente López, Quintana Roo: 113,541.74	Quintana Roo: 113,541.74
Marítima: Dos Bocas, Paraíso, Tabasco: 138,007.52	Tabasco: 138,007.52
Marítima: Altamira, Tamaulipas: 198,536.86	Tamaulipas: 198,536.86
Marítima: Coatzacoalcos, Veracruz: 334,287.56	Chiapas: 334,287.56
Marítima: Tuxpan, Veracruz: 1,553,905.54	Ciudad de México: 518,438.09
	Hidalgo: 178,938.48
	Estado de México: 446,654.53
	Puebla: 385,977.59
	Tlaxcala: 23,896.84
Marítima: Veracruz, Veracruz: 456,195.63	Veracruz: 456,195.63
Marítima: Progreso, Yucatán: 191,297.18	Campeche: 53,425.12
	Yucatán: 137,872.06
Total imported supply (maritime): 3,575,641.39	Total national demand: 3,575,641.39

Prepared by the author using the output of results.

The results for the open-economy scenario showed that only 28.94% (11 out of 38) of the customs entry points through which wheat could be imported were optimal, while the remaining customs points were not advantageous due to their distance from the producing states, which implies higher transportation costs and, consequently, renders them suboptimal.

Three relevant observations emerged from the analysis:

- Tuxpan, Veracruz, was the most important customs entry point, distributing 1,553,905.54 tons, equivalent to 40% of total imports.
- When Tuxpan, Veracruz, and Coatzacoalcas are considered together, the state of Veracruz concentrated 61% of imported wheat inflows.
- The second most relevant state was Tamaulipas, accounting for 13% of imports through Altamira and Ciudad Miguel Alemán.

Figure 2 illustrates this logistical pattern.

When domestic supply and imported supply were combined, the total demand of 5,871,122.15 tons was fully satisfied, of which domestic production contributed 33.6%, whereas imports accounted for 66.4%, equivalent to 2 out of every 3 tons consumed in Mexico.

This finding is consistent with other studies indicating that Mexico systematically ranks among the world's largest wheat importers, with a high structural dependence on foreign trade (Doperto & Michelena, 2011; Gutiérrez, 2023).

Structural and geopolitical risks (requested expansion)

The model reveals that the system depends heavily on a limited number of customs entry points. If Tuxpan or Veracruz were to experience logistical blockages, meteorological

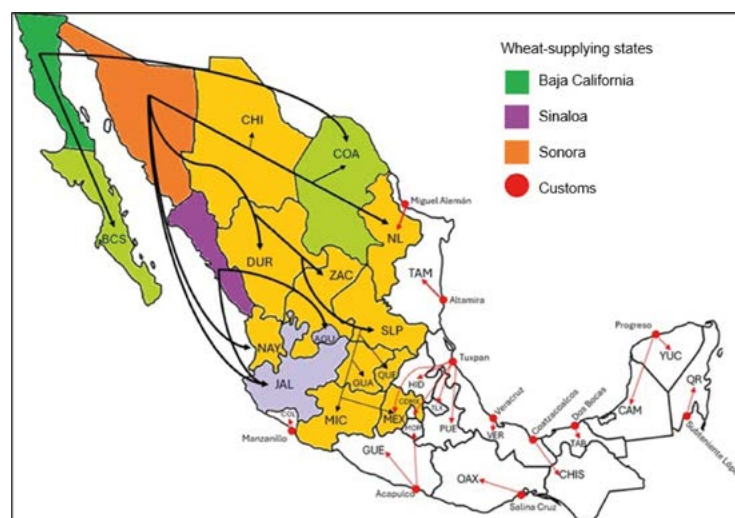


Figure 2. Wheat distribution network in Mexico under an open-economy scenario. Source: Prepared by the authors based on the model output results.

events, failures in port infrastructure, or increases in tariffs, wheat supply would be critically compromised.

The Russia-Ukraine war has demonstrated how geopolitical shocks can disrupt international wheat prices and availability, severely affecting importing countries (Gutiérrez, 2023).

In addition, the global context of inflation, economic slowdown, and trade tensions further increases the vulnerability of the Mexican system, which depends on specific ports and highly concentrated maritime routes.

Sensitivity analysis (added)

To evaluate the stability of the model, variations in domestic production can be tested:

- –10% production: the deficit would increase significantly and would require a greater use of maritime customs entry points, possibly incorporating some currently suboptimal ones.
- +10% production: imports would decline, but the general structure would remain unchanged, as Mexico would still depend on external supply.

The system is highly sensitive to agricultural yields, which is consistent with the agronomic literature.

CONCLUSIONS

The results showed that, under both the closed-economy and open-economy scenarios, Mexico does not produce sufficient wheat to satisfy its domestic demand, which makes dependence on imports unavoidable and compromises national food security. The country's total demand could only be met through a combination of 33.6% domestic supply and 66.4% imports, thereby evidencing a structural dependence on foreign trade.

The central variable of the model was the distance between origins and destinations, employed as the principal criterion in the simplex method to identify optimal routes. This approach made it possible to detect efficient distribution patterns under the logistical principle of carrying out the greatest number of activities with the smallest possible amount of resources. Under the closed-economy scenario, Sonora emerged as the predominant supplier, although total supply was able to cover the demand of only 14 states, leaving significant deficits, particularly in the State of Mexico. In contrast, under the open-economy scenario, the system exhibited greater coverage capacity due to the inflow of imports, with Veracruz consolidating its position as the most important logistical node in the country for the distribution of imported wheat.

Nevertheless, the model presents important methodological limitations. By considering distance as the sole optimization factor, it excludes real transportation costs, infrastructure availability, customs congestion, seasonal variability, and climatic or sanitary risks that could alter the optimal routes. Likewise, the model is static and does not incorporate fluctuations in productivity, international prices, or geopolitical shocks.

Based on these findings, it is suggested that decision-makers:

- Strengthen logistical infrastructure in key states such as Veracruz, Tamaulipas, and Sonora in order to reduce costs and vulnerabilities in distribution.
- Promote regionally differentiated public policies aimed at diversifying wheat production toward states with agroclimatic potential, thereby reducing concentration in the northwest.
- Advance risk-mitigation strategies, such as strategic reserves, forward contracts, and monitoring systems to respond to international crises.

As future lines of research, it would be pertinent to:

- Integrate real transportation costs, transit times, and road and port infrastructure.
- Incorporate dynamic analyses that include supply and demand projections.
- Evaluate scenarios involving productivity increases in emerging states.
- Conduct a more comprehensive sensitivity analysis considering variations in international prices, port closures, or climatic shocks.

Taken together, this study contributes to the analysis of wheat logistics in Mexico by showing, through linear programming, how the current configuration of production and the port system conditions the viability of national supply. The model demonstrates that, even under conditions of optimal distribution, wheat self-sufficiency is not attainable without reconfiguring domestic production and strengthening strategic logistical infrastructure.

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Physicochemical and Microbiological Assessment of Fishmeal

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ABSTRACT

Objective: To evaluate the quality of fishmeal obtained from two fish species (trout and carp) through its physicochemical and microbiological characterization, and to verify its compliance with established regulatory standards and its potential use in animal feed.

Design/methodology/approach: The fish samples consisted of rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio communis* L.). The freshness of the raw material was determined, and it was processed to obtain a fishmeal-type product for subsequent physicochemical and microbiological assessment.

Results: The trout and carp used as raw materials for fishmeal production scored 96 and 93.2 respectively, classifying them as top-quality fresh fish. Physicochemical analysis indicated that the moisture and protein content for trout fishmeal was 4.53% and 60.3%, respectively, while for carp fishmeal the moisture and protein content was 6.4% and 53%, respectively. In addition, the microbiological analysis indicated that *Salmonella* spp. and coliforms were not found in either fishmeal sample, and only low counts of aerobic mesophiles, fungi, and yeasts were present.

Limitations on study/implications: According to the results obtained, the species of fish and processing conditions in the production of fishmeal influence the physicochemical and microbiological properties, which suggests consideration in future research.

Findings/conclusions: The fishmeal obtained from trout and carp complied with the regulatory framework in terms of microbiological and physicochemical profile, with potential use in animal feed.

Keywords: Fishmeal production, feed, carp, trout, microbiological quality.

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INTRODUCTION

Fish is a highly perishable food product due to various intrinsic factors (high water activity, nutrients, and a near-neutral pH); its shelf life is limited by factors such as enzymatic, chemical, and microbiological deterioration (de Paiva Soares & Gonçalves, 2012; García *et al.*, 2022). Fish consumption is considered important for human nutrition and health due to its contribution of nutrients such as lipids (omega-3 fatty acids), proteins (high biological



value and digestibility), vitamins, and minerals, with a weekly recommended consumption of three to four servings per week (de Paiva Soares & Gonçalves, 2012; Fuertes Vicente *et al.*, 2014; Restrepo-Betancurt *et al.*, 2016; Cortés-Sánchez *et al.*, 2024). According to FAO, the world production of fish through fishing and aquaculture activities in 2024 was 184.5 million tons, which 164.6 million tons were destined for direct human consumption, with a *per capita* consumption of 20.7 kg; meanwhile, the remaining 20.8 million tons of production were directed to non-food uses such as the production of fishmeal and oil (FAO, 2024). Among the species of importance in aquaculture activities are the common carp and the rainbow trout, which at the global level in continental aquaculture were reported for the year 2020 an estimated production of 4236.3 million tons and 739.5 million tons respectively (FAO, 2022). Fishing and aquaculture activities represent a globally important production chain, which often generates large quantities of fish that may consist of different species of small fish, unwanted catch products or by-products of the process (heads, skins, scales, skeletons and viscera), with significant economic, health and negative environmental impacts caused by rejects and accumulation (Hleap & Gutierrez, 2017; Jiménez *et al.*, 2022). The use of whole fish or by-products from different species can lead to the generation of products with high nutritional value, such as fishmeal (Hleap & Gutierrez, 2017; Chonta, 2022; FAO, 2022). It is estimated that in 2020, 27% of global fishmeal production came from the use of by-products and 73% from whole fish (FAO, 2022).

Fishmeal is produced worldwide using different technologies and is the main form of cleansing of non-edible fishing and residues of the fillet and preservation industries (Silva Ortiz, 2003).

Fishmeal is considered the product of whole fish or by-products from processing such as filleting, which may or may not include oil extraction, and treatment with antioxidants (Cabello *et al.*, 2013; Quijije-Mero *et al.*, 2019). The general production process initially involves chopping or grinding the fish, cooking it at 100 °C for 20 minutes, pressing and/or centrifuging the product to extract some of the oil, and finally drying it to a maximum moisture content of 10% (Crispín-Sánchez *et al.*, 2019; FAO, 2022). Fishmeal can be used for various purposes, such as plant nutrition, acting as an organic fertilizer due to its high nitrogen and ash content (Monares-Gallardo *et al.*, 2012), and as a basic ingredient in the production of feed for livestock and companion animals (ruminants, rabbits, fish, crustaceans, pigs, and poultry) due to its high nutritional value in protein and omega-3 fatty acids (Cabello *et al.*, 2013; Quijije-Mero *et al.*, 2019; Chonta, 2022; FAO, 2022). It benefits animal production by reducing costs due to faster growth, improving nutrition and fertility, and decreasing the risk of disease (Chonta, 2022). In Mexico, fishing and aquaculture are economically important. In 2023, a total live weight production of 2,150,294 tons was recorded, with the main species produced being sardines, tuna, shrimp, carp, trout, among others. 1,511,332 tons were primarily destined for direct human consumption, with a *per capita* consumption of 14.68 kg and an additional 634,752 tons were used indirectly, meaning the fish was used to obtain fishmeal for animal feed (CONAPESCA, 2023). Carp and trout are important species in Mexico's aquaculture and fisheries production. Both activities are sources of employment and contribute to social subsistence and economic development. In 2023, carp production reached a peak of 14,816 tons for both capture

fisheries and aquaculture, with the state of Hidalgo being the main producer; while total trout production was 2,748 tons, with the state of Veracruz contributing the most nationally. (CONAPESCA, 2023).

Thus, various species of fish inhabit the coasts, inland waters and are produced in various aquaculture systems in Mexico. It is estimated that 60% of a fish's contents are used for food production, while the remaining 40%, consisting of fins, scales, skeleton, and head, is discarded. However, in various regions of Mexico, fishmeal and fish oil are obtained and used in livestock farming (Jiménez *et al.*, 2022).

Therefore, the objective of this research was to evaluate the quality of fishmeal obtained from two fish species (trout and carp) through its physicochemical and microbiological characterization, in order to verify its compliance with established regulatory standards and potential use in animal feed.

MATERIALS AND METHODS

Sample Collection

The samples consisted of three batches of rainbow trout (*Oncorhynchus mykiss*) weighing 813 ± 34 g, from aquaculture centers in the Lerma region and surrounding areas in the State of Mexico, and three batches of common carp (*Cyprinus carpio communis* L.) weighing 1292.6 ± 326 g, caught in Lake Chignahuapan, located in the municipality of Almoloya del Río, State of Mexico. The samples obtained were immediately transported under aseptic and refrigerated conditions to the laboratory for freshness analysis, processing into fishmeal under good hygiene practices, and subsequent physicochemical and microbiological assessment of the product.

Methodology

Samples of fresh whole carp and trout were evaluated for their sensory attributes to determine their quality grade according to Mexican standard (NMX-FF-002-SCFI-2011). This indicates that the product rating is based on a point deduction system starting from a base of 100. The final product rating is obtained by adding up all the applied deductions and subtracting the sum from the base score (Table 1). Subsequently, the fish were gutted, washed, and processed to obtain fishmeal according to Ayala-Pérez *et al.*, 2015, with modifications such as cooking at 90 °C for 20 minutes and drying at a controlled temperature of 90 °C in an oven (Memmert Model 30-1060) until reaching a final moisture content of less than 10%, followed by grinding (using a manual iron mill). The yield of fishmeal obtained was calculated using the following formula:

$$\text{yield \%} = (\text{weight of fishmeal produced} / \text{weight of fresh fish}) \times 100$$

The product obtained was subjected to the corresponding physicochemical analysis of pH (NOM-F-317-S-1978), moisture (NOM-116-SSA1-1994), proteins (NOM-F-68-S-1980), lipids (NMX-F-545-1992), ash (NMX-F-066-S-1978), carbohydrates (by difference), granulometry: 5% retained on #2 sieve with nominal opening of 2 mm (NMX-Y-013-1998-SCFI; COVENIN (1979) 1482-79), rancidity (Cabello *et al.*, 2013)

microbiological assessment was carried out using traditional methods with nutrient, differential and/or selective culture media by pour plate count, enrichment and selective isolation corresponding to total aerobic mesophilic bacteria (NOM-092-SSA1-1994), total coliforms (NOM-113-SSA1-1994), fungi and yeasts (NOM-111-SSA1-1994) and *Salmonella* spp. (NOM-114-SSA1-1994) respectively. Physicochemical and microbiological studies were performed in triplicate on independent batches of fishmeal obtained.

Analysis of data

Data analysis was performed using a one-way analysis of variance (ANOVA), following normality tests (Kolmogorov-Smirnov, $\alpha=0.05$) and Bartlett's test for homoscedasticity ($\alpha=0.05$). Significant differences ($p<0.05$) were determined using Tukey's multiple comparisons test ($p<0.05$). Data expressed as percentages were subjected to arcsine square root transformation (Zar, 2010). All tests were performed using SigmaStat V3.1 statistical software (2004).

RESULTS AND DISCUSSION

Quality grade of raw materials intended for fishmeal production

For trout and carp used as raw materials in the production of fishmeal, the total freshness scores were 96 ± 5.4 and 93.2 ± 4.4 , respectively, indicating, according to the Mexican standard (NMX-FF-002-SCFI-2011), that they are first-quality fresh fish (Table 1), the

Table 1. Sensory evaluation attributes to determine the quality grade of fish (NMX-FF-002-SCFI-2011).

Factor	Variation	Deduction
Smell of meat	Fermented grass (light), moisture.	0
	Fermented (penetrating), sweetish herb.	4
	Fecal-ammoniacal, rotten fruit (light).	8
	Ammoniacal rotten fruit (penetrating).	16
Meat Consistency	Resistance to digital and elastic pressure.	0
	The fingerprint pressure print disappears slowly, elastically.	2
	With little elasticity, fingerprints persist for extended periods.	8
	When the skin is flaccid, fingerprints do not disappear.	16
General appearance of the skin	Shiny, lifelike appearance, transparent mucus, attached scales.	0
	Loss of shine, slightly dark mucus, with detachment of some scales.	2
	Slight loss of color, sticky mucus, loss of scales.	4
	Dull, ashy, dull, milky or greenish mucus, without scales.	8
Eyes	Convex pupil, shiny black or slightly diminished.	0
	Flat pupil, slightly opaque cornea.	2
	Flat pupil and opaque cornea.	6
	Pupil totally reduced, cornea milky.	8
Gills	Bright pink, blood red or brick red color, well differentiated gill lamellae.	0
	Purple, blood red, brick red, some flakes stick together, smell of the sea.	2
	Purple, blood red, brick red, slightly discolored, loss of sea smell.	6
	Yellowish, dark brown, discolored, sour smell.	8

standard indicates that any product with less than 85 points is out of specification. Fish freshness is defined as the combination of multiple nutritional and sensory attributes that deteriorate rapidly in stages following capture or harvesting (processing, storage, transport, distribution, and marketing). The rate of spoilage is affected by factors such as fish type, capture conditions, temperature, water activity, pH, among others (Fuertes Vicente *et al.*, 2014; García *et al.*, 2022). Fish freshness is a key characteristic in the food industry and influences consumers' willingness to purchase the product. Sensory analysis is the oldest and most reliable method for assessing fish freshness and it is considered a tool that allows the evaluation of freshness-related attributes (color, odor, appearance, among others) (de Paiva Soares & Gonçalves, 2012; García *et al.*, 2022). Among the characteristics of sensory evaluation is its frequent use in the fishing industry, facilitating the assessment of batches of raw materials or finished products, as well as for sanitary evaluations (de Paiva Soares & Gonçalves, 2012; Prado-Toledo *et al.*, 2022). These tests are minimally invasive, easy to perform, do not involve the destruction of the sample, allow for the estimation of shelf life, are low-cost, and offer a comprehensive evaluation of fresh fish (de Paiva Soares & Gonçalves, 2012; García *et al.*, 2022).

It has been noted that the quality, yield, and nutritional value of fishmeal depend on factors such as the type of fish used, fish freshness, cooking temperature, drying, and storage conditions (Silva Ortiz, 2003; Ionita, 2022; FEDNA, 2025). Fish freshness can be affected by temperature and storage time conditions both after capture and before processing. From the moment of capture, fish are susceptible to autolytic and microbiological changes due to the activity of enzymes and microorganisms present on their surfaces and in their digestive system, leading to spoilage (Cruz *et al.*, 2000; Silva Ortiz, 2003). Therefore, spoiled fish can generate various undesirable compounds, including biogenic amines, which can affect the safety, nutritional value, digestibility, and sensory characteristics of a final product such as fishmeal (Cruz *et al.*, 2000; FEDNA, 2025).

Physicochemical assessment of fishmeal

In fishmeal production, the yield obtained from trout was $20 \pm 0.6\%$ and $23 \pm 0.9\%$ for carp. These values match with those reported by various studies on the yield of fishmeal production, estimated at around 20% (Tornes & George, 1970), 21% (Osorio, 2014), and 22% (Sandbol, 1993). The physicochemical analysis of the fishmeal obtained from trout and carp indicated a moisture content of 4.53% and 6.4%, respectively, which is lower than the maximum specification established by Mexican standard NMX-Y-013-1998-SCFI (animal feed-fish meal-specifications), Ecuadorian standard INEN 472. 1988-04 (fish meal-for animal consumption-Requirements) and Venezuelan standard COVENIN (1979) 1482-79 (Requirements for fishmeal), which specify a maximum of 10%. Fishmeal is primarily valued for its protein content. For the fishmeal obtained from trout and carp, the protein content was 60.3% and 53%, respectively, which was lower than the minimum content specified by Mexican standard NMX-Y-013-1998-SCFI of 62% (which will be used in this study as the main standard of comparative reference). However, according to Venezuelan standard COVENIN (1979) 1482-79, which specifies a protein content of 55 to 65% in fishmeal, only trout meal, with 60.3% protein, met this standard and was

therefore classified as Class B. Similarly, according to Ecuadorian standard (INEN 472. 1988-04), which indicates a protein content of 60% in fishmeal, only trout meal also met this specification with 60.3% (Table 2). Likewise, the quality specifications for fishmeal according to the Animal Health Service of the Republic of Argentina indicate a classification based on protein content, with first quality being those with a minimum of 60% protein and second quality those with a minimum of 40% protein (Flores, 2012). Considering the above, the trout and carp meal obtained is considered to be of first and second quality, respectively, according to the Animal Health Service of the Argentine Republic. It should be noted that standards applied to fishmeal are diverse and may not always have homology in quality specifications. This would require harmonization by the different regulatory entities for this type of product in terms of quality criteria, methods of analysis, units used, certification systems, among others.

The lipid content in trout and carp meal was 12.4% and 21%, respectively, exceeding the maximum specifications of the standards (NMX-Y-013-1998-SCFI), (INEN 472. 1988-04), and COVENIN (1979) 1482-79 (Table 1). Likewise, the lipid values obtained in the trout and carp meals are higher than the quality specifications for fishmeal according to the lipid content guidelines of the Animal Health Service of the Republic of Argentina, which indicate a maximum of 8% for first-quality meals and a maximum of 10% for second-quality meals (Flores, 2012). The differences in lipid content of the meals may be due to the production process and the type of raw material used. It has been established that fat and moisture content influence the shelf life of these products and if these are not controlled, spoilage due to rancidity and/or microbial growth can occur (Cabello *et al.*, 2013). Furthermore, the importance of fish lipids lies in their high nutritional value due to their omega-3 fatty acid content (eicosapentaenoic acid (EPA), docosapentaenoic acid, and docosahexaenoic acid (DHA), in addition to their prophylactic and therapeutic properties under certain nutritional conditions and diseases. Species such as trout have been reported to contain 4.5 g of EPA and 8.6 g of DHA per 100 g of lipids, respectively (Valenzuela *et al.*, 2012).

Table 2. Physicochemical assessment of fishmeal trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*).

Parameter	Trout	Carp	Mexican Standard (NMX-Y-013-1998-SCFI)	Ecuadorian Standard (INEN 472. 1988-04)	Venezuelan Standard COVENIN (1979) 1482-79
pH	6.6±0.01a	6.61±0.02a	NS	NS	≥5
Moisture (%)	4.53±0.05b	6.4±0.4a	10%	10%	10%
Protein (%)	60.3±0.6a	53±3.8b	62%	60%	55-65%
Lipids (%)	12.4±0.12b	21±0.76a	12%	10%	10-13%
Ash (%)	8.4±0.08b	13.8±0.36a	16%	16%	18-20%
% Carbohydrates (%)	14.2±1.8a	5.8±0.7b	NS	NS	NS
Rancidity meq O ₂ /kg	3±0.2b	4.5±0.71a	NS	20	13
Granulometry	0.69 ± 0.02 %a	0.46 ± 0.02 %b	5% retention in 2 mm opening mesh	2% retention in 2 mm opening mesh	0% retention in 2 mm opening mesh

NS=Not Specified. The standard does not present a specific criterion.

Mean ± standard deviation. n=3. Different letters per column indicate statistical differences (p<0.05).

Trout and carp meal had an ash content of 8.4% and 13.8%, respectively, which is lower than the maximum specification of 16% set by the NMX-Y-013-1998-SCFI and INEN 472.1988-04 standards, as well as the COVENIN (1979) 1482-79 standard, which specifies a content of 18% to 20% (Table 2). A high ash content in fishmeal can indicate a high proportion of bones in the raw material, which affects the digestibility and quality of the meal (Cabello *et al.*, 2013). Furthermore, the ash content of fishmeal is relevant in plant nutrition, where its alternative use as an organic fertilizer and complement to inorganic fertilizers has been noted due to its nitrogen and ash content (Monares-Gallardo *et al.*, 2012). On the other hand, the presence of carbohydrates in fishmeal is due to their presence mainly in the fish muscle where they are found as glycogen and constituents of nucleotides, which are a source of ribose released due to post-mortem autolytic changes (Huss, 1999).

The pH of the trout and carp meals obtained was around 6.6 (Table 1), complying with COVENIN (1979) 1482-79 and INEN 472 (1988-04) standards. It has been noted that the pH value should not be less than 5, as it is an indicator of the degree of chemical and physical reactions occurring in the meals (Cuéllar, 2021). Storage conditions can affect fishmeal quality, triggering chemical reactions and physical changes that can contribute to product degradation and cause a decrease in pH (Cabello *et al.*, 2013). The rancidity level in trout meal was 3 meq O₂/kg and in carp meal 4.5 meq O₂/kg. These values were lower than the peroxide value specifications of 20 and 13 meq O₂/kg established by INEN 472.1988-04 and COVENIN (1979) 1482-79, respectively (Table 1). High peroxide values can occur due to packaging defects in the fishmeal, which allow oxygen or light to penetrate. Inadequate storage conditions for the raw material also contribute to lipid deterioration (Cabello *et al.*, 2013). It has also been noted that the fishmeal production and drying process influences the oxidation of lipids present (Ackman, 1996). Regarding animal feed, the quality of fishmeal and fish oil indicates that the peroxide value recommended for feeding fish such as Salmonids should be <5 meq O₂/kg (Tacon, 1989).

The particle size analysis of the trout and carp meals obtained indicated a retention percentage lower than that specified by the NMX-Y-013-1998-SCFI and INEN 472.1988-04 standards (5% and 2%, respectively), but higher than that indicated by the COVENIN (1979) 1482-79 standard, which specifies 0% retention on a 2 mm opening mesh/sieve (Table 1). It has been mentioned that particle size is a basic parameter for the food industry in the analysis of physical properties of fishmeal, especially in food standardization and formulation processes, as well as in identifying raw material requirements (Campabadal & Celis, 1996; Dussán-Sarria *et al.*, 2019). In animal husbandry, the composition and physical properties of feed, such as uniformity and particle size, have a significant influence on digestive physiology, feed conversion, and productivity (Farfán-López *et al.*, 2015), as well as in pelleting and extrusion processes for the feed production intended for livestock (Campabadal & Celis, 1996).

Related research has indicated that fishmeal generally has a chemical composition of 60 to 72% protein, 5 to 12% fat, and 10 to 20% ash (Chonta, 2022). Cabello *et al.* (2013) reported, in a proximate analysis of fishmeal, a protein content of 60 to 52%, ash of 24 to 26%, lipids of 4 to 11.7%, and moisture of 4.8 to 6.7%. Quijje-Mero *et al.* (2019) indicates

that fishmeal, on average, contains 60% to 72% protein, 5% to 12% fat, and a maximum moisture content of 9%. Finally, Rojas (2005) indicates that fishmeal, on average, contains a minimum of 65% protein, a maximum of 12% fat, 10% moisture, and 15% ash. On the other hand, Sancho-Hernandez and Herrera-Ramirez (1996) obtained fishmeal presenting the product with 8.9% moisture and 69.9% protein, noting that a positive aspect in the use of fish protein in animal feed is the protein content, which should be a minimum of 50%.

It is important to note that the chemical composition of fish can be influenced by various factors such as species, season, migratory behavior, sexual maturation, feeding cycles, diet, environment, size, and genetic traits, among others (Huss, 1999). Therefore, fish as a raw material for obtaining products such as fishmeal, along with processing parameters, can affect its composition and quality (Graü de Marín *et al.*, 2011; Cabello *et al.*, 2013; Quijije-Mero *et al.*, 2019).

Considering the above, it should be noted that the two different species used in this study, their origins (wild-caught carp and farmed trout), and the processing method for obtaining fishmeal, which did not involve pressing or centrifuging, are just some of the factors that may have influenced the physicochemical parameters found, such as the high lipid content in both fishmeal samples, which exceeds the levels stipulated by regulatory standards for these products. This excess of lipids, beyond the nutritional and energy value it provides, can also promote rancidity, reducing the quality and shelf life of the fishmeal for use in animal feed.

Within the fishmeal industry, quality criteria are based on chemical composition, including protein, lipids, moisture, ash, and salt. One of the main parameters is protein content, which is better the higher its value, especially when high-nutrient diets are required (Sandbol, 1993). Fishmeal production can be carried out using different raw materials, which influences the composition of the final product. It has been observed that some fishmeal made from fish by-products tend to have a lower protein content and a higher ash content compared to fishmeal obtained from whole fish (Sandbol, 1993; FAO, 2022). It has been pointed out, for example, that oily fish has a higher protein content than white fish (Ionita, 2022). Most of the fishmeal produced is used in animal feed, and its quality is primarily determined by its protein and lipid content, moisture, and the absence of pathogenic microorganisms, among other factors (Silva Ortiz, 2003). The main constituent protein in fishmeal, which is easily digestible, has a good proportion of essential amino acids such as methionine, cysteine, lysine, threonine and tryptophan with high digestibility value, in addition to having low antigenicity, making it convenient for the formulation of feeds (Graü de Marín *et al.*, 2011; Chonta, 2022; FEDNA, 2025).

Fishmeal microbiological assessment

Table 3 presents the results of the fishmeal microbiological analysis. The absence of *Salmonella* and total coliforms in both fishmeal is noteworthy, as is the low proportion of total aerobic mesophiles, fungi and yeasts with 135 CFU/g, and <10 CFU/g in trout meal and <10 CFU/g, and <10 CFU/g in carp meal respectively. Therefore, the fishmeal (trout and carp) meets the microbiological parameters of Mexican and international

Table 3. Microbiological assessment of fishmeal trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*).

Analysis	Trout	Carp	Ecuadorian standard (INEN 472. 1988-04)	Venezuelan Standard COVENIN (1979) 1482-79	Mexican Standard NMX-Y-013-1998-SCFI
<i>Aerobic mesophiles</i>	135 ± 6.3 CFU/g	<10 CFU/g	10 ⁶ CFU/g	NS	NS
Total coliforms	Absence *	Absence *	10 ⁴ CFU/g	NS	Absence
<i>Salmonella</i> spp.	Absence in 25g	Absence in 25g	Absence in 25 g	Absence in 25 g	Absence in 25g
Fungi and yeasts	<10 CFU/g	<10 CFU/g	10 ⁴ CFU/g	Absence	NS

NS: The standard does not present a specific criterion. CFU: colony-forming units. *No growth at 10⁻¹ dilution.

standards. In fishmeal, microbiological quality is evaluated by counting or detecting the presence of various pathogens such as *Salmonella*, *Shigella*, *Vibrio cholerae*, coliforms like *Escherichia coli*, and fungal organisms (Silva Ortiz, 2003; Crispín-Sánchez *et al.*, 2019; Ubaqui, 2021). Given that the fishmeal production process involves cooking and drying, which inactivate microorganisms and spoilage enzymes and even when the raw material is in a highly deteriorated state, it is considered that microbiological contamination should not be present in the final product (Silva Ortiz, 2003; Chonta, 2022). However, in stages subsequent to drying, contamination can occur due to product handling by the operator, inadequate or nonexistent cleaning and disinfection procedures, as well as poor hygiene conditions during fishmeal storage (Silva Ortiz, 2003; Chonta, 2022). In this study, the processing of fish to obtain fishmeal, including handling, packaging (at room temperature), and subsequent storage, was carried out following good hygiene practices. Likewise, the microbiological analysis was performed under aseptic conditions for handlers, materials, equipment, and the work area. This contributed to the low microbial load present in the trout and carp fishmeal.

It should be noted that stages involving heat treatment in the fishmeal production process allow for the control of microbial contamination of these products, but the quality of their constituents can be affected when prolonged temperatures and times are involved. Therefore, alternatives for the microbiological control of fishmeal, such as the use of organic acids, have been proposed (Crispín-Sánchez *et al.*, 2019).

Among the zoonotic pathogens to be evaluated in fishmeal, *Salmonella* spp. is the most prominent in sanitary control due to its economic impact on human and animal health (Silva Ortiz *et al.*, 2003). *Salmonella* belongs to the Enterobacteriaceae family, inhabits the intestinal tract of humans and animals, and consists of Gram-negative, motile bacteria. It grows at pH 4-9 and temperatures of 8-45 °C, is heat-sensitive, and dies at temperatures of 70 °C or higher; it is resistant to drying and can survive for years in dust and dirt (Crispín-Sánchez *et al.*, 2019). Furthermore, this pathogen has been identified as one of the main agents responsible for cases of foodborne illness worldwide (Alerte *et al.*, 2012; Espinosa *et al.*, 2014; Soto *et al.*, 2016).

The main use of fishmeal is in the development of balanced feeds for aquaculture and livestock activities, making the control of this pathogen in feed particularly important, as its purpose is to protect the human food chain from contamination by infected animals (Crispín-Sánchez *et al.*, 2019). In fishmeal, fungal contamination can lead to the presence

of fungi and mycotoxins responsible for serious human and animal health problems from the consumption of contaminated food and feed. Fishmeal with a moisture content above 10%, subjected to inadequate drying or improper storage, becomes a substrate for the growth of various fungal species such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Mucor*, some of which produce mycotoxins (Graü de Marín *et al.*, 2011). Coliform bacteria are found throughout nature, including in humans and animals; coliform bacteria are Gram-negative, aerobic or facultative anaerobic bacilli that ferment lactose (Campuzano *et al.*, 2015). In food, they are considered indicators of quality and hygiene, reflecting inadequate hygiene practices in food handling, manufacturing, and preservation (NOM-113-SSA1-1994; Campuzano *et al.*, 2015). This microbial group assesses the microbiological quality of a product, the efficiency of sanitary and hygienic practices for equipment, and the sanitary quality of the water used in food processing (NOM-113-SSA1-1994).

Aerobic mesophilic bacteria are a group of microorganisms capable of growing at around 35 °C. Their count estimates the total bacterial population, reflecting the sanitary quality of a food product and the handling and hygiene conditions of the raw materials (ANMAT, 2014; Campuzano *et al.*, 2015). High counts may indicate high contamination of the raw materials, poor hygienic handling during processing, the possible presence of pathogens, and a short shelf life for the product (ANMAT, 2014). In studies related to the microbiological analysis of fishmeal, Sancho-Hernandez & Herrera-Ramirez (1996) reported that fishmeal made from bycatch of shrimp catches showed a total bacterial count of 1.4×10^5 CFU/g and a fungal and yeast count of 3×10^2 CFU/g, indicating hygienic handling in the fishmeal production process. Therefore, it was considered suitable for use in formulating chicken diets.

Meanwhile, Lúquez-Pérez & Hleap-Zapata (2020) in their microbiological analysis of fishmeal obtained from viscera for use in broiler chicken feed, reported that the fishmeal had microbiological values adjusted to what is allowed in the Colombian Technical Standard (NTC 685/2004) including the absence of *Salmonella*, mesophilic count $\leq 10 \times 10^4$ CFU/g, coliforms $\leq 10 \times 10^2$ CFU/g, fungi and yeasts $\leq 10 \times 10^2$ CFU/g, concluding that the fishmeal obtained is an alternative for animal feed.

The fishmeal obtained from trout and carp, evaluated for its physicochemical profile, presented a moisture content of less than 10%, as stipulated by the regulatory framework (NMX-Y-013-1998-SCFI, INEN 472.1988-04 and COVENIN (1979) 1482-79) focused on physicochemical specifications for fishmeal used in animal feed. However, regarding protein content, one of the main parameters considered in the quality of fishmeal, the carp meal did not meet the established requirements for protein content; while the trout meal only failed to meet the specification of the NMX-Y-013-1998-SCFI standard, as its protein values were below the stipulated level.

The trout and carp fishmeal obtained and microbiologically evaluated met the microbiological specifications (*Salmonella*, aerobic mesophilic bacteria, and total coliforms) of the regulatory framework (NMX-Y-013-1998-SCFI, INEN 472.1988-04, and COVENIN (1979) 1482-79) focused on microbiological specifications for fishmeal used in animal feed.

The lipid content of the carp meal obtained was above the limits established by the regulatory framework NMX-Y-013-1998-SCFI, INEN 472.1988-04, and COVENIN (1979) 1482-79. While the trout meal presented levels within the established COVENIN (1979) 1482-79 standard, it exceeded the specifications of the NMX-Y-013-1998-SCFI and INEN 472.1988-04 standards.

The carp and trout fishmeal obtained showed values for ash content, rancidity index, and particle size (granulometry) in accordance with the established regulatory framework (NMX-Y-013-1998-SCFI, INEN 472.1988-04, and COVENIN (1979) 1482-79), which focuses on physicochemical and microbiological specifications for fishmeal used in animal feed.

The carp and trout used as raw materials for fishmeal production were classified as first-class or fresh according to the specifications of standard NMX-FF-002-SCFI-2011, and rancidity index values were obtained below those stipulated in the regulatory framework (INEN 472.1988-04 and COVENIN (1979) 1482-79). The freshness and quality of the raw material are important for obtaining high-quality fishmeal, since fish, due to its lipid content and greater spoilage when used for fishmeal production, may exhibit higher rancidity levels, affecting stability and shelf life.

The protein, lipid, ash, and peroxide value content of trout and carp fishmeal, and their differences, can be associated with several factors, including the species or origin of the fish, as well as the fishmeal production process. The results obtained allow us to understand the physicochemical profile and microbiological quality of value-added products derived from the use of two aquatic resources widely produced and marketed in Mexico, primarily for human consumption.

CONCLUSIONS

Fishmeal is of primary interest in animal feed. The fishmeal obtained in this study meets the microbiological specifications of the regulatory framework established for fishmeal intended for animal feed. However, the same cannot be said for compliance with physicochemical specifications regarding lipid and protein content for carp fishmeal. Thus, trout meal can be considered potentially useful in animal feed formulations, while carp meal, only partially meeting the physicochemical specifications, should be considered with caution for this purpose.

The study presents several limitations, including the species, origin, and versatility of processing conditions in obtaining fishmeal, which may be associated with the final physicochemical and microbiological characteristics of the product. Therefore, further studies are needed to obtain more information on the conditions for obtaining and optimizing fishmeal from these two species and the effect on its physicochemical and microbiological properties in order to meet specifications for use in animal feed.

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Organic mulch as a strategy to improve the health and productivity of Calafate (*Berberis microphylla* G. Forst) in south-central Chile

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ABSTRACT

Objective: Calafate (*Berberis microphylla* G. Forst) is a shrub with berries of high functional value that is cultivated in early stages in Chile. Fundamental agronomic management practices, such as physical weed control, have been scarcely evaluated. This study analyzed, over two seasons (2022 and 2024), the effect of different types of mulch.

Design/methodology/approach: The treatments of no mulch (CK), hazelnut shell (HAZ), oat straw (OAT), and geotextile (GEO) were evaluated on plant health indicators (photosystem II quantum yield, stomatal conductance, leaf area index, and foliar pigments) and productivity in an orchard in south-central Chile.

Results: In 2022, OAT and GEO significantly increased leaf development and chlorophyll content, registering increases of up to 115% and 108%, respectively, compared to the control, without significant increases in fruit production. In 2024, the mulches did not significantly alter leaf pigments (chlorophyll, anthocyanins, flavonoids); however, OAT and GEO significantly increased yield and fruit weight, reaching increases of up to 85% and 75%, respectively, compared to the control. The number of seeds per fruit did not vary between treatments ($P > 0.05$).

Findings/conclusions: OAT and GEO improve physiological performance and plant health in the short term and, cumulatively, promote productivity in subsequent seasons.

Keywords: Pigments; antioxidants; yield; berries; chlorophyll.

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INTRODUCTION

The Calafate (*Berberis microphylla* G. Forst), is a species of the genus *Berberis*, which can grow up to 1.5 meters and has small, spiny and evergreen leaves, yellow flowers grouped in clusters and blue fruits with five to seven seeds (Silva *et al.*, 2020). This wild species grows in cool climates and moist soils, and is commonly found associated with temperate forests in the Patagonian region of Chile and Argentina (Silva *et al.*, 2020). Calafate has been shown to have high edaphoclimatic adaptability, being able to grow and fruit in extreme and warm climates (Radice *et al.*, 2018). For example, in Chile, Calafate has been identified in the Maule Region, where maximum temperatures can reach up to 32 °C in summer (Agrometeorología, 2025).

In the last 20 years, research in Calafate has had a notable increase due to the nutritional content and phenolic profile of its fruits beneficial to human health, which



can have up to four times greater antioxidant capacity than the blueberry (Rodoni *et al.*, 2014). For this reason, public and private initiatives have developed its nascent cultivation for small-scale commercial purposes, currently reporting an area of 0.24 ha (Natural Resources Information Center, 2022). However, information on plant management to assess its physiological state and fruit production is still scarce, requiring experimentation with key practices used in fruit orchards, such as weed control.

Soil cover, or mulch, is a practice that is being implemented more frequently in fruit orchards, due to its benefits as a physical suppressor of weeds, allowing the replacement of herbicides (Paušič *et al.*, 2021), and for favoring biodiversity and the activity of microbial communities, which contribute to the nutrition and productivity of plants (Rodoni *et al.*, 2014). However, it has been shown that the effects on plant physiology and yield are strongly determined by the type of mulch used. For example, in an apple orchard, it was shown that highly degradable organic materials such as cereal straw, when more easily incorporated into the soil, can significantly improve fertility through changes in pH, organic matter and nutrients such as P and N, generating improvements in the plant (Selvalakshmi *et al.*, 2022). On the other hand, in some cases, black synthetic mulches that absorb solar radiation can lead to a decrease in biological activity and soil fertility with repercussions for the plant (Meyer *et al.*, 2021). In cultivated Calafate, mulches were previously evaluated by Betancur *et al.* (2023), who demonstrated that mulch suppressed weeds and significantly improved soil moisture retention, increasing soil biological activity by up to 38% compared to plants without mulch. Despite this evidence, a significant knowledge gap remains regarding the effects of different mulch types on plant health components such as physiology, morphology, leaf pigments, and fruit quality.

This study aimed to determine the effect of different soil mulch types (no mulch, hazelnut shell, oat straw, and geotextile) on plant health components of Calafate (stomatal conductance, photosystem II yield, chlorophyll concentration, anthocyanins, and flavonoids) and productivity (total fruit yield, weight, and size). The hypothesis suggests that oat straw mulch treatment, being easier to degrade, will significantly improve the plant's physiological response and concentrate more pigments such as chlorophyll, which will have a favorable impact on fruit production. This effect is attributed to the ability of cereal straw to improve soil fertility by providing organic matter and promoting microbial activity (Selvalakshmi *et al.*, 2022). On the other hand, we hypothesize that hazelnut shell and geotextile treatments, being less degradable, do not significantly influence the plant's pigment content, but will improve fruit production due to greater soil moisture retention (Betancur *et al.*, 2023). The results of this study allow us to determine the types of soil mulch that offer the greatest vegetative and productive benefits to Calafate, a species that produces fruit with functional properties for human health.

MATERIALS AND METHODS

Location, soil, and climate conditions of the study site

The study was conducted in an experimental orchard in Calafate, located at the Adventist University of Chile, Ñuble Region, Chile (36° 31' S; 71° 54' W). The soil corresponded to the Andisol order, which was characterized by a chemical analysis.

The climate was temperate Mediterranean, characterized by cold, wet winters with temperatures and precipitation of 21.1 °C and 528.4 mm, respectively, between April and July; summers, meanwhile, had temperatures of 40 °C and low precipitation of 24.4 mm (INIA, 2024).

Planting and management of the Calafate crop

Plants originating from southern Chile and propagated from seed in 2015 were established in 2017 in a 352-plant orchard with a spacing of 3 m between rows and 1 m within rows. At establishment, a base fertilization was applied for pest control. This consisted of 150 g of urea (45% N), 200 g of triple superphosphate (46% P₂O₅), and 200 g of potassium sulfate (50% K₂O). Water replenishment was standardized for all treatments, and the daily potential evapotranspiration of the crop (ETc) was estimated using the methodology suggested by Romero *et al.* (2010).

Establishment of the trial and experimental design

In 2017, weeding was carried out around the Calafate plants, and subsequently, soil mulches were applied with a minimum thickness of 5 cm and a 1 m diameter around each plant. The organic mulches consisted of dry oat straw and European hazelnut shells, while the geotextile used was composed of black polyester and polypropylene. The oat straw mulch was renewed annually due to its rapid decomposition, while the hazelnut shell mulch was renewed every four years. The experimental design of the trial corresponded to a randomized complete block design, where the experimental unit consisted of the average of two Calafate plants.

Evaluation of physiological variables and foliar pigments

Stomatal conductance and photosystem II peak quantum yield were measured during the 2022 growing season (December) at four times of day (09:00 h, 12:00 h, 15:00 h, and 18:00 h). Stomatal conductance was measured using a portable SC-1 porometer (Decagon Devices, Washington, DC, USA), as described by Pinto-Morales *et al.* (2022). Photosystem II peak quantum yield was measured with a portable OS-5p fluorometer (Opti-Sciences, Hudson, NH, USA), as indicated by Pinto-Morales *et al.* (2022). Leaf area index was measured at 12:00 h with an AccuPAR LP-80 ceptometer (Decagon Devices Inc., Washington, DC, USA), also following the recommendations of Pinto-Morales *et al.* (2022). In addition, the surface temperature of the leaf and mulch (°C) was measured using a CTR1000 portable infrared thermometer (WIKA Instruments S.A.U., Barcelona, Spain).

Between September and December 2024, once a week on clear days at 12:00 PM, the foliar pigment content of Calafate leaves was evaluated. The concentration of chlorophyll, flavonoids, anthocyanins, and the nitrogen-flavonol index were measured by the fluorescence ratio at wavelengths F660 nm/F325 nm and F660 nm/F525 nm, respectively. A portable multipigment analyzer, model MPM-100 (Opti-Sciences, Hudson, NH, USA), was used for this purpose, following the same criteria used for the physiological variables.

Productivity and fruit yield assessment

In 2022 and 2024, fruit yield (kg plant^{-1}) was measured by manually harvesting the total production per plant, 130 days after full bloom, during December. The harvested fruits were then weighed on a Gravimetrics 360 ES Series analytical balance (Precisa Gravimetrics AG, Dietikon, Switzerland). Each fruit was also characterized individually by measuring the weight of ten selected fruits and determining their polar diameter with calipers, as described by Betancur *et al.* (2023). In 2024, seed weight was also included. Ten fruits were randomly selected, each fruit was manually opened, and the seeds were extracted, weighed individually, and the average weight was calculated.

Statistical analysis

An analysis of variance (ANOVA) was performed, and Fisher's LSD test was applied to compare treatments, considering a significance level of 0.05. Statistical analysis was performed using InfoStat software (Di Rienzo *et al.*, 2020). To evaluate the relationships between vegetative and productive variables of the Calafate plant affected by the types of mulch, a principal component analysis (PCA) was performed, applying a multivariate analysis of variance (MANOVA) with a significance level of $p=0.05$ using RStudio software, version R 4.5.1 (R Core Team, 2024).

RESULTS AND DISCUSSION

Leaf and mulch temperature, 2022 season

Leaf and mulch temperature measurements taken during 2022 indicated significant differences between treatments. Leaf temperature (Figure 1a) increased progressively throughout the day, reaching its peak at 3:00 PM, coinciding with the period of greatest solar radiation. The mulch treatments (HAZ, GEO, and OAT) exhibited the lowest leaf temperatures at the time of highest temperature (3:00 PM) compared to the control (CK), being 12%, 16%, and 19% lower, respectively. Mulch temperature (Figure 2b) showed a similar pattern, increasing from the early morning hours until the end of the evaluation

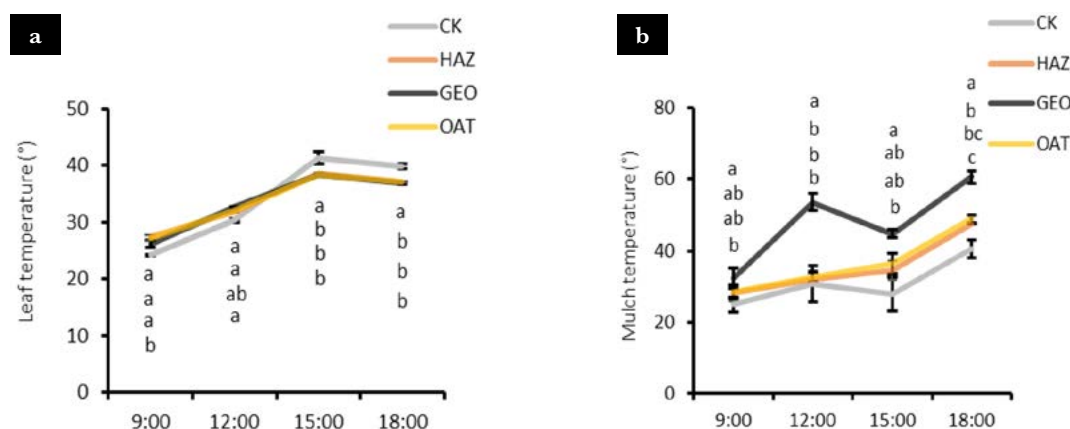


Figure 1. Leaf temperature (a) and mulch (b) 2022 season. CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Different lowercase letters indicate significant differences between treatments according to Fisher's LSD test ($p<0.05$). Mean $\pm$ standard error ($n=4$). Bars represent the experimental error for each treatment.

period. The GEO treatment had the highest mulch temperature at the end of the day (6:00 PM) compared to the other treatments, being 18% higher than HAZ, 30% higher than OAT, and 44% higher than CK.

Plant physiological parameters, 2022 season

Regarding the plant's physiological variables evaluated during 2022, the results indicated that the GEO and OAT treatments favored some variables compared to the other treatments (CK and HAZ). The leaf area index (LAI) (Figure 2a) was significantly higher with OAT and GEO than with HAZ (115%) and CK (85%). For the chlorophyll content index (SPAD) (Figure 2b), GEO showed the highest average value, being approximately 108% higher than CK and 85% higher than HAZ. The photosystem II quantum yield (F_v/F_m) (Figure 2c) decreased during the hours of highest radiation (3:00 PM), followed by a recovery towards the end of the day, but no significant differences ($p > 0.05$) were observed between treatments. Stomatal conductance (Figure 2d) showed an increase around midday (12:00 PM), although without significant differences between treatments. Average stomatal conductance values ranged between 180 and 350 $\text{mmol m}^{-2} \text{s}^{-1}$.

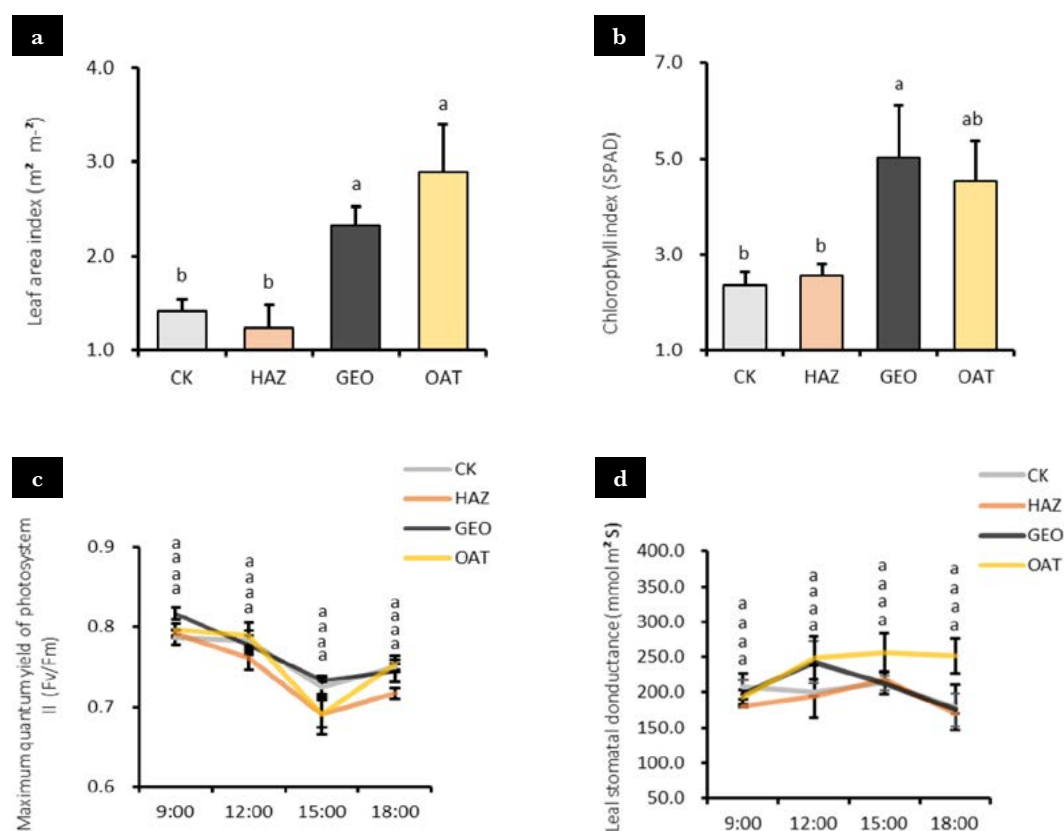


Figure 2. Plant physiological parameters measured in the 2022 season: a) leaf area index, b) chlorophyll index, c) photosystem II quantum yield, and d) stomatal conductance. Treatments: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Different lowercase letters indicate significant differences between treatments according to Fisher's LSD test ($p < 0.05$). Mean $\pm$ standard error ($n=4$). Bars represent the experimental error for each treatment.

Fruit performance parameters 2022

The fruit parameters analyzed in 2022 showed no statistically significant differences between treatments. Regarding fruit yield (Figure 3a), the GEO treatment showed the highest average value, reaching approximately $1.9 \text{ kg plant}^{-1}$, followed by OAT, while the control (CK) registered the lowest yield, at approximately $0.8 \text{ kg plant}^{-1}$. Despite this trend, there were no statistically significant differences ($p > 0.05$) between treatments. Regarding the average weight of 10 fruits (Figure 3b), the values ranged from 2.5 g to 2.9 g, with no significant differences between treatments. As for the morphological dimensions of the fruit, both the equatorial diameter (Figure 3c) and the polar diameter (Figure 3d) showed average values between 6.5 and 7.5 mm, respectively.

Analysis of plant and fruit variables 2022

Principal component analysis (PCA; Figure 4) showed that the first two components explained 57.5% of the total variation. Some trends were observed with the mulch treatments; for example, the GEO treatment was slightly associated with higher yield and fruit weight, while OAT was linked to physiological variables such as leaf area index and chlorophyll content. In contrast, CK and HAZ clustered more closely, reflecting similar physiological responses. However, PERMANOVA analysis showed no significant differences between treatments ($F = 2.099$; $p = 0.133$), with high overlap observed between groups.

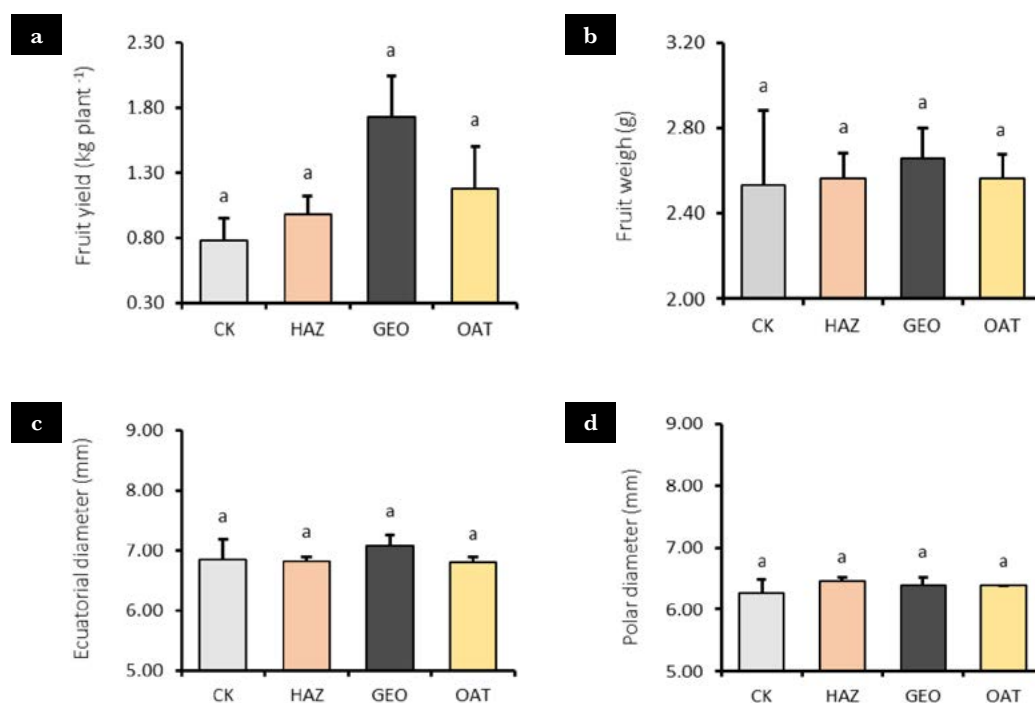


Figure 3. Fruit parameters measured in the 2022 season: a) fruit yield, b) average weight of 10 fruits, c) equatorial diameter, and d) polar diameter. Treatments: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Different lowercase letters indicate significant differences between treatments according to Fisher's LSD test ($p < 0.05$). Mean $\pm$ standard error ($n = 4$). Bars represent the experimental error for each treatment.

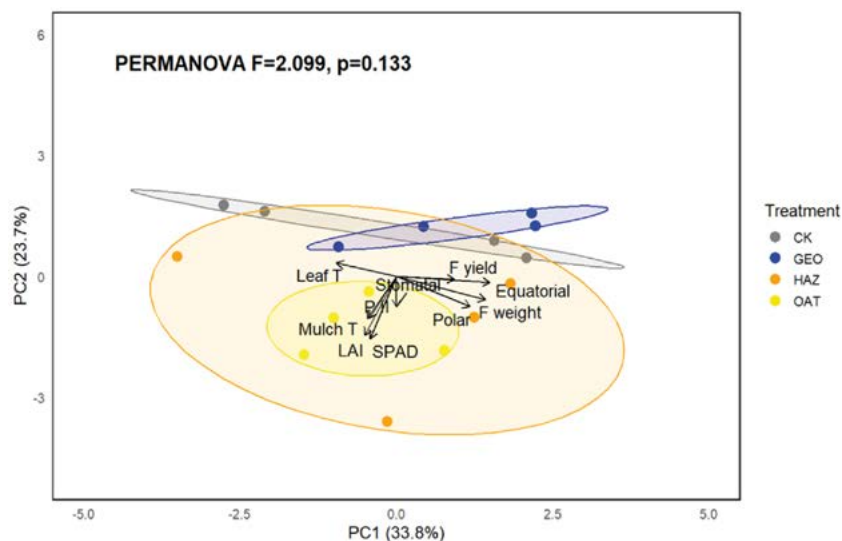


Figure 4. Fruit parameters measured in the 2022 season. Treatments: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Leaf T=leaf temperature; Mulch T=mulch temperature; LAI=leaf area index; SPAD=chlorophyll index; Stomatal=stomatal conductance; PII=photosystem II maximum quantum yield; Polar=polar diameter; Equatorial=equatorial diameter; F yield=fruit yield per plant.

Leaf pigment parameter, 2024 season

The averaged foliar pigments evaluated, chlorophyll (ChlM; Figure 5), flavonoids (FIVM; Figure 5b), anthocyanins (AnthM; Figure 5c) and nitrogen-flavonol index (NFI; Figure 5d), did not have significant differences between treatments.

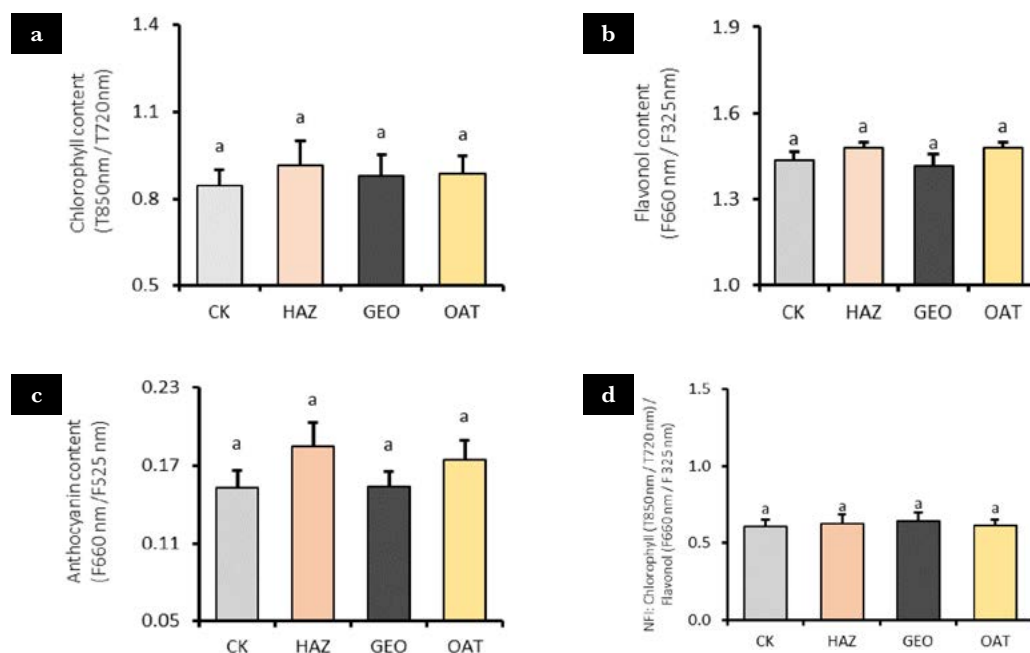


Figure 5. Leaf pigment variables: a) chlorophyll measurement, b) flavonoid measurement, c) anthocyanin measurement, d) flavonol nitrogen index. Treatments: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Different lowercase letters indicate significant differences between treatments according to Fisher's LSD test ($p < 0.05$). Mean $\pm$ standard error ($n=4$). Bars represent the experimental error for each treatment.

Fruit parameters for the 2024 season

Regarding the behavior of fruit variables in 2024, fruit yield (Figure 6) was statistically higher with HAZ, GEO, and OAT compared to the control, with increases of 75%, 85%, and 60%, respectively. Fruit weight (Figure 6b) was statistically higher with GEO and OAT compared to CK, being up to 41% and 31% higher, respectively. As for fruit dimensions, the equatorial diameter (Figure 6c) and polar diameter (Figure 6d) did not change significantly due to the treatments, with averages close to 5.5 mm for both equatorial and polar diameters.

Seed parameter season 2024.

The fruit seed variables (Figures 7 and 7b) showed no statistically significant differences ($p>0.05$) between treatments. The average seed weight ranged from 0.47 g to 0.59 g, while the average number of seeds per fruit ranged from 6.5 to 7.3.

Analysis of plant and fruit variables 2024

In the PCA (Figure 8), the first two principal components explained 50.8% of the total variation. GEO and OAT cover types maintained their association with higher yields and fruit weight, as in 2022, while physiological variables (such as leaf pigments) showed a more diffuse distribution without defined clustering. However, no significant differences were detected between treatments ($F=0.49$; $p=0.87$), with high overlap between groups.

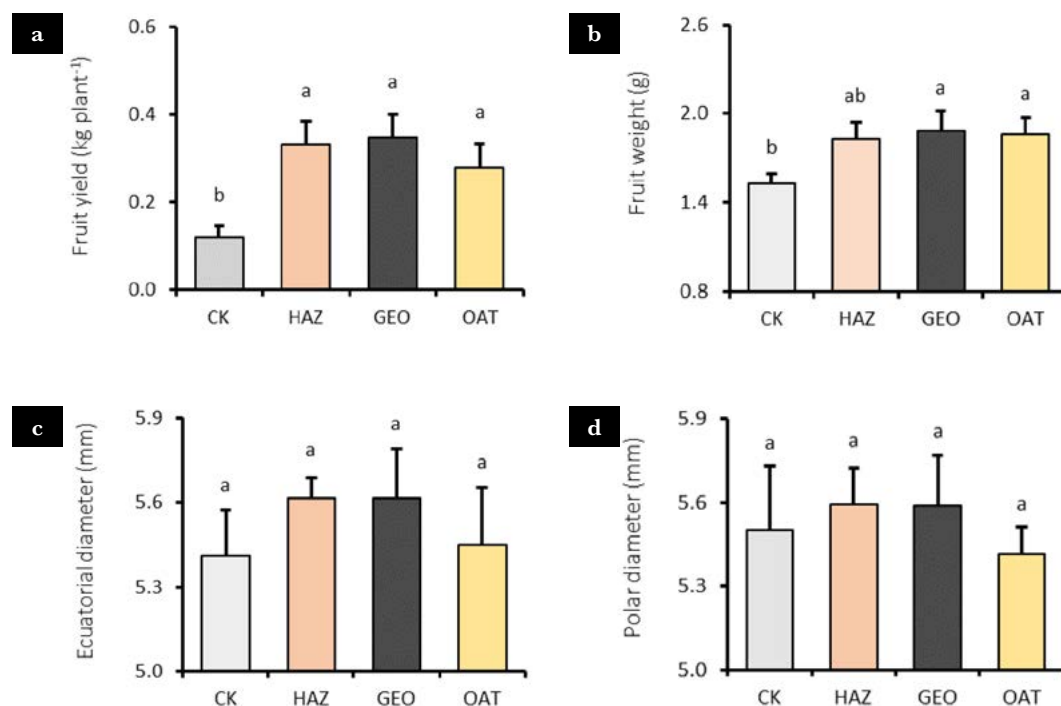


Figure 6. Fruit parameters for the 2024 season: a) yield, b) fruit weight, c) equatorial diameter, d) polar diameter. Treatments: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Different lowercase letters indicate significant differences between treatments according to Fisher's LSD test ($p<0.05$). Mean $\pm$ standard error ($n=4$). Bars represent the experimental error for each treatment.

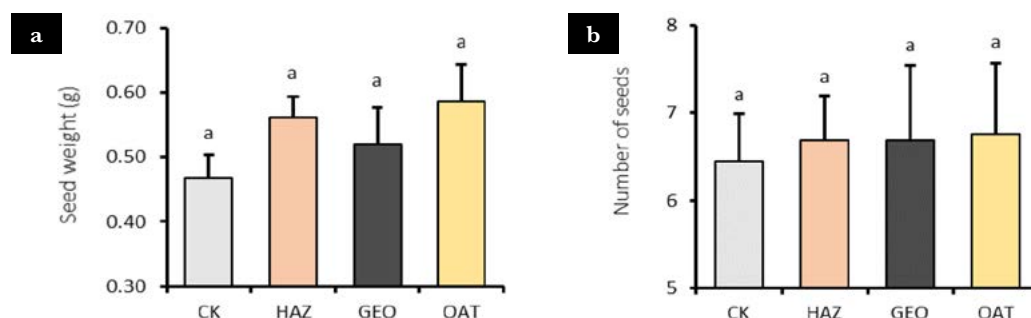


Figure 7. Seed measurements, 2024 season: a) seed weight, b) number of seeds. Treatments: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. Different lowercase letters indicate significant differences between treatments according to Fisher's LSD test ($p < 0.05$). Mean $\pm$ standard error ($n=4$). Bars represent the experimental error for each treatment.

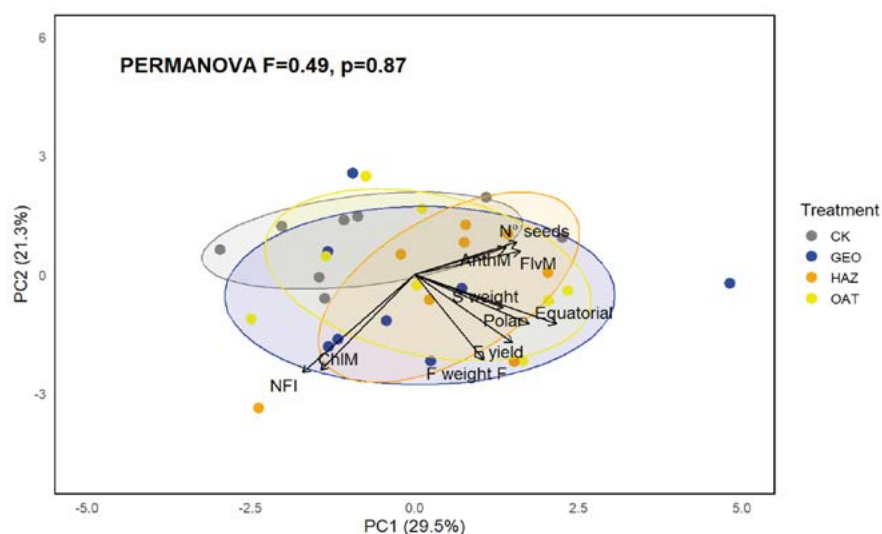


Figure 8. Seed measurements, 2024 season: CK=control, HAZ=hazelnut shell, GEO=geotextile, and OAT=oat straw. N° seeds=number of seeds; AnthM=anthocyanins; FlvM=flavonoids; Polar=polar diameter; Equatorial=equatorial diameter; F yield=fruit yield per plant; F weight F=fruit weight; ChlM=chlorophyll; NFI=flavonol nitrogen index.

This study evaluated the effect of different types of mulch on physiological variables and fruit production of Calafate (*Berberis microphylla*) over two seasons (2022 and 2024). The results showed that OAT and GEO mulches affected the plant's physiological functioning in the first season (2022), but no significant differences were observed in the 2024 season. In terms of production, OAT and GEO resulted in higher fruit yields in the second season (2024). These results are consistent with those reported for other fruit crops, where different mulches can initially modulate the plant's photosynthetic activity, and in subsequent seasons, this translates into improvements in yield and/or fruit quality (Zhang *et al.*, 2021; Betancur *et al.*, 2023). These findings are relevant because they show that agronomic management through the use of mulch has effects both at the plant level and on the characteristics of the fruit.

Effect of mulches on plant and fruit 2022

In the 2022 growing season, chlorophyll content and leaf area index were sensitive to mulch use, with the highest responses observed primarily with GEO and OAT. Surface mulches have been shown to modify the soil microclimate by increasing soil temperature, conserving moisture and improving nutrient availability, favoring the development of foliar biomass and the accumulation of photosynthetically active area (Chauhan *et al.*, 2023). The improved physiological performance observed under GEO is consistent with reports indicating that synthetic or semi-synthetic mulches can generate a more stable root environment, promoting water and nutrient efficiency, and sustaining higher levels of photosynthetic pigments (Li *et al.*, 2022). Likewise, organic mulches such as OAT maintain high levels of organic matter in the soil and suppress weeds, which also improves the availability of resources to promote vegetative growth (El-Beltagi *et al.*, 2022; Betancur *et al.*, 2023). In this study, the principal component analysis (PCA) of the 2022 season, although it did not detect significant differences, showed patterns of association between GEO and fruit weight and yield variables, while OAT was mainly linked to physiological variables such as leaf area index and chlorophyll index.

In the 2022 season, other physiological parameters such as photosystem II quantum efficiency and stomatal conductance did not vary significantly between treatments. However, under conditions where water availability is not critically limiting, mulch may not generate marked differences in photosystem II quantum efficiency or conductance, even though it does improve the root microenvironment (Zhang *et al.*, 2014; Guo *et al.*, 2021; Betancur *et al.*, 2023). This is consistent with what has been observed in blueberries and other berries managed with different mulch systems and supplemental irrigation, where the plant maintains a relatively stable physiology at the level of photoprotection even under contrasting treatments (Betancur *et al.*, 2022; Retamal-Salgado *et al.*, 2023; Guo *et al.*, 2021). Even so, a diurnal variation was observed in photosystem II quantum efficiency and conductance where they decreased around the point of maximum radiation (close to 3:00 pm) and recovered towards the end of the afternoon. This pattern is typical of transient photochemical stress due to high radiation and leaf temperature, followed by partial recovery when conditions moderate, and has been described in Calafate as well as in other fruit trees in areas with high summer radiation (Arena *et al.*, 2020; Betancur *et al.*, 2023).

Regarding fruit yield in 2022, there were no significant differences between mulch treatments. In other fruit trees, it has been described that the productive response to mulch can take several seasons to become apparent, especially in young orchards (Zhang *et al.*, 2021). Despite this, the absence of yield differences could also be affected by other factors such as the genetics of the plant material, pruning management, and nutrition, which can mask the direct effect of mulch on the number of fruits per plant (Betancur *et al.*, 2023). On the other hand, the lack of effect of mulch on fruit yield is more consistent in soils of low fertility, unlike in our study (Romero-Román *et al.*, 2021; Pinto-Morales *et al.*, 2022).

Effect of mulches on plant and fruit 2024

In the 2024 growing season, foliar pigments such as chlorophyll, anthocyanin, and flavonoid content were not affected by the different mulches. Since pigments like

anthocyanins typically increase under stress conditions, the lack of differences between mulches indicates that none of the conditions generated severe physiological stress in the plant (Arena *et al.*, 2020; Guo *et al.*, 2021; Retamal-Salgado *et al.*, 2023). Multivariate analysis using PCA reinforced this interpretation, although without significant differences. In terms of yield, in the 2024 growing season, plant weight and individual fruit weight increased significantly with OAT and GEO. Mulches have been shown to significantly improve yield through better soil moisture conservation and nutrient availability (El-Beltagi *et al.*, 2022; Betancur *et al.*, 2023). Specifically, OAT provided more highly degradable organic material that can improve soil organic matter and phosphorus content, which has been associated with more efficient fruit filling in berries (Sun *et al.*, 2017; Duan *et al.*, 2022). Meanwhile, GEO, with its black color, is able to stabilize and increase soil temperature, limit weed competition, and improve water use by the plant—effects that translate into increased fruit yields (Chauhan *et al.*, 2023; Betancur *et al.*, 2023). This study also measured the number of seeds per fruit and showed no significant effect from the use of mulch. This result is consistent with what has been observed in other perennial fruit trees, where soil management through mulch tends to affect fruit filling and quality more than seed formation, which is a genetic factor (Zhang *et al.*, 2014; Retamal-Salgado *et al.*, 2023).

Our results demonstrate that OAT is more beneficial for both the plant and the fruit. From a sustainability perspective, this is relevant because the use of plant residues such as cereal straw has been proposed as a management practice that simultaneously controls weeds, reduces soil water loss, and improves microbial activity, thus decreasing the need for external inputs (El-Beltagi *et al.*, 2022). This type of organic mulch has also been linked to increases in soil microbial biomass, basal respiration, and key enzymatic activities associated with the nitrogen and carbon cycles (Li *et al.*, 2022).

Future perspectives of the study

The results of this study demonstrated that the effects of mulch can be cumulative across seasons, beginning with physiological improvements that are subsequently reflected in increased fruit weight and yield per plant. This has also been described in other perennial fruit trees such as grapevines, apples, and peaches, where mulching gradually improves soil properties, reduces weed competition, and, over time, enhances fruit productivity and quality (Giese *et al.*, 2014; Sharma *et al.*, 2023; Mairata *et al.*, 2023). However, future studies should evaluate the effects of mulch over consecutive seasons to fully capture the physiological and productive dynamics under mulch. In this study, the orchard evaluated is still young, so yield per plant may increase as the orchard progresses toward physiological and reproductive maturity, which could amplify the differences between mulch treatments in future seasons (Pinto-Morales *et al.*, 2022; Radice *et al.*, 2018). Given that these results demonstrate benefits from the use of reusable organic mulches such as OAT and HAZ, future research should also evaluate other types of mulch made from organic products and residues on cultivated and native plants such as Calafate.

CONCLUSIONS

This study demonstrated that mulches, especially OAT and GEO, significantly improved the plant's physiological processes in the first instance (2022 season), increasing the leaf area index and chlorophyll content by more than 100% compared to the control. Subsequently (2024 season), Calafate fruit production increased significantly by over 70% with these treatments (OAT and GEO) compared to the control. Although HAZ had a less pronounced effect compared to OAT and GEO, it showed moderately superior productivity compared to the control. Given that these results demonstrate the benefits to the plant from the use of reusable organic mulches, particularly OAT, future research should evaluate other types of mulch made from organic products on native plants cultivated as Calafate. This study demonstrates that mulches are useful for enhancing Calafate productivity and represent an environmentally responsible alternative.

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Prioritization of areas degraded by forest fires for rehabilitation

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ABSTRACT

Objective: To assess fire severity in affected areas through spectral analysis and geographic information systems in order to identify priority areas for restoration and strengthen the sustainable management of natural resources.

Design/methodology/approach: Field and remote sensing data from areas affected by forest fires were used to determine and classify fire severity using the differenced Normalized Burn Ratio (dNBR) spectral index, thereby enabling the identification and quantification of priority areas exhibiting the greatest severity. In addition, an analysis of the topographic factors influencing fire regimes was incorporated to establish management and prevention strategies.

Results: The period from November to April accounts for the highest number of events, with 90% corresponding to surface fires, mainly triggered by anthropogenic activity. The most severely affected sites, which require rehabilitation, are associated with pine and fir forest ecosystems, slopes of 31° (difficult access and greater fire spread), a southeastern aspect of 135° (high solar exposure), and transitional zones between forested areas and high-mountain grasslands.

Limitations on study/implications: The availability of open-access data, remote sensors, and spectral analysis constitutes a strategic resource; however, these tools may present technical limitations that affect accuracy. Accordingly, future research directions are proposed to evaluate the dynamics of ecological recovery and thereby strengthen methodological robustness.

Findings/conclusions: Anthropogenic pressure and climate change intensify the degree of impact, thereby compromising ecosystem functions. Therefore, differentiated rehabilitation measures are required, supported by policy instruments that reinforce territorial governance oriented toward environmental security and productive sustainability.

Keywords: Fire severity, ecological restoration, erosion processes, geospatial modeling, sustainability.

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INTRODUCTION

Humankind appropriates nearly one-third of the Earth's potential net primary production for food, feed, fiber, timber, and energy (IPCC, 2022), thereby constituting a fundamental basis for human subsistence and well-being. However, this same proportion of terrestrial surface is also subject to degradation, as evidenced by soil erosion rates in



agricultural fields that are ten times higher under no-tillage systems and more than one hundred times higher under conventional tillage than the natural rate of soil formation (IPCC, 2022).

In addition, climate change exacerbates land degradation, with increasingly intensified effects, as exemplified by forest fires of greater intensity and extent. These account for five percent of the burned land area and contribute more than 80 percent of greenhouse gas (GHG) emissions, with unsustainable land use, deforestation, rising temperatures, and changes in plant community patterns, among other factors, being the principal drivers (IPCC, 2020).

As a consequence of climate change, forest fires have markedly increased in frequency, intensity, and hazard over the last decade, resulting in alterations in forest fuel dynamics, species composition, vegetation structure, and soil moisture. Depending on these factors, fires generate direct impacts, such as biodiversity loss and soil degradation, as well as indirect effects, including soil erosion and water contamination, thereby affecting ecological processes (FAO, 2019).

Within the Mexican context, 1998, 2011, 2017, and 2022 stand out as the most critical years in terms of damaged area. During the 2012-2022 period, the affected area increased from 347 thousand ha to 660 thousand ha, with the greatest concentration occurring in central Mexico (CONAFOR, 2022). In 2023, 7,611 fires were recorded over an area exceeding one million hectares, with Jalisco and the State of Mexico being the most affected federal entities. During 2024, the Volcanes region (Ixtapaluca, Tlalmanalco, Amecameca, Chalco, among others) was among the areas most severely affected (PROBOSQUE, 2024).

After a fire, soil becomes highly susceptible to erosion processes due to its exposure to wind and water, which leads to soil material loss, reduced water infiltration, increased surface runoff, and hydrophobicity (Caon *et al.*, 2014). Moreover, post-fire harvesting of burned timber may further increase vulnerability to erosion and soil degradation because of the use of heavy machinery and log dragging operations (García-Orenes *et al.*, 2017).

With regard to soil, nutrient content increases in the uppermost surface layers owing to ash deposition, nutrient mineralization, and the formation of stable structures. However, over time, nutrient levels decline because of volatilization, transformation, and removal by gravity and wind. Their retention therefore requires site stabilization through the implementation of post-fire measures aimed at limiting erosion, surface runoff, and wind-driven ash removal (Caon *et al.*, 2014).

Forest ecosystems are among the planet's most important natural resources because they contribute to climate change mitigation and constitute the principal carbon sink (Rojas *et al.*, 2021). Accordingly, post-fire forest management represents a critical step toward ecosystem recovery. Such management includes interventions such as the treatment of burned trees, soil protection, and practices specifically oriented toward ecosystem restoration through the improvement of its components or processes (García-Carmona *et al.*, 2023).

Although severity indices derived from remote sensing are widely used, their systematic application for prioritizing restoration actions at the regional scale remains insufficient or heterogeneous (Key & Benson, 2006; Chuvieco *et al.*, 2019). In the specific case of the State of Mexico, there is limited integration of spatial analyses of forest fire severity based

on objective and standardized criteria for delimiting priority rehabilitation areas, which in turn constrains decision-making for sustainable post-fire management.

Thus, in a context marked by the intensification of wildfires and climate change, the objective of this contribution was to analyze areas impacted and degraded by forest fires in the State of Mexico through the determination of fire severity, in order to contribute to the delimitation of priority sites for rehabilitation and, consequently, to the sustainable use of natural resources. This study is guided by the following question: how is forest fire severity distributed across the State of Mexico, and which areas should be prioritized for rehabilitation?

MATERIALS AND METHODS

Source of information and study area

This was a quantitative study; therefore, data collection was conducted through two complementary approaches: the use of Geographic Information Systems (GIS) and remote sensing, and field surveys for validation in municipalities of the Volcanes region, State of Mexico (Amecameca, Chalco, Ixtapaluca, and Tlalmanalco) (Figure 1), one of the areas most affected by forest fires in the state (PROBOSQUE, 2024). The study was based on the severity of all fires recorded between January 1, 2020, and December 31, 2024.

For the GIS component, the following were used: 1) polygons of the affected areas, 2) land use and vegetation maps from INEGI Series VII (2024), 3) multispectral Landsat satellite images of the affected areas, selected for their spatial resolution, radiometric quality, and spectral availability, 4) a Digital Elevation Model (DEM), and 5) the political-administrative boundaries of the municipalities (INEGI, 2024).

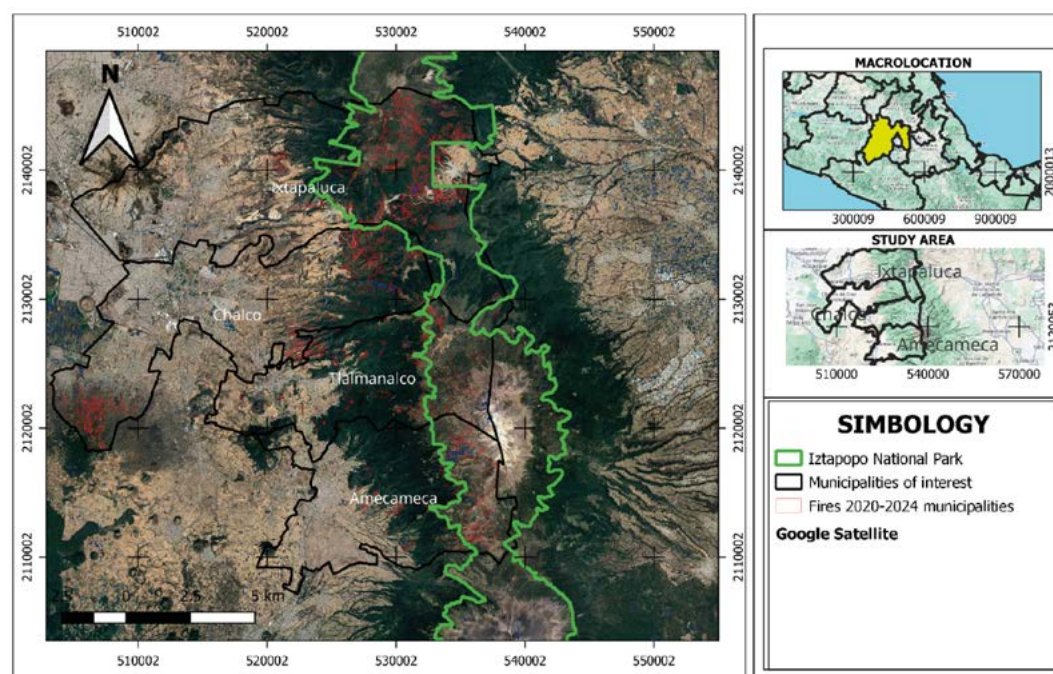


Figure 1. Municipalities of the Volcanes Region affected by fires during 2020-2024. Source: Prepared by the authors based on field information.

For input 3, pre- and post-fire images were obtained.

Systematization of information

To determine fire severity, the dNBR (differenced Normalized Burn Ratio) spectral index was used, based on pre- and post-fire satellite images and field sampling (Vales *et al.*, 2020; Zhu *et al.*, 2006). The use of dNBR is supported by its spectral sensitivity to fire impact (pre- and post-fire spectral difference), empirical validation and standardization (correlations between dNBR values and the severity of ecological effects), as well as its availability and reproducibility through open-access satellite data (Hudak *et al.*, 2011; Parente *et al.*, 2014).

First, the NBR was calculated, one of the most widely used indices in burned-area mapping, through the near-infrared and shortwave infrared bands (NIR and SWIR, respectively) (Key & Benson, 2006; van Gerrevink & Veraverbeke, 2021). The NIR (near-infrared) band highlights vegetation health and structure, since living plants reflect more light in this portion of the spectrum than burned vegetation.

In turn, the SWIR (shortwave infrared) band reflects plant moisture and water content; dry and burned areas, with lower moisture, reflect more light than healthy vegetation. Their combined use makes it possible to identify burned areas and zones with live vegetation. These are defined as follows:

$$dNBR = NBR_{pre} - NBR_{post}$$

$$NBR = (NIR - SWIR) / (NIR + SWIR)$$

where: *dNBR*=Differenced Normalized Burn Ratio; *NBR*=Normalized Burn Ratio; *NIR*=Near-infrared band; *SWIR*=Shortwave infrared band; *NBR_{pre}*=Pre-fire *NBR* value; *NBR_{post}*=Post-fire *NBR* value.

Based on the model values, the level of fire severity was then classified in order to locate and quantify priority areas, that is, those with the greatest severity (Table 1). Higher values indicate greater damage severity (vegetation loss), whereas values close to zero or negative indicate unburned areas or vegetation regrowth (Lutes, 2006).

Table 1. dNBR values.

Value	Description
< -0.250	High post-fire regrowth
-0.250 to -0.100	Low post-fire regrowth
-0.100 to 0.100	Unburned or stable areas
0.100 to 0.270	Low severity
0.270 to 0.440	Moderate-low severity
0.440 to 0.660	Moderate-high severity
> 0.660	High severity

Source: Prepared by the authors based on Lutes (2006).

In addition, based on the DEM, topographic factors influencing fire regimes, such as aspect and slope (Table 2), were analyzed (Gholami *et al.*, 2021). Both factors make it possible to identify priority intervention areas, define differentiated management strategies, and prevent secondary impacts (Tiwari *et al.*, 2020).

RESULTS AND DISCUSSION

Characterization of the burned areas

The study area recorded 623 fires during the period from January 1, 2020, to December 30, 2024, affecting a total area of 17,000 hectares; Ixtapaluca was the most affected municipality, with 5,200 hectares. More than 50% of the damaged area corresponds to the Iztaccíhuatl-Popocatepetl National Park, which is recognized as a Protected Natural Area (Figure 2). The months from November to April concentrate the highest number of events, with 90% classified as surface fires, spreading from the ground surface up to 1.5 meters in height.

Table 2. Topographic parameters analyzed.

Factor	Description
Aspect	Aspect, or slope exposure, determines the amount of incident solar radiation and influences soil temperature and moisture; therefore, it affects fire severity and vegetation resilience (Ibarra-Bonilla <i>et al.</i> , 2024).
Slope	Slope influences fire spread and behavior (Taylor <i>et al.</i> , 2020), as well as erosion processes and surface runoff (Shakesby & Doerr, 2006).

Source: Prepared by the authors based on field information.

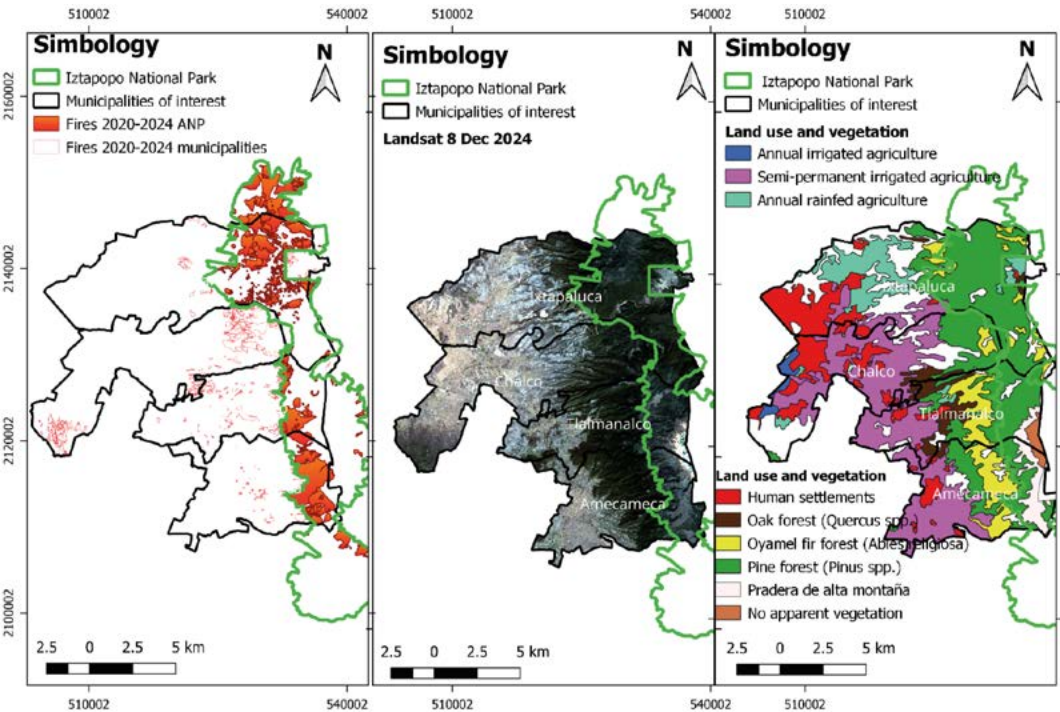


Figure 2. Municipalities of the Volcanes Region affected by fires during 2020-2024. Source: Prepared by the authors based on field information.

The phenomenon analyzed may occur at any time of the year; however, it depends on the presence of combustible material, its moisture content, and prevailing meteorological conditions. Fire severity during the summer has increased over recent decades, and within a context of climatic warming, in the absence of strategic management, further increases are likely to occur (Alvarez *et al.*, 2024).

According to land-use cover, the burned areas correspond predominantly to pine forest, fir forest, high-mountain grassland, and agricultural land, thereby revealing a multifactorial vulnerability that is further amplified under climate change conditions. Fir forest constitutes an ecologically sensitive relict, highly vulnerable to variations in temperature and drought. It develops under specific moisture and altitudinal conditions; however, global warming is forcing it toward increasingly higher altitudinal limits.

The impact on high-mountain grasslands is particularly critical, since these ecosystems function as carbon sinks and water reservoirs. More broadly, the severe degradation of high-mountain forests by fire increases their vulnerability to climate change; the loss of forest cover accelerates soil erosion and surface runoff, which in turn compromises water capture. Regarding agricultural areas and pine forests, fire recurrence underscores the risk posed by prolonged droughts and by a vicious cycle of wildfires, whereby dry vegetation and crop residues become new sources of fuel.

The analysis revealed that illegal land-use change and uncontrolled agricultural and livestock burning constitute the main triggers; that is, the phenomenon is a direct consequence of human actions (Figure 3), whereby changes in land use and land cover, together with anthropogenic activities, promote the occurrence of forest fires (Pereira *et al.*, 2020).

dNBR and classification of the burned areas

The analysis of the fire severity index (dNBR) made it possible to distinguish healthy vegetation from burned areas and revealed that the greatest damage occurred in pine and fir forest zones, both within the Protected Natural Area (PNA) and in its surrounding areas (Figure 4).

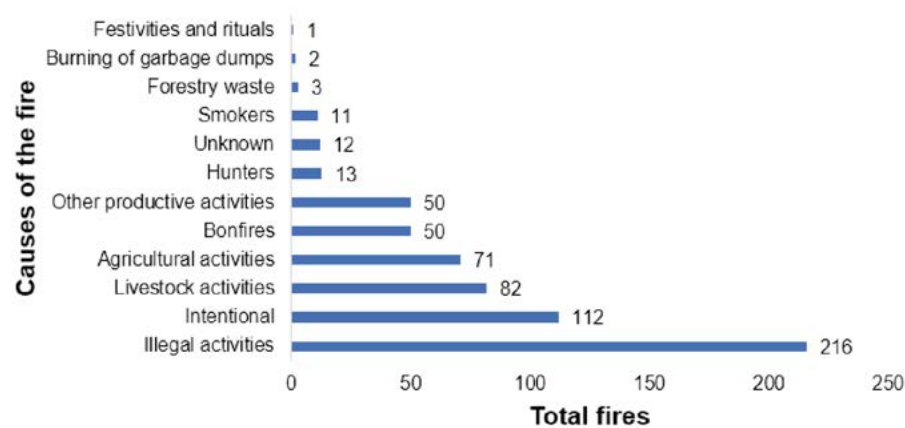


Figure 3. Main causes of fires in the Volcanes Region, 2020-2024. Source: Prepared by the authors based on field information.

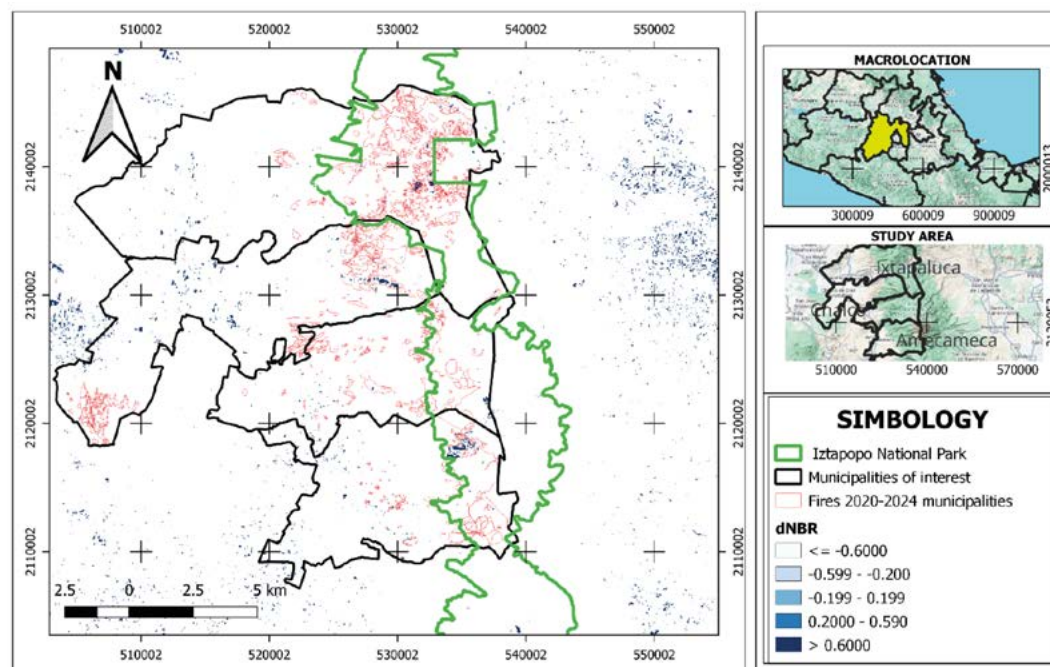


Figure 4. Fire severity in the Volcanes Region, 2020-2024. Source: Prepared by the authors based on Landsat satellite imagery.

The results indicate that 88% of the fires recorded during the analysis period exhibited low severity (5,955 ha), 11% showed moderate severity (8,896 ha), and only 2% presented high severity (2,241 ha), thereby identifying these areas as priority sites for intervention; these are located in Amecameca and Tlalmanalco, respectively. These events were primarily associated with campfires and livestock-related activities, with direct impacts on pine forests and high-mountain grasslands. Notably, part of the burned area is located within the Protected Natural Area (PNA).

Knowledge of dNBR values can contribute to sustainable forest management, which is essential for forest sustainability, particularly by focusing on severity mitigation and forest resilience (Fernandes, 2013). However, forest fire management has historically emphasized suppression measures rather than strategic management, which has further contributed to the large-scale accumulation of fuel loads (Castellnou *et al.*, 2019; Moreira *et al.*, 2020).

Topographic factors

The highest severity values (dNBR) were recorded in southeast-facing areas (135°) (Figure 5), which typically receive high morning solar radiation, thereby accelerating the drying of surface fuels and increasing their flammability during the most critical hours of the day. These areas present an average slope of 31°, under which fire spreads rapidly and intensely. Under such conditions, ground-based suppression becomes particularly hazardous, as these zones are characterized by extreme topographic vulnerability.

Terrain aspect, as a component of topography, constitutes a consistent and context-dependent predictor of fire severity. It influences species composition, vegetation structure,

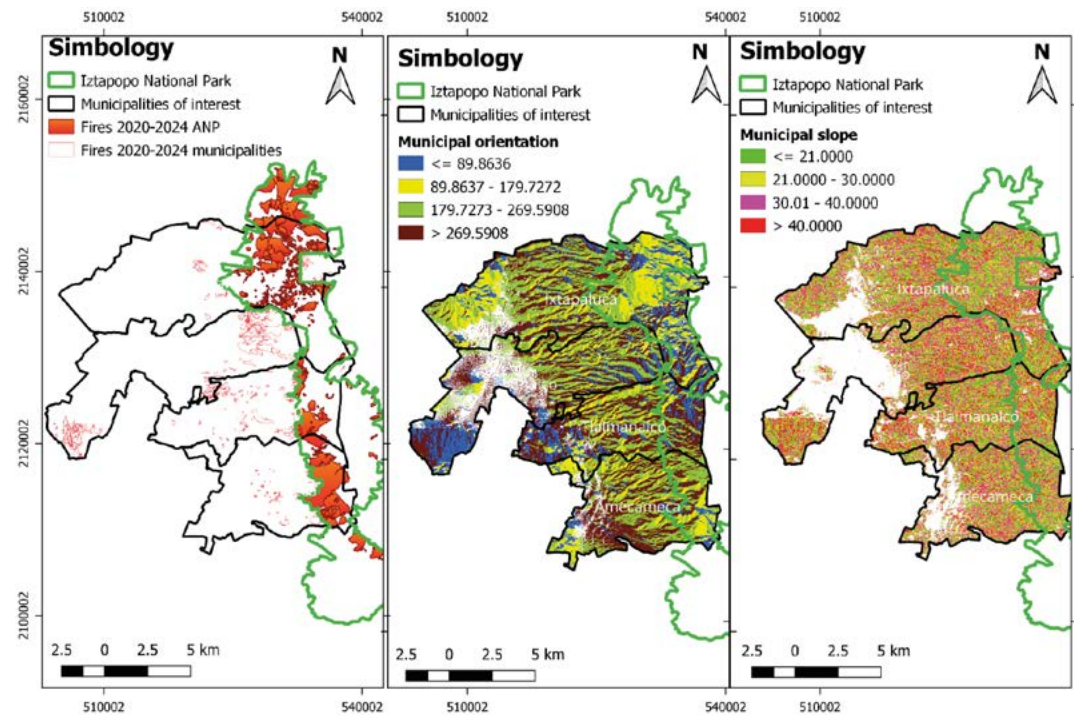


Figure 5. Aspect and slope of fires in the Volcanes Region, 2020-2024. Source: Prepared by the authors based on Landsat satellite imagery.

and biodiversity patterns. North-facing slopes exhibit greater biomass, cover, height, and species diversity, which is consistent with the higher soil nutrient content on these slopes and the insolation processes affecting southern exposures (Yang *et al.*, 2020). A southeastern aspect (135°) is associated with fires that cause greater structural damage to the ecosystem (Estes *et al.*, 2017).

Likewise, on slopes of 31° , fire spreads rapidly and intensely, since this factor determines both fire propagation and fire intensity (Ortiz-Mendoza *et al.*, 2024). Under these conditions, ground-based suppression is particularly hazardous because these areas present extreme topographic vulnerability. The concentration of damage in terrains with these characteristics not only compromises forest regeneration, but also increases the risk of post-fire erosion and landslides.

The analysis of fire severity levels, land use, and topographic variables is consistent with the fact that, in recent years, forest management objectives have expanded to enhance ecosystem resilience under future climate conditions (Franklin & Johnson, 2012). In this context, strengthening strategies that prioritize smart fire management, together with policies based on payments for ecosystem services, represents a policy pathway capable of incentivizing forest stewardship and preventing forest fires (Lecina-Díaz *et al.*, 2023).

The incorporation of topographic variables, particularly slope and aspect, strengthens this analysis by explaining the spatial heterogeneity of fire severity, given that steeper slopes and south-southwest exposures tend to experience greater water stress and more rapid fire spread. Accordingly, the observed severity results from the interaction between

topographic conditions and fire dynamics, thereby increasing the risk of degradation in specific geomorphological contexts.

A proactive approach constitutes a compelling alternative to defensive and reactive strategies. In this case, institutional strengthening and intergovernmental coordination are essential elements for consolidating integrated fire management. Addressing priority areas requires a territorial duality based on coordination between municipalities and the administration of the Protected Natural Area (PNA). Such coordination contributes to resource optimization and ensures an efficient emergency response, supported by permanent prevention, surveillance, and restoration mechanisms tailored to territorial conditions.

For areas outside the PNA, strengthening regulations on agricultural and livestock burning, together with the implementation of surveillance programs and reporting mechanisms for illicit activities, is crucial. Within the PNA, investment in and reinforcement of monitoring brigades, as well as the harmonization of regulatory and local planning instruments, may constitute effective starting points.

Strengthening fire management and combating illicit and criminal activities are imperative, since human action constitutes the principal trigger of the phenomenon and of vulnerability to fire. In addition, climate change increases the probability of occurrence (Ribeiro *et al.*, 2021), fragmenting the landscape and generating impacts on wildlife, water quality, and ecosystem services (Basso *et al.*, 2020; Legarreta-Miranda *et al.*, 2021; Taboada & Calvo, 2021).

The findings of this contribution translate into technical criteria for the allocation of public resources, the delimitation of critical areas, and the design of forest restoration and soil conservation programs. Likewise, the use of standardized satellite information facilitates the replicability and periodic updating of the diagnosis, thereby strengthening decision-making at both the municipal and regional scales.

The identification of spatial patterns provides a solid basis for risk management and makes it possible to focus prevention and active restoration strategies on slopes prone to maximum severity (Lecina-Díaz *et al.*, 2023). An approach grounded in mitigation and prevention, rather than in the exclusion of fire, is both logical and pragmatic, with a greater likelihood of reducing its negative socioeconomic and ecological effects (Moreira *et al.*, 2020).

dNBR analysis makes it possible to interpret the relative intensity of damage to vegetation cover and, consequently, the degree of post-fire degradation, since it reflects spectral changes associated with the loss of aboveground biomass and canopy alteration. However, it depends to a large extent on Landsat's spatial resolution (30 m), the presence of cloud cover, and the temporal selection of pre- and post-fire imagery, which may lead to the underestimation of small or low-severity fires. Because it does not capture belowground impacts or delayed effects, it should be regarded as a proxy for severity rather than a direct measurement of ecological damage; therefore, its interpretation as ecological degradation must be complemented according to vegetation type, fire regime, and ecosystem resilience capacity.

This contribution makes it possible to prioritize rehabilitation areas, identify fires with greater average impact, and guide differentiated restoration actions. In particular, fires with high dNBR values on steep slopes may be considered priority cases because of their greater risk of erosion and soil loss, in line with adaptive post-fire management approaches.

CONCLUSIONS

The burned areas requiring priority rehabilitation are those exhibiting the highest levels of fire severity ($\text{dNBR} > 0.66$), steep slopes (31°), and a southeastern aspect. These conditions reflect the relationship between the recurrence and intensity of forest fires and the pressures imposed by climate change. Anthropogenic action constitutes the principal trigger of these events, thereby compromising the resilience capacity of temperate forests, particularly in mountainous areas characterized by high ecological and social vulnerability. As part of rehabilitation, differentiated actions are recommended on the basis of topographic variables, accompanied by environmental education programs, mechanisms for surveillance and monitoring of illicit activities, and the design of policy instruments such as economic incentives and regulations that consolidate territorial governance by articulating environmental security and productive sustainability. As a methodological contribution, the study demonstrates the feasibility of integrating fire-aggregated dNBR and topographic variables to characterize severity at the regional scale, an approach that is replicable through satellite imagery and standardized basic cartography. The findings support the formulation of hypotheses regarding the relationship among severity, topography, and fire recurrence, as well as the temporal monitoring of vegetation recovery through multitemporal series. The availability of open-access satellite data constitutes a strategic resource for the academic community. However, such data may present technical limitations that affect accuracy; consequently, future lines of research are proposed, including predictive risk models and monitoring systems designed to evaluate the dynamics of ecological recovery.

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Passiflora edulis peel extracts applied as an edible coating on strawberry (*Fragaria* × *ananassa*)

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ABSTRACT

This study evaluated the antioxidant and antifungal potential of ethanolic extracts obtained from passion fruit (*Passiflora edulis*) peel and their application as an active edible coating for extending the postharvest life of strawberry (*Fragaria* × *ananassa*). Peels from two botanical forms of *Passiflora edulis* (*P. edulis* f. *flavicarpa* and *P. edulis* f. *edulis*) and one additional species (*Passiflora ligularis*) were characterized in terms of color, extraction yield, and antioxidant activity using DPPH and ABTS assays. The antifungal activity of *P. edulis* peel extract against *Rhizopus stolonifer* was evaluated through a radial growth inhibition at concentrations ranging from 500 to 3000 ppm. The extract was incorporated into a carboxymethylcellulose-based edible coating and applied to strawberries by immersion. During 17 days of storage, color (CIELab parameters) and weight loss were monitored. *Passiflora edulis* showed the highest extraction yield and antioxidant capacity, particularly by the ABTS method. The extract exhibited a strong dose-dependent antifungal effect, with higher concentrations showing a marked reduction of mycelial growth under *in vitro* conditions. Coated strawberries showed greater stability in color parameters (L*, a*, and b*) and significantly reduced weight loss compared to the control, indicating delay in some physical changes associated with senescence. These results suggest that *P. edulis* peel, an agro-industrial by product, represents a promising source of bioactive compounds for the development of functional edible coatings capable of improving strawberry postharvest quality.

Key words: *Passiflora edulis*; edible coating; strawberry; antioxidant activity; antifungal control.

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INTRODUCTION

Among the most perishable fruits traded globally are berries, prized for their high antioxidant properties. In this regard, the strawberry (*Fragaria* × *ananassa*) is a fruit in high commercial demand due to its numerous sensory characteristics, nutritional value, and nutraceutical properties. This has led to a surge in demand and rapid expansion of



the strawberry industry in recent years, solidifying its position as a vital component of the global fruit sector (Hernández-Martínez *et al.*, 2023). However, its shelf life is limited due to its high metabolic activity, water activity, and nutrient content, resulting in a high susceptibility to fungal growth and proliferation during post-harvest storage (Saleh & Abudieyh, 2022). Therefore, preserving the fruit using various conservation techniques is essential.

Several studies have incorporated coatings as a strategy to improve the post-harvest preservation of berries. These coatings act as a protective barrier, helping to reduce various factors such as moisture loss, respiration rate, and microbiological deterioration caused by fungi and bacteria, thus extending shelf life. Furthermore, different natural compounds with biological activity (essential oils, antioxidants, antimicrobials, extracts) have been incorporated into the coatings, demonstrating greater efficacy, primarily in terms of microbiological control (Colin-Alvarez *et al.*, 2025).

In addition to the above, there is a growing interest in utilizing agro-industrial waste as a source of bioactive compounds. In this regard, the peels of *Passiflora* spp., particularly *Passiflora edulis*, are of high interest due to their high content of phenolic compounds and flavonoids (Rentería-Ortega *et al.*, 2023), with antioxidant, antimicrobial, and antifungal properties. Several studies have reported that extracts obtained from this biomass exhibit antifungal activity against microorganisms associated with food spoilage.

However, despite numerous studies in the literature reporting on the antioxidant capacity and bioactive composition of byproducts from various *Passiflora* varieties, as well as their application in the manufacture of edible films and coatings, there is currently limited information on the incorporation of *Passiflora* peel extracts into active coating systems specifically designed to control microorganisms such as *Rhizopus stolonifer* in the post-harvest stage. Therefore, this study evaluates the antioxidant and antifungal potential of *Passiflora* peel extracts and their application as a functional component in a carboxymethylcellulose based edible coating to extend the fruit's shelf life.

MATERIALS AND METHODS

Materials

Strawberry fruits (*Fragaria* × *ananassa*) were purchased from a local market in San Felipe del Progreso, State of Mexico, Mexico. The fruits were selected based on commercial maturity and uniform external quality attributes, including similar size, homogeneous red surface coloration, and absence of visible mechanical damage or fungal decay.

For the antifungal capacity tests, the fungus *Rhizopus stolonifer* was used and maintained in potato dextrose medium at room temperature until use in the trials.

In addition, passion fruit samples corresponding to two botanical forms of *Passiflora edulis* (*P. edulis* f. *flavicarpa* and *P. edulis* f. *edulis*) and one additional species (*Passiflora ligularis*) were obtained from a local market in Atlacomulco de Fabela, State of Mexico, Mexico. The fruits were selected at a semi-ripe stage based on external color and absence of visible deterioration. Because the plant material was obtained from commercial sources, no formal botanical voucher specimen or batch code was available.

Methods

Sample preparation

The fruit peel was manually removed and cut into approximately 0.5 cm×0.5 cm pieces. Subsequently, the material was dried in a food dehydrator (Rommelsbacher DA 900) at 35 °C for 12 h until reaching approximately 3% moisture content. The dried material was then ground using a coffee blade mill (Masterchef By Huken MK-CG-SS) and further subjected to manual size reduction using a mortar and pestle to obtain a finer powder. The resulting material was sieved through a series of progressively finer meshes, and the fraction corresponding to particles smaller than 1 μm was collected for subsequent extraction.

Soxhlet extraction

The extraction was performed using the Soxhlet method, as described by Castillo-Membreno & Ramírez, (2006), with modifications. Ten grams of the previously prepared sample were weighed, transferred to a cellulose cartridge, and placed in the extraction apparatus. Then, 250 mL of 96% ethanol was used as solvent, and the extraction was carried out under continuous reflux conditions for 5.5 h. The heating plate was set at approximately 180 °C to ensure that the solvent reached and maintained its boiling point (≈78 °C), thereby promoting continuous evaporation-condensation cycles within the Soxhlet system.

To remove the solvent, the extract was concentrated by distillation for 1 h. Finally, the concentrate was transferred to an amber bottle, and the residual solvent was allowed to evaporate under room conditions. The obtained extracts were stored at 4 °C under refrigeration until further analysis.

Extraction yield

The extraction yield was calculated from the mass of the concentrated extract obtained after partial solvent evaporation, using the following equation.

$$Performance(\%) = \frac{Mass\ of\ extract\ obtained(g)}{Initial\ dry\ sample\ mass(g)} \times 100 \quad (1)$$

CIE Lab Color Analysis

Color analysis was performed using the CIE L*a*b* system, following the guidelines of the International Commission on Illumination (CIE), with a Precision Colorimeter V2.0 colorimeter previously calibrated with black and white standards. For solid samples (fruits of *Fragaria* × *ananassa* and passionflower peels), measurements were taken directly on the surfaces, whereas for liquid samples (peel extracts), measurements were taken in glass cuvettes of constant thickness to avoid background interference. In both cases, at least three readings were taken per sample at different points, and the average L*, a*, and b* values were reported.

Antioxidant capacity

DPPH

The DPPH assay was performed using a 150 $\mu\text{mol/L}$ solution prepared in methanol:water (80:20). The calibration curve was constructed with Trolox, and the extracts were evaluated from a 10 mg/mL stock solution diluted to 0-250 μL . In a microplate, 20 μL of sample was mixed with 180 μL of DPPH, incubated in the dark for 40 min, and the absorbance was measured at 515 nm using a Multiskan Go reader (Thermo Fisher Scientific).

The results were expressed as μmol Trolox equivalents per gram of extract ($\mu\text{mol TE/g}$), calculated from the calibration curve considering the dilution factor, the initial extract concentration, and the mass of extract used. This normalization ensured comparability between samples, and all measurements were performed within the linear response range of the DPPH assay (Bobo-García *et al.*, 2015).

ABTS $\cdot^+$

The ABTS $\cdot^+$ radical was generated by mixing equal parts of ABTS stock solutions (7 mmol/L) and potassium persulfate (2.45 mmol/L), which were incubated in the dark for 12-16 h. Before the assay, the solution was diluted with distilled water to an absorbance of 0.700 at 754 nm. The calibration curve was constructed using Trolox, and the extracts were prepared from a 10 mg/mL stock solution, diluted to 0-250 μL . For the analysis, 250 μL of diluted ABTS solution was mixed with 50 μL of the sample, and the absorbance was measured at 754 nm after 6 min using a microplate reader (Multiskan Go, Thermo Fisher Scientific).

The results were expressed as μmol Trolox equivalents per gram of extract ($\mu\text{mol TE/g}$), calculated from the calibration curve considering the dilution factor, the initial extract concentration, and the mass of extract used. This approach ensured that the reported antioxidant capacity corresponded to the intrinsic activity of the extract rather than to the volume of sample analyzed, and that measurements were performed within the linear range of the assay (Leite *et al.*, 2011).

Inoculum preparation and spore quantification

The fungus *Rhizopus stolonifer* was cultured in Petri dishes potato dextrose agar (PDA) for 15 days. After this time, 10 mL of sterile distilled water were added to each plate and the mycelium was gently scraped using a sterile glass rod to release the spores.

Subsequently, the resulting suspension was homogenized and diluted to obtain a 1:100 dilution. Spore counting was performed using a Neubauer chamber and an optical microscope, considering the four large quadrants, according to the method described by Meneses *et al.* (2001).

The spore concentration was expressed as spores per milliliter (spores/mL)

Preparation and application of the coating on strawberry (*Fragaria* $\times$ *ananassa*)

The edible coating was prepared following the methodology described by Mamani (2019) with some modifications, using a polysaccharide matrix composed of carboxymethylcellulose (CMC, 0.4 g), glycerol (1.2 mL), Tween 80 (0.8 mL), and sterile

distilled water (76.8 mL). *Passiflora edulis* extract was incorporated at different concentrations (500, 1000, 1500, 2000, 2500, and 3000 ppm) to obtain six different coatings.

The components were mixed and stirred for 40 min until a homogeneous solution was obtained. These concentrations were selected to evaluate their effect on the inhibition of mycelial growth of *Rhizopus stolonifer*. The coating was applied by immersion, as reported by Dai *et al.* (2025), with some modifications. The strawberries were completely submerged in the coating solution for 5 s and subsequently placed on racks to remove excess coating prior to inoculation.

***In vivo* antifungal assay on strawberries**

The antifungal activity of the coatings was evaluated *in vivo* using strawberries (*Fragaria* × *ananassa*) artificially inoculated with *Rhizopus stolonifer*. Four fruits were used per treatment, with four independent replicates. The fruits were randomly assigned to the treatments. The coating was applied by immersion (5 s); the fruits were inoculated on the intact surface by spraying a suspension of *R. stolonifer* spores. A total volume of 690 µL was applied per treatment (distributed in three sprays), equivalent to approximately 2325 spores. A 10-minute resting period was allowed between inoculation and coating application to ensure proper inoculum adhesion.

The treated fruits were stored at 8 °C, and fungal growth was monitored over time. Two control conditions were included: (i) uncoated fruit (UC) and (ii) fruit coated with the polymeric matrix (CMC) without extract (CC), in order to differentiate the effect of the matrix from the bioactive compound. In addition, treatments with different concentrations of extract were evaluated to determine their antifungal activity.

Minimum Inhibitory Concentration of *P. edulis* extracts against *Rhizopus stolonifer*

Radial growth test in solid medium

The antifungal activity of *Passiflora edulis* peel extract against *Rhizopus stolonifer* was evaluated using a radial growth assay on potato dextrose agar (PDA). The method involved sterilizing the PDA, then adding the extract at different concentrations (500, 1000, 1500, 2000, 2500, and 3000 ppm) and mixing under sterile conditions until complete homogenization. The solution was then poured into sterile Petri dishes, allowed to solidify, and inoculated by placing a disc of SR mycelium in the center.

The dishes were stored at 25 °C to evaluate growth by measuring the diameter of the mycelial growth on different days (1, 2, 3, and 7). The minimum concentration required to inhibit visible mycelial growth was determined based on the reduction of radial growth compared to the control. The percentage of mycelial growth inhibition was calculated using the following equation:

$$\text{Inhibition of micelial growth (\%)} = \frac{T - Tr}{T} \times 100$$

where: *T*: radial growth of the control (mm); *Tr*: radial growth of the treatment (mm).

Experimental design and statistical analysis

All trials were performed in triplicate using a completely randomized design. The data obtained were subjected to analysis of variance (ANOVA) to evaluate the effect of the different concentrations of *Passiflora* spp. extract. For the postharvest evaluation, a two-factor ANOVA was applied considering treatment (coating concentration) and storage time (days) as independent factors, as well as their interaction (Day $\times$ Treatment). Each sampling day was analyzed using independent fruit samples. When significant differences were detected, means were compared using Tukey's multiple comparisons test, considering a significance level of $p \leq 0.05$. Results are expressed as mean $\pm$ standard deviation. Statistical analysis was performed using Minitab software, version 22.

RESULTS AND DISCUSSION

Peel color and extracts

The color determination is shown in Table 1, revealing significant differences in the CIELab parameters of the peels of the three *Passiflora* species. These differences indicate that, beyond external morphological variations, there are distinct physicochemical characteristics associated with each species.

Passiflora edulis f. *flavicarpa* exhibited the highest L* and b* values, reflecting a lighter and more yellow coloration, has been described in the literature as characteristic of yellow-colored varieties, although pigment composition was not evaluated in this study. In contrast, *P. edulis* f. *edulis* showed the lowest L* values, corresponding to a darker coloration, which has been reported in previous studies as a characteristic of darker-colored peels (Fonseca *et al.*, 2022). However, it is important to note that the present study did not include a direct quantification of phenolic compounds or detailed phytochemical profiling; therefore, these associations should be interpreted with caution.

In this context, the observed differences in color are discussed only as measurable physicochemical attributes. Nevertheless, in this study, such interpretations are limited to indirect evidence derived from color parameters.

This trend was maintained after the ethanolic extraction process (Table 2), where the extract of *Passiflora edulis* f. *edulis* retained lower lightness values compared to the other

Table 1. CIELab color of passionflower fruit peel.

Species	L*	a*	b*
<i>Passiflora edulis</i> f. <i>flavicarpa</i>	66.92 $\pm$ 9.06 ^a	1.29 $\pm$ 0.27 ^b	38.70 $\pm$ 7.00 ^b
<i>Passiflora edulis</i> f. <i>edulis</i>	32.98 $\pm$ 3.39 ^b	15.08 $\pm$ 3.76 ^a	10.27 $\pm$ 1.39 ^c
<i>Passiflora ligularis</i>	59.02 $\pm$ 2.32 ^a	15.74 $\pm$ 2.40 ^a	54.60 $\pm$ 4.39 ^a

Table 2. CIELab color from ethanolic extracts of passionflower peel.

Species	L*	a*	b*
<i>Passiflora edulis</i> f. <i>flavicarpa</i>	38.37 $\pm$ 2.69 ^a	18.69 $\pm$ 4.67 ^a	21.30 $\pm$ 6.59 ^a
<i>Passiflora edulis</i> f. <i>edulis</i>	23.82 $\pm$ 0.62 ^c	11.27 $\pm$ 3.55 ^b	4.91 $\pm$ 1.39 ^b
<i>Passiflora ligularis</i>	31.67 $\pm$ 4.17 ^b	12.33 $\pm$ 5.21 ^b	8.14 $\pm$ 3.57 ^b

species. This indicates that the color characteristics of the raw material were preserved after extraction under the conditions evaluated. However, the relationship between extract color and its metabolic composition or antioxidant potential was not directly evaluated and should not be inferred beyond the observed colorimetric behavior.

Although previous studies have reported correlations between extract color intensity and bioactive compound content in active coating systems, such relationships depend on specific compositional analyses. Therefore, in the present work, the observed differences in color should be interpreted strictly as physicochemical descriptors rather than direct indicators of bioactive compound concentration or functional activity.

Ethanol extraction yield

Regarding extraction yield, Figure 1 shows that the highest extraction yield was obtained with the *P. edulis* f. *edulis* variety, attributed to the high metabolic density of the tissue and the fact that the *P. edulis* f. *edulis* peel concentrates polyphenols, flavonoids, pectins, and other secondary metabolites associated with structural and chemical defense functions (Fonseca *et al.*, 2022). A higher yield means that a greater load of bioactive compounds is obtained per unit of residue, improving the technological viability of the process. Furthermore, when these extracts are used as part of protective barriers, the likelihood of the coating having a better physiological impact on the fruit increases. In accordance with the above, Shahdadi *et al.* (2025) reported, in active coatings on strawberries, that the systems efficacy depends largely on the effective load of functional compounds incorporated into the polymer matrix.

Antioxidant activity in ethanolic extracts of passionflower peel

The results obtained from the different antioxidant activity tests using the DPPH method (Figure 2) revealed that sample *Passiflora edulis* f. *edulis* with a value of $176 \pm 3.54 \mu\text{mol TE/g}$, followed by *Passiflora edulis* f. *flavicarpa* with a concentration of $130.5 \pm 12.37 \mu\text{mol TE/g}$. Conversely, the *Passiflora ligularis* sample ($62.40 \pm 4.24 \mu\text{mol TE/g}$) showed a significant difference from the other samples ($p=0.002$) according to Tukey's test. It is important to note that these quantifications were optimized by working within an extract concentration



Figure 1. Yield (%) of ethanolic extraction from passionflower peel.

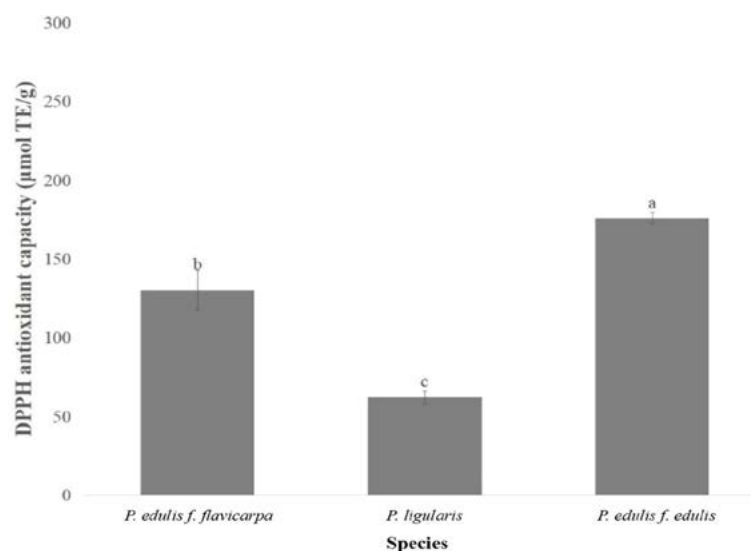


Figure 2. Antioxidant capacity determined by the DPPH method in *Passiflora* spp. peel extracts. Different letters above the bars indicate significant differences between samples ($p=0.002$).

range (100 to 250 mg/mL) that ensured linearity of the response. The concentration of 100 mg/mL was determined to be the most appropriate to prevent DPPH radical saturation, ensuring that absorbance readings remained within the methods detection limits and preventing underestimation of activity per gram of sample (Molyneux, 2004; Kedare & Singh, 2011). This is possibly because the measured antioxidant capacity can vary with the ratio of DPPH radical to antioxidants and with the experimental conditions used, which can affect the accessibility of antioxidants to the radical in complex matrices or at high concentrations of compounds (Prior & Schaich, 2005).

Furthermore, in the ABTS method (Figure 3), the observed trend was different ($p<0.000$), with sample *P. ligularis* exhibiting the highest antioxidant capacity at $266\pm 14.1 \mu\text{mol}$

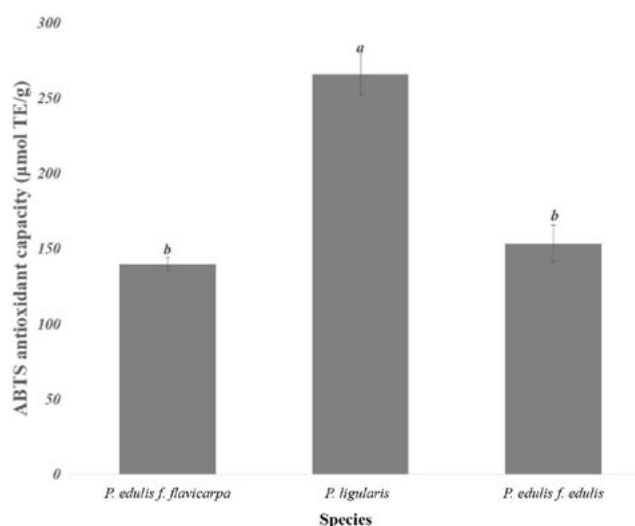


Figure 3. Antioxidant capacity determined by the ABTS method in *Passiflora* spp. peel extracts. Different letters above the bars indicate significant differences between samples ($p=0.000$).

TE/g. This value significantly exceeded those recorded for *P. edulis* f. *edulis* ($153.68 \pm 12.05 \mu\text{mol TE/g}$) and *P. edulis* f. *flavicarpa* ($139.93 \pm 4.35 \mu\text{mol TE/g}$). According to Tukey's test, *P. ligularis* ($p < 0.000$) different significance from *P. edulis* f. *flavicarpa* and *P. edulis* f. *edulis* (Figure 3).

The numerical superiority of the ABTS values over those of DPPH is consistent with that reported by Floegel *et al.* (2011). It is attributed to the ABTS radicals capacity to react with a wider range of hydrophilic and lipophilic compounds present in the peel matrix. This difference in reactivity suggests that *Passiflora* spp. extracts possess a diverse phytochemical profile, and certain compounds, possibly hydrophilic, exhibit a greater affinity for the electron-transfer mechanism of the ABTS assay (Magalhaes *et al.*, 2014).

Several studies on *Passiflora* spp. by-products have reported that the peel contains higher concentrations of phenolic compounds and flavonoids than the pulp and seeds, resulting in superior antioxidant activity. For instance, the peel of *P. maliformis* has shown antioxidant capacities of $23.21 \pm 1.16 \mu\text{mol TE/g}$ (ABTS) and $11.87 \pm 0.59 \mu\text{mol TE/g}$ (DPPH). In comparison, *Passiflora edulis* f. *edulis* has yielded values of $33.45 \pm 2.50 \mu\text{mol TE/g}$ and $15.29 \pm 0.76 \mu\text{mol TE/g}$ for ABTS and DPPH, respectively (Taborda *et al.*, 2021). These findings align with the ABTS > DPPH pattern observed in the present study and highlight *P. edulis* f. *edulis* as the species with the highest antioxidant potential. This trend suggests that the phytochemical profile of *P. edulis* f. *edulis* peel possesses a greater affinity for the ABTS radical cation than for the DPPH radical.

Furthermore, when comparing these results with studies on conventional matrices, primarily *Passiflora* pulp, it is evident that the antioxidant capacity of the external tissue (peel) significantly surpasses that of the pulp. A study investigating the pulp of various *Passiflora* species cultivated in Ecuador reported ABTS values from 30.32 ± 0.06 to $43.00 \pm 0.70 \mu\text{mol TE/g}$, with *P. edulis* f. *flavicarpa* demonstrating the highest response (Viera *et al.*, 2022). These results confirm that *Passiflora* peel is a superior source of antioxidant compounds. Free radicals are unstable, highly reactive species derived primarily from oxygen, nitrogen, and sulfur, which can lead to the formation of Reactive Oxygen Species (ROS[•]) and Reactive Nitrogen Species (RNS), and Reactive Sulfur Species (RSS[•]) (Carocho & Ferreira, 2013). The ABTS assay is a broad-spectrum method that interacts with these species due to its sensitivity to both hydrophilic and lipophilic antioxidants. *In vitro* studies have shown its effectiveness in neutralizing the peroxy (ROO[•]) and hydroxyl (OH[•]) radicals (Koroleva *et al.*, 2014). Consequently, the high ABTS values obtained in this study suggest that *Passiflora* spp. extracts possess a significant potential to neutralize physiologically relevant radicals, potentially reducing lipid peroxidation and oxidative stress.

This bioactivity is further supported by research on other *Passiflora* tissues. For example, tea derived from *P. edulis* leaves yielded an ABTS concentration of $19.2 \pm 0.50 \mu\text{mol TE/g}$, with *in vivo* studies demonstrating an increase in glutathione reductase activity. This suggests that the bioactive compounds present in the genus can prevent ROS formation and reduce oxidative stress by enhancing endogenous antioxidant defenses (Da Silva *et al.*, 2013). Overall, these findings demonstrate that *Passiflora* tissues exhibit consistent and robust antioxidant activity across different matrices and methodologies.

***In vitro* antifungal activity of *Passiflora edulis* peel extract against *Rhizopus stolonifer*: minimum inhibitory concentration and mycelial growth inhibition**

Figure 4 shows the results obtained regarding the growth of *Rhizopus stolonifer* over time. The radial growth of the mycelium demonstrates that the antifungal effect of the *Passiflora edulis* peel extract against the pathogen depends on the dose used. In the control sample, almost complete colonization of the Petri dish was observed from the first few days, while a higher concentration of the extract progressively reduced mycelial growth. At 3000 ppm, growth remained practically nonexistent until day 7, indicating almost total and sustained inhibition, and concentrations of 2500-3000 ppm also showed minimal growth. In contrast, at ≤ 2000 ppm, a progressive recovery in growth was observed, indicating a loss of the antifungal effect.

Based on the absence of visible growth at the end of the assay, the *in vitro* MIC (potato dextrose agar) was determined to be 3000 ppm, consistent with reports for *R. stolonifer* in agar dilution assays (Colín-Álvarez *et al.*, 2025). This behavior demonstrates that insufficient doses allow for the recovery or growth of the pathogen as has been documented in *in vitro* and *in vivo* studies in strawberry (Triunfo *et al.*, 2023; Shahdadi *et al.*, 2025).

In addition to the MIC determination, the percentage of mycelial growth inhibition was calculated to describe the magnitude and temporal stability of the antifungal effect at each concentration. The mycelial growth of *Rhizopus stolonifer* showed a dose-dependent antifungal effect of *Passiflora edulis* peel extract. At concentrations of 2500 ppm or higher, inhibition rates close to 100% were observed by day 7 (2500 ppm, $\approx 99.4\%$; 3000 ppm, $\approx 99.6\%$), whereas lower doses lost efficacy over time (2000 ppm, $\approx 87.6\%$; 1500 ppm, $\approx 31.4\%$). This behavior is consistent with the definition of the MIC in agar dilution assays; in this regard, Colín-Álvarez *et al.* (2025) reported an MIC of 500 ppm for cinnamon essential oil against *R. stolonifer*.

In contrast, the hydroalcoholic extract evaluated in this study required higher concentrations to achieve total inhibition, likely due to differences in composition and diffusion in the medium (Saleh & Abu-Dieyeh, 2022; Fonseca *et al.*, 2022). Furthermore, the loss of efficacy at inhibitory doses confirms that insufficient concentrations allow growth recovery, which helps explain the lower efficacy observed under *in vivo* conditions compared to *in vitro* assays (Triunfo *et al.*, 2023; Shahdadi *et al.*, 2025).

In vivo* antifungal effect of *Passiflora edulis* extract based coatings on strawberries artificially inoculated with *Rhizopus stolonifer

Regarding the effect of *Passiflora edulis* extract (PEE) as part of the coating applied to strawberries (*Fragaria* $\times$ *ananassa*), similar to the *in vitro* study, it showed a concentration-dependent antifungal effect against *Rhizopus stolonifer* in strawberries stored at 8 °C (Figure 5). At low concentrations (500-1500 ppm), only partial development of the pathogen was observed, while at concentrations of 2000-2500 ppm, growth was postponed until later stages of storage. At a concentration of 3000 ppm, the greatest inhibition was observed, with no visible growth until day 17. However, the coating was not able to postpone fruit deterioration, as bleeding appeared, causing weight loss, suggesting that physiological deterioration may precede fungal colonization.

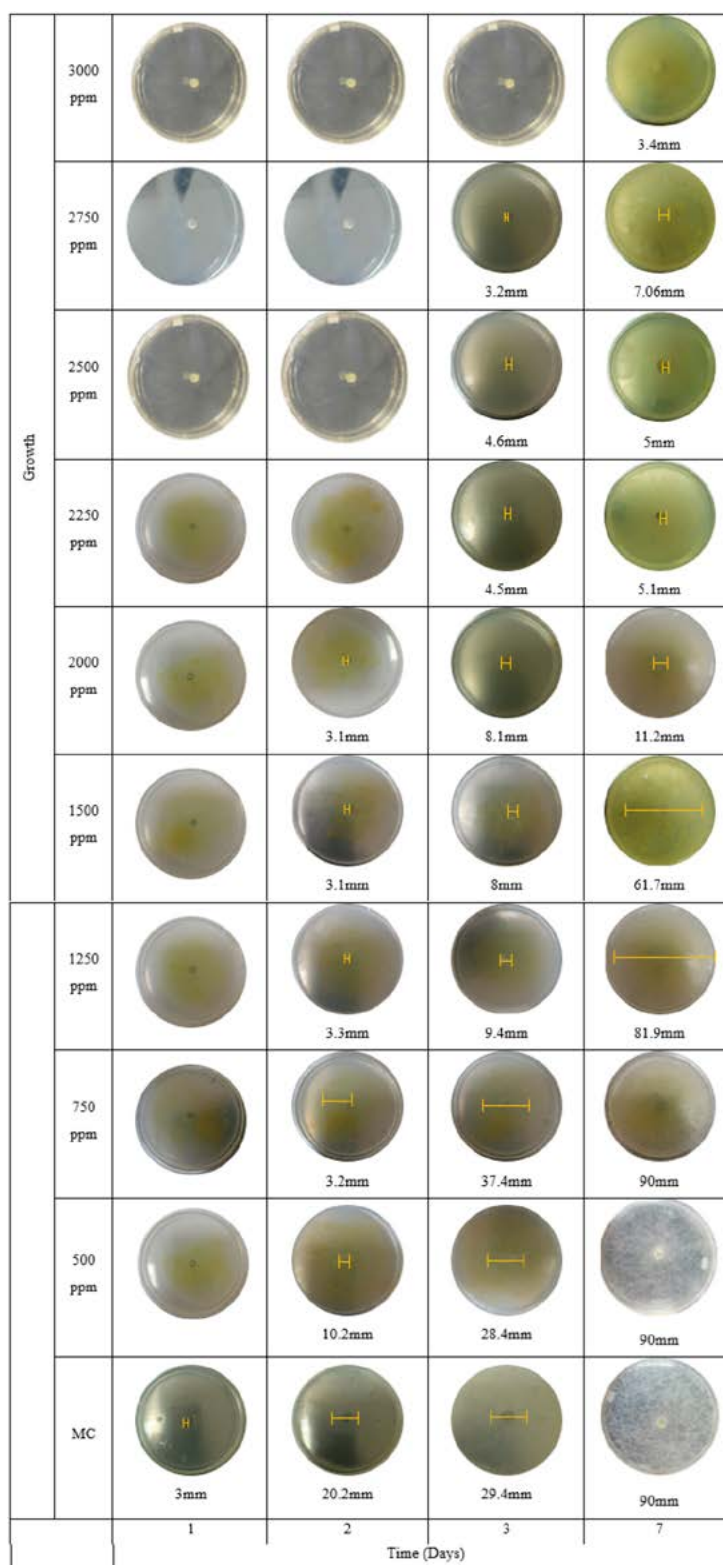


Figure 4. Monitoring of radial growth of *Rhizopus stolonifer* mycelium against *Passiflora edulis* extracts.

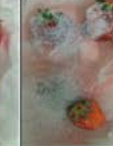
	Day										
Treatment	1	2	3	6	7	8	9	10	13	15	17
Uncoated Control (U.C)											
Coated Control (C.C)											
Treatment 1 (T1) 1000ppm											
Treatment 2 (T2) 1500ppm											
Treatment 3 (T3) 2000ppm											
Treatment 4 (T4) 2500ppm											
Treatment 5 (T5) 3000ppm											

Figure 5. Visual evolution of strawberries infected with *Rhizopus stolonifer* and coated with an edible coating formulated with *Passiflora edulis* extract during storage.

Regarding the control samples, deterioration was observed from day 3, confirming the fruit’s high susceptibility to *Rhizopus stolonifer* (ER). This behavior is consistent with reports for active coatings on strawberries, where efficacy depends strongly on the dosage of the incorporated bioactive agent (Triunfo *et al.*, 2023; Shahdadi *et al.*, 2025), and with studies showing that low concentrations allow *Rhizopus* to recover, while high doses suppress its growth for a longer period (Colín-Álvarez *et al.*, 2025; Saleh & Abu-Dieyeh, 2022). Overall, the results confirm that EPE acts as an active system whose efficacy is critically dose dependent.

CIELab strawberry color with *Passiflora edulis* extract coating

In the analysis of the CIELab parameter, strawberry color was found to be a highly sensitive attribute during storage, as the fruit’s physiological processes involve changes in

pigments, oxidative state, and epidermal integrity. In this study, L^* , a^* , and b^* all changed with respect to time and coating type, confirming that color was influenced by both storage time and treatment conditions. This behavior is consistent with that described for strawberries coated with active systems (González-Cuello *et al.*, 2022; Shahdadi *et al.*, 2025; Feng *et al.*, 2024).

Parameter L^*

Regarding the L^* parameter, a treatment-dependent response was observed during storage (Figure 6). Specifically, in the control group, L^* decreased more irregularly and markedly, with progressive darkening during storage, as reported by Feng *et al.* (2024). In contrast, the coated fruits maintained more stable L^* values which may indicate that the coating contributed to delaying visible darkening under the evaluated conditions (Shahdadi *et al.*, 2025; González-Cuello *et al.*, 2022). This greater stability is consistent with that reported for strawberries coated with active Aloe vera-based systems enriched with rosemary (Shahdadi *et al.*, 2025).

Parameter a^*

The a^* parameter (reddish tones) showed a time- and treatment-dependent response. In the control samples (without extract), the fruits exhibited uneven variations, with a^* initially intensifying and subsequently showing accelerated pigment degradation during senescence (Feng *et al.*, 2024). In contrast, coated fruits displayed more stable a^* trajectories, which may reflect a more gradual change in color during storage, which is consistent with reports for active systems (Shahdadi *et al.*, 2025; González-Cuello *et al.*, 2022).

Although anthocyanin degradation has been associated in the literature with oxidative processes (Feng *et al.*, 2024), in the present study no direct measurements of oxidative status, antioxidant activity within the fruit, or interactions between the extract and the coating matrix were performed. Therefore, the observed color stability should be interpreted based on the experimental results, without attributing it to a specific biochemical mechanism.

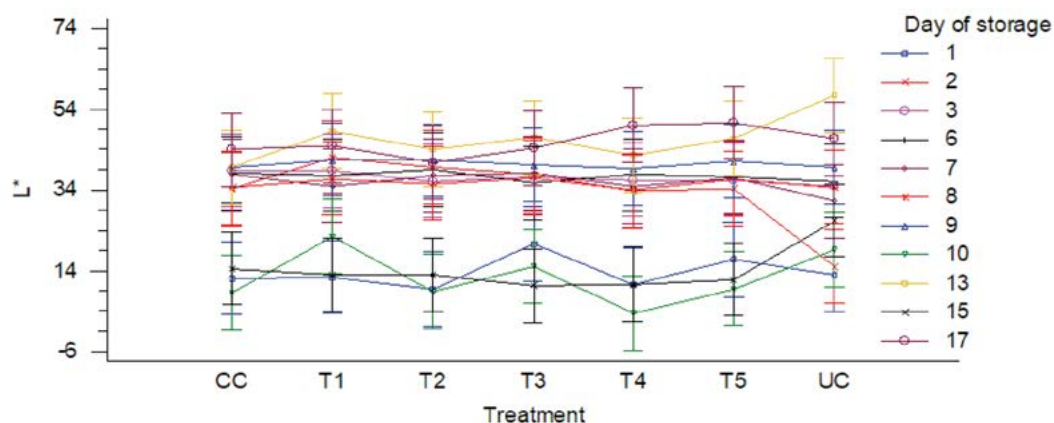


Figure 6. Day $\times$ Treatment Interaction on the L^* Parameter over 17 Days of Shelf Life of Strawberries Coated with *Passiflora edulis* Extracts.

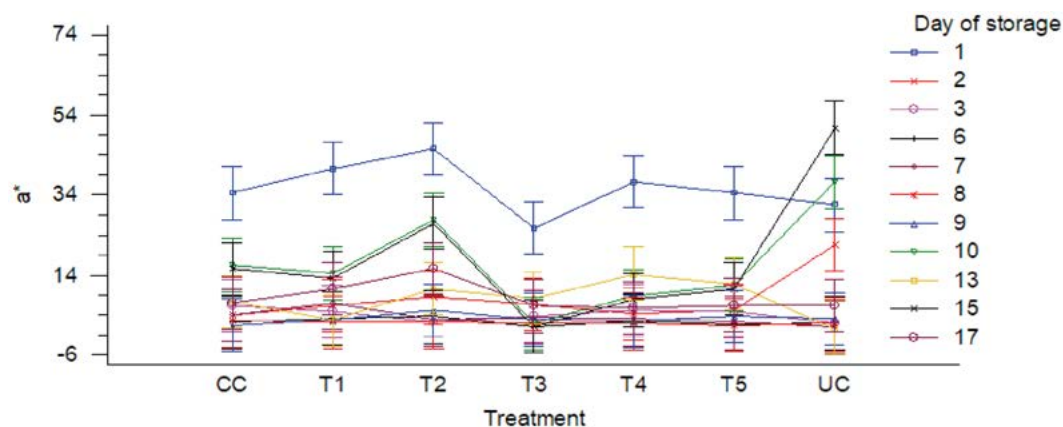


Figure 7. Day $\times$ Treatment Interaction on the a^* Parameter over 17 Days of Shelf Life of Strawberries Coated with *Passiflora edulis* Extracts.

Parameter b^*

The same behavior was observed as in the a^* parameter, with b^* showing a time- and treatment-dependent response. Similarly, in the control samples, a greater dispersion in the b^* values was observed, showing a greater loss of chromatic purity during storage (González-Cuello *et al.*, 2022).

While coated fruits exhibited more stable b^* values, showing a delay in hue changes during storage, these variations reflect possible chemical changes in the pigments and modifications in the tissue microstructure, which can affect the interaction of light with the fruit surface (Feng *et al.*, 2024). This behavior is consistent with what has been reported for coated strawberries, where coated systems have been associated with improved color stability during storage (Shahdadi *et al.*, 2025; González-Cuello *et al.*, 2022).

Weight loss kinetics in strawberries coated with *Passiflora edulis* extract during 17 days of shelf life

The weight loss of the control sample (15.16 to 8.92 g; $\approx 41.2\%$) demonstrated that strawberries are highly susceptible to dehydration, associated with their high respiration

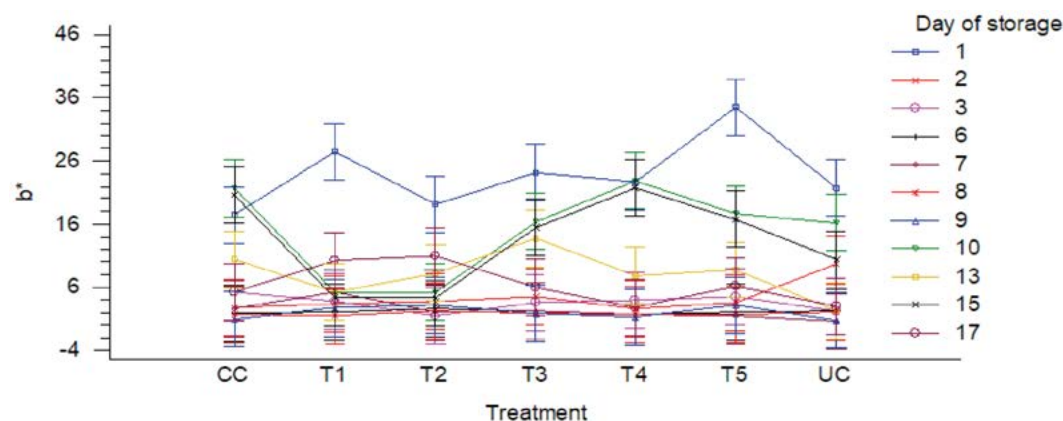


Figure 8. Day $\times$ Treatment Interaction on the b^* Parameter over 17 Days of Shelf Life of Strawberries Coated with *Passiflora edulis* Extracts.

rate, intense transpiration, and inefficient cuticle (Figure 9). These results are consistent with studies conducted under more severe conditions, where losses of up to 53.62% have been observed in just a few days, while coatings significantly reduce this process (Triunfo *et al.*, 2023), even under refrigeration (González-Cuello *et al.*, 2022), and in different coating systems (Saleh & Abu-Dieyeh, 2022; da Silveira *et al.*, 2025; Shahdadi *et al.*, 2025). Based on the above, the results confirm that coating with *P. edulis* extract reduced fruit dehydration. Furthermore, weight loss was found to be directly related to microbial deterioration, as dehydration promotes loss of turgor and the formation of microfractures that facilitate fungal colonization, as shown in Figure 5. In this regard, research has demonstrated that coatings simultaneously reduce water loss and fungal growth (Saleh & Abu-Dieyeh, 2022; Triunfo *et al.*, 2023; Colín-Álvarez *et al.*, 2025; Shahdadi *et al.*, 2025). This effect is probably due to the combined action of a physical mechanism (semipermeable barrier to water vapor and gases) and a biological one, associated with the antioxidant and antifungal activity of the *P. edulis* extract (Lam & Ng, 2009; Fonseca *et al.*, 2022).

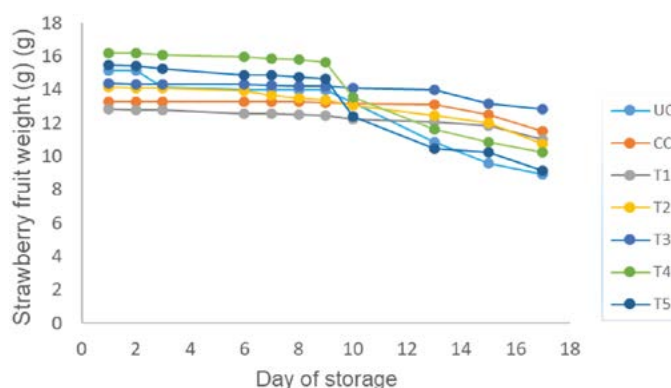


Figure 9. Weight loss of strawberries coated with *Passiflora edulis* ethanolic extract during 17 days of shelf life.

CONCLUSIONS

The research confirmed that the peel of *Passiflora edulis*, an agro-industrial residue, is a significant source of bioactive compounds with antioxidant and antifungal activity. Of the species evaluated, *P. edulis* had the highest extraction yields and also exhibited the highest antioxidant capacity; therefore, it was selected for the formulation of the edible coating. Regarding the ethanolic extract, it showed an antifungal effect against *Rhizopus stolonifer* under *in vitro* conditions, with the effect being strongly concentration-dependent. A minimum inhibitory concentration of 3000 ppm was identified using the radial growth assay. The loss of inhibitory efficacy at low concentrations demonstrated that low or insufficient doses lead to a recovery of fungal growth.

In the *in vivo* study, the incorporation of *P. edulis* extract into an edible coating applied by immersion to strawberries delayed fungal growth during refrigerated storage, reduced weight loss, and maintained greater stability of color parameters compared to the control. These results indicate that the coating helps delay senescence and postharvest-deterioration processes in the fruit.

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Determination of the phytochemical and biofungicide characteristics of Peruvian pepper tree (*Schinus molle*) against *Neofusicoccum* sp. that damages post-harvest avocados (*Persea americana* Mill)

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ABSTRACT

Objective: To evaluate the effect of leaf, stem, and fruit plant extracts of Peruvian pepper tree (*Schinus molle*) on *Neofusicoccum* sp.

Design/Methodology/Approach: Methanol extractions of Peruvian pepper tree were used to determine its phytochemical profile. The inhibition caused by the plant extracts was evaluated at a 0.01, 0.03, and 0.07% concentration, using the in vitro poisoned agar technique against the phytopathogen isolated from post-harvest avocados.

Results: Saponins, coumarins, total phenols, flavonoids, and antioxidant activity recorded positive results. Stem obtained better ABTS inhibition results ($92.7 \pm 0.8\%$), while the leaves recorded a better DDPH inhibition result ($88.2 \pm 0.24\%$). The *in vitro* assays showed the effects of the extracts on *Neofusicoccum* sp., higher concentrations (0.07%) had a better mitigation effect. The inhibition of the pathogen fluctuated between 43.8 and 49.5% for all the plant extracts.

Study Limitations/Implications: Methanol was used as a solvent to obtain *S. molle* extracts.

Findings/Conclusions: The resulting inhibition was associated with a pseudo-quantitative approach that helped to understand the effect of bioactives against this phytopathogen and to include *Schinus molle* as an alternative within a biorational plan for the phytosanitary protection of crops.

Keywords: extracts, bioactives, phytopathogens, biological control, diseases.



INTRODUCTION

Mexico is the main producer and exporter of avocado (*Persea americana* Mill.) worldwide. Most of the avocados produced in Mexico come from the Pacific central region (Jalisco, Michoacán, and Nayarit), which accounts for 96% of the domestic production (Herrera-González *et al.*, 2024). Avocado is a climacteric fruit and, consequently, its preservation and commercialization to distant markets is difficult, because its consumption quality, shelf life, and commercial value decrease (Ramírez-Gil *et al.*, 2020). Post-harvest deterioration of avocado is mainly caused by fungal diseases, such as anthracnose and peduncle ringing. The symptoms of these diseases particularly appear during the ripening process and increase due to mechanical damage, physiological disorders, unappropriated storing temperatures, long refrigeration periods, harvest season, and fruitage (Arpaia *et al.*, 2018; Bowen *et al.*, 2018). *Neofusicoccum* sp. was previously reported in avocados by Molina-Gayosso *et al.* (2012). For their part, Sasia *et al.* (2023) isolated *Neofusicoccum* sp. from plants with bacterial canker symptoms in stems, which impacted 60% of avocado plantations. *Neofusicoccum* sp. is the third most frequently found fungi in avocado plantations. Modern agriculture depends on the use of chemical pesticides to control phytopathogens. This situation has gradually resulted in pest resistance, changes in soil microbial diversity, and environmental pollution (Jin-Lian *et al.*, 2016). Custode *et al.* (2023) pointed out that, in order to tackle this problem, natural solutions such as plant extracts are being researched.

Currently, new technological initiatives and trends have resulted in bioproducts such as bioinsecticides, bioherbicides, bioacaricides, bionematicides, and biofungicides. These alternatives include stimulants of the plant defenses, biological control techniques, and natural byproducts obtained from plants and microorganisms (Sharma and Malik, 2012; Isman and Grieneisen, 2014; Ordanza-Beneitez, 2017). Biological control involves the use of beneficial organisms and their products, such as secondary metabolites that reduce the effects of pathogens on plants and promote a favorable response (García-Espejo *et al.*, 2016).

Plants synthesize variable concentrations of different secondary metabolites and perhaps use them as defense mechanism. They belong to different groups, including essential oils, alkaloids, coumarins, steroids, phenols, flavonoids, glycoside, rubber, iridoids, lignans, mucilage, pectin, quinones, saponins, tannins, and terpenoids (Paumier *et al.*, 2018 and Pinard *et al.*, 2019).

These substances are synthesized as a plant response to fungi, bacteria, and pest attacks, as well as allelopathic effect, pollution, and adverse effects resulting from climatic factors, and herbivores. These chemical defenses can be found in different concentrations in nature and depend on the type of metabolite, the characteristics of the plant, and the conditions to which the plant is subjected (Reyes-Silva *et al.*, 2020). The species *Schinus molle* L. is variously known by people of the Americas as pepper, Peruvian pepper, American pepper, false pepper, molle, molli, anacahuita, or pirul (Enersis, 2014). This plant has several biological effects, including antioxidant, anti-inflammatory, hypotensor, analgesic, antispasmodic, antifungal, and antitumor properties (Zamora, 2007, and Rebolledo, 2020). The phytochemical profile of the

leaves, stems, and fruit of *Schinus molle* and its effect on *Neofusicoccum* sp. were identified under *in vitro* conditions. Consequently, the aim was to include this plant as a biorational management alternative.

MATERIALS AND METHODS

Production of the Peruvian pepper tree (*Schinus molle*) extract

The first stage of this research was to obtain the Peruvian pepper tree extract from different parts of the tree (leaves, stems, and fruits); consequently, the research team collected samples from the nearby plots of La Barca, Jalisco (20° 21' 20.5" N, 102° 26' 13.3" W), prioritizing undamaged and pest- and disease-free plant material (Figure 1). This plant material was processed in the Phytopathology Lab of the Centro Universitario de la Ciénega, La Barca, Jalisco, University of Guadalajara.

The plant material was collected from the nearby plots of La Ciénega region, Jalisco, Mexico. The plant material was carefully divided into leaves, stems, and fruits. Half a kilogram of each plant material was collected. The plant material was washed with running water. Subsequently, it was washed with distilled water. Finally, it was left to dry under natural abiotic conditions until the appropriate drying was achieved (temperature and moisture, sun or shade).

Each sample was lyophilized in a Nutrebule equipment to obtain fine particles. The technique proposed by Del Toro-Sánchez *et al.* (2015) was used in the extraction process. Three-point-zero g of a given lyophilized plant part (leaves, stems, and fruits) were poured into Corning® tubes. Afterwards, 30 mL of methanol were added to the tubes and the mixture was allowed to rest for 24 h. Subsequently, it was homogenized for 30 s in an ULTRA-TURRAX® (T 25 DS1 digital homogenizer). Then, the mixture was sonicated for 15 minutes, at 4 °C and at 4,000 rpm, using a centrifuge machine (Heraeus Megafuge 16R, Thermo Fisher Scientific, Waltham, MA, USA). The supernatant was filtered in a round-bottom flask, restricting the light. The process was repeated with the precipitate. Once the process concluded, the yield of the extract was determined during the methanolic phase; the solvent was evaporated using the rotavapor system (Heidolph Rotavapor, 4003 VAC Senso T), reporting extract grams per dry sample grams (gE/gms). The total extract was recovered adding 10 mL of methanol and, subsequently, it was refrigerated at 5 °C.



Figure 1. *Schinus molle* plant material used to obtain the extracts: leaf (A); stem (B); fruit (C).

Phytochemical profile based on qualitative tests

Saponin identification (foam testing)

The method proposed by García *et al.* (2009) and Sánchez *et al.* (2010) was used to determine saponin content. A 1:9 dilution rate (1 mL of crude extract and 9 mL of distilled water) was poured into a test tube. The mixture was vigorously shaken by hand for 30 s. Afterwards, the mixture rested for 15 min. The results depended on the height of the foam: <5 mm: (–), negative content; 5-10 mm: (+), low content; 10-15 mm: (+), moderate content; >15 mm: (++), high content.

Coumarin identification

A 1:9 dilution from the crude extract (1 mL of crude extract and 9 mL of distilled water) was used to identify coumarins. Two mL of this solution were poured into a test tube with a cap. Afterwards, a strip of filter paper soaked in NaOH (0.06 g mL^{-1}) was placed inside the tube, avoiding contact with the crude extract dilution. Subsequently, the tube was heated in a Bunsen burner until it produced enough vapors. To verify the accumulation of coumarins, the paper was observed under a transilluminator UV chamber (Labnet, model: TM-26). If the filter paper showed fluorescent dots, the sample was positive (García *et al.*, 2009; Sánchez *et al.*, 2010).

Alkaloid identification

To identify the alkaloid content, 6 mL of extract and 6 mL of 10% HCL were poured into a test tube and boiled for 5 minutes. Afterwards, the mixture was cooled, filtered, and transferred to two tubes. A drop of Dragendorff's reagent was added to one of the tubes. The result was positive when the Dragendorff's reagent formed an orange precipitate.

Tannin identification

To identified the tannin content, 0.7 g of the lyophilized sample were added to a flask, along with 200 mL of potassium ferrocyanide $\text{K}_4[\text{Fe}(\text{CN})_6]$. The final mixture had a concentration of 0.004 M. The mixture was kept at 100 rpm during 15 minutes, without light. Subsequently, 20 mL of 0.008 M ferric chloride (FeCl_3) (García *et al.*, 2009; Sánchez *et al.*, 2010) were added. Changes in color were considered as a positive result: dark green indicated condensed tannins, while blue meant hydrolysable tannins.

Total phenol identification

The Folin Ciocalteu assay was used to determine the phenol content (Prior *et al.*, 2005, Mullen *et al.*, 2007). Twenty-five μL of the Folin 1 N solution were added to 10 μL of crude extract. Afterwards, the mixture rested in the refrigerator for 5 minutes. Subsequently, 25 μL of 20% Na_2CO_3 and 140 μL of distilled water were added to obtain a final volume of 200 μL . The mixture rested for half an hour and, then, absorbance was calculated at 760 nm with a Thermo Fisher Scientific™ FI-01620 Microplate Reader. The standard gallic acid in methanol was used to draw a curve (0-1 mg/mL). The results were expressed as mg of gallic acid per g of dry sample (mg EAG/gms). All the measurements were carried out in triplicate.

Total flavonoid determination

The colorimetric method proposed by Venu *et al.* (2012) was used to determine the total flavonoid content. Eighty μL of an aluminum trichloride - ethanol solution (20 g L^{-1}) were added to 80 μL of the extract. The mixture was shaken for 30 s and, afterwards, it was covered with Parafilm and left in the dark for one hour, at 25 °C. Subsequently, the mixture was shaken for 30 s and absorbance was measured at 415 nm with a Thermo Fisher Scientific™ FI-01620 Microplate Reader. Standard quercetin in methanol was used to draw a curve. The results were expressed as mg of quercetin per g of fresh sample (mg EQ/gmf).

Antioxidant capacity determined by ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid)

The ABTS radical was prepared following the methodology described by Re *et al.* (1999). Nineteen-point-three mg of ABTS were dissolved in 5 mL of distilled water. Meanwhile, 0.0378 g of potassium persulphate were weighted and mixed with 1 mL of water. Afterwards, 88 μL of the persulphate solution were added to the ABTS. This mixture was left in the dark for 12 hours. An adjusted solution was prepared at a 0.7 ± 0.01 absorbance and a wavelength of 734 nm in a Thermo Fisher Scientific™ FI-01620 Microplate Reader. Twenty μL of the sample were added to 270 μL of the prepared cationic radical solution. After a 30 minutes rest, absorbance reached 734 nm. The assay was carried out in triplicate and the results were expressed in inhibition percentages.

$$\text{Inhibition \%} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100 \quad (1)$$

Antioxidant capacity determined by DPPH (2,2-Diphenyl-1-Picrylhydrazyl)

One-point-five mg of the DPPH radical was weighted and dissolved in 50 mL of methanol, which was adjusted to a 0.7 ± 0.01 absorbance, in a 515 nm wavelength (Molyneux, 2004). Two-hundred μL of the radical were taken from this mixture, along with 20 μL of sample. Subsequently, the mixture rested for 30 minutes in the dark and was measured at a 515 nm wavelength, using a Thermo Fisher Scientific™ FI-01620 Microplate Reader (Molyneux, 2004). The assay was carried out in triplicate and the results were expressed in inhibition percentages, using the ABTS equation.

Isolation of *Neofusicoccum* sp. from post-harvest avocados (*Persea Americana* Mill)

The methodology proposed by Ceja *et al.* (2000) was used to isolate the pathogen from post-harvest avocados. The fruits were subjected to a disinfestation process with 2% sodium hypochlorite for 3 minutes. Afterwards, they were washed with sterile distilled water and, finally, approximately 1 cm cuts were made in the epicarp. The cuts were placed in Petri dishes with potato dextrose agar (PDA) and chloramphenicol (1 mL of antibiotic/L of the PDA medium, obtained from a 50 mg antibiotic/mL of ethanol). The Petri dishes were

incubated at 25 ± 1 °C until the mycelium appeared (2-4 days). The growths were purified in new Petri dishes with PDA, isolating individual colonies with the hyphal head technique. The *Neofusicoccum* sp. isolated strains were identified with an optical microscope (model CX311RTSF, Olympus, Tokyo, Japan) with a 40X augmentation.

***In vitro* antifungal analysis of the extract of leaves, stems, and fruits of *Schinus molle* used against *Neofusicoccum* sp.**

The method proposed by Jiménez *et al.* (2006) was used in this analysis. The PDA culture medium was poisoned at different concentrations (0.07, 0.03, and 0.01%). Each dose was applied in the previously sterilized and melted PDA medium, at 30-45 °C. Once the medium was jellified, an active growth of *Neofusicoccum* sp. was inoculated in the center of the dish with a plug. Three replicates per treatment were made. The dishes were incubated at 25 ± 1 °C for seven days. Subsequently, the diameters of the treated and control colonies were measured to calculate the inhibition percentage using the following formula:

$$\% = \frac{DCC - DTC}{DCC} \times 100 \quad (2)$$

where: *DCC*: diameter of the control colony; *DCT*: diameter of the treated colony.

Statistical analysis

All the experiments had three repetitions. Data were evaluated using a multifactorial analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) test, with a 95% reliability. All the analysis were conducted with the Statgraphics Centurion XVI software (StatPoint Technologies, Inc., Warrenton, VA, USA).

RESULTS AND DISCUSSION

Collection and yield determination of leaves, stems, and fruits extract of *Schinus molle*

The yield from the extracts of each plant section was not very diverse; however, they were diverse enough to conduct the *in vitro* assay. The amount of plant material followed the fruit>stem>leaf order (Table 1). The fruit extract recorded the highest content (43%), while the leaf extract recorded the lower value (34%). Alfaro-Pérez *et al.* (2018) pointed out that the quality of the plant extracts depends on the collection site. The growing conditions of the crops have a significant influence on the production of secondary metabolites

Table 1. Yield of the *Schinus molle* extract.

Vegatable sample	Initial weight (g)	Extract (g)	Yield gE/gms	Yield (%)
Leaf	3	1.02	0.34	34
Stem	3	1.16	0.38	38
Fruit	3	1.3	0.43	43

g: grams; gE/gms: extract grams per dry sample grams; %: percentage.

—i.e., the bioactive compounds obtained in the plant extracts. In addition, they can have antibacterial and antifungal properties.

For their part, Holopainen *et al.* (2018) and Estrada-Jiménez *et al.* (2019) pointed out that the presence of secondary compounds is related with the age of the plant material, the defense mechanisms, the content of primary nutrients, and the influence of the soil and weather. The complexity of the ecosystem —particularly weather and soil— in which the plants develop has a significant influence on the active principles and the secondary compounds, which have a marked effect on the plants: the lack and excess of these elements can cause stress, leading to the synthesis of these substances.

In this context, Amyrgialakiu *et al.* (2014) and Caprioti *et al.* (2014) mentioned that the quantitative and qualitative yield of the extraction mainly depends on the polarity of the solvent used in the process. However, no standardized method or solvent has been defined for this process. They both depend on the chemical composition of the extracted compounds, amount and position of the hydroxyl group, and molecular size. In addition, other factors such as solvent concentration, temperature, contact time, particle size, and mass-solvent ratio must be taken into account. The Peruvian pepper tree yield obtained in this research was good.

Phytochemical profile of methanol extracts of leaves, stems, and fruits of Peruvian pepper tree

The analysis conducted to determine the chemical bioactive compounds (Table 2) in the leaf, stem, and fruit methanol extracts showed that stems were the only extract that recorded saponin content. The low content of saponins is likely the result of the absence of fungal or bacterial agents in the tree during the sampling stage (February-March); consequently, the tree was not subjected to stress. Gongóra *et al.* (2022) pointed out that saponins are secondary metabolites naturally produced by plants as a consequence of biotic stress. In addition, saponins can potentially be used in different areas of the industry, particularly in food technology, health, and agriculture.

Güçlü-Üstündağ and Mazza (2007) reported that extracts with saponins are used to improve agricultural productivity because they stimulate plant growth and function as insecticides and fungicides.

Table 2. Phytochemical profile of the methanol extracts of leaves, stems, and fruits of Peruvian pepper tree.

secondary metabolite	Plant fraction		
	Sheet	Stem	Fruit
Saponins	(−)	(+)	(−)
Coumarins	(+)	(+)	(+)
Alkaloids	(−)	(−)	(−)
Tannins	(+); condensable	(+); hidrolyzable	(+); hidrolyzable
Total phenols	(+)	(+)	(+)
Flavonoids	(+)	(+)	(+)

(+): positive; (−): negative.

Meanwhile, the three different leaf, stem, and fruit methanol extracts recorded a positive coumarin content. Oliveros *et al.* (2011) recorded coumarin levels in three extraction processes, using the infusion method. Although this process is not very aggressive, they reported significant coumarin quantities.

For its part, no alkaloids were found in any of the Peruvian pepper extracts. These results match the findings of Ferreira *et al.* (2023), who did not find alkaloids in all the tests conducted with ethanol extracts of *S. weinmanniifolia* leaves. Nevertheless, these results do not mean that alkaloids were not present in the plant.

Each extract obtained a positive tannin content. Leaf extract recorded condensed tannins, while stem and fruit extracts recorded hydrolysable tannins (Table 2). Jin *et al.* (2013) pointed out that, on the one hand, condensed tannins have different physical and chemical properties. In addition, once they are consumed, they have different biological properties: antioxidant, chemotherapeutic, anti-inflammatory, and anti-microbial. On the other hand, water-soluble tannins have a carbohydrate nucleus (mainly glucose). They are susceptible to hydrolysis under physiological conditions, enabling the gradual release of primary components.

For their part, Okuda *et al.* (2011) mentioned that tannins can be produced in higher amounts under adverse environmental conditions —*i.e.*, these secondary metabolites defend the plants against predators (herbivores) and perform antimicrobial activities.

Meanwhile, the total phenol contents were 6.8, 17.1, and 30.1 mg EAG/g for leaf, stem, and fruit extracts, respectively. Polyphenols and phenolic compounds are widely distributed among plants. These are natural molecules of the secondary metabolism of the plants that include >8,000 different compounds. They are a large group of chemical substances, with different structures, chemical properties, and biological activities (Valencia *et al.*, 2017). Phenols are usually researched due to their different functions such as nutrient assimilation, protein synthesis, enzymatic activity, photosynthesis, formation of structural components, and defense against adverse environmental factors (pathogens and insects) (Manach *et al.*, 2004). In addition, phenols are known for their outstanding antioxidant capacity (Gomez *et al.*, 2016).

The flavonoid contents recorded 4.6, 3.2, and 1.8 mg/EQ/g for leaf, stem, and fruit extracts, respectively. Agudelo *et al.* (2013) reported flavonoids in the extract of *S. weinmanniifolia* leaves. Their results match the reports of other studies about similar species, which recorded flavonoids found in methanol extracts of *Schinus longifolius* leaves and *Schinus molle* leaves and fruits (López *et al.*, 2017). Wimalaratne *et al.* (1996) pointed out that Peruvian pepper tree leaves have tannins, alkaloids, flavonoids, spheroidal saponins, sterols, terpenoids, rubber, resin, and >20 essential oils. Likewise, Lannacone and Alvarino (2010) reported that *S. molle* ethnobotanical extracts subjected to a blank test include a mixture of active substances of *S. molle* leaves that could have synergy and antagonist effects. The metabolites found in this study proved that the edaphoclimatic conditions favored the methanol expression of these secondary bioactives, as a result of the interaction between genotype and environment. Growing Peruvian pepper tree —a natural extract source for the industry, agriculture, and pharmacopoeia sectors— plays a key role in the determination of its beneficial potential.

Antioxidant capacity

The antioxidant capacity was determined using the ABTS and DPPH (synthetic radicals) methods. The results were expressed in inhibition percentages. Secondary metabolites produced by plants include a wide range of antioxidants (Siddartha *et al.*, 2022). The analyzed extracts of Peruvian pepper tree recorded high inhibition percentages, revealing the bioactive content that provides their antioxidant capacity to the extracts (Figure 2). The inhibition of the methanol extracts in the radical ABTS reached 90.8 ± 0.4 , 91.8 ± 0.04 , and $92.7 \pm 0.8\%$ for leaf, fruit, and stem extracts, respectively. Meanwhile, the highest inhibition resulting from the radical DPPH reached $88.2 \pm 0.24\%$ (leaf extract), followed by $87.7 \pm 0.15\%$ (fruit), and $84.9 \pm 0.30\%$ (stem). According to the results of the ANOVA, extract, radical, and dilution factors, as well as other interactions, recorded significant effects on inhibition ($p \leq 0.05$). Mendoza (2011) recorded 69.44% (leaves), 56.23% (stems), and 32.84% (fruits) antioxidant capacity applying the free radical capture DPPH test to the hydroalcoholic extract of *Schinus molle*.

The different inhibition percentages obtained with the free radical DPPH method applied by Mendoza (2011) and this research team could be the result of the solvents used to develop the extracts. Meanwhile, ABTS recorded the highest inhibition percentages out of the two free radical methods (ABTS and DPPH) used in this research to determine the antioxidant capacity of the extracts. According to Villaño *et al.* (2007), the inhibition percentage difference between the two free radical methods could be the result of the affinity of the compounds of the extracts. Consequently, ABTS had the highest affinity, because the leaf, stem, and fruit extracts recorded a $>91\%$ inhibition. These results match the findings of Ibarra *et al.* (2018), who subjected the leaf extract of *P. auriculata* to the same free radical methods (ABTS and DPPH).

In vitro antifungal analysis of leaf, stem, and fruit extracts from *Schinus molle* against *Neofusicoccum* sp.

Isolation of the *Neofusicoccum* sp. phytopathogen

Post-harvest avocados with necrotic lesions were used to obtain the pathogen. The method consisted in repeatedly extracting the rotten mesocarp (Figure 3A). The microscopic (Figure

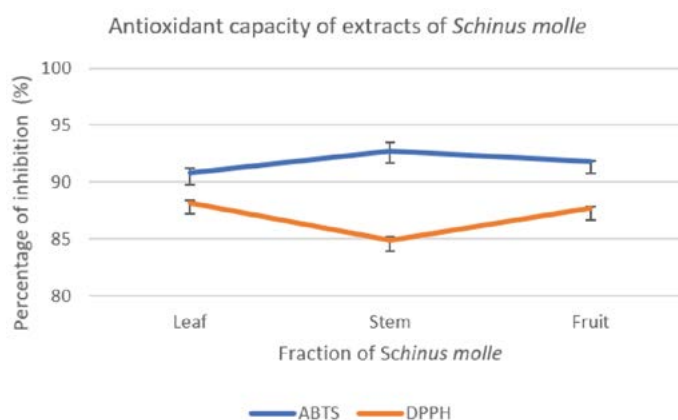


Figure 2. Antioxidant capacity of the Peruvian pepper tree leaf, stem, and fruit extracts.

3C) and macroscopic (Figure 3B) characteristics of the selected colonies were analyzed. In order to confirm its presence, the fungus was grown in potato dextrose broth and pine needles (Figure 3D). The aim was to observe the conidia (Figure 3E). The colonies with similar morphology to the colonies found in the bibliography were chosen. Consequently, the axenic strain (Figure 3B) that was used in the subsequent *in vitro* experiments was obtained.

***In vitro* evaluation of the Peruvian pepper tree (*Schinus molle*) extracts against *Neofusicoccum* sp.**

The *in vitro* poisoned agar technique showed that the biofungicide effect depends on the content of the bioactives (Figure 4) and, consequently, the crude extract effect on *Neofusicoccum* sp. was mitigated with a higher plant extract concentration (0.07%).

López-López *et al.* (2022a) inoculated post-harvest avocados with *Neofusicoccum* sp. and reported necrotic lesions in 83.3% of the fruit. In addition, these authors pointed out that the confrontation of *Neofusicoccum* sp. and an antagonist during the *in vitro* tests resulted in a more aggressive colonization of the pulp, accelerating ripening and increasing colonization in conspicuous areas. Figure 5 shows that leaf, stem, and fruit extracts recorded the higher inhibition percentages (43.8-49.5%) with a 0.07% concentration. These results can indicate a quantitative association with the high phenol content in the leaf extract and, simultaneously, in the stem extract.

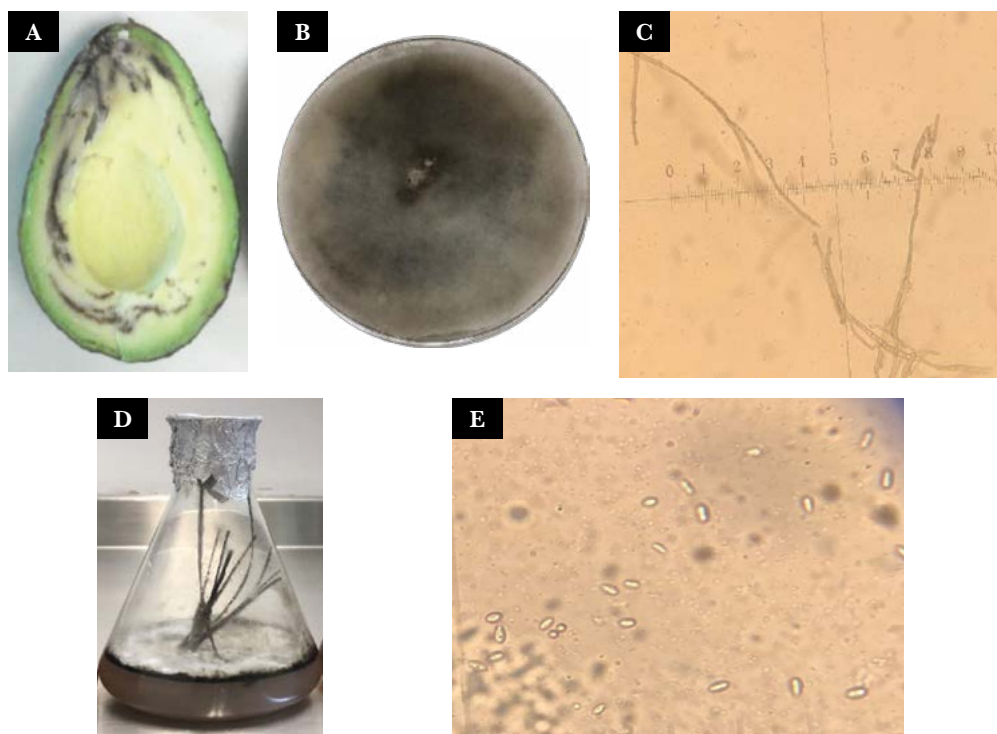


Figure 3. A) Avocado colonized by *Neofusicoccum* sp.; B) *Neofusicoccum* sp. development after 10 growing days in PDA; C) *Neofusicoccum* sp. hyphae at 40 X; D) Active growth of *Neofusicoccum* sp. in potato dextrose broth with pine needles; E) *Neofusicoccum* sp. conidia at 40 X.

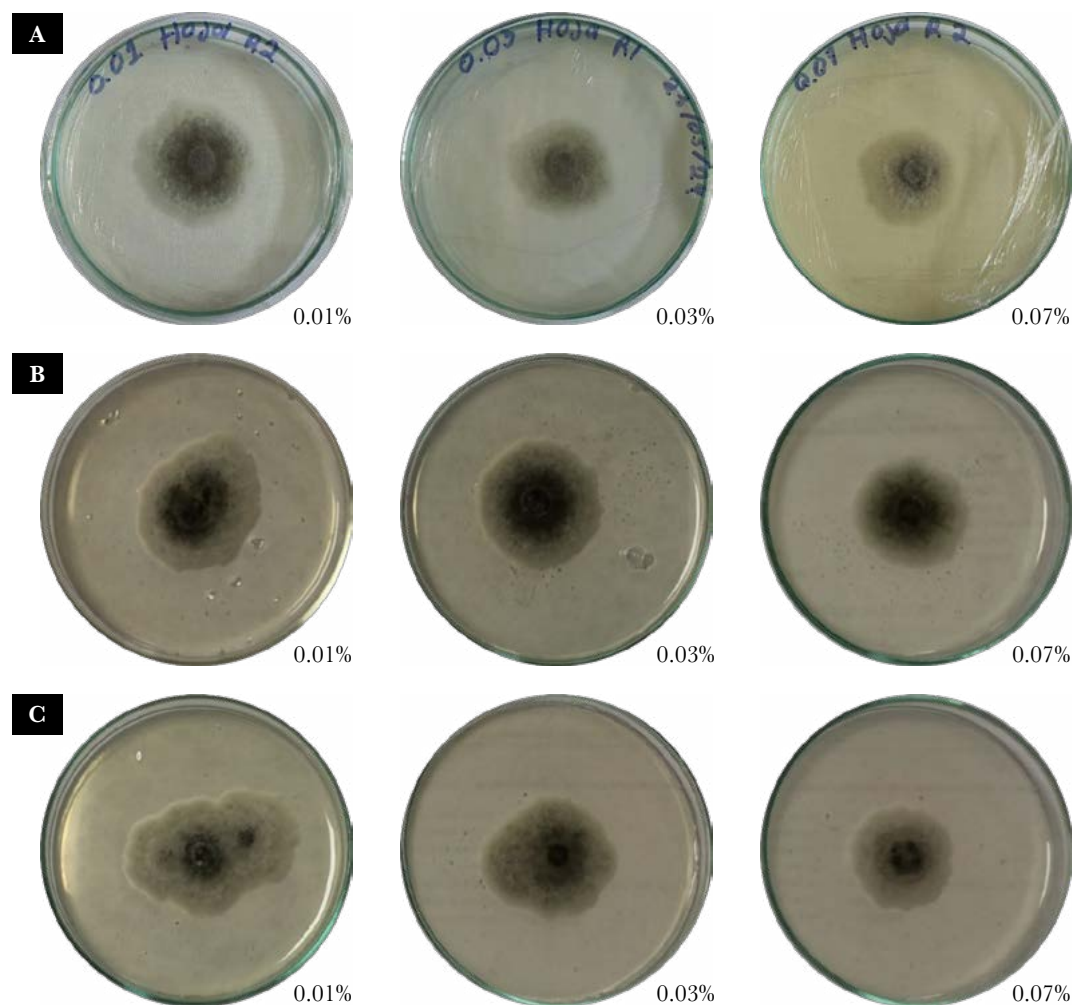


Figure 4. *In vitro* evaluation of the Peruvian pepper tree extract inhibition against *Neofusicoccum* sp., carried out on a poisoned PDA medium, at 0.01, 0.03, and 0.07% concentrations. A: leaves; B: stems; C: fruits.

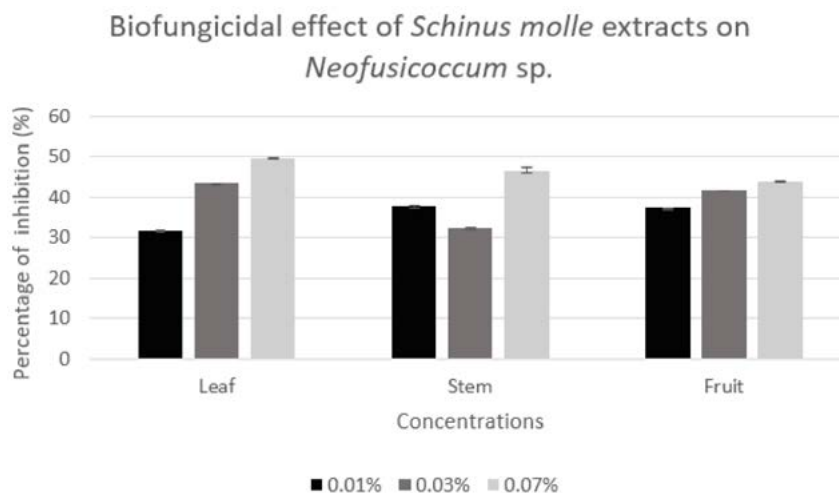


Figure 5. Evaluation of the effect of three concentrations of the bioactive content of *Schinus molle* leaf, stem, and fruit extracts. Different letters show significant differences ($p < 0.05$).

Meanwhile, lower concentrations of flavonoids were mostly found in both leaf and stem extracts. Nevertheless, they provide an understanding of the bioactive effects on *Neofusicoccum* sp. The stem extract mainly recorded positive qualitative results, increasing the potential production of an *in vitro* inhibition boost against the phytopathogen. The inhibition recorded by the three extracts, at a 0.07% concentration, was statistically significant ($p < 0.05$). The phytopathogen inhibition has a high potential to control diseases caused by fungi in post-harvest avocados (López-López *et al.*, 2022b). In addition, Hernandez *et al.* (2019) pointed out the importance of conducting a biological effectiveness evaluation with plants grown under different conditions. This recommendation will validate their application under field conditions.

Meanwhile, the 0.03% concentration recorded a 43.3, 41.6, and 32.2% inhibition for leaf, fruit, and stem extracts, respectively. Finally, the 0.01% concentration obtained results ranging from 31 to 37% for the three extracts. The inhibition statistical analysis of both the 0.03 and 0.01% concentrations was statistically significant ($p < 0.05$).

Aguilar *et al.* (2013) reported that the use of ethanol and oil extracts of Peruvian pepper tree against *C. gloeosporioides* resulted in a 300 mg L^{-1} inhibition. Meanwhile, Ávila-Sosa *et al.* (2011) used a lower concentration of ethanol extracts and obtained 400 mg L^{-1} , showing that Peruvian pepper tree had a higher inhibitory effect with lower concentrations. Despite their low inhibition percentages, all the extracts used in this research undeniably had secondary metabolites with high antimicrobial activity in their chemical composition. The bioactive expression of each extract of Peruvian pepper tree had a changing behavior. Storing time could be taken into account to determine the phytochemical profile, in order to establish its influence on the qualitative and quantitative reduction of the bioactive.

CONCLUSIONS

Traditional methods were used to isolate and identify *Neofusicoccum* sp. in post-harvest avocados. *Schinus molle* produces phytochemical compounds with a 49.5% antifungal inhibition, at a 0.07% concentration, against *Neofusicoccum* sp. based on the phytochemical quantitative profile, the fruit extract recorded the highest phenol content and the leaf extract obtained the highest flavonoid content, while the stem extract recorded the highest quality. The ABTS synthetic radical recorded the highest antioxidant capacity (92.7% inhibition) in the stem extract. *S. molle* can be included in a biorational plan for the phytosanitary protection of crops, as a result of its bioactive content in the leaf extract. The tests conducted using the extracts have proven the existence of biological alternatives to control *Neofusicoccum* sp. under controlled conditions; however, the said extracts should be tested under open field conditions. Understanding their behavior under competition with other microorganisms, as well as the influence of environmental factors, is fundamental.

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Trends in scientific research in *Yucca* spp.

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ABSTRACT

Objective: To analyze the performance and trends of research on *Yucca* spp. at the international level through a bibliometric approach.

Design/methodology/approach: Publications indexed in SCIE, SSCI, and ESCI (1904-2024) were analyzed using unidimensional and multidimensional bibliometric indicators, as well as network visualization tools to evaluate literature growth, author collaboration, and thematic evolution.

Results: A total of 1,266 documents were retrieved, showing sustained growth with a significant increase in the last five years. Collaboration networks with influential authors were identified, along with five main research lines: ecology and evolution, ruminal fermentation, steroidal saponins, metabolite biosynthesis, and applications in sustainable materials.

Limitations on study/implications: The analysis was limited to documents indexed in SCIE, SSCI, and ESCI, which may exclude relevant research published in other databases or non-indexed sources.

Findings/conclusions: The study reveals a transition from taxonomic and ecological research toward applications in biotechnology and materials science. Bibliometric analysis proves useful for identifying emerging trends and priority areas, contributing to the sustainable use of *Yucca* spp.

Keywords: *Yucca* sp., bibliometrics, scientific production, collaboration networks.

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INTRODUCTION

Low-income populations are concentrated primarily in rural areas, where they depend on activities related to agriculture, livestock farming, and the harvesting of non-timber forest resources (FAO, 2010). Although these resources constitute an important source of income, they are often marginalized in public policies and development strategies (Shackleton *et al.*, 2015), and the limited information on their chemical composition and biological assessment restricts their comprehensive use.



Non-timber forest resources have played a fundamental role in traditional medicine, food, and crafts, representing a significant source of economic sustenance in rural areas (Meinhold and Darr, 2019). In Mexico, semi-arid ecosystems cover 70.79 million hectares (Villavicencio-Gutiérrez *et al.*, 2020) and harbor species of high value, including the genus *Yucca* L., comprised of 40-50 species native to North and Central America (Hochstätter, 2004). Around 30 species are recorded in the country, of which 53% are endemic (García-Mendoza, 2004).

Yucca species have been historically used in the production of food, fibers, cosmetics, and medicines (Patel, 2012). Archaeological evidence documents their use in rope and textile making for over two thousand years (Pearlstein, 2008), as well as medicinal applications (Efferth, 2007). They are also recognized as sources of saponins and polyphenols with bioactive properties (Jiménez *et al.*, 2021). However, research has focused on a limited number of species and metabolites, leaving gaps in the comprehensive knowledge of the genus.

In this context, a bibliometric analysis of the scientific output on *Yucca* spp. indexed in SCIE, SSCI, and ESCI of the WoS Core Collection was conducted for the period 1904-2024, with the purpose of evaluating its growth, identifying the main institutions and thematic areas, and characterizing the documentary dynamics of the field.

MATERIALS AND METHODS

Data Collection

To compile the scientific literature on *Yucca* spp., the SCIE, SSCI, and ESCI databases of the WoS Core Collection were consulted, selected for their coverage of high-impact scientific journals and their usefulness in bibliometric studies. The search was conducted in July 2024, considering the title, abstract, and keyword fields, using the term “*Yucca*” and applying exclusion criteria to remove records not directly related to the genus. The document types selected were: Article, Letter, Note, and Early Access.

Initially, 809 documents were retrieved using the title search (TI=“*Yucca*”), which were then subjected to a manual cleaning process, excluding records associated with terms such as “*Yucca Mountain*,” “*Manihot esculenta*,” and “*Arabidopsis*,” among others. After this review, 652 relevant documents were obtained. Subsequently, a thematic search was conducted (TS=“*Yucca*”), yielding 627 records which, after cleaning, were reduced to 614 relevant documents.

This strategy allowed for the differentiation of documents that directly address the topic in the title from those where it appears secondarily in the abstract or keywords. The final records were reclassified by year of publication (1904-2024) and document type for the development of bibliometric indicators.

Statistical Processing

1,266 bibliographic records were retrieved from the Web of Science database (accessed July 31, 2024). The records were exported in RIS format and processed in Zotero for preliminary cleaning and organization, as well as in Microsoft Excel® 2010 for the calculation of initial descriptive indicators.

Subsequently, the complete records, including cited references, were exported in plain text format and analyzed using the Bibliometrix package to generate unidimensional and multidimensional bibliometric indicators. The unidimensional indicators evaluated included: growth in scientific output, document type, language of publication, most productive authors, and countries with the highest number of publications.

Regarding multidimensional indicators, the concentration and dispersion of the literature were analyzed by applying Bradford's Law proposed by Samuel C. Bradford (1934), as well as the productivity of authors by country, the thematic evolution and the relationship between authors and lines of research based on Keyword Plus.

The construction of collaborative networks between authors and countries, citation analysis between journals, co-citation, and keyword co-occurrence were performed using VOSviewer (v.1.6.18), generating bibliometric maps and clusters to visualize structural patterns and thematic trends.

The variables analyzed included number of articles, year of publication, authors per document, citations per article and per author, document type, and collaborative networks. Finally, abstracts and, when necessary, full texts were reviewed to validate the thematic relevance of the 1,266 documents included in the study.

RESULTS AND DISCUSSION

Growth of the literature (1904-2024)

The analysis of the period 1904-2024 (120 years) showed sustained growth in scientific production on *Yucca* spp., with a positive trend supported by a coefficient of determination $R^2=0.6964$. During the first third of the period (1904-1944), production was incipient, with fewer than five documents per year. Between 1945 and 1984, a period of moderate expansion was observed, with averages of between 5 and 15 publications per year. From 1985 onward, growth accelerated, with between 15 and 55 documents per year recorded in most years (Figure 1).

Similar to what Urbizagástegui Alvarado (2016) noted in his analysis of Bradford's Law, the sustained growth of scientific production has also been documented in *Dactylopius coccus* (Bravo-Vinaja and Méndez-Gallegos, 2022). As with *Yucca* spp., research on this species has

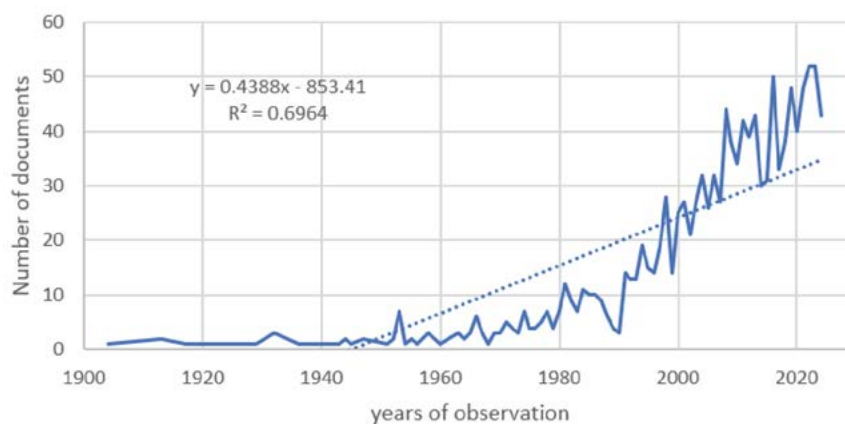


Figure 1. Growth of literature (1904-2024) on *Yucca* spp.

experienced progressive expansion in recent decades, associated with the growing interest in sustainability, agro-industry, and the biology of organisms with economic and ecological relevance. This trend confirms the growth patterns described in bibliometric studies applied to plant resources and species of productive interest. Regarding document type, research articles constitute the predominant form of scientific communication (94.3%), while early access documents represent only 0.23% of the total (Table 1).

Documents in which the search term appeared in the title represented 51.5% of the total, while the remaining 48.5% were located in the abstract and keywords. This distribution indicates that more than half of the publications explicitly address *Yucca* spp. as the central focus of the study, suggesting a high degree of thematic specificity.

Regarding document type, the results show a trend consistent with previous bibliometric studies. Urbizagástegui Alvarado (2016) reports that scientific articles constitute 86% of the publications analyzed under Bradford’s Law, while Bravo-Vinaja and Méndez-Gallegos (2022) identify a proportion of 95.3% in the literature on *Dactylopius coccus*. Similarly, in the case of *Yucca* spp., journal articles emerge as the predominant format. This pattern confirms the central role of the scientific article as the main vehicle for communicating peer-validated knowledge, favoring its visibility, circulation, and impact in the academic community.

Publication language

Regarding the language of publication, English was the predominant language in the scientific production on *Yucca* spp., representing 95.5% of the total documents analyzed. In contrast, publications in German, Hungarian, Turkish, Czech, Indonesian, Lithuanian, and Polish each accounted for less than 2% (Table 2).

This marked hegemony of English demonstrates its consolidation as the lingua franca of international scientific communication, even in a field of study primarily associated with Latin American regions. The predominance of this language favors the global visibility of the results, but also reflects the editorial dynamics of journals indexed in international databases.

The analysis of scientific production on *Yucca* spp. reveals a pattern consistent with previous studies regarding the language of publication. Similar to the findings reported by Urbizagástegui Alvarado (2016), who identified that 86% of documents related to Bradford’s Law are published in English, and to those noted by Bravo-Vinaja and Méndez-Gallegos

Table 1. Types of documents obtained from SCIE, SSCI and ESCI (first 20) with the most published documents (1904-2024) on *Yucca* spp.

Types of documents	Total	%
Scientific article	1195	94.39
Note	29	2.29
Letter	20	1.58
Brief article	19	1.50
Early access	3	0.23
Total	1266	100

Table 2. Publication languages of documents on *Yucca* spp., published (1904-2024) in journals.

Languages	Documents	%
English	1209	95.50
Spanish	21	1.66
Russian	14	1.11
Japanese	5	0.40
Portuguese	4	0.31
French	3	0.23
7 languages with fewer than 3 publications	10	0.78
Total	1266	100

(2022), who found that 92.7% of the literature on *Dactylopius coccus* is in English, the present study shows that 95.5% of articles on *Yucca* spp. are also published in English. This high proportion confirms the consolidation of English as the lingua franca of international scientific communication. Its predominance not only facilitates the dissemination and visibility of findings within the global academic community but also reflects the editorial dynamics of journals indexed in international databases.

Distribution of Publications by Journal: concentration and dispersion of scientific literature

The results show a high concentration of scientific output in a small number of journals. In total, 88 journals (15.60%) account for 50.07% of the analyzed documents, while the remaining 49.03% are distributed among 476 journals (84.39%). Furthermore, 61.52% (347 journals) published only one document on *Yucca* spp., reflecting a marked dispersion on the periphery of the field.

Table 3 shows the 20 most productive journals, representing 3.54% of the 564 registered titles and accounting for 24.48% of the publications. Among those with more than 15 documents, Animal Feed Science and Technology, American Journal of Botany, Oecologia, Journal of Animal Science, Phytochemistry, and Asian-Australasian Journal of Animal Sciences stand out. This behavior is consistent with the core-periphery structure described by Bradford’s Law.

The JCR category indicated in the table corresponds to the one in which the journal achieves its highest position according to the impact factor (IF). *IF 2021, the most recent available; **IF 2000 and IF 1999, the latest reported values for the corresponding journals. Among the leading journals that have published research on the genus *Yucca* are Animal Feed Science and Technology, American Journal of Botany, and Ecology, which together account for approximately 8% of all articles in the field. These journals are indexed in the Journal Citation Reports (JCR) and are located in categories related to ecology, biology, and food science, which reinforces their relevance as established channels for disseminating scientific information on *Yucca* spp. Their ranking enhances the visibility, editorial rigor, and international reach of the research published in this area.

Table 3. Journals indexed in SCIE, SSCI, and ESCI (top 20) with the most published documents (1904-2024) on *Yucca* spp.

Journal	Documents number	Documents (%)	Impact factor (2023)	Category Journal of Citation Reports	Position in the JCR category/ Quartile in the Category
Animal feed science and technology	41	3.24	2.5	Agriculture, Animal and Dairy Science	13/80, 1
American journal of botany	33	2.61	2.4	Plant Science	96/265, 2
Oecology	27	2.13	2.3	Ecology	87/195, 2
Journal of animal science	21	1.66	2.7	Agriculture, Animal and Dairy Science	10/80, 1
Phytochemistry	21	1.66	3.2	Plant Science	68/265, 2
Asian-australasian journal of animal sciences	17	1.34	2.7 *	Agriculture, Animal and Dairy Science	17/62, 2
Journal of dairy science	15	1.18	3.7	Agriculture, Animal and Dairy Science	7/80, 1
animals	13	1.03	2.7	Veterinary Science	16/167, 1
Oikos	12	0.95	3.1	Ecology	56/195, 2
Southwestern naturalist	12	0.95	0.2	Biodiversity Conservation	64/65, 4
Journal of arid environments	11	0.87	2.6	Ecology	73/195, 2
Livestock science	11	0.87	1.8	Agriculture, Animal and Dairy Science	25/80, 2
Poultry science	11	0.87	3.8	Agriculture, Animal and Dairy Science	6/80, 1
Dutch Botanical Act	10	0.79	1.3**	Plant Science	39/137, 2
Plant	10	0.79	3.6	Plant Science	56/265, 1
Industrial crops and products	9	0.71	5.6	Agronomy	8/125, 1
Journal of agricultural and food chemistry	9	0.71	5.7	Multidisciplinary Agriculture	7/89, 1
Journal of the science of food and agriculture	9	0.71	3.3	Multidisciplinary Agriculture	16/89, 1
Khimiya prirodnikh soedinenii	9	0.71	0.2***	Organic Chemistry	46/48, 4
Nature	9	0.71	50.5	Multidisciplinary Sciences	1/134, 1
544 journals with less than 9 publications	956	75.51	Not applicable	Not applicable	NA
Total	1266	100			

Prolific Authors

The most prolific authors on *Yucca* spp. are Olle Pellmyr (University of Idaho), David M. Althoff (Syracuse University), and Tim A. McAllister (University of Manitoba). Pellmyr has focused his research on ecology and evolutionary biology, particularly on the coevolution between *Yucca* and pollinating moths of the genus *Tegeticula*. Althoff has explored insect-plant interactions from an ecological and evolutionary perspective, examining the role of competition, plant chemistry, genetics, and global change in mutualisms. McAllister, for his part, has conducted research focused on sustainability and livestock production, addressing feeding strategies, the rumen microbiome, and greenhouse gas mitigation.

Taken together, the most influential publications on *Yucca* spp. It reveals two predominant thematic axes: on the one hand, coevolution and ecological interactions with pollinating moths; and on the other, productive applications linked to animal nutrition and sustainability. These approaches demonstrate the convergence between basic research focused on mutualism and reciprocal adaptation and applied studies oriented toward agricultural systems. Details of authors with 20 or more documents, as well as the classification of their output according to the presence of search terms in the title, abstract, or keywords, are presented in Table 4.

It was observed that seven of the eight most productive authors published papers addressing *Yucca* spp., treating the topic as their main focus, since the search terms for information included it in the title of most of their studies. These studies centered on *Yucca* spp. research in environmental science and ecology, focusing on its adaptation and role in arid ecosystems. In agriculture, its applications in crops and sustainability were explored. In microbiology, its interactions with soil microorganisms were analyzed. In plant science, its physiology and adaptations were investigated.

Finally, in chemistry, the composition of its bioactive compounds was studied. Among these authors, Olle P., David M. Althoff, and Tim A. McAllister from the USA stood out. Conversely, only one author included the topic in the abstract and keywords of their papers, specifically the search terms for the topic under study. This means that *Yucca* represents the main theme addressed in these documents, which include environmental science and ecology, agriculture, microbiology, chemistry, cultivation and production,

Table 4. Authors with 20 or more documents (1904-2024) related to *Yucca* spp.

Author	Documents	H-index	Citations	documents with search concepts found in the title	documents with search concepts found in the abstract and keywords	Institution	Research topics
Olle Pellmyr	51	38	2479	45	6	University of Idaho	Environmental Sciences and Ecology
David M. Althoff	29	22	667	25	4	Syracuse University	Environmental Sciences and Ecology
Tim A. McAllister.	25	67	1376	8	17	University of Manitoba	Agriculture and Microbiology
Jim Leebens-Mack	22	68	1080	16	6	University of Georgia	Plant Sciences
Segraves, K. A.	22	24	575	20	2	Archbold Biology Station	Environmental Sciences and Ecology
Wieslaw Oleszek	21	49	772	17	4	Institute of Soil Science and Plant Cultivation	Chemistry
Ether Kemertelidze	20	19	239	18	2	Tbilisi State Medical University	Chemistry
Smith, C. I. Edvard	20	15	536	13	7	University of Liverpool	Chemistry

as well as physiological aspects. On the other hand, the three authors with the highest H-index in the SCIE were: Jim Leebens-Mack, from the University of Georgia (68 in the WoS Core Collection), Tim A. McAllister, from the University of Manitoba (SCI 67), and Wieslaw O., from the Institute of Soil Science and Plant Cultivation (SCI 49). In general, studies on cassava focus on coevolution and ecological interactions with their pollinating moths, evaluating how reciprocal adaptations and environmental factors influence these mutualistic relationships.

The “Docs. title” and “Docs. keywords” columns in Table 4 indicate the number of documents in which the search terms appear in the title, abstract, and keywords, or in the abstract and keywords. The colors indicate whether the author’s work addressed the topic as a primary or secondary focus, according to the strategy applied for the period 1904-2024 (Table 4).

Cooperation between authors

Thirty-two research clusters with no interaction between them were identified, highlighting a fragmented structure in the field. However, two main clusters focused on studies of *Yucca* spp. stand out (Figure 2).

The first cluster is comprised of David M. Althoff, Olle Pellmyr, Robert A. Raguso, Kari A. Segraves, Göran P. Svensson, M. Balcázar-Lara, and C. J. Huth, affiliated with institutions such as Syracuse University, the University of Colima, Vanderbilt University, the University of Idaho, Cornell University, and Lund University in the United States, Mexico, and Sweden. This group focuses primarily on studies of evolutionary ecology and plant-insect mutualism.

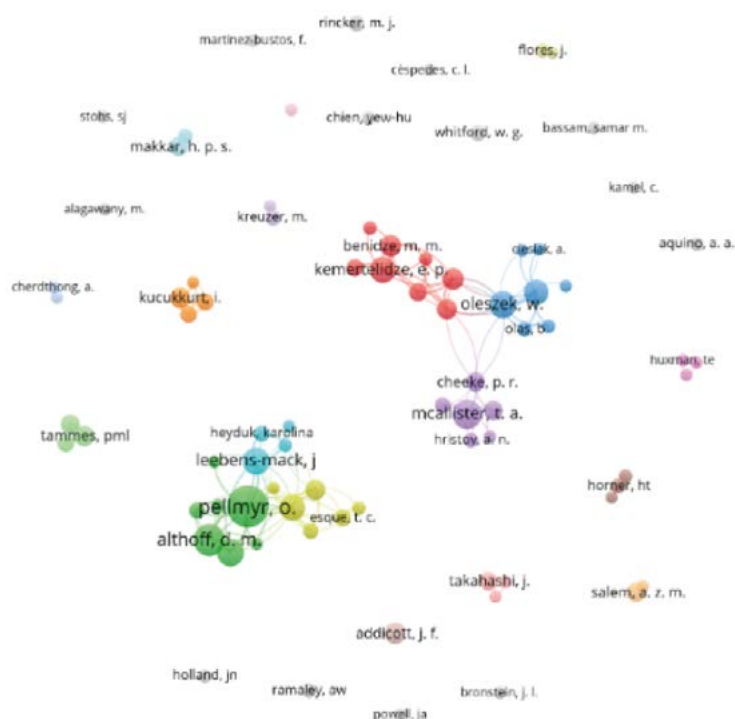


Figure 2. Visualization of research clusters on *Yucca* spp. using VOSviewer software.

The second cluster includes Benidze, M. M., Kemertelidze, E. P., Montoro, P., Piacente, S., Pizza, C., Pkheidze, T. A., and Skhirtladze, A. V., affiliated with Tbilisi State Medical University, the University of Salerno, the TSMU I. Kutateladze Institute of Pharmacochemistry, and Ivane Javakhishvili State University of Tbilisi, in Georgia and Italy. This cluster focuses on research related to chemical properties and secondary metabolites.

Although no interaction was observed between clusters, a consolidated collaboration exists within each one. Three subgroups were identified in Cluster 1, notably including Jim Leebens-Mack and Smith, C. I., along with Althoff, whose research is linked to Environmental Science and Ecology. Cluster 2 also includes three subgroups, led by Tim A. McAllister, Wiesław Oleszek, and Kemertelidze, focused on plant chemistry and production applications. Together, these two clusters comprised six of the eight most prolific authors, confirming the existence of specialized groups that structure the development of the field.

Co-authorship Index

During the analyzed period (1904-2024), the overall average was 4.13 authors per document, reflecting a high level of collaboration in scientific production on *Yucca* spp. However, this indicator has varied significantly over time. Between 1904 and 1980, the co-authorship index was lower, averaging 1.96 (Table 5).

For the period 2001-2010, the co-authorship index was 3.94, a value comparable to that reported by Bravo-Vinaja and Méndez-Gallegos (2023), who recorded indices of 3.59 and 3.75 for the decades 1990-1999 and 2000-2009, respectively, in research on *Dactylopius coccus* Costa (Hemiptera: Dactylopiidae), carminic acid, and its derivatives. Likewise, these results are consistent with those documented in Mexico by the Scientific and Technological Advisory Forum (2006), which reported an average of 3.96 in agricultural sciences for the period 1990-2004. Taken together, these comparisons demonstrate a sustained trend toward increased scientific collaboration, suggesting a progressive transition toward more integrated and multidisciplinary research approaches.

Countries with the Most Publications

The country-by-country analysis revealed that the United States leads scientific production with 31.75% of the total documents, followed by Mexico with 9.00%. The People’s Republic of China and India also had a significant share within the analyzed

Table 5. Co-authorship Index for documents related to *Yucca* spp. (1904-2024).

Time (years)	Co-authorship index
1904 to 1980	1.96
1981 to 1990	2.20
1991 to 2000	2.90
2001 to 2010	3.94
2011 to 2020	4.86
2021 to 2024	5.30

set. The “Other” category grouped 372 documents distributed across 75 countries, with fewer than 34 publications each, representing 29.38% of the total (Table 6). Overall, these results show a structure characterized by a strong concentration in a small number of countries, accompanied by a broad international base with smaller contributions. This pattern demonstrates both the leadership of certain nations in the field and the increasing internationalization of research on *Yucca* spp.

The concentration of publications in countries such as the United States, Mexico, and the People’s Republic of China suggests greater institutional capacity, funding, and consolidation of research lines focused on *Yucca* spp. In contrast, the dispersion of production among the remaining 75 countries, with small individual contributions, demonstrates broad but asymmetrical international participation. This pattern reflects a global structure characterized by centers of scientific leadership and a periphery with emerging contributions, highlighting the need to strengthen international cooperation networks that foster a more balanced integration of knowledge in the field of *Yucca* spp.

Publishing Institutions

The ten institutions with more than 15 documents accounted for 16.43% of all publications on *Yucca* spp. Among them, the University of Idaho stands out with 2.92% of the registered documents. Of the top ten institutions, seven are located in the United States, one in Canada, one in Mexico, and one in Saudi Arabia. This distribution confirms the predominance of North American institutions in the scientific output of this field and is linked to the leadership of English-speaking countries, which helps explain the high proportion of publications in English (Table 7).

Research topics

The joint keyword analysis performed in VOSviewer using bibliographic records and the LingLog normalization method, visualized according to the number of occurrences

Table 6. Countries with the Most Publications (1904-2024) on documents related to *Yucca* spp.

Country	Documents	%
USA	402	31.75
Mexico	114	9.00
People’s Republic of China	86	6.79
Egypt	48	3.79
Canada	46	3.63
Poland	43	3.40
Japan	42	3.32
Turkey	40	3.16
Italy	38	3.00
India	35	2.76
Other	372	29.38
Total	1266	100.00

Table 7. Institutions publishing documents on *Yucca* spp., published (1904-2024).

Institution	Country	Number of citations	Documents	%
University of Idaho	USA	1168	37.00	2.92
Agriculture and Agri-Food Canada	CANADA	1751	27.00	2.13
National Autonomous University of Mexico	MEXICO	581	23.00	1.82
Syracuse University	USA	389	21.00	1.66
University of Georgia	USA	616	21.00	1.66
U.S. Geological Survey	USA	320	17.00	1.34
Vanderbilt University	USA	750	17.00	1.34
King Saud University	Saudi Arabia	353	15.00	1.18
University of California, Riverside	USA	368	15.00	1.18
Willamette University	USA	314	15.00	1.18
1101 institutions			1,058.00	83.57

(considering five or more per author keyword) in the SCIE, SSCI, and ESCI (Keyword Plus) databases, revealed that the research focused on the topics covered by the five clusters related to *Yucca* (Figure 3). These clusters range from the evolution and pollination of the Agavaceae family, through the impact of *Yucca schidigera* on growth performance and digestibility in animals, to ruminal fermentation and methane production. Studies on the chemical properties of *Yucca*, such as steroidal saponins, and the biosynthesis of compounds in *Yucca* plants were also included. The studies applied various techniques to delve deeper into these aspects and contribute to the knowledge of *Yucca* and its importance in animal nutrition, ecology, and plant chemistry. Among the most relevant topics is the red cluster, focused on the Agavaceae family, which addresses the evolution, diversity, and pollination of plants such as *Yucca*, as well as their ecological interactions and coevolutionary processes with pollinators (Kato *et al.*, 2003; Pellmyr & Augenstein, 1997). The green cluster analyzes the effects of *Yucca schidigera* on growth performance and animal digestibility, including ruminal fermentation and ammonia reduction in feed (San Martín & Briones, 2000; Wallace, 2004). Closely related, the blue cluster delves into ruminal fermentation and methane production, highlighting the influence of *Yucca schidigera* and other additives on methanogenesis and emissions mitigation (Busquet *et al.*, 2005; Wallace, 2004).

The yellow cluster focuses on steroidal saponins present in *Yucca*, especially their antimicrobial activity and their application in animal feed and health (Wallace, 2004; Wallace *et al.*, 2002). Finally, the purple cluster addresses the biosynthesis of plant compounds, such as auxins and other metabolites, and their influence on plant growth and development (Patra *et al.*, 2006; Youle & Huang, 1981). Together, these clusters demonstrate the multidisciplinary nature of *Yucca* research, integrating evolutionary ecology, animal nutrition, and plant chemistry.

Research trends over the years

Keyword analysis allows us to identify the main lines of research developed in this field. To examine thematic evolution, 413 keywords present in 1266 articles were analyzed.

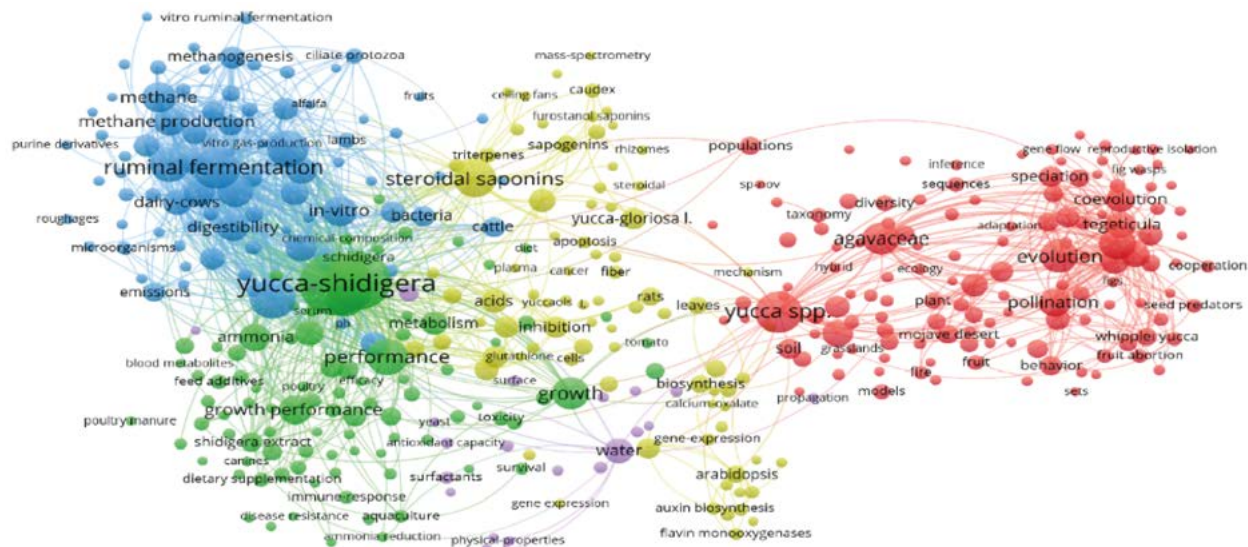


Figure 3. Joint word analysis by author and database keywords with five or more occurrences, in publications (1904-2024) on *Yucca* spp.

Figure 4 shows the network map organized by year of publication, where the size of the nodes indicates the frequency of occurrence of each keyword and the colors represent the groups according to their co-occurrence.

Five main clusters were identified that reflect the evolution of knowledge about *Yucca*: Cluster 5 (royal blue, 1904-2000): Brings together the oldest studies, focused on taxonomy, diversity, traditional uses and early applications in food and biomaterials.

Cluster 2 (blue, 2000-2009): Focused on evolution, pollination, and coevolution within the Agavaceae family, highlighting ecological and genetic interactions. Cluster

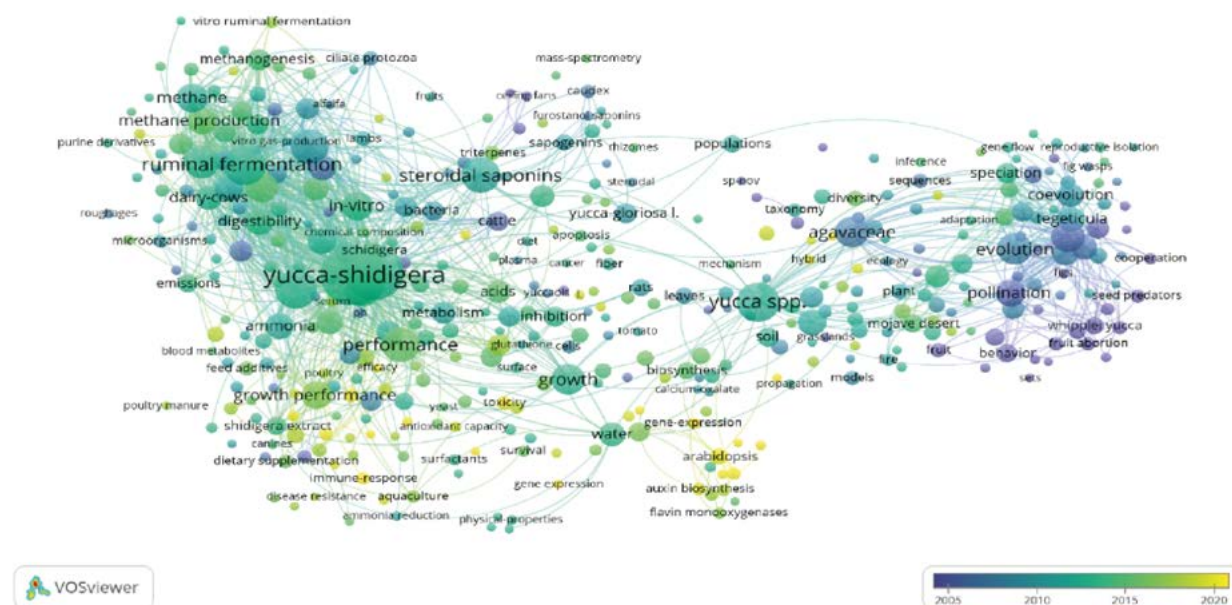


Figure 4. Evolution of Research Topics in *Yucca* (1904-2024): Analysis of Keywords and Co-occurrence in Academic Publications.

1 (aqua green, 2010-2015): Focused on *Yucca schidigera*, extracts, ruminal fermentation, and steroidal saponins, with an emphasis on digestibility and methane reduction in animal feed.

Cluster 4 (lime green, 2015-2019): Focused on the biosynthesis of compounds in *Yucca* and their relationship to plant growth and development, as well as applications in improving crop yields. Cluster 3 (yellow, 2019-2024): Represents the most recent research, focused on the characterization of *Yucca schidigera*, ruminal fermentation, and the mitigation of greenhouse gas emissions through the regulation of methanogenesis. Together, these clusters demonstrate a transition from taxonomic and descriptive studies toward applied and environmental approaches with greater productive and ecological impact.

Over time, research lines have evolved toward the development of value-added applications for the use of wild plants as biomaterials and in food. Likewise, production and utilization strategies for *Yucca* species have been strengthened, aimed at optimizing the sustainable use of these natural resources. In parallel, scientific activity has addressed its management from multiple perspectives, integrating its role in animal feed, ecology, and plant chemistry.

Recent advances in yucca research: analysis of the most cited articles in the last five years

The citation structure of articles published between 2019 and 2021 included five studies with an average of 64 citations (Table 8). The most cited article was “Experimental and computational investigation of sound absorption performance of sustainable porous material: *Yucca gloriosa* fiber” by Soltani *et al.* (2020) with 80 citations, which evaluated the acoustic properties of its fiber as a sustainable porous material for sound absorption.

With the same number of citations (80), the study “Saponin-Induced Shifts in the Rumen Microbiome and Metabolome of Young Cattle” by Wang *et al.* (2019) stood out. This study analyzed the effect of saponins on rumen microbiota and the metabolome in calves, providing relevant evidence in animal microbiology and nutrition.

The most cited study was “The regulatory roles of *Yucca* extract on the growth rate, hepatorenal function, histopathological alterations, and immune-related genes in common

Table 8. The five most cited articles on *Yucca* in the last five years. Abbreviated title TD Journal Category.

Author	Year	Citations (number)	Abbreviated title	Documnt type	Journal	Category
Soltani <i>et al.</i>	2020	80	Sound absorption of Yucca gloriosa fiber	Art.	Applied Acoustics	Sustainable Materials
Wang <i>et al.</i>	2019	80	Saponin shifts in rumen microbiome of cattle	Art.	Frontiers in Microbiology	Microbiology
Dawood <i>et al.</i>	2021	55	Yucca extract under ammonia stress in carp	Art.	Aquaculture	Aquaculture
Grodsky & Hernandez	2020	54	Solar development effects on desert plants	Art.	Nature Sustainability	Ecology and Sustainability
Zazharskyi <i>et al.</i>	2019	53	Antimicrobial activity of plant extracts	Art.	Biosystems Diversity	Biotechnology and Pharmacognosy

carp exposed with acute ammonia stress” by Dawood *et al.* (2021), with 55 citations. This study evaluated the impact of yucca extract on the growth and health of common carp under ammonia stress, highlighting its application in aquaculture. The text abruptly shifts to a different topic: Taken together, these studies demonstrate the relevance of the genus *Yucca* in areas ranging from applied acoustics to biotechnology and aquaculture, highlighting its potential as a natural resource in research focused on sustainability and efficient resource management.

Main Contributions to Yucca Stability: Analysis of Highly Cited Articles

The most cited articles from the period 1904-2024 demonstrate key advances in understanding mutualism and its stability, as well as in biotechnological applications for agriculture and livestock.

The most cited work was that of Bull and Rice (1991) (378 citations), which analyzes the mate choice model in the relationship between moths and yuccas, demonstrating how this mechanism promotes cooperation and penalizes non-cooperative individuals. Similarly, Pellmyr and Huth (1994) (334 citations) explain the stability of mutualism through the selective abort of flowers with a high egg load, which promotes efficient pollination.

In the field of biotechnology, Krishnamurthy *et al.* (2014) (216 citations) stand out for the synthesis of nanomaterials derived from *Yucca* with catalytic po-tential, demonstrating the use of natural compounds in new technologies. Furthermore, several studies cited in Table 9, such as Wallace *et al.* (2002) (211 citations), analyze the use of plant extracts to

Table 9. Most cited articles on *Yucca* (1904-2024).

Author	Year	Citations (number)	Abbreviated title	Documnt type	Journal	Category
Benchaar <i>et al.</i>	2007	221	Essential oils and rumen fermentation in dairy cows	Article	J. Dairy Sci.	Veterinary Medicine and Animal Nutrition
Bodas <i>et al.</i>	2012	270	Plant metabolites and methane reduction	Article	Anim. Feed Sci. Technol.	Veterinary Medicine and Metabolism
Bull & Rice	1991	378	Evolution of cooperation mechanisms	Article	J. Theor. Biol.	Evolutionary Biology
Busquet <i>et al.</i>	2006	323	Plant extracts and rumen fermentation	Article	J. Dairy Sci.	Veterinary Medicine and Metabolism
Hu <i>et al.</i>	2005	202	Tea saponin and rumen fermentation	Article	Anim. Feed Sci. Technol.	Veterinary Medicine and Metabolism
Krishnamurthy <i>et al.</i>	2014	216	Yucca-mediated gold nanomaterials	Article	Nano Res. Lett.	Nanotechnology
Patra <i>et al.</i>	2006	206	Plant extracts and in vitro methanogenesis	Article	Anim. Feed Sci. Technol.	Veterinary Medicine and Metabolism
Pellmyr & Huth	1994	334	Stability of yucca–moth mutualism	Article	Nature	Evolutionary Ecology
Wallace	2004	311	Antimicrobial plant metabolites	Article	Proc. Nutr. Soc.	Pharmacognosy
Wallace <i>et al.</i>	2002	211	Natural products and rumen manipulation	Article	Asian-Australas. J. Anim. Sci.	Veterinary Medicine and Metabolism

modify ruminal fermentation and reduce methane production, contributing to improved feed efficiency and a decreased environmental impact of livestock production.

CONCLUSIONS

During the period 1904-2024, research on *Yucca* spp. showed sustained growth, with a notable increase in the last five years, which accounted for 22.35% of publications. In 2023-2024, 95 articles (7.50%) were published in 13 languages, predominantly English (95.49%). The United States leads in scientific production, followed by Mexico, China, Japan, and Canada, and also consolidates the main international collaboration networks. Among the most prominent institutions are the University of Idaho and Agriculture and Agri-Food Canada, while Olle Pellmyr and David M. Althoff are the most prolific authors. The most frequently used keywords were *Yucca schidigera*, saponins, *Yucca* spp., ruminal fermentation, and evolution, identifying five main lines of research: saponins and extracts, mutualism, rumen, pollination, and evolutionary studies. The thematic evolution reveals a transition from taxonomic and ecological approaches toward applications in animal nutrition, sustainability, biotechnology, and methane emission mitigation. Although the study is limited to the exclusive use of Web of Science and a predominantly quantitative approach, the results allow for the identification of trends, collaborative dynamics, and emerging areas, contributing to guiding future research and scientific cooperation strategies.

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