

AGRO PRODUCTIVIDAD



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Año 19, Volumen 19, Número 5, mayo 2026, Agro productividad es una publicación mensual editada por el Colegio de Postgraduados. Carretera México-Texcoco Km. 36.5, Montecillo, Texcoco, Estado de México. CP 56264. Tel. 5959284427. www.colpos.mx. Editor responsable: Dr. Jorge Cadena Iñiguez. Reservas de Derechos al Uso Exclusivo No. 04-2017-031313492200-203. ISSN: 2594-0252, ambos otorgados por el Instituto Nacional del Derecho de Autor. Responsable de la última actualización de este número, M.C. Valeria Abigail Martínez Sias. Fecha de última modificación, 266 de junio de 2026.

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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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Forest inventory using proximal remote sensors, in a plantation of *Cedrela odorata* L. and *Hevea brasiliensis* Müll. Arg.

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Citation: Hernández-Moreno, J. A., Ordóñez-Prado, C., Hernández-Ramos, A., Cortés-Sánchez, B. G., Buendía-Rodríguez, E., & Martínez-Márquez, C. A. (2026). Forest inventory using proximal remote sensors, in a plantation of *Cedrela odorata* L. and *Hevea brasiliensis* Müll. Arg. *Agro Productividad*. <https://doi.org/10.32854/0xrkbj79>

Academic Editor: Jorge Cadena Iniguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: January 5, 2026.

Accepted: April 19, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 3-14.

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ABSTRACT

Objective: To evaluate the accuracy of the iPhone 15 Pro[®] LiDAR sensor for measuring diameter at breast height (DBH) and photogrammetry with unmanned aerial vehicle (UAV) for estimating tree height.

Methodology: The research was conducted in a 17-year-old mixed plantation. DBH was measured with a diameter tape, and tree height with a clinometer. These measurements were compared with data from the LiDAR sensor and UAV photogrammetry. Agreement was assessed using linear regression (R^2 , RMSE) and the intraclass correlation coefficient (ICC).

Results: High agreement was observed between traditional methods and remote sensing. For DBH, the comparison yielded $R^2=0.95$, RMSE=1.47 cm, and ICC=0.964. For height, the results showed $R^2=0.96$, RMSE=0.64 m, and ICC=0.981.

Study limitations/implications: The study was conducted in a plantation with regular spacing. The results should be validated under different conditions (species and stand structures). The slight systematic underestimation of DBH using LiDAR, attributed to the sensor's resolution, justifies its operational use.

Findings/conclusions: Remote sensors provided accuracy comparable to that of traditional techniques, with superior operational efficiency. iPhone LiDAR is viable for DBH measurement, and UAV photogrammetry is suitable for height estimation in commercial inventories. Their integration allows for automated, georeferenced digital databases for advanced spatial analysis.

Keywords: Forest parameters, Augmented reality, Free software, Mobile LiDAR sensor.

INTRODUCTION

Forest inventories describe the quantity, size, and quality of trees in a forest, as well as other characteristics of the area where they grow and develop (Liang *et al.*, 2016).

They also serve as a basis for planning the sustainable management of forest resources, estimating timber volume, biomass, carbon, and biodiversity, and evaluating the dynamics of forest ecosystems and identifying changes over time with the greatest possible accuracy (Hernández-Moreno *et al.*, 2024).

Traditionally, data and information for forest inventories are obtained using manual equipment, tools, or devices, the most common being diameter measuring tapes or calipers for diameter at breast height (DBH), or clinometers for total height (TH). In practice, this is time-consuming, labor-intensive, costly, and prone to multiple measurement errors (Liang *et al.*, 2016; Hernández-Moreno *et al.*, 2025). The above requires alternative methods, such as the use of remote sensing devices to obtain tree parameters (Liang *et al.*, 2016; Hernández-Moreno *et al.*, 2025). Currently, the availability of devices and the combination of remote sensors offer innovative alternatives for this purpose.

Terrestrial LiDAR (Light Detection and Ranging) and digital photogrammetry using unmanned aerial vehicles (UAVs) have demonstrated potential for the accurate estimation of structural parameters of forest ecosystems (Liang *et al.*, 2016). However, their operational adoption at small, medium, and large scales has been limited by the high cost and technical complexity of professional equipment (Mokroš *et al.*, 2018). This difference has driven the development and evaluation of low-cost proximal remote sensing technologies, such as LiDAR sensors on mobile devices or low-cost UAVs with high-resolution RGB cameras. These technologies have multiple applications and will provide an unprecedented amount of tree/local-level information on ecosystem-specific physical and biological processes and their dynamics (Pierrat *et al.*, 2025).

In particular, LiDAR sensors integrated into mobile devices, along with low-cost unmanned aerial vehicle (UAV) platforms, represent a promising and affordable alternative for generalizing the use of precision forestry technologies, making them more accessible and cost-effective for foresters, technicians, academics, students, and researchers (Mokroš *et al.*, 2018; Fassnacht *et al.*, 2024). Currently, forest inventories need reliable measurement alternatives using low-cost devices or sensors that increase efficiency, accuracy, and the quality of field data. Devices such as smartphones or tablets with low-cost LiDAR and UAVs, originally designed for other sectors, are being adapted for forestry applications, offering an alternative to improve traditional methods in the face of satellite remote sensing technologies (Fassnacht *et al.*, 2024; Zhou *et al.*, 2025; Pierrat *et al.*, 2025).

In this context, the objectives of this research were: 1) To evaluate the accuracy and efficiency of the LiDAR sensor in an iPhone 15 Pro[®] for measuring diameter at breast height (DBH), and 2) To determine the reliability of UAV photogrammetry for estimating the heights of individual trees, compared to traditional methods, in a mixed tropical forest plantation.

MATERIALS AND METHODS

Area of study and experimental design

The research was conducted in a 0.87-hectare mixed forest plantation of *Cedrela odorata* L. and *Hevea brasiliensis* Müll. Arg., established 17 years ago, located between

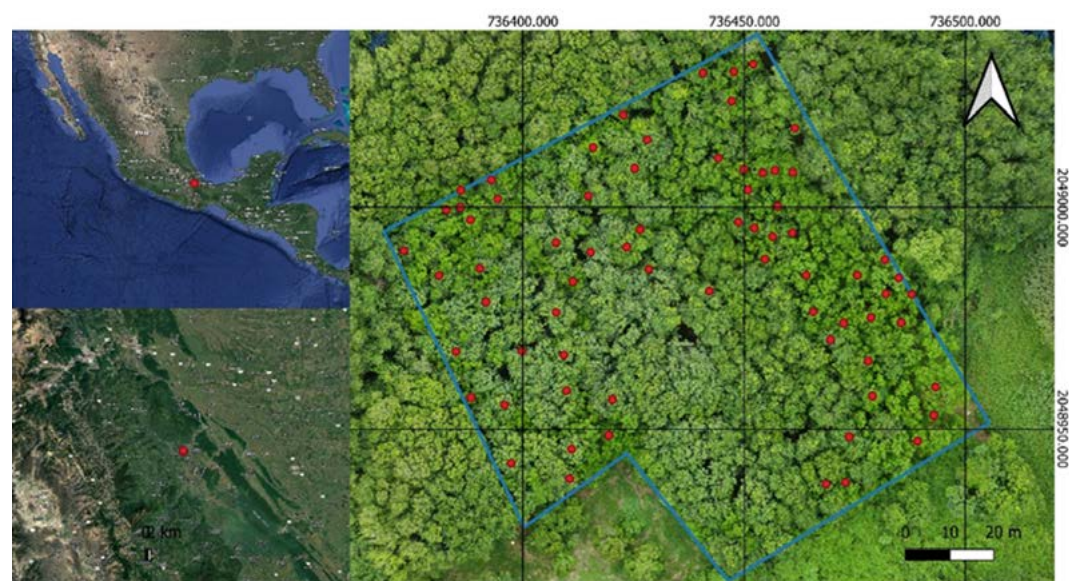
coordinates 18° 31' 5.98" N and 96° 45' 36.20" W, in the “El Palmar” Experimental Field of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), in Tezonapa, Veracruz (Figure 1). The plantation has a systematic design with a spacing of 3×4 m (density of 833 trees/ha), and a composition of 60% *C. odorata* and 40% *H. brasiliensis*.

Acquisition of field data and data using remote sensors

The experimental unit consisted of 70 individuals, 44 *C. odorata* trees and 26 *H. brasiliensis* trees, with $DBH \geq 15$ cm, which were selected by stratified random sampling to ensure the representativeness of the population, with respect to the heterogeneity of the mixed commercial forest plantation.

Reference measurements were performed using traditional methods. DBH was measured with a diameter tape, and total height (TH) was determined using a Suunto® clinometer, taking two reciprocal readings per tree (north-south), averaging them to minimize errors.

Subsequently, the iPhone 15 Pro® LiDAR sensor was used with the Polycam® application (Polycam, Inc., 2025) to generate point clouds and 3D models of each tree in “.las” format. The app utilizes the device’s three cameras as well as the LiDAR sensor (Mokroš *et al.*, 2018; Hernández-Moreno *et al.*, 2024). Each tree was scanned from multiple angles at a distance of 3-5 m, circling the tree in a 360° scan to ensure the quality of the point cloud. It should also be noted that the maximum range of the LiDAR sensor is 5 m (Mokroš *et al.*, 2018; Hernández-Moreno *et al.*, 2024); therefore, a 2 m pole



● Study trees
 ■ Red cedar-rubber plantation polygon
 Google Hybrid

Location of the mixed tropical forest plantation (*Cedrela odorata* – *Hevea brasiliensis*), in the Mexican humid tropics, High Mountains Region

“El Palmar” Experimental Field, INIFAP, Tezonapa, Veracruz.

Figure 1. Location map of the study area, INIFAP, E. F. El Palmar, Tezonapa, Veracruz, Mexico.

was also used to ensure greater scanning range towards the upper parts of the trunk. The device was mounted on a DJI Osmo Mobile 7[®] stabilizer to ensure more stable and higher-quality point cloud scans (Figure 2).

To obtain the photogrammetric data, a protocol of three consecutive flights was executed using the commercial DJI Mini 3 Pro[®] UAV equipped with a 48 MP RGB camera. The flight design was based on validated methodologies for high-precision forest inventories (Luppichini *et al.*, 2025; Ordóñez-Prado *et al.*, 2025) and configured to optimize canopy cover and detail. Each flight consisted of a double grid trajectory (Figure 2), with three different flight heights (45 m, 55 m and 65 m) and with three different camera tilts (90° and oblique at 80° and 65°) between missions, obtaining 371 images for subsequent processing.

This multimodal strategy combines vertical geometry for the accurate generation of elevation models with oblique angles that better capture the lateral structure of the trunk and crown (Luppichini *et al.*, 2025; Ordóñez-Prado *et al.*, 2025). All flights were scheduled with a minimum overlap of 80% frontal and 70% lateral to ensure the construction of a dense point cloud. For high-precision georeferencing, six ground control points (GCPs) were distributed and taken on stable features in the study area using an EFIX 7[®] dual-frequency differential GNSS receiver, achieving horizontal and vertical accuracies with a ground resolution ≥ 2 cm/pixel.

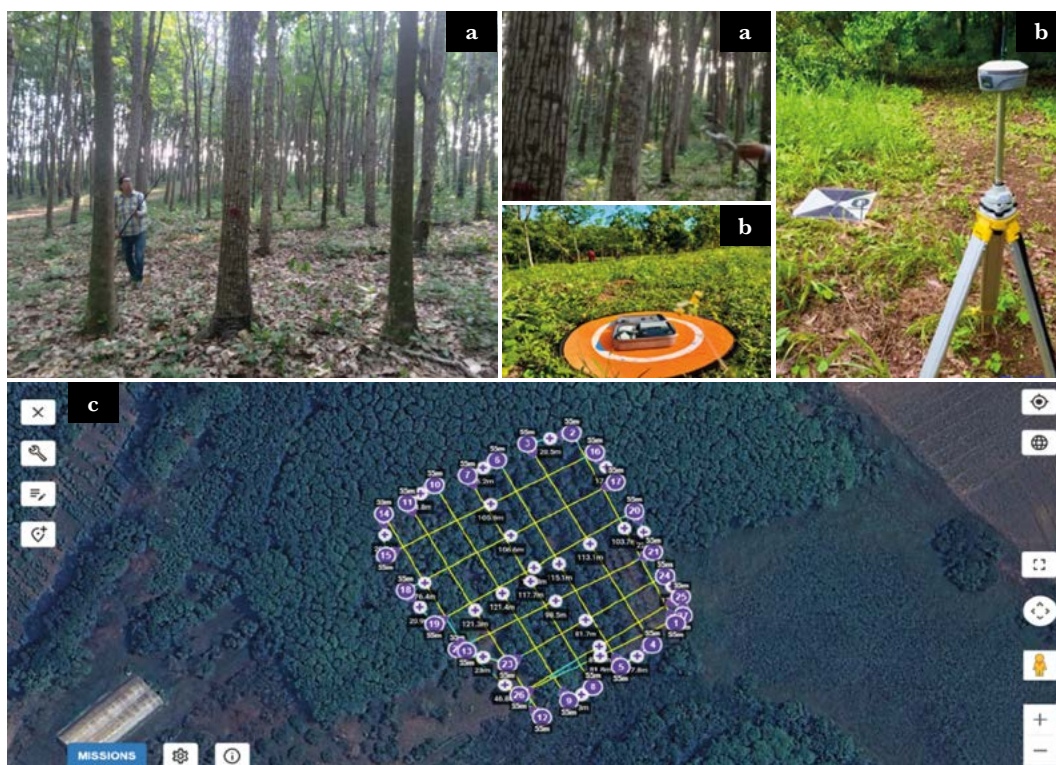


Figure 2. Data acquisition process: a) LiDAR scanning with mobile device, b) UAV flight for photogrammetry with PCT, c) Example of flight mission.

Data processing and statistical analysis

Estimation of DBH

Once the point clouds, individual 3D models of the basal section of the trunk of between 6-7m, of each tree were obtained by scanning with the LiDAR sensor of the iPhone 15 Pro® (Figure 3), the processing was automated by means of a script developed in the R programming environment (R Core Team, 2025).

The algorithm implemented with the lidR (Roussel *et al.*, 2020) and conicfit (Gama & Chernov, 2015) packages executed the following steps for each “.las” file, which represents each of the 70 trees:

- *Filtering of the DBH slice:* Each point cloud was filtered to isolate points between 1.2 and 1.4 meters above ground level, thus defining the slice corresponding to the diameter at breast height (1.3 m). This step discards noise from branches, leaves, or understory vegetation (Figure 4).
- *Geometric fit and diameter calculation:* A least-squares circle fitting algorithm was applied to the filtered point cloud to find the cylinder that best fits the spatial distribution of the points, using Pratt’s method (Gama & Chernov, 2015). The diameter of the fitted circle (multiplied by 100 to convert to centimeters) was recorded as the LiDAR-estimated diameter at point (DBH_LiDAR). Metrics for the quality of the fit were calculated, including the number of points used from the cloud, the center, the radius, and the root mean square error (RMSE) of the points to the fitted circle (Figure 4).
- *Quality control and visualization:* The script incorporated visualization functions that generated, for each tree, a 2D graphic showing the distribution of points in the slice and the adjusted geometric circle, along with a 3D graphic of the point cloud (Figure 4). This visual output allowed for immediate verification of the scan quality and the fit, facilitating the identification of errors caused by slices with too few points or atypical trunk geometry.



Figure 3. Example of individual “.las” point cloud (3D model) of the basal section of the trunk of a *C. odorata* tree (View in the Polycam app).

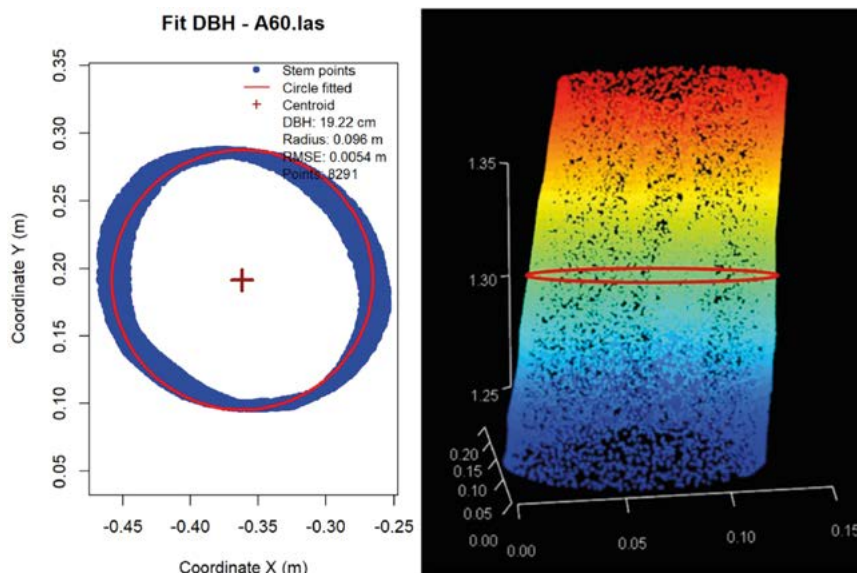


Figure 4. Example of graphical output in R, of the LiDAR processing script for a tree (A60), showing: Left) Plan view (2D) of the points of the trunk slice and the geometric circle adjusted to estimate the DBH. Right) 3D view of the point cloud filtered between 1.2 and 1.4 m in height., for the DBH adjustment.

The quantitative results (DBH_LiDAR, adjustment error, center coordinates) were automatically compiled into a digital table for further statistical analysis.

Photogrammetry with UAV

Photogrammetric processing was performed using Agisoft Metashape[®] software (v. 2.0), following the standard workflow with PCT, to generate a dense point cloud, a high-resolution digital terrain model (DTM), and finally an orthomosaic with a resolution of 1.8 cm/pixel (Luppichini *et al.*, 2025).

The total heights of the 70 trees studied were estimated from the following products: digital terrain model (DTM) and dense point cloud, generated by photogrammetry with UAVs (Figure 5). For this purpose, an automated processing workflow was implemented in R, using the lidR package (Roussel *et al.*, 2020).

First, the point cloud was normalized to remove terrain topography using an irregular triangulation (TIN) algorithm based on points classified as ground, using the CSF filter (Zhang *et al.*, 2016). This normalization allowed us to obtain the heights above ground (Z_b) for all points, *i.e.*, the base of all trees (Figure 6). Subsequently, a canopy height model (CHM) was generated with a spatial resolution of 0.3 m, using the pixel-to-maximum-value method (Zhang *et al.*, 2016). The precise location of each tree within the CHM was determined using the known coordinates of the 70 sampled individuals. For each location, the maximum height from the normalized point cloud (Z_{crown}) was extracted within a defined search radius (buffer=2 m), corresponding to the total height estimated by the UAV (TH_VANT). This procedure ensured that the Z_{crown} measurement would align with the Z_b measurement, for each of the 70 trees, ensuring that both measurements were made on the correct tree (Figure 6).

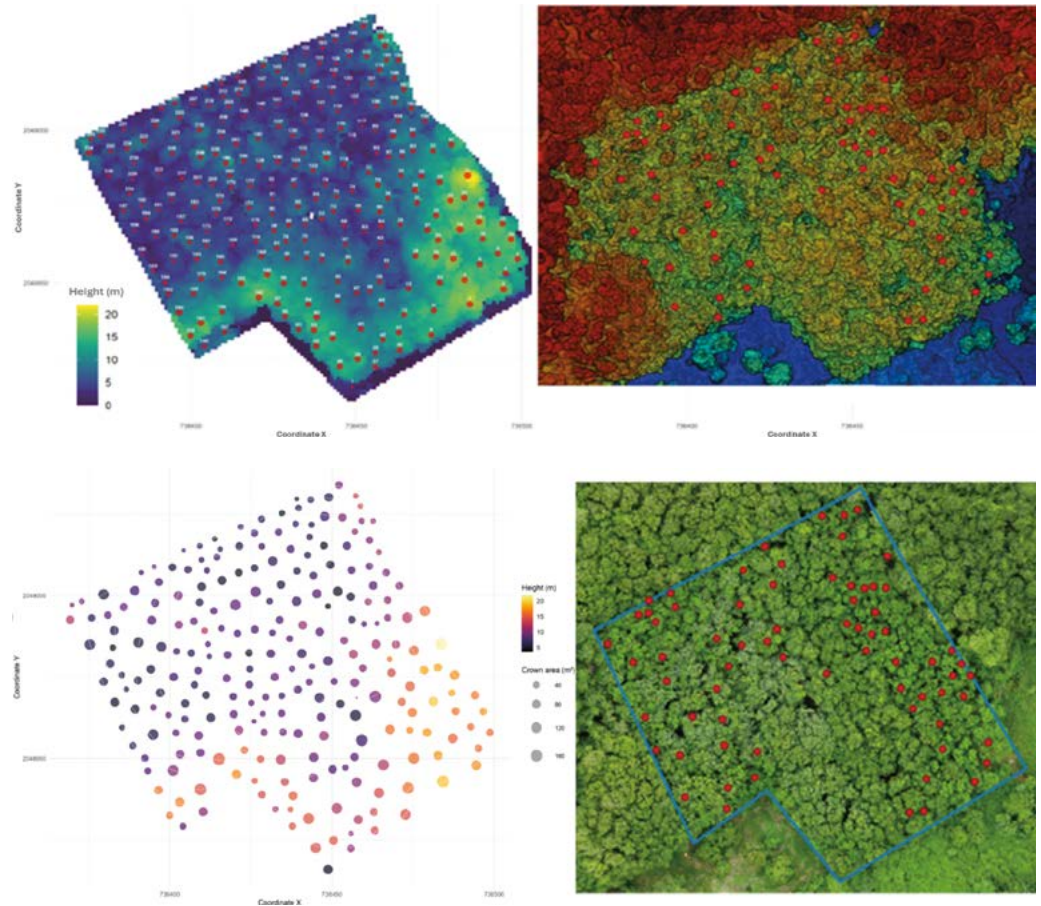


Figure 5. Left) Location and total heights of all trees in the plantation. Right) DTM and point cloud, showing the location of the 70 trees studied.

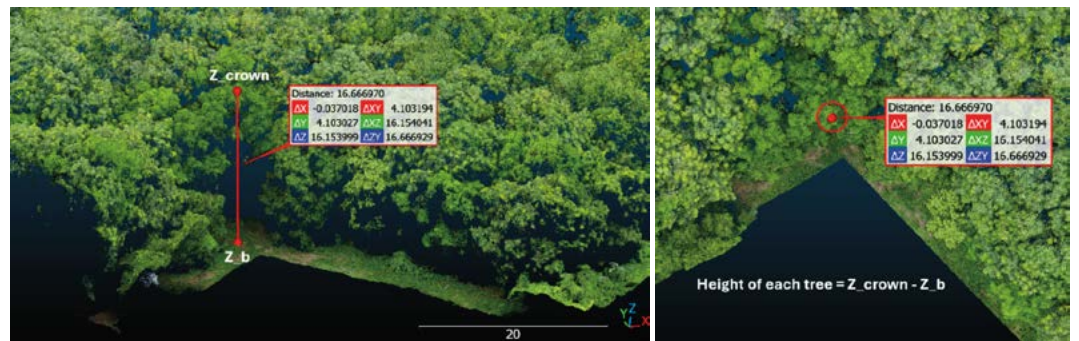


Figure 6. Obtaining the heights of the crown (Z_{crown}) and the base of the tree (Z_b), using point cloud and DTM.

For quality control, advanced visualizations were generated (3D graphs, 2D maps, histograms) and all data were exported to standard formats (.csv, shapefile) for further analysis in GIS.

Statistical analysis

To determine the agreement between traditional methods (tape measure, clinometer) and proximal remote sensors (mobile LiDAR, UAV), a simple linear regression model was used. The coefficient of determination (R^2) and the root mean square error (RMSE) were calculated, the latter being a primary measure of accuracy (White *et al.*, 2016). Additionally, the intraclass correlation coefficient (ICC) was calculated using a two-way model and absolute agreement to measure the reliability and absolute agreement between the methods; values greater than 0.90 indicate high reliability (White *et al.*, 2016).

RESULTS AND DISCUSSION

The results demonstrated a high correlation between traditional and alternative methods (Figures 7 and 8). For DBH, the regression showed an $R^2=0.95$ ($p<0.001$), $RMSE=1.47$ cm, and $ICC=0.964$, indicating excellent agreement. The calibration equation obtained was: $DBH_{\text{traditional}}=1.93+0.963\times DBH_{\text{LiDAR}}$ (Figure 7). For height, $R^2=0.96$ ($p<0.001$), $RMSE=0.64$ m, and $ICC=0.981$ were obtained. The relationship was: $Height_{\text{clinometer}}=0.09+0.998\times TH_{\text{VANT}}$ (Figure 8). Residual analyses confirmed the linearity and homoscedasticity of the relationships, validating the applicability of the calibration models. The graphical analysis of the comparison in DBH measurement by species revealed greater accuracy in *H. brasiliensis*, compared to *C. odorata*, probably attributable to differences in trunk architecture and presence of buttresses in cedar (Figure 7).

This result, attributed to the trunk architecture, aligns with the findings of Lau *et al.* (2019), who documented that structures such as buttresses in some species can introduce errors in LiDAR measurements. However, in this case, the error was minimal, as the trees belong to a plantation that has undergone consistent maintenance and experienced normal growth. Competition with other trees has resulted in straight trunks with no lower branches (Lau *et al.*, 2019).

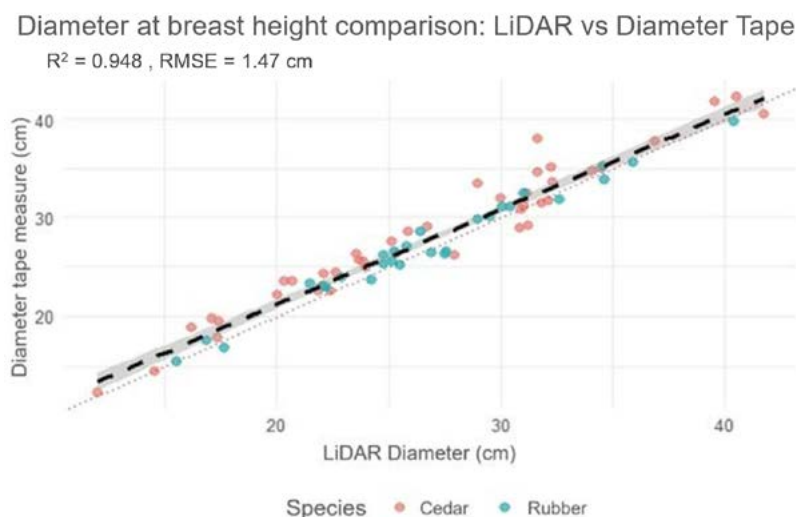


Figure 7. Comparison between DBH measurements taken using remote sensing (LiDAR) and the traditional method (measuring tape).

These results surpass those reported by Wallace *et al.* (2016) in Eucalyptus plantations ($R^2=0.83$), suggesting that mobile LiDAR technology may be technically viable for operational forestry applications. The slight systematic underestimation of LiDAR (Figure 7) is consistent with the literature, attributable to sensor resolution and processing algorithms (Mokroš *et al.*, 2018; Hernández-Moreno *et al.*, 2024).

Regarding heights, we can observe a slight difference in the accuracy of height estimation between species (Figure 8), with greater accuracy observed in *H. brasiliensis*. This can be attributed to ecophysiological and structural factors (Disney, 2019). First, the crown structure, since *C. odorata* typically develops a dense and stratified crown, even wider than *H. brasiliensis*, especially in plantations with wide spacing like the one studied (3×4 m), where competition is lower (Disney, 2019). This foliage density can limit the effective penetration of photogrammetric techniques, making it difficult for the algorithms to accurately identify the true apex of the tree (Ma *et al.*, 2021). In contrast, *H. brasiliensis* in its productive stage usually maintains a more open crown and a more defined upper canopy, facilitating its detection by photogrammetry (Ma *et al.*, 2021). Secondly, the measurement period (May, prior to the rains) and the phenology could have been a critical factor, since *C. odorata*, a deciduous species, had less foliage, in a state of partial defoliation, while *H. brasiliensis* is a species with more persistent foliage (Neuville *et al.*, 2021). According to Neuville *et al.* (2021), this difference in phenology generates variation in height estimation using photogrammetry, because image correlation algorithms are based on the density, color, and texture of the canopy or foliage. Finally, the composition of the plantation (60% *C. odorata*) presents an irregular and complex canopy. This, according to Neuville *et al.* (2021), increases the overall error in canopy modeling for all species, affecting more noticeably the species or species with a more complex architecture.

Remote sensors proved to be valid and accurate tools, showing high agreement, offering advantages in measurement time (reduction of $\sim 67\%$) and data consistency (Table 1), with potential to improve forest inventory in plantations.

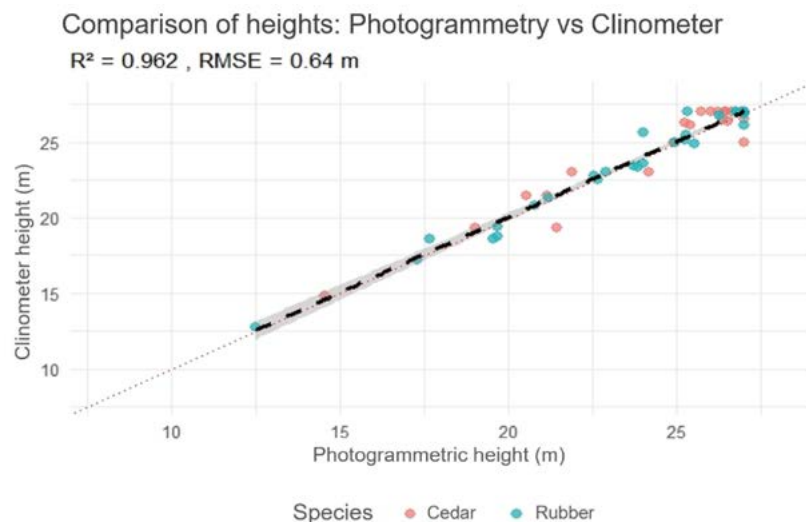


Figure 8. Comparison of total height measurements obtained using a remote sensor (UAV) and the traditional method (clinometer).

Table 1. Time spent (in hours) and number of people, to measure the inventory parameters of 70 trees using iPhone 15 pro[®], UAV, measuring tape and clinometer.

Device	iPhone 15 Pro [®]	UAV	Total	Measuring tape	Clinometer	Total
Measured parameters	DBH	TH	Both	DBH	TH	Both
Number of people	1*	1*	1	2**	2**	2**
Time used (h)	0.45	3.25	3.70	1.75	4.65	6.40
People per hour	0.45	3.25	3.70	3.50	9.30	12.80

UAV: unmanned aerial vehicle; DBH: diameter at breast height; TH: total height.

Photogrammetric technology presented significant advantages in terms of operational safety and visual documentation capacity, eliminating risks associated with field measurements in rugged terrain. Liang *et al.* (2016) reported similar accuracies (RMSE=0.9–1.4 m) in natural forests, validating the robustness of the method in different forest contexts. Likewise, Hernández-Moreno *et al.* (2024) obtained accuracies of $R^2=0.99$ and RMSE=0.657 cm for diameter at breast height (DBH); and $R^2=0.98$ and RMSE=0.369 m for total area (TA). The implementation of remote sensors reduced the measurement time per tree from 8.5 minutes (traditional method) to 3.2 minutes (remote sensors), representing an approximately 67% improvement in time efficiency. This gain translates into a significant reduction in operating costs and the capacity for extensive sampling (Table 1).

The application, adaptation and adoption of the combination of these tools and devices in commercial plantations presents the following advantages (Mokroš *et al.*, 2018; Hernández-Moreno *et al.*, 2024; Zhou *et al.*, 2025):

- *Cost reduction:* A ground-based LiDAR, as well as a high-end UAV, can cost tens of thousands of dollars, not including specialized software and training. In contrast, an iPhone with a LiDAR sensor and a low-cost UAV, plus the use of open-source software, represent an investment of less than a third of that; they have the capacity to generate dense point clouds, which are operationally useful for obtaining reliable measurements, and are also accessible to producers, foresters, technicians, students, academics, and even small and medium-sized businesses.
- *Operational efficiency and scalability:* These low-cost technologies will allow for faster, automated data collection of each tree in the plantation, which can then be stored digitally. For example, a single operator with a UAV can cover tens of hectares in one flight, and a person with an iPhone can measure hundreds of trees per day, compared to traditional methods that require more than one operator (Table 1).
- *Accuracy:* Unlike traditional estimates, these technologies generate a digital, georeferenced, quantifiable, and, depending on the frequency, permanent record of the plantation, which can be used for continuous monitoring. This will allow for more accurate calculations of volume, biomass, and carbon (*e.g.*, applicable to carbon market management).

All of the above will facilitate forest inventories for silvicultural management in tropical forest plantations. However, for the applicability and adoption of these methodologies to be reliable for foresters, technicians, or decision-makers, rigorous validation is necessary, such as that of the present study, which provides statistically sound evidence necessary for decision-makers in commercial plantations to confidently and certainly integrate these low-cost tools.

CONCLUSIONS

The low-cost proximal remote sensors evaluated demonstrated precision and accuracy comparable to traditional methods, with substantial advantages in operational efficiency and data quality. The iPhone 15 Pro[®] LiDAR sensor is a viable tool for diameter measurement in commercial forest inventories, while photogrammetry with control points provides reliable height estimates. The combination of sensors enabled the automatic generation of georeferenced digital databases, eliminating transcription errors and facilitating advanced spatial analysis. This feature is particularly valuable for long-term monitoring in commercial forest plantations. Validation of these methods under different site conditions and with different tree species is recommended, as well as the application of artificial intelligence techniques for the automated processing of LiDAR and photogrammetric data together.

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Flower induction in MD-2 pineapple using growth regulators and leaf pruning to obtain off-season fruit

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ABSTRACT

Objective: To prevent natural flowering of pineapple using concentrations of CPA and pruning levels, as well as to determine the flowering efficiency of MD-2 pineapple covered with shade netting, with the application of ethylene, in order to obtain off-season fruit.

Design/methodology/approach: The experiment to prevent flowering was a randomized block design with four replications, in split plots; a: 0, 75, and 128 mg L⁻¹ of 2,3-chlorophenoxypropionic acid (CPA) and; b: 0, 30, and 50% pruning; treatments were applied on November 6, 2022. Flowering percentage was measured in February and April 2022. The experiment on floral induction consisted of: a) plants covered with shade netting and b) plants in open air; 18 samplings were performed with 20 plants; inflorescences were counted and analyzed using Student's t-test ($\alpha=0.05$); the per-hectare dose was applied three times with 2 kg of ethylene with 20 kg of activated charcoal.

Results: Pruning decreased natural flowering, and the response improved when combined with 75 mg L⁻¹ of CPA. At 128 mg L⁻¹ of CPA, excellent results were obtained, but the dose can be reduced to achieve the same effect when combined with pruning. The treatment without shade netting had 94% floral induction efficiency, while the one covered with shade netting had 49%.

Study limitations/implications: These results can be applied in Veracruz and Oaxaca, Mexico, in plantations with plastic mulch.

Findings/conclusions: Pruning prevented flowering, and its effect was enhanced with the lower concentration of CPA; additionally, shade netting reduced flowering when ethylene was applied during periods of high temperatures.

Keywords: *Ananas comosus*, floral inhibition, floral differentiation, shade netting.

Citation: Rebolledo-Martínez, A., Rebolledo-García, R. L., López-Vázquez, M. E., Peralta-Antonio, N., Murillo-Capetillo R., & Nataren-Velázquez, J. (2026). Flower induction in MD-2 pineapple using growth regulators and leaf pruning to obtain off-season fruit. *Agro Productividad*. <https://doi.org/10.32854/dn96ja76>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: January 13, 2026.

Accepted: April 24, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 15-25.

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INTRODUCTION

Pineapple (*Ananas comosus* var. *comosus*) is among the top five tropical fruit crops in the world, and in Mexico, 22,000 ha are established annually with a production of 1,292,000 tons of fruit (SIAP, 2025). The crop generates around 35,000 annual jobs, both direct and indirect, and an economic output of 34 billion pesos. Eighty percent of the pineapple in



Mexico corresponds to the MD-2 hybrid, 15% to the Cayena Lisa cultivar, and the rest to other cultivars such as 'Champaka F153', 'Cabezona', and some landraces such as 'Coita' from Chiapas, 'Criolla de Guerrero', and 'Criolla de Nayarit', among others (Rebolledo-Martínez *et al.*, 2025).

This species is one of the few that respond to exogenous ethylene application to stimulate floral differentiation, allowing harvest scheduling throughout the year (Bartholomew *et al.*, 2003; Cunha, 2005). If flowering occurs naturally, it is promoted by factors that increase ethylene production, including the physiological and nutritional status of the plant, varietal sensitivity, photoperiod, temperature, radiation, and stress, among others (Bartholomew, 2018; Kuan *et al.*, 2005; Rabie *et al.*, 2011).

This situation makes flowering one of the main problems for producers in pineapple-growing regions worldwide, mainly caused by short days, long nights, and low temperatures during winter, provided that the plant has sufficient size and age (Rebolledo *et al.*, 2016; Farid *et al.*, 2015).

Mexico is no exception; the pineapple-growing region is located around 18° north latitude, where winter meets several of these factors, promoting the occurrence of untimely natural flowering, which becomes more pronounced at higher latitudes.

From November to February, minimum temperatures generally reach around 17 °C; this causes approximately 25% of the production to be concentrated in June and July. This overproduction leads to a market gap in which fruit prices drop significantly, resulting in decreased profitability for producers. On the other hand, a period of fruit scarcity is observed during September and October, representing a window of opportunity for producers to obtain the best pineapple prices (Rebolledo *et al.*, 2016; Uriza *et al.*, 2018).

To attempt to minimize this problem, the effect of different inhibitors that block the ethylene biosynthesis pathway and, consequently, flowering has been evaluated (Sanford and Bartholomew, 1981; IRFA, 1987; Scott, 1992; Rebolledo *et al.*, 1997); including research using genetic engineering to identify promising transgenic lines that prevent natural flowering inhibition (Trusov and Botella, 2006). Evidence shows that silver nitrate, at concentrations ranging from 30 to 180 mg L⁻¹, divided into six applications at 15-day intervals and starting two months before floral differentiation, reduces or delays flowering; the same applies to silver thiosulfate, which, although more economical, has phytotoxic effects on the crop (IRFA, 1987). However, in Mexico, in 'Cayena Lisa', silver nitrate only achieved 47% floral inhibition, while gibberellic acid and bromacil had no inhibitory effect (Rebolledo *et al.*, 1998). In another study, Scott (1992) evaluated CPA (2,3-chlorophenoxypropionic acid) and Paclobutrazol at various doses, where the best treatments of both products resulted in only 8.2% and 28% natural flowering, respectively, while the untreated controls reached between 48% and 55% flowering. In Mexico, in 'Cayena Lisa', with 100 mg L⁻¹ of active ingredient CPA 8%, divided into three equal applications starting at the beginning of October and spaced 15 days apart, flowering was inhibited up to 90% without causing plant burns, with more favorable responses in higher planting densities, younger and better-nourished plants; a single application of 100 mL L⁻¹ resulted in 32% plant mortality due to burns (Rebolledo *et al.*, 1997; Rebolledo *et al.*, 2000).

CPA was also evaluated in the MD-2 pineapple hybrid, and it was found that concentrations of 130 mg L^{-1} with four equal applications spaced 15 days apart, starting at the end of October, in plants weighing 2.02 kg at the time of growth regulator application, inhibited flowering by more than 95%, compared to 19% in the control; the 130 mg L^{-1} single-application treatment had the highest percentage (100%) of non-flowering plants, but resulted in 16% of plants with burns caused by CPA, which is undesirable (Rebolledo *et al.*, 2021).

Recently, in Mexico, it has been discovered that horizontal leaf pruning has a positive effect in preventing natural flowering in pineapple (Rebolledo *et al.*, 2022), where it was found that 30% pruning in MD-2 achieved 74% floral inhibition; however, when this pruning level was combined with half of the recommended CPA dose (66 mg L^{-1}) in two applications, floral inhibition was very close to 100%, compared to 16% in the control, in plants weighing 1.98 kg. That is, pruning allowed a 50% reduction of CPA, and it is also an environmentally friendly practice.

López *et al.* (2023) found that 30% pruning combined with the application of the inhibitor product aminovinylglycine hydrochloride (AVG) reduced natural flowering by 80% compared to the control; while, compared to the treatment without pruning plus AVG, natural flowering was reduced by 49%. Likewise, Rebolledo-García *et al.* (2024) obtained conclusive results, close to 95% flowering control, compared to 16% in the control, with pruning levels of 60% and 90% in a plastic mulch planting system, far superior to 30% pruning, when pruning was performed at the end of October in plants weighing 1.7 kg; plants pruned at 90%, although they did not flower, experienced delayed development, which did not occur in 60% pruning. The same authors achieved values close to 100% with a single application of 128 mg L^{-1} CPA, without causing leaf burns.

On the other hand, plants that do not flower naturally must reach an adequate weight (2.5-3.2 kg) to induce flowering in March, April, and early May in order to obtain off-season fruit from late August to October. Consequently, flowering induction in pineapple is achieved through the application of growth regulators that act on ethylene synthesis, the compound responsible for floral differentiation (Rebolledo *et al.*, 2016). Floral induction in pineapple using calcium carbide, Ethrel, and ethylene gas is a common practice in nearly all plantations in Mexico. Ethylene gas has been the most widely used in recent years due to its high efficiency and the ability to be applied mechanically; this practice is carried out using 2 kg ha^{-1} of ethylene gas in two applications, mixed with 20 kg of activated charcoal per 3,000 liters of water (Rebolledo *et al.*, 2016).

With the use of 50/50 black shade netting placed over the pineapple plants to prevent radiation damage, many producers avoid removing the netting to apply the flowering inducer, expecting the same results as when the product is applied directly to the plant (Uriza *et al.*, 2018); however, not removing the netting could reduce flowering efficiency, depending on the conditions at the time of application, plant weight and age, nutrition, ambient temperature, among others (Bartholomew and Sanewski, 2018).

Based on the above, the objective was to prevent natural flowering of pineapple using CPA concentrations and pruning levels, as well as to determine the flowering efficiency of

MD-2 pineapple covered with shade netting with ethylene application, in order to obtain off-season fruit.

MATERIALS AND METHODS

Experiment 1: Inhibition of natural flowering: The experiment was established in the pineapple-growing region of Bajo Papaloapan, in the municipality of Juan Rodríguez Clara, in the locality Ejido 5 de Mayo (17° 50' 22.9" N, 95° 22' 50.6" W at 190 m above sea level); the coldest period is from December to February, with an average temperature of 19 °C, and the hottest period is from April to June, with an average maximum temperature of 37 °C. The climate of the area is classified as warm subhumid (Aw0) with a rainy season from June to October and a dry season from January to May. The soil is a dystric cambisol, predominantly sandy-loam in texture, poor in organic matter (1.54%) and nutrients, with a pH of 5.2 and electrical conductivity of 0.06 dS/m.

The plantation was established in February 2021 with MD-2 pineapple propagative material called “gallo” or slip, weighing 500 g, at a planting density of 41,000 plants per hectare, in raised beds of 1.2 m apart from center to center, with two rows of pineapple per bed and 40 cm between plants. Prior to establishment, the land was cleared, left fallow, harrowed twice with a heavy harrow, and the beds were constructed; immediately afterward, the soil was covered with black plastic film, gauge 150. Agronomic management of the crop was carried out according to the technical recommendations for the region of this study (Rebolledo *et al.*, 2016).

The experimental design was a randomized block design with four replications, and treatments arranged in split plots; for factor a, the levels were concentrations of 2,3-chlorophenoxypropionic acid (CPA 8%) applied in a single application: 0, 75, and 128 mg L⁻¹, while for factor b, the levels were horizontal leaf pruning: 0, 30, and 50% of plant height. The experimental unit consisted of four pineapple rows of 6 m (14.4 m²).

The treatments were applied on November 16, 2021, when the plants weighed an average of 2.3 kg. Leaf pruning was performed with a machete, and CPA was applied using manual backpack sprayers at a dose of 50 mL of solution, spraying the entire plant. The percentage of natural flowering and the percentage of non-flowering plants were measured on February 2 and April 19, 2022. Fruits from natural flowering matured in June, while plants that did not flower naturally were induced with ethylene in May and harvested in October 2022. Analysis of variance ($\alpha=0.05$) and Tukey's multiple range tests ($p\leq 0.05$) were performed using the Statistical Analysis System (SAS), while figures and statistical regression models were generated using SigmaPlot version 12.0.

Experiment 2: Flowering induction was conducted in the pineapple-growing area known as Los Robles, in the municipality of Medellín, Veracruz, Mexico (18° 56' 13" N; 96° 11' 38" W). Average minimum and maximum temperatures ranged between 23 °C and 33 °C, respectively, with temperatures exceeding 35 °C recorded during May, June, and July; the rainy season began in early June. The soil had a pH of 5.7, 2.14% organic matter, sandy-loam texture with 13% clay, 73% sand, and 14% silt. Concentrations of nitrate N, P-PO₄³⁻, S-SO₄²⁻, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, Zn²⁺, Cu²⁺, Mn²⁺, and B³⁺ were 64.0, 3.0, 23.7, 135.0, 2280.0, 610.0, 46.7, 1.6, 1.1, 7.9, and 0.26 ppm, respectively.

The study was conducted in an MD-2 pineapple plantation with 600 g slips in June 2021, planted in two rows per bed at a density of 55,000 plants per hectare, with 100% of the soil previously covered with black plastic film, gauge 150. In April 2022, to prevent sunburn damage, 50/50 black shade netting was placed over the plants. At the time of floral induction treatment application on July 11 at 22:00 h, shade netting was removed from part of the plantation (0.5 ha), while the rest of the crop (2 ha) remained covered. The dose used for floral induction in both treatments was 2 kg of ethylene gas per-hectare per application, mixed with 20 kg of activated charcoal in 4,000 L of water.

Three applications were carried out, spaced two days apart. Fifty days after the treatment for floral differentiation, 18 samplings were conducted for each treatment, with 20 plants per sampling, counting the number of inflorescences per plant, which allowed determination of the percentage of pineapple flowering obtained in each treatment. Plant height and weight were also evaluated. Statistical analysis was performed using Student's t-test for independent samples at a 95% confidence interval, carried out with SigmaPlot version 12.0.

RESULTS AND DISCUSSION

Experiment 1. Floral inhibition

Table 1 presents the results of flowering for each of the evaluated treatments on February 2. Statistically significant differences ($\alpha=0.05$) were observed for each level of both factors, but the same was not true for the interactions. CPA concentrations were statistically equal among themselves and higher than the control, which by that date already had 7.6% of plants flowering; pruning levels were also statistically equal, but different from the control, which showed 6.3% of plants with flowering.

At these dates, the initiation of the floral differentiation process is just beginning, occurring approximately 40 days before the emergence of floral primordia, which took place at the end of December; however, environmental conditions will cause this phenomenon to continue in the rest of the plants (Bartholomew, 2018), according to the evaluated treatments, as shown in Figure 1. By April 19, the pineapple plants had completed their winter natural flowering process, with more than 90% flowering, and only the inhibitory treatments showed their response to this phenomenon.

On this sampling date, April 19, analysis of variance showed statistically significant differences in both factors and their interactions ($\alpha=0.05$). CPA concentrations of 128 and

Table 1. Percentage of non-flowering MD-2 pineapple plants under different CPA concentrations and leaf pruning levels on February 2. Different letters indicate statistically significant differences.

CPA concentration (mg L ⁻¹)	Value of leaf pruning (%)			
	30	50	0	Mean
128	100	100	100	100 a
75	100	100	96.6	98.7 ab
0	97.72	95.15	84.4	92.4 b
Mean	99.2 a	98.38 a	93.69 b	97.1

CV (%)=4.73; Tukey \leq 0.05.

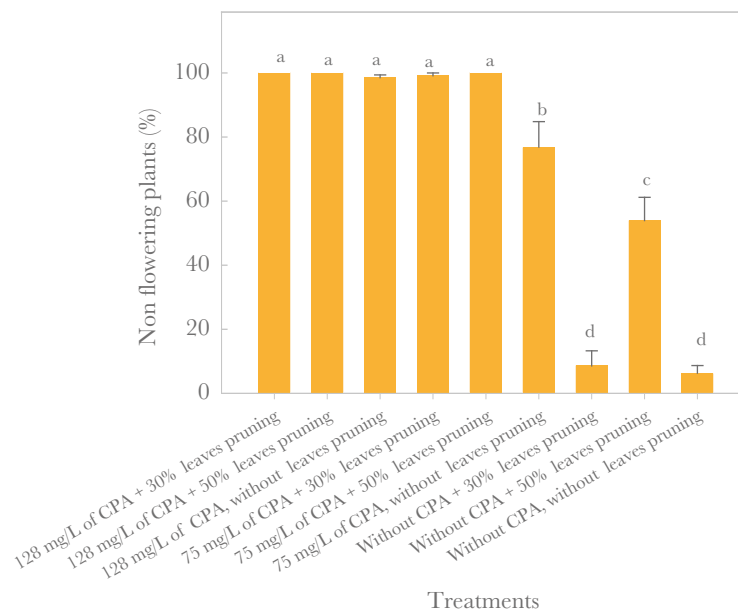


Figure 1. Percentage of non-flowering MD-2 pineapple plants under different CPA concentrations at three leaf pruning levels. Different letters indicate statistical differences between treatments (Tukey ≤ 0.05) within each pruning level at each CPA concentration. Bars represent \pm standard error. Coefficient of variation = 11.09%.

75 mg L⁻¹ were statistically equal and differed from the control, which had 22.9% non-flowering plants, in contrast with 92% and 99% obtained with 75 and 128 mg L⁻¹ CPA, respectively. Regarding factor b, the three pruning levels were statistically different from each other, with percentages of non-flowering plants of 60.5, 84.6, and 69.2 for the 0%, 50%, and 30% pruning treatments, respectively (Table 2).

Interaction values are presented in Figure 1; the CPA concentration of 128 mg L⁻¹ showed statistically equal values ($\alpha=0.05$) at the three pruning levels; that is, with this applied dose, this practice is not required. CPA at 75 mg L⁻¹ is more efficient when combined with 30% or 50% pruning, since 99% non-flowering plants were achieved, compared to 76.8% obtained without the CPA/pruning combination. The control without CPA within the pruning levels showed that, statistically ($\alpha=0.05$), the best treatment was 50% pruning with 54% non-flowering plants, while in the other two levels of this factor the values were 7.4% on average. Regarding the results for CPA concentration and pruning percentage, through

Table 2. Percentage of non-flowering MD-2 pineapple plants at different CPA concentration levels with the average of pruning levels, and percentage of natural flowering at different pruning levels with the average of CPA concentration levels, in the April 19 sampling.

CPA concentration (mg L ⁻¹)	Value of non-flowering plants (%)	Value of leaf pruning (%)	Value of non-flowering plants (%)
128	99.50 a	30	69.2 a
75	91.98 a	50	84.6 b
0	22.92 b	0	60.5 c

Different letters indicate statistically significant differences (Tukey ≤ 0.05).

regression, each of these factors was fitted to a second-order ($r^2=0.9570$) and fourth-order polynomial ($r^2=0.9570$), respectively. Figure 2 shows that, as CPA concentration increases, the response increases; however, for pruning levels, the effect begins after 40% pruning (Figure 3).

These results are closely related to those obtained in other studies: regarding CPA, it was applied in a single dose and no plant damage was observed, as reported by Rebolledo *et al.* (1997) in ‘Cayena Lisa’ with 100 mg L^{-1} and Rebolledo *et al.* (2021) with 128 mg L^{-1} in ‘MD-2’; although these authors worked in these experiments only with the vegetative material called ‘clavo’ or ‘stem shoot’ (buds that emerge from the stem) with plantations established at the beginning of summer, whereas in this study suckers called ‘gallo’ or ‘slip’ (buds that emerge from the peduncle) planted in February were used; that is, plants of

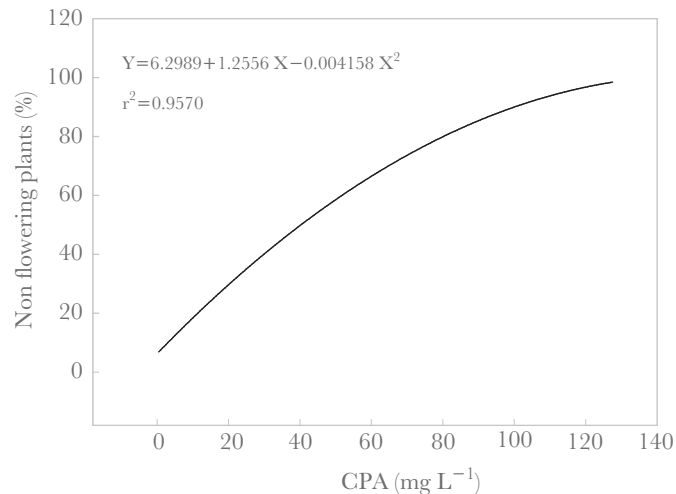


Figure 2. Second-degree polynomial line fit and regression equation between CPA concentration and the percentage of non-flowering MD-2 pine-apple plants.

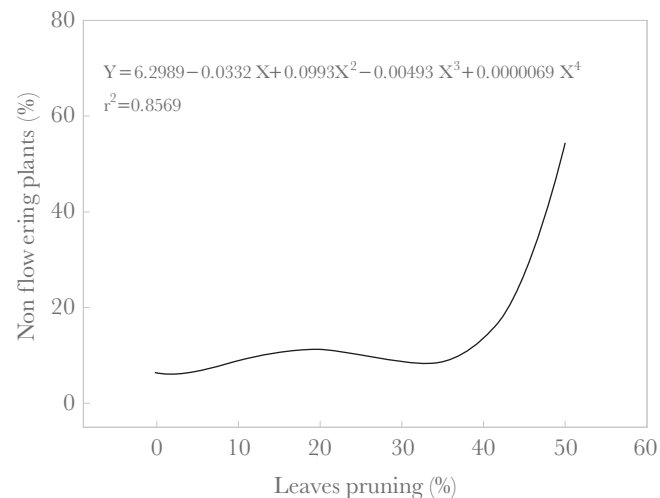


Figure 3. Fourth-degree polynomial line fit and regression equation between pruning levels and the percentage of non-flowering MD-2 pineapple plants.

greater age and size (2.3 kg). However, Rebolledo-García *et al.* (2024) obtained the same results as in this study when using 128 mg L^{-1} in a single dose, without finding leaf burns, in plantations established in July with ‘clavos’.

Pruning had an important effect, since at the 50% level flowering was significantly reduced; if pruning is more severe, the response improves, as it has been found that 90% pruning has a 100% effect but delays growth by approximately three months; likewise, 60% pruning has been found to achieve more than 90% reduction in flowering (Rebolledo-García *et al.*, 2024). In this study, 50% pruning in combination with CPA at 75 mg L^{-1} produced the best result, coinciding with Rebolledo *et al.* (2022) and Rebolledo-García *et al.* (2024), who found that CPA doses can be reduced by 50% when pruning is applied, which implies savings in application and less environmental damage due to agrochemical use.

Experiment 2

There was a statistically significant difference between the evaluated treatments for floral induction in pineapple. Better results were obtained when ethylene gas was applied to plants without shade netting compared to those that had shade netting (Figure 4).

Keeping the plant covered with 50/50 shade netting reduces the efficiency of ethylene gas entry into the plant, since much of the solution first comes into contact with the netting; secondly, the application period is the most difficult for the plant to undergo organogenic changes toward flowering, due to the occurrence of the longest days of the year and high temperatures that favor vegetative growth and lower ethylene production, as mentioned by Bartholomew and Sanewski (2018).

For the plant height variable, the treatment with shade netting was statistically higher (Figure 5), which is explained by the reduction of solar radiation in that environment (Uriza *et al.*, 2018); shade netting produces shading that stimulates apical meristem growth and the

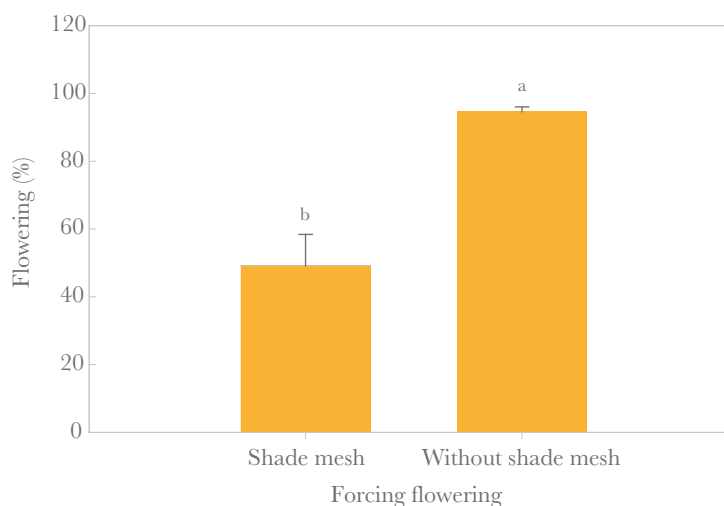


Figure 4. Percentage of flowering of MD-2 pineapple in two floral induction treatments applied to plants with shade netting and without netting. Bars, \pm standard error; different letters represent statistically significant differences ($p < 0.01$).

plant continues its vegetative growth, although this could be compensated by the reduction in temperature that occurs in the controlled environment created by the netting; however, under the conditions of this study this phenomenon was not observed. On the other hand, plant weight may have influenced flowering efficiency (Figure 6), since large plants tend to respond less to floral induction (Rebolledo *et al.*, 2016); however, no statistically significant differences were observed between treatments.

Finally, when the plant is highly developed and there is soil moisture at field capacity due to the beginning of the rainy season, and flowering induction is intended mainly during June and July, shade netting should be removed before applying the floral induction treatment.

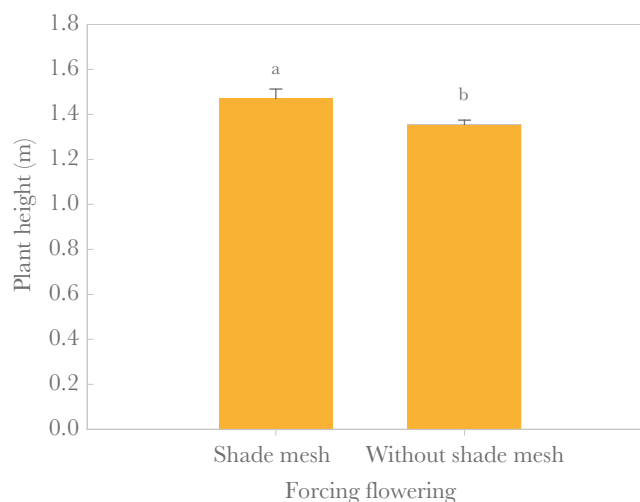


Figure 5. Plant height of MD-2 pineapple in two floral induction treatments applied to plants with shade netting and without netting. Bars, \pm standard error; different letters represent statistically significant differences; $p=0.049$.

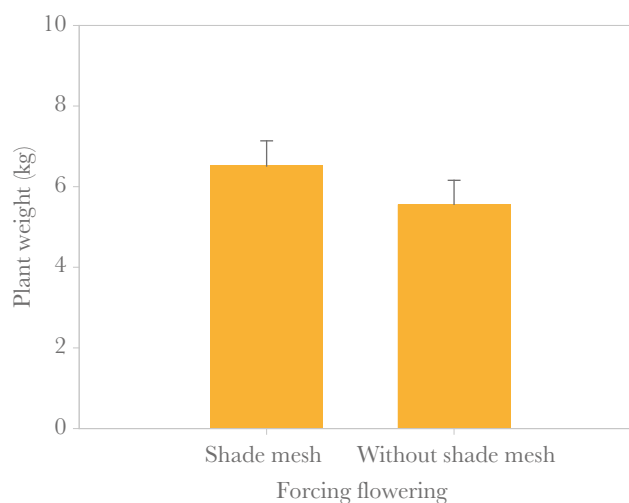


Figure 6. Plant weight of MD-2 pineapple in two floral induction treatments applied to plants with shade netting and without netting. Bars, \pm standard error; no statistically significant difference was observed, $p = 0.312$.

CONCLUSIONS

Pruning significantly reduced natural flowering, and the response improved when it was combined with the application of 75 mg L⁻¹ CPA. The 128 mg L⁻¹ CPA dose in a single application, although it produced good results, can be reduced by half to achieve the same effect when combined with pruning. When ethylene gas was applied in the treatment without shade netting, 94% floral induction efficiency was obtained, while in the treatment with shade netting only 49% efficiency was achieved. By inhibiting flowering and subsequently forcing it, it is possible to obtain off-season production.

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Physiology of the recalcitrant seed of chayote (*Sechium edule* [Jacq.] Sw.) and its role in postharvest fruit deterioration

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ABSTRACT

Objective: To integrate existing knowledge on the physiology of recalcitrant chayote seeds and their interaction with fruit tissues, highlighting their impact on conservation alternatives and postharvest life.

Methodology: A systematic review of the scientific literature related to the recalcitrant seed of *Sechium edule* was conducted, focusing on studies published over the last 25 years. The review addressed fruit and seed biology and physiology, conservation practices in germplasm banks, and postharvest management alternatives for reducing premature seed germination.

Results: Chayote has a recalcitrant seed characterized by high sensitivity to dehydration and low temperatures, which hinders conventional storage. This characteristic represents a major challenge for conservation programs, making the maintenance of living plant collections essential for preserving the biodiversity of the genus *Sechium*. Moreover, this same trait reduces the postharvest life of the fruit. Therefore, postharvest treatments such as wax coatings, 1-MCP application, and cold storage are viable alternatives for extending shelf life. The knowledge generated also provides valuable information for designing more efficient and sustainable postharvest strategies and contributes to the conservation and appropriate use of species with recalcitrant seeds, which cannot be preserved using conventional seed storage methods.

Implications: The physiology of recalcitrant chayote seeds has direct implications for postharvest fruit deterioration because their high metabolic activity promotes premature germination and vivipary, while also hindering long-term conservation.

Conclusions: The recalcitrant seed is not only a challenge for long-term conservation but also the main physiological determinant of postharvest deterioration in chayote fruit. Despite these limitations, viable conservation alternatives are available, including *in vivo*, *in situ*, and *in vitro* approaches.

Keywords: recalcitrant seed, germplasm conservation, germplasm banks, postharvest management, fruit-seed coupling.

Citation: Ramírez-Rodas, Y. C., Gayosso-Rosales, L. Y., Arévalo-Galarza, Ma. de L., Cadena-Iñiguez, J. & Riviello-Flores, M. de la L. (2026). Physiology of the recalcitrant seed of chayote (*Sechium edule* [Jacq.] Sw.) and its role in postharvest fruit deterioration. *Agro Productividad*. <https://doi.org/10.32854/2yth6z73>

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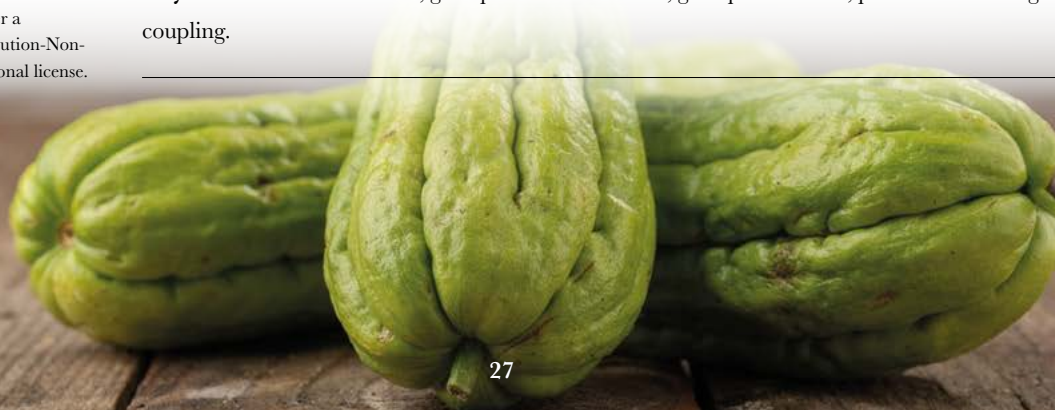
Received: January 23, 2026.

Accepted: April 17, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 27-42 .

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INTRODUCTION

Seeds play a fundamental role in plant reproduction and the preservation of biodiversity; moreover, they are essential for food systems. However, not all seeds can be stored in the same way. Some seeds, after reaching physiological maturity and a low moisture content, can be stored for long periods without losing viability. These seeds, known as orthodox seeds, have the ability to enter a state of dormancy, during which they remain viable until favorable conditions for germination such as water, light, and oxygen are present. This period of dormancy involves complex physiological, genetic, and environmental processes.

In contrast, recalcitrant seeds are characterized by their high water content at the time of dispersal and their inability to tolerate desiccation or low temperatures, which prevents long-term storage, unlike orthodox seeds (Bewley *et al.*, 2013; Walters *et al.*, 2013). This type of seed is common in tropical, subtropical, and forest species, such as avocado (*Persea americana*), cacao (*Theobroma cacao*), mango (*Mangifera indica*), and oaks (*Quercus* spp.), among others (Normah *et al.*, 2019; Priyanka *et al.*, 2021). The physiological characteristics of these seeds pose significant challenges for long-term ex situ conservation and postharvest management. In this context, chayote seeds (*Sechium edule*) are recalcitrant, which limits their long-term conservation. For this reason, chayote plants are mostly conserved using in vivo methods, which allow long-term preservation of genetic diversity (Barrera-Guzmán *et al.*, 2021).

In addition, seed type directly influences the postharvest management of the endocarpic fruit because, once the seed reaches physiological maturity, germination begins inside the fruit. This process can occur as early as 13 days after harvest, coinciding with the period when the fruits are being marketed. This phenomenon not only negatively affects the fruit's appearance but also facilitates pathogen entry, increases moisture loss, and significantly reduces shelf life. The objective of this review is to integrate existing knowledge on the physiology of recalcitrant chayote seeds and their interaction with fruit tissue, highlighting their impact on conservation strategies and postharvest life.

MATERIALS AND METHODS

A systematic review of the scientific literature related to *Sechium edule* seeds was conducted, focusing on publications from the last 25 years. The databases consulted were Google Scholar, Scopus, SciELO, and Redalyc. Studies addressing fruit and seed biology and physiology, germplasm conservation methods, conservation practices in germplasm banks, and alternative approaches to postharvest fruit management were prioritized. The exclusion criteria included articles published outside the established timeframe, opinion papers, essays, and theses (Figure 1).

RESULTS

Physiological basis of recalcitrance in chayote seeds

Characteristics of orthodox and recalcitrant seeds

Seeds are the means by which plants ensure their survival; however, successful germination depends on their tolerance to desiccation. The terms *orthodox* and *recalcitrant* began to be used in the 1970s to describe seed tolerance to desiccation and low temperatures

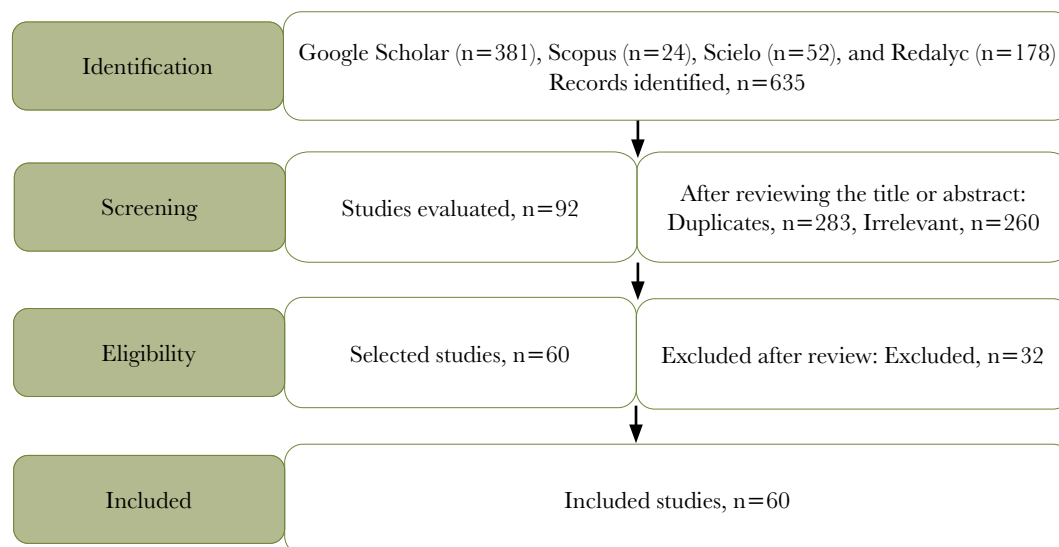


Figure 1. PRISMA flow diagram of the information selection process.

during conservation. Based on these characteristics, seeds are classified into three groups: orthodox, intermediate, and recalcitrant (Barbedo *et al.*, 2013; Bewley *et al.*, 2013; Walters *et al.*, 2013) (Table 1).

Orthodox seeds can reach equilibrium and survive under limited moisture conditions, with moisture contents as low as 5%. Desiccation tolerance is acquired progressively during seed maturation. Once seeds have completed maturation, including embryo morphogenesis and reserve accumulation, they enter a period of dormancy characterized by minimal metabolic activity, which is maintained until favorable conditions allow germination (Berjak and Pammenter, 2013). Intermediate seeds are partially tolerant to desiccation; however, they lose viability when their moisture content ranges between 10% and 12% (Tweddle *et al.*, 2003).

In contrast, recalcitrant seeds are generally found in tropical species, are larger than 1 cm, have high water content, and do not tolerate desiccation at moisture levels of 30%-50%. This sensitivity may be related to low levels of protective proteins involved in preventing damage during desiccation. Because they undergo only partial dehydration, these seeds maintain active metabolism and continue developing by initiating germination after

Table 1. Plant species with orthodox, intermediate, and recalcitrant seeds.

Seed Types	Plant Species	Reference
Orthodox	Maize (<i>Zea mays</i>), oat (<i>Avena sativa</i>), wheat (<i>Triticum aestivum</i>), common bean (<i>Phaseolus vulgaris</i>), cucumber (<i>Cucumis sativus</i>), among others.	Solberg <i>et al.</i> (2020)
Intermediate	Oil palm (<i>Elaeis guineensis</i>), papaya (<i>Carica papaya</i>), coffee (<i>Coffea</i> sp.), citrus (<i>Citrus</i> sp.), among others.	Sharmela <i>et al.</i> (2019); Ballesteros <i>et al.</i> (2021)
Recalcitrant	Cacao (<i>Theobroma cacao</i>), mango (<i>Mangifera indica</i>), rambutan (<i>Nephelium lappaceum</i>), mangosteen (<i>Garcinia mangostana</i>), chayote (<i>Sechium</i> spp.), among others.	Normah <i>et al.</i> (2019); Priyanka <i>et al.</i> (2021)

reaching physiological maturity. This characteristic hinders long-term storage; therefore, these species are maintained as living plants in germplasm banks (Magnitskiy and Plaza, 2007; Kalemba and Pukacka, 2012).

Because recalcitrant seeds are sensitive to desiccation, they are vulnerable to rapid viability loss shortly after harvest (Berjak and Pammenter, 2013). However, in chayote, the seed remains inside the fruit throughout development, which helps maintain embryo viability. When fruits with recalcitrant seeds detach from the mother plant, the seeds have high moisture content, are metabolically active, and lack some mechanisms required to acquire desiccation tolerance. These limitations may include insufficient accumulation of vacuolar proteins that provide mechanical resistance against cellular collapse, as well as limited starch and lipid reserves, molecules commonly reported in orthodox seeds (Delahaie *et al.*, 2013; Berjak and Pammenter, 2013).

Biology and physiology of chayote seeds

Botanically, the chayote fruit is a fleshy berry that grows on a peduncle and is typically fleshy or fleshy with fibers surrounding the single seed located in the central-basal cavity of the fruit. The seed is flattened and large (3.47 cm) and consists of two cotyledons and a soft testa, 1.5 mm thick, that surrounds the embryo (Figure 2). At harvest, corresponding to horticultural maturity, the seed has not yet reached morphophysiological maturity because the cotyledons are only 25% developed and other embryonic components are not yet evident (Ramirez-Rodas *et al.*, 2023).

Chayote seeds are among the 10%-20% of angiosperms that produce recalcitrant seeds (Walters *et al.*, 2013). Because of their recalcitrant nature, these seeds do not undergo developmental interruption. Therefore, when the fruit reaches physiological maturity and the seed has completed its development, germination begins, particularly if the fruit experiences preharvest or postharvest stress. As a reproductive strategy, germination may

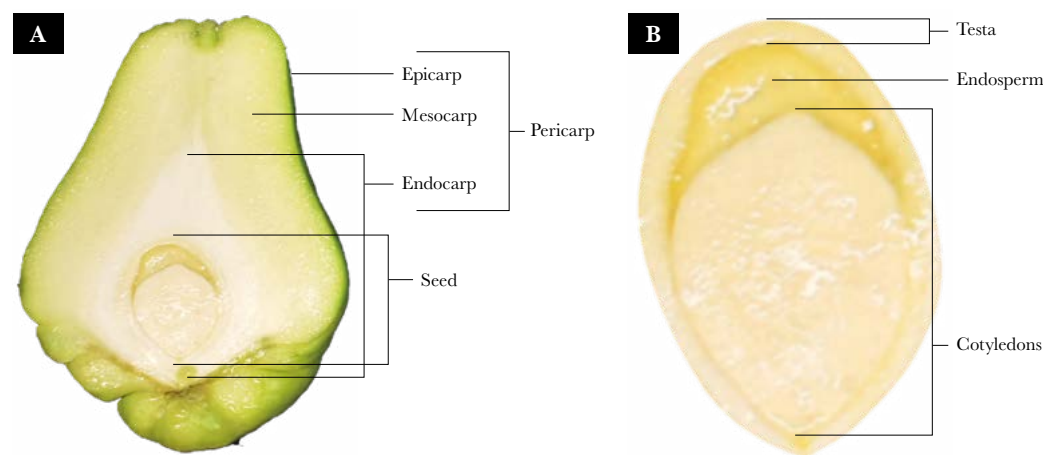


Figure 2. Morphology of the fruit and seed of chayote var. *virens levis*. Morphological structure of the fruit, composed of the pericarp epicarp, mesocarp, and endocarp and the seed located in the central-basal region of the fruit (A). Morphological structure of the seed, consisting of the testa, gelatinous endosperm, and two cotyledons, from the outermost to the innermost layer, respectively (Ramirez-Rodas *et al.*, 2023).

occur even while the fruit remains attached to the plant (Chauhan *et al.*, 2018). It has been observed that when chayote vines bearing fruits are damaged or wilted, germination is induced in the attached fruits (Figure 3).

Morphophysiological changes associated with chayote seed development occur both before and after fruit harvest (Figure 4). Following pollination and fertilization, chayote fruit development begins from an inferior ovary. From this stage, fruit growth follows a simple sigmoidal curve, similar to the developmental patterns observed in squash (*Cucurbita pepo* L.), cucumber (*Cucumis sativus*), and pineapple (*Ananas comosus* L. Merr. cv. Sarawak) (Oloyede *et al.*, 2013; Soloman *et al.*, 2016; Liu *et al.*, 2020).

The cell division stage (0-9 days after anthesis, DAA) is characterized by slow seed growth and the initial accumulation of gelatinous endosperm. During the cell elongation stage (9-18 DAA), endosperm presence increases, along with evident cotyledon growth. In the final stage of seed development prior to harvest, the growth rate declines, and the fruit reaches horticultural maturity, making it ready for harvest. At this point, the seed remains immature, with cotyledons developed to approximately 25%.

After harvest, the seed continues its development and reaches physiological maturity around 10 days later, at which point the cotyledons fill the seed cavity. Subsequently, germination begins, leading to testa rupture and the basal opening of the fruit, a phenomenon known as vivipary. This process is evidenced by the growth of a new seedling within the fruit and ultimately results in a loss of fruit quality during the postharvest stage (Figure 4).

Vivipary and hormonal regulation during germination

Chayote fruits lose commercial quality, causing consumers to perceive them as less attractive. This quality loss is associated with factors such as dehydration, pathogen presence, physiological maturity, and, primarily, vivipary (Romero-Velázquez *et al.*, 2015; Ramirez-Rodas *et al.*, 2023; Rivera-Ponce *et al.*, 2024). Vivipary is observed during the first week after harvest at room temperature (24 °C). However, when fruits are stored

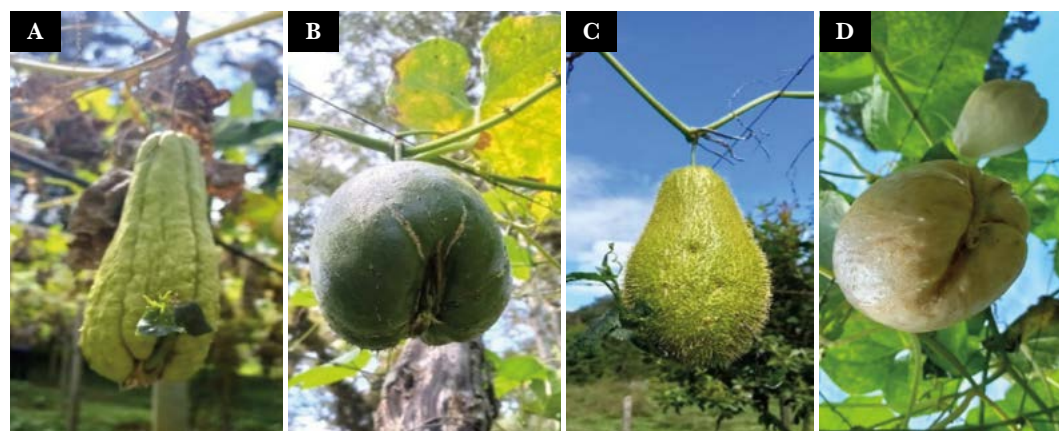


Figure 3. Vivipary in different varieties of chayote fruits at physiological maturity. Vivipary in *nigrum maxima* (A), *nigrum xalapensis* (B), *nigrum spinosum* (C), and *virens levis* (D), showing visible shoot emergence in fruits attached to the mother plant.

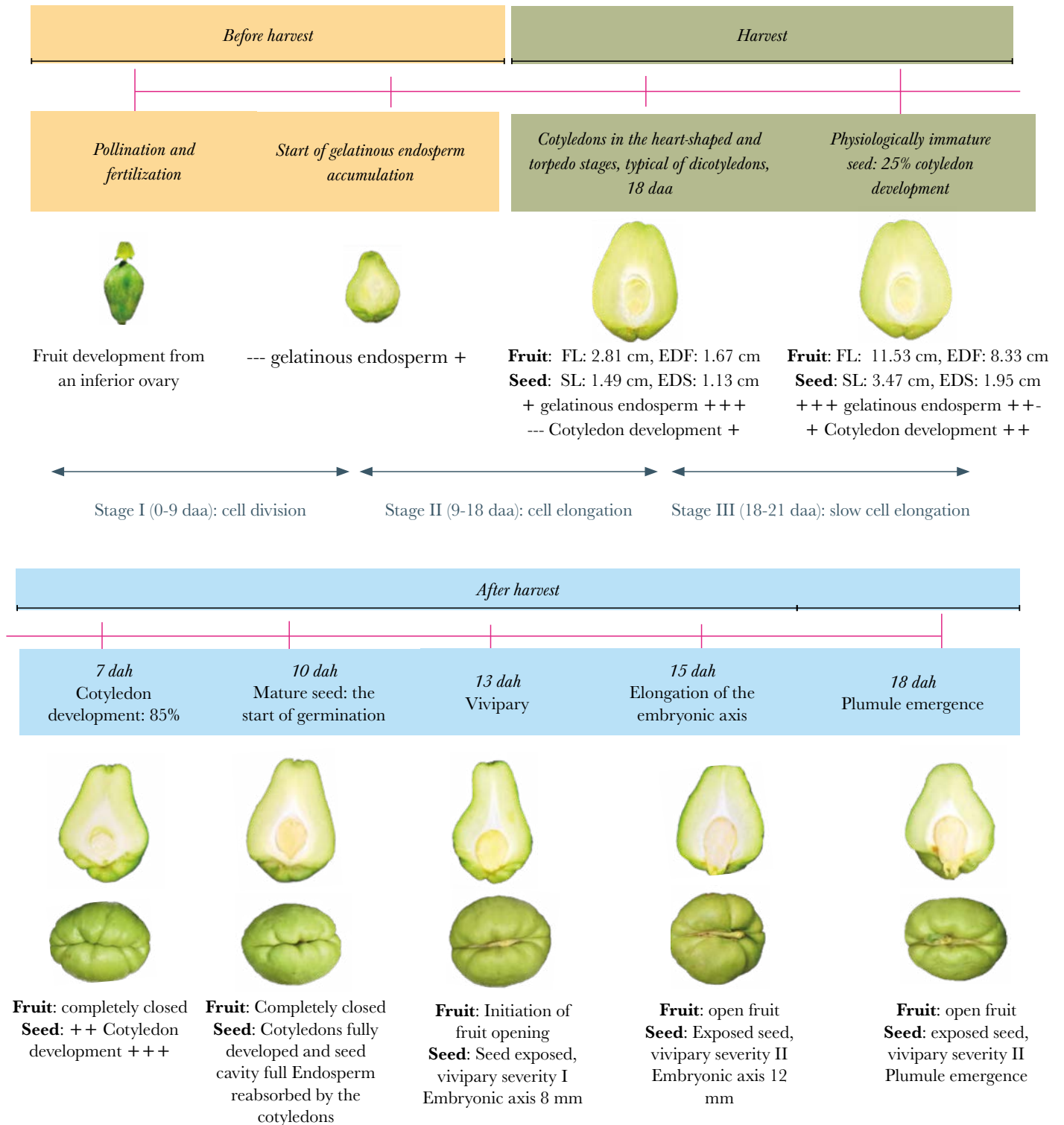


Figure 4. Morphophysiological changes in chayote seeds var. *virens levis* during development, from anthesis to vivipary. Before harvest, fruit and seed development consists of three stages: cell division, cell elongation, and slow cell growth. At harvest, the fruit is at horticultural maturity, whereas the seed remains physiologically immature. During postharvest, the seed reaches physiological maturity at 10 days, and vivipary is observed at 13 days, with basal opening of the fruit. daa=days after anthesis, dah=days after harvest, FL=fruit length, EDF=equatorial diameter of the fruit, SL=Seed length, EDS=equatorial diameter of the seed.

at 13 °C, it is observed at 13 days after harvest, whereas at 7 °C, it appears more rapidly. As an endocarpic fruit with a recalcitrant seed, chayote continues its development after reaching physiological maturity, allowing germination, fruit opening, and visible growth of the new seedling (Ramírez-Rodas *et al.*, 2021; Ramírez-Rodas *et al.*, 2023).

Vivipary and hormonal regulation during germination

Chayote fruits lose commercial quality as consumers perceive them as less attractive due to dehydration, pathogen presence, physiological maturity, and, primarily, vivipary (Romero-Velázquez *et al.*, 2015; Ramírez-Rodas *et al.*, 2023; Rivera-Ponce *et al.*, 2024). Vivipary is observed during the first week after harvest at room temperature (24 °C). However, when fruits are stored at 13 °C, it is observed at 13 days after harvest, whereas at 7 °C, it appears earlier. As an endocarpic fruit with a recalcitrant seed, chayote continues developing after reaching physiological maturity, allowing germination, fruit opening, and visible growth of the new seedling (Ramírez-Rodas *et al.*, 2021; Ramírez-Rodas *et al.*, 2023).

This phenomenon also occurs in papaya, mamey sapote (*Pouteria sapota*), tomato (*Solanum lycopersicum*), maize, *Arabidopsis thaliana*, pecan (*Carya illinoensis*), coconut (*Cocos nucifera*), chili pepper (*Capsicum annuum*), mango, and other species (Cruz and Deras, 2000; Farnsworth, 2000; Shareefa *et al.*, 2014; Wood, 2015; Cota-Sánchez, 2018; Krishna *et al.*, 2020; Garg and Garg, 2021). However, in these fruits, vivipary does not necessarily affect appearance, whereas in chayote it causes fruit deterioration by facilitating pathogen entry, altering appearance, and ultimately reducing shelf life (Figures 5A-5C).

Protection against desiccation in seeds, particularly orthodox seeds, is considered to arise from the production of molecular antioxidants and carbohydrates, as well as metabolically inert proteins that are highly soluble in water. The production of these potential protective compounds is induced in both orthodox and recalcitrant seeds during late embryogenesis; however, this response appears to be more pronounced in orthodox seeds (Walters *et al.*, 2008).

In orthodox seeds, embryo viability during dormancy has been associated with a reduction in water volume within the vacuoles (Magnitskiy and Plaza, 2007; Méndez-Ferreira *et al.*, 2013). During desiccation, these seeds acquire adaptive mechanisms that prevent cellular deterioration. These mechanisms may be related to the accumulation

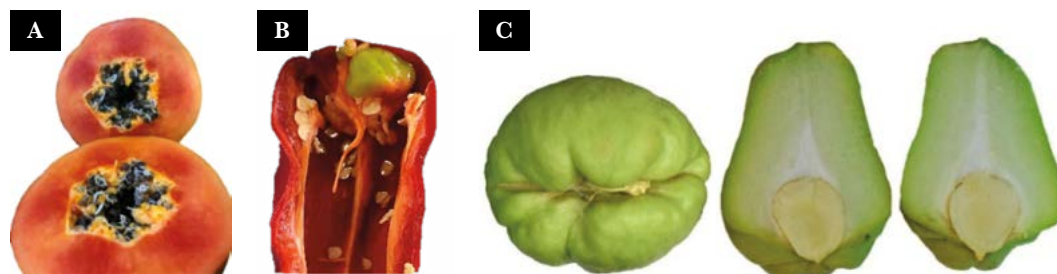


Figure 5. Vivipary in fruits of different species. Postharvest vivipary in papaya (A) and chili pepper (B), species in which fruit appearance is not affected because the fruit remains completely closed. Vivipary in chayote (C), showing the basal opening of the fruit.

of disaccharides, such as trehalose and sucrose, and oligosaccharides, such as raffinose, which help stabilize membranes through hydrogen bonding and cellular vitrification. In addition, during this period, orthodox seeds accumulate antioxidant compounds, heat shock proteins (HSPs), and late embryogenesis abundant (LEA) proteins. These LEA proteins are small, hydrophilic, intrinsically disordered, and thermostable, and they exhibit antioxidant activity, help remove reactive oxygen species, prevent the degradation of other proteins, and form hydrogen bonds that stabilize membranes (Berjak and Pammenter, 2013; Méndez-Ferreira *et al.*, 2013; Farooq *et al.*, 2022).

Dormancy and germination processes are closely related to phytohormones, including gibberellins, ethylene, and abscisic acid, as well as their synergistic and antagonistic interactions (Kucera *et al.*, 2005). During dormancy in orthodox seeds, abscisic acid (ABA) regulates the accumulation of storage reserves, including proteins, lipids, and carbohydrates; inhibits embryo growth; and induces the production of late embryogenesis abundant (LEA) proteins and heat shock proteins (HSPs), which provide protective functions to other proteins during seed desiccation (Kermode, 2005).

Plants accumulate ABA through phloem transport or through synthesis within the seed itself, which occurs during the intermediate stage of seed development. Among the transcription factors that regulate seed development are *abscisic acid insensitive 3 (ABI3)*, a central ABA-related transcription factor, and *FUSCA 3 (FUS3)*, a gene involved in seed development. These factors promote reciprocal activation, and ABA accumulation inhibits gibberellin (GA) biosynthesis, thereby maintaining dormancy (Sano and Marion-Poll, 2021).

When seeds encounter the conditions required to break dormancy, the levels of certain phytohormones change: ABA decreases, whereas GA levels increase and promote germination. In addition, GAs activate genes involved in the biosynthesis of enzymes such as endo- β -1,4-mannanase, endo- β -1,3-glucanase, and expansins in the endosperm. They also stimulate embryonic cell expansion, regulate the activity of *replication protein A1 (RPA1)*, which is found in actively dividing tissues, and activate hydrolytic enzymes that degrade starch (Kucera *et al.*, 2005; Corbineau *et al.*, 2014; Miransari and Smith, 2014). Other phytohormones, including ethylene, auxins, jasmonic acid (JA), brassinosteroids (BRs), and cytokinins (CKs), also participate in promoting germination. In addition to GAs, ethylene interacts antagonistically with ABA and synergistically with GAs, contributing to dormancy release (Farooq *et al.*, 2022).

Unlike orthodox seeds, in which LEA proteins accumulate more abundantly and are considered important protective molecules during desiccation, recalcitrant seeds show reduced abundance of these proteins. In this regard, Delahaie *et al.* (2013) characterized the cotyledon proteomes of *Castanospermum australe* (recalcitrant) and *Medicago truncatula* (orthodox). They identified transcripts for 16 homologs of 17 LEA genes for which polypeptides were detected in *M. truncatula* seeds. For 12 LEA genes, the corresponding polypeptides were absent or strongly reduced in *C. australe* cotyledons compared with *M. truncatula* seeds.

Recalcitrant seeds generally contain limited amounts of ABA during development and higher levels of GAs during germination, accompanied by other phytohormones that act

synergistically or antagonistically (Romero-Rodríguez *et al.*, 2018). In the specific case of chayote, Ramirez-Rodas *et al.* (2024) described the complex hormonal relationship in the seed before and during germination, at 7 and 10 days after harvest, using metabolomic data. They highlighted that ethylene, gibberellins, and their precursors synergistically accelerate seed germination.

Delayed seed germination is attributed to the differential accumulation of indole-3-acetic acid, indole-3-butyric acid, abscisic acid, and their precursors, which stimulate their own biosynthesis. Therefore, the antagonistic behavior of these two phytohormonal groups gibberellin ethylene and auxin abscisic acid accelerates or delays chayote seed germination, respectively, by mutually restricting their biosynthesis.

Physiology and postharvest response of chayote as a non-climacteric fruit

Harvested agricultural products are living plant organs with high water content and active metabolism. Therefore, when metabolic processes accelerate, postharvest life becomes shorter. Physiological changes, such as respiration, transpiration, ripening, and senescence, may occur slowly or rapidly and are influenced by environmental conditions, including temperature, humidity, and physical damage stress, as well as genetic factors, phytohormones, diseases caused by phytopathogens, and whether the fruit is climacteric or non-climacteric (Fukano and Tachiki, 2021). Climacteric fruits show a characteristic increase in respiration and ethylene production, accompanied by postharvest ripening. Examples include tomato, avocado (*Persea americana*), apple (*Malus domestica*), peach (*Prunus persica*), mango, pear (*Pyrus communis*), and banana (*Musa paradisiaca*). In contrast, non-climacteric fruits do not show a relevant increase in respiration or ethylene production. However, after harvest, at consumption maturity, they begin senescence. Examples include cucumber, strawberry (*Fragaria* spp.), blackberry (*Rubus ulmifolius*), watermelon (*Citrullus lanatus*), pineapple (*Ananas comosus*), grape (*Vitis vinifera*), citrus fruits, and chayote (Chen *et al.*, 2018; Kou *et al.*, 2021).

Chayote fruit is classified as non-climacteric, with a low respiration rate ($0.86\text{-}1.3 \text{ kg}^{-1} \text{ s}^{-1} \text{ CO}_2$) and minimal ethylene production after harvest, approximately $3.5\text{-}5 \text{ ng kg}^{-1} \text{ s}^{-1}$ (Cadena-Iñiguez *et al.*, 2006). In addition, a dependence between the fruit and the seed has been observed. Therefore, once the fruit is harvested, it enters senescence, making it necessary to develop preharvest and postharvest strategies to extend fruit shelf life. To counteract senescence caused by dehydration, chilling injury, blistering, and other phytosanitary disorders, and especially to delay vivipary, several studies have been conducted in different chayote varieties (Figure 6). Vivipary in chayote cannot be attributed solely to fruit deterioration; rather, it results from a physiological interaction between the seed and the fruit. Unlike most fleshy fruits, chayote contains a large, recalcitrant, nondormant seed that remains metabolically active. The fruit supplies water, gases, and hormonal signals to the seed, highlighting the importance of postharvest treatments for controlling vivipary. Although these treatments are not applied directly to the seed, they modify the physiological environment of the fruit.

To counteract vivipary, low-temperature storage and modified atmospheres have been used to slow fruit and seed metabolism. Edible coatings have also been applied to

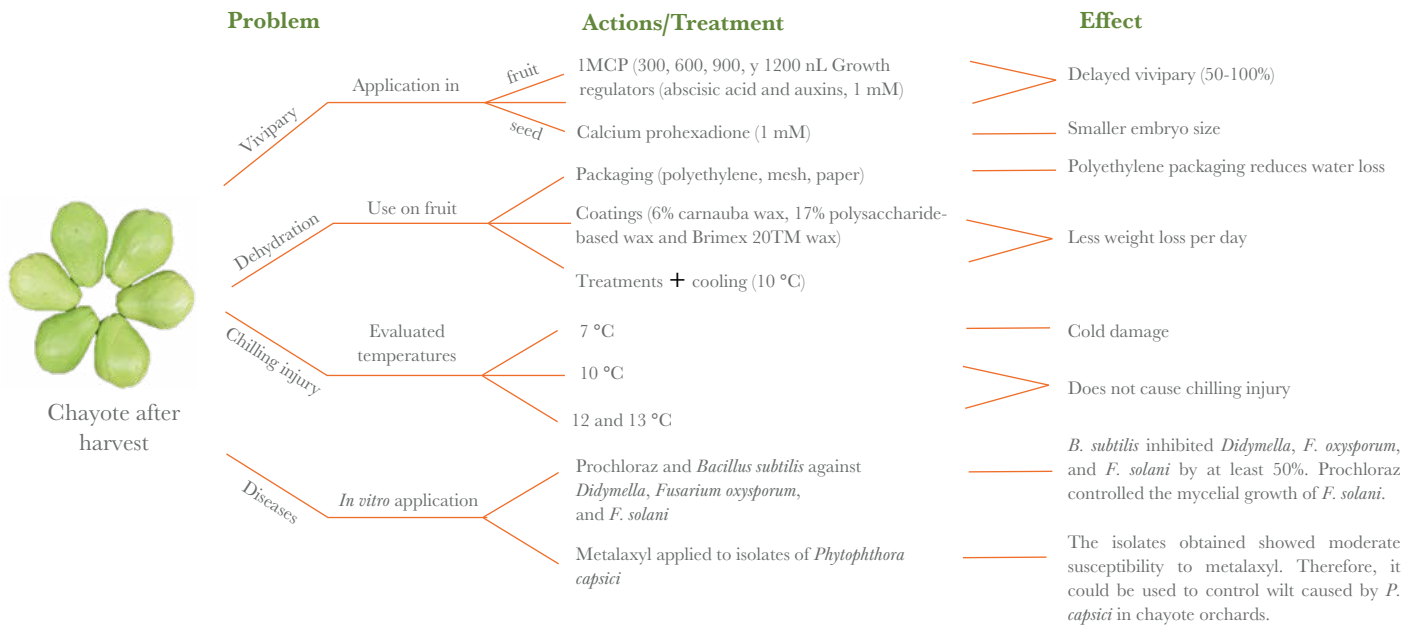


Figure 6. Postharvest management of chayote fruits. The four main problems affecting postharvest fruit quality are shown: vivipary, dehydration, chilling injury, and diseases, along with their respective management actions or treatments and effects.

regulate gas exchange within the fruit. Because vivipary is influenced by modulation of the hormonal balance, treatments such as 1-methylcyclopropane (1-MCP) have been evaluated. Due to its structural similarity to 1-aminocyclopropane-1-carboxylic acid (ACC), the ethylene precursor, 1-MCP competes for ethylene-related action sites and has a high affinity for ethylene receptors (ETRs), making it a direct competitor for ethylene perception (Balaguera-López *et al.*, 2021). Cadena-Iñiguez *et al.* (2006) applied 1-MCP at 300, 600, 900, and 1,200 nL L⁻¹ to chayote fruits var. *virens levis*. The treatments delayed vivipary in more than 90% of fruits after 31 days after harvest, consisting of 28 days at 10 °C followed by 3 days without refrigeration. Similarly, Ramírez-Rodas *et al.* (2021) applied 600 nL L⁻¹ 1-MCP to fruits of the varieties *virens levis*, *nigrum spinosum*, and *nigrum xalapensis*. After 21 days after harvest, consisting of 14 days at 13 °C followed by 7 days at 24 °C, vivipary was delayed by 50%, 87%, and 100%, respectively. Calcium prohexadione (1 mM) has also been injected directly into chayote seeds to inhibit gibberellin (GA) synthesis, resulting in smaller embryos (~80 mm) after 35 days of fruit storage at 25 °C (Aung *et al.*, 2004).

The application of abscisic acid and auxins also delays vivipary in chayote fruits var. *virens levis* by affecting the biosynthesis of ethylene, gibberellins, and their precursors. Ramirez-Rodas *et al.* (2024) applied 2-chloroethylphosphonic acid, an ethylene-releasing compound, at 1.4 mM; GA₃ at 0.6 mM; ABA at 1 mM; and auxins, including indole-3-acetic acid and indole-3-butyric acid, at 1 mM to *S. edule* var. *virens levis* fruits. A control group treated with distilled water was also included. Fruits were evaluated at 0, 7, 10, 13, and 18 days at 21 ± 1 °C and 65% relative humidity. Exogenous application of 2-chloroethylphosphonic acid and GA₃ accelerated germination and vivipary, whereas auxins and abscisic acid delayed both processes.

Implications for germplasm conservation

Conservation of orthodox and recalcitrant seeds

The conservation of plant species depends on the type of seeds they produce. Accessions are stored as different types of collections. Orthodox seeds can be conserved in germplasm banks in the short and medium term, between 10 and 20 years, as active collections stored under controlled refrigeration conditions of 0-5 °C and 10%-30% relative humidity. They can also be conserved long term, for more than 20 years, as base collections under controlled freezing conditions of –18 to –20 °C and 10%-30% relative humidity (Rao *et al.*, 2007). These conditions allow collections to remain metabolically slow but viable over time. In Mexico, several germplasm banks store orthodox seeds from different species, such as maize, chili pepper, common bean, squash, rice, wheat, roselle, and sesame. These include the National Genetic Resources Center (CNRG), the Southern Orthodox Seed Germplasm Bank of the National Institute for Forestry, Agricultural, and Livestock Research (INIFAP), the Seed Germplasm Bank of the International Maize and Wheat Improvement Center (CIMMYT), and the National Plant Germplasm Bank of Chapingo Autonomous University, among others. Once seeds reach physiological maturity, they are dehydrated to ensure storage and enter a period of dormancy until conditions become favorable for germination.

In contrast, because recalcitrant seeds do not tolerate desiccation, alternative *ex situ* conservation methods are used, including the following:

- **Cryopreservation of embryonic axes.** This technique requires partial drying to prevent damage caused by ice crystal formation, allowing the vitrification of the remaining intracellular water when cooling rates are sufficiently rapid. Recalcitrant seeds are generally too large to allow the drying and cooling rates required for vitrification; therefore, excised embryonic axes are used (Pammenter and Berjak, 2014; Berjak and Pammenter, 2014). However, this storage method is not widely used because of seed complexity, maintenance costs, and short-term viability limitations.
- ***In vitro* conservation.** Plant tissue culture techniques allow plant material to be preserved under controlled conditions. These methods maintain plantlets in *in vitro* germplasm banks, free of pathogens, in reduced space, at low cost, and under controlled conditions that facilitate short- and long-term management of plant material (Morales, 2015).
- ***In vivo* conservation.** This approach involves maintaining collections in germplasm banks, botanical gardens, nurseries, or even in the cultivation area itself, including *in situ* conservation with cooperating producers (Fernández *et al.*, 2023).

Conservation of chayote diversity in México

Conservation techniques

Techniques for conserving chayote diversity include a set of *in situ* and *ex situ* strategies focused on preservation, management, and use. Common crop varieties are generally maintained *in situ* as living plants by producers within traditional agricultural systems and are part of conservation practices (Priyanka *et al.*, 2021). Different cultivated species

and varieties of *Sechium* have been conserved in family backyard gardens because they contribute to food supply and income generation. To date, this has been the most effective approach for conserving chayote diversity *in situ* in Mexico (Cadena-Iñiguez *et al.*, 2010).

Common crop varieties are generally maintained *in situ* as living plants by producers within traditional agricultural systems, making this an important conservation practice (Priyanka *et al.*, 2021). Different cultivated species and varieties of *Sechium* have been conserved in family backyard gardens because they contribute to food supply and income generation. To date, this approach remains the most effective method for conserving chayote diversity *in situ* in Mexico (Cadena-Iñiguez *et al.*, 2010).

For *ex situ* conservation, *in vitro* conservation represents a strategic alternative for safeguarding chayote genetic diversity. Several studies have reported *in vitro* protocols for the regeneration of *S. edule* plant material through organogenesis (Alvarenga-Venutolo *et al.*, 2007; Cruz-Martínez *et al.*, 2017; Soto-Contreras *et al.*, 2022), as well as shoot regeneration and callusgenesis in *S. compositum*, a wild chayote species (Riviello-Flores *et al.*, 2024). These approaches support the conservation of plant material and provide an important source of natural products, such as phenolic compounds and cucurbitacins (Cadena-Iñiguez *et al.*, 2024). In addition, techniques such as clonal micropropagation, axillary bud culture, and conservation under slow-growth conditions have been widely used for *in vitro* conservation of other species. These methods allow genotypes to be maintained in reduced spaces and under controlled conditions, thereby decreasing risks associated with environmental and phytosanitary factors (Engelmann, 2011). However, the application of these methodologies in *Sechium* still faces challenges, including the optimization of genotype-specific protocols, evaluation of the genetic stability of regenerated material, and possible occurrence of somaclonal variation (Benson, 2008).

Regarding institutional actions for chayote conservation, the Interdisciplinary Research Group on *Sechium edule* in Mexico (GISEM) has designed a national program for the rescue, conservation, and research of infraspecific variation in *S. edule*, including wild species, cultivated varieties, and hybrids. Most of the morphological, structural, biochemical, and genetic characterization has been carried out, enabling the identification of varietal descriptors and the establishment of the *Sechium edule* Germplasm Bank (BANGESE) in 2005-2007, located in Huatusco, Veracruz. BANGESE currently contains more than 100 accessions from 10 Mexican states, as well as accessions from Guatemala and Costa Rica (Cadena-Iñiguez *et al.*, 2010).

At BANGESE, accessions of domesticated chayote belonging to *S. edule* are arranged according to varietal groups. Wild accessions of *S. edule*, *S. compositum*, and *S. chinantlense* are located in understory areas, which provide greater protection from excessive irradiation, drought, and herbivore attack. In addition, BANGESE includes an area for improved germplasm, such as bitter varieties and hybrids. Currently, the GISEM interdisciplinary group is working to achieve *in vitro* conservation of this plant germplasm. This effort aims to provide a backup of chayote diversity and preserve it for long periods in the *in vitro* germplasm banks of the National Center for Genetic Resources and Productivity (CNRG-INIFAP).

CONCLUSIONS

Chayote is a vegetable crop with distinctive characteristics due to its recalcitrant seed, which creates specific challenges for crop conservation and postharvest management. The physiological characteristics of its seed require high humidity and controlled temperature conditions to maintain viability, making long-term storage difficult. Therefore, its diversity is conserved mainly through methods used in producers' backyard systems, *in vivo* germplasm banks, and some *in vitro* collections. Another important area of opportunity is postharvest fruit management, as strategies are needed to minimize physical damage, prevent pathogen proliferation, and delay dehydration and vivipary. Given that chayote is a commercially important vegetable crop for both national markets and export, developing technologies that help maintain fruit quality for longer periods is essential.

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Productive performance of native chili peppers (*Capsicum* spp.) adapted to greenhouse conditions

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ABSTRACT

Objective: To evaluate the productive performance of native chili pepper genotypes from Teticic, municipality of Olinalá, Guerrero, under greenhouse conditions.

Design/methodology/approach: The experiment was established in a greenhouse under a completely randomized design. The treatments consisted of seven chili pepper genotypes (Cascabel, Gallo gallina mediano, Gallo gallina grande, Larguillo chico, Larguillo grande, Mochiteco, and Serrano) from Teticic, municipality of Olinalá, Guerrero, with six replicates. Fruit size was evaluated based on polar diameter, equatorial diameter, and individual fruit weight. Yield per plant was assessed as number of fruits and fresh and dry fruit weight per plant. Fruit quality was evaluated based on firmness, total number of seeds, number of viable and empty seeds, and total seed weight (g) per fruit.

Results: The Mochiteco genotype produced the highest number of fruits per plant (607.50). The Gallo gallina grande genotype exceeded the fresh fruit weight of Mochiteco, Gallo gallina mediano, and Cascabel by 41.9, 44.3, and 47.5%, respectively. The Serrano genotype had 46.3% greater dry fruit weight than Cascabel. The greatest polar diameter was observed in Gallo gallina mediano, Serrano, and Larguillo grande, whereas the greatest equatorial diameter was found in Larguillo grande and Gallo gallina grande. The highest individual fruit weight was recorded in Gallo gallina grande and Gallo gallina mediano. Gallo gallina grande produced 27.02, 47.2, and 53.01% more seeds than Larguillo grande, Mochiteco, and Larguillo chico, respectively. Gallo gallina grande and Gallo gallina mediano produced more viable seeds, whereas Serrano produced more empty seeds than Larguillo grande and Mochiteco.

Limitations on study/implications: Chili pepper productivity was conditioned by phenotypic variation and the environmental conditions during crop growth. As a result, some genotypes adapted well to greenhouse conditions, whereas others showed a less favorable response.

Findings/conclusions: The cultivation of native chili peppers from Teticic, Olinalá, Guerrero, under greenhouse conditions represents an alternative for their conservation and potential use.

Keywords: *Capsicum*, genotype, yield, quality, seed

Citation: Espinosa-Rodríguez, M., Antúnez-Ocampo, O. M., Guzmán-Olea, I., Flores-Hernández, L. A., Maldonado-Peralta, M. D.A., Sabino-López, J. E. (2026). Productive performance of native chili peppers (*Capsicum* spp.) adapted to greenhouse conditions. *Agro Productividad*. <https://doi.org/10.32854/g2byfh27>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: January 18, 2026.

Accepted: April 15, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 43-53.

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INTRODUCTION

Chili pepper cultivation (*Capsicum* spp.) originated in the Americas, where the greatest morphological diversity of this species is found (Rodríguez, 2018). Notable species include *C. baccatum*, *C. chinense*, *C. pubescens*, *C. frutescens*, and *C. annuum*; the latter was domesticated and propagated in Mexico (Aguirre and Muñoz, 2015). Among the main genotypes cultivated in Mexico are Jalapeño, Serrano, Habanero, Ancho, Mulato, Pasilla, and Piquín (Salinas *et al.*, 2010). In 2020, Mexico reported a production of 3,324,260 t, accompanied by an increase in yield per hectare from 13.86 t ha⁻¹ in 2005 to 21.65 t ha⁻¹ in 2019 (SADER, 2021). In 2021, a total area of 14,235,129.40 ha was planted with this crop in Mexico (DGSIAP, 2021). Chili pepper production in Mexico is closely linked to the development of protected agriculture through the use of structures and coverings such as shade houses, greenhouses, and macro tunnels, among others. In this context, in 2019, the average chili pepper yield reported under protected agriculture was 12.3 kg m⁻², compared with 1.8 kg m⁻² in open-field production (SIAP, 2020). This indicates that the cultivation of commercial as well as native chili pepper varieties under protected systems favors increased yield (Escamirosa-Tinoco *et al.*, 2021). In addition, chili pepper production is influenced by genotype, climate, soil fertility, crop management, pest and disease control, and harvest and drying processes, whereas fertilization can generate secondary effects that may alter plant resistance or tolerance to biotic and abiotic stress factors (Hernández *et al.*, 2021).

The use of native varieties is attributed to their adaptation to diverse climates and soil types, which has contributed to their successful and widespread geographic distribution (Aguirre and Muñoz, 2015). Native chili pepper varieties have greater acceptance in local and regional markets, where they are consumed mainly as fresh and dried fruits to season a variety of traditional dishes (SAGARPA, 2016). However, most native materials are grown under rainfed conditions and are exposed to phytosanitary problems and environmental factors that directly influence crop productivity (Aguilar *et al.*, 2010).

One strategy to improve production yield and quality is the use of fertigation, container cultivation, and agronomic management practices that promote plant development and fruit set, thereby increasing productive potential and profitability (Bahena-Delgado *et al.*, 2012). In this way, local chili pepper varieties may gain greater national and international importance, with the possibility that producers will conserve and reproduce their native materials through seed storage techniques, since some do not select fruits with outstanding size and quality for seed production (Carrillo *et al.*, 2009; Andueza-Noh *et al.*, 2017). This suggests the need for studies focused on productivity in order to determine their economic importance and productive potential (Meneses-Lazo *et al.*, 2018).

In the Mountain region of Guerrero, Mexico, there is substantial genetic and phenotypic diversity among native chili peppers of economic and food importance due to their high demand and local consumption. These peppers are indispensable in the preparation of typical regional dishes, where this crop is regarded as a symbol of cultural identity, as in the rest of Mexico, which has contributed to its social and economic importance since the domestication of the crop (Herrera *et al.*, 2018). In this regard, DGSIAP (2021) reported for the state of Guerrero a planted area of 1,426.35 ha and a harvested area of 1,424.35 ha,

with a production of 9,696.40 t and a yield of 6.81 t ha⁻¹. According to previous records, some chili pepper genotypes cultivated in the Mountain region of Guerrero include Serrano, Gallo gallina, and chile Gordo (Aguilar *et al.*, 2010). Therefore, the objective of this study was to evaluate the productive performance under greenhouse conditions of chili pepper genotypes from the Mountain region of Guerrero.

MATERIALS AND METHODS

Germplasm collection and experimental site

Visits were made to farmers' fields in 2020, and fruits from seven chili pepper genotypes (Table 1) were collected in the community of Teticic, municipality of Olinalá, Guerrero, Mexico (17° 52' 02" N, 98° 50' 54" W; 1,273 m). The fruits were placed in perforated brown paper bags and transported to the multipurpose laboratory of the Faculty of Agricultural and Environmental Sciences, Tuxpan campus, of the Autonomous University of Guerrero (18° 20' 38" N, 99° 30' 04" W; 775 m). Subsequently, the morphological characteristics of the fruits of each genotype were recorded (Table 1), after which the seeds were extracted and stored in airtight containers at room temperature (26.1 °C).

Seedbed establishment and transplanting

In 2021, seeds of each genotype were sown in 200-cell polypropylene trays filled with peat. One seed was placed in each cell at an approximate depth of 0.5 cm and covered with the same substrate. Irrigation was applied twice daily using well water. Transplanting was carried out 48 days after sowing (DAS), when the seedlings had reached approximately 15 cm in height and had developed four to five true leaves. One plant was placed per pot consisting of a 12 L black polyethylene bag filled with forest soil (pH: 7.11, EC: 4.78 dS m⁻¹, OM: 28%, bulk density: 0.35 g cm⁻³, N: 188, P: 22.3, K: 136, Ca: 3978, Mg: 715, S: 82.9, Fe: 3.79, Zn: 3.13, Mn: 17.2, Cu: 0.11, and B: 0.21 mg kg⁻¹). Planting density was 4 plants m², arranged in a ridge-vent greenhouse covered with white polyethylene (70% transmittance).

Treatments and experimental design

The collected genotypes, considered as treatments, were Cascabel, Gallo gallina mediano, Gallo gallina grande, Larguillo chico, Larguillo grande, Serrano, and Mochiteco.

Table 1. Fruit characteristics of chili pepper genotypes from Teticic, Olinalá, Guerrero.

Genotype	Diameter (cm)		Individual dry fruit weight (g)
	Polar	Equatorial	
Cascabel	2.32	2.39	0.95
Gallo gallina mediano	8.19	3.63	5.69
Larguillo grande	10.05	1.97	4.11
Serrano	7.96	1.60	2.50
Mochiteco	4.07	1.37	0.95
Gallo gallina grande	8.75	4.46	6.83
Larguillo chico	10.22	3.31	3.02

These were distributed in the greenhouse under a completely randomized design with six replicates. The experimental unit consisted of one pot containing one plant.

Crop management

Crop agronomic management consisted of daily irrigation in the morning and afternoon, with an average of 4 L plant⁻¹ day⁻¹, using the universal nutrient solution proposed by Steiner (1984) (Table 2). Its concentration was modified according to the crop's water requirements and phenological stage, beginning with an EC of 0.5 dS m⁻¹ during the vegetative stage and increasing to 2.0 dS m⁻¹ during fruiting. Pest prevention and control were carried out using chromatic traps and the application of ecological products (Escaminosa-Tinoco *et al.*, 2021). Harvest began 133 days after transplanting (DAT), when the fruits turned red. The fruits were then collected, placed in perforated brown paper bags, and transported to the multipurpose laboratory of the Faculty of Agricultural and Environmental Sciences, Tuxpan campus, Autonomous University of Guerrero, for measurement and drying.

Evaluated variables

Yield

Yield was quantified as the number of fruits and the fresh and dry weight (g) of fruits per plant over seven weeks of harvest, using an ISOLAB[®] Laborgeräte GmbH LS-EJ-2200AS balance. Fruit drying was carried out in perforated paper bags, which were placed in a forced-air drying oven (Riossa[®], model HCF-62D) at 75 °C for 72 h.

Fruit and seed quality

In 30 fresh fruits randomly selected from each genotype, fruit size was measured based on polar diameter (cm), from the base of the peduncle to the apical end of the fruit, and equatorial diameter (cm), at the middle part of the fruit, using an electronic caliper (Stainless Hardened[®]). Individual fruit weight (g) was determined with an ISOLAB[®] Laborgeräte GmbH balance, model LS-EJ-2200AS. Firmness (kg cm⁻²) was measured at the equatorial midpoint of the fruit using a penetrometer (Truper[®], model FDV-30) with a conical tip. The total number of seeds, viable seeds, and empty seeds per fruit, as well as total seed weight per fruit (g), were quantified using the previously described balance. The obtained values were subjected to analysis of variance and Tukey's mean comparison test ($\alpha=0.05$) using SAS[®], version 9.0.

Table 2. Ion concentration in Steiner's nutrient solution for the fertigation of native chili pepper genotypes.

Concentration of the nutrient solution (%)	NO ₃ ⁻	H ₂ PO ₄ ⁻	SO ₄ ⁻	K ⁺	Ca ²⁺	Mg ²⁺
	meq L ⁻¹					
25	3.00	0.25	1.75	1.75	2.25	1.00
50	6.00	0.50	3.50	3.50	4.50	2.00
75	9.00	0.75	5.25	5.25	6.75	3.00
100	12.00	1.00	7.00	7.00	9.00	4.00

RESULTS AND DISCUSSION

Yield

Differences ($p \leq 0.05$) were detected in the cumulative fruit yield per plant among the evaluated genotypes. Mochiteco produced the highest number of fruits per plant (607.50) compared with the other evaluated materials. In contrast, Cascabel and Serrano produced a similar number of fruits (Figure 1). These differences can be attributed to variability among genotypes, since some produce more fruits than others and differ in fruit size (Ramírez-Meraz *et al.*, 2015). This is consistent with the existence of numerous chili cultivars and races grouped into morphotypes with genetic variability that is transmitted from one generation to another within their populations under different environments (Rodríguez, 2018), which influences productivity. Similar results were reported by López-Gómez *et al.* (2017) in Habanero chili genotypes, in which the highest average was 425 fruits plant⁻¹.

In contrast, Moreno *et al.* (2014) reported 40 fruits plant⁻¹ in Hungarian chili grown in sand with a nutrient solution. Similarly, Monge and Loria (2018), working with sweet pepper cv. FBM-9, recorded 29.71 fruits plant⁻¹ under greenhouse conditions with pruning. This confirms that fruit production in chili crops is conditioned by various environmental factors and the production technology used, both of which modify fruit yield (Alemán *et al.*, 2018). In the same vein, Sandoval-Rangel *et al.* (2011) reported values ranging from 221.03 to 433.18 fruits plant⁻¹ in Piquín chili.

Cumulative fresh and dry fruit yield showed differences ($p \leq 0.05$) among the evaluated genotypes. The greatest fresh fruit weight was observed in Gallo gallina grande, which exceeded Mochiteco, Gallo gallina mediano, and Cascabel by 41.9, 44.3, and 47.5%, respectively. Meanwhile, Gallo gallina grande, Serrano, and Larguillo grande and chico showed similar fresh fruit weights, ranging from 451.8 to 667.5 g plant⁻¹ (Figure 2). In terms of dry fruit yield, only Serrano (124.5 g plant⁻¹) stood out, exceeding Cascabel

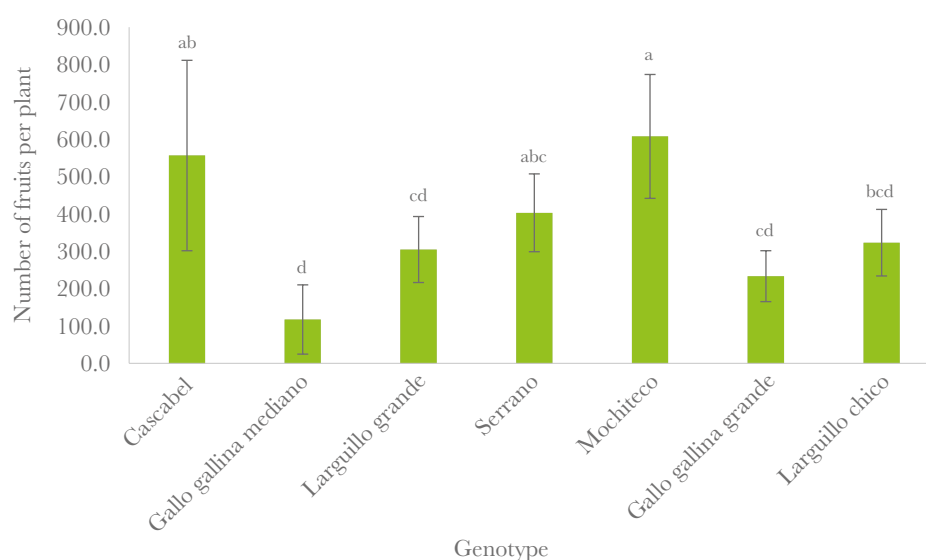


Figure 1. Number of fruits per plant in native chili pepper genotypes grown under greenhouse conditions. Means followed by the same letter are not statistically different (Tukey, $\alpha = 0.05$). LSD=248.15.

(66.8 g plant⁻¹), which was the genotype with the lowest dry fruit yield, by 46.3%. In the remaining genotypes, dry weight ranged from 86.0 to 103.5 g plant⁻¹ (Figure 3).

This behavior is related to the type and size of fruits produced by each genotype (broad, thick, or slender) (Aguilar *et al.*, 2010), as well as to the adaptive response of the plant materials to the production system and the environmental conditions that prevailed during growth (Galeote-Cid *et al.*, 2022). In this regard, chili pepper production in Mexico is characterized by the use of commercial varieties with high productive potential; however,

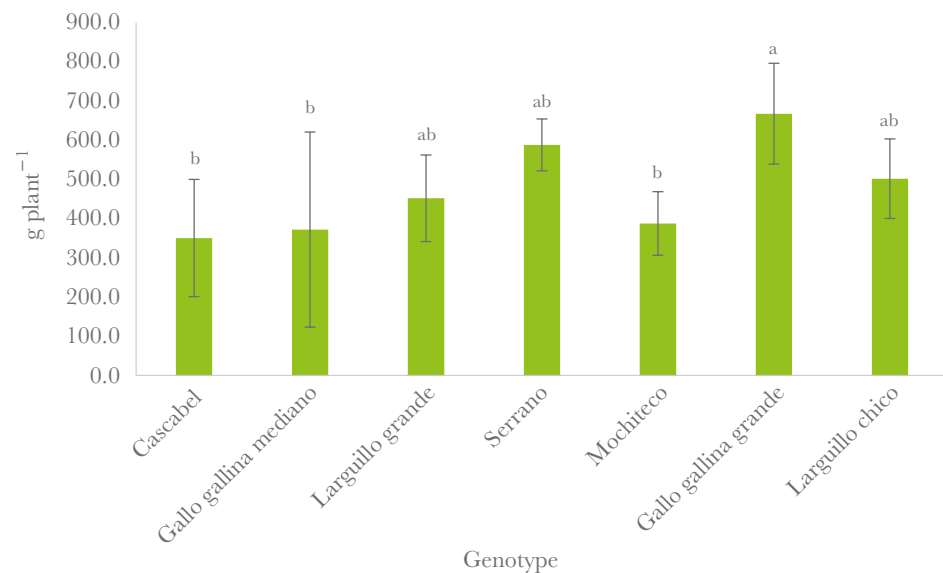


Figure 2. Fresh fruit weight per plant in native chili pepper genotypes grown under greenhouse conditions. Means followed by the same letter are not statistically different (Tukey, $\alpha=0.05$). LSD=249.98.

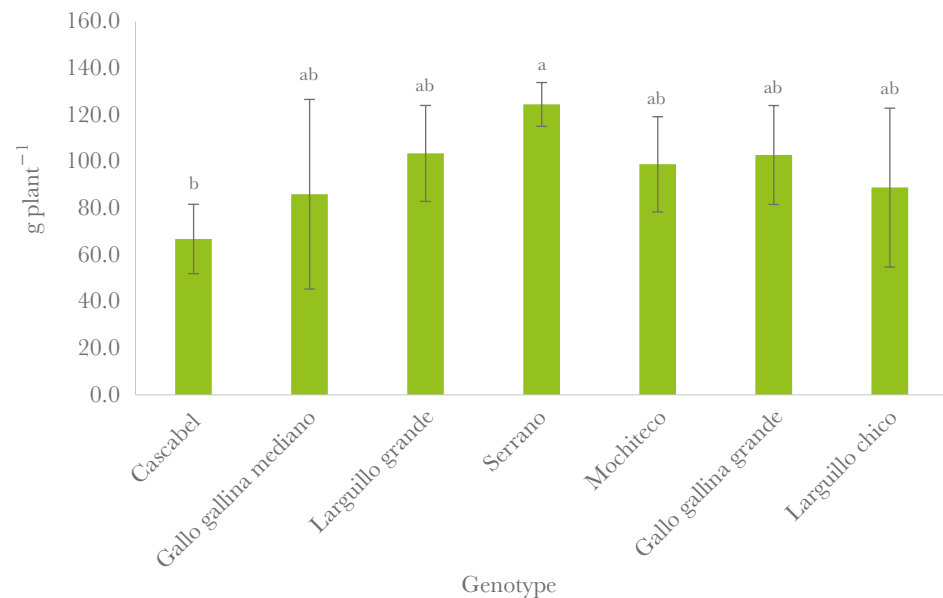


Figure 3. Dry fruit weight per plant in native chili pepper genotypes grown under greenhouse conditions. Means followed by the same letter are not statistically different (Tukey, $\alpha=0.05$). LSD=45.308.

their use contributes to biodiversity loss in regions where native chili peppers are cultivated (Aguilar *et al.*, 2010). In improved varieties, yield has been reported to increase when the crop is established under greenhouse and hydroponic conditions. For example, fresh fruit yields of 1,220 to 1,518 g plant⁻¹ have been obtained in Serrano chili (*Capsicum annuum* L.) using nutrient solution and substrates as the growing medium (Cruz-Crespo *et al.*, 2014). Likewise, Sandoval-Rangel *et al.* (2011) reported yields of 130.91 g plant⁻¹ in Piquín chili (*Capsicum annuum* var. *glabriusculum*). It is important to note that fresh and dry yield in chili crops increases as the harvest period is extended (Azofeifa and Moreira, 2004). However, the determining factor in yield is the genotype, together with adequate root and shoot growth within the crop production system (Galeote-Cid *et al.*, 2022). Differences in yield between field production and protected systems for native chili peppers are due to the fact that greenhouse coverings modify environmental conditions inside the structure, such as radiation, temperature, and relative humidity, among others. This favors plant growth and may increase fruit yield by more than 600% compared with field production (Escamirosa-Tinoco *et al.*, 2021). In addition, protected systems allow better agronomic management, including pest and disease control. In this regard, San Juan *et al.* (2018) reported similar fresh fruit yields in chile de Agua (1,450 g m⁻²) and Huacle chili (1,700 g m⁻²) under protected conditions; in contrast, dry fruit yield was affected by the production system, with Huacle chili (474 g m⁻²) tripling the yield of chile de Agua (157 g m⁻²).

Fruit and seed quality

In relation to fruit quality, differences ($p \leq 0.05$) were found in fruit size and firmness (Table 3). Larguillo grande, Larguillo chico, Serrano, and Gallo gallina grande produced longer fruits, with values ranging from 7.73 to 8.15 cm. Likewise, Gallo gallina grande, Gallo gallina mediano, and Larguillo grande showed values from 8.10 to 14.19 cm for fruit width; however, Gallo gallina grande produced wider fruits than Larguillo chico, Cascabel, Serrano, and Mochiteco. Fruit size influenced individual fruit weight, such that Gallo gallina grande and Gallo gallina mediano produced fruits with the greatest individual weights, with values of 17.28 and 20.92 g, respectively. This diversity in fruit dimensions

Table 3. Fruit quality of native chili peppers from Teticic, Olinalá, Guerrero, grown under greenhouse conditions.

Genotype	Length	Width	Individual weight (g)	Firmness (kgf cm ⁻²)
	cm			
Cascabel	2.40 d	5.26 bc	3.17 c	1.72 a
Gallo gallina mediano	6.71 b	8.10 ab	17.28 a	1.63 ab
Larguillo grande	8.15 a	10.68 ab	6.64 bc	2.03 a
Serrano	7.73 a	1.46 c	9.70 b	1.69 ab
Mochiteco	3.97 c	1.06 c	3.20 c	2.01 a
Gallo gallina grande	7.62 ab	14.19 a	20.92 a	2.12 a
Larguillo chico	8.05 a	6.93 b	7.57 b	1.15 b
DMS:	0.98	6.73	4.29	0.56

Means followed by the same letter within the same column are not statistically different (Tukey, $\alpha=0.05$). LSD: least significant difference.

and weight among chili pepper genotypes is due to the genetic characteristics of the populations themselves (Galeote-Cid *et al.*, 2022); it may also be associated with adaptation to the climatic and edaphic conditions, as well as the crop management practices provided at the production sites, whether in the field or under greenhouse conditions (San Juan *et al.*, 2018).

Meanwhile, the results obtained in this study exceed the values reported by Sanjuan-Martínez *et al.* (2022) in Chile de Agua, Coxtle, Huacle amarillo, Huacle negro, Pasilla Mixe, and Tabiche, which ranged from 4.93 to 5.74 cm in length and from 4.27 to 4.80 cm in width. In contrast, individual dry fruit weight ranged from 4.48 to 5.56 g in Huacle chili (San Juan *et al.*, 2019). Likewise, Vázquez *et al.* (2010) reported fruit weights of 6.9 to 12.2 g in 19 Serrano chili varieties, values that fall within the range observed for Serrano (9.70 g) and Larguillo chico (7.5 g) in this study. Similarly, Tapia-Vargas *et al.* (2016) reported polar and equatorial diameters of 2.92 and 2.44 cm, respectively, and a fruit weight of 29.2 g, values similar to those obtained in the present study.

With respect to fruit firmness, Gallo gallina grande, Larguillo grande, and Mochiteco showed similar values; however, all three exceeded the firmness detected in Larguillo chico. These results were lower than those reported by Vázquez *et al.* (2010), who evaluated Serrano chili varieties (98 to 159 N cm⁻²), a physical trait influenced by variety. Firmness is an important quality variable because fruit shelf life depends largely on it (López-Salazar *et al.*, 2019).

Differences ($p \leq 0.05$) were also detected in the number of total, empty, and viable seeds, as well as in seed weight per fruit (Table 4). The Gallo gallina grande, Gallo gallina mediano, Cascabel, and Serrano genotypes produced a similar number of seeds per fruit. However, Gallo gallina grande exceeded the number of seeds in Larguillo grande, Mochiteco, and Larguillo chico by 27.02, 47.2, and 53.01%, respectively. The same pattern was observed for the number of viable seeds, in which Gallo gallina grande and Gallo gallina mediano were similar, but both exceeded the remaining genotypes. In contrast, Mochiteco and Larguillo chico had the lowest number of viable seeds per fruit. By comparison, the number of empty seeds differed only between Serrano and Larguillo grande and Mochiteco, the

Table 4. Seed quality of native chili peppers from the Mountain region of Guerrero, grown under greenhouse conditions.

Genotypes	Seeds			Seed weight (g)
	Total	Viables	Empty	
Cascabel	91.13 ab	86.96 ab	4.56 ab	0.60 bc
Gallo gallina mediano	109.86 ab	106.60 a	3.46 ab	0.94 a
Larguillo grande	82.10 bcd	80.50 ab	1.60 b	0.64 b
Serrano	87.26 abc	80.33 ab	6.63 a	0.70 b
Mochiteco	59.26 cd	58.40 bc	1.20 b	0.50 bc
Gallo gallina grande	112.50 a	107.36 a	5.20 ab	1.07 a
Larguillo chico	52.86 d	47.63 c	5.70 ab	0.38 c
DMS:	29.61	28.95	4.61	0.23

Means followed by the same letter within the same column are not statistically different (Tukey, $\alpha = 0.05$). LSD: least significant difference.

latter two being similar to each other. The Serrano genotype had 75.86 and 81.90% more empty seeds than Larguillo grande and Mochiteco, respectively. Seed weight per fruit also differed among genotypes; the seed weight of Larguillo grande and Gallo gallina mediano was similar, but greater than that of the other materials. In contrast, Cascabel, Mochiteco, and Larguillo chico showed the lowest seed weight per fruit.

In this regard, Castillo-Aguilar *et al.* (2019) reported 59 and 131 seeds per fruit in two Xcat ik chili ecotypes. The same authors noted that differences in seed size and number are common even within the same genotype. Similarly, San Juan *et al.* (2019) quantified between 173 and 203 seeds in Huacle chili fruits, with seed weights ranging from 1.0 to 1.25 g. Hernández-Verdugo *et al.* (2012) reported 15.1 seeds and a seed weight of 2.9 mg in fruits from 19 wild chili populations (*Capsicum annum* var. *glabriusculum*). In this context, the differences found in seed number and seed weight per fruit in the present study are attributed to phenotypic variation among the evaluated chili peppers. These variations are also influenced by the environment in which the materials are introduced (Hernández-Verdugo *et al.*, 2008; Hernández-Verdugo *et al.*, 2012), because some genotypes adapted rapidly to the environmental conditions that prevailed in the greenhouse, whereas others did not show a favorable response (San Juan *et al.*, 2018; Escamirosa-Tinoco *et al.*, 2021).

CONCLUSIONS

The productive performance of native chili peppers from Teticic, Olinalá, Guerrero, showed differences in fruit size, yield per plant, and fruit quality, which can be attributed to genetic variability and the environmental conditions that prevailed in the greenhouse during crop growth. The Mochiteco genotype stood out for producing the highest number of fruits. The Gallo gallina grande, Gallo gallina mediano, Larguillo chico, and Larguillo grande genotypes showed greater fresh fruit weight, whereas the Serrano genotype was characterized by greater dry fruit weight. Therefore, the diversity of native chili pepper genotypes from Teticic, Olinalá, Guerrero, cultivated under greenhouse conditions, is important for their conservation and potential use.

ACKNOWLEDGMENTS

The authors thank the producers of Teticic, municipality of Olinalá, Guerrero, Mexico, for providing access and support for germplasm collection during the conduct of this research.

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Effect of selected treatments for glyphosate substitution in the chemical weed control of Persian lime (*Citrus latifolia* Tanaka)

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ABSTRACT

Objective: To determine the effectiveness of selected treatments for weed control compared to glyphosate in a Persian lime plantation.

Design/methodology/approach: The experiment was established in Ignacio de la Llave, Veracruz, in August 2024. A randomized complete block design with six treatments and four replications was used: glyphosate at 907.5 g ha⁻¹, diuron + paraquat at 1,600 + 500 g ha⁻¹, indaziflam + paraquat at 75 + 500 g ha⁻¹, indaziflam + glufosinate-ammonium at 75 + 300 g ha⁻¹, bromacil/diuron at 1,600/1,600 g ha⁻¹, and a control with no application. Weed control was evaluated at 10, 20, 30, 40, and 50 days after application (DAA).

Results: At 50 DAA, the most effective treatments for controlling *Ruellia ciliatiflora* Hook were indaziflam + paraquat at 75 + 500 g ha⁻¹, bromacil/diuron at 1,600/1,600 g ha⁻¹, and indaziflam + glufosinate-ammonium at 75 + 300 g ha⁻¹. *Paspalum dilatatum* Poir was effectively controlled with bromacil/diuron at 1,600/1,600 g ha⁻¹ and diuron + paraquat at 1,600 + 500 g ha⁻¹. The best treatments for complete weed control were bromacil/diuron at 1,600/1,600 g ha⁻¹ and indaziflam + paraquat at 75 + 500 g ha⁻¹.

Limitations on study/implications: The effectiveness of the evaluated treatments may vary depending on the weed species present.

Findings/conclusions: Bromacil/diuron at 1,600/1,600 g ha⁻¹ and indaziflam + paraquat at 75 + 500 g ha⁻¹ can be used as alternatives to glyphosate for weed control in Persian lime plantations.

Keywords: *Ruellia ciliatiflora*, *Paspalum dilatatum*, herbicides.

Citation: Esqueda-Esquivel, V. A., Enríquez-Quiroz, J. F., & Matilde-Hernández, C. (2026). Effect of selected treatments for glyphosate substitution in the chemical weed control of Persian lime (*Citrus latifolia* Tanaka). *Agro Productividad*. <https://doi.org/10.32854/7aqjzq91>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: January 17, 2026.

Accepted: April 21, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 55-64.

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INTRODUCTION

In Mexico, the state of Veracruz is the second-largest producer of lime, surpassed only by Michoacán. In Veracruz, Persian lime (*Citrus latifolia* Tanaka) is the main cultivated species, and its production area has increased significantly during the present century. While 17,495 ha were planted in 2000, the cultivated area increased more than threefold

by 2024, reaching 52,930 ha (SIAP, 2025). This crop is an important source of direct and indirect employment and has a high benefit-cost ratio (Franco-Valderrama *et al.*, 2022).

Pests, diseases, and weeds are the main biotic constraints affecting Persian lime and citrus crops in general. In newly established plantations or during the early stages of crop development, inadequate and untimely weed control can reduce fruit yield by up to 33% due to competition for water, light, and nutrients (Martinelli *et al.*, 2017; Otieno, 2020). In mature plantations, weeds hinder agricultural operations and may serve as hosts for pests and diseases (Brown *et al.*, 2014; Onen *et al.*, 2018). For these reasons, efficient weed management is necessary, especially during critical stages of crop development and management or during periods of limited moisture availability (Bernardes *et al.*, 2021).

Although mechanical, chemical, and biological methods are available for weed control in citrus crops (Mía *et al.*, 2020; Matilde-Hernández *et al.*, 2024), the application of non-selective post-emergence herbicides, mainly glyphosate, is deeply rooted in Veracruz. Glyphosate can control annual and perennial weeds without leaving soil residues (Alcántara-de la Cruz *et al.*, 2021). However, because glyphosate does not prevent the emergence of new weed seedlings, it is generally applied three to four times per production cycle, depending on climatic conditions. Its continuous use for more than 15 years in citrus plantations in the municipalities of Cuitláhuac and Martínez de la Torre, Veracruz, resulted in the evolution of glyphosate-resistant biotypes of sprangletop grass [*Leptochloa virgata* (L.) P. Beauv.] (Pérez-López *et al.*, 2014). Currently, a total of seven weed species resistant to glyphosate have been reported in citrus orchards in Mexico (Heap, 2025).

In addition to the problems associated with the presence of herbicide-resistant weed biotypes, in 2015 the International Agency for Research on Cancer classified glyphosate as probably carcinogenic to humans (IARC, 2017). However, based on independent evaluations conducted by the United States Environmental Protection Agency, it was concluded that when glyphosate is used according to label instructions, it does not pose risks to human health and is not likely to cause cancer in humans (EPA, 2025). Consequently, substantial discrepancies still exist among scientists regarding the magnitude and significance of this issue (Tarazona *et al.*, 2017; Van Bruggen *et al.*, 2018).

In 2020, the Federal Government of Mexico published a decree in the Official Gazette of the Federation aimed at “gradually substituting the use, acquisition, distribution, promotion, and importation of the chemical substance known as glyphosate and the agrochemicals containing it as an active ingredient, with sustainable and culturally appropriate alternatives that maintain production while ensuring safety for human health, the country’s biocultural diversity, and the environment” (SEGOB, 2020).

In the search for chemical, physical, and organic alternatives to replace this herbicide in different crops and production systems, from 2021 to 2024 the Federal Government of Mexico, through SENASICA and INIFAP, funded the research and validation project entitled “Alternatives to the Use of Glyphosate for Weed Control in Mexico,” which consisted of 24 activities or subprojects. Activity 12, “Control Alternatives to Replace Glyphosate in Citrus Plantations in the State of Veracruz,” focused on identifying chemical control alternatives for Persian lime and orange crops.

In Persian lime cultivation, four experiments were established from 2021 to 2023 in the municipalities of Tlalixcoyan and Medellín, Veracruz, where a total of 19 treatments were evaluated, including both chemical herbicides and some natural products that, in uncultivated fields, had shown weed control comparable to glyphosate (Esqueda-Esquivel *et al.*, 2025). From these treatments, the four most effective were selected. Therefore, the objective of this study was to determine the effectiveness of the selected treatments for weed control compared with glyphosate in a Persian lime plantation at an early stage of development.

MATERIALS AND METHODS

On August 2, 2024, an experiment was established in a Persian lime plantation located in the community of Barrio de los Evodios, municipality of Ignacio de la Llave, Veracruz (18° 42' 49.906" N, 95° 58' 29.241" W, at 8 m above sea level). Trees were planted at a spacing of 5 m between rows and 5 m between trees within the row. The plantation was six months old, and the trees were between 1 and 1.5 m in height. A randomized complete block design with six treatments and four replications was used.

The following treatments were evaluated: 1. Glyphosate at 907.5 g ha⁻¹ (regional check), 2. Diuron + paraquat at 1,600 + 500 g ha⁻¹, 3. Indaziflam + paraquat at 75 + 500 g ha⁻¹, 4. Indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹, 5. Bromacil/diuron at 1,600/1,600 g ha⁻¹, and an untreated control. The "+" sign between two herbicides indicates a tank mixture, whereas the "/" sign indicates a commercially formulated mixture. An adjuvant, ADP (alkyl phenol), was added to treatments 1 through 5 at a rate of 2.5 mL L⁻¹ of water used.

The experimental units were located between two tree rows and measured 4 m in width and 10 m in length. A 0.5 m strip was left on each side and used as a weedy lateral control during weed control evaluations. Herbicides were applied using a Honda WJR2525T[®] motorized backpack sprayer equipped with a boom containing four 8003 flat-fan nozzles, delivering 291 L of spray solution per hectare. Applications were directed toward the weeds, which showed vigorous growth due to favorable soil moisture conditions.

Before treatment application, weed population density by species was determined using 1 m × 1 m quadrats randomly placed within the four untreated control plots, and plant height was measured in five plants of each species. At 10, 20, 30, 40, and 50 DAA, visual evaluations were conducted for weed control by dominant species, overall weed control, and toxicity to Persian lime. In both cases, a percentage scale (0-100%) was used. For weed control, 0 indicated that the treatments had no effect on the weeds, whereas 100 indicated complete weed destruction. For toxicity to Persian lime trees, 0 indicated that the treatments caused no damage to the crop, whereas 100 indicated complete plant death (Singh *et al.*, 2012). Analyses of variance were performed using arcsine-transformed data, as recommended by Dey and Pandit (2020), and Tukey's test ($p \leq 0.05$) was used for mean separation. For clarity, the following section presents the original weed control data, together with the significance groupings obtained from the mean comparisons of the transformed data.

RESULTS AND DISCUSSION

Three weed species belonging to three botanical families were identified in the experimental field. A weed population density of 950,000 plants ha⁻¹ was recorded, with *Ruellia ciliatiflora* Hook and *Paspalum dilatatum* Poir. identified as the dominant species. Together, these species accounted for 98.15% of the total weed density present in the experimental field (Table 1).

At 10 DAA, indaziflam + paraquat at 75 + 500 g ha⁻¹ provided slightly more than 99% control of *R. ciliatiflora*, which was statistically superior to the remaining treatments, whose control levels ranged from 75 to 85%, with no significant differences among them. This treatment also showed the highest levels of control of *P. dilatatum*, together with diuron + paraquat at 1,600 + 500 g ha⁻¹ and bromacil/diuron at 1,600/1,600 g ha⁻¹. Meanwhile, the control levels obtained with glyphosate at 907.5 g ha⁻¹ and indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹ ranged from 70 to 75%, and were statistically similar. For overall weed control, indaziflam + paraquat at 75 + 500 g ha⁻¹ achieved 95% control, which was statistically similar to the 87.5% effectiveness obtained with diuron + paraquat at 1,600 + 500 g ha⁻¹. Glyphosate at 907.5 g ha⁻¹ provided only slightly more than 70% control, similar to that obtained with bromacil/diuron at 1,600/1,600 g ha⁻¹ and indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹ (Table 2). During the study, none of the treatments caused toxicity to Persian lime trees (data not shown).

At 20 DAA, indaziflam + paraquat at 75 + 500 g ha⁻¹ maintained almost complete control of *R. ciliatiflora*, which was significantly superior to the remaining treatments, although indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹ and bromacil/diuron

Table 1. Scientific name, botanical family, population density, and average height of weed species at the experimental site. Barrio de los Evodios, municipality of Ignacio de la Llave, Veracruz, 2024.

Scientific name	Botanical family	Density (plants ha ⁻¹)	Average height (cm)
<i>Ruellia ciliatiflora</i> Hook	Acanthaceae	602,500	9.10
<i>Paspalum dilatatum</i> Poir	Poaceae	330,000	17.40
<i>Cyperus eragrostis</i> Lam.	Cyperaceae	17,500	17.43
Total		950,000	

Table 2. Effect of treatments on weed control by species and overall weed control (%) at 10 DAA.

Treatment (g ha ⁻¹)	<i>R. ciliatiflora</i>	<i>P. dilatatum</i>	Total
Gly (907.5)	76.25 b	73.75 bc	72.50 bc
Diu + Par (1,600 + 500)	83.75 b	98.00 a	87.50 ab
Ind + Par (75 + 500)	99.25 a	91.25 a	95.00 a
Ind + Glu (75 + 300)	82.50 b	70.00 c	70.00 c
Bro/Diu (1,600/1,600)	80.00 b	90.00 ab	72.50 bc
Control without application	0.00 c	0.00 d	0.00 d

Gly=Glyphosate, Diu=Diuron, Par=Paraquat, Ind=Indaziflam, Glu=Glufosinate ammonium, Bro=Bromacil. Letters to the right of the control values indicate Tukey's test ($p \leq 0.05$). Values followed by the same letter are not statistically different. Comparisons were made among treatments for each variable.

at 1,600/1,600 g ha⁻¹ provided control levels of 90 and 97%, respectively. With glyphosate at 907.5 g ha⁻¹ and diuron + paraquat at 1,600 + 500 g ha⁻¹, control was around 85%. Control of *P. dilatatum* ranged from 90 to 99% with the same treatments identified at 10 DAA, whereas with glyphosate at 907.5 g ha⁻¹ and indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹, control was lower than 80 and 70%, respectively. For the overall weed population, indaziflam + paraquat at 75 + 500 g ha⁻¹, diuron + paraquat at 1,600 + 500 g ha⁻¹, and, for the first time, bromacil/diuron at 1,600/1,600 g ha⁻¹ were the treatments with the highest and statistically similar levels of control. Glyphosate at 907.5 g ha⁻¹ and indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹ provided control levels ranging from 70 to 75% (Table 3).

At 30 DAA, indaziflam + paraquat at 75 + 500 g ha⁻¹ still maintained almost complete control of *R. ciliatiflora*, and was statistically superior to the remaining treatments, although bromacil/diuron at 1,600/1,600 g ha⁻¹ and indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹ achieved control levels greater than 90%. Control of this species with glyphosate at 907.5 g ha⁻¹ was close to 85%, whereas with diuron + paraquat at 1,600 + 500 g ha⁻¹ it was lower than 80%. For *P. dilatatum*, both diuron + paraquat at 1,600 + 500 g ha⁻¹ and bromacil/diuron at 1,600/1,600 g ha⁻¹ showed 99% control, and were statistically superior to the remaining treatments. Indaziflam + paraquat at 75 + 500 g ha⁻¹ provided control close to 90%, whereas control with glyphosate at 907.5 g ha⁻¹ and indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹ was poor. For the overall weed population, indaziflam + paraquat at 75 + 500 g ha⁻¹ and bromacil/diuron at 1,600/1,600 g ha⁻¹ achieved control levels greater than 90%, and were statistically superior to the remaining treatments. Diuron + paraquat at 1,600 + 500 g ha⁻¹ provided control close to 85%, whereas glyphosate at 907.5 g ha⁻¹ achieved slightly more than 70% control, and indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹ only 65% control (Table 4).

At 40 DAA, indaziflam + paraquat at 75 + 500 g ha⁻¹, bromacil/diuron at 1,600/1,600 g ha⁻¹, and indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹ provided control of *R. ciliatiflora* ranging from 90 to 100%, and were statistically similar to each other and superior to the remaining treatments. Control with glyphosate at 907.5 g ha⁻¹ was slightly greater than 70%, significantly exceeding the approximately 50% control provided by diuron + paraquat at 1,600 + 500 g ha⁻¹. For *P. dilatatum*, only bromacil/diuron at 1,600/1,600 g

Table 3. Effect of treatments on weed control by species and overall weed control (%) at 20 DAA.

Treatment (g ha ⁻¹)	<i>R. ciliatiflora</i>	<i>P. dilatatum</i>	Total
Gly (907.5)	84.25 c	76.25 b	74.50 bc
Diu + Par (1,600 + 500)	85.00 c	99.00 a	87.50 ab
Ind + Par (75 + 500)	99.25 a	91.25 a	95.00 a
Ind + Glu (75 + 300)	90.00 bc	66.25 b	71.25 c
Bro/Diu (1,600/1,600)	97.00 b	98.75 a	95.50 a
Control without application	0.00 d	0.00 c	0.00 d

Gly=Glyphosate, Diu=Diuron, Par=Paraquat, Ind=Indaziflam, Glu=Glufosinate ammonium, Bro=Bromacil. Letters to the right of the control values indicate Tukey's test ($p \leq 0.05$). Values followed by the same letter are not statistically different. Comparisons were made among treatments for each variable.

Table 4. Effect of treatments on weed control by species and overall weed control (%) at 30 DAA.

Treatment (g ha ⁻¹)	<i>R. ciliatiflora</i>	<i>P. dilatatum</i>	Total
Gly (907.5)	83.75 cd	65.00 c	72.50 bc
Diu + Par (1,600 + 500)	77.50 d	99.00 a	83.75 b
Ind + Par (75 + 500)	99.25 a	89.75 b	93.00 a
Ind + Glu (75 + 300)	91.75 bc	57.50 c	65.00 c
Bro/Diu (1,600/1,600)	95.50 b	99.00 a	96.00 a
Control without application	0.00 e	0.00 d	0.00 d

Gly=Glyphosate, Diu=Diuron, Par=Paraquat, Ind=Indaziflam, Glu=Glufosinate ammonium, Bro=Bromacil. Letters to the right of the control values indicate Tukey's test ($p \leq 0.05$). Values followed by the same letter are not statistically different. Comparisons were made among treatments for each variable.

ha⁻¹ and diuron + paraquat at 1,600 + 500 g ha⁻¹ maintained control levels greater than 95%, and were statistically superior to the remaining treatments, whereas control with indaziflam + paraquat at 75 + 500 g ha⁻¹ was slightly greater than 85%, and significantly superior to glyphosate at 907.5 g ha⁻¹ and indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹, which showed the lowest levels of control. Overall weed control with bromacil/diuron at 1,600/1,600 g ha⁻¹ was 96%, whereas indaziflam + paraquat at 75 + 500 g ha⁻¹ provided control close to 90%, although both treatments were statistically similar. In the remaining chemical treatments, control ranged from 55 to 65% (Table 5).

Finally, at 50 DAA, indaziflam + paraquat at 75 + 500 g ha⁻¹, bromacil/diuron at 1,600/1,600 g ha⁻¹, and indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹ maintained control of *R. ciliatiflora* ranging from 90 to 100%, and were statistically similar and superior to glyphosate at 907.5 g ha⁻¹ and diuron + paraquat at 1,600 + 500 g ha⁻¹, which only outperformed the untreated control. At this evaluation time, bromacil/diuron at 1,600/1,600 g ha⁻¹ and diuron + paraquat at 1,600 + 500 g ha⁻¹ still maintained control levels greater than 90% of *P. dilatatum*. With indaziflam + paraquat at 75 + 500 g ha⁻¹, control was close to 85%, and significantly superior to that obtained with indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹ and glyphosate at 907.5 g ha⁻¹, which were the chemical treatments with the lowest levels of control. Regarding the overall weed population, control with bromacil/diuron at 1,600/1,600 g ha⁻¹ was 95.5%, whereas with

Table 5. Effect of treatments on weed control by species and overall weed control (%) at 40 DAA.

Treatment (g ha ⁻¹)	<i>R. ciliatiflora</i>	<i>P. dilatatum</i>	Total
Gly (907.5)	71.25 b	52.50 c	63.75 b
Diu + Par (1,600 + 500)	52.50 c	98.50 a	63.75 b
Ind + Par (75 + 500)	99.25 a	86.00 b	87.75 a
Ind + Glu (75 + 300)	91.25 a	46.25 c	55.00 b
Bro/Diu (1,600/1,600)	94.25 a	98.75 a	96.00 a
Control without application	0.00 d	0.00 d	0.00 c

Gly=Glyphosate, Diu=Diuron, Par=Paraquat, Ind=Indaziflam, Glu=Glufosinate ammonium, Bro=Bromacil. Letters to the right of the control values indicate Tukey's test ($p \leq 0.05$). Values followed by the same letter are not statistically different. Comparisons were made among treatments for each variable.

indaziflam + paraquat at 75 + 500 g ha⁻¹ it was 85.5%, although both were statistically similar. With indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹ and glyphosate at 907.5 g ha⁻¹, control was around 50% (Table 6).

Although the ban on glyphosate use published in the Official Gazette of the Federation in 2020 was based mainly on risks to human health, most regulatory agencies worldwide have indicated that the proper use of glyphosate does not pose risks to human health or the environment (Alcántara-de la Cruz *et al.*, 2021). Likewise, studies have shown that the risk of glyphosate (Hunter and Blecker, 2019) and other pesticides being inhaled or coming into contact with the skin or eyes of applicators is significantly reduced when protective clothing is used (Yarpuz-Bozdogan, 2018; Naksata *et al.*, 2020). It is also known that environmental contamination problems occur mainly when doses higher than those recommended and multiple applications during the crop cycle are used (Yamada *et al.*, 2009; Hanke *et al.*, 2010). Therefore, regulating glyphosate application in terms of dosage, number of applications, and use of protective clothing would be more appropriate than banning it.

The increasing occurrence of glyphosate-resistant weed biotypes in different crops and countries is a matter of concern. As of December 2025, 388 glyphosate-resistant biotypes belonging to 61 weed species have been reported in 30 countries (Heap, 2025), and it is considered that additional cases still remain undocumented. For this reason, it is very important to have alternative chemical control options, such as those identified in this experiment, that can be used in situations where glyphosate-resistant weed biotypes are already present, or where this herbicide has been applied for many years and there is a high risk of their appearance. Rotation with herbicides having different modes of action reduces selection pressure on individual sites of action and eliminates resistant biotypes already present or delays their appearance (Beckie, 2006; Norsworthy *et al.*, 2012).

In this experiment, it was determined that the best alternatives to glyphosate for overall weed control were bromacil/diuron at 1,600 + 1,600 g ha⁻¹ and indaziflam + paraquat at 75 + 500 g ha⁻¹. The first treatment is applied in preemergence or early postemergence and can maintain efficient control of grass and broadleaf weeds for more than 90 days without causing toxicity to citrus crops (Singh and Tucker, 1988). In two experiments conducted in Persian lime orchards in the state of Puebla, Alcántara-de la

Table 6. Effect of treatments on weed control by species and overall weed control (%) at 50 DAA.

Treatment (g ha ⁻¹)	<i>R. ciliatiflora</i>	<i>P. dilatatum</i>	Total
Gly (907.5)	61.25 b	37.50 c	48.75 b
Diu + Par (1,600 + 500)	43.75 b	96.00 ab	53.75 b
Ind + Par (75 + 500)	99.25 a	83.50 b	85.50 a
Ind + Glu (75 + 300)	90.75 a	40.00 c	50.00 b
Bro/Diu (1,600/1,600)	93.75 a	98.00 a	95.50 a
Control without application	0.00 c	0.00 d	0.00 c

Gly=Glyphosate, Diu=Diuron, Par=Paraquat, Ind=Indaziflam, Glu=Glufosinate ammonium, Bro=Bromacil. Letters to the right of the control values indicate Tukey's test ($p \leq 0.05$). Values followed by the same letter are not statistically different. Comparisons were made among treatments for each variable.

Cruz *et al.* (2019) determined that at 75 DAA, control of hairy beggarticks (*Bidens pilosa* L.) and *L. virgata* with glyphosate at 1,080 g ha⁻¹ was around 40%, whereas when the bromacil + diuron mixture at 1,200 + 1,200 g ha⁻¹ was added, control increased to nearly 90%. This indicates that this mixture may be useful for controlling weed species that show resistance to glyphosate. However, it should be applied only once during the crop cycle because bromacil has very high mobility in the soil and, under conditions of high rainfall, may leach, reducing the weed control period and increasing the risk of groundwater contamination (Gomez de Barreda *et al.*, 1993).

Regarding the indaziflam + paraquat treatment, the first herbicide is applied only in preemergence, and its effectiveness may vary according to the dose, soil texture, and weed species present (Amim *et al.*, 2014). Esqueda-Esquivel (2012) found that at a dose of 50 g ha⁻¹, control of *L. virgata* at 75 DAA ranged from 90 to 95%, whereas the control provided by diuron at 1,600 g ha⁻¹ was only around 40%. However, when comparing the effectiveness of indaziflam alone, indaziflam + rimsulfuron, and rimsulfuron alone, Faber *et al.* (2016) determined that six months after application, weed control was significantly greater when the mixture of both herbicides was applied compared with their individual application. In Persian lime plantations developed exclusively under rainfed conditions, it is almost impossible to apply indaziflam in preemergence because weeds are continuously present. Under these conditions, the indaziflam + paraquat mixture could be very useful, since paraquat would control emerged weeds, whereas indaziflam would prevent the emergence of new weed seedlings for an extended period of time. In Mexican lime cultivation in the state of Colima, Orozco-Santos and García-Mariscal (2023) determined that this mixture, at doses of 100 and 400 g ha⁻¹, provided efficient weed control for six months. In addition, herbicide costs were reduced by 45% compared with the regional standard treatment, consisting of six glyphosate applications. Lozano-Contreras and Avilés-Baeza (2025) indicated that the same treatment, applied at doses of 200 + 800 g ha⁻¹ in orange cultivation in Yucatán, in addition to providing efficient weed control up to 75 DAA, favored the vegetative development of the trees by increasing canopy diameter, stem thickening, and shoot production. This represents an additional advantage over glyphosate application, since in Brazil it has been demonstrated that the repeated use of this herbicide can significantly reduce tree vigor and fruit production in citrus crops, greatly decreasing their financial viability (Martinelli *et al.*, 2022).

Although in this experiment the mixtures of diuron + paraquat at 1,600 + 500 g ha⁻¹ and indaziflam + glufosinate ammonium at 75 + 300 g ha⁻¹ did not show the same effectiveness in overall weed control or duration of control as the two treatments previously mentioned, with other weed species they could also represent alternatives for glyphosate replacement. Therefore, it is recommended to carry out an exploratory application of the four treatments in a small area of the orchard before applying any of them to the entire plantation area.

It is very important to indicate that, in order to avoid toxicity to citrus crops with any herbicide treatment, contact of the spray solution with the foliage or trunk of the trees should be avoided, especially in newly established plantations or during early stages of development (Kanissery *et al.*, 2021).

CONCLUSIONS

The formulated mixture of bromacil/diuron at a dose of 1,600/1,600 g ha⁻¹ and the tank mixture of indaziflam + paraquat at a dose of 75 + 500 g ha⁻¹ are efficient alternatives to replace glyphosate for weed control in Persian lime cultivation.

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Seed Quality and Germination Dynamics in Small-Grain Cereals

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ABSTRACT

Objective: To determine the quality and germination dynamics of seeds of rye cv. Elbon, wheat cv. Valles, and triticale cv. T5.

Design/methodology/approach: Seeds were separated by size (small and large) and subjected to different conditioning treatments (accelerated aging). Study variables included the number of pure seeds, inert material, other seeds, thousand-seed weight, bulk density, moisture content, germination percentage, emergence speed, mean germination time, germination rate, germination speed coefficient, and germination uncertainty and synchrony indices. Analysis of variance was conducted using a randomized factorial design with four replications for each species and seed size. Means were compared using Tukey's test ($P < 0.05$).

Results: The rye lot exhibited a long shelf life and medUIm vigor, while wheat and triticale seeds were short-lived and of low vigor. In all three species, large seeds were more vigorous than small seeds; however, seed size did not affect the temporal distribution of germination. Aging reduced germination percentage, emergence speed, germination rate, and synchrony index, while increasing mean germination time and the uncertainty index.

Limitations on study/implications: None

Findings/conclusions: Large seeds of rye, wheat, and triticale exhibit higher vigor, while seed aging negatively affects germination dynamics.

Keywords: accelerated aging, germination, seed size, *Secale cereale* L., *Triticum aestivum* L., X *Triticosecale* Wittmack

Citation: Domínguez-Martínez, P. A., Mendoza-Pedroza, S. I., Pro-Martínez, A., Torres-Hernández, G., Vaquera-Huerta, H., & Alejos-de la Fuente, J. I. (2026). Seed Quality and Germination Dynamics in Small-Grain Cereals. *Agro Productividad*. <https://doi.org/10.32854/f0jezm27>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: January 28, 2026.

Accepted: April 22, 2025.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 65-73.

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INTRODUCTION

The use of high-quality germplasm is crucial during the initial stage of any crop, as it enables successful seedling establishment (Steiner *et al.*, 2019). Seed quality encompasses physical, physiological, genetic, and sanitary attributes (Bewley *et al.*, 2013; Carranza-



González *et al.*, 2022). These attributes must be in balance for a grain lot to be classified as high quality (Costa *et al.*, 2021). Among the parameters that determine seed quality, physical quality is particularly relevant and includes aspects such as purity and grain size. Genetic quality ensures that the seed maintains a high degree of similarity to the original germplasm. Likewise, sanitary quality focuses on ensuring that the grain is free of pests and diseases. Finally, physiological quality refers to the germinative capacity and vigor of the germplasm under field conditions at the time of sowing (Delouche, 1985).

Crop yield and efficient resource use depend on the successful establishment of seedlings; seed vigor determines germination capacity and the rapid, uniform establishment of seedlings under diverse agroclimatic conditions (Finch-Savage & Bassel, 2016). Seeds reach their maximum vigor when the mother plant attains physiological maturity (Delouche, 1985; Costa *et al.*, 2021). However, grain is harvested only once its moisture content falls below 14% (harvest maturity) to prevent mechanical damage during harvest and storage (Copeland & McDonald, 2001). Between physiological and harvest maturity, seeds remain on the mother plant and are exposed to adverse environmental conditions that may reduce their quality (Copeland & McDonald, 2001; Costa *et al.*, 2021). Moreover, seeds are rarely sown immediately after harvest; consequently, they may be exposed to suboptimal conditions during storage (Copeland & McDonald, 2001; Domínguez-Martínez *et al.*, 2024a).

Germination is a developmental response of an individual seed that occurs at a specific moment; however, within a lot or treatment, seeds may germinate at different times (Orchard, 1977; Kader, 2005). For example, two seed lots may exhibit the same germination percentage, yet one may complete germination four days earlier than the other. This demonstrates that germination percentage alone is insufficient for comparing seed lots. To address this issue, various methods and techniques have been proposed. Time, rate, uniformity, and synchrony are parameters that can be measured to describe the dynamics of the germination process (Kader, 2005; Ranal & Santana, 2006).

Physical quality, physiological quality, and germination dynamics may be affected in seed lots after a storage period. Based on this, the objective of this study was to determine the quality and germination dynamics of rye cv. Elbon, wheat cv. Valles, and triticale cv. T5 seeds.

MATERIALS AND METHODS

This study was conducted at the Colegio de Postgraduados, Montecillo Campus. The site is located at an altitude of 2,250 m and has a temperate subhumid climate with summer rainfall, classified by García (1998) as Cb(wo)(w)(i)g. The mean annual temperature is 15 °C, and the average annual precipitation is 645 mm (García, 1998).

Three seed lots classified as “Declared” category were sampled. The first lot corresponded to rye cv. Elbon, the second to wheat cv. Valles, and the third to triticale cv. T5. For each lot, four primary samples were obtained and mixed to form a 1-kg composite sample. The composite sample was homogenized in the laboratory, and from it a 500-g working sample was extracted to determine the physical and physiological quality and the vigor of the seeds.

Moisture content was determined using the direct method from a 300-g sample collected separately for each species. In the laboratory, two 10-g subsamples (per species) were taken and placed in aluminum containers with lids and known weights. The containers with the seed samples were placed in a drying oven at 130 °C for two hours. Moisture content was calculated using the following formula:

$$\% \text{moisture} = \left(\frac{P_2 - P_3}{P_2 - P_1} \right) \times 100$$

where: P_1 corresponds to the weight of the container and its lid (g), P_2 is the weight of the container, lid, and seed (g), and P_3 is the weight of the container, lid, and seed after oven drying. For the determination to be accepted as valid, the difference in moisture percentage between the two subsamples of each species had to be less than 0.2%.

Physical quality was determined based on the amount of pure seed (PS), inert matter (IM), other seeds (OS), thousand-seed weight (TSW), bulk density (BD), and moisture content (MC), following the methodologies proposed by AOSA (2014). Physiological quality was evaluated through germination percentage (GP), emergence speed (ES; Maguire, 1962), and the accelerated aging test (Delouche & Baskin, 1973), using the modifications proposed by Huber *et al.* (1982) and Kim *et al.* (1985).

Mean germination time (MGT), mean germination rate (MGR), germination speed coefficient (GSC), germination uncertainty index (UI), and germination synchrony index (SI) were calculated to describe the germination dynamics of the different seed lots (Ranal & Santana, 2006).

Seeds were separated by size using a 2.38-mm sieve. All seeds retained on the sieve were classified as large (L), while those that passed through the mesh were considered small (S). Conditioning treatments were applied to 800 seeds of each size: 400 seeds underwent accelerated aging (AA), and the remaining 400 were left without aging (NA). The combination of the two factors (size \times conditioning) resulted in four treatments, with four replications of 100 seeds per species.

The germination test for each species was conducted in a 2.5 m² seedbed using sterilized sand as the substrate. The distribution of treatments and replications was randomized within each seedbed. Each replication was sown in four rows, with 25 seeds per row, placed at a depth of 2 cm and spaced 3 cm apart within and between rows.

Analysis of variance for physiological quality variables was performed using a randomized factorial experimental design, with four replications for each species and seed size. Means were compared using Tukey's test ($P < 0.05$). Data analysis was conducted using SAS software v. 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Seeds classified as "Declared" or "Commercial" must comply with the standards established for the "Certified" category (SNICS, 2014). In the present study, only the wheat seed lot met the minimum requirement of 98% pure seed, whereas rye and triticale

did not comply with this standard (Table 1). All three lots exceeded the maximum allowed limit (1%) for inert matter. The regulation allows 0.02% for other seeds; in this regard, the rye and triticale lots did not meet this requirement.

The TSW of rye was 8 to 15 g lower than that of wheat and triticale. Rye also had a lower bulk density compared to the other two species. Authors such as Voloshchuk *et al.* (2023) compared the TSW of three varieties of each species and reported that rye consistently exhibits a lower TSW than wheat and triticale. Furthermore, Aprodu and Banu (2016) found that rye grains are smaller and less compact, which explains why its TSW and BD are lower than the values observed in wheat and triticale. Although rye grains were smaller compared to wheat and triticale, the values obtained in the present study fall within the range reported for the species. Hansen *et al.* (2004) found that among 19 rye genotypes, TSW ranged from 20 to 39 g, while BD ranged from 70.2 to 80.6 kg hL⁻¹. In another study, Carranza-González *et al.* (2022) evaluated 24 wheat genotypes and reported that TSW varied between 29 and 48 g and BD ranged from 76 to 82 kg hL⁻¹. The values obtained for wheat cv. Valles in the present study fall within the aforementioned ranges. The interaction of genetic, epigenetic, and environmental factors influences grain development, which ultimately determines seed yield and quality (Wang & Sun, 2021). In general, large seeds are preferred because they have better-developed embryos, a greater amount of reserves to support early growth (Carvalho & Nakagawa, 2000), and better field performance; however, results regarding germination, emergence, and growth vary among species (Zareian *et al.*, 2013). Additionally, evidence indicates that selecting cultivars with higher TSW can increase grain yield (Paquini *et al.*, 2016).

Seeds from all three lots had moisture contents below the upper limit of 13% established for the Declared category (SNICS, 2014). Moisture content plays a key role in seed deterioration or preservation during storage (Pomeranz, 1992; Ranganathan & Groot, 2023). Harrington (1972) proposed, as a general rule, that for grains with 5 to 14% moisture, shelf life doubles each time moisture content decreases by 1%.

In the rye lot, large (L) seeds germinated 8% more and emerged at a higher rate (2.6 plants day⁻¹) than small (S) seeds (Table 2). Likewise, mean germination time was higher in the small seeds (0.3 days) compared to the large seeds. The mean germination rate of large seeds was 0.01 plants day⁻¹ higher than that of small seeds. Seed size had no significant effect ($P < 0.05$) on the germination speed coefficient or on the germination uncertainty and synchrony indices. On the other hand, the aging treatment reduced germination and emergence rate by approximately 11% (5.5 plants day⁻¹) in the small seeds. Additionally, mean germination time was 0.9 days longer in seeds subjected to accelerated aging.

Table 1. Physical quality of rye cv. Elbon, wheat cv. Valles, and triticale cv. T5 seeds.

	PS ¹ (%)	IM (%)	OS (%)	TSW (g)	BD (kg hL ⁻¹)	MC (%)
Rye cv. Elbon	97.11	2.05	0.84	21.38	76.34	6.50
Wheat cv. Valles	98.80	1.20	0.00	30.25	77.22	8.00
Triticale cv. T5	97.23	2.55	0.22	36.63	77.22	8.50

¹PS=pure seed, IM=inert matter, OS=other seeds, TSW=thousand-seed weight, BD=bulk density, and MC=moisture content.

Table 2. Germination percentage (GP), emergence speed (ES), mean germination time (MGT), mean germination rate (MGR), germination speed coefficient (GSC), and germination uncertainty (UI) and synchrony (SI) indices of rye cv. Elbon seeds classified by size and subjected to different conditioning treatments.

Source of variation		GP (%)	VE (plants d ⁻¹)	MGT (d)	MGR (plants d ⁻¹)	GSC	UI	SI
Size	S ¹	82.0 ^b	17.7 ^b	4.8 ^a	0.21 ^b	29.2	1.3	0.5
	L	90.0 ^a	20.3 ^a	4.5 ^b	0.22 ^a	28.8	1.0	0.5
Conditioning	AA	80.2 ^b	16.2 ^b	5.1 ^a	0.20 ^b	29.9	1.6 ^a	0.4 ^b
	NA	91.7 ^a	21.7 ^a	4.2 ^b	0.23 ^a	28.1	0.8 ^b	0.6 ^a
Interaction	S × AA	74.7	14.2	5.4 ^a	0.18	29.2	1.7	0.4
	S × NA	89.2	21.1	4.3 ^c	0.23	29.1	0.9	0.6
	L × AA	85.7	18.2	4.8 ^b	0.21	30.5	1.4	0.4
	L × NA	94.2	22.4	4.2 ^c	0.24	27.0	0.6	0.7

¹S=small seed, L=large seed, AA=seeds subjected to accelerated aging, and NA=non-aged seeds. Values presented for seed size and conditioning correspond to the mean of eight observations. Means for the interaction of factors are based on four observations. ^{a,b} Different letters within a column and source of variation indicate statistically significant differences, while the absence of letters indicates no significant difference (Tukey; P≤0.05).

The interaction of both factors had the greatest effect on the small aged seeds (S × AA), increasing mean germination time by 0.6 days compared to large aged seeds. The mean germination rate was higher in the non-aged treatment (0.03 plants day⁻¹) compared to the aged treatment. Finally, the germination uncertainty index in aged seeds was twice that of the non-aged seeds, whereas synchrony was higher (0.2) in the latter group.

Germination of small seeds in the wheat lot was 23.7% lower than that of large seeds. Among the conditioning treatments, the difference (42.9%) favored the non-aged seeds (Table 3). L and NA seeds emerged 4.5 and 9.6 plants day⁻¹ faster than S and AA seeds, respectively. The size × conditioning interaction was significant for ES, and it was observed that aging negatively affected this variable regardless of seed size; however, the most pronounced reduction occurred in small aged seeds. Significant differences (P<0.001) for mean germination time were found only between conditioning treatments, with aged seeds taking 1.7 days longer to germinate.

For mean germination rate, significant differences were observed between conditioning treatments (P<0.001) and for the interaction (P=0.028). Non-aged seeds germinated at a rate 0.05 plants day⁻¹ higher than aged seeds. Additionally, the negative effect of the AA treatment was of similar magnitude in both seed size categories, and large non-aged seeds (L × NA) had a higher germination rate (0.05 plants day⁻¹) compared to small non-aged seeds (S × NA). Greater germination uncertainty (UI) was found in aged seeds (AA), while synchrony (SI) was higher in non-aged seeds (NA) (P<0.001).

Seed size, conditioning, and the interaction of factors were significant (P<0.001) for GP, ES, MGT, and MGR (Table 4). Small seeds (S) germinated less (9.5%) and at a lower rate (2.7 plants day⁻¹) than L seeds. The aging treatment (AA) reduced GP and ES by 21% (6.3 plants day⁻¹). Small seeds in combination with accelerated aging (S × AA) showed the most severe reduction in germination percentage and emergence speed. MGT increased

Table 3. Germination percentage (GP), emergence speed (ES), mean germination time (MGT), mean germination rate (MGR), germination speed coefficient (GSC), and germination uncertainty (UI) and synchrony (SI) indices of wheat cv. Valles seeds classified by size and subjected to different conditioning treatments.

Source of variation		PG (%)	VE (plants d ⁻¹)	MGT (d)	MGR (plants d ⁻¹)	GSC	UI	SI
Size	S ¹	36.3 ^b	6.6 ^b	6.1	0.16	31.4	1.7	0.3
	L	60.0 ^a	11.1 ^a	6.0	0.17	28.1	1.7	0.4
Conditioning	AA	26.7 ^b	4.0 ^b	6.9 ^a	0.14 ^b	30.7	2.1 ^a	0.2 ^b
	NA	69.6 ^a	13.6 ^a	5.2 ^b	0.19 ^a	28.8	1.3 ^b	0.5 ^a
Interaction	S × AA	14.5	2.2 ^d	6.8	0.14 ^c	32.0	1.9	0.2
	S × NA	58.2	11.0 ^b	5.5	0.18 ^b	30.8	1.5	0.4
	L × AA	39.0	5.8 ^c	7.0	0.14 ^c	29.4	2.2	0.2
	L × NA	81.0	16.3 ^a	5.0	0.19 ^a	26.9	1.2	0.5

¹S=small seed, L=large seed, AA=seeds subjected to accelerated aging, and NA=non-aged seeds. Values presented for seed size and conditioning correspond to the mean of eight observations. Means for the interaction of factors are based on four observations. ^{a,b}Different letters within a column and source of variation indicate statistically significant differences, while the absence of letters indicates no significant difference (Tukey; P≤0.05).

Table 4. Germination percentage (GP), emergence speed (ES), mean germination time (MGT), mean germination rate (MGR), germination speed coefficient (GSC), and germination uncertainty (UI) and synchrony (SI) indices of triticale cv. T5 seeds classified by size and subjected to different conditioning treatments.

Source of variation		PG (%)	VE (plants d ⁻¹)	MGT (d)	MGR (plants d ⁻¹)	GSC	UI	SI
Size	S ¹	59.9 ^b	11.8 ^b	5.6 ^a	0.18 ^b	29.1 ^b	1.8	0.3
	L	69.4 ^a	14.5 ^a	5.1 ^b	0.20 ^a	33.1 ^a	1.7	0.4
Conditioning	AA	54.1 ^b	10.0 ^b	5.9 ^a	0.17 ^b	31.0	1.9 ^a	0.3 ^b
	NA	75.1 ^a	16.3 ^a	4.8 ^b	0.21 ^a	31.2	1.5 ^b	0.4 ^a
Interaction	S × AA	44.3 ^c	7.3 ^c	6.4 ^a	0.16 ^c	28.0	2.0	0.3
	S × NA	75.5 ^a	16.3 ^a	4.8 ^c	0.21 ^a	30.1	1.6	0.4
	L × AA	64.0 ^b	12.7 ^b	5.3 ^b	0.19 ^b	34.0	1.9	0.4
	L × NA	74.8 ^a	16.2 ^a	4.8 ^c	0.21 ^a	32.3	1.5	0.4

¹S=small seed, L=large seed, AA=seeds subjected to accelerated aging, and NA=non-aged seeds. Values presented for seed size and conditioning correspond to the mean of eight observations. Means for the interaction of factors are based on four observations. ^{a,b}Different letters within a column and source of variation indicate statistically significant differences, while the absence of letters indicates no significant difference (Tukey; P≤0.05).

by 0.5 days in small seeds, 1.1 days with EA, and the interaction of factors was significant (P<0.001). Regarding MGR, the opposite behavior to MGT was observed for both factors and their interaction. Large seeds showed a germination speed coefficient 4% higher than the S seeds.

For the germination uncertainty and synchrony indices, differences were only found between conditioning treatments (P<0.001 and 0.004, respectively). Uncertainty increased

by 0.4 when seeds were subjected to AA. Conversely, synchrony was higher (0.1) in seeds that were not conditioned.

In México, as previously established, commercial seeds must comply with the regulations corresponding to the Certified category. Regarding germination, the cereal seed grading rule sets 90% as the minimum (SNICS, 2014). In this case, only in the rye lot were large (L), non-aged (NA), and L × NA seeds found to meet or exceed this requirement. On the other hand, the accelerated aging treatment (AA) negatively affected germination in all three species; however, the most considerable damage was observed in wheat cv. Valles. In this regard, Delouche and Baskin (1973) mention that a seed lot maintaining more than 80% germination after accelerated aging (AA) is considered long-lived, whereas lots failing this criterion are considered short-lived.

Mathias and Coelho (2021) classified soybean seed lots as high vigor when they retained $\geq 85\%$, medUIm vigor 70-84%, and low vigor $< 70\%$ of their germination after artificial aging treatment. In general terms, the rye lot in the present study can be considered long-lived with medUIm vigor. In contrast, wheat and triticale seeds were short-lived and of low vigor. However, these findings contradict previous evidence indicating that rye has lower longevity compared to other small-grain cereals (Peters *et al.*, 1964; Specht *et al.*, 1997).

Emergence speed is a methodology that allows comparison of vigor among samples from the same seed lot (Maguire, 1962). In this regard, high values indicate that those samples are more vigorous than the others (Ranal & Santana, 2006). In contrast to the results of the present study, in a barley cv. Brennus lot, small seeds emerged at a higher speed than large seeds; moreover, the negative effect of aging was more pronounced on small seeds (Domínguez-Martínez *et al.*, 2024b). In another study, seed size and its interaction with the aging treatment did not have a significant effect on VE in oat seeds cv. Turquesa (Domínguez-Martínez *et al.*, 2024a).

Germination is a response of seed development that occurs over time; however, within a lot or treatment, individual seeds may respond (germinate) at different times (Talská *et al.*, 2020). Consequently, germination percentage alone is insufficient for comparative analysis between datasets. Therefore, various methods and techniques have been proposed to measure germination and address this issue. Time, rate, uniformity, and synchrony are aspects that can be measured to describe the dynamics of the germination process (Kader, 2005; Ranal & Santana, 2006). Mean germination time (MGT) indicates the day on which the mean germination occurs; consequently, a low MGT value reveals that that seed population reaches germination more quickly compared to others (Orchard, 1977). Mean germination rate (MGR) is calculated as the reciprocal of MGT ($1/\text{MGT}$); thus, high values indicate a higher daily germination rate in that population (Ranal & Santana, 2006). The germination speed coefficient (GSC) shows the rate at which germination occurs (Jones & Sanders, 1987). This value increases as the number of germinated seeds rises and the time required for germination decreases (Talská *et al.*, 2020). On the other hand, UI is an adaptation of the Shannon index that measures the degree of uncertainty associated with the distribution of relative germination frequency; in other words, it measures the dispersion of germination over time (Ranal & Santana, 2006). Conversely, SI describes the level of overlap in germination, that is, how concentrated this process is over time (Ranal &

Santana, 2006). Seed lots are preferably characterized by low UI values and high SI values to promote rapid establishment and uniformity of crops.

CONCLUSIONS

The rye lot evaluated in the present study was long-lived with medium vigor. Wheat and triticale seeds were short-lived and of low vigor. In all three species, large seeds were more vigorous than small seeds. Large seeds of the three species germinated more and at a higher speed compared to small seeds; however, seed size had no effect on the distribution of germination over time. Aging significantly reduced germination, emergence speed, mean germination rate, and synchrony index, while increasing mean germination time and germination uncertainty.

ACKNOWLEDGMENTS

The authors thank the LGAC: Efficient Livestock with Biotechnological Precision, Sustainable Wellbeing, and Climate Change for the support and facilities provided for the completion of this work.

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Correlation between second-tier bank credit and agricultural GDP in Mexico

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ABSTRACT

Objective: to evaluate the impact that the operation of second-tier bank loans has had on Mexico's agricultural Gross Domestic Product (GDP).

Design/Methodology/Approach: a set of 1360 data records corresponding to the operation of the discounted credit and the one guaranteed by the Trusts Instituted in Relation to Agriculture (in Mexico, FIRA) were used, as well as the agri-food Gross Domestic Product (GDP) reported by the Bank of Mexico (BANXICO). These records were grouped into 40 four-month periods between March 2013 and December 2022. The information was presented in constant values to be comparable; it was analyzed through a correlation model with the stepwise procedure in R statistical software.

Results: a 0.1471 positive impact on agricultural GDP growth was observed for each unit of credit drawn down by FIRA with a determination coefficient $R^2=0.973$. This impact derives from FIRA's large participation in the agri-food sector; and it was also highly influenced by seasonality in agricultural cycles.

Limitations/ Implications of the study: by shortening the periods of time analyzed, the results can be affected by external factors as happened with the recent global economic contraction generated by the COVID-19 pandemic in 2020 and whose effects have lasted for three years.

Findings/Conclusions: FIRA has fulfilled its character within development banking by adapting its policies and services to the economic conditions of the moment. And for adjusting to those that are required, within its financial model, to maintain and promote the placement of the credit necessary for the operation and growth of agricultural companies in Mexico.

Keywords: agricultural credit, development banking, FIRA (Mexico's Trusts in relation to Agriculture).

Citation: Aguilar-Soler, J. C., Torres-Bermúdez, E. G., Pérez-Elizalde, S., & López-Reyna, M. del C. (2026). Correlation between second-tier bank credit and agricultural GDP in Mexico. *Agro Productividad*. <https://doi.org/10.32854/agrop.v17i12.2853>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: March 6, 2024.

Accepted: April 6, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 75-81.

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INTRODUCTION

Agricultural enterprises in Mexico present strong challenges, basically generated by the reconfiguration of the population towards urban areas and their demographic growth, climate change and change in eating habits, as well as the disaggregation of agricultural income towards other productive activities (Cartón, 2009; FIRA, 2020). In this new environment, a more productive and innovative agriculture is required (González & Orrantia, 2006; FIRA, 2020). It is also required to maintain an availability of economic resources wherever there is a high demand; second-tier credits must play an active role there.

However, the risks inherent to agricultural activity, such as variations in production yields, price fluctuations, and natural disasters, as well as asymmetric information and high transaction costs, cause a decreased motivation towards the activity of development banks to be able to serve the sector (Acevedo & Delgado, 2002). So greater efforts have been focused on addressing lower-risk activities, such as consumer and government credit.

Although this ignores the favorable impact of agricultural activity on the national economy (Clavellina, 2013; Cruz & Mayren, 2014; Torre & Flores, 2020).

As a response to this problem, development banking (De la Vega *et al.*, 2014) emerges as an instrument specialized in mitigating risks by providing guarantees and other support to financial intermediaries that favor inclusion in more entrepreneurial credits. Such is the case of FIRA, as a second-tier bank specialized in meeting the financing needs of agriculture in Mexico. In addition to incentivize the placement of credits, FIRA seeks to diversify them by training credit subjects and incorporating new financial intermediaries to achieve greater incidence in this sector (Del Ángel, 2005 & Huidobro, 2012).

The importance of the agricultural sector lies in the fact that it is the starting engine of the economy (Cruz & Mayren, 2014), since in addition to supplying food to the population, it is a generator of labor and wealth to other productive sectors. This means it is both a primary consumer of manufacturing and industrial companies, and a supplier of raw materials. This sector is currently identified as the main source of foreign exchange in the trade balance of the country. It is important to recognize the impact of development banking on the generation of wealth and the capitalization of the sector (Espinoza & Martínez, 2017) and its future challenges. For example, according to the National Agricultural Survey (INEGI, 2019) only 8.4% of agricultural companies have access to credit.

For all of the above, the objective of this study was to measure, through a regression model, the impact that the credit of FIRA (Mexico's Trusts instituted in relation to Agriculture) and the use of their guarantees, both have had on the agri-food Gross Domestic Product -GDP between 2013 and 2022.

MATERIALS AND METHODS

The research was based on the compilation and analysis of 1360 records of FIRA's credit operation and the quarterly value of agricultural GDP, which make up the time series for the period 2013 to 2022. Figures presented in billions of Mexican pesos (\$ MXN). The first group of data comes from the information provided by FIRA, divided into three categories and updated to constant values until December 2022. The first category corresponds to the credit operation of each regional division of FIRA in Mexico, Northwest, North, Central, South and the Yucatan Peninsula.

The second category refers to financed activity, primary, industry, commerce and services. And the third category corresponds to the operational records: Funding (F), Guarantee without Funding (GSF) and Paid Guarantees (GP). Because the GPs correspond to loans with recovery problems, they are outside the objective of this study, which is only to measure the impact of the dynamic resource. GP was thus discarded, and the variables of Funding and Guarantees without Funding were grouped. Funding indicates the FIRA resources used directly by financial intermediaries to grant credits (as discounts). On the other hand, the Guarantee without Funding refers to the resources granted by financial intermediaries with resources from their own treasury, but which are covered by a FIRA guarantee (induced credit).

The third category corresponds to the information obtained from the Bank of Mexico, presented in constant values until December 2022. This group is made up of credit portfolios and national GDP. The credit portfolios are made up of the Commercial Banking portfolio, Total current credit portfolio to the private sector, Total current portfolio by credit destination, Companies and individuals with business activity, in the agricultural, forestry and fishing sectors. For its part, the quarterly GDP for the years 2013 to 2022, also in constant values until December 2022, is broken down into primary (agriculture), secondary (manufacturing) and tertiary (services and trade) activities. For this study, in order to limit the impact of FIRA and relate it to GDP, only the value corresponding to primary activities was considered.

The hypothesis proposes to demonstrate that FIRA, in its role as a development bank, positively affects agri-food GDP, by promoting investment activities and a better regional distribution of resources.

The data used for the construction of the model are described below:

- Trim (X): Quarter of the year (X=1-4);
- CASP: Total current credit portfolio by credit destination companies and individuals with business activity: Agriculture, forestry and fishing;
- CCVT: Total outstanding credit portfolio to the private sector;
- CCEM: Total current credit portfolio for companies and individuals with business activity;
- FGSF: FIRA Funding and Guarantees without Funding for primary activity (100); Industry (200), Trade (300) and Services (400) by Regional and Total;
- PIB: Gross Domestic Product (GDP);
- PIAP: Domestic Product in Primary Activity;
- PIPT: Total Domestic Product.

The model was adjusted with the use of the ordinary least squares method in the statistical package R; a selection of significant variables was made based on the adjusted R^2 value. With the stepwise procedure, those variables with a greater incidence in magnitude on the model were identified, to verify the obtaining of the expected signs.

RESULTS AND DISCUSSION

As a result, the following model was obtained:

$$PIAP_t = \beta_0 + \beta_1 COVID_t + \beta_2 T_t^{(2)} + \beta_3 T_t^{(3)} + \beta_4 T_t^{(4)} + \beta_5 t + \beta_6 FGDFTOT_t + \varepsilon_t, t = 1, \dots, T$$

where: *PIAP*, PIB primary activities; *COVID*, indicator variable of the pandemic period caused by the SARS-Cov2 virus; $T^{(j)}$, indicator variable for the quarter j , $j=2, 3, 4$; T , tendency; *FGDFTOT*, funds from the Trusts Instituted in Relation to Agriculture – in Mexico FIRA; and ε , experimental error (0-mean and σ^2 -variance).

It is shown that determining variables in the behavior of agricultural GDP in the period evaluated were Funding, the total amount guaranteed by FIRA (FGDFTPT), and the effect of the quarter of the year Trim (Q). The covid variable was included to evaluate the impact it had on the economic recession of 2020, and to determine how macroeconomic factors may affect the behavior of Mexico's agriculture credit.

As it can be seen, regression is significant ($p \leq 0.05$) with a coefficient of determination $R^2 = 0.973$; that is, 97% of the observed variability is explained by the adjusted model. In addition, all the effects were significant and with the expected signs.

The Covid pandemic had a negative effect (-10640) on PIAP. While the quarterly effects were all statistically significant and are expressed as differences compared to the first quarter. The fourth quarter (Q4) was the one in which a greater increase in the PIAP was observed compared to the first quarter. The effects of the trend variables (t) and FGSFTOT were statistically significant with $p \leq 0.0001$ and $p \leq 0.05$, respectively.

The adjusted model has a high predictive power, with a correlation between the values observed, in a test set (40%) and those predicted 0.98. This means that the prediction of the estimated future behavior of these data has a 98% probability, they will behave similarly to the actual observed data (Figure 1).

Similarly, through the graphical analysis of effects (Figure 2) it is seen how the PIAP varies as a function of FGFTOT with a high confidence interval; observable by the proximity of the processed data to the line estimated according to the model.

The quarters of the year had a significant effect on the PIAP behavior, which showed increases in quarters 1, 2 and 4. This can be explained by the behavior of agricultural cycles and their demand for credit; since 55% of FIRA's operation is allocated to agriculture (FIRA, 2022). Thus, 90% of the harvest of the spring-summer cycle (PV) is concentrated in January; while the planting and demand for inputs of this cycle occurs between March and April. On the other hand, in the autumn-winter cycle (OI), 64% of the harvest occurs during May and June, but its planting and demand for inputs is highest in the months of October and November (FIRA, 2023) (Figure 3).

Results are consistent and expected, according to the findings by other authors. Huidobro (2012), for example, pointed out the fundamental role of development banks in

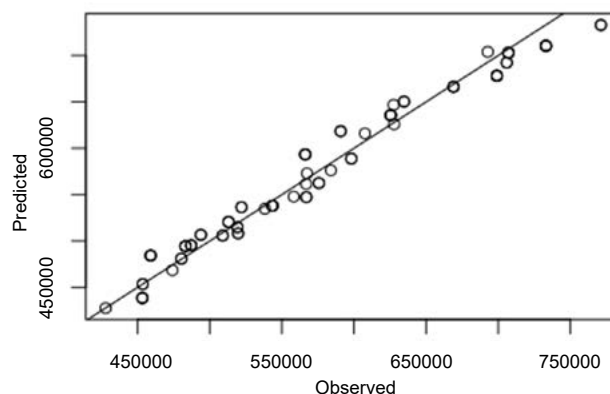


Figure 1. Prediction of the behavior of expected GDP vs. real GDP.
Source: elaborated by the authors for this study.

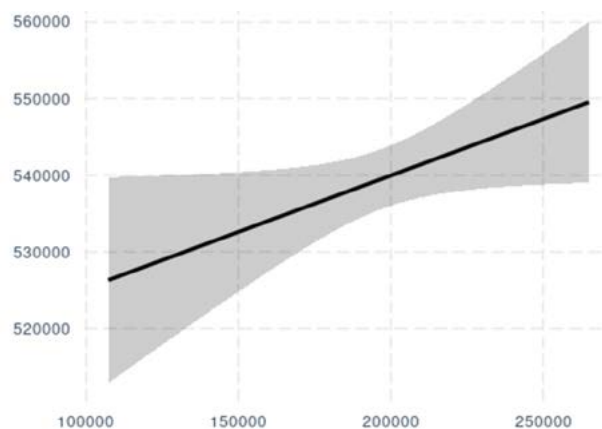


Figure 2. Confidence interval of the impact of the FGSFTOT on the PIAP.
Source: elaborated by the authors for this study.

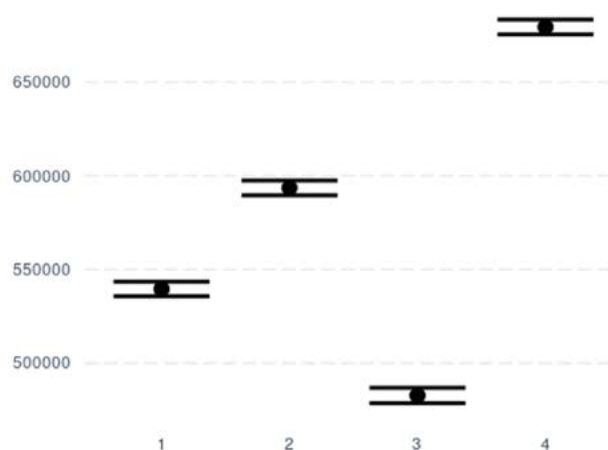


Figure 3. Impact of the quarters of the year on the PIAP.
Source: elaborated by the authors for this study.

providing solutions to long-term financing needs, in order to contribute to the economic development of companies. A fact that leads to the generation of wealth, growth, increase in the labor force and with it, the economic gross income to the country, which is measured as an increase in GDP.

Lara (2021) in his research, highlighted the importance of the entry over time of those companies that had access to financing with FIRA resources. While Espinoza and Martínez (2017) highlighted the positive role played by development bank credit in increasing agriculture GDP analyzed from 1970 to 2010.

However, there is still a long way to go, therefore, according to De la Vega *et al.* (2014), development banks have sought their permanence towards increasing their revenues through higher financing costs. Because of this, second-tier banking remains at short results in terms of depth and scope, since it has not generated enough impact to grow support for the most unprotected producers. Nor for more innovative projects, but still focuses its efforts on servicing short-term loans, added to the low interest of banks in supporting loans

to the primary sector (Almeraya, 2011). In addition, the lack of mechanisms for attention to producers and credits, plus the asymmetry of the markets, all of which limits the interest of producers with fewer resources to apply for a loan or even approaching the banks.

CONCLUSIONS

The Covid pandemic (2020-2021) had a negative effect on the GDP of primary activities. The gross domestic product at the end of the evaluation period was 10,640 units lower than that at the beginning, in March 2020. In the second and fourth quarters, increases were observed in the GDP of primary activities; while in the third quarter a decrease was observed. This seasonality effect is explained by the demand for resources exerted by the agricultural cycles, in which there is a high demand combined both for harvesting work and for the continuous start of sowing in the autumn-winter and spring-summer cycles.

In the study period, a quarterly increase of 2531 units in the GDP of primary activities was observed, which shows a positive trend in the timeline. Regarding the impact that the FIRA resource has on agricultural GDP, the model showed that for each credit unit drawn by FIRA, a positive impact of 0.1471 can be observed on agricultural GDP. This confirms the important role that FIRA plays in this development banking sector. As well as the need to establish strategies with companies and the government to continue growing operations in support of the agricultural sector.

ACKNOWLEDGEMENTS

To the Trusts Instituted in Relation to Agriculture – FIRA (in Mexico) for the facilities provided for access to their information. To the Universidad Iberoamericana Campus Santa Fe, Mexico City and the Colegio de Postgraduados, in Montecillo, State of Mexico, for their support to this study.

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Description of the physical characteristics of the eggs from creole hens and commercial eggs

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ABSTRACT

Objective: to compare the external and internal physical characteristics and yolk color of eggs with white, brown or blue shells from creole hens, and the commercial eggs with white and brown shells from hens bred in layer-farms, which are sold in the region of Mezquital Valley (Hidalgo), Mexico.

Design/Methodology/Approach: eggs from creole hens were collected in traditional markets (called tianguis in Mexico) customarily placed in the Mezquital Valley (Hidalgo), Mexico. The colors of the shell were blue (n=100), white (n=100), brown (n=100), in addition, pieces of eggs with white shell (n=100) and brown shell (n=100) were purchased in retailer stores. In those stores no eggs with blue shells were found. Variables evaluated in the eggs were weight, area, volume, shape index, length and width, whites and yolk weight; also, color intensity of the yolk was evaluated with the DSM™ Yolk color fan.

Results: the blue egg in this study presented ($p \leq 0.05$) the highest weight (61 *vs.* 53, ± 0.66 g), area (72 *vs.* 65, ± 0.67 cm²) and volume (56 *vs.* 48, ± 0.60 cm³) compared to the creole white egg. Creole brown egg, as well as commercial white and commercial brown eggs had a similar shape index (78, 79 and 77, ± 0.65 %). Egg length was also similar; no significant differences were found ($p > 0.05$). However, the creole white egg had the smallest egg width compared to the other types of egg. The blue egg had the highest weight of whites, and the creole white egg the lowest, compared to the other types of egg (36 *vs.* 30, ± 0.51 g). Creole blue, commercial white and commercial brown eggs had similar yolk weight (19, 18 and 18, ± 0.52 g). The commercial white and commercial brown eggs had the highest color intensity in the yolk (10.3 and 11.3, ± 0.15 DSM™ Yolk color fan), whereas the creole white egg had the least colorful yolk (7.4 ± 0.15 DSM™ Yolk color fan).

Limitations/Implications of the study: backyard poultry farming in the state of Hidalgo is limited by the loss of rural areas and creole hens are displaced by commercial breeds.

Findings/Conclusions: the blue egg from backyard creole hens was larger than the commercial egg, while the creole white egg was the smallest. However, this characteristic does not affect nutritional quality. Commercial white egg and commercial brown egg had the highest color intensity in the yolk. The color of the yolk is due to the carotenoids present in the yolk that are beneficial for human health.

Keywords: creole hens, commercial eggs, blue shell, physical characteristics, yolk color.

Citation: Rodríguez-Ortega, L. T., Hernández-Guzmán, F. J., Pro-Martínez, A., Zárate Contreras, D., Leyva-Jiménez, H., & Rodríguez-Ortega, A. (2026). Description of the physical characteristics of the eggs from creole hens and commercial eggs. *Agro Productividad*. <https://doi.org/10.32854/cyramb69>

Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: February 25, 2025.
Accepted: April 16, 2026.
Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 83-91.

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INTRODUCTION

Eggs are an inexpensive and accessible source of protein for low-income families in Mexico. Creole hens are an important genetic resource that must be conserved and reproduced, since they have hardiness to survive in difficult places, also resistance to parasites and diseases (Toalombo *et al.*, 2019). Eggs from creole hens can be found for sale in traditional markets or retailer stores in towns or neighborhoods (Jerez-Salas *et al.*, 2009). Those eggs are an important source of income for low-income families in Mexico. Shades of color of shell are very varied; shells can be white, brown, blue or green (Rodríguez-Ortega *et al.*, 2020). Although stores and shops only sell commercial white and commercial brown eggs from specialized hens, also known layer-farm hens.

Creole hens range freely in the backyards of rural households, fed on insects, wild plants, seeds, and sometimes small amounts of concentrate. This domestic production system provides a state of well-being in the hens. However, in this type of farming, there is little management of parasites and diseases, which results in low production and high mortality. It seems necessary to promote backyard poultry farming; but the increase in urban areas has generated a reduction in small production units. This has caused the populations of creole hens, turkeys, ducks and geese to be severely reduced. The objective of this research was to compare the external and internal physical characteristics and yolk color of the eggs from creole hens with white, brown, or blue shells, and the commercial eggs of specialized hens with white and brown shells which are sold in the region of the Mezquital Valley (Hidalgo), Mexico.

MATERIALS AND METHODS

The study was done at the UPFIM-Poultry Unit under the Polytechnic University “Francisco I. Madero” (UPFIM), which is located in Tepatepec (Hidalgo), Mexico. Egg pieces from creole hens were collected in the tianguis (the traditional Mexican street-market) of Actopan (Hidalgo) and in the tianguis of Tepatepec, in the municipality of Francisco I. Madero (Hidalgo), Mexico. Eggs were collected from creole hens with white (n=100), brown (n=100) and blue (n=100) shells, and eggs with white (n=100) and brown (n=100) shells were purchased in local retailer stores, no eggs with blue shells were found in those stores.

External physical characteristics

In a refrigerator, the collected egg was stored at 10 °C until analysis, the external physical characteristics were egg weight (P), length (L) and width (A), these were measured with a digital vernier (Model HER-411, STEREN, Mexico, Figure 1) with a measurement range of 0 to 150 mm and resolution of 0.1 mm. The shape index (Si) was obtained according to Rodríguez-Ortega *et al.* (2021), with the formula $Si = A/L \times 100$, the volume (V; cm³) was calculated as described by Illescas-Cobos *et al.* (2022), $V = 0.913 \times P$; and area (A; cm²) according to Rodríguez-Ortega *et al.* (2021), with $A = 4.558 \times P$.

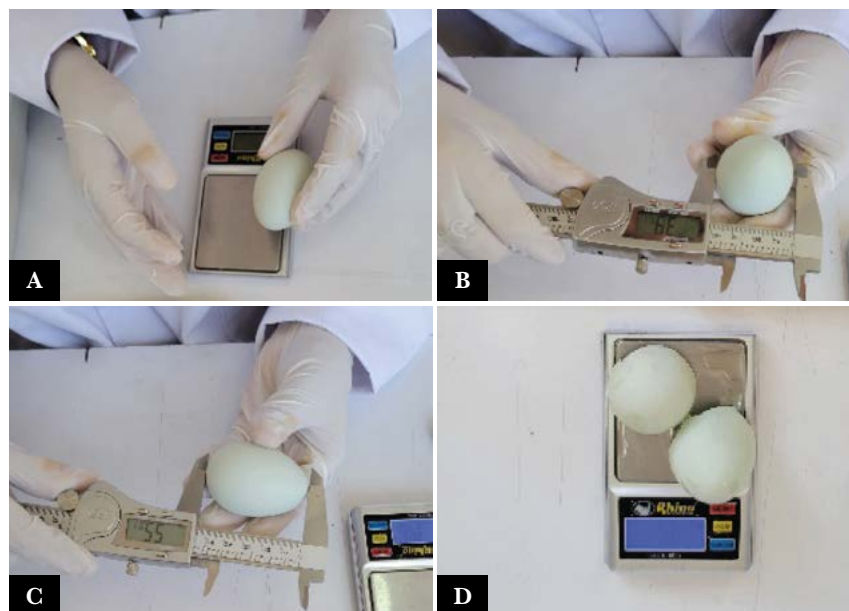


Figure 1. Measurement of egg pieces; A: egg weight, B: width, C: length, and D: shell weight.

Internal physical characteristics

The internal physical characteristics evaluated were yolk weight, whites weight, and yolk color. The weight of the egg whites and yolk weight were recorded with a digital scale with a capacity of 200 g and a resolution of 0.01 g (Model MH-200, MKS Tools brand, China). Yolk color was determined visually, by comparing the color of the yolk with a value in the DSM™ Yolk color fan (Figure 2). Two evaluators agreed on the color that matched the one in the color fan. Colors of the fan range from 1 (the lightest) to 15 (the most orange). The color fan provides a convenient and economical way to assess yolk coloration.

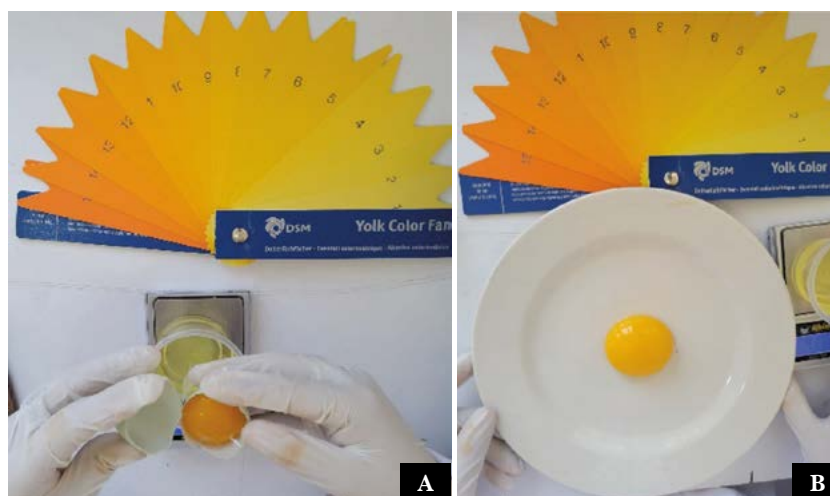


Figure 2. Evaluation of yolk color with the DSM™ Yolk color fan.

Statistical analysis

Egg weight, length, width, shape index, area, volume, whites weight, yolk weight, relative weight of whites to yolk, and yolk color were analyzed with the PROC GLM procedure. The means were compared with the Tukey test in SAS[®] (SAS Institute, Inc., 2013). The color of the yolk was analyzed with the GLM and PROC FREQ procedure of SAS[®] (SAS Institute, Inc., 2013). The PROC CORR procedure was used to determine the relation of egg weight to the weight of whites and shell weight.

RESULTS AND DISCUSSION

Egg weight

The size and color of eggs are physical characteristics that attract the consumer. The blue color of the shell is due to biliverdin, a natural pigment that is deposited in the shell gland (Hernández-Guzmán *et al.*, 2021). The blue egg had the highest weight ($p \leq 0.05$) compared to the other types of egg, whereas the white egg from creole hens [Creole-white] was the smallest ($p \leq 0.05$) compared to the others (Figure 3).

Consumers prefer large and heavy pieces of egg, however, the size of the egg depends on the breed, age and diet of the hens (Hernández-Guzmán *et al.* 2021). Juárez-Caratachea *et al.* (2010) reported that the egg from creole hens is smaller than the egg from commercial breeds. However, internal quality is similar to that of the commercial egg. Such results coincide with those found in this study, commercial white and commercial brown eggs were larger than the white egg [Creole-white] from creole hens (Figure 3).

Oñate-Mancero *et al.* (2020) reported that eggs of creole hens are less heavy, weight does not exceed $50 \text{ g} \pm 4.97$; in our study those results coincided only with the weight of the creole white egg. The Official Mexican Standard (NOM-FF-127-SCFI-2016) classified chicken eggs as follows; over 64 g they are considered extra-large; large 60-64 g; medium 55-60 g; small 50-55 g; and marble, less than 50 g. According to this classification in the Mexico's Official Gazette of the Federation (DFO, 2004), the creole white egg was labelled as small, and all the other types of eggs were large (Figure 3).

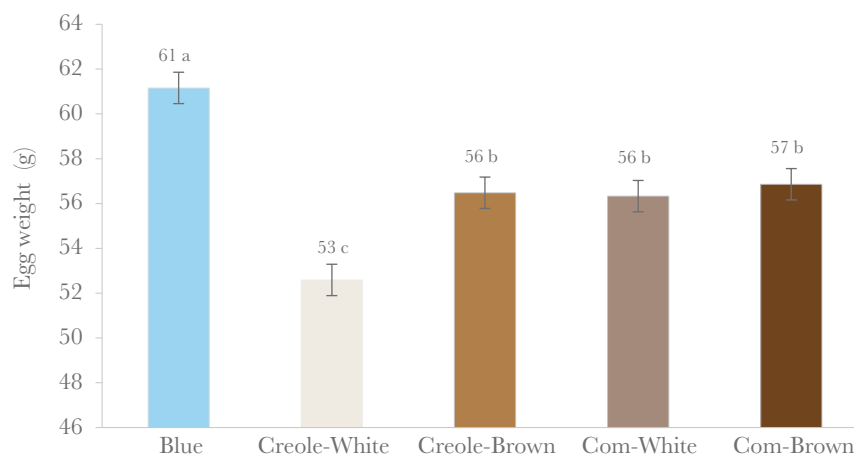


Figure 3. Weight of eggs; blue (Azul), creole white (Creole-White), creole brown (Creole-Brown), commercial white (Com-White) and commercial brown (Com-Brown). Different letters indicate significant differences (Tukey; $p \leq 0.05$).

Egg Area

Egg area and volume are two important geometric variables for the poultry industry. Because large egg pieces make packing difficult (Narushin, 2005) and the calculation of those variables is not easy due to the sui generis shape of the egg. The eggs are not circular or elliptical, they are ovoid; the rounded wider end is called the base, and the pointed end is called the tip. They can be classified into four groups by form, oval, pyriform, circular and elliptical (Nishiyama, 2012). Eggs with blue shells had the largest area ($p \leq 0.05$), while the white egg from creole hens had the lowest area value. The pieces of creole brown, commercial white and commercial brown eggs had a similar ($p > 0.05$) area (Figure 4). These results are in agreement with those reported by Hloko *et al.* (2022), who evaluated the egg area of intensively bred hens and recorded values between 64.96 cm^2 and $71.16 \text{ cm}^2 \pm 0.26$.

Egg volume

Eggs from any bird begin to lose weight as soon as they are laid, but their volume and area do not change during storage or incubation (Karabulut, 2021). Volume is a variable that is related to egg weight and shell strength; estimation of area and volume can help to reduce losses due to transportation, storage and packaging. Larger eggs should be packed in materials that withstand the external forces exerted during grading and transport (Altuntas and Sekeroglu, 2008). The blue egg had the highest volume, while the lowest was observed in the creole white egg (Figure 5). The pieces of creole brown, commercial white and commercial brown eggs had ($p \leq 0.05$) similar volume (Figure 5). Results in this study indicate that egg area and egg volume increased as the egg weight increased. This is in agreement with Rodríguez-Ortega *et al.* (2021), who observed that the volume of white eggs from Bovans White commercial hens was in the range from 59 to 62 cm^3 .

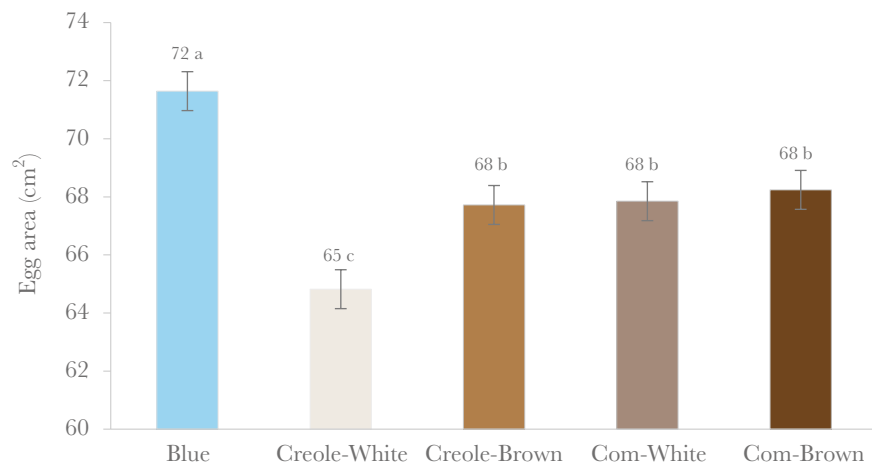


Figure 4. Egg area; blue, creole white (Creole-White), creole brown (Creole-Brown), commercial white (Com-White) and commercial brown (Com-Brown). Different letters indicate significant differences (Tukey; $p \leq 0.05$).

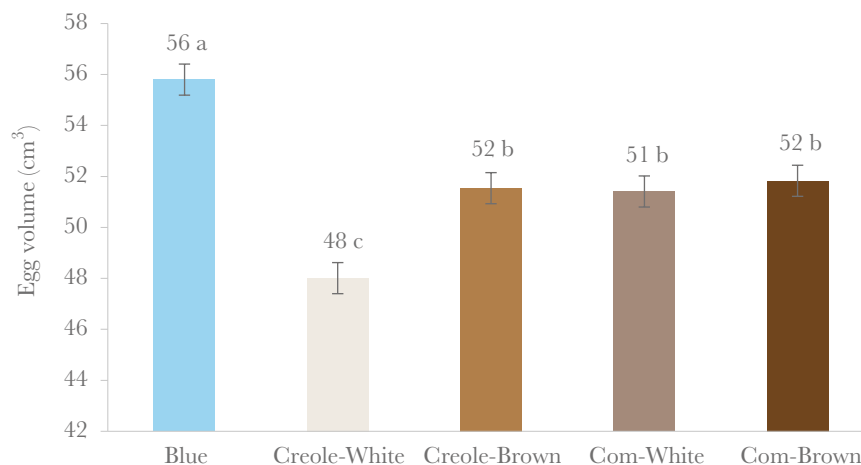


Figure 5. Eggs volume; blue, creole white (Creole-White), creole brown (Creole-Brown), commercial white (Com-White) and commercial brown (Com-Brown). Different letters indicate significant differences (Tukey; $p \leq 0.05$).

Egg length and egg width

Length and width of eggs are important physical characteristics in the poultry industry. An increase in egg length can generate friction with the packaging material thus causing shell breaks. Egg length was similar ($p > 0.05$) in the egg types evaluated (blue, 5.8 cm; creole white, 5.6 cm; creole brown, 5.7 cm; commercial white, 5.9 cm; and commercial brown, 5.9 cm, ± 0.29). However, there were significant differences ($p \leq 0.05$) in egg width. The creole white egg presented the smallest ($p \leq 0.05$) width value (4.1 cm \pm 0.02), whereas width average value of eggs, blue, creole brown, commercial brown and commercial white, were similar (4.3 cm \pm 0.02) for all the other types ($p > 0.05$). The values in this study are similar to those observed by Hernández-Guzmán *et al.* (2021), who reported that the length of the blue egg from Araucana hens was 5.73 cm \pm 0.26, and the width 4.33 cm \pm 0.16.

Shape Index

The shape index (Si) is the relation of egg width to egg length. Creole brown, commercial white and commercial brown eggs presented a similar ($p \leq 0.05$) shape index; whereas, creole white and blue eggs had the lowest ($p \leq 0.05$) value of shape index (Figure 6). Shape index is a variable with which the thickness of the shell can be predicted. Romera *et al.* (2022) observed that the shape index is correlated with shell strength, the higher the shape index value the lower the shell strength. Our results could indicate that commercial egg pieces are less durable than blue and creole white eggs (Figure 6).

Whites weight and yolk weight of the eggs

The egg inside is composed of whites and yolk, and on the outside by the shell, whose main function is to protect the inside content (Rodríguez-Ortega *et al.*, 2020). Blue eggs had the highest ($p \leq 0.05$) whites weight, while the creole white egg had the lowest (Table 1). The weight of whites in this study coincides with that reported by Rodríguez-Ortega *et al.* (2025), who reported the weight of whites in a range from 30 to 35 g.

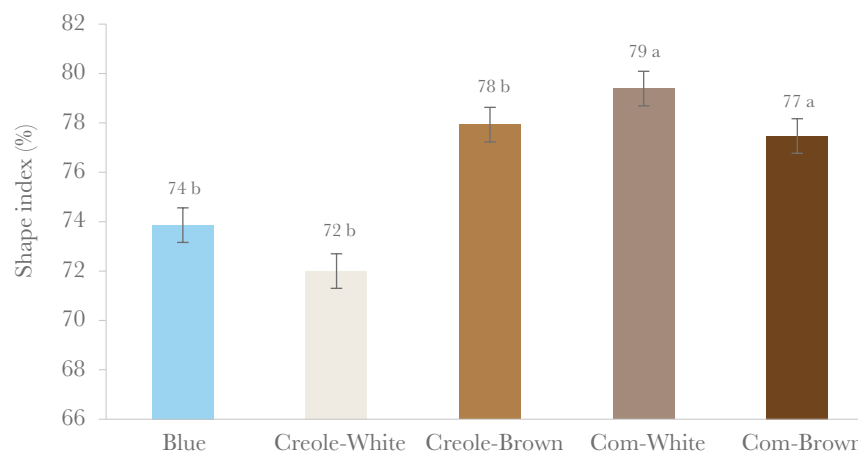


Figure 6. Shape index of eggs; blue, creole white (Creole-White), creole brown (Creole-Brown), commercial white (Com-White), and commercial brown (Com-Brown). Different letters indicate significant differences (Tukey; $p \leq 0.05$).

The highest yolk weight was found in blue eggs, commercial white and commercial brown eggs; whereas, the lowest yolk weight was observed in creole brown and creole white eggs (Table 1). The yolk weight in this study was similar to that observed by Rodríguez-Ortega *et al.* (2021), who found an average $17.59 \text{ g} \pm 0.07$ value of yolk weight in the eggs from commercial Bovans White hens. Finally, shell weight was similar in all the types of egg evaluated (Table 1).

Yolk color

Consumers prefer yolks with intense color, because people attribute to those a higher concentration of nutrients and greater freshness. The color of the yolk depends on the hen's diet. The yellow color of eggs yolk is due to the carotenoids present in the diet (Aparicio *et al.*, 2018). Lutein and zeaxanthin are the carotenoids with the highest presence in the yolk, these pigments are beneficial for human health, due to their antioxidant and anti-inflammatory effects (Rodríguez-Rodríguez *et al.*, 2021). Commercial white and commercial brown eggs had the most colorful yolks ($p \leq 0.05$) compared to the other types of eggs (Figure 7). This result could be due to the consumption of commercial feed enriched with pigment.

Table 1. Weights of the whites, yolk and shells of eggs; blue, creole white, creole brown, commercial white and commercial brown, in Hidalgo, Mexico.

	White (g)	Yolk (g)	Egg shell (g)
Blue	36 a	19 a	8.0 a
Creole white	30 c	15 b	7.6 a
Creole brown	32 b	15 b	7.6 a
Commercial white	32 b	18 a	8.0 a
Commercial brown	33 b	18 a	7.8 a
Standard error	0.511	0.525	0.212
p-value	0.001	0.001	0.0584

Different letters within a column indicate significant differences (Tukey; $p \leq 0.05$).

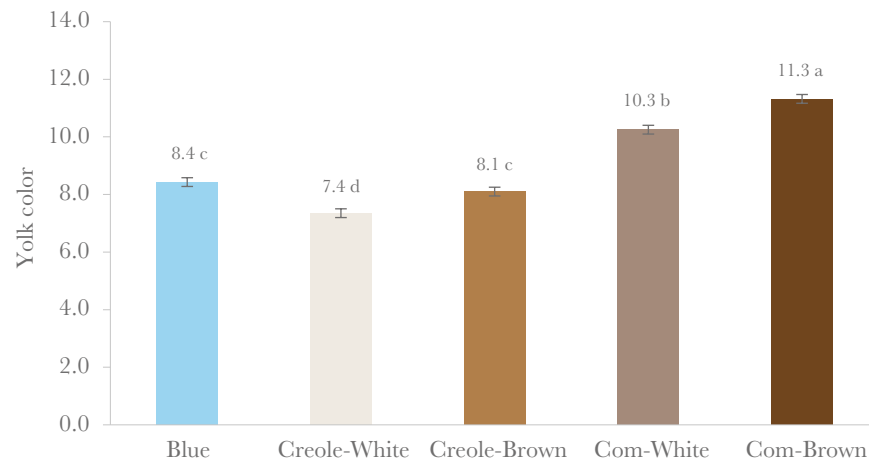


Figure 7. Yolk color in eggs; blue, creole white (Creole-White), creole brown (Creole-Brown), commercial white (Com-White) and commercial brown (Com-Brown). Different letters indicate significant differences (Tukey; $p \leq 0.05$).

The commercial feed used for hens in layer-farms contains added pigments with the goal of increasing yolk color. The creole white, creole brown and blue eggs presented less intense ($p \leq 0.05$) yolk color compared to the commercial eggs. This is probably due to backyard creole hens are fed on a diet with a minimum of commercial feed, but higher in kitchen residues such as bread, tortilla and some vegetables. Creole hens spend much of the day ranging in the wild, looking for insects or seeds to satisfy their physiological needs, thus consuming low concentrations of carotenoids.

CONCLUSIONS

The egg with blue shell from hens in backyard poultry was larger than the commercial eggs, whereas the creole white egg was the smallest. However, this characteristic did not affect nutritional quality. The commercial white and commercial brown eggs had the highest color intensity in the yolk. That color is due to the carotenoids present in the yolk that are beneficial for human health.

Creole hens are a source of income from the sale of eggs and meat for low-income families in the Mezquital Valley (Hidalgo), Mexico. For this reason, it is considered necessary to promote the development of more backyard poultry farming, thus preventing creole hens from being completely displaced by commercial breeds.

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Arbuscular mycorrhizal fungi improve water use, nutrient uptake, and fruit quality in strawberries

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ABSTRACT

Objective: To evaluate the water expenditure of two strawberry cultivars (Albion and San Andreas) inoculated with three arbuscular mycorrhizal fungi (AMF) grown in two substrates. Moreover, to determine their efficiency in the strawberry plant nutrition and fruit quality.

Design/Methodology/Approach: Five treatments were established: plants inoculated with *Funneliformis geosporum*, *F. mosseae* BEG25, and *F. mosseae* Mich; with conventional management (M. conv), and control. Water consumption, photosynthetic pigments, plant macro- and micronutrient concentrations, soluble sugar concentration, titratable acidity, pH, and Brix degrees in fruits, and mycorrhizal colonization and glomalin concentration were analyzed.

Results: In both substrates, the lowest water consumption was observed in plants inoculated with *F. mosseae* Mich. Plants inoculated with *F. mosseae* BEG25 had similar foliar and fruit concentrations of almost all nutrients analyzed to those of plants with the M. conv treatment. Fruits from inoculated plants had higher Brix degrees, total sugars, glucose, and citric acid than these parameters in control plants.

Limitations/Implications of the study: Although inoculated plants received 40% less nutrient solution (with fertilizer cost savings of US\$461 per ha), these showed improved plant nutrition and fruit quality.

Findings/Conclusions: AMF inoculation is a promising strategy for strawberry production under greenhouse conditions, with savings in water and fertilizer use.

Keywords: Arbuscular mycorrhiza, moisture retention, climate change.

Citation: Corona-Sánchez, J. E., Camacho-Negrete, R. C., Carrillo-González, R., Santillán-Galicia, Ma. T., & González-Chávez, Ma. del C. Á. (2026). Arbuscular mycorrhizal fungi improve water use, nutrient uptake, and fruit quality in strawberries. *Agro Productividad*. <https://doi.org/10.32854/n3yt1r29>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: August 7, 2025.

Accepted: March 25, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 93-119.

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INTRODUCTION

Food safety and food sovereignty are among the most important challenges faced by humanity, and aggravated by global climate change, growing demographics, and urbanization (Abbas *et al.*, 2025). Climate change promotes droughts and reduces water availability for agriculture (Sosa-Hernandez *et al.*, 2019). Agriculture consumes approximately 70% of the water withdrawn from aquifers (Mendelsohn, 2016). Low water availability for agricultural production reduces crop yields since water (approximately 80%) is the main component of 90% of fruits and vegetables (Popkin *et al.*, 2010).



Strawberries (*Fragaria × ananassa* Duch) are a high-demand product due to their flavor and nutritional value (Mikiciuk *et al.*, 2019). Strawberries are rich in nutrients, organic acids, phenolic compounds, flavonoids, and anthocyanins (Urün *et al.*, 2021), making them a functional food with high antioxidant activity. According to Giampieri *et al.* (2015), these metabolites help prevent chronic (such as diabetes and hypertension) and degenerative diseases (like cancer). A serious concern is that strawberry cultivation requires large amounts of water and is highly susceptible to drought (Raturi *et al.*, 2023). This vulnerability is intensified in countries with limited water availability (Audefroy, 2015). Low water availability restricts crop development and productivity, leading to unviable open-field production. Therefore, producers will be gradually forced to abandon open-field food production and opt for technologies such as greenhouse conditions, which predominantly use substrates (Gruda *et al.*, 2019).

Greenhouse production offers clear advantages over soil-based production, including an extended growing season, easier harvesting, and reduced soil-borne diseases. Moreover, the main advantage of greenhouse production is that it provides greater control over irrigation regimes, although it may require more water than traditional agriculture (Robinson-Boyer *et al.*, 2016). Therefore, alternatives must be explored to retain moisture in substrates, increase plant water availability, and improve food quality.

Peat moss is a common component of substrate formulations in protected agriculture (Krueger *et al.*, 2018). However, its extensive agricultural use has a negative environmental impact: extracting peat moss from wetlands impedes CO₂ fixation in these areas. This important ecological function regulates the water cycle and climate (Oberpaur *et al.*, 2010); therefore, it is required to find alternative substrate components with a lower environmental impact. Conversely, rice husk, a byproduct generated in large quantities worldwide, does not affect plant growth (Kumar *et al.*, 2013). In Mexico, annual rice production is expected to reach 350 million tons (SAGARPA, 2017). It is estimated that one ton of husk is generated for every four tons of rice, equivalent to approximately 87.5 million tons of this byproduct per year. This material contributes to the circular economy because of its status as an agricultural residue, and is also a more economical option than peat moss. This makes it an affordable alternative for producers that may reduce production costs and environmental impact.

Inoculation with arbuscular mycorrhizal fungi (AMF) is a biotechnological tool in plant production. These microorganisms form one of the most ubiquitous symbioses in the ground plant community (Smith and Read, 2010; Kuyper and Jansa, 2023). AMF hyphae function as an extended root system that increases the surface area and soil exploration volume, increasing nutrient uptake (Finlay, 2008; Begum *et al.*, 2019). Moreover, AMF modify the extension of the root system, impacting soil structure (Daynes *et al.*, 2013) by forming aggregates and thereby enhancing water retention (Augé *et al.*, 2001; Akter *et al.*, 2024). This suggests that inoculating crops with AMF could lead to more efficient water use. The effect of AMF on strawberry production has been widely recognized (Chávez and Ferrera-Cerrato, 1990; Bona *et al.*, 2015), but their role in substrate moisture retention has been poorly studied. Therefore, this study aimed: 1) To evaluate the water expenditure of two strawberry cultivars

(Albion and San Andreas) inoculated with arbuscular mycorrhizal fungi (AMF) in two growth substrates, and 2) To determine the efficiency of three AMF in the nutrition of strawberry plants and their fruit quality. The hypotheses according to objectives were: 1) Inoculation of strawberry plants with at least one of the AMF decreases water expenditure in strawberry plants, 2) Strawberry plants inoculated with at least one of the AMF increase their fruit quality.

MATERIALS AND METHODS

Experimental design and setup

Two experiments were established to determine the effect of AMF inoculation on water consumption. The first experiment used substrate 1, with the following composition: 50% vermiculite, 25% peat moss, and 25% coconut fiber. The second experiment used substrate 2, consisting of 50% vermiculite, 25% rice husk, and 25% coconut fiber. The first experiment was conducted from March to August 2023, while the second experiment was conducted from July to December 2023. Both experiments were conducted in the same greenhouse, but climatic conditions varied. Therefore, comparisons between the results of the two substrates were not possible, and the results are presented separately for the two substrates tested.

The experiments were established under a completely randomized design. For each experiment, we used two strawberry cultivars: Albion and San Andreas. Each of these cultivars was either 1) inoculated with *Funneliformis geosporum*, 2) inoculated with *Funneliformis mosseae* Mich, 3) inoculated with *Funneliformis mosseae* (BEG25), 4) grown under conventional management (M. conv), or 5) grown as a control treatment (without fungal inoculation). Each treatment was replicated five times, resulting in 50 experimental units for each experiment. In both experiments, all treatments were irrigated twice a week with tap water and once with Hoagland nutrient solution during the growth stage and until flowering, except M. conv, which was irrigated with Hoagland solution twice a week and once with tap water. Similarly, in both experiments, all treatments were irrigated as the M. conv during the early flowering stage until harvest. For inoculated treatments, the nutrient solution was a modified Hoagland solution (Miller and Kitt, 1992), low in phosphorus ($20 \mu\text{M KH}_2\text{PO}_4$) to prevent inhibition of AMF establishment (root colonization) and fungal functionality. The M. conv treatment involved a complete Hoagland solution for nutrient management in strawberry plants to mimic conventional management and contrast the potential plant growth and nutritional benefits of AMF, even when using a nutrient solution with lower phosphorus concentrations. Under this management perspective, the results will compare treatments independently of differences in the nutrient solution tested.

Field capacity (FC, water retained at 30.4 kPa) and permanent wilting point (PWP, water retained at 1,519.9 kPa) were determined for both substrates in the pressure pot and pressure membrane, respectively (Richards, 1956). Bulk density (ρ_b) with the test tube method. With the FC, PWP, and ρ_b data, readily available water (RAW) was calculated with the following equation:

$$RAW = \frac{FC - PWP}{100} \times Z \times \rho_b \times 0.2$$

where: *RAW* is readily available water, *FC* is field capacity (%), *PWP* is the permanent wilting point (%), *Z* is the depth of the substrate in the pot (cm), ρ_b is bulk density (g cm^{-3}), and 0.2 is the depletion factor for strawberries.

Equation 1 determined the volume necessary to have 100% RAW. To quantify the water expenditure of the strawberries in both substrates, we determined substrate moisture using a moisture meter (Lincoln, model 6400/6405). When the % of RAW decreased, water or nutrient solution was added to reach 100% RAW. These adjustments were performed every third day for each experimental unit in both experiments.

Nutrient concentration in leaves and fruits

In both experiments, the concentration of nutrients in the leaf (middle leaves) and fruit was determined by wet digestion of the tissue, according to Jones and Case (1990). The plant material (0.5 g) was weighed and placed in digestion tubes added with 1 mL of 30% hydrogen peroxide and 4 mL of the acid mixture of $\text{H}_2\text{SO}_4\text{-HClO}_4$ (4:1 ratio). Then, the tubes were placed in the digestion oven at 240 °C until the samples became transparent. Finally, the samples were made to 25 mL and filtered with Whatman No. 42 paper. N was quantified in the digestion solution by the Microkjeldahl method, as described by Isaac and Johnson (1976). P concentration was quantified following Murphy and Riley's method (1962) with a Varian UV-visible spectrophotometer (Cary 50). K concentration was measured by flammetry (Chapman and Pratt 1982). Ca, Mg, Fe, Cu, and Zn were quantified using a Perkin Elmer model 3110 atomic absorption spectrometer (Oliveira *et al.*, 2010). The economic analysis was carried out using direct cost analysis, considering the reduction of costs due to input savings according Corella (2020). Hence, the volume of nutrient solution saved (L ha^{-1}) was multiplied by the average cost of fertilizers ($\text{US\$ L}^{-1}$).

Chemical variables of the fruits

Titrate acidity was determined by the method of the Association of Official Analytical Chemists (AOAC; Boland, 1990). Ten grams of fresh fruit pulp were weighed and liquefied with 50 mL of distilled water. Then, an aliquot of 5 mL was titrated with 0.01N NaOH, which was previously added with three drops of phenolphthalein as an indicator. The percentage of titrate acidity (TA) was calculated according to the following equation. The pH was determined in the same extract with an ORION model 701 A potentiometer.

$$TA = \frac{(\text{NaOH volume used})(\text{NaOH Normality})(\text{mEq of Acid})(V)}{(\text{sample weight})(\text{aliquot volume})} \times 100$$

where: *TA* is the percentage of titrate acidity, NaOH volume is given in mL, *mEq* of the acid correspond to the weight of 1 mEq of the acid found in higher proportion in the fruit

(citric acid=0.064), V is the volume used for liquefied the fresh fruit, the sample weight is given in g, and the aliquot volume is given in mL.

The content of total soluble solids (TSS) was determined by the method of Boland (1990), which consisted of placing two drops of strawberry fruit juice in a digital refractometer (ATAGO, PAL⁻¹ China) previously calibrated with distilled water. The results were reported in Brix degrees (°Brix).

Measurement of soluble sugars in fruit

The extraction of sucrose, glucose, and fructose was conducted according to Ogiwara *et al.* (1999). Ten grams of fresh fruit pulp were liquefied with 50 mL of distilled water. The samples were heated in a microwave oven for 40 s and centrifuged at 12,000 rpm for 10 min. Then, 5 μ L of each sample was mixed with 200 μ L of a solution composed of 25 mM-HCl HEPES (pH=8.0), 50 mM KCl, 1 mM ATP, 3 mM MgCl₂, 0.3 mM NAD, and 1U mL⁻¹ yeast hexokinase. Basal absorbance was determined with a microplate reader (Multiskan FC microplate photometer, Thermo Scientific, USA) at 340 nm. In the second stage, 1 U mL⁻¹ of glucose 6-phosphate dehydrogenase was added to the mixture, and stable absorbance was determined at 340 nm. For the fructose extraction, 1 U mL⁻¹ of phosphoglucose isomerase was added, followed by 1 U mL⁻¹ invertase. Glucose and fructose were quantified in the same reaction. In this sequence of reactions, sugars are stoichiometrically transformed to glucose 6-phosphate (G6P) and then to 6-phosphogluconate. The sample concentrations were calculated using the equation from a linear calibration curve created with known concentrations of glucose (5 mM), fructose (5 mM), and sucrose (2.5 mM) (Bernal *et al.*, 2005). Finally, the total sugar concentration included glucose, fructose, and sucrose concentrations.

Photosynthetic pigments

Photosynthetic pigments were measured in the fruiting stage of the strawberry plants. Five circular segments of 5 mm diameter were randomly cut from the mature leaves of the main branch of each experimental unit. The segments were placed in 5 mL of 80% (v/v) acetone. Extraction was performed in the dark at 4 °C until the leaf circles became transparent. The concentration of photosynthetic pigments was determined in a Varian UV-visible spectrophotometer at three wavelengths: 663.2 nm, 646.8 nm, and 470 nm. Carotenoids and chlorophyll content were calculated according to the equations of Lichtenthaler (1987).

$$\text{Chlorophyll } a \text{ (mg g}^{-1}\text{)} = \left[(12.25 \times A_{663.2}) - (2.79 \times A_{646.8}) \right] \left[\frac{\text{volume (mL) of 80\% acetone}}{\text{weight (g) of the five segments}} \right]$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1}\text{)} = \left[(21.50 \times A_{646.8}) - (5.10 \times A_{663.2}) \right] \left[\frac{\text{volume (mL) of 80\% acetone}}{\text{weight (g) of the five segments}} \right]$$

$$\begin{aligned}
 \text{Total chlorophyll (mg g}^{-1}\text{)} &= \left[\frac{(7.15 \times A_{663.2}) + (18.71 \times A_{646.8})}{\left[\frac{\text{volume (mL) of 80\% acetone}}{\text{weight (g) of the five segments}} \right]} \right] \\
 \text{Carotenoids (mg g}^{-1}\text{)} &= \left[\frac{\left((1000 \times A_{470}) - (1.82 \times A_{Chla}) - (85.02 \times A_{Chlb}) \right)}{198} \right] \\
 &\quad \left[\frac{\text{volume (mL) of 80\% acetone}}{\text{weight (g) of the five segments}} \right]
 \end{aligned}$$

Mycorrhizal colonization

Four representative root samples were obtained for each experimental unit using a hole-punch tool. Strawberry roots were washed and cut into 1 cm segments, which were cold clarified with 3% KOH for 10 d to remove root pigmentation. Then, they were immersed in 3% HCl for 15 min and stained with 0.5% trypan blue in 50% glycerin for three d (Phillips and Hayman, 1970). The percentage of total colonization by AMF was determined according to Koske and Gemma (1989) on permanent preparations of root segments, which were observed under a Leica optical microscope (DM750 model, 40x objective). The frequency of AMF structures was quantified by considering the presence or absence of arbuscules, vesicles, and hyphae. AMF frequency was calculated with the following formula:

$$\% \text{ total colonization} = \frac{\text{Number of colonized fields}}{\text{Total observed fields}} \times 100$$

Glomalin concentration in the root

For methodological purposes, glomalin is referred in this study as a group of proteins produced by AMF. Total glomalin in the root (TGR) and glomalin in the substrate may improve the substrate structure. However, the organic substrate components interfered with glomalin extraction, resulting in an overestimation of the values (data not shown). Therefore, only TGR was evaluated. Strictly speaking, TGR refers to the concentration of glomalin-like proteins extracted from the root. To measure this concentration, we placed 1 g of root and 8 mL of 20 mM sodium citrate pH=7.0 in 20 mL glass tubes. Subsequently, the samples were sterilized in the autoclave at 121 °C for 1 h. The supernatant volume was measured and stored at 4 °C. Roots remaining at the bottom of the tubes underwent repeated extraction (with 8 mL of sodium citrate and autoclaving) until the reddish-brown color in the samples disappeared. The supernatant of each sample was stored in the same bottle. Finally, the total volume was measured, and the samples were stored at 4 °C (Gonzalez-Chavez *et al.*, 2004). Protein concentration was determined using a calibration curve with bovine serum albumin as a standard solution. This solution was prepared from

a 1 mg mL⁻¹ solution in saline phosphate buffer at pH=7.4, with Bradford reagent as a dye in a 1:4 ratio with deionized water. Before the assay, the stored extracts were shaken in a vortex for 1 min. Then, 40 µL of Bradford reagent and 160 µL of the protein extract from the roots were added to the microplate trays in triplicate, allowing to react for 1 min, and then shaken (Bradford, 1976). A control sample without protein extract was run simultaneously. The standard calibration curve was linear ($R^2=0.9729$), with a sensitivity range from 0 to 1 mg mL⁻¹. The absorbance was measured at 595 nm using a microplate reader, and the results were expressed as mg of glomalin per g of root.

Statistical analysis

The experimental data were tested for normality and homogeneity of variance using the Shapiro-Wilks ($\alpha=0.05$) and Bartlett ($\alpha=0.05$) tests, respectively. Data that did not meet these assumptions were transformed using the Box-Cox method. The lambda coefficients (λ) obtained in the transformations were: Foliar nitrogen in peatmoss of the Albion strawberry variety ($\lambda=1.9$), and in the San Andreas variety ($\lambda=1.4$). Foliar potassium in Peatmoss of the San Andreas variety ($\lambda=0.8$). Sucrose concentration in Peatmoss of Albion variety ($\lambda=0.15$). Water use was analyzed using repeated measures. Before the analysis, the assumptions of normality were corroborated with the Shapiro-Wilks ($\alpha=0.05$), and homogeneity of variance with the Levene test ($\alpha=0.05$). A marginal violation of the homogeneity assumption was detected ($p=0.045$); however, because the experimental design was completely balanced, the ANOVA is considered robust to this deviation, and the validity of the F test is maintained (Box, 1954). Additionally, the sphericity test (Mauchly) showed a significant violation ($p=0.012$) in the Substrate 1—San Andreas cultivar. Therefore, the Greenhouse-Geisser correction was applied to the degrees of freedom to validate the F test (Greenhouse & Geisser, 1959).

Analysis of variance, followed by multiple comparisons using Tukey's test ($\alpha=0.05$) was performed. For the other variables (photosynthetic pigments, leaf and fruit nutrients, °Brix, titratable acidity, soluble sugars, glomalin, and mycorrhizal colonization), we performed an analysis of variance and multiple comparisons of means using Tukey's test ($\alpha=0.05$). Principal component analysis (PCA) was executed for the following data: micro and macronutrient concentrations in fruit and leaf, chemical variables of the fruits, water use, TGR concentration, photosynthetic pigment concentrations, soluble sugars, and percentage of AMF colonization. The PCA was calculated from a correlation matrix because the measurement scales differed for each variable; therefore, it ensured that all data had the same weight (Greenacre *et al.*, 2022). The applicability of PCA was validated using Bartlett's sphericity test. Statistical analyses were performed with R statistical software version 4.1.1 for Windows.

RESULTS AND DISCUSSION

Water use per strawberry plant

The water use was similar during the first month in both cultivars (Albion and San Andreas), and substrates 1 and 2 (Figure 1). From the second month onward, statistical differences were observed between treatments, in both substrates and cultivars. In

substrate 1, plants of cultivar Albion inoculated with *F. mosseae* Mich had the lowest water use compared to the other treatments from April to August. In cultivar San Andreas, plants inoculated with AMF had the lowest water expenditure in April and May ($\alpha=0.05$). Meanwhile, plants inoculated with *F. mosseae* Mich in June and July showed significantly lower water use than the M. conv and control treatments. Moreover, in August, the water use of the plants inoculated with *F. mosseae* Mich differed from that of the control plants. In substrate 2, during August, September, October, and December, plants of the Albion cultivar inoculated with *F. mosseae* Mich showed the lowest water use compared to those of the M. conv and control treatments. In November, significant differences were only observed in M. conv plants in terms of water use. Meanwhile, during August and October, plants of the San Andreas cultivar inoculated with *F. mosseae* Mich had lower water use compared to M. conv, control, and *F. geosporum* inoculated plants. In September, the water use of plants inoculated with *F. mosseae* Mich was lower than that of plants inoculated with M. conv and those inoculated with *F. geosporum*. In November, plants inoculated with *F. mosseae* Mich had lower water use than plants in the M. conv and control treatments. Finally, in December, plants inoculated with *F. mosseae* Mich had lower water use than plants with the control treatment.

The differences in water use between treatments from the second month onward could be attributed to the functional establishment of the mycorrhizal symbiosis in strawberry plants.

Taylor and Harrier (2001) observed that strawberries established a mycorrhizal symbiosis 25 to 28 d after inoculation. Moreover, Andrade *et al.* (1998) reported that the establishment of AMF in the growth substrate often occurs within the first five to ten weeks. In addition, Augé *et al.* (2001) noted that the influence of AMF on plant physiology becomes significant over time. However, the benefits of this symbiosis are specific and dependent on both cultivars and AMF strains (Sinclair *et al.*, 2014). This could explain why treatments inoculated with *F. geosporum* and *F. mosseae* BEG25 showed different water use than those of plants inoculated with *F. mosseae* Mich.

Overall, *F. mosseae* Mich produced the lowest water use in our strawberry plants. For the Albion cultivar, calculating the total water consumption of the entire six months for each treatment revealed that the plants inoculated with *F. mosseae* Mich in substrate 1 required 5.2 L, 2.2 L, 3.8 L, and 3.5 L less water than the plants inoculated with *F. geosporum*, *F. mosseae* BEG25, and the M. conv and control treatments, respectively. In cultivar San Andreas, the water savings were 1.9 L, 2.2 L, 5.1 L, and 5.6 L for the same treatments. Moreover, plants inoculated with *F. mosseae* Mich of cultivar Albion in substrate 2 saved 4.5 L, 2.1 L, 6.5 L, and 5.5 L compared to plants inoculated with *F. geosporum* and *F. mosseae* BEG25; whereas cultivar San Andreas saved 6.2 L, 2.9 L, 7.2 L, and 6.8 L compared to plants with M. conv and control treatments.

Some studies have shown that the substrates of AMF-inoculated plants retain more moisture than those of non-inoculated plants, which could decrease water use in plants. Bitterlich *et al.* (2018) observed that *Solanum lycopersicum* plants inoculated with *F. mosseae* increased moisture retention in the growth substrate (sand and vermiculite in a 1:1 v/v ratio) compared to non-inoculated plants. Pauwels *et al.* (2020) demonstrated that *Medicago*

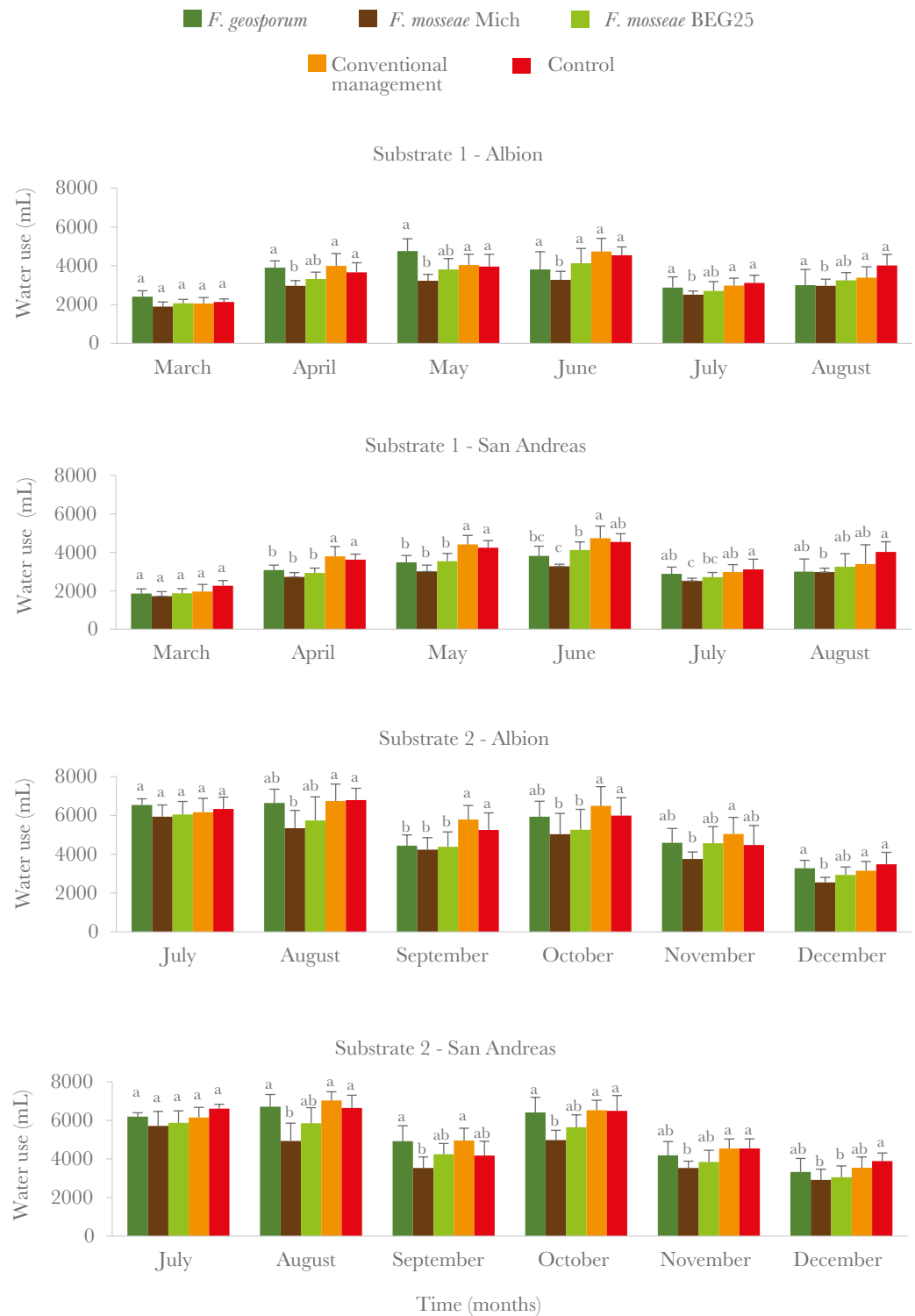


Figure 1. Monthly water use during the experiment by two strawberry cultivars grown in two substrates and five treatments n=10. The mean and standard deviation are shown. Different letters indicate significant differences among the five treatments in each evaluation per month.

truncatula plants inoculated with *Rhizophagus irregularis* had higher water retention in a sand and zeolite substrate (1:1 v/v) compared to non-inoculated plants. In both investigations, the authors reported that the higher moisture retention in substrates with inoculated plants is due to the AMF's ability to improve the substrate's structure.

In substrate 1, both strawberry cultivars inoculated with *F. mosseae* Mich had an average water saving of less than 6 L compared to the control over the six-months. In substrate 2, the same treatment saved less than 7 L compared to the control. Under commercial greenhouse conditions for strawberries, the average plant density is 7 per m², equivalent to 50,000 plants per ha (Alvarado-Chávez *et al.*, 2020). Based on the water use results from substrate one of the Albion and San Andreas cultivars inoculated with *F. mosseae* Mich, it is estimated that water savings of 38,000 L and 50,000 L, respectively, occur compared to the control. In substrate 2, for the cultivars Albion and San Andreas, water use savings were estimated at 65,000 L and 71,000 L, respectively. These data suggest that AMF can significantly improve water use efficiency in strawberry cultivation. This improvement could be relevant and beneficial under a climate change scenario, where water availability is uncertain. Therefore, the results support that AMF are a viable alternative for efficient water use in agriculture. Since agriculture consumes up to 70% of the water extracted from aquifers (FAO, 2019), plant farmers urgently need this alternative. Future research should address how chemical and physical substrate properties influence AMF establishment, water dynamics, and *viceversa*.

Photosynthetic pigments

In substrate 1, control plants from cultivar Albion presented lower concentrations of chlorophyll *a*, total chlorophyll, and carotenoids compared to the other treatments. This trend was also observed in the San Andreas cultivar, where control plants showed lower chlorophyll *a* and carotenoid concentrations. No significant difference was observed between treatments in chlorophyll *b* concentration in both cultivars (Table 1).

In the Albion cultivar, the average concentration of chlorophyll *a*, total chlorophyll, and carotenoids in the inoculated and M. conv treatments were 53%, 39%, and 38% higher than the control plants. In the cultivar San Andreas, the average concentrations of chlorophyll *a* and carotenoids in the same treatments were 41% and 21% higher compared to the control plants. For total chlorophyll, the two *F. mosseae* isolates exhibited that were at least 36% higher than those of the control plants.

In substrate 2, control plants of the Albion cultivar showed a trend of lower concentrations of chlorophyll *b*, total chlorophyll, and carotenoids. Plants inoculated with the two isolates of *F. mosseae* showed 84% higher chlorophyll *a* concentration than control plants. The average chlorophyll *b*, total chlorophyll, and carotenoid concentrations of the inoculated and M. conv treatments were 37%, 64%, and 46% higher than the control plants. In addition, in the San Andreas cultivar, inoculated plants showed the highest concentration of chlorophyll *b*, total chlorophyll, and carotenoids. On average, the concentrations of the three inoculated treatments were 65%, 73%, and 55% higher than those of the control plants.

Mikiciuk *et al.* (2019) found higher concentrations of chlorophyll *a* (2.63 mg g⁻¹) and total chlorophyll (3.50 mg g⁻¹) in strawberry plants inoculated with *Rhizophagus intraradices*,

Table 1. Chlorophyll *a*, *b*, total and carotenoids concentration (mg g^{-1} FW) in strawberry leaves from two cultivars (Albion and San Andreas) grown in two substrates.

Photosynthetic pigments	Treatments									
	Substrate 1					Substrate 2				
	<i>F. geosporum</i>	<i>F. mosseae</i> Mich	<i>F. mosseae</i> BEG25	Conventional management	Control	<i>F. geosporum</i>	<i>F. mosseae</i> Mich	<i>F. mosseae</i> BEG25	Conventional management	Control
	Albion					San Andreas				
Chlorophyll <i>a</i>	1.09±0.17 a	1.11±0.19 a	1.08±0.22 a	1.01±0.20 a	0.70±0.22 b	1.09±0.19 a	1.16±0.20 a	1.18±0.22 a	1.11±0.19 a	0.80±0.21 b
Chlorophyll <i>b</i>	0.46±0.10 a	0.46±0.06 a	0.49±0.12 a	0.44±0.13 a	0.40±0.01 a	0.48±0.07 a	0.53±0.12 a	0.49±0.15 a	0.46±0.16 a	0.43±0.13 a
Total chlorophyll	1.55±0.25 a	1.57±0.23 a	1.56±0.30 a	1.45±0.22 a	1.10±0.27 b	1.57±0.21 b	1.68±0.28 a	1.67±0.30 a	1.56±0.27 b	1.23±0.28 c
Total Carotenoids	0.37±0.06 a	0.40±0.07 a	0.39±0.11 a	0.34±0.11 a	0.27±0.08 b	0.40±0.11 a	0.44±0.13 a	0.41±0.07 a	0.40±0.08 a	0.34±0.08 b
	Albion					San Andreas				
Chlorophyll <i>a</i>	1.20±0.18 b	1.24±0.24 ab	1.31±0.22 a	1.19±0.20 b	0.69±0.27 c	1.30±0.19 a	1.31±0.22 a	1.32±0.28 a	1.10±0.15 a	0.74±0.30 b
Chlorophyll <i>b</i>	0.52±0.13 a	0.53±0.09 a	0.55±0.13 a	0.49±0.12 a	0.38±0.12 b	0.50±0.09 a	0.51±0.10 a	0.48±0.11 ab	0.42±0.11 b	0.30±0.10 c
Total chlorophyll	1.71±0.25 a	1.77±0.27 a	1.86±0.28 a	1.68±0.25 a	1.07±0.24 b	1.80±0.26 a	1.82±0.30 a	1.80±0.32 a	1.52±0.22 b	1.04±0.21 c
Total Carotenoids	0.57±0.11 a	0.59±0.13 a	0.62±0.13 a	0.57±0.11 a	0.40±0.09 b	0.67±0.12 a	0.65±0.11 a	0.63±0.13 ab	0.56±0.10 b	0.42±0.10 c

Table shows average and standard deviation from 5 replicates. Different letters represent a statistical difference in photosynthetic pigment for each treatment, according to the Tukey test ($\alpha=0.05$).

compared to the chlorophyll *a* concentration in our strawberry plants. However, the carotenoid concentration (0.39 mg g^{-1}) is comparable to our results with both strawberry varieties in substrate 2.

Furthermore, Lachinani *et al.* (2023) observed even higher concentrations of chlorophyll *a* (7.11 mg g^{-1}), chlorophyll *b* (5.76 mg g^{-1}), total chlorophyll (12.87 mg g^{-1}), and carotenoid concentration (3.22 mg g^{-1}) in strawberry plants inoculated with *R. irregularis*. Other studies have observed an increase in chlorophyll *a*, concentration, total concentration, and carotenoid concentration in inoculated strawberry plants compared to control plants. Baslam *et al.* (2013) explained this observation on the basis that AMF stimulate metabolic pathways responsible for synthesizing these pigments. Photosynthetic pigment synthesis is known to have a positive correlation with nutrients (Khan *et al.*, 2022). In agreement with this, our plants in the M. conv treatment received 40% more nutrient solution compared to the plants with other treatments. The increase in chlorophyll *a* and carotenoids is particularly beneficial for strawberry plants because chlorophyll *a* converts solar energy into chemical energy through electron transfer (Luo *et al.*, 2022), which increases plant metabolism and improves growth and photosynthetic rate (Khan *et al.*, 2022). Conversely, carotenoids can promote resistance to biotic and abiotic stresses in plants (Vafadar *et al.* 2014). Therefore, inoculated plants have better metabolic conditions since they show higher concentrations of photosynthetic pigments.

Foliar nutrient concentration

Similar to photosynthetic pigment concentrations, both cultivars in substrate 1 had lower macronutrient and micronutrient concentrations in the control, except for Fe (Table 2). In the Albion cultivar, the highest foliar P concentration was found in inoculated plants (average of 714.1 mg kg^{-1} dry weight) compared to M. conv (479.8 mg kg^{-1}) and control plants (293.7 mg kg^{-1}). In the San Andreas cultivar, the average leaf concentration in inoculated plants was similar to that of plants with M. conv (747 mg kg^{-1}) but higher than control plants (304 mg kg^{-1}). Plants of cultivar Albion inoculated with *F. mosseae* BEG25 had an N concentration 71% higher than control plants and 4% higher than plants with M. conv. In cultivar San Andreas, plants inoculated with *F. mosseae* BEG25 had 85% higher leaf N concentration, while M. conv plants had 64% higher leaf N concentration, this compared to control plants. In the Albion cultivar, the plants inoculated with *F. mosseae* BEG25 and M. conv increased their K leaf concentration by 42% on average compared to the control, while in the San Andreas cultivar, the increase was 32%. In addition, within the same cultivar and under the same treatments, we observed a 79% increase in leaf Mg concentration compared to the control plants. However, in the San Andreas cultivar, the plants inoculated with *F. mosseae* BEG25 showed the highest increase in foliar Mg (119%) compared to the control plants and those inoculated with *F. geosporum*.

In the Albion cultivar, the foliar Ca concentration was 57% higher in plants with the M. conv treatment compared to control plants, while in the cultivar San Andreas with the same treatment, the foliar Ca concentration was 31% higher than in control plants. In both cultivars, the leaf concentrations of Zn and Mn were similar between treatments but higher than those of the control plants. Moreover, in the Albion cultivar, the lowest leaf

Table 2. Effect of five treatments in the concentration of micro- and macronutrients in the leaves of strawberry plants grown in two substrates

Nutrients	Treatments									
	<i>F. geosporum</i>	<i>F. mosseae</i> Mich	<i>F. mosseae</i> BEG25	Conventional management	Control	<i>F. geosporum</i>	<i>F. mosseae</i> Mich	<i>F. mosseae</i> BEG25	Conventional management	Control
	Substrate 1					Substrate 2				
	Albion					San Andreas				
N (%)	2.0±0.2 b	2.0±0.3 b	2.4±0.1 a	2.3±0.2 ab	1.4±0.2 c	2.5±0.6 a	2.0±0.6 b	2.6±0.2 a	2.3±0.2 ab	1.4±0.5 b
P (mg kg ⁻¹)	786±110 a	739±141 a	616±88 ab	480±45 b	294±82 c	853±138 a	623±92 a	614±87 a	747±195 a	304±76 b
K (g kg ⁻¹)	20.4±2 b	18.6±3 b	25.6±3 a	22.1±2 a	16.7±2 c	20.3±2 b	22.4±3 a	23.5±3 a	21.0±3 a	16.8±2 c
Ca (g kg ⁻¹)	15.0±1 b	15.0±2 b	16.6±1 ab	19.6±2 a	12.5±0.6 c	15.1±1 a	14.9±2 a	15.1±3 a	16.6±4 a	12.6±1 b
Mg (g kg ⁻¹)	6.2±1 b	6.6±1 b	9.4±2 a	9.7±1 a	5.3±1 c	6.0±1 c	7.4±1 b	11.2±3 a	7.3±2 b	5.1±1 c
Cu (mg kg ⁻¹)	8.5±1 a	7.1±1 ab	10.0±2 a	9.9±2 a	1.6±0.5 c	4.9±1 b	6.5±2 a	12.2±5 a	7.2±3 a	2.1±1 c
Mn (mg kg ⁻¹)	79.9±13 a	103.2±12 a	106.5±23 a	98.1±28 a	51.9±12 b	90.9±22 a	85.1±17 a	110.0±28 a	101.7±22 a	44.4±17 b
Fe (mg kg ⁻¹)	189.7±26 a	185.3±30 a	209.5±35 a	178.1±11 a	178.3±12 a	184.0±42 a	213.5±63 a	193.7±63 a	201.4±35 a	185.9±44 a
Zn (mg kg ⁻¹)	91.3±4 a	92.6±10 a	100.9±18 a	96.8±12 a	53.6±5 b	92.4±22 a	92.8±18 a	124.1±23 a	96.9±9 a	60.6±9 b
Nutrients	Reference values*									
	Albion					San Andreas				
	Substrate 1					Substrate 2				
	Albion					San Andreas				
N (%)	2.0 - 0.1 b	2.0±0.2 b	2.3±0.2 a	2.0±0.1 b	1.0±0.1 c	2.1±0.2 a	2.2±0.4 a	2.0±0.1 a	2.0±0.1 a	1.1±0.1 b
P (mg kg ⁻¹)	665±53 a	582±56 a	604±49 a	613±55a	347±70 b	615±61 a	632±82 a	680±44 a	665±76 a	419±52 b
K (g kg ⁻¹)	24.0±2 ab	23.2±1 ab	26.2±1 a	23.5±1 ab	20.3±1 b	24.1±2.4 a	25.1±1.5 a	26.9±1.4 a	24.5±1.6 a	19.2±1.4 b
Ca (g kg ⁻¹)	9.2±0.6 ab	10.6±1 a	11.0±0.7 a	11.8±0.2 a	8.7±0.4 b	10.3±0.2 b	10.0±0.3 b	11.3±0.6 a	11.8±1 a	8.4±1.3 c
Mg (g kg ⁻¹)	5.9±0.3 b	6.0±0.9 ab	6.2±1.0 ab	7.4±0.7 a	3.2±0.8 c	6.3±0.4 a	5.9±0.8 ab	6.6±0.4 a	6.0±0.2 ab	4.3±1.8 b
Cu (mg kg ⁻¹)	4.2±0.7 b	5.2±0.6 a	6.9±2.0 a	4.9±1.0 b	2.7±1.0 c	3.7±0.5 b	4.2±0.7 b	6.2±0.9 a	4.4±0.7 b	2.5±0.1 c
Mn (mg kg ⁻¹)	65.6±2 a	65.2±3 ab	69.5±2 a	66.2±3 a	60.6±3 b	65.3±2 b	65.2±1 b	69.7±2 a	65.3±1 b	57.4±1 c
Fe (mg kg ⁻¹)	149.8±5 a	158.6±12 a	161.0±6 a	159.4±13 a	135.0±6 b	166.2±15 a	168.4±14 a	171.8±8 a	164.0±17 a	141.7±14 b
Zn (mg kg ⁻¹)	61.1±2 b	59.2±1 b	66.8±1 a	60.8±1 b	52.6±4 c	57.1±2 b	57.8±2 ab	61.1±3 a	56.7±1 b	49.3±2 c
N (%)	2.0 - 3.0	P (mg kg ⁻¹)	3000 - 4000	K (g kg ⁻¹)	13 - 18	Ca (g kg ⁻¹)	10 - 22	Mg (g kg ⁻¹)	2.8 - 4.2	
Cu (mg kg ⁻¹)	2.6 - 4.9	Mn (mg kg ⁻¹)	65 - 320	Fe (mg kg ⁻¹)	85 - 200	Zn (mg kg ⁻¹)	11 - 20			

n=5. Different letters show a statistical difference in nutrients concentration between treatments, based on the Tukey test ($\alpha=0.05$). *Reference nutrient values for strawberry leaves according to Bolda *et al.* (2012).

Cu concentration was observed in control plants; conversely, in the San Andreas cultivar, the lowest concentration corresponded to control plants and plants inoculated with *F. geosporum*.

In substrate 2, all control plants of both cultivars had the lowest foliar concentration of nutrients evaluated. Treated plants in the Albion and San Andreas cultivars had, on average, 77% and 54% higher P concentrations than control plants, respectively. The same trend was observed in the San Andreas cultivar regarding the foliar concentrations of K and N; the treated plants, whether inoculated or treated with *M. conv*, had 30% and 88% more of these nutrients. Moreover, in the Albion cultivar, the plants inoculated with *F. mosseae* BEG25 had the highest concentration of K (26.2 mg kg⁻¹) and N (2.3%). Plants treated with *M. conv* and inoculated with *F. mosseae* BEG25 had a higher average leaf Ca concentration (11.4 mg kg⁻¹) than control plants (8.7 mg kg⁻¹). In the San Andreas cultivar, the same treatments resulted in an average Ca concentration of 11.5 mg kg⁻¹, while the control had 8.4 mg kg⁻¹. Plants inoculated with *F. mosseae* BEG25 had higher leaf Zn concentrations than control plants, 26% more in the Albion cultivar and 23% more in the San Andreas. In the Albion cultivar, the highest leaf concentration of Mn was observed in plants inoculated with *F. mosseae* BEG25 and *M. conv*, while the highest leaf concentration of Cu was found in plants inoculated with both mycorrhizal isolates of *F. mosseae*. In contrast, in the San Andreas cultivar, plants inoculated with *F. mosseae* BEG25 had the highest leaf concentrations of Cu and Mn, 148% and 21% higher, respectively, than control plants. Finally, the foliar concentration of Fe for both cultivars was 16% higher in treated plants compared to control plants.

Other authors have also observed higher concentrations of certain nutrients in inoculated strawberry plants compared to non-inoculated plants. Haghshenas *et al.* (2024) found higher concentrations of P (5,300 mg kg⁻¹), K (27 g kg⁻¹), and N (2.86%) in the leaves of strawberry plants inoculated with *Glomus mosseae* (currently, *F. mosseae*) compared to control plants. Taylor and Harrier (2001) quantified higher concentrations of Cu (9 mg kg⁻¹), Mn (391 mg kg⁻¹), and Zn (33 mg kg⁻¹) in the leaves of strawberry plants colonized with *Glomus clarum* (currently, *Rhizophagus clarus*) compared to non-inoculated plants. Regarding the leaf concentration of Ca and Mg, the present investigation is one of the first to demonstrate differences in these elements in AMF-inoculated strawberry leaves, highlighting the importance of mycorrhization in the increased absorption of these macronutrients.

Based on the reference values reported by Bolda *et al.* (2012; Table 2), the control plants show foliar N and Mn deficiencies in both cultivars and substrates, but Cu deficiency in the Albion cultivar in both substrates. For the San Andreas cultivar, only substrate 2 had Cu deficiency. Ca deficiencies were also observed in control plants of both cultivars growing in substrate 2. These data show that strawberry plants responded favorably to inoculation and the *M. conv* treatment. Regarding leaf P concentration, inoculated and *M. conv* plants had higher P concentrations than control plants ($\alpha=0.05$) in both substrates and cultivars. However, the P concentration in all treatments was lower than the reference value of 3000 mg kg⁻¹; this could result from all treatments being irrigated with a Hoagland solution low in phosphorus.

The higher concentration of nutrients in treatments inoculated with *F. mosseae* and *F. geosporum* is due to AMF facilitating the absorption of these nutrients by plants (Khaliq *et al.*, 2022). Nutrient absorption is essential for plant growth since an adequate concentration of nutrients in leaves is crucial for optimal plant development (Trejo-Téllez and Gómez-Merino, 2014). It is worth noting that although the M. conv treatment included the application of 40% more nutrient solution, the nutritional quality of the plants was not higher than that of plants inoculated with *F. mosseae* Mich. In addition, as mentioned earlier, the treatment inoculated with *F. mosseae* Mich saved up to 7 L of Hoagland solution compared to M. conv during the period evaluated. Based on fertilizer prices for Mexico in 2023, we estimated savings of US\$0.0027 per 7 L of nutrient solution. Hence, the potential input saving for substrate 1 was estimated to be US\$292 ha⁻¹ for the Albion cultivar and US\$304 ha⁻¹ in the San Andreas cultivar. For substrate 2, the projected savings were US\$461 and US\$440 ha⁻¹, respectively. This estimation was based on direct cost analysis and assuming a density of 50,000 plants ha⁻¹, which occurs in the commercially strawberry production.

Nutritional analysis of strawberry fruits

Fruit Fe concentration was similar in plants from both cultivars and substrates. The highest fruit P concentration was observed in plants from the M conv treatment in both cultivars and substrates (Table 3). In the Albion cultivar with substrate 1, the concentration of Mg, Mn, Zn, and N was similar in all fruits except those obtained from control plants, which had the lowest concentration of these nutrients. The highest concentrations of Ca and K were observed in plants inoculated with *F. mosseae* BEG25. In the Albion cultivar with substrate 2, there were no differences between treatments for Mg, Mn, and Ca concentrations, except for the fruits of control plants, which presented the lowest concentrations of these nutrients. Also, the highest Zn concentration was observed in fruits from plants inoculated with *F. mosseae* BEG25 and M. conv. The highest concentration of K was observed in fruits from plants inoculated with the two isolates of *F. mosseae*. Fruits from plants inoculated with *F. geosporum*, *F. mosseae* BEG25, and M. conv had the highest N content. The highest P concentration was observed in plants of the Albion cultivar under the M. conv treatment in both substrates. Similar Zn and Ca concentrations were observed in the fruit across all treatments in the San Andreas cultivar with substrate 1, except for the control plants.

The N fruit content was also similar among treatments, but was lower in the control plants. In substrate 1, the highest concentration of Mg in the fruit was observed in plants inoculated with *F. mosseae* BEG25. In contrast, the K concentration was similar in the fruit of plants inoculated with the two isolates of *F. mosseae* and M. conv. In the San Andreas cultivar with substrate 2, the fruits of plants inoculated with *F. mosseae* BEG25 and M. conv had the highest concentration of Mg and K.

In general, we found positive effects on strawberry fruit nutrition due to the inoculation with *Funneliformis* isolates, resulting from AMF's role in increasing the nutritional quality of fruits (Castellanos Morales *et al.*, 2010; Mikiciuk *et al.*, 2019). Parada *et al.* (2019) observed higher concentrations of K (60.3 g kg⁻¹) and Mg (13.7 g kg⁻¹) in fruits from strawberry

Table 3. Concentration of macro and micronutrients in strawberry fruits of two cultivars (Albion and San Andreas) grown in two substrates.

Nutrients	Treatments												
	<i>F. geosporum</i>		<i>F. mosseae</i> Mich		<i>F. mosseae</i> BEG25		Conventional management	Control	<i>F. geosporum</i>	<i>F. mosseae</i> Mich	<i>F. mosseae</i> BEG25	Conventional management	Control
	Substrate 1					Substrate 2							
	Albion					San Andreas							
N (%)	0.9±0.1 a	0.9±0.1 a	0.9±0.1 a	1.0±0.1 a	0.6±0.0 b	0.8±0.1 a	0.8±0.1 a	0.9±0.1 a	0.9±0.1 a	0.8±0.1 a	0.9±0.1 a	0.9±0.1 a	0.6±0.1 b
P (mg kg ⁻¹)	106±23 c	133±32 bc	160±13 ab	181±37 a	40±14 d	106±22 b	140±35 b	168±46 ab	163±35 a	140±35 b	168±46 ab	163±35 a	35±14 c
K (g kg ⁻¹)	15.1±2 c	16.1±1 bc	20.2±3 a	19.0±2 ab	12.7±1 d	15.4±2 b	20.7±3 a	22.2±4 a	19.2±1 a	20.7±3 a	22.2±4 a	19.2±1 a	12.2±2 c
Ca (g kg ⁻¹)	2.2±0.5 b	2.6±0.5 ab	3.6±0.6 a	3.3±1.2 ab	1.5±0.4 b	2.4±0.6 a	2.0±0.4 a	2.5±0.8 a	2.4±0.6 a	2.0±0.4 a	2.5±0.8 a	2.4±0.6 a	1.4±0.3 b
Mg (g kg ⁻¹)	1.5±0.1 a	1.5±0.1 a	1.7±0.1 a	1.6±0.1 a	1.1±0.1 b	1.6±0.1 b	1.5±0.1 bc	1.8±0.1 a	1.7±0.1 ab	1.5±0.1 bc	1.8±0.1 a	1.7±0.1 ab	1.3±0.2 c
Mn (mg kg ⁻¹)	30.0±7 a	32.7±6 a	40.5±13 a	47.5±10 a	21.5±8 b	30.4±2 bc	37.4±3 b	45.0±8 a	46.0±13 a	37.4±3 b	45.0±8 a	46.0±13 a	20.6±10 c
Fe (mg kg ⁻¹)	28.4±6 a	32.1±4 a	35.3±4 a	29.3±6 a	23.5±5 a	36.7±4 a	42.2±7 a	45.5±8 a	36.2±6 a	42.2±7 a	45.5±8 a	36.2±6 a	26.3±2 a
Zn (mg kg ⁻¹)	17.7±4 a	16.0±3 a	20.7±2 a	19.0±5 a	10.0±1 b	17.8±5 a	16.8±4 a	19.4±6 a	17.7±6 a	16.8±4 a	19.4±6 a	17.7±6 a	8.4±3 b
	Albion					San Andreas							
N (%)	1.0±0.1 a	0.8±0.1 b	1.0±0.1 a	1.0±0.1 a	0.5±0.1 c	1.2±0.2 a	1.3±0.1 a	1.3±0.2 a	1.3±0.1 a	1.3±0.1 a	1.3±0.2 a	1.3±0.1 a	0.7±0.1 b
P (mg kg ⁻¹)	120±27 c	129±28 b	159±21 b	239±27 a	70±42 c	129±27 b	137±25 b	149±35 b	219±42 a	137±25 b	149±35 b	219±42 a	87±28 c
K (g kg ⁻¹)	23.2±0.4 b	24.4±0.8 a	24.9±0.5 a	24.2±0.6 ab	18.3±0.4 c	24.3±0.4 b	24.8±0.7 b	26.2±0.4 a	26.0±0.5 a	24.8±0.7 b	26.2±0.4 a	26.0±0.5 a	19.2±0.5 b
Ca (g kg ⁻¹)	2.2±0.3 a	2.1±0.0 a	2.4±0.2 a	2.2±0.3 a	1.6±0.0 b	2.0±0.1 b	1.9±0.1 b	2.5±0.1 a	2.1±0.1 b	1.9±0.1 b	2.5±0.1 a	2.1±0.1 b	1.5±0.3 c
Mg (g kg ⁻¹)	2.0±0.1 a	1.7±0.2 a	2.1±0.1 a	2.0±0.2 a	1.2±0.2 b	2.4±0.3 ab	2.2±0.1 b	2.5±0.2 a	2.6±0.2 a	2.2±0.1 b	2.5±0.2 a	2.6±0.2 a	1.5±0.2 c
Mn (mg kg ⁻¹)	38.7±1 a	39.6±2 a	40.2±1 a	41.3±3 a	30.5±2 b	41.2±1 a	38.7±1 b	41.3±1 a	40.3±1 ab	38.7±1 b	41.3±1 a	40.3±1 ab	33.6±1 c
Fe (mg kg ⁻¹)	30.5±3 a	30.2±1 a	31.2±1 a	30.1±4 a	28.7±1 a	27.6±2 a	28.8±2 a	28.9±2 a	29.7±3 a	28.8±2 a	28.9±2 a	29.7±3 a	27.3±3 a
Zn (mg kg ⁻¹)	32.7±1 ab	29.6±2 b	35.9±1 a	34.0±5 a	24.3±4 c	32.1±3 ab	31.5±0 b	36.0±2 a	34.3±3 ab	31.5±0 b	36.0±2 a	34.3±3 ab	24.5±2 c
	Reference values [†]												
N (%)	1	P (mg kg ⁻¹)	2500	K (g kg ⁻¹)	17.5	Ca (g kg ⁻¹)	1.84						
Mg (g kg ⁻¹)	1.35	Mn (mg kg ⁻¹)	40	Fe (mg kg ⁻¹)	28.26	Zn (mg kg ⁻¹)	11.9						

n=5. Different letters indicate significant differences when comparing nutrient concentrations between treatments. [†] Reference values were estimated from element concentrations in fresh-weight strawberries (USDA 2023), assuming that 100 g of fresh weight contains 90.8% water.

plants inoculated with *Claroideoglomus claroideum* compared to fruits from plants without inoculation. Castellanos Morales *et al.* (2010) showed higher concentrations of K (185 g kg⁻¹) and Cu (2.8 mg kg⁻¹) in strawberry fruits that were inoculated with *Glomus intraradices* (currently, *R. intraradices*) compared to fruits from uninoculated plants. Importantly, the present study is one of the first to report higher concentrations of Mn, Zn, Ca, and N in fruit from strawberry plants inoculated with AMF compared to non-inoculated plants. Fruit from plants inoculated with *F. geosporum*, *F. mosseae* Mich, *F. mosseae* BEG25, and *M. conv* had higher concentrations of Fe, Zn, Ca, and Mg than reference values (USDA, 2023), 1.3, 1.7, 1.7, 1.9, and 1.3 times, respectively.

In both substrates and cultivars, only plants from the *F. mosseae* BEG25 and *M. conv* treatments exceeded the reference fruit Mn concentration, with 1.2 times the value. Similarly, K concentration in fruit was higher than the reference value in plants inoculated with *F. mosseae* BEG25 and *M. conv* of the Albion cultivar. The concentration of K was also higher in plants inoculated with *F. mosseae* Mich in the cultivar San Andreas with substrate 1. In substrate 2, the concentrations of Fe, Zn, Ca, Mg, and K in the AMF-inoculated treatments and the *M. conv* treatment were at least 1.2, 3.0, 1.3, 1.5, and 1.4 times higher compared to the values reported in the USDA. Regarding the San Andreas cultivar, the concentrations of these elements were at least 1.1, 3.3, 1.4, 1.9, and 1.5 times higher than those in other cultivars, respectively.

A higher concentration of nutrients in strawberry fruits increases their nutritional value and makes them potentially beneficial for consumer health. Nutrients from strawberry fruits perform specific functions in the body. For example, Fe is involved in the synthesis of deoxyribonucleic acid, as well as electron and oxygen transport (Abbaspour *et al.*, 2014). At the cellular level, Zn is critically involved in proliferation, differentiation, and apoptosis (Costa *et al.*, 2023). Ca is indispensable for the growth and development of an optimal skeletal structure (Ciosek *et al.*, 2021), while Mg is necessary for energy metabolism, as well as muscle contraction and relaxation (Ciosek *et al.*, 2021). Maintaining a diet rich in K helps reduce cardiovascular disease mortality (Muiesan *et al.*, 2023). Therefore, the knowledge generated in this study regarding the impact of AMF inoculation on strawberry fruit nutrition is relevant for producing more nutritious food to promote human health.

Physical and chemical components of the fruit

In addition to the visual appearance of strawberry fruits, chemical components such as sweetness and acidity are important variables that influence consumer preference (Cecatto *et al.*, 2013). In both cultivars and substrates, fruits from strawberry plants with *M. conv* and inoculated with AMF presented higher citric acid percentages and °Brix compared to the fruits of control plants ($\alpha=0.05$; Table 4). Cordeiro *et al.* (2019) observed that higher °Brix and citric acid percentages in strawberry fruits were positively correlated with better plant nutrition and higher photosynthetic yield, which was corroborated in the present investigation. Todeschini *et al.* (2018) observed that citric acid concentration (0.46%) was higher in fruits of plants inoculated with *R. irregularis* compared to non-inoculated plants. In the cultivar Camino Real, Cordeiro *et al.* (2019) quantified a 1.2 times higher percentage of citric acid with respect to control plants

Table 4. Effect of five treatments in citric acid content, pH and Brix degrees of fruits of two strawberry cultivars grown two substrates.

Chemical composition	Treatments									
	Substrate 1					Substrate 2				
	<i>F. geosporum</i>	<i>F. mosseae</i> Mich	<i>F. mosseae</i> BEG25	Conventional management	Control	<i>F. geosporum</i>	<i>F. mosseae</i> Mich	<i>F. mosseae</i> BEG25	Conventional management	Control
	Albion					San Andreas				
Citric acid (%)	1.14±0.1 a	1.23±0.1 a	1.15±0.2 a	1.18±0.2 a	0.84±0.1 b	1.14±0.2 a	1.12±0.1 a	1.23±0.1 a	1.12±0.1 a	0.83±0.1 b
pH	3.29±0.1 b	3.30±0.1 b	3.32±0.0 b	3.38±0.1 ab	3.42±0.0 a	3.26±0.1 b	3.30±0.1 b	3.29±0.1 b	3.33±0.1 ab	3.42±0.0 a
° Brix	7.52±0.3 a	7.86±0.2 a	7.63±0.4 a	7.93±0.5 a	6.46±0.4 b	7.98±0.3 a	7.78±0.6 a	8.14±0.6 a	7.73±0.4 a	6.49±0.4 b
	Albion					San Andreas				
Citric acid (%)	1.15±0.1 a	1.04±0.1 a	1.09±.1 a	1.08±0.1 a	0.85±0.1 b	1.13±0.1 a	1.13±0.0 a	1.18±0.0 a	1.11±0.1 a	0.97±0.1 b
pH	3.91±0.1 b	3.94±0.1 b	3.97±0.1 b	3.96±0.1 b	4.14±0.1 a	3.95±0.1 b	4.02±0.1 ab	3.94±0.1 b	3.91±0.2 b	4.18±0.1 a
° Brix	7.80±0.2 a	7.88±0.1 a	7.98± 0.1 a	8.00±0.4 a	6.94±0.4 b	8.00±0.1 a	8.02±0.2 a	8.00±0.2 a	7.96±0.2 a	7.52±0.2 b

n=5. Different letters show a statistical difference between treatments, based on the Tukey test ($\alpha=0.05$)

when strawberry plants were inoculated with a consortium of eight AMF species. A higher concentration of citric acid in strawberry fruits benefits human health because it is associated with antioxidant and anti-inflammatory effects in cells (Singh *et al.*, 2022). Importantly, fruits from inoculated plants or plants treated with *M. conv* presented an ideal °Brix content ($\geq 7\%$) for foods that have not been modified since harvest (Cecatto *et al.*, 2016). Ansari *et al.* (2018) recorded an increase in °Brix (12%) in strawberry fruits obtained from plants inoculated with *R. intraradices* compared to strawberry fruits from non-inoculated plants (4%). Most consumers prefer strawberries with higher °Brix, as this increases the sweetness of the fruit (Cecatto *et al.*, 2013).

Fruit pH differed between treatments ($\alpha=0.05$). In general, we observed that in the two substrates and cultivars, fruits from inoculated and *M. conv* treated plants were more acidic than those from control plants. Similar results were reported by Cekic and Yilmaz (2011), Bona *et al.* (2015), and Todeschini *et al.* (2018). According to Cordeiro *et al.* (2019), fruit with a lower pH is better appreciated by consumers who prefer acidic fruits. Although strawberries contain citric, malic, ellagic, and salicylic acid, Zhang *et al.* (2021) indicated that citric acid is responsible for approximately 92% of the total acidity. This was corroborated by the fact that the treatments with the highest concentration of citric acid also had the most acidic fruit.

In both substrates and cultivars, significant differences were observed in glucose and total sugars ($\alpha=0.05$); the fruits of control plants had the lowest concentrations (Figure 2). Fruits from plants inoculated with *F. mosseae* BEG25 in cultivars Albion and San Andreas with substrate 1 had the highest concentration of glucose (29.5 and 28.5 mg g⁻¹, respectively) and total sugar (53.3 and 57.1 mg g⁻¹, respectively); this in comparison with control plants. Contrary to control plants, plants inoculated with *F. mosseae* BEG25 in substrate 2 from the Albion cultivar presented the highest concentrations of glucose and total sugar in the fruit which were 29.78 and 53.50 mg g⁻¹, respectively.

In the San Andreas cultivar, the highest glucose concentration (29.24 mg g⁻¹) was observed in fruit from plants inoculated with *F. mosseae* BEG25, while the fruits from plants with *M. conv* treatment had a higher total sugar concentration (52.80 mg g⁻¹) in both cases compared with fruits from non-inoculated plants.

Bona *et al.* (2015) reported that strawberry plants inoculated with a consortium of five AMF increased their fruit sucrose (10.86 mg g⁻¹) and total sugar (70 mg g⁻¹) content compared to fruits from non-inoculated plants (1.35 and 1.20 times, respectively). In contrast, Castellanos Morales *et al.* (2010) observed no significant differences in the concentration of glucose, fructose, and sucrose in strawberries from plants either inoculated with *G. intraradices* (currently, *R. intraradices*) or non-inoculated. The differences in strawberry fruit sugar concentrations in inoculated *vs.* non-inoculated plants can be attributed to the influence of AMF species on the metabolic pathways of sugars (Azcón-Aguilar and Barea, 1992; Wang and Wu, 2023); sugar exchange between the plant and the fungus modifies carbohydrate metabolism in the plant and its storage in the fruit (Noceto *et al.*, 2021). Plants inoculated with *F. mosseae* BEG25 showed similar concentrations of sugars in the fruit of plants with the *M. conv* treatment. This demonstrates that plants inoculated with this fungus promoted sugars production in the

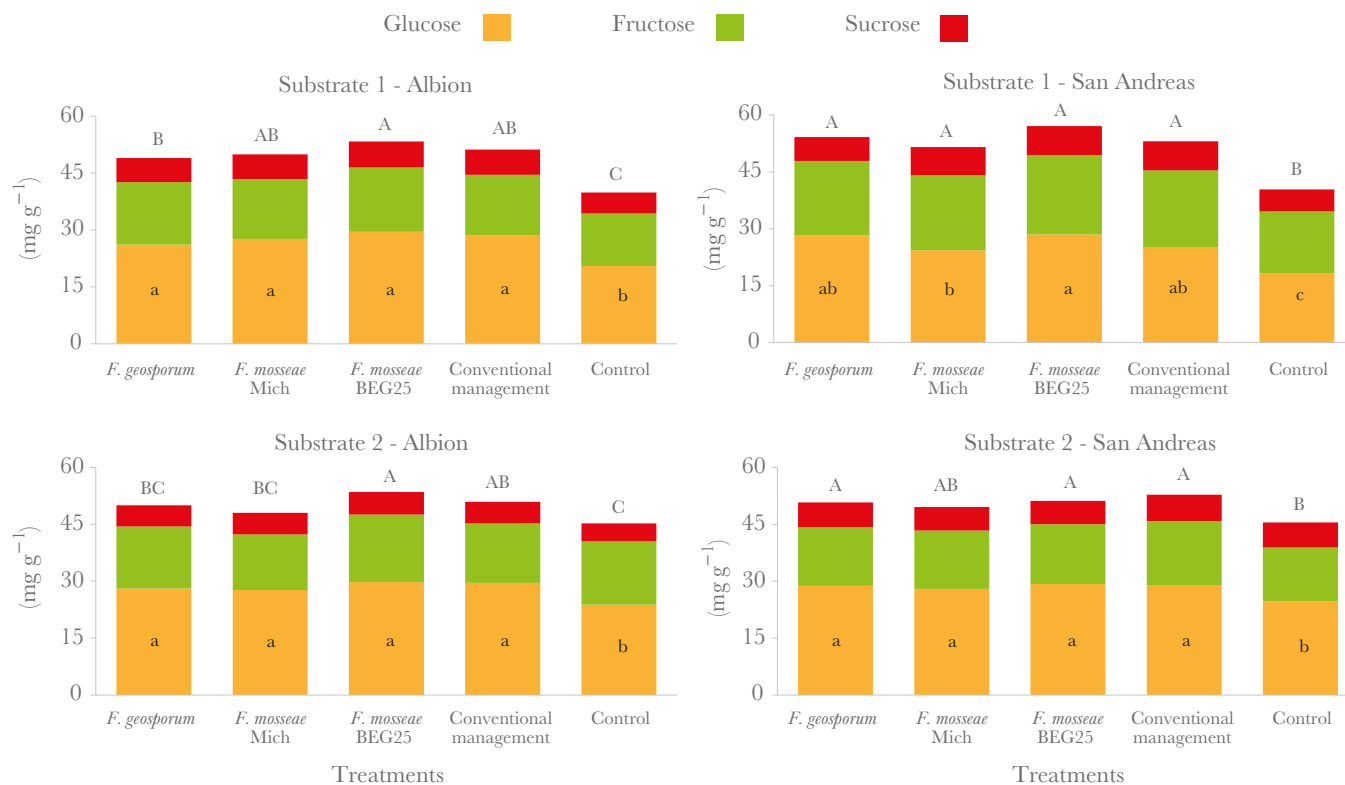


Figure 2. Concentrations of glucose, fructose, sucrose, and total sugar in fruits of two strawberry cultivars grown in two substrates and five treatments, $n=5$. Lowercase letters show differences between glucose concentrations, and uppercase letters indicate differences in total sugar concentration. No significant differences were observed between fructose and sucrose concentrations.

strawberry fruit, even though the inoculated plants received 40% less nutrient solution. Haghshenas *et al.* (2024) also observed that the nutrient solution strength in the control plants can increase the concentration of sugars in the fruit of strawberry plants and behave similarly to inoculated plants.

Mycorrhizal colonization

AMF colonized all the roots of our inoculated plants, while control plants showed no mycorrhizal structures. Hyphae and vesicles were observed on the strawberry roots, regardless of the cultivar and substrate. For substrate 1, plants from the Albion cultivar showed a colonization interval of 35-37%, while those from the San Andreas cultivar had a colonization interval of 31- 39%. Conversely, in substrate 2, the interval was 35-37% in the Albion cultivar and 36-37% in the San Andreas cultivar. These percentages are within the range reported by Taylor and Harrier in strawberries (2001), which indicates 20-40% of mycorrhizal colonization with *G. clarum* (currently, *R. clarus*). However, other studies with strawberry plants have reported higher percentages of colonization. Robinson-Boyer *et al.* (2016) quantified 55% of colonization with *F. mosseae*. Castellanos Morales *et al.* (2010) observed a colonization interval of 65-80% with *G. intraradices* (currently, *R. intraradices*). The different colonization percentages could be associated with nutrient availability and environmental conditions specific to each study. In addition, colonization

depends on the AMF species that colonize the roots (Taylor and Harrier, 2001), which can lead to differences even in the same strawberry variety. Importantly, the percentage of colonization is not a direct indication of mycorrhizal benefits (Wang *et al.*, 2023). Although the colonization percentages found in the present study were within the range reported by other authors, it is necessary to acknowledge that the benefits of mycorrhizal colonization depend on several factors. Proper management and optimal conditions may potentiate the benefits of symbiosis.

Total glomalin concentration in roots

In both cultivars and substrates, the roots of plants inoculated with *F. mosseae* Mich presented higher TGR concentration ($\alpha=0.05$) than other inoculated plants (Figure 1S). In contrast, no TGR was detected in the roots of *M. conv* treatment and control plants. This is the first study reporting TGR measurement in strawberries; however, Wu *et al.* (2016) showed that root glomalin production varies among mycorrhizal species due to physiological differences and different colonization strategies. Wu *et al.* (2016) found 0.06 mg g^{-1} of TGR in trifoliolate orange tree roots inoculated with *F. mosseae*. Singh *et al.* (2013) noted that glomalin production benefits soil structure and positively affects the host plant, enhancing nutrient absorption and stress resistance. Moreover, glomalin can contribute to reducing CO_2 and N_2O , as it constitutes an important C and N reserve, suggesting that the inoculation with AMF in agricultural production systems can contribute to mitigate the effects of climate change (Rillig *et al.*, 2001).

Recently, Alptekin *et al.* (2025) reexamined the molecular nature of glomalin and observed a more positive immunological response, when using the monoclonal antigen Mab32B11, to an unidentified polysaccharide than to the commonly considered glycoprotein. Therefore, they suggested renaming glomalin to glomalose. Interestingly, the extraction method (as used in the present research) and functional attributes of glomalin are similar to those of glomalose.

This study highlights that inoculating strawberry plants with AMF benefits plant growth and physiology. However, future research is needed to explore interactions between different AMF and strawberry cultivars, as well as different substrates. It is also necessary to analyze the mechanisms involved in glomalin (glomalose) production, not only in the root but also in the soil, as well as the effects on moisture retention, to increase these benefits. This protein (carbohydrate) contributes to the formation and stabilization of aggregates in the soil due to its recalcitrance and hydrophobic nature (Wright and Anderson, 2000). In the present study, glomalin (glomalose) may play a similar effect to that of soil in substrates, decreasing water expenditure in plants inoculated with *F. mosseae* Mich. However, this aspect requires further investigation.

Principal Component Analysis

Figure 3 (A) shows that, for substrate 1, the first two components explain 79.2% of the total variability (66.7% and 12.5% for components 1 and 2, respectively). In substrate 2, these components explained 66.0% and 12.3% of the total variability, respectively (Figure 3 B). The PCA allowed us to identify the influence of substrates and treatments in obtaining

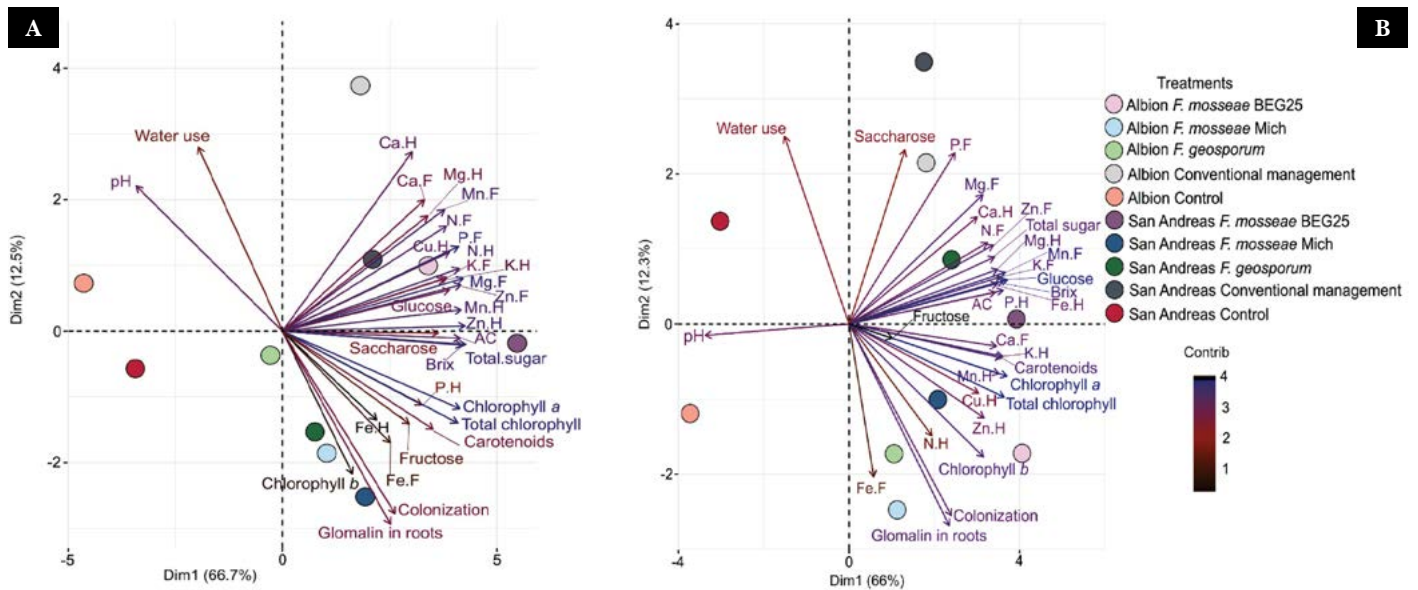


Figure 3. Principal component analysis of two strawberry cultivars (Albion and San Andreas) grown in substrate 1 (A) and substrate 2 (B) towards mycorrhiza inoculation.

FeF=iron in fruit, MnF=manganese in fruit, ZnF=zinc in fruit, CaF=calcium in fruit, MgF=magnesium in fruit, PF=phosphorus in fruit, KF=potassium in fruit, NF=nitrogen in fruit, CuL=copper in leaves, MnL=manganese in leaves, FeL=iron in leaves, ZnL=zinc in leaves, MgL=magnesium in leaves, CaL=calcium in leaves, KL=potassium in leaves, PL=phosphorus in leaves, NL=nitrogen in leaves, Brix=Brix degrees, pH=pH of the fruit, AC=titratable acidity in fruit

plants or fruits with specific, more desirable characteristics. The main trends that occurred in both substrates were as follows: 1) In substrate 1, no variable (vector) was associated with the control treatment. In contrast, different vectors were associated with the AMF-inoculated treatments and the *M. conv* treatment.

In substrate 2, the control treatments in both cultivars were associated with the pH vector, corroborating that fruits from control plants were less acidic.

2) In substrate 1, plants of both cultivars inoculated with *F. mosseae* Mich, as well as those of cultivar San Andreas inoculated with *F. geosporum* were associated with TGR, chlorophyll b, and percentage of colonization.

Notably, the vectors for TGR and percent colonization were oriented in the opposite direction to those for water use and pH. This suggests that higher TGR concentration and colonization percentage contribute to lower water expenditure, favoring fruit acidification in those treatments. In substrate 2, in the Albion cultivar, inoculation of plants with *F. mosseae* Mich and *F. geosporum* were also positively related to TGR and colonization but negatively related to water use.

3) In substrate 1, plants of the San Andreas cultivar inoculated with *F. mosseae* BEG25 showed a positive association with the fruit vectors of total sugar, sucrose, and °Brix. In substrate 2, plants of the cultivar San Andreas inoculated with *F. mosseae* BEG25 showed a positive relation with glucose and °Brix in the fruit. Conversely, plants treated with *M. conv* were only related to sucrose concentration in the fruit. This suggests that these treatments produced sweeter fruits, a characteristic preferred by consumers (Fan *et al.*, 2021). 4) In substrate 1, plants of the Albion cultivar inoculated with *F. mosseae* BEG25, as well as

those of the San Andreas cultivar with *M. conv* were positively associated with nutrient vectors in fruit (Ca, Mn, N, P, K, and Mg) and leaves (Mg, Cu, and K). In substrate 2, plants of cultivar Albion inoculated with *F. mosseae* BEG25, as well as those of San Andreas cultivar inoculated with *F. mosseae* Mich were positively associated with chlorophyll *b* concentration and leaf nutrients (N, Zn, and Cu). Those inoculated with *F. geosporum* were positively associated to nutrients in fruit (Mg, N, Zn, Mn, and K) and leaf (Ca, Mg, and P). Higher nutrient content in strawberry fruits benefits both producers and consumers. While producers improve crop nutrition for optimal production (Trejo-Télez and Gómez-Merino, 2014), consumers value nutrient-rich fruits that fulfill specific bodily functions (Yahia *et al.*, 2017).

CONCLUSION

Inoculation of strawberry plants with *F. mosseae* Mich was the most effective in reducing water use in both cultivars and substrates during the six-month evaluation period. This evidences its potential as a viable alternative for improving water-use efficiency in agriculture under climate change conditions. The reduction in water use is particularly relevant given the pressure that agriculture exerts on water resources and the declining availability of high-quality water. The PCA showed that mycorrhizal colonization and TGR were negatively associated with plant water use and varied according to cultivar, mycorrhizal fungus, and substrate. Compared to the *M. conv* treatment, plants inoculated with *F. mosseae* BEG25 yielded no differences under reduced fertilization conditions (which were used to avoid inhibiting AMF root colonization and to evaluate fungal effectiveness), mainly in nutrient uptake in the leaf (N, P, K, Ca, Mg and K, Cu, Mn and Zn) and fruit (N, P, K, Ca, Mg, Mn and Zn). This represents fertilizer savings of up to US\$461 (per ha) in the production of greenhouse-grown strawberries. The inoculation also enhanced fruit nutrient concentration, Brix degrees, total sugar and glucose concentrations, and the percentage of citric acid, all parameters related to flavor and consumer preference. Therefore, AMF inoculation contributes to physiological, economic, and ecological benefits and should be implemented in the production of strawberry plants. Further research is needed to evaluate other substrates and more efficient irrigation methods. In addition, the assessment of fungal consortia used in this study or other AMF is required to understand their compatibility, effects on water use efficiency as well as the nutritional and nutraceutical quality of strawberry fruits, particularly under increasingly extreme climatic scenarios.

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Aspects, strategies and challenges for banana producers in Mexico towards sustainable cultivation

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Citation: Lungo-Rodríguez, A. J., Juárez-López, A. L., Vences-Martínez, J. A., Antúnez-Ocampo, O. M., Rosas-Acevedo, J. L., De La Llata-López, M. E. & Herrera-Navarrete, R. (2026). Aspects, strategies and challenges for banana producers in Mexico towards sustainable cultivation. *Agro Productividad*. <https://doi.org/10.32854/a8s9wd89>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: September 29, 2025.

Accepted: March 19, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 121-134.

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ABSTRACT

Objective: to analyze the strategies and challenges faced by banana producers in southern Mexico, from the key aspects that contribute to the achievement of the Sustainable Development Goals (SDGs).

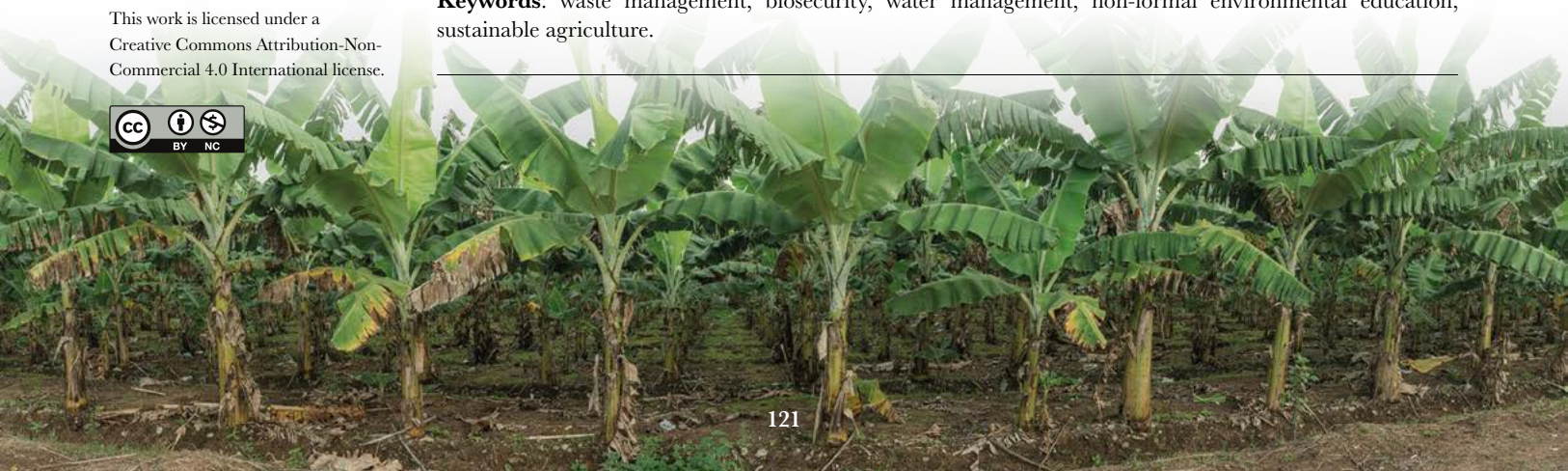
Design/Methodology/Approach: from a cross-sectional descriptive design, a survey was applied, with five sections and 40 items, to 42 banana producers. As well as in-depth interviews (according to the snowball technique) with key informants from three localities in the municipality of Tecpan de Galeana, in the state of Guerrero, Mexico.

Results: most producers operate in small family units and only 33% have some formal training, the rest learn empirically and develop productive activities by trial-and-error. A wide gap was identified between sustainable practices (best management) and practices applied in the field, which were related to the key aspects analyzed. These are training, biosecurity measures, waste management, use of traditional cultivation techniques, and water management for irrigation activities. With the information collected and these determinants, differences in the agricultural practices of the producers interviewed were identified in the three localities.

Limitations/implications of the study: the size of the interviewed population was small due to the restrictions of the COVID-19 pandemic, during the period in which the study was conducted. In addition, it was identified that the state does not have an official registry of banana producers; although its annual production is estimated at the state scale.

Findings/Conclusions: hereby the proposed strategies we highlight are the promotion of composting practices, use of organic fertilizers and improvements in irrigation infrastructure. As well as the intervention with a non-formal environmental education program to raise awareness and transfer knowledge that promotes more sustainable agricultural practices.

Keywords: waste management, biosecurity, water management, non-formal environmental education, sustainable agriculture.



INTRODUCTION

The agricultural sector worldwide is of great relevance for socioeconomic development, because it contributes directly to two of the Sustainable Development Goals (SDGs): no poverty and zero hunger, towards ensuring food safety (Gumbi *et al.*, 2023). The United Nations (UN) recognizes that agricultural activity is crucial to achieving the SDGs, especially when it has a sustainable approach (Cao & Solangi, 2023). Sustainable agriculture arises to reduce environmental impacts related to the leaching of chemicals into water, and the loss of soil quality (He *et al.*, 2023); as well as those related to traditional irrigation systems and the use of agrochemicals.

Several studies have shown success in the adoption of sustainable practices in agriculture; one province in Ecuador carried out the implementation of agroecological practices with producers formed in groups or organizations. Mature organizations have more experience and have presented better results that are reflected in more sustainable production and improved living conditions in rural communities (Alava *et al.*, 2020).

In Australia, the transition from capitalist to sustainable agriculture has generated a peasant social movement that demonstrates an interest in the environment, food sovereignty and public policies (Jonas & Gressier, 2025). On the other hand, there have been cases that link health to traditional agricultural practices; for instance, in Mexico, the presence of organochlorine pesticides was found in blood samples from pregnant women, agricultural soils, and surficial bodies of water (Arce-Estrada *et al.*, 2025).

The transition of agriculture in Mexico towards healthy food production with sustainable practices focuses on strategies to counteract environmental deterioration and its adaptation to climate change (Castellanos-Gutiérrez *et al.*, 2021, Vázquez *et al.*, 2021). Therefore, the objective of this study was to analyze the strategies and challenges faced by banana producers in southern Mexico, from the key aspects that contribute to the achievement of the Sustainable Development Goals (SDGs).

METHODOLOGY

Study design

The study was based on the analysis of information with a cross-sectional descriptive design, because its application was in a certain timeline under complex circumstances due to restrictions of the COVID-19 pandemic, between December 2020 and January 2021. However, the interaction period with the producers was larger; as it began before the study to build bonds of trust, and the interaction continued after the study was conducted.

Participants

The study involved 42 banana growers from three localities in Tecpan de Galeana (Guerrero), Mexico including key informants. The selection of participants was done through convenience sampling (Kleeberg & Ramos, 2009), for reasons of accessibility and availability of producers during the period of restrictions. To minimize selection bias, producers from the three localities with diversity in age, experience and educational level were included.

In addition, the sample was complemented with the ‘snowball’ technique to include producers recommended by the key informants, thus enriching the representativeness of the sample. It was noteworthy that producers expressed their agreement to participate in the survey and interviews, as well as giving their consent to share their agricultural ideas, experiences, and practices (Table 1).

Survey design

A questionnaire was designed to collect specific information, the construction of the items was guided by the themes and the objective of the research (Borromeo-García, 2023). Once the questionnaire was formulated, it was reviewed by a committee of five environmental experts and a pilot test was applied to three producers to calculate the time of application, as well as to verify the understanding and relevance of the questions. Afterwards, adjustments were made to the questions for a second review, and finally the questionnaire was applied to the selected interviewees.

The questionnaire was divided into five dimensions to address 40 items related to the factors identified as the main challenges that slow down socioeconomic development in the

Table 1. Sociodemographic characteristics of banana producers in three localities of Tecpan de Galeana (Guerrero), Mexico.

Data record description	Number of producers	%
Localities		
El Súchil	20	47.6
Tenexpa	15	35.7
Tetitlán	7	16.7
Gender		
Male	41	97.6
Female	1	2.40
Age (years)		
18-40	3	7.10
41-60	20	47.6
Older than 60	19	45.2
Level of Education		
No schooling	7	16.7
Primary (Elementary)	17	40.5
Secondary (Junior High School)	8	19.0
Senior High School	7	16.7
College (undergraduate)	3	7.10
Cultivation experience (years)		
5-10	9	21.4
11-20	8	19.0
21-30	18	42.9
More than 30	7	16.7

agricultural sector (Gambart *et al.*, 2020; Gumbi *et al.*, 2023). In this context, those factors also affect banana cultivation (Table 2).

The interviews were applied to key informants from the three locations under study, using the participant observation technique with in-depth interviews (Vázquez-López *et al.*, 2018). They addressed questions focused on land use, cultivation practices and security measures. Each interview was conducted on the plantations, a space where producers felt more comfortable sharing their experiences and perceptions.

Data collection and analysis

For the application of the questionnaire in the cultivation plots, individual technical visits were scheduled. Research in the field made it possible to verify the crop production system, waste management, both agrochemical containers and green waste, as well as water use and the type of irrigation. The data obtained were systematized and analyzed in Excel[®] Microsoft[™] 2019 version. Due to the sample size (n=42) and the design of the study, descriptive statistics were chosen to analyze the patterns of responses in the practices and perceptions of the producers. No inferential tests were performed; a qualitative analysis was complementary to the interviews and allowed triangulation to deepen the quantitative findings and increase the internal validity of the study.

Interviews were scheduled through socialization with key informants, intentionally selected for their greater experience in banana cultivation. This intervention allowed us to delve into the relevant and determining aspects of the study. The interview was analyzed from an interpretive paradigm approach, which is based on the subjectivities of the participant. This allowed us to understand a vision of the world through the perspective of each person individually (Miranda & Ortiz, 2020).

Table 2. Structure of the questionnaire applied to 42 banana producers interviewed in Tecpan de Galeana (Guerrero), Mexico.

Dimension	Description	Items	Response type
Sociodemographic	It addresses issues such as age, gender, schooling and trade of the participants.	5	Open and multiple selection
Training	Collect participation by courses, the number of courses taken, and their level of importance to the participant.	5	Open and dichotomous (Yes/No)
Biosecurity and sanitary measures	It focuses on questions about the use of personal protective equipment (PPE) during and after agriculture activities, as well as an exploration of health status.	10	Binary numerical response (0 & 1)
Solid waste management	It investigates the treatment of that waste generated in the plot, from plastic agrochemical containers to the green waste generated during planting (harvest and post-harvest).	10	Open and binary numerical response (0 & 1)
Agricultural techniques, technologies and water use	It collects information on planting techniques that contribute to sustainable development, including the use of water for crop irrigation.	10	Open and binary numerical response (0 y 1)

In each interview, photos and videos were taken, as well as notes, a qualitative ethnographic research technique. Some notes were made privately during the intervention, with a thoughtful composition of the content that revealed information on land use and management, safety measures and environmentally friendly practices in banana cultivation. Then, the main arguments that best explained the context and environmental behavior were extracted to validate the findings, detect patterns or discrepancies.

Study area

The three localities under study are in the municipality of Tecpan de Galeana, in the Costa Grande geo-economic region of the state of Guerrero (Mexico) at coordinates 17° 13' 21" N and 100° 37' 57" W (INEGI, 2020). According to the Ministry of Economy (SE, 2020) in Mexico, Tecpan de Galeana has a population of 65 237 inhabitants (Figure 1).

In addition to agriculture, most of the population is engaged in retail, temporary accommodation services, and food and beverage preparation, as well as manufacturing (SE, 2020). These particularities allowed this study to be conducted during the CovID-19 pandemic (2020-2021). The number of producers interviewed was reduced due to limited interaction between people, due to the sanitary restrictions imposed by the Federal Government. However, the intervention with the 42 banana producers identified important points for future comparative studies. In Mexico, there is no updated record of the number of banana producers at the state level. This is due to the lack of organization in formal farmer associations, since most are small producers organized by independent family groups.

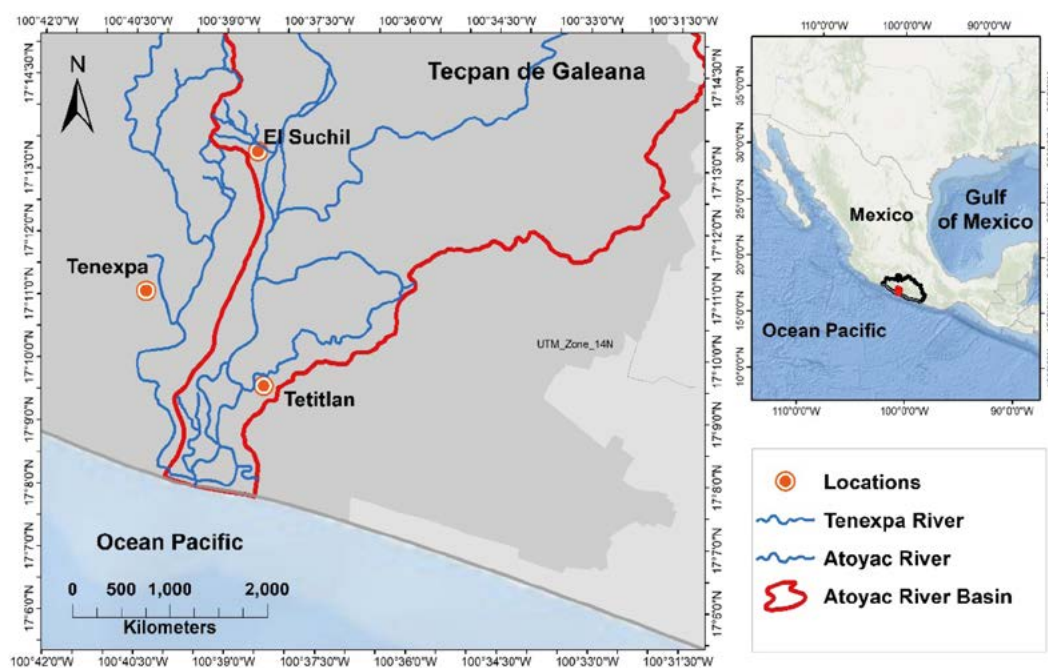


Figure 1. Banana producing localities (El SÚchil, Tenexpa and Tetitlán) in Tecpan de Galeana (Guerrero), Mexico and their water supply network.

RESULTS AND DISCUSSION

Overall, the findings showed a wide gap between sustainable practices and actual field practices related to key aspects of sustainable banana cultivation in three locations in southern Mexico.

Key aspects for sustainable cultivation

Farmer training

Most farmers implement weeding, fertilization and application of herbicides, fungicides and other agrochemicals with insufficient training. It is worth mentioning that doing this type of activity with limited knowledge is a health risk and a threat to the environment. For this reason, training through talks and courses is a key aspect in the agricultural sector, and in this case farmers in the three localities under study received training. Regarding the training, annual talks were identified in which most farmers acquired knowledge about vermicompost, the use of herbicides and the management of post-harvest residues (Figure 2A).

Figure 2B shows evidence of training received through courses that addressed various topics such as fertilizers, herbicides, fungicides, safety measures, and final disposal of wastes. Data showed a minimum participation in the courses of no more than 38%, since, of 42 producers, only 14 confirmed having attended at least one course. While the rest of the participants have acquired their learning through the experience of family or friends, through verbal exchange of knowledge. Other producers stated that they used the trial-and-error method in the application of fertilizers and agrochemicals on their plots.

These findings coincide with studies such as those by Sánchez-Gervacio *et al.* (2021) and Mukta *et al.* (2022), who stressed that the mere transfer of knowledge is insufficient if it is not complemented by economic incentives, continuous technical support, and non-formal environmental education strategies. Likewise, Xian-Kang & Hong-Song (2024) reported that training from a technical perspective can encourage the adoption of technologies, especially those related to the efficient use of water in agriculture.

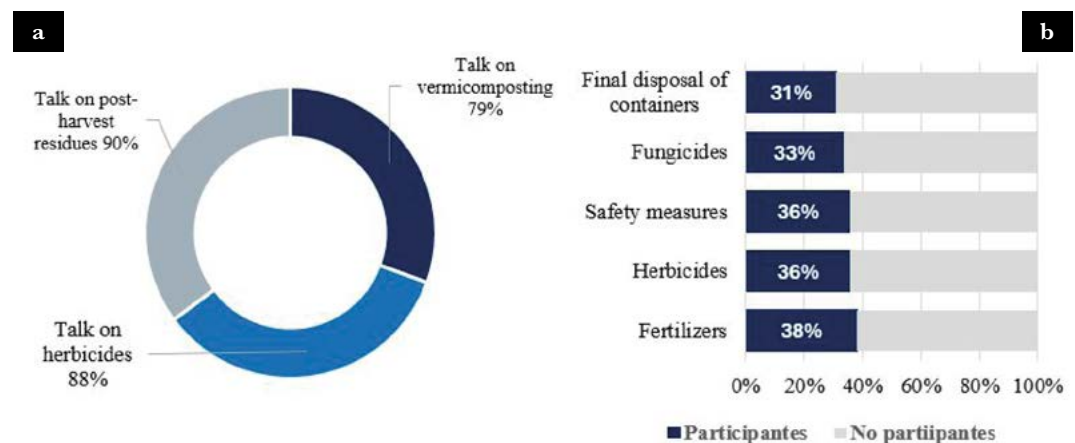


Figure 2. Participation of banana growers in a: talks on environmental issues and agricultural practices, and b: training courses on Agriculture in El Súchil, Tenexpa and Tetitlán, localities of Tecpan de Galeana (Guerrero), Mexico.

Biosecurity and sanitary practices

The first three items made a greater contribution (62%) from producers in biosecurity actions, although they partially complied with protocols, prioritizing health protection with the use of gloves, boots and masks. The rest of the participants (38%) stated that they did not use any type of safety equipment to carry out their activities in the field, they only practiced hand washing and changing clothes (Table 3).

Safety habits were confirmed during field trips and interviews. In fact, one of the producers commented “*I like to give conditions to my workers; but they argue that they do not like to feel uncomfortable, and that they would only accept a suit that clears their view, like a windshield.*” Of their own volition, these workers managed their activities with minimal protection in a high-risk context. When discussing contamination problems with producers in the three localities of Tecpan de Galeana, only a minority (14.3%) acknowledged they have presented health problems such as skin irritation and headaches after applying agrochemicals (Figure 3). However, solving this situation is complex; *i.e.*, one of the banana producers argued that they acted like this “*because they had no other option, they live off the work of the land*”.

Another important aspect is health and biosecurity measures. Responses showed that the use of personal protective equipment in the field is considered uncomfortable; and some use it inappropriately or incompletely. In this regard, Sánchez-Gervacio *et al.* (2021) noted that farmers should be trained in the correct use of personal protective equipment (PPE)

Table 3. Safety actions and hygiene measures of banana producers in the localities of Tecpan de Galeana (Guerrero), Mexico.

Biosecurity actions	%
Use of gloves, boots, masks, hand washing and changing clothes	28.6
Use of gloves, boots, masks and hand washing	21.4
Use of boots, masks and hand washing	12.0
Hand washing and changing clothes	19.0
Only hand washing	19.0

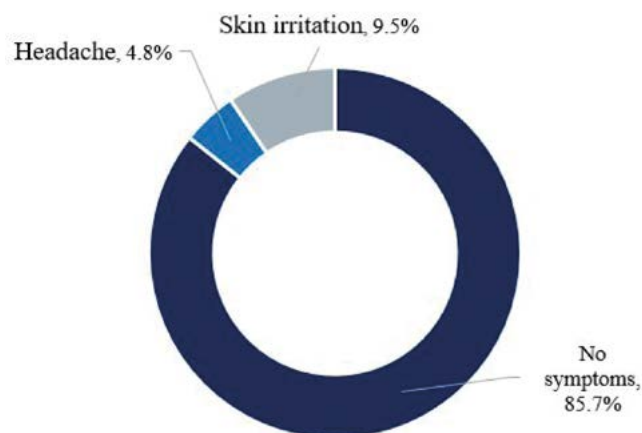


Figure 3. Health effects of banana producers generated by the application of agrochemicals in Tecpan de Galeana (Guerrero), Mexico.

and stressed the importance of using it despite the discomfort it causes in hot climates. The real impact is that direct contact with agrochemicals can cause from just discomfort such as vomiting, headache or drowsiness, to seizures, congenital malformations, and chronic health problems such as cancer or peripheral neuropathies that can lead to death. Hence the importance of adopting biosecurity measures, mainly in the management of fertilizers and agrochemicals, due to their high-risk effects on human health (Harizanova-Bartos & Stoyanova, 2018; Sharifzadeh *et al.*, 2019).

Agriculture waste management

Banana crop residues are generated in different stages: planting, harvesting and post-harvest. Organic waste is often placed in the soil to be disintegrated as organic matter. In the post-harvest stage, the residues of the cut (banana stem or rachis and banana waste) are handled. In this last stage, producers obtain economic income from the sale of the fruit, and the waste generated in the previous stages is considered of minor importance, therefore, they do not keep track of it.

In all three localities, the main practice observed in agricultural waste management is composting (70.6%); another common practice is the burning of agricultural waste. The use of this waste to prepare feed for livestock also occurs; this use in Tetitlán represents 14%, and in Tenexpa it is done by 7% of the interviewed producers (Table 4).

Another way to take advantage of the agricultural waste generated by the cuttings or shoots of the banana plant is to distribute them throughout the plantations. So that through its natural degradation a compost is generated useful for the recovery of nutrients in the soils thus fertilized. Even depositing them in the soil as ridges has been observed to help reduce erosion in banana orchards. And another type of waste that is generated in banana cultivation is agrochemical packaging, which generates a problem of contamination in soils and bodies of water when disposed of improperly. In this case, the town of El Súchil showed an outstanding participation, as 50% of the interviewees reported that they participate in an efficient control of agrochemical containers, by returning them to the distributor or supplier (Figure 4).

It is important to note that the favorable response in the town of El Súchil is due to a direct interaction with the agrochemical supplier and their business location close to the town. In addition, there is a collection center for these by-products, supported by dissemination through packaging collection campaigns and an agreement with other civil associations; that is, there is citizen participation for this collection.

Table 4. Management of agriculture residues by banana producers in three localities of the municipality of Tecpan de Galeana (Guerrero), Mexico.

Actions in waste management	El Súchil (%)	Tenexpa (%)	Tetitlán (%)
Composting	80	60	72
Burning	20	27	14
Preparing livestock feed	0	7	14
Transport of harvest	0	6	0
Totals	100	100	100

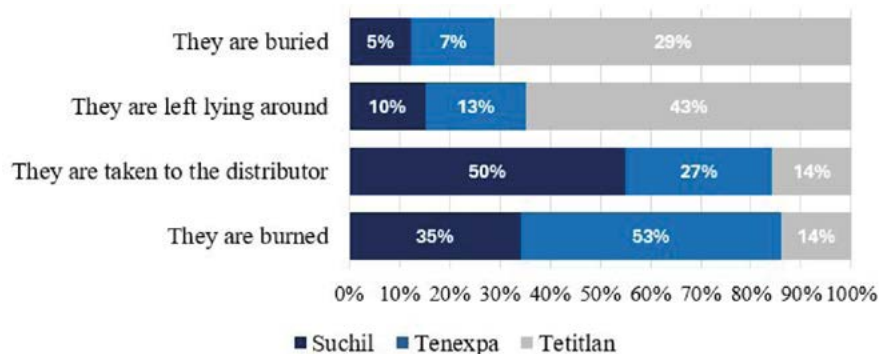


Figure 4. Disposal of agrochemical containers used by banana producers in three localities, in Tecpan de Galeana (Guerrero), Mexico.

In contrast to the other localities, 53% of the producers in Tenexpa burn the containers, which generates air pollution. In Tetitlán, 43% throw them away and accumulate them in an area of their orchards, with the risk of contaminating the soil and groundwater. Therefore, there is a need to raise awareness among banana producers to implement correct management of agrochemical containers in the locality.

In regard to the management of agricultural waste, burning is an activity that if not controlled can cause serious fires, and even authorities try to impose sanctions on this common practice. However, Luna-Celino & Kainer (2024) suggested that local governments provide technical assistance and training to apply controlled burns, which also incorporate the knowledge and experience of farmers on fire and wind management as factors. Likewise, Kouman *et al.* (2009) shared another use of the waste, in this case the covers of the banana pseudostems, as these can be used to transport the banana from the orchard to the ripening facilities, or to treatment and packaging points.

Strategies and challenges for environmental-friendly cultivation

Traditional farming techniques

In agricultural techniques, some traditional aspects are outstanding, such as family influence on production decisions, the way in which cultural practices are made, and the exchange of knowledge. All this influences the adoption of good practices, which come from the transmission of intergenerational experience from parents to children or from exchange with other producers. For example, cultural practices are of great importance in the elimination of tissues that limit the productive potential and quality of bananas, through the removal of leaves and the removal of some sprouts. The latter involves leaving the plant (offshoot) with greater vigor, so that it develops better and eliminating the others that compete for nutrients and space. Another example is the taping that serves to identify the degree of ripeness, as well as the practice of bagging the bunches to avoid damage caused by harmful fauna and placing a wooden pitchfork (in the form of a slingshot, with the double point-ends at the top) to support the plant so that it does not bend.

In the three localities, most decisions in the countryside are preferably made through family, ejido or communal agreements and exchanges. Similarly, land loans and transactions

are generated and validated more by family or neighborhood exchanges and agreements than by the participation of banks or the free competition of capital in the land market. This can be exemplified by the opinion of one of the producers who commented “*to make agreements for the purchase and sale of land, you negotiate first with relatives, then with neighbors, and finally with foreigners.*” Mukta *et al.* (2022) stated that culture changes continuously, as well as the members of the group to which they belong. For example, younger generations among producers tend to be more sensitive to environmental issues.

Water technologies and use

The problems faced by banana producers in the municipality of Tecpan are diverse, but one of the main difficulties is the large volume of water demanded by the production of bananas and other fruits. In terms of their irrigation practices, 18% of the producers surveyed indicated that they use sprinkler and drip irrigation, while 82% prefer flood systems despite water scarcity in some areas. Only in times of low water supply (dry season) do they try drip and sprinkler irrigation techniques, or a combination of both (Figure 5).

In Tecpan de Galeana, banana production takes place in an area irrigated by the Tecpan River (Figure 1). The water collection from the river is distributed through an infrastructure, such as an irrigation canal, which benefits the towns of El Súchil and Tenexpa. During the dry season, both localities supplement their irrigation needs with water extracted from artesian wells with connection to drip and sprinkler irrigation systems. While in Tetitlán, farmers rely exclusively on groundwater, extracted from deep wells, and try to use water efficiently through drip and sprinkler irrigation systems.

The maintenance of water infrastructure depends largely on public investment and community work. According to one of the producers, “*the last investment with Government support was in 2012 when a one-kilometer pipeline was dredged and rehabilitated in the town of El Súchil. Every year, we make the desilting work and installation of a stone-dam that distributes water to the canals in the months of June-July and November-December.*” The implementation of best management practices for banana cultivation in Tecpan de

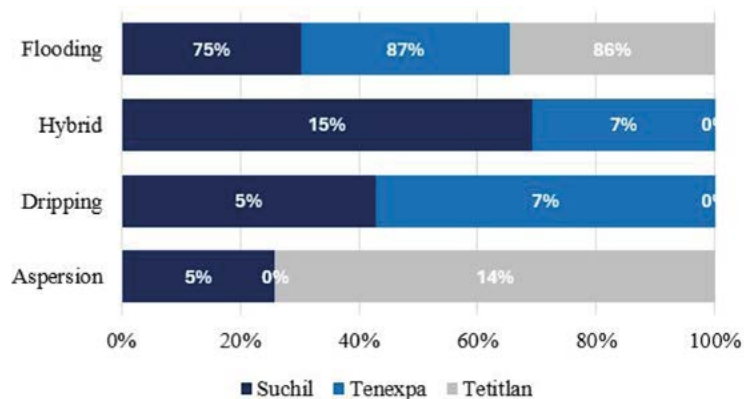


Figure 5. Types of irrigation systems for the cultivation of bananas and other crops in the localities of El Súchil, Tenexpa and Tetitlán, in Tecpan de Galeana (Guerrero), Mexico.

Galeana is a challenge for many producers, since many of them demand investment, technology and knowledge to guarantee effectiveness, since the application requires specialized soil and hydrology studies, as well as previous experience for pest control and water use.

Although the study reveals some good practices with the use of water, these represent a challenge for small farmers due to the cost and the specialized personnel required for their installation. The use of modern agricultural technologies, such as drip or sprinkler irrigation systems, can effectively alleviate the problem of water resource scarcity (Xian-Kang & Hong-Song, 2024). To achieve this, the United Nations Food and Agriculture Organization (FAO) (2020) recommended investing in irrigation systems or modernizing and rehabilitating existing ones to use water in agriculture more efficiently.

Consensus on environmentally friendly agricultural strategies

During the study, several talks were held with producers where they shared experiences aimed at environmental-friendly practices, such as the use of agricultural waste, the production of organic fertilizers and biosecurity measures that include water conservation. The proposals that resulted from this consensus are described (Table 5).

Table 5. Consensual proposals with banana producers in the municipality of Tecpan de Galeana (Guerrero), Mexico.

Challenges	Strategies	Intervention	Themes for EE
Soil degradation from intensive use of agrochemicals	Studies that consider the degree of soil fertility and the use of organic fertilizers at affordable prices.	Analysis of soil fertility and development of a plan that includes the use of waste.	<ul style="list-style-type: none"> • Importance of soil fertility studies. • Use of waste for soil recovery.
Use of PPE	Adjust protective equipment to make it more comfortable. Campaigns for rising awareness on the use of PPE in farmlands.	Health brigades from research institutions.	<ul style="list-style-type: none"> • PPE for farmers. • Proper use of PPE. • Risks and consequences for farmer health.
Agriculture Waste Management	Use of organic waste to produce vermicomposting and leachates.	Studies of leachate components demonstrate usefulness.	<ul style="list-style-type: none"> • Waste use. • Adding value to crop residues.
Proper handling/ disposing of agrochemical containers	Training in triple washing. Reporting on the packaging management chain with suppliers.	Implementation of a Non-Formal Environmental Education Program (NFEEP)	<ul style="list-style-type: none"> • Hazardous waste. Health effects, soil and water pollution.
Water management for irrigation	Awareness campaigns, state support for the acquisition and training of new technology for efficient use of water.	Implementation of a Non-Formal Environmental Education Program (NFEEP).	<ul style="list-style-type: none"> • Development of contents on the water cycle and environmental care to avoid water pollution.

EE: environmental education; PPE: personal protective equipment; NFEEP: non-formal environmental education program.

The results showed that in localities with strong solidarity, farmers tend to share knowledge, forming a broad local network committed to minimizing pesticide use. Building consensus on sustainable agricultural practices through the exchange of experiences can be complex, although the results are often favorable and conducive to the common good. According to Kumar *et al.* (2023) Each region has a particular method to implement various activities, which involves cropping systems and the management of their residues; as well as the use of available local resources and the socioeconomic conditions of farmers. For example, Mukta *et al.* (2022) pointed to an experience with farmers in Bangladesh, who solved their problems related to pest management in their crops by using community knowledge from nearby sources, such as neighbors, friends, farmer family, local suppliers, and officials visiting the community.

Although this study was limited in scope, with a focus on banana crop producers, some of their socio-environmental dynamics were identified. Therefore, for a deeper understanding regarding the adoption of sustainable practices for the crop studied, it is convenient to extend the evaluation to other types of crops. To obtain information on other good practices in aspects of training, biosecurity measures and use of agricultural residues. Also, to analyze other key aspects that serve as support for the formulation of agricultural programs, from a scientific context for decision-makers.

A larger study could reveal more information if the implementation of a non-formal environmental education program is added to raise awareness and enhance the knowledge acquired from the experiences of producers. This would encourage and promote a change towards safer and more environmentally friendly agricultural practices in the medium term, and experiment subsequently with the appropriation of new practices or technologies aimed at sustainable agriculture.

Findings brought evidence of a wide gap between sustainable practices and practices applied in the field, in aspects such as training, biosecurity measures, and agricultural waste management, which includes agrochemical packaging, in which inadequate practices persist. Among the suggested strategies, we highlighted the promotion of composting practices, the use of organic fertilizers, which are essential to move towards a more sustainable agriculture. Challenges were observed in the use of personal protective equipment and the improvement of irrigation infrastructure, since both require campaigns to raise awareness to generate changes in overall attitudes. Although the changes imply an economic investment that most of small producers are not yet willing to make.

CONCLUSIONS

Although banana producers present challenges in implementing sustainable cultivation, they also present opportunities if they organize themselves into groups or guilds. Consensus among producers identified viable practices for sustainable agriculture. However, Government involvement is key to driving these advances and positioning the region as a leader in banana production. It is important to note that an investment in non-formal environmental education programs (NFEPP) can encourage the use of environmental-friendly practices. This is not only for banana cultivation, but also for other types of

crops. An environmental education program can be a driver that attracts investment and economic development to the region.

ACKNOWLEDGMENTS

To all banana producers who participated in the survey and shared their experiences and good practices in banana cultivation.

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Evaluation of pregermination treatments as potential promoters of germination and development in tomato (*Solanum lycopersicum* L.)

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ABSTRACT

Objective: To evaluate diverse pregermination treatments aimed at accelerating, synchronizing, and enhancing seed germination, as well as promoting the development of vigorous tomato seedlings, by assessing the efficacy of hydropriming methods, chemical compounds, plant extracts, and bioprimering strategies.

Design/Methodology/Approach: Germination rate, velocity, and temporal dynamics were determined using variables such as total germination, maximum daily germination, germination interval, and time to 50 % germination (T_{50}). Seedling vigor was assessed through measurements of stem and root length, in addition to dry matter accumulation. An analysis of variance (ANOVA) was conducted under a completely randomized design for each evaluated variable.

Results: Several treatments significantly enhanced the germination potential of tomato seeds and effectively improved seedling morphological attributes. Nevertheless, none of the treatments succeeded in modifying the temporal dynamics of the germination process.

Study Limitations/Implications: Future research should focus on evaluating the most relevant findings of this study under productive conditions and in relation to resistance against environmental stressors.

Findings/Conclusions: Hydropriming and bioprimering with understudied *Bacillus* and *Pseudomonas* species exerted positive effects on germination and seedling vigor indicators, respectively. Moreover, garlic, onion, and chili extracts demonstrated favorable effects on both germination and seedling development parameters.

Keywords: priming, bioprimering, plant extracts, germination, development.

Citation: Hernández-Escalona, R., Carrillo-Castañeda, G. M., Cruz-Izquierdo, S., & Antúñez-Ocampo, O. M. (2026). Evaluation of pregermination treatments as potential promoters of germination and development in tomato (*Solanum lycopersicum* L.). *Agro Productividad*. <https://doi.org/10.32854/n0wayb16>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: October 10, 2025.

Accepted: March 28, 2025.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 135-150.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the second most important horticultural crop worldwide. It is cultivated on nearly five million hectares and exceeds 190 million tons in annual global production (FAOSTAT, 2023), with approximately 3.5 million tons produced in Mexico (SIAP, 2023). Tomato is also among the most widely used ingredients in gastronomy, as it constitutes a rich source of lycopenes, vitamin C, and phenolic compounds with notable antioxidant properties that are relevant to human health (Rodríguez *et al.*, 2024).

Seed germination efficiency and, consequently, production levels may be constrained by factors such as seed-coat hardness, natural aging, endogenous inhibitors, and unfavorable environmental conditions (Lamichhane *et al.*, 2018). Within this framework, the production of vigorous and uniform seedlings is a decisive determinant of productive success, as it directly influences survival, vegetative growth, and final yield. This scenario has fostered the development of pregermination treatments based on the controlled manipulation of physical, chemical, and biological factors to optimize germination and enhance seedling vigor. Among the most widely implemented strategies are priming treatments, including distinct variants such as hydropriming, chemical priming with solutes, and biopriming, all of which can improve germination performance and subsequent development (Dhal *et al.*, 2022). More recently, plant extracts rich in flavonoids, organosulfur compounds, and carotenoids have also been explored; however, substantial gaps remain regarding their allelopathic properties and underlying modes of action (Da Silva Carvalho *et al.*, 2021).

Systematic research on pregermination treatments is essential for developing technologies that optimize tomato seedling production by improving germination, vigor, disease resistance, and crop establishment. Elucidating the mechanisms associated with each treatment enables the selection of the most effective strategies under diverse production conditions. Accordingly, this study aimed to evaluate different pregermination treatments on tomato germination and the development of vigorous seedlings using priming techniques and assays with plant extracts, with the purpose of identifying the most effective strategies.

MATERIALS AND METHODS

The experiments were conducted between January 2024 and June 2025 at the facilities of Colegio de Postgraduados, Montecillo Campus, Texcoco, State of Mexico. Saladette tomato seeds, cultivar Río Grande (2020 lot), marketed by “<http://www.lasemilleria.com>”, were used.

Pregermination Treatments

Hydropriming

Hydropriming was evaluated for its effects on germination. This treatment involves seed hydration for a defined period without allowing radicle protrusion, followed by drying until the initial dry weight is reattained (Pandey *et al.*, 2022). In the present study, 100 seeds (0.22 g) were hydrated for 24 h, dried for 1 h using a fan, and then stored. Seed weight was monitored periodically until it matched the initial dry weight. The duration of this treatment and that used in the chemical-compound experiment was established based on the water-uptake curve, which indicated that seeds required 27 h to germinate.

Plant extracts

Germination

Aqueous extracts were prepared from fresh samples of five plant species: garlic (*Allium sativum* L.), onion (*Allium cepa* L.), habanero chili (*Capsicum chinense* Jacq.), ginger (*Zingiber officinale* Roscoe), and aloe vera (*Aloe vera* L.), at concentrations of 15, 25, 50, and 75 % in

16 mL volumes. Water and mortars were pre-cooled to 16 °C and -18 °C, respectively. For each plant material, 0.21 g was weighed and macerated for 3-4 min (garlic, chili, onion) or 5-6 min (ginger, aloe vera), gradually adding water. Each extract was adjusted to the required concentration according to Table 1.

Development

Based on germination tests, two of the most promising extracts were selected: 25% chili pepper extract (CH25) and 50% onion extract (O50). Three treatments were established: seeds soaked in CH25, seeds soaked in O50, and a control soaked in distilled water (I). An additional untreated control (T) was also included. For each treatment, 20 seeds were soaked for 24 h in 10 mL of the corresponding extract, and the control was soaked in distilled water (I).

Chemical compounds

Five chemical compounds were selected based on their prevalence in the seed conditioning literature to evaluate their effects on germination: acetylsalicylic acid ($C_9H_8O_4$ or ASA) at concentrations of 0.06 and 0.12%, potassium nitrate (KNO_3) at 1 and 3%, copper sulfate ($CuSO_4$) at 1 and 2%, calcium chloride ($CaCl_2$) at 2 and 3%, and sodium chloride ($NaCl$) at 0.5 and 1%. Initially, the concentrations matched those reported in the literature, and based on the results, the concentration was adjusted for the second

Table 1. Volumes of plant extracts and water needed to obtain the concentrations of the experiment based on a total volume of 16 mL.

Required Concentration (%)	Extract Volume (mL)	Water Volume (mL)
15	2.4	13.6
25	4	12
50	8	8
75	12	4

Table 2. Amounts of substance needed to prepare the solutions for the experiment based on a total volume of 16 mL.

Chemical substance	Solution concentration (%)	Amount of substance (g)
ASA	0.06	0.01
	0.12	0.02
KNO_3	1	0.16
	3	0.48
$CuSO_4$	1	0.16
	2	0.32
$CaCl_2$	2	0.32
	3	0.48
NaCl	0.5	0.08
	1	0.16

stage of the experiment. The treatment consisted of soaking the seeds in the chemical compounds in solution for 24 h prior to germination; 16 mL of solution were prepared for each substance. To prepare the solutions, the quantities of each substance described in Table 2 were weighed, water was added, and the mixture was stirred until completely dissolved. For each concentration, the seeds were placed in Petri dishes, the solutions were added, and the seeds were incubated for 24 h. Finally, they were thoroughly washed with distilled water and placed in the germination chamber.

Biopriming

Biopriming was selected to evaluate seedling development responses. This technique is based on exposing seeds to microorganisms with beneficial properties (Rendón and Gómez, 2018). In this experiment, strains from the collection of the Cellular Biology Laboratory at Colegio de Postgraduados, Montecillo Campus, were used. *Pseudomonas* spp. and other species associated with tomato and maize roots were evaluated. Selection of *Pseudomonas* strains was based on their ability to grow and maintain fluorescence under increasing salinity in the culture medium. Strains from other species were selected solely according to salinity tolerance. Each strain preserved in test tubes was streaked using an inoculation loop onto Petri dishes containing King's B medium with salinity levels of 1, 2, and 3% (Table 3). Fluorescence was assessed using an ultraviolet (UV) lamp. The best-performing strains were then inoculated onto Petri dishes with King's B medium and incubated at 26-28 °C for 24 h. The following day, bacterial suspensions were prepared in test tubes containing sterile water. Each suspension was adjusted to a standardized turbidity of 0.90 at an optical density of 660 nm using a spectrophotometer. Twenty seeds per treatment and the control were placed in sterile Gerber jars. For seed coating, 10 mL of bacterial suspension was added to each jar and allowed to stand for 15 minutes. The seeds were then removed and sown immediately.

Germination assays

For each treatment and control, 100 seeds were used and distributed into four Petri dishes (25 seeds per dish). Then, 4 mL of water, plant extract, or solution depending on the treatment was added to each dish, always over three sheets of Sanitas paper. The dishes

Table 3. Bacterial strains evaluated in tomato development tests.

Species	Code	Origin	Type
Unidentified	RJ6	Tomato	Rhizospheric
<i>Bacillus albus</i>	RJ12	Tomato	Rhizospheric
<i>Bacillus paranthracis</i>	RJ13	Tomato	Endophytic
<i>Bacillus pacificus</i>	RJ16	Tomato	Endophytic
<i>Bacillus amyloliquefaciens</i>	RJ25	Tomato	Rhizospheric
<i>Pseudomonas hunanensis</i>	1M	Maize	Endophytic
<i>Pseudomonas fungipugnans</i>	3L	Maize	Rhizospheric
<i>Pseudomonas fungipugnans</i>	4L	Maize	Rhizospheric
<i>Enterobacter bugandensis</i>	5L	Maize	Rhizospheric

were incubated at 26-28 °C, and the number of germinated seeds was recorded daily. A seed was considered germinated when a visible radicle protrusion (≥ 2 mm) was observed outside the seed coat. The Petri dish was considered the experimental unit ($n=4$).

Germination data analysis

From the recorded data, the Cumulative Germination (CG) curve was plotted; expressed as the cumulative sum of the mean daily germination percentages of the four Petri dishes used in each treatment. For each treatment, the TG was calculated as the mean final germination percentage of the four Petri dishes. The TG results are presented with their respective standard deviations (SD, equation 1) and confidence intervals (CI, equation 2) for a 95% confidence level, calculated using the four replicate values.

$$SD = \sqrt{\frac{\sum_{i=1}^N (x_i - \bar{x})^2}{N}} \quad (1)$$

$$CI = \bar{x} \pm z(\sigma / \sqrt{N}) \quad (2)$$

where: N denotes the sample size; x_i represents the individual observations within each treatment; \bar{x} is the sample mean; and z is the critical value associated with the selected confidence level.

In addition, T_{50} the time required for 50% of the viable seeds to germinate was estimated using the CG data and the slope of a linear function model, according to the following formulas:

$$m = \frac{y_2 - y_1}{x_2 - x_1}$$

$$y = mx + n$$

where: m is the slope of a line; x_n and y_n these are coordinates representing intercept points along a straight line (in this case, x corresponds to time and y represents the germination value at that time), and n denotes the y-intercept of the line.

The germination interval was also determined as the elapsed time between the onset and completion of germination (D_0 - D_f). Graph construction and data processing were performed using Microsoft Excel (Microsoft Office suite).

Development assays

The seeds were sown in sterile substrate in polystyrene germination trays, which remained in the greenhouse for 30 to 35 days; the approximate time before transplanting (Gómez-Salazar *et al.*, 2023). The sample size was 10 plants per treatment, where each plant represented an experimental unit ($n=10$). The measured variables were stem length, root length, and dry matter weight of the seedlings. To determine dry matter weight, the

seedlings were first placed in an oven for 24 h at 43 °C, and then their weight was checked every hour until it stabilized.

Statistical analyses

The treatments evaluated for developmental variables were distributed in a completely randomized design with 10 replicates per treatment. The data for each vigor variable were subjected to a one-way Analysis of Variance (ANOVA). When the ANOVA indicated significant differences ($p < 0.05$), the treatment means were separated using Tukey's Honestly Significant Difference (HSD) test at $p = 0.05$. Prior to the ANOVA, assumptions (Normality and Homogeneity of Variances) were verified. All analyses were performed using R version 4.4.3.

RESULTS AND DISCUSSION

Pregermination Treatments

Hydropriming

Seed germination benefited from this treatment, as it increased total germination (TG) to 90%, with improved standard deviations and confidence intervals. Synchrony and germination rate also improved, as evidenced by a high maximum daily germination (MDG; 64%) and a lower T_{50} (39.7 h), even though the overall germination period extended by one additional day under the treatment (Table 4). The germination process began and proceeded more actively following a typical temporal pattern, with no appreciable modifications, as reflected by the cumulative germination (CG) curve (Figure 1).

Table 4. Germination parameters of tomato seeds subjected to hydropriming and the control treatment.

	TG (%)	MDG (%)	T_{50} (h)	D_0-D_f (d)	SD	CI
Control	86	40	47.8	6	7.65	± 7.49
Hydropriming	90	64	39.7	7	5.16	± 5.05

TG: total germination; MDG: maximum daily germination; T_{50} : time to 50% germination of viable seeds; D_0-D_f : germination time interval; SD: standard deviation of TG; CI: confidence interval of TG.

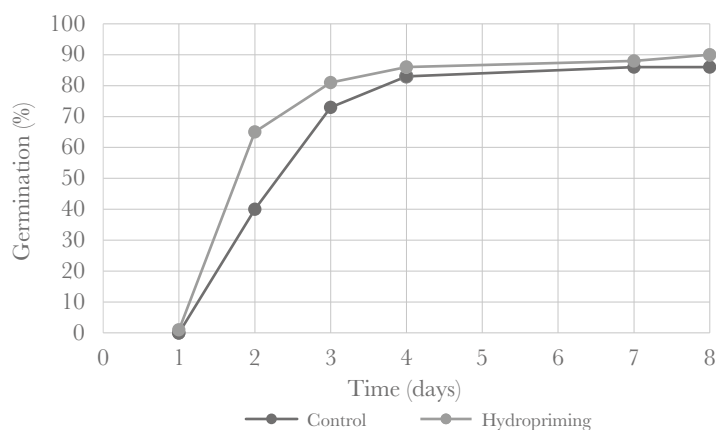


Figure 1. Germination behavior of tomato seeds subjected to hydropriming and control. Cumulative germination percentage determined at the indicated times.

Hydropriming promotes advancement to a late point within Phase II of the triphasic germination pattern, during which critical metabolic processes occur, including increases in the ATP/ADP ratio, mitochondrial repair, protein synthesis, and enzyme activation (Lemmens *et al.*, 2019). Consequently, upon rehydration, seeds exhibit clear advantages: more efficient metabolism, embryo enlargement, and endosperm weakening, thereby reducing the time and resources required for radicle protrusion (Chen and Arora, 2012). In aged seeds, moisture loss can lead to membrane damage and diminished enzymatic activity; controlled hydration can partially restore germination potential (Rendón and Gómez, 2018).

Natural Extracts

Germination

The results identified chili extracts at 15 and 25%, garlic extract at 25%, and onion extract at 50% as the most effective treatments for improving germination. The primary effects of the extracts were observed on total germination (TG) and process synchrony, as all treatments exceeded the control values for these parameters. However, only the 25% garlic and 25% chili extracts increased germination rate, as indicated by improved T_{50} values relative to the control. Notably, the 25% extracts yielded consistent outcomes, exhibiting favorable performance across multiple parameters (Table 5). The cumulative germination (CG) curve captured the key phases of the typical temporal germination pattern of tomato seeds (Figure 2).

Table 5. Germination parameters of tomato seeds subjected to different plant extracts and the control treatment.

	Control	Extracts (%)									
		15%					25%				
		Garlic	Onion	Chili	Ginger	Aloe	Garlic	Onion	Chili	Ginger	Aloe
TG (%)	86	84	90	95	86	90	91	94	93	92	91
MDG (%)	37	45	41	54	48	42	43	37	41	48	42
T_{50} (h)	54.5	62.1	58.5	59.3	62.5	61.6	49.8	54.6	52.1	58.0	50.5
D_o-D_f (d)	7	7	7	6	7	6	7	8	6	8	7
SD	5.16	7.57	9.52	3.82	6.92	9.52	3.82	5.16	2.00	8.64	5.03
CI	±5.05	±7.41	±9.32	±3.74	±6.78	±9.32	±3.74	±5.05	±1.96	±8.46	±4.92
		50%					75%				
TG (%)	86	89	92	81	83	93	84	89	90	85	85
MDG (%)	37	52	54	42	52	51	56	45	42	49	38
T_{50} (h)	54.5	44.5	58.2	60.2	62.5	62.3	59.1	47.7	50.1	58.7	51.0
D_o-D_f (d)	7	6	6	7	6	6	6	7	8	8	7
SD	5.16	8.24	3.26	11.01	11.94	8.86	3.26	6.00	4.00	5.03	5.03
CI	±5.05	±8.07	±3.19	±10.78	±11.70	±8.68	±3.19	±5.87	±3.91	±4.29	±4.29

TG: total germination; MDG: maximum daily germination; T_{50} : time to 50% germination of viable seeds; D_o-D_f : germination time interval; SD: standard deviation of TG; CI: confidence interval of TG.

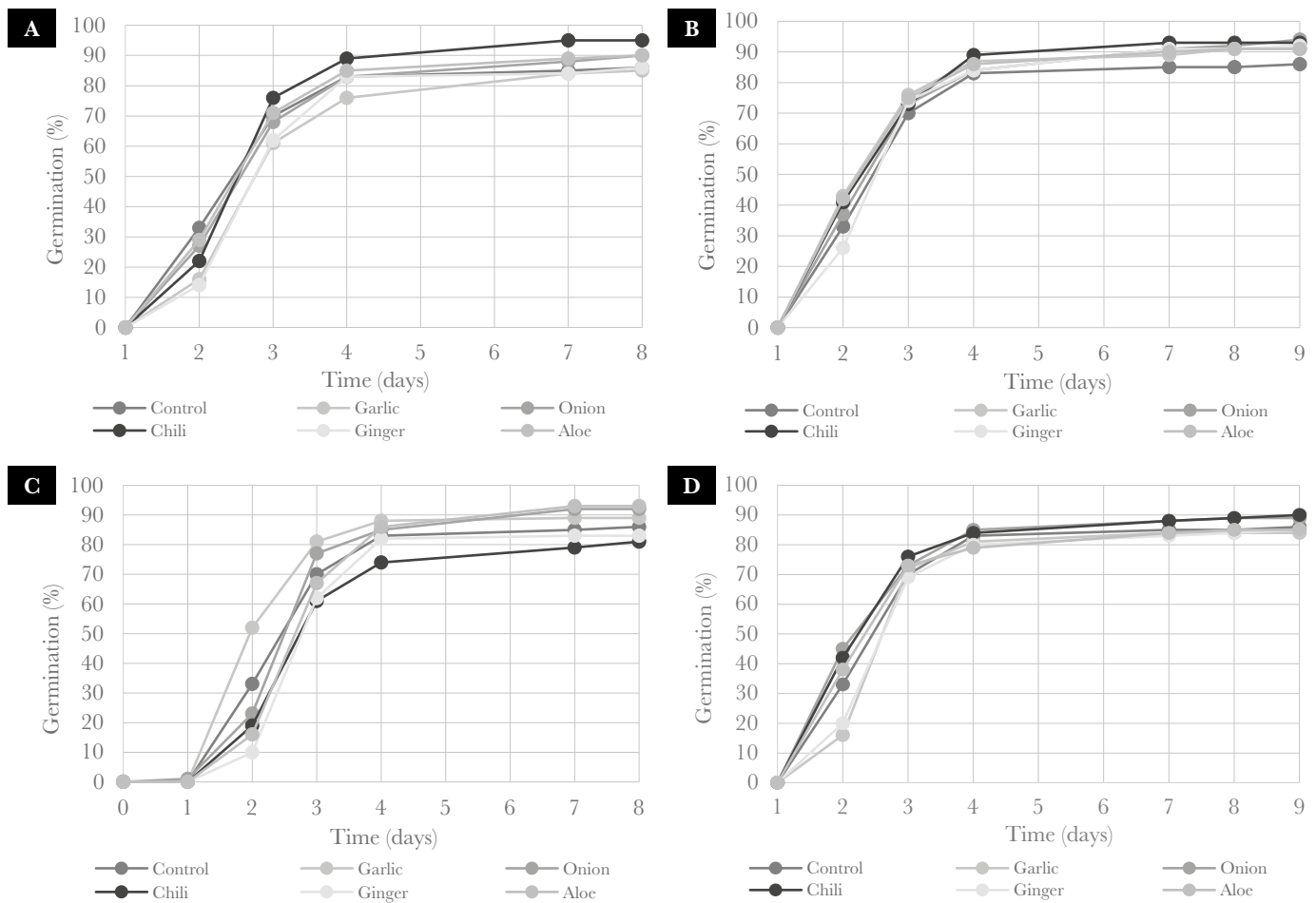


Figure 2. Germination behavior of tomato seeds exposed to different plant extracts and the control treatment. Cumulative germination percentages at the indicated time points for extracts at 15% (A), 25% (B), 50% (C), and 75% (D) concentrations.

Plant extracts exhibited a hormetic effect, a phenomenon in which a stress factor recognized for its toxicity at high doses elicits stimulatory responses at lower concentrations (Agathokleous *et al.*, 2023). Onion extract was among those that displayed this response, a logical outcome given its richness in diverse bioactive compounds, including organosulfur compounds, phenolic acids, flavonoids, minerals, and amino acids (Sagar *et al.*, 2022). Additionally, phytosterols present in onion act as precursors of brassinosteroids, which may promote germination by reducing seed sensitivity to abscisic acid (Pareek *et al.*, 2017; Finch-Savage, 2013).

Regarding the effect of chili extract on plants, there are differing opinions; It has been shown that it does not affect the germination of *Ipomoea purpurea* L. (Del Rosario García-Mateos *et al.*, 2013); while studies in *Capsicum annuum* demonstrated negative effects of the extract on seed germination (Barchenger and Bosland, 2016). In this work, the chili extract enhanced germination performance. Capsaicin, one of its principal bioactive compounds, is a nitrogen compound that may serve as a precursor for amino acid synthesis during the activation of germinative metabolism (Finch-Savage, 2013).

Additionally, chili contains quercetin, a distinctive subclass of flavonoid known to participate in the regulation of diverse physiological processes, including germination (Singh *et al.*, 2021).

Regarding garlic, organosulfur compounds are particularly abundant, especially allicin, which exhibits high permeability across biological membranes. This compound functions as a signaling molecule capable of modulating endogenous hormonal balance by increasing auxin and gibberellic acid levels, while allelochemicals act as natural biostimulants that trigger reactive oxygen species signaling pathways (Hayat *et al.*, 2020).

Development

Chili extract at 25% (CH25) and onion extract at 50% (O50) were evaluated for development variables. Statistical analysis revealed significant differences between treatments and the control across all three evaluated variables. For stem and root length, no significant differences were detected between treatments, although CH25 (8.16 cm) and O50 (8.30 cm), respectively, exhibited slightly superior values. In contrast, differences in dry matter weight were more pronounced; treatments were separated into three distinct groups, and imbibition (I) outperformed the remaining treatments, exceeding the control by more than 40% (Table 6).

Imbibition performed adequately, and the combination of imbibition with the bioactive compounds present in the extracts further enhanced stem and root length; however, no significant differences were detected among the extract treatments. The marked differences observed in dry matter weight provide evidence of structural changes in the root system, likely associated with increased secondary root formation. Lemmens *et al.*, (2019) concluded that seed prehydration is effective not only in enhancing germination; they also showed that, after sowing, primed seeds absorb water and reactivate metabolism more rapidly, thereby improving establishment and vigor.

Regarding the extracts, chili contains carotenoids that act as precursors of strigolactones, which have the capacity to stimulate root development (Cortes *et al.*, 2019), as well as quercetin, which plays a key role in activating plant physiological traits such as growth and photosynthesis (Singh *et al.*, 2021). In onion, flavonoids and phenolic compounds are among the most abundant constituents and are noteworthy due to their active involvement in regulating plant growth and mediating defense responses (Rodríguez *et al.*, 2024).

Table 6. Results of Tukey's test for comparing means to evaluate the treatments with plant extracts applied to tomato seeds for development.

Stem length(cm)			Root length (cm)			Dry matter weight (mg)		
Treatment	Mean	Group	Treatment	Mean	Group	Treatment	Mean	Group
CH25	8.16	a	O50	8.30	a	I	108.30	a
O50	7.81	a	CH25	8.05	a	O50	97.95	ab
I	7.55	a	I	7.99	a	CH25	91.95	b
T	6.14	b	T	6.31	b	T	74.10	c

Chemical compounds

The CuSO₄ solutions at 1 and 2% were identified as the most effective treatments. These solutions primarily improved total germination (TG), whereas the remaining germination variables were not substantially modified relative to the control (Table 7). The cumulative germination (CG) curves followed similar trajectories consistent with the typical temporal germination pattern of tomato (Figure 3).

Table 7. Germination parameters of tomato seeds subjected to different chemical compound solutions and the control treatment.

Variables	Solutions (Concentration %)					
	0	0.5	0.06	2	1	1
TG (%)	Control	NaCl	AAS	CaCl ₂	KNO ₃	CuSO ₄
TG (%)	86	85	88	89	87	91
MDG (%)	40	47	36	40	46	41
T ₅₀ (h)	50.8	58.5	54.0	53.1	46.7	53.6
D ₀ -D _f (d)	7	6	6	7	6	6
SD	5.16	8.24	3.26	5.03	2.00	3.82
CI	±5.05	±8.07	±3.19	±4.92	±1.96	±3.74
	0	1	0.12	3	3	2
TG (%)	86	84	80	86	89	90
MDG (%)	40	36	33	30	43	39
T ₅₀ (h)	50.8	55.3	53.1	60.0	57.2	51.8
D ₀ -D _f (d)	7	7	7	7	7	6
SD	5.16	3.26	14.37	10.58	5.16	2.70
CI	±5.05	±3.19	±14.08	±10.36	±5.05	±2.64

TG: total germination; MDG: maximum daily germination; T₅₀: time to 50% germination of viable seeds; D₀-D_f: germination time interval; SD: standard deviation of TG; CI: confidence interval of TG.

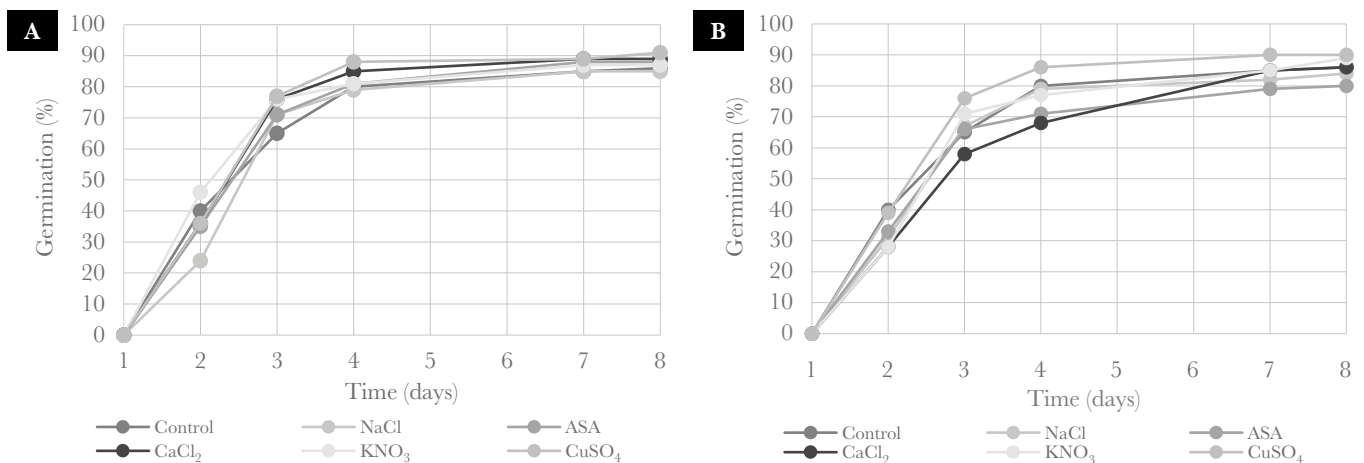


Figure 3. Germination behavior of tomato seeds exposed to different chemical compound solutions and the control treatment. Cumulative germination percentages (A and B) across the indicated time points. (A) NaCl 0.5%, acetylsalicylic acid (C₉H₈O₄; ASA) 0.06%, CaCl₂ 2%, KNO₃ 1%, and CuSO₄ 1% solutions. (B) NaCl 1%, ASA 0.12%, CaCl₂ 3%, KNO₃ 3%, and CuSO₄ 2% solutions.

Copper (Cu) is an essential element for plants; nonetheless, the available evidence regarding its use is contradictory. Iqbal *et al.*, (2018) reported toxic effects of Cu-derived compounds, including growth inhibition and reduced germination. Conversely, the findings of De la Cruz *et al.* (2023) in pepper indicate that CuSO₄ supplementation can stimulate regenerative processes in cotyledons, enhancing embryo production and promoting germination.

This apparent discrepancy may be explained by differential sensitivity among species or by excessively high application concentrations. Carrillo *et al.* (2005) support this hypothesis by showing considerable variation in Cu accumulation among different roselle (*Hibiscus sabdariffa* L.) varieties, suggesting that differential responses may constitute genotype-specific protective mechanisms. The observation that CuSO₄ is not universally lethal and that, at certain concentrations, it can stimulate germination suggests the existence of an optimal range in which Cu functions as an essential micronutrient before reaching toxic thresholds.

As demonstrated in this study, germination onset and critical points such as the day of maximum MDG and the interval of highest germinative activity follow a nearly invariable temporal germination pattern, consistent with the results reported by Michelin *et al.* (2016). Identifying the most effective treatment depends on multiple factors, and TG alone is often not a sufficiently comprehensive indicator. Parameters such as MDG, T₅₀, and the germination interval (D₀-D_f) must also be considered. If the objective is to determine the most efficient treatment, it should also confer greater speed and synchrony to the germination process; only then can establishment success and seedling survival be effectively improved (García and González, 2021).

Biopriming with tomato-associated bacteria

Analysis of variance for treatments involving *Bacillus* strains isolated from tomato roots revealed statistically significant differences among treatments for the evaluated development variables, albeit with specific considerations for root length and dry matter weight. For root length, the assumption of homogeneity of variances was not met ($p=0.01$); therefore, an arcsine transformation was applied to data normalized as proportions. For dry matter weight, a Box-Cox transformation was required ($\lambda=-0.3$). In both cases, the respective transformations corrected the assumption violations, and the analyses of variance continued to indicate significant treatment effects. The means presented in Table 8 are reported using the original (untransformed) data. Among the evaluated strains, RJ16 consistently performed best across the three variables, showing no major differences relative to RJ25 and RJ13, yet maintaining superior values in all cases (Table 8).

These bacteria belong to the group commonly referred to as Plant Growth-Promoting Bacteria (PGPB) (Castaño *et al.*, 2021b). The mechanisms of action of PGPB are typically classified as direct and indirect. Direct mechanisms include the biosynthesis of phytohormones and vitamins, as well as stimulation of germination. Indirectly, PGPB can synthesize antibiotics and fungicidal compounds, produce siderophores, and act as biological control agents against phytopathogens (Álvarez-García *et al.*, 2020). The genus *Bacillus* is dominant among endophytic bacteria (Cochard *et al.*, 2022), which are known

Table 8. Results of Tukey's test for comparing means to evaluate the inoculation of tomato seeds on development with tomato root-associated bacteria.

Stem length (cm)			Root length (cm)			Dry matter weight (g)		
Treatment	Mean	Group	Treatment	Mean	Group	Treatment	Mean	Group
RJ16	6.95	a	RJ16	5.95	a	RJ16	76.62	a
RJ25	6.91	ab	RJ13	5.30	ab	RJ25	70.06	ab
RJ6	6.90	ab	RJ25	5.13	ab	RJ13	65.93	bc
RJ13	6.85	ab	RJ12	4.82	bc	RJ12	65.37	bc
RJ12	6.80	ab	T	4.53	bc	RJ6	64.06	bc
T	5.55	b	RJ6	3.81	c	T	62.37	c

to establish intimate associations with plants, may be vertically transmitted, and can act through the secretion of minute concentrations of metabolites (Álvarez-García *et al.*, 2020). This could help explain the pronounced effects observed for *Bacillus pacificus* (RJ16) and *Bacillus paranthracis* (RJ13).

Regarding *B. pacificus* (RJ16), information on its effects in tomato is limited. Kouam *et al.* (2023b) reported that it increased stem length and the dry weight of roots and shoots. Ma *et al.* (2024) demonstrated that it enhances chlorophyll content and promotes root development in *Arabidopsis thaliana* and *Medicago sativa*, with the latter effect also corroborated in the present study. In these species, it also exhibited the capacity to mitigate drought damage. *Bacillus amyloliquefaciens* (RJ25), another strain with strong performance, has been extensively studied in tomato as a biocontrol agent (Chou *et al.*, 2022; Imran *et al.*, 2021). As a PGPB, Imran *et al.* (2021) confirmed that it increases germination and improves root and shoot length, with results comparable to those reported by Ali *et al.* (2025b). *Bacillus paranthracis* (RJ13) is a recently described species with limited information available; however, it belongs to a group closely related to *Bacillus cereus* (Campos-Avelar *et al.*, 2025), a species known to promote both aerial and root development in tomato (Andrade-Sifuentes *et al.*, 2022).

Biopriming with Maize-associated bacteria

Analysis of variance for treatments with *Pseudomonas* and *Enterobacter* strains isolated from maize roots, evaluated on tomato seedling development variables, revealed statistically significant differences in stem length and dry matter weight, whereas no differences were detected for root length. For stem length, the reference treatment was 1M, which was slightly higher than 3L and 4L, although differences were modest. Dry matter weight exhibited a different pattern: 5L and 3L were the best treatments, with weight increases of up to 75% relative to the control. Treatment 3L showed the most stable performance, ranking second for both evaluated variables, yet it did not differ significantly from the top-performing treatments (Table 9).

Pseudomonas fungipugnans (3L), *Pseudomonas humanensis* (1M), and *Enterobacter bugandensis* (5L) were the most effective treatments for promoting vegetative development, with *P. fungipugnans* (3L) showing particularly strong performance. The effects of *Pseudomonas* strains derived from maize roots on tomato seedlings were previously demonstrated by

Table 9. Results of Tukey's mean comparison test for evaluating the effect of inoculating tomato seeds on seedling development with bacteria associated with maize roots.

Stem length (cm)			Dry matter weight (g)		
Treatment	Mean	Group	Treatment	Mean	Group
1M	8.81	a	5L	148.50	a
3L	8.81	a	3L	144.30	a
4L	8.61	a	4L	128.25	ab
5L	8.47	ab	1M	117.95	ab
T	6.98	b	T	84.85	b

Rojas-Solís *et al.* (2016), who reported increases in hypocotyl and root length, as well as seedling fresh weight. *Pseudomonas* spp. are described as major components of the endophytic community in maize roots, with functions linked to plant growth promotion through the production of compounds such as NH_3 , siderophores, auxins, and extracellular enzymes (Singh and Goodwin, 2022). Similar effects have also been documented following inoculation of tomato seeds with species from this genus (Pandey and Gupta, 2020). The pronounced increase in dry matter weight, despite the absence of significant differences in root length, provides evidence of variation in root system development. This suggests modifications in lateral rooting and an increase in secondary roots traits that are critical for nutrient acquisition from the soil (Chen *et al.*, 2022).

P. fungipugnans is a recently described species for which a potential antifungal effect has been hypothesized (Schnyder *et al.*, 2025); however, the present work demonstrates that it may also exert additional effects associated with the promotion of vegetative development. With respect to *P. hunanensis*, its performance as a PGPB in tomato infested with *Fusarium oxysporum* under saline stress conditions has been previously validated (Verma *et al.*, 2024). Likewise, *E. bugandensis* has been reported to promote growth in maize and wheat under water and salinity stress (Tian *et al.*, 2025; Verma *et al.*, 2024).

CONCLUSIONS

Hydropriming increased and accelerated germination. Chili, garlic, and onion extracts exhibited concentration-dependent biostimulant effects, evidencing a hormetic phenomenon in which low concentrations improved both germination and vegetative development. CuSO_4 was the best-performing chemical-solution treatment, although its effects were not outstanding. Biopriming results confirm the relevance of host-microorganism specificity in beneficial interactions, as well as cross-effects of maize-associated bacteria acting on tomato. Moreover, the outcomes obtained with recently described species expand current knowledge regarding their biological effects.

This study provides scientific evidence to support the development of pregermination treatment protocols for tomato. Integrating physical (hydropriming), allelopathic (plant extracts), and biotechnological (biopriming) approaches offers a robust theoretical basis for more efficient production systems. Improved understanding of hormetic mechanisms and response specificity facilitates scale-up toward commercial applications, contributing to the development of sustainable technologies for modern agriculture.

ACKNOWLEDGMENTS

This work was supported by funding from the Secretariat of Science, Humanities, Technology and Innovation (SECIHTI) through a scholarship, as well as by the facilities and equipment of COLPOS (Colegio de Postgraduados Montecillo Campus).

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Addition of *Pichia guilliermondii* yeast to diets for finishing pigs (50-100 kg)

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ABSTRACT

Objective: To determine whether the inclusion of *Pichia guilliermondii* in finishing diets improves productive performance, carcass characteristics, blood metabolites, and modulates the gut microbiota in pigs.

Design/methodology/approach: The study was conducted using a completely randomized design with 28 hybrid pigs (Yorkshire × Landrace × Duroc), with seven replicates per treatment. Four levels of additive inclusion in the diet were evaluated: T0: 0% (control), T1: 0.1%, T2: 0.2%, and T3: 0.3%. The following variables were analyzed: body weight, average daily feed intake (ADFI), average daily gain (ADG), feed gain ratio (FGR), and fat-free lean gain (FFLG). Carcass characteristics included backfat thickness (BFT), Longissimus dorsi muscle area (LMA), and lean meat percentage (LMP). Blood metabolites included total cholesterol (TC) and plasma urea (PU). Fecal bacterial populations of lactic acid bacteria and coliforms were also evaluated.

Results: The inclusion of *Pichia guilliermondii* to finishing pig diets had no effect on most of the variables evaluated, except for an increase in plasma cholesterol levels.

Limitations on study/implications: The physiological stage of pigs is a factor that makes modulation of the gut microbiota difficult.

Findings/conclusions: The inclusion of *Pichia guilliermondii* in finishing diets is not an effective strategy for improving production efficiency or modifying the intestinal microbiota during the finishing stage of fattening pigs, although cholesterol concentration increased in response to supplementation during the Finishing II stage.

Keywords: Yeast, finishing pigs, postbiotic, nutrition, bacteria.

Citation: Ahedo-Quero, E. A., Figueroa-Velasco, J. L., Martínez-Aispuro, J. A., Sánchez-Torres, M. T., Cordero-Mora, J. L., Crosby-Galván, M. M. & Miranda-Romero, L. A. (2026). Addition of *Pichia guilliermondii* yeast to diets for finishing pigs (50-100 kg). *Agro Productividad*. <https://doi.org/10.32854/5gs7c456>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: October 27, 2025.

Accepted: March 21, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 151-165.

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INTRODUCTION

The swine industry is continuously searching for nutritional technologies that improve growth performance and animal health. Among these strategies are the inclusion of inorganic minerals such as copper, recognized for its effects on growth and immunity



(Espinosa and Stein, 2021); the use of phytobiotic additives such as hydrolyzable tannins with antioxidant and antimicrobial properties (Aguirre-Meza *et al.*, 2016); the incorporation of enzymes such as lysozyme, which acts as a natural antimicrobial agent (CIAP, 2015); and the application of probiotics (Ayala *et al.*, 2022). Among the most commonly used probiotics are lactic acid bacteria such as *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* (Morales-Oñate and Morales-Oñate, 2020), as well as yeasts such as *Saccharomyces* and *Pichia guilliermondii* (Coda *et al.*, 2013; Sampath *et al.*, 2023).

In response to the growing problem of antimicrobial resistance, the use of yeast-derived probiotics has been proposed as a strategy to promote growth, improve digestion, and enhance the immune system. Specifically, *Saccharomyces cerevisiae* has demonstrated the ability to modulate the intestinal microbiota, promote nutrient absorption, and improve the overall health status of animals, resulting in better feed conversion, greater weight gain, and a reduction in enteric diseases (Pang *et al.*, 2022). The effectiveness of these effects depends on factors such as the strain used, the method of administration, and environmental conditions (Sampath *et al.*, 2023).

On the other hand, *Pichia guilliermondii* has been identified as a yeast with antimicrobial, antioxidant, and antifungal potential, capable of surviving in environments with low nutrient availability and low pH, it also shows immunomodulatory properties and the ability to improve intestinal health and productive performance in monogastric animals (Cardozo *et al.*, 2018). *Pichia guilliermondii* is an inactivated whole-cell yeast with the capacity to chelate enteric pathogens and modulate the host immune system. The cell wall of *P. guilliermondii* is rich in β -glucans and mannans; activation of immune receptors by these components leads to modulation of the immune response. Due to its smaller size, the surface area per unit weight of *P. guilliermondii* is four times greater than that of *Saccharomyces cerevisiae*, allowing a greater binding capacity to pathogens such as *Salmonella enterica* and *Escherichia coli* (Cardozo *et al.*, 2018).

Supplementation of *P. guilliermondii* to sows during gestation and lactation has been shown to improve sow performance at farrowing, including a greater number of piglets born and higher birth weight, as well as improved offspring performance after weaning, reflected in higher weaning weights and better performance during the starter phase. However, the beneficial effects of dietary supplementation with *P. guilliermondii* at different production stages may be inconsistent, as some studies in piglets from supplemented sows have reported no differences in weight gain or feed intake during the lactation and weaning phases, and even adverse effects on feed efficiency have been observed (Oguy and Thayer, 2024).

Because there is evidence that supplementation with *Pichia guilliermondii* in finishing pigs may improve the modulation of intestinal immune homeostasis, reduce systemic inflammation, and decrease the metabolic diversion of energy and amino acids toward immune maintenance, we hypothesized that these effects could be reflected in improvements in intestinal microbiota and growth performance. Therefore, the objective of the present study was to evaluate the use of *Pichia guilliermondii* postbiotic in diets for finishing pigs (50-100 kg BW) and its effects on growth performance, intestinal microbiota, and blood metabolite levels.

MATERIALS AND METHODS

The study was conducted at the Swine Unit of the Experimental Farm of the Colegio de Postgraduados, Montecillo Campus, located in the municipality of Texcoco, State of Mexico (98° 48' 27" W and 19° 48' 23" N, at an altitude of 2,245 m above sea level). The climate is temperate with summer rainfall, with a mean annual temperature of 15.9 °C and an average annual precipitation of 644.8 mm (García de Miranda, 2004).

Animal management was carried out in strict compliance with the Regulations for the Use and Care of Animals for Research of the Colegio de Postgraduados (COBIAN, 2023), under protocol number COBIAN/010/24. A total of 28 hybrid pigs (Yorkshire × Landrace × Duroc), including both sexes (gilts and barrows), were used in the experiment. The animals entered the study with an average initial body weight of 50.51 ± 1.89 kg. The pigs were housed in individual pens measuring 1.2×1.5 m with the following characteristics: flooring consisted of solid concrete partially covered with plastic slats for liquid drainage. Water was provided *ad libitum* through a nipple drinker, and feed was offered *ad libitum* using a hopper feeder.

Cleaning of the facility and inspection of the pigs' health status were carried out daily throughout the experimental period to ensure animals welfare.

Treatments and diets

An experiment with four treatments was designed, consisting of a basal diet (with a standard protein level) supplemented with three increasing levels of inactivated *Pichia guilliermondii* yeast (considered a postbiotic). The inactivated *P. guilliermondii* yeast was obtained from the commercial product CitriStim[®] (Pancosma, ADM). CitriStim[®] is an inactivated whole-cell yeast primarily obtained as a by-product of citric acid production. The inclusion levels were selected according to the manufacturer's recommendations. The treatments were as follows: T0: control; T1: 0.1%; T2: 0.2%; and T3: 0.3%. The ingredient composition of each experimental diet is detailed in Tables 1 and 2. The diets were formulated using common ingredients in swine nutrition, including corn, soybean meal, synthetic amino acids (L-lysine, DL-methionine, L-threonine, and L-tryptophan), supplemented with vitamin and mineral premixes. Nutrient levels were established according to the requirements recommended by the National Research Council (NRC, 2012). Diet formulations for all treatments were calculated to be isoproteic and isoenergetic using the Solver tool in Excel (version 365, Microsoft Corporation, 2019).

Variables and samples collection

To validate the nutritional composition of the experimental diets (control and treatments supplemented with *Pichia guilliermondii*), a proximate chemical analysis of the Finishing I and Finishing II diets was performed. The analyzed parameters were determined according to AOAC methods (2012; 2023) and the system proposed by Van Soest *et al.* (1991).

Growth performance: The body weight of each pig was individually recorded at the beginning and end of each stage (Finishing I and II) to determine average daily gain (ADG) and final body weight (FBW). Average daily feed intake (ADFI) was also recorded. Feed:gain ratio (FGR) was calculated using ADG and ADFI values.

Table 1. Ingredients and calculated nutritional composition of experimental diets for pigs in the Finishing I stage (50-75 kg) supplemented with *Pichia guilliermondii* yeast.

Ingredient	T0 (0%)	T1 (0.1%)	T2 (0.2%)	T3 (0.3%)
Corn grain	82.39	82.31	82.23	82.15
Soybean meal	15.31	15.23	15.15	15.07
Soybean oil	0.09	0.14	0.20	0.25
L-Lysine	0.25	0.26	0.26	0.26
DL-Methionine	0.00	0.00	0.00	0.00
L-Threonine	0.00	0.01	0.01	0.01
L-Tryptophan	0.01	0.01	0.01	0.01
Calcium carbonate	1.12	1.12	1.11	1.11
Ortophosphate	0.01	0.01	0.01	0.01
Mycotoxins sequestrant	0.40	0.40	0.40	0.40
Vitamins premix	0.03	0.03	0.03	0.03
Minerals premix	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30
Yeast	0.00	0.10	0.20	0.30
Estimated Nutritional Content (%)				
ME (Mcal kg ⁻¹)	3.27	3.27	3.27	3.27
Crude Protein	13.00	13.00	13.00	13.00
Calcium	0.50	0.50	0.50	0.50
Phosphorus	0.30	0.30	0.30	0.30
Lysine	0.61	0.61	0.61	0.61
Threonine	0.37	0.37	0.37	0.37
Tryptophan	0.10	0.10	0.10	0.10
Arginine	0.65	0.65	0.65	0.64
Histidine	0.37	0.37	0.37	0.37
Isoleucine	0.41	0.41	0.41	0.41
Leucine	1.34	1.34	1.33	1.33
Valine	0.49	0.49	0.49	0.48
Methionine + Cystine	0.36	0.36	0.35	0.35
Determined Nutritional Content (%)				
Dry Mater	87.13	86.89	86.96	86.96
Ashes	2.27	2.23	2.77	2.76
Crude Protein	12.92	14.63	13.96	14.23

^A Provided per kg of feed: vitamin A, 15,000 IU; vitamin D3, 2,500 IU; vitamin E, 37.5 IU; vitamin K, 2.5 mg; thiamine, 2.25 mg; riboflavin, 6.25 mg; niacin, 50 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.0375 mg; biotin, 0.13 mg; choline chloride, 563 mg; pantothenic acid, 20 mg; folic acid, 1.25 mg.

^B Provided per kg of feed: Fe, 150 mg; Zn, 150 mg; Mn, 150 mg; Cu, 10 mg; Se, 0.15 mg; I, 0.9 mg; Cr, 0.2 mg.

Carcass Characteristics: Body composition variables were measured using the real-time ultrasound device Sonovet 600 equipped with a 3.5 MHz convex transducer (Medison Inc., Cypress, CA, USA). Measurements taken at the level of the tenth rib included backfat thickness (BFT) and *Longissimus dorsi* muscle area (LMA) at the beginning and end of

Table 2. Ingredients and calculated nutritional composition of experimental diets for pigs in the Finishing II stage (75-110 kg) supplemented with *Pichia guilliermondii* yeast.

Ingredient	T0 (0%)	T1 (0.1%)	T2 (0.2%)	T3 (0.3%)
Corn grain	85.57	85.555	85.54	85.52
Soybean meal	12.71	12.621	12.53	12.44
Soybean oil	0.00	0.000	0.00	0.00
L-Lysine	0.17	0.173	0.18	0.20
DL-Methionine	0.00	0.00	0.00	0.00
L-Threonine	0.00	0.00	0.00	0.00
L-Tryptophan	0.00	0.00	0.00	0.00
Calcium carbonate	0.99	0.99	0.99	0.99
Ortophosphate	0.04	0.04	0.04	0.05
Mycotoxins sequestrant	0.10	0.10	0.10	0.10
Vitamins premix	0.03	0.03	0.03	0.03
Minerals premix	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30
Yeast	0.00	0.10	0.20	0.30
Estimated Nutritional Content (%)				
ME (Mcal kg ⁻¹)	3.28	3.28	3.27	3.27
Crude Protein	12.00	12.00	12.00	12.00
Calcium	0.45	0.45	0.45	0.45
Phosphorus	0.30	0.30	0.30	0.30
Lysine	0.55	0.55	0.55	0.55
Threonine	0.33	0.33	0.33	0.33
Tryptophan	0.09	0.09	0.09	0.09
Arginine	0.59	0.58	0.58	0.58
Histidine	0.35	0.34	0.34	0.34
Isoleucine	0.37	0.37	0.37	0.37
Leucine	1.28	1.28	1.28	1.27
Valine	0.45	0.45	0.45	0.45
Methionine + Cystine	0.34	0.34	0.33	0.33
Determined Nutritional Content (%)				
Dry Mater	87.14	86.70	87.13	87.25
Ashes	4.37	4.55	4.40	3.37
Crude Protein	15.30	13.54	13.05	12.53

^A Provided per kg of feed: vitamin A, 15,000 IU; vitamin D3, 2,500 IU; vitamin E, 37.5 IU; vitamin K, 2.5 mg; thiamine, 2.25 mg; riboflavin, 6.25 mg; niacin, 50 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.0375 mg; biotin, 0.13 mg; choline chloride, 563 mg; pantothenic acid, 20 mg; folic acid, 1.25 mg.

^B Provided per kg of feed: Fe, 150 mg; Zn, 150 mg; Mn, 150 mg; Cu, 10 mg; Se, 0.15 mg; I, 0.9 mg; Cr, 0.2 mg.

each stage. These data were used to calculate fat free lean gain (FFLG) and lean meat percentage (LMP) using procedure 5 proposed by Burson and Berg (2001).

Blood metabolites: For the determination of blood metabolites, blood samples were collected from each pig on the last day of each experimental stage. Blood samples were

obtained by puncture of the anterior vena cava using heparinized Vacutainer[®] tubes (BD Vacutainer Systems, NJ, USA). The tubes were identified and stored on ice for transport to the laboratory. Once in the laboratory, the samples were centrifuged using a Sigma 2-16k centrifuge (Germany) at $1,788 \times g$ for 20 min to separate the plasma from the cellular fraction. The plasma from each sample was transferred into properly labeled 2 mL polypropylene Eppendorf tubes. Plasma samples were stored at $-20\text{ }^{\circ}\text{C}$ until biochemical analysis. Serum levels of total cholesterol and urea were analyzed as indicators of metabolic status. Determinations were performed using commercial enzymatic kits specific for each metabolite (SPINREACT[®]: Cholesterol-LQ and Urea 37). Absorbance readings were obtained using a visible-light spectrophotometer (Beckman DU65 spectrophotometer) at the following wavelengths: cholesterol, 505 nm; and urea, 510 nm.

Fecal Microbial Populations: Two samplings were carried out, one at the end of each fattening stage (Finishing I and Finishing II), to evaluate the dynamics of intestinal microbial populations using the viable plate count method. Fecal samples were obtained by direct rectal stimulation using a sterile lubricated glove to collect the feces. Immediately after collection, samples were placed in sterile bags, identified, and stored in a cooler for transport and immediate laboratory analysis. Serial dilutions were prepared to obtain concentrations of 10^{-4} , 10^{-5} , and 10^{-6} , which were used for spread plating. The surface spread plate technique was performed using a glass spreader, inoculating $100\ \mu\text{L}$ of the selected dilutions in duplicate onto Petrifilm[™] plates. MacConkey agar was used to quantify total coliforms and *Escherichia coli*, whereas MRS agar was used to quantify lactic acid bacteria. All plates were incubated at $29\text{ }^{\circ}\text{C}$. Petrifilm[™] plates were monitored and quantified for seven days until no further colony growth was observed.

After incubation, colonies were counted on plates containing between 30 and 300 colony-forming units (CFU). To standardize the results, the dry matter (DM) content of each fecal sample was determined by drying an aliquot in an oven at $50\text{ }^{\circ}\text{C}$ until constant weight was achieved. Final bacterial counts were expressed as CFU per gram of dry matter (CFU/g DM), and data were transformed to base-10 logarithms (Log_{10}) for statistical analysis.

Statistical Analysis

The study was conducted under a completely randomized design with four treatments using a total of 28 experimental units during the two fattening periods (Finishing I and Finishing II), with seven replicates per treatment in each stage. The number of replicates was established considering a coefficient of variation (CV%) of 6% for average daily gain (ADG) and a minimum detectable difference of 10% (Johnston *et al.*, 2003). The CV information for ADG in finishing pigs was obtained from previous studies conducted within our research line.

The experimental unit consisted of one pig housed in an individual pen, ensuring the statistical independence of observations for the proper application of analysis of variance and avoiding confounding effects due to competition or social interaction. Statistical analyses were performed using SAS (SAS Institute Inc., 2002).

The effects of treatments on growth performance, carcass characteristics, blood metabolites, and bacterial populations were analyzed using analysis of variance (ANOVA) through the GLM (General Linear Model) procedure. Subsequently, Tukey's honestly significant difference (Tukey's HSD) test was used to compare treatment means at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Finishing I stage

Growth performance

Treatments supplemented with the *P. guilliermondii*-based postbiotic did not induce statistically significant changes ($P > 0.05$) in any of the growth performance variables evaluated during the Finishing I period, including final body weight (FBW), average daily feed intake (ADFI), average daily gain (ADG), and feed:gain ratio (FGR). Average daily gain remained between 0.72 and 0.80 kg d⁻¹ across all groups. Lean meat gain was also similar, ranging from 0.265 to 0.280 kg d⁻¹ ($P = 0.89$), as shown in Table 1.

This lack of response has previously been reported in finishing pigs, where direct supplementation with *P. guilliermondii* during advanced production phases does not always result in improved growth performance (Bass *et al.*, 2022). This contrasts with studies in piglets, in which the yeast has demonstrated potential to improve feed conversion and weight gain, effects attributed to the modulation of the microbiota and improved nutrient digestibility in the immature intestine (de Paula *et al.*, 2022; Thayer *et al.*, 2023). The absence of effects during the finishing stage may be associated with the greater physiological and microbial stability of adult pigs, or because the inclusion levels used were insufficient to generate significant benefits during a phase in which nutritional demands for growth are high.

Carcass Characteristics

Consistent with the growth performance results, the main carcass parameters measured at the end of the experimental period showed no significant differences among treatments ($P > 0.05$). Specifically, final *Longissimus dorsi* muscle area and final backfat thickness were statistically similar among treatments. Final lean meat percentage also remained unchanged ($P = 0.69$). These results suggest that the postbiotic, at the inclusion levels evaluated, did not alter nutrient partitioning in a manner that favored lean tissue deposition over adipose tissue during this critical growth phase.

Other studies have reported a positive carryover effect following maternal supplementation, indicating greater lean meat gain and improved carcass performance in the offspring (Bass *et al.*, 2019; Thayer *et al.*, 2023). The similarity in carcass results observed in the present study reinforces the idea that the effectiveness of the additive may depend on the production stage or the route of supplementation.

Blood metabolites

Analysis of blood metabolites revealed that plasma urea concentration (PU) remained unchanged among treatments ($P = 0.72$). PU is an indicator of amino acid catabolism;

therefore, the absence of differences suggests that inclusion of the postbiotic did not affect protein utilization efficiency or nitrogen balance in the pigs, which may explain the lack of effect on LMA (Montoya *et al.*, 2018; Bass *et al.*, 2019).

In contrast, a significant effect on total cholesterol concentration (TC) was detected ($P=0.0036$) in response to dietary supplementation with *P. guilliermondii*. Pigs in T2 reached the highest TC concentration ($180.57 \text{ mg dL}^{-1}$), which was significantly higher than those observed in T1 ($144.23 \text{ mg dL}^{-1}$) and T3 ($135.35 \text{ mg dL}^{-1}$). This result contrasts with the hypocholesterolemic effects commonly reported for probiotic yeasts.

The use of yeasts and yeast cell wall products in pig diets has generally been associated with reductions in total serum cholesterol and LDL cholesterol levels compared with control groups, since *P. guilliermondii* may act as a modulator of the microflora and enhance the binding of pathogenic bacteria, thereby indirectly influencing lipid metabolism through improvements in digestive health (Marrero *et al.*, 2014; Ruiz *et al.*, 2017; Tenea *et al.*, 2021).

The increase in TC observed in T2, in the absence of an increase in final backfat thickness, suggests that the postbiotic did not promote lipid accumulation in subcutaneous adipose tissue, but rather modified cholesterol turnover and circulation in the serum. It is possible that the inclusion of *P. guilliermondii* affected bile salt reabsorption at the intestinal level. Reduced bile salt reabsorption forces the liver to synthesize new bile salts from circulating cholesterol, a process that typically lowers TC levels. If the postbiotic modulated this process, it may have caused a transient accumulation of lipoproteins in the plasma (García *et al.*, 2006; Montoya *et al.*, 2018).

Microbial variables

The results of the microbiological analysis showed no significant differences ($P>0.05$) among treatments for any of the variables measured during Finishing I, as shown in Figure 1.

Lactic Acid Bacteria (LAB) Counts

LAB counts were not affected by inclusion of the postbiotic ($P=0.72$), with values ranging from 9.09 to 9.58 Log CFU g DM⁻¹. These results indicate that *P. guilliermondii* yeast did not exert a modulatory effect on the microbiota sufficient to significantly increase LAB populations in the digestive tract of finishing I pigs. The microbial stability of adult pigs, which already possess an established microbiota, may mask the stimulatory effects commonly observed in piglets. Although *P. guilliermondii* strains have demonstrated the ability to stimulate bacterial growth in *in vitro* systems, their *in vivo* performance depends on the specific interaction with the diet and the ecological niche already occupied within the gastrointestinal tract (Ruiz, 2016; Montoya *et al.*, 2018).

Coliforms

Coliform bacteria also showed no significant differences among treatments ($P=0.70$), with values ranging from 7.46 to 8.39 Log CFU g DM⁻¹. The absence of a reduction in coliform counts in the supplemented groups contrasts with the competitive exclusion

Table 3. Growth performance variables in pigs during the Finishing I stage.

Variable	T0	T1	T2	T3	EEM	P lineal
Initial BW (Kg)	47.25	50.57	51.28	50.42	5.15	-
Final BW (Kg)	79.71	84.57	81.71	82	13.93	0.89
ASFI (Kg d ⁻¹)	2.48	2.52	2.57	2.47	0.11	0.95
ADG (Kg d ⁻¹)	0.77	0.80	0.72	0.75	0.02	0.65
FGR	3.25	3.15	3.56	3.31	0.10	0.11
FFLG (Kg d ⁻¹)	0.28	0.28	0.27	0.27	0.01	0.89
LMAi (cm ²)	20.77	21.51	22.60	21.93	1.39	0.82
LMAf (cm ²)	30.97	31.18	33.48	32.14	2.55	0.77
LMPi	42.13	42.19	42.66	42.57	3.18	0.92
LMPf	39.86	39.22	40.37	40.10	3.43	0.69
BFTi (mm)	0.81	0.77	0.78	0.75	0.01	0.35
BFTf (mm)	1.3	1.21	1.24	1.25	0.02	0.74
Blood Metabolites (mg dL ⁻¹)						
TC	156.74 ab	144.23 b	180.57 a	135.35 b	4.55	0.0036
PU	20.07	23.29	19.31	20.51	4.7	0.72

T0: Control; T1: Standard protein + 1 kg t⁻¹; T2: Standard protein + 2 kg t⁻¹; T3: Standard protein + 3 kg t⁻¹. BWi: Initial body weight; BWf: Final body weight; ADFI: Average daily feed intake; ADG: Average daily gain; FGR: Feed:gain ratio; FFLG: Fat Free Lean gain; LMAi: Initial *Longissimus dorsi* muscle area; LMAf: Final *Longissimus dorsi* muscle area; LMPi: Initial lean meat percentage; LMPf: Final lean meat percentage; TC: Total cholesterol; PU: Plasma urea nitrogen. a, b, c Different letters indicate statistically significant differences among means (P≤0.05).

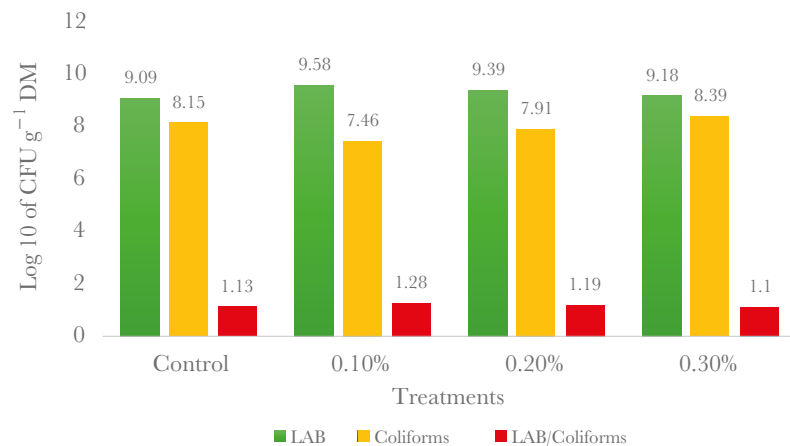


Figure 1. Effect of supplementation with the postbiotic (*P. guilliermondii*) on the concentrations of lactic acid bacteria, coliforms (LAB), and the ratio in the intestine of pigs during the Finishing I stage. Treatment means or main effects with different letters indicate statistical differences (P<0.05). CFU g⁻¹ DM. Ratio: LAB/Coliforms.

activity expected from yeasts such as *P. guilliermondii* (Tenea *et al.*, 2021). These results indicate that the postbiotic did not exert a direct suppressive effect on pathogens, or that its antimicrobial activity was insufficient to displace the pre-existing coliform populations during this stage. Studies involving *P. guilliermondii* have focused primarily on modulation of the immune response (Bass *et al.*, 2019) and improvement of overall intestinal health (de

Paula *et al.*, 2022); however, the ability to reduce coliform load in the intestine of finishing I pigs was not demonstrated in the present experiment.

LAB/Coliform Ratio

The LAB/coliform ratio was similar among all groups ($P=0.61$), ranging from 1.10 to 1.28. This ratio is considered a key and sensitive indicator of intestinal microbial balance and mucosal health. A higher ratio (reflecting a greater proportion of lactic acid bacteria relative to coliforms) indicates a more favorable intestinal environment associated with improved nutrient absorption and a lower load of toxic metabolites produced by Gram-negative bacteria. The lack of modulation observed in the present study indicates that the postbiotic was not able of exerting sufficiently strong competitive exclusion to stimulate the growth of LAB populations (Montoya *et al.*, 2018).

Finishing II Stage: growth performance

During the Finishing II phase, inclusion of the *P. guilliermondii* postbiotic did not produce changes in BWf, ADFI, ADG, or FGR ($P>0.05$). FFLG was similar among all treatments, as shown in Table 4 ($P=0.88$).

The absence of response in Finishing II pigs agrees with the findings of Bass *et al.* (2022), who reported that the effectiveness of this additive is expressed as a carryover effect when administered during gestation and lactation, resulting in piglets with greater body weight and improved performance during the nursery stage, although these benefits dissipate toward the finishing phase (Bass *et al.*, 2019; Thayer *et al.*, 2023). In finishing pigs, digestive physiology is already mature and, consequently, the microbiome is more stable; therefore, the postbiotic is unable to generate a significant impact on intestinal colonization and modulation.

Carcass characteristics

Carcass variables measured at the end of Period II showed no significant differences among treatments ($P>0.05$). Both LMAf (37.57 to 39.95 cm²), BFTf (1.50 to 1.65 mm), and LMPf (38.81 to 39.87%) were statistically similar among treatments. This similarity in carcass composition was expected given the absence of effects on FFLG and ADG. If the additive does not influence nutrient absorption in favor of muscle deposition over adipose tissue, carcass indicators remain unchanged (Bass *et al.*, 2022). Although the mechanism of action is associated with improvements in intestinal health and immunity, the translation of these effects into improvements in carcass quality during the finishing phase is uncommon. Thayer *et al.* (2023) evaluated carcass responses to direct supplementation or carryover effects and found that carcass characteristics are generally not affected by the inclusion of yeast in pig diets. This confirms that, during the final stages of growth, body composition is highly determined by genetics and dietary energy density.

Blood Metabolites

Analysis of blood metabolites during Period II revealed that TC and PU concentrations were similar among all groups. PP remained between 20.31 and 24.78

mg dL⁻¹ (P=0.68), whereas TC concentrations ranged from 178.04 to 190.60 mg dL⁻¹ (P = 0.90).

The similarity in plasma urea indicates that protein utilization remained constant, which is consistent with the absence of effects on FFLG and a stable PU value indicates that the postbiotic did not improve absorption efficiency during the final weeks of the fattening period (Montoya *et al.*, 2018).

Regarding TC, the absence of significant differences during this period (P=0.90) contrasts with the modulation observed during Period I (P=0.0036). Although *P. guilliermondii* biomass has the capacity to assimilate cholesterol *in vitro* due to the β -glucan composition of its cell wall (Tenea *et al.*, 2021), the *in vivo* dynamics are more complex. During this finishing phase, pigs have greater body weight and a higher growth rate, which may reflect a mechanism of metabolic adaptation or greater homeostatic stability in pigs with a fully developed physiological system (Marrero *et al.*, 2014). Unlike piglets, finishing pigs have already established a balance between hepatic cholesterol synthesis and biliary excretion.

It is possible that the postbiotic dose was insufficient to increase bile salts binding, since the mechanism involved in cholesterol reduction is the inhibition of bile salts reabsorption, forcing the liver to utilize serum cholesterol for the synthesis of new bile salts. Therefore, in animals with greater metabolic body weight, the effect of the postbiotic on hepatic and serum cholesterol recirculation is minimal, which may explain the lack of a significant response during Finishing II period.

Table 4. Productive performance variables in pigs during the Finishing II stage.

Variable	T0	T1	T2	T3	EEM	P lineal
BWi (Kg)	79.71	84.57	81.71	82	1.39	-
BWf (Kg)	97.85	102.57	101.28	98.57	2.15	0.92
ADFI (Kg d ⁻¹)	2.97	3.05	3.11	2.99	0.16	0.92
ADG (Kg d ⁻¹)	0.86	0.85	0.93	0.78	0.03	0.60
FGR	3.75	3.60	3.41	3.88	0.61	0.71
FFLG (Kg d ⁻¹)	0.29	0.31	0.33	0.31	0.01	0.88
LMAi (cm ²)	30.90	31.18	33.48	32.14	2.55	0.77
LMAf (cm ²)	37.57	38.36	39.35	39.95	3.73	0.89
LMPi	39.86	39.22	40.37	40.10	3.43	0.69
LMPf	38.81	38.85	39.40	39.87	2.32	0.52
BFTi (mm)	1.2	1.22	1.24	1.22	0.03	0.97
BFTf (mm)	1.65	1.58	1.5	1.52	0.07	0.73
Blood Metabolites (mg dL ⁻¹)						
TC	190.60	188.12	178.04	187.64	11.48	0.90
PU	23.48	20.31	24.78	23.51	5.06	0.68

T0: Control; T1: Standard protein + 1 kg t⁻¹; T2: Standard protein + 2 kg t⁻¹; T3: Standard protein + 3 kg t⁻¹; BWi: initial body weight; BWf: final body weight; ADFI: Average daily feed intake; ADG: Average daily gain; FGR: Feed:gain ratio; FFLG: Fat free lean gain; LMAi: Initial *Longissimus dorsi* muscle area; LMAf: Final *Longissimus dorsi* muscle area; LMPi: Initial lean meat percentage; LMPf: Final lean meat percentage; BFTi: Initial back fat thickness; BFTf: Final back fat thickness; TC: Total cholesterol; PU: Plasma urea nitrogen concentration. a, b, c Different superscript indicates statistical difference (P≤0.05). Letters are shown only when differences were detected.

Lactic acid bacteria counts

The population of lactic acid bacteria quantified on MRS agar did not differ significantly among treatments, as shown in Figure 2 ($P=0.48$), with values ranging from 8.90 (T2) to 9.66 (T0) CFU g DM⁻¹. The absence of a positive response in LAB populations indicates that *P. guilliermondii* did not act as a trophic agent for the beneficial microbiota. Although *P. guilliermondii* may contain metabolites capable of stimulating fermentation, finishing pigs possess a more stable and consolidated microbiome that is resistant to low-level modulation by non-viable additives (Gutiérrez-Acosta *et al.*, 2016). Fermentation in the large intestine of these animals already generates optimal production of short-chain fatty acids (SCFA), indicating that the impact of inactivated yeast on promoting additional LAB growth is minimal.

Coliforms

Coliform counts on MacConkey agar also showed no significant differences among experimental groups ($P=0.69$). Values ranged from 6.73 CFU g DM⁻¹ (T0) to a maximum of 7.55 CFU g DM⁻¹ (T3), without a clear reduction trend attributable to the postbiotic. Yeast in the form of cell wall components or metabolites acts as an immunostimulant through binding to pathogen adhesion sites in the small intestine. However, in the large intestine of finishing pigs, from which the samples were obtained, the microbiota has already reached a high degree of complexity and density.

The stability of coliform populations is consistent with the lack of effects on ADG, since pathogen reduction is a primary pathway for improving performance in young animals (Bass *et al.*, 2019; Zhao and Yang, 2025).

LAB/Coliform Ratio

The LAB/coliform ratio was similar among all treatments, with values of 1.45 in T0 and 1.22 in T3. This ratio confirms that the *P. guilliermondii* postbiotic did not alter the

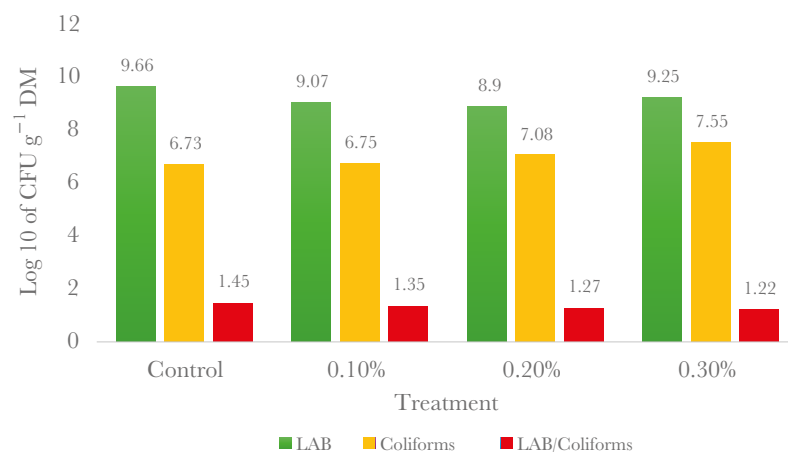


Figure 2. Effect of supplementation with the postbiotic (*P. guilliermondii*) on the concentrations of lactic acid bacteria (LAB), coliforms, and their ratio in the intestine of pigs during the Finishing II stage. Treatment means or main effects with different letters indicate statistical differences ($P<0.05$). CFU g⁻¹ DM. Ratio: LAB/Coliforms.

overall ecological balance of the microbiota. Since neither LAB populations increased nor coliform counts decreased individually, the ratio remained similar to that observed during Period I.

These findings suggest that, for *P. guilliermondii* to exert beneficial effects during the final stages of fattening, modulation should occur indirectly as a carryover effect from supplementation during gestation, lactation, or the piglet phase, when microbiome functionality and immunity are improved during critical developmental stages (Bass *et al.*, 2022; Thayer *et al.*, 2022; Choi, 2023). The impact of the yeast during the finishing stages appears to be marginal when health status and microbiota are already optimized.

CONCLUSIONS

This experiment demonstrated that supplementation with the postbiotic derived from *Pichia guilliermondii* in finishing pigs exhibits only marginal effectiveness when administered during the finishing stage of fattening pigs. The results showed no impact on the analyzed variables, with the exception of increased total cholesterol during the Finishing I stage in pigs supplemented with the postbiotic, providing an indirect evaluation of the additive-host interaction in physiologically mature animals.

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Comparative evaluation of meat production potential in hair sheep crossbreeds under tropical conditions

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ABSTRACT

Objective: To evaluate the effect of genetic group on productive performance and carcass characteristics of hair sheep under tropical conditions.

Design/methodology/approach: Male lambs from Dorper × Pelibuey (DpPb), Katahdin × Pelibuey (KtPb), and Charollais × Pelibuey (ChPb) crossbreeds were finished under confinement and fed a commercial diet containing 15% crude protein and 2.76 Mcal of metabolizable energy (ME)/kg of dry matter (DM). Dry matter intake (DMI), average daily gain (ADG), and feed conversion ratio (FCR) were recorded. Carcass characteristics and morphometric measurements were evaluated at a live weight of 45 kg. Data were analyzed using mixed linear models.

Results: No significant differences ($P > 0.05$) were detected among genetic groups for productive performance. Mean DMI was 0.955 kg DM/animal/day, ADG was 0.270 kg/animal/day, and FCR was 4.0. However, the DpPb group exhibited greater carcass weight, larger *Longissimus dorsi* muscle area, and higher backfat thickness ($P < 0.05$). No differences were observed among crossbreeds for morphometric measurements.

Limitations on study/implications: Variations in productive performance reported in previous studies may be associated with diet composition, environmental conditions, and the genetic background of the animals.

Findings/conclusions: Differences in carcass traits were mainly associated with live weight at slaughter, whereas no differences in productive performance were observed among genotypes. The Dorper × Pelibuey crossbreed can be considered a functional option for intensive tropical production systems aimed at increasing carcass weight.

Keywords: Hair sheep, crossbreeding, carcass yield, morphometric traits, intensive production.

Citation: Méndez-Valdez, G. R., Ortiz-de la Rosa, B., Cantón- Castillo, J. G., Piñeiro-Vásquez, Á. T., & Ramón-Ugalde, J. (2026). Comparative evaluation of meat production potential in hair sheep crossbreeds under tropical conditions. *Agro Productividad*. <https://doi.org/10.32854/4pn7bd86>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: November 7, 2025.

Accepted: March 19, 2025.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 167-174.

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INTRODUCTION

Sheep meat production in tropical regions faces limitations associated with heat stress, high parasite burden, and variability in the availability and quality of feed resources, all of which negatively affect animal productive performance. In this context, sheep production



systems are primarily based on hair breeds and their crossbreeds, owing to their greater adaptation to tropical environments, hardiness, and productive stability (Chay *et al.*, 2019; Portillo *et al.*, 2024). The use of terminal crossbreeding between adapted breeds and breeds specialized in meat production represents a viable strategy to increase productive efficiency and improve carcass characteristics in tropical sheep production systems. However, the productive response depends on the genetic potential of the sire breed, particularly in relation to growth rate, muscle conformation, adipose tissue deposition, and degree of maturity at slaughter (Cloete *et al.*, 2012; Lunesu *et al.*, 2023). Among the breeds commonly used as terminal sires, Dorper, Katahdin, and Charollais exhibit relevant productive differences. Dorper is characterized by early maturity, high weight gain, and good carcass conformation (Cloete *et al.*, 2000; Macías *et al.*, 2010; Souza *et al.*, 2016); Katahdin shows later growth and lower fat deposition, resulting in leaner carcasses (Vázquez *et al.*, 2011); whereas Charollais stands out for its high growth potential and muscular development (Ivanov *et al.*, 2016), although its performance may be affected by lower adaptation to tropical conditions (López *et al.*, 2016). The Pelibuey breed is widely used as a maternal base in tropical areas because of its hardiness, environmental adaptability, and reproductive stability (Rosas *et al.*, 2022; De La Cruz *et al.*, 2025). Despite the increasing use of these crossbreeds in intensive systems, information simultaneously comparing the productive performance and carcass characteristics of Dorper × Pelibuey, Katahdin × Pelibuey, and Charollais × Pelibuey lambs under controlled tropical conditions remains limited. This situation hinders technical decision-making for sire selection in crossbreeding programs aimed at improving the productivity and profitability of sheep production systems (Cedeño *et al.*, 2023).

In this context, it has not been clearly established whether differences in productive performance and carcass characteristics are primarily attributable to the paternal genetic group or to live weight at slaughter when animals are managed under the same nutritional and husbandry scheme. The hypothesis proposed is that Dorper × Pelibuey lambs exhibit greater average daily gain and superior carcass characteristics compared with Katahdin × Pelibuey and Charollais × Pelibuey genotypes, as a result of their greater growth capacity and tissue deposition. Based on the foregoing, the aim of the present study was to evaluate the productive performance and carcass characteristics of Dorper × Pelibuey, Katahdin × Pelibuey, and Charollais × Pelibuey lambs raised under an intensive system in tropical conditions, with the purpose of generating technical information to support sire selection in crossbreeding programs aimed at efficient and profitable sheep meat production.

MATERIALS AND METHODS

Study location

The study was conducted from May to September 2024 and comprised two complementary experiments: the first focused on the evaluation of productive performance and the second on the analysis of carcass characteristics. Both experiments were carried out at the Livestock Research Unit of the Tecnológico Nacional de México, Campus Conkal, Yucatán, Mexico (21° 07' N, 89° 49' W), at an altitude of 8 m above sea level. According to the Köppen classification modified by García (2004), the regional climate

is classified as tropical subhumid (Aw), with an approximate mean annual rainfall of 900 mm, concentrated mainly between June and November, and a mean annual temperature of 26.5 °C (Duch, 2002).

Productive performance

Eighteen intact male lambs, 3 to 4 months of age, with an average initial live weight (\pm SD) of 28.0 ± 6.4 kg, were used. The lambs originated from Dorper \times Pelibuey (Dp \times Pb), Katahdin \times Pelibuey (Kt \times Pb), and Charollais \times Pelibuey (Ch \times Pb) crosses, with six replicates per genotype. The animals were housed in individual pens measuring 1.20×1.50 m, equipped with a shaded area, drinker, and feeder. The diet consisted of a commercial feed (Table 1) formulated with 15.0% crude protein (CP) and 2.76 Mcal of metabolizable energy per kg of dry matter (Mcal ME/kg DM), designed to achieve an estimated daily gain of 200-250 g/animal (Huerta, 2001). Before the beginning of the experiment, the animals underwent internal deworming with ivermectin (Sanfer) and received a vitamin complex containing vitamin B12 and phosphorus (Vitabecid; Pisa). Subsequently, a 14-day adaptation period to the diet and housing conditions was established. The experiment lasted 84 days. Animals were weighed after a 16-h fasting period at the beginning of the study and subsequently every 14 days. Feed was offered twice daily, at 08:00 and 16:00 h, and intake was recorded daily by weighing the feed offered and refused. At the end of the experiment, the lambs reached an average live weight (\pm SD) of 47.8 ± 6.3 kg. The variables evaluated were dry matter intake (DMI), average daily gain (ADG), and feed conversion ratio (FCR).

Carcass characteristics evaluation

Twenty-four carcasses from intact male lambs, 3 to 4 months of age, corresponding to the Ch \times Pb, Dp \times Pb, and Kt \times Pb crossbreeds, were evaluated, with eight replicates per genotype. The animals received the same diet, health management, and housing conditions described in the previous trial. Upon reaching an average live weight (\pm SD) of 45.7 ± 7.5 kg, the animals were transported to the slaughter facility of the Decentralized Municipal Agency Abastos de Mérida, Yucatán. Slaughter was performed after a 16-h feed restriction period, in accordance with the Mexican Official Standard NOM-033-SAG/ZOO-2014. Live weight at slaughter (LWS), hot carcass weight (HCW), and cold carcass weight (CCW) were recorded to calculate hot carcass yield (HCY) and cold carcass yield (CCY). The carcasses were refrigerated at 4 °C for 24 h, after which a cut was made between the 12th and 13th ribs to measure backfat thickness, according to the Mexican Standard NMX-FF-106-SCFI-2006. The *Longissimus dorsi* (LD) muscle area was determined using a gridded plastic template, following the protocol described by The Ohio State University Extension (2011). Likewise, the following carcass morphometric measurements were recorded: carcass length (CL), from the scapulohumeral joint to the ischial tuberosity; leg length (LL), from the proximal region of the femur to the tibiotarsal joint; leg width (LW), at the widest portion of the leg; thorax length (TL), from the scapulohumeral joint to the last rib; and thorax width (TW), measured at the widest portion of the chest at the sternum level.

Table 1. Ingredients and chemical composition of the experimental diet.

Component	DM basis (%)
Whole sorghum	23.66
Ground corn	39.71
Soybean meal	11.80
Soybean hulls	13.50
Sugarcane molasses	5.00
Calcium carbonate	2.80
Nutritional additives	0.84
Common salt	0.80
Urea	0.80
Sodium bicarbonate	0.40
Ammonium sulfate	0.15
Trace minerals	0.43
Vitamins A, D, and E	0.06
Total	100.00
Dry matter (%)	88.70
Crude protein (%)	14.80
Calcium (%)	0.52
Phosphorus (%)	0.31
Metabolizable energy (Mcal/kg DM)*	2.76

Bromatological and mineral analyses were performed at the Bromatology Laboratory of the Tecnológico Nacional de México, Campus Conkal, Yucatán, Mexico. *Estimated according to NRC (2007).

Experimental design and statistical analysis

Productive performance data were analyzed using a repeated-measures mixed linear model, considering paternal genetic group as a fixed effect, measurement period as the within-subject factor, and initial weight as a covariate. Model fitting was performed by restricted maximum likelihood (REML) using the PROC MIXED procedure of the SAS statistical package (SAS Institute Inc., 2003). Before the analysis, the assumptions of residual normality and homogeneity of variances were verified through graphical and statistical tests. Multiple comparisons were performed using Tukey's test, with a significance level of $\alpha=0.05$. Variables related to carcass characteristics were analyzed using a general linear model (GLM), considering paternal genetic group as the main fixed effect, through the PROC GLM procedure of SAS (SAS Institute Inc., 2003). In this case, the assumptions of residual normality and homoscedasticity were also corroborated before mean comparison, which was performed using Tukey's test ($\alpha=0.05$).

RESULTS AND DISCUSSION

Productive performance

The productive performance results of the lambs are presented in Table 2. No significant differences ($P>0.05$) were detected among genetic groups for the variables evaluated. On

average, the animals recorded a dry matter intake (DMI) of 0.955 kg DM/animal/day, an average daily gain (ADG) of 0.270 kg/animal/day, and a feed conversion ratio (FCR) of 4.0. These values fall within the ranges reported for hair-breed lambs fed under tropical conditions with diets containing 14 to 17% crude protein (Ríos-Rincón *et al.*, 2014; Lima *et al.*, 2022).

The absence of differences among genotypes suggests that, under the same intensive system and with an isoenergetic and isoproteic diet, differential genetic potential is not fully expressed in short-term growth variables. In this regard, the similarity in ADG indicates that the Dorper × Pelibuey genotype did not exhibit a significant productive advantage during the fattening phase evaluated, which is consistent with previous reports indicating that nutritional homogeneity reduces variability among crossbreeds (Macías *et al.*, 2010; Lima *et al.*, 2022).

Other studies have reported higher dry matter intake values (1.3-1.5 kg/animal/day) in similar crossbreeds fed total mixed diets with a higher proportion of fiber and alfalfa forage, which increases intake but does not necessarily translate into greater weight gains because of increased maintenance energy expenditure and lower efficiency of energy utilization (Sileshi *et al.*, 2021; Macdonald *et al.*, 2021).

In contrast, the ADG values observed in the present study are comparable to those reported by Rosas *et al.* (2022) in Charollais × Pelibuey and Dorper × Pelibuey lambs managed under similar feeding schemes, thereby reinforcing that the performance observed is consistent with intensive tropical production systems.

Carcass characteristics

Significant differences ($P < 0.05$) were observed among genetic groups for live weight at slaughter and hot and cold carcass weights (Table 3), with higher values recorded in Dp × Pb lambs. However, these differences should be interpreted primarily as an effect of the greater final weight attained at slaughter, rather than as a direct expression of independent genetic superiority, since no previous differences in ADG were detected during the growth phase. Consistent with this, hot and cold carcass yields did not differ significantly ($P > 0.05$) among genotypes and fell within the ranges reported for hair breeds under tropical conditions (Weiss *et al.*, 2015; Rosas *et al.*, 2022). This indicates a similar efficiency of carcass utilization among crossbreeds when management and diet are homogeneous. Therefore, an advantage in percentage yield cannot be attributed to the Dp × Pb genotype, but only to the absolute carcass weight. The *Longissimus dorsi* muscle area was greater ($P < 0.05$) in the Dp × Pb and Ch × Pb genotypes than in Kt × Pb, which is closely associated with

Table 2. Effect of genetic group on the productive performance of confined lambs.

Variables	DpPb	KtPb	ChPb	P-value	SEM
Dry matter intake (kg/animal/day)	0.975	0.984	0.905	0.577	0.219
Average daily gain (kg/animal/day)	0.259	0.274	0.278	0.866	0.062
Feed conversion ratio	3.777	4.235	4.017	0.473	1.155

DpPb=Black-headed Dorper × Pelibuey; Kt × Pb=Katahdin × Pelibuey; ChPb=Charollais × Pelibuey; SEM=standard error of the mean.

the higher slaughter weight and the degree of physiological development achieved, rather than with intrinsic differences in muscle growth efficiency (Vázquez *et al.*, 2011; Souza *et al.*, 2016). Similarly, the greater backfat thickness observed in Dp × Pb is explained by its higher final weight and greater degree of physiological maturity, in agreement with the positive relationship among weight, fatness, and tissue development reported in sheep (Prache *et al.*, 2022; Van der Merwe *et al.*, 2022). Conversely, the lower backfat values observed in Kt × Pb reflect a later growth pattern and lower lipid deposition, consistent with the leaner profile of Katahdin lambs (Phillips *et al.*, 2023).

Carcass morphometric measurements did not show significant differences ($P > 0.05$) among the genetic groups evaluated (Table 4). Values for carcass length, leg length, and thorax width were within the ranges reported for hair-breed lambs managed under intensive tropical production systems (Macías *et al.*, 2010; Cedeño *et al.*, 2023; Martínez, 2024).

Since the observed differences were not statistically significant, it is not possible to attribute a direct productive or commercial impact to the slight numerical variations among genotypes. In this context, the morphometric similarity indicates that, under controlled feeding and management conditions, the external conformation of the carcass is primarily influenced by live weight at slaughter rather than by the genetic group *per se*. Thus, although Dp × Pb lambs attained greater absolute carcass weights, linear proportions remained stable among genotypes, suggesting a comparable morphostructural adaptation of hair-breed crossbreeds to intensive tropical production systems.

Table 3. Effect of genetic group on the carcass characteristics of lambs.

Variables	DpPb	Kt Pb	ChPb	P-value	SEM
Live weight at slaughter (kg)	53.60 a	41.79 b	41.75 b	0.014	7.22
Hot carcass weight (kg)	28.98 a	20.52 b	23.19 b	0.013	4.22
Cold carcass weight (kg)	28.83 a	20.00 b	23.07 b	0.009	4.15
Hot carcass yield (%)	53.86 a	49.10 a	55.94 a	0.107	5.81
Cold carcass yield (%)	53.62 a	47.85 a	55.64 a	0.058	5.78
Backfat thickness (mm)	5.60 a	3.00 b	4.12 ab	0.015	1.28
<i>Longissimus dorsi</i> muscle area (cm ²)	18.45 a	12.71 b	18.34 a	0.017	3.54

Dp×Pb=Black-headed Dorper × Pelibuey; Kt×Pb=Katahdin × Pelibuey; Ch×Pb=Charollais × Pelibuey; SEM=standard error of the mean. Values with different superscript letters indicate statistical differences ($P < 0.05$).

Table 4. Effect of genetic group on the morphometric characteristics of lamb carcasses.

Variables (cm)	DpPb	KtPb	ChPb	P-value	SEM
Carcass length	92.80	86.00	88.81	0.13	5.09
Leg length	19.90	21.80	19.35	0.15	2.32
Leg width	22.72	23.10	23.18	0.84	1.49
Thorax length	27.90	30.96	26.73	0.21	4.39
Thorax width	80.90	75.56	76.24	0.16	4.86

Dp×Pb=Black-headed Dorper × Pelibuey; Kt×Pb=Katahdin × Pelibuey; Ch×Pb=Charollais × Pelibuey; SEM=standard error of the mean.

CONCLUSIONS

Under the conditions evaluated, the Dorper × Pelibuey crossbreed exhibited greater absolute carcass weights, a larger *Longissimus dorsi* muscle area, and greater fat cover compared with the Katahdin × Pelibuey and Charollais × Pelibuey crossbreeds, with no differences in average daily gain, percentage carcass yield, or morphometric proportions. These results indicate that carcass performance was primarily associated with the live weight attained at slaughter under an intensive system with a homogeneous diet. Therefore, the Dorper × Pelibuey crossbreed may represent a functional alternative for intensive tropical production systems aimed at increasing carcass weight, considering that its productive advantage is conditioned by nutritional management and the final weight achieved.

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Bioactive management alternatives for *Sclerotinia minor* Jagger in lettuce (*Lactuca sativa*)

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ABSTRACT

Objective: To determine the effect of biological control agents and plant extracts on the sclerotia of *Sclerotinia minor* through *in vitro* and *in vivo* trials.

Design/Methodology/Approach: Through *in vitro* and *in vivo* trials, analyses were performed on sclerotia treated with bioactive products, including the percentage of germination, parasitic colonization, disease incidence, and fresh plant weight.

Results: *Trichoderma harzianum* completely inhibited the growth of *S. minor* *in vitro* through mycoparasitism and enzymatic degradation. *In vivo*, it reduced disease incidence to 20% and resulted in a lower percentage of germination and sclerotia produced post-trial, whereas the control treatments reached 100% infection. Sclerotial germination only occurred in the presence of the host plant. *Trichoderma* maintained a stable population in the soil (10^4 CFU/g), demonstrating high persistence. In contrast, *Bacillus* spp. showed lower efficacy, with high percentages of sclerotial germination (92.5-97.5%). Organic matter slightly increased disease incidence by creating favorable conditions for the pathogen.

Limitations/Implications: Due to the biology of the pathogen, it is necessary to determine more precisely the biological interactions occurring in the plant rhizosphere to better understand the action of the biological agents.

Findings/Conclusions: *Trichoderma harzianum* establishes itself as a viable alternative for the management of *S. minor* in lettuce, reducing sclerotia viability through mycoparasitism and competition. Its implementation allows for a reduction in the dependence on synthetic fungicides and contributes to more sustainable production, minimizing the development of fungicide resistance.

Keywords: Biocontrol, sclerotia, white mold, mycoparasitism, variability.

Citation: López-García, A. A., Gómez-Rodríguez, O., Ayala-Escobar, V., Nava-Díaz, C., & Leyva-Mir Santos, G. (2026). Bioactive management alternatives for *Sclerotinia minor* Jagger in lettuce (*Lactuca sativa*). *Agro Productividad*. <https://doi.org/10.32854/7xddq468>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: November 18, 2025.

Accepted: March 3, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 175-189.

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INTRODUCTION

Lettuce (*Lactuca sativa* L.) is cultivated worldwide. By 2022, Mexico ranked as the ninth largest producer of this vegetable, with a production volume of 558,032.88 tons (FAO, 2022). According to the Agri-Food and Fisheries Information Service, as of November 2024, Mexico reported a production of 494,710.85 tons, with the principal producing states being Guanajuato (138,668.54 tons), Zacatecas (78,524.67 tons), Puebla (73,835.59 tons), Aguascalientes (57,657 tons), and Baja California (30,422.50 tons) (SIAP, 2024).

Lettuce is a crop affected by numerous organisms, particularly phytopathogens such as *Botrytis cinerea* (gray mold), *Bremia lactucae* (downy mildew), *Fusarium*, *Rhizoctonia*, *Phytophthora* (root diseases), *Sclerotinia sclerotiorum*, and *Sclerotinia minor* (white mold or soft rot) (INIA, 2016). *S. sclerotiorum* and *S. minor* are regarded as highly destructive pathogens in this production system (Arias *et al.*, 2007; Macioszek *et al.*, 2023). Owing to the pathogenesis of the fungus and the nature of the crop, the concept of disease severity is not applicable; rather, disease incidence predominates, since once the pathogen initiates its pathogenic process, the infection can no longer be halted.

The morphology of *S. minor* has been described as tuberculoid sclerotia with a black sclerotic rind composed of two to six deep layers and a prismatic texture, originating from medullary cells, with irregular contours and dimensions ranging from 0.5 to 2 mm (Rakesh *et al.*, 2016; Petkova *et al.*, 2024). These structures are rough in texture and angular in shape (Laemmlen, 2001). This pathogen has been reported to infect approximately 400 hosts (Wu *et al.*, 2008).

Disease onset occurs during the eruptive phase of the *S. minor* sclerotium through the myceliogenic mode. The mycelium comes into contact with lettuce stems and senescent leaves, penetrating the plant tissue. Subsequently, through the action of hydrolytic enzymes, degradation of the vascular bundle takes place, resulting in impaired water and nutrient transport, which ultimately causes desiccation and complete wilting of the plant (Hao *et al.*, 2007; Arias *et al.*, 2007; Hayes *et al.*, 2010).

Its management is considered particularly challenging because sclerotia can survive for up to 20 years (Bullock *et al.*, 1980; Blancard, 2002; Crutcher *et al.*, 2017; Petkova *et al.*, 2024). For the preventive management of diseases caused by *Sclerotinia* species, synthetic fungicides are used in Mexico and worldwide, including fluazinam, fluopyram, fluxapyroxad, trifloxystrobin, pyraclostrobin, and boscalid. Combinations of these active ingredients have been reported to reduce disease incidence by up to 60% (Matheron and Porchas, 2009). However, their efficacy may be affected by pathogen pressure, climatic conditions, application methods, and, above all, by pathogen-acquired resistance resulting from the excessive use of these fungicides (Petkova *et al.*, 2024). To date, no lettuce varieties resistant to these pathogens have been identified (Chitrampalam *et al.*, 2008; Mamo *et al.*, 2019; Petkova *et al.*, 2024), although partial resistance has been reported among different cultivated lettuce varieties (Grube and Aburomia, 2004).

This situation complicates the management of these pathogens; therefore, investigating the effect of natural extracts and biological control agents could provide an ecologically sound strategy to reduce the resistant propagules present in the soil and diminish disease incidence. Accordingly, the objective of this study was to determine the effect of biological control organisms and plant extracts on the sclerotia of *Sclerotinia minor*.

MATERIALS AND METHODS

In vitro assay

Inoculum Collection and Reproduction

The inoculum was collected from fields in the municipality of San Francisco Putla (latitude: 19° 07' 54" N, longitude: 99° 37' 50" W), in the Toluca Valley. Sclerotia were

extracted from plants exhibiting the characteristic symptoms and signs of the disease, surface-disinfested with 70% ethanol for 10 s, and rinsed three times with distilled water. Subsequently, they were placed on sterile blotting paper and transferred in groups of four sclerotia to Petri dishes containing PDA culture medium (4.0 g potato starch, 20.0 g dextrose, and 15.0 g agar per liter of solution) for inoculum reproduction.

Treatments

Eight commercial products, including biological organisms and natural extracts (Table 1), were used. Of these, only Baktillis, Apolo, and Timorex Gold are authorized on the label for use in lettuce. These products were evaluated under *in vitro* conditions in order to biologically assess the capacity of each treatment to inhibit fungal growth and development.

In 52-mm-diameter Petri dishes containing PDA, two sclerotia were placed 1 cm apart from each other; the sclerotia exhibited fungal mycelium on their surface. For treatments 1 to 5 (Table 1), the products were directly inoculated onto the sclerotia. For treatments 6, 7, and 8 (Table 1), since they consisted of natural extracts, the active ingredient was incorporated into the PDA culture medium before the sclerotia were placed onto this substrate. The highest dose recommended on the manufacturers' labels for each product was used. Sterile distilled water was sprayed onto the positive control. After inoculation, observations were made every 3 days for 15 days to monitor *S. minor* mycelial growth. Each product included five experimental units with two sclerotia each, for a total of 10 replicates per product. It should be noted that none of these products is labeled for the management of *Sclerotinia minor* Jagger; however, they are commonly used in vegetable production systems.

Transfer of Sclerotia After Treatment Application

Fifteen days after inoculation, the experimental units were opened, and each sclerotium was transferred to a new Petri dish containing PDA to observe, for an additional 15 days, the effect of the biological control organisms or the residual activity of the plant extracts on sclerotial germination capacity. Thirty days after the initial sclerotial inoculation, a qualitative assessment was conducted by evaluating mycelial growth and sclerotial production.

Table 1. Treatments and doses of bioactive products used in the *Sclerotinia minor*-*Lactuca sativa* pathosystem under *in vitro* conditions.

Treatment	Active ingredient	Product	Concentration (CFU spores mL ⁻¹ or g ⁻¹)	Dose (g or mL L ⁻¹)
1	<i>Bacillus amyloliquefaciens</i> strain MBI	Serifel	5.5×10^{10}	5
2	<i>Bacillus subtilis</i>	Baktillis	1×10^{12}	7.5
3	<i>Trichoderma harzianum</i> strain KRL-AG2	PHC T-22	1×10^7	2.27
4	<i>B. subtilis</i> , <i>T. harzianum</i> , <i>Streptomyces lydicus</i>	Apolo	CC*	5
5	<i>Melaleuca alternifolia</i> oil	Timorex Gold	0.2225 g	3.75
6	<i>Swinglea glutinosa</i> extract	Ecoswing	868 g a.i.	5
7	<i>Larrea tridentata</i> extract	Org-fung	40 g a.i.	5
8	<i>Bacillus subtilis</i> strain SP 83	Fungi free	5.5×10^9	5

*Combination of concentrations.

***In vivo* assay**

Preparation of Experimental Units

Field soil was subjected to double pasteurization at 15 psi for 4 h each cycle and subsequently mixed with peat moss at a 4:1 ratio (pasteurized field soil:peat moss). Pots measuring 30 × 30 cm were filled with approximately 0.625 kg of the soil mixture, and one lettuce seedling of the Great Lake variety was transplanted into each pot.

Using the sclerotium-trap method (Stazzonelli *et al.*, 2017), as adapted by Arrúa Alvarenga and Aquino Jara (2013) with modifications, 10 sclerotia of *S. minor* were placed into a microporous fabric bag, which was then buried in each pot. A total of 240 bags were prepared and placed individually in each pot at a depth of 5 cm. From the sclerotia reproduced during the *in vitro* stage, four randomly selected sclerotia were placed in each Petri dish containing PDA culture medium, with a total of two Petri dishes. These were incubated at 28 °C.

Application of Bioactive Products and Organic Matter

For product application, a 1000 mL suspension was prepared for each treatment. Subsequently, 25 mL of each suspension was applied to each pot as a drench. For the organic matter treatment, pasteurized bat guano (previously registered for commercial sale) was used at a dose of 25 mL of active ingredient per liter of water and incorporated into the substrate. Both the bioactive products and the organic matter were applied at 25 mL per experimental unit every 7 days for one month.

All experimental units were fertilized every 5 days with a 20-30-10 (N-P-K) fertilizer at a dose of 3 g L⁻¹ of water, beginning on day 1 after transplanting and continuing until 45 days after transplanting (head formation stage). The experimental units were maintained under open-field conditions in order to simulate field conditions and naturally expose the pathogen to the environment. Irrigation was applied every 2 days, and temperature and relative humidity were monitored using a HOBO PRO data logger, model U23-00A, programmed to record data every 30 min.

Experimental Design

The assay was established under a completely randomized design with three factors (treatments, plant, and organic matter) in order to determine the effect of each on the epidemiological system of *L. sativa* L.-*S. minor* and to identify the physical, biological, or environmental conditions influencing disease incidence. The treatment factor included six levels (five products and an untreated control), the plant factor had two levels (with or without plant), and the organic matter factor also had two levels (with or without organic matter), resulting in a total of 24 possible combinations. Each combination consisted of 10 replicates, for a total of 240 experimental units.

Post-assay analysis of sclerotial germination

Eighty days after lettuce transplantation, the plants were weighed, and five bags were selected from each of the established combinations as a representative sample of the 10 experimental units per combination. These were labeled and processed in the laboratory.

From each bag, four sclerotia were selected and placed into 2 mL Eppendorf tubes containing 1 mL of sterile distilled water. Sclerotial washing was performed by vortexing for two periods of 30 s each, after which the sclerotia were decanted onto Whatman® grade 1 filter paper (90 mm diameter) placed in a glass funnel, and excess water was removed with the aid of burners. Subsequently, the four sclerotia were transferred to Petri dishes containing PDA culture medium. Sclerotial germination was evaluated 11 days after sowing (DAS).

Colonization by biological organisms

For the recovery of organisms from the soil, samples were taken from five pots out of the 10 experimental units of each combination to avoid excessive sample processing, except for those receiving treatment 5 (*Melaleuca alternifolia* oil). PDA culture medium was used for samples containing *Trichoderma* spp., whereas nutrient agar was used for samples containing bacteria. Extraction was performed using 1 g of soil sample in 10 mL of sterile distilled water, followed by serial dilutions to a concentration of 10^{-4} for *Bacillus* spp. strains and 10^{-2} for *Trichoderma* spp. strains. Finally, 100 μ L of each dilution was plated onto the respective culture medium and spread with a bacteriological loop. The plates were incubated at 28 °C. To obtain CFU g^{-1} of soil, the standard colony-forming unit counting equation was used as follows:

$$\frac{UFC}{g} = \frac{N \times D}{V} \times weight\ factor$$

where: N =number of colonies; D =dilution factor; V =volume plated on the dish (mL).

The count was adjusted by the weight factor ($0.1\ g\ mL^{-1}$), since the initial preparation consisted of 1 g of soil mixed with 10 mL of water. Because only a single dilution was performed, the results should be interpreted as point estimates. Since the bacteria were extracted from a previously sterilized medium, each of the products containing the bacterial strains used was plated at a concentration of 10^{-6} so that colony counts could be performed based on their morphology.

Determination of *Trichoderma* parasitism

To determine the mode of action, Petri dishes containing nutrient agar were used, and two points were marked at a distance of 5 cm from the center of each dish. On one side, a PDA fragment containing mycelium and sclerotia of *S. minor* was inoculated, while on the opposite equidistant point, a PDA fragment bearing a colony of each of the two *Trichoderma* strains was placed under sterile conditions. After seven days, observations were made of the effect of *Trichoderma* mycelium on the mycelium of *S. minor* under light microscopy.

Statistical analysis

The principal response variable used to evaluate treatment effects was fresh lettuce weight. Since fresh weight data did not conform to a normal distribution (Shapiro-Wilk,

$p < 0.05$), a nonparametric Scheirer-Ray-Hare test was applied for a factorial design with two factors: “Treatment” (6 levels) and “Organic Matter” (2 levels). This test is a useful statistical tool when nonparametric data analysis is required, that is, when the assumptions of normality, homogeneity of variance, and independence are not met. The test was used to analyze the independent effects of the factors as well as their interaction. All data analyses were performed in R v4.3.1 (R Core Team, 2025).

RESULTS AND DISCUSSION

In vitro assay

It was observed that treatments 1, 2, 3, 4, and 8 (Table 2; Figure 1) showed no mycelial growth from the sclerotia upon exposure to the products, nor after sclerotial transfer (Figure 3). In contrast, treatments 5, 6, and 7 (Table 2; Figure 2), which corresponded to natural extracts, exhibited the presence of cottony mycelium as well as the formation of new sclerotia even before transfer was performed. The treatments that showed no mycelial growth were selected for the *in vivo* assay.

In vivo assay

Four days after sclerotial sowing, germination and mycelial growth were observed in the viability test, confirming that the sclerotia placed in the bags retained the capacity to germinate and initiate the infection cycle.

In the assay, a maximum disease incidence of 30% was observed, whereas the absolute control exhibited 100% incidence (Figures 4 and 5).

Through the Scheirer-Ray-Hare test, a highly significant effect of treatments on fresh lettuce weight was detected ($H = 42.215$, $df = 5$, $p < 0.0001$). However, neither organic matter ($H = 0.002$, $df = 1$, $p = 0.964$) nor the interaction between treatments and organic matter ($H = 1.086$, $df = 5$, $p = 0.955$) showed significant effects. Dunn’s *post hoc* test with Holm adjustment revealed that treatment T6 (absolute control) differed significantly from all other treatments (T1-T5) ($p < 0.001$ in all cases), whereas no statistically significant differences were found among treatments T1 to T5 ($p > 0.05$ for all comparisons). These results indicate that treatment T6 (absolute control) produced a significantly lower fresh

Table 2. Germination of *Sclerotinia minor* sclerotia after exposure to different biocontrol products.

Treatment	Active ingredient	15 days after sclerotial sowing	
1	<i>Bacillus amyloliquefaciens</i> strain MBI	–	–
2	<i>Bacillus subtilis</i>	–	–
3	<i>Trichoderma harzianum</i> strain KRL-AG2	–	–
4	<i>B. subtilis</i> , <i>T. harzianum</i> , <i>S. lydicus</i>	–	–
5	<i>Melaleuca alternifolia</i> oil	+	+
6	<i>Swinglea glutinosa</i> extract	+	+
7	<i>Larrea tridentata</i> extract	+	+
8	<i>Bacillus subtilis</i> strain SP 83	–	–

Positive germination (+); negative germination (–).

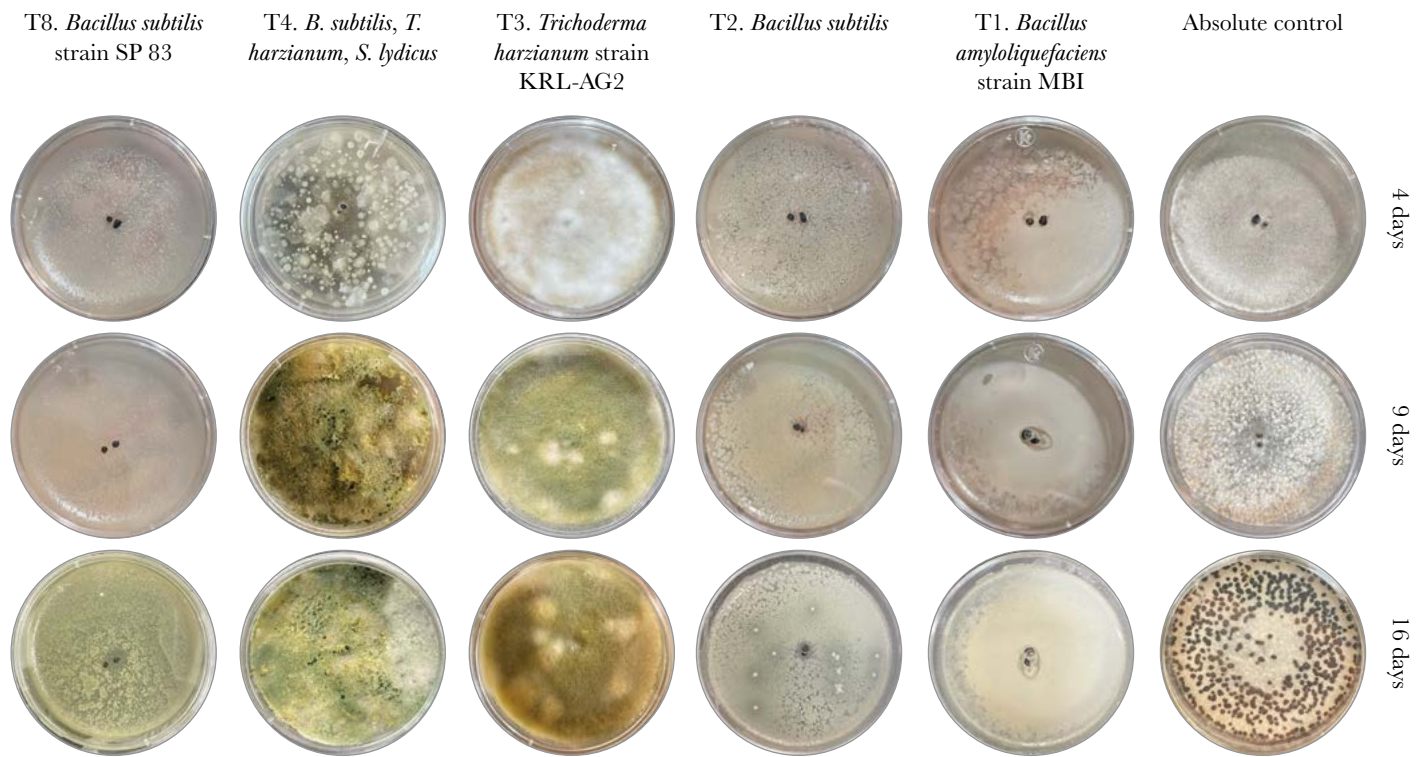


Figure 1. Growth after sowing of treatments composed of biological organisms.

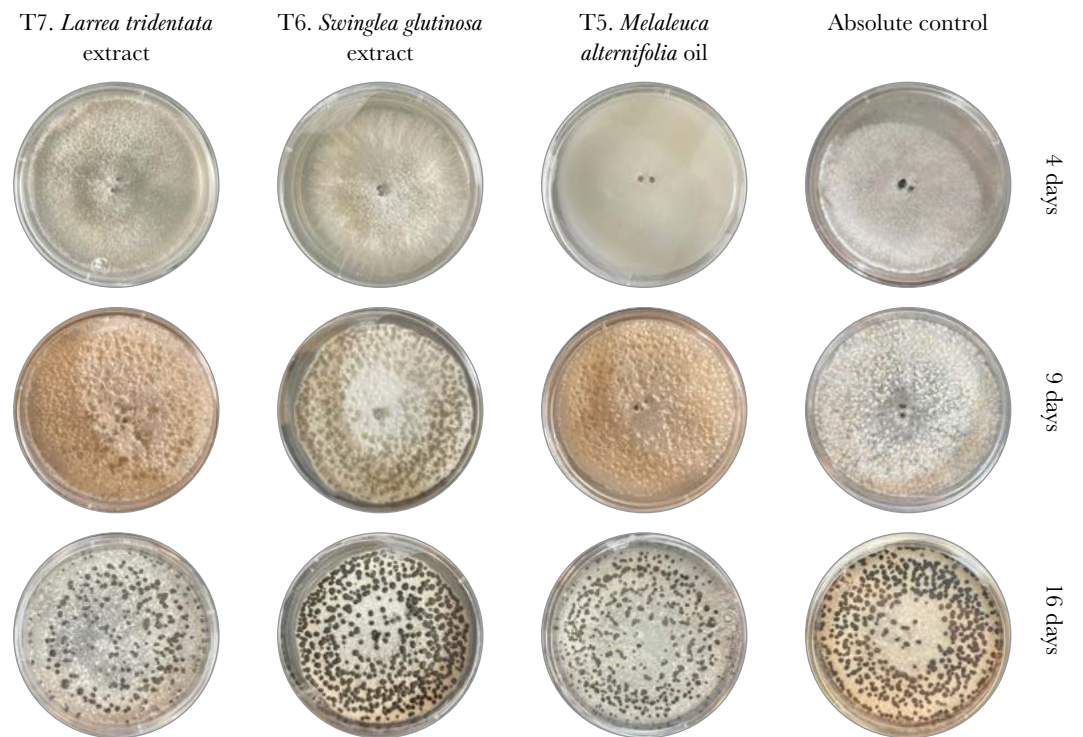


Figure 2. Growth after sowing of treatments composed of natural extracts and oils.

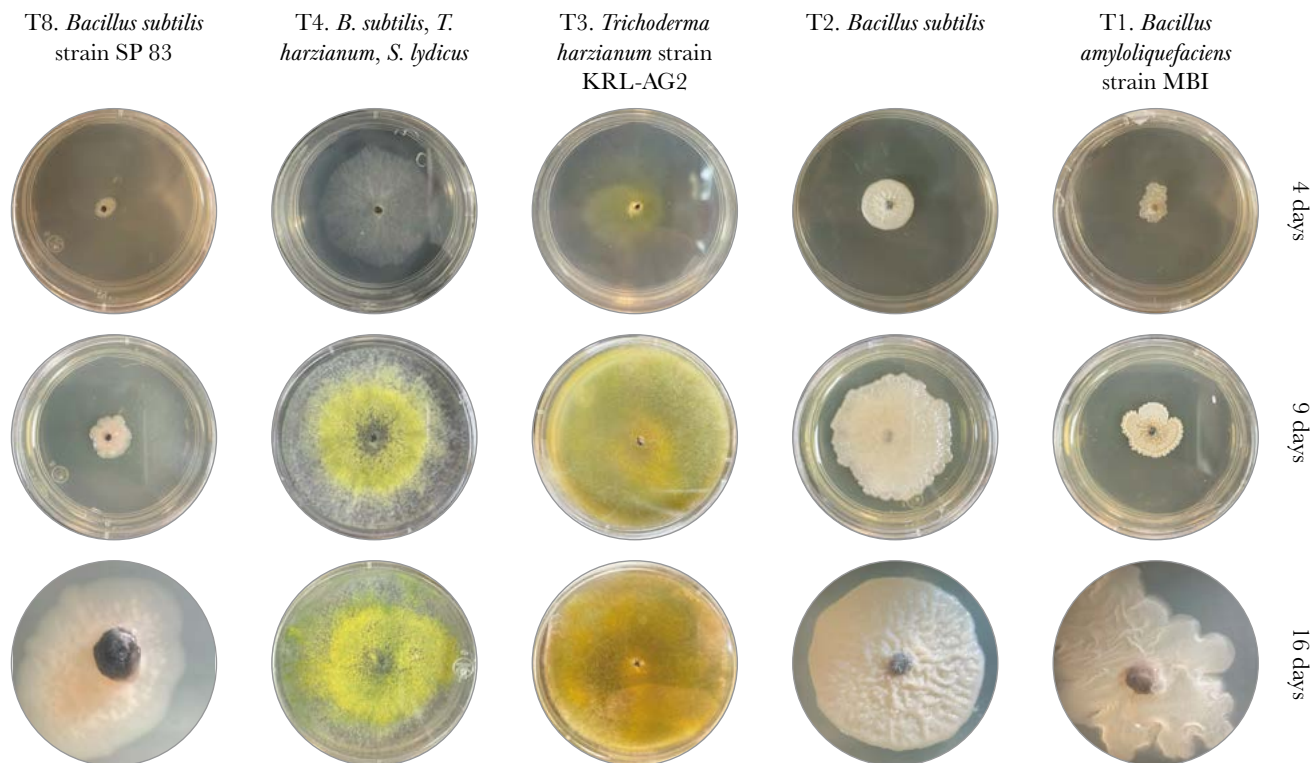


Figure 3. Sclerotia exposed to bioactive products that were reseeded without germination.

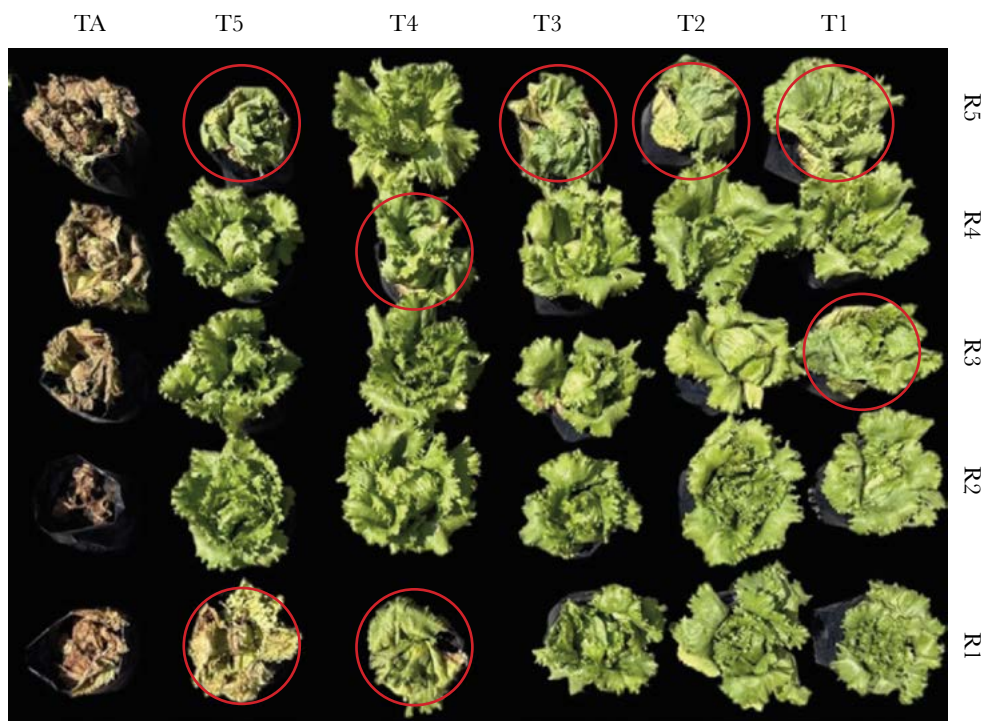


Figure 4. Incidence of white mold in a five-treatment factorial trial, without application of organic matter and with the presence of plants 80 days after transplanting. Absolute control (TA), *Bacillus amyloliquefaciens* (T1), *Bacillus subtilis* (T2), *Trichoderma harzianum* strain KRL-AG2 (T3), *B. subtilis*, *T. harzianum*, *S. lydicus* (T4), *Melaleuca alternifolia* oil (T5).

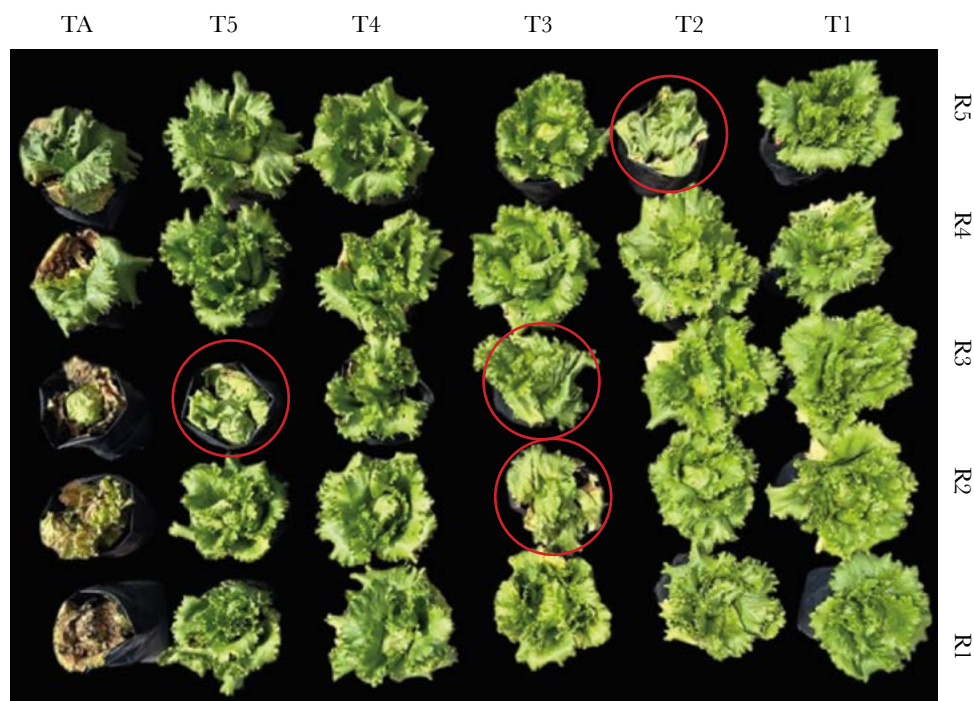


Figure 5. Incidence of white mold in a five-treatment factorial trial, without application of organic matter and with the presence of plants 80 days after transplanting. Absolute control (TA), *Bacillus amyloliquefaciens* (T1), *Bacillus subtilis* (T2), *Trichoderma harzianum* strain KRL-AG2 (T3), *B. subtilis*, *T. harzianum*, *S. lydicus* (T4), *Melaleuca alternifolia* oil (T5).

weight than all other treatments (T1-T5). The absence of significant differences among treatments T1 to T5 suggests that all of them exerted a similar effect on fresh lettuce weight, which was superior to that of control T6. Likewise, the lack of a significant interaction between treatments and organic matter indicates that treatment effects were consistent regardless of whether organic matter was applied; nevertheless, given the biology of this pathosystem, this factor may contribute to the establishment of plant-pathogen or bioactive organism-pathogen interactions. Studies conducted by Abawi and Grogan (1979) indicate that *S. minor* may benefit from soils with high organic matter content, as this contributes to greater moisture retention and nutrient availability, which may explain the high incidence observed in the experimental units supplied with organic matter. This finding is consistent with reports on strains of *Bacillus amyloliquefaciens* used for the control of *Sclerotinia* species, in which *B. amyloliquefaciens* did not produce an additional increase in the normal growth variables of lettuce, even though it had the capacity to produce auxins (Sabaté *et al.*, 2018). The absence of interaction may also be associated with the number of experimental units, suggesting that a larger sample size may be required than that used in this assay.

Post-Assay Germination and Sclerotial Production

It is worth noting that no germination was observed in sclerotia located in experimental units where no plant was present, thereby reinforcing the findings of Abawi and Grogan (1979), Hao *et al.* (2007), and Arias *et al.* (2007), who reported that germination of *S. minor* requires the microclimate generated between the lettuce canopy and the soil surface.

For the sclerotial germination variable, germination percentage data were also analyzed using a nonparametric approach because the assumptions of normality were not met (Shapiro-Wilk, $p < 0.05$). The Scheirer-Ray-Hare analysis revealed a highly significant effect of treatments ($H = 89.65$, $df = 4$, $p < 0.001$), whereas no significant effects were detected for organic matter ($H = 29.34$, $df = 114$, $p = 1$) or for the interaction between these factors. Multiple comparisons using Dunn's test with Holm adjustment revealed significant differences among treatment groups ($p < 0.001$). Specifically, T3 (*B. subtilis*, *T. harzianum*, *S. lydicus*) and T4 (*Trichoderma harzianum* strain KRL-AG2) showed a significant reduction in sclerotial germination compared with the other treatments. In particular, T4 exhibited the lowest germination values and was statistically different from T1, T2, T5, and the absolute control (T6). Treatments T1, T2, T5, and T6 did not differ significantly from one another ($padj > 0.05$ in all comparisons), maintaining high germination percentages ($> 75\%$).

Trichoderma (T3 and T4) was able to reduce germination to below 10% in most replicates, and this effect was independent of organic matter addition ($p > 0.05$). The absence of significant differences between T3 and T4 suggests that both treatments constitute viable alternatives for inhibiting the germination of *S. minor* sclerotia, although T4 showed a tendency toward greater consistency in this effect. These findings are in agreement with Rivera-Méndez *et al.* (2015), who studied the interaction between *Sclerotium cepivorum* and *Trichoderma* to determine the potential of this organism as a biological control agent and to reduce disease incidence. Their results showed lower mortality in blocks where *Trichoderma* was applied, associating this outcome with its antagonistic capacity against soilborne propagules.

Regarding the number of sclerotia produced (Table 3), the Scheirer-Ray-Hare test revealed highly significant differences among treatments in sclerotial production ($p < 0.0001$). However, no significant effects were detected for the presence or absence of organic matter ($p = 0.276$) or for the interaction between treatments and organic matter ($p = 0.291$), indicating that treatment efficacy was independent of the organic matter factor. Dunn's post hoc analysis with Holm adjustment showed the same trend observed for germination percentage, in which treatments T3 (*B. subtilis*, *T. harzianum*, *S. lydicus*) and T4 (*Trichoderma harzianum*) were the most effective ($padj = 0.115$). Treatments T3 and T4 did not differ significantly from each other ($padj = 0.115$), while both exhibited a favorable contrast with the absolute control T6. In contrast, treatments T2 (*Bacillus amyloliquefaciens*) and T5 (*Melaleuca alternifolia* extract) were significantly inferior to the absolute control. Treatment T1 (*Bacillus subtilis*) showed an intermediate performance, being superior to T2 and T5 ($padj < 0.0001$), but without significant differences relative to the most effective treatments or the control ($padj > 0.05$). These results indicate that only the treatments containing *Trichoderma* (T3 and T4) were capable of significantly reducing sclerotial production, with an effect independent of organic matter addition.

Colonization of Biological Organisms

The presence of the applied organisms was confirmed, and their persistence varied according to the product as well as the presence or absence of plants and organic matter (Figures 6 and 7). *Bacillus subtilis* and *Bacillus amyloliquefaciens* showed low concentrations

Table 3. Number of sclerotia cm² from sclerotia exposed to bioactive ingredients.

Treatment	Number of sclerotia per cm ²			
	a	b	c	d
Witness	19.20±0.96	20.20±1.80	20.40±1.28	21±1.58
<i>B. subtilis</i>	17.60±1.16	16±1.48	18.80±0.86	18.60±1.28
<i>B. amyloliquefaciens</i>	21.40±1.20	13.20±1.15	19.40±1.20	15.40±0.74
<i>B. subtilis</i> , <i>T. harzianum</i> , <i>S. lydicus</i>	0	2.80±1.74	1.0±1.0	0
<i>T. harzianum</i> strain KRL-AG2	0	1.20±1.20	0	5.20±2.26
<i>Melaleuca argentifolia</i> oil	18.80±0.73	14.16±0.87	19±0.94	18.40±1.07

a: treatments with plant and organic matter, b: treatments with plant without organic matter, c: treatments without plant and with organic matter, d: treatments without plant and without organic matter.

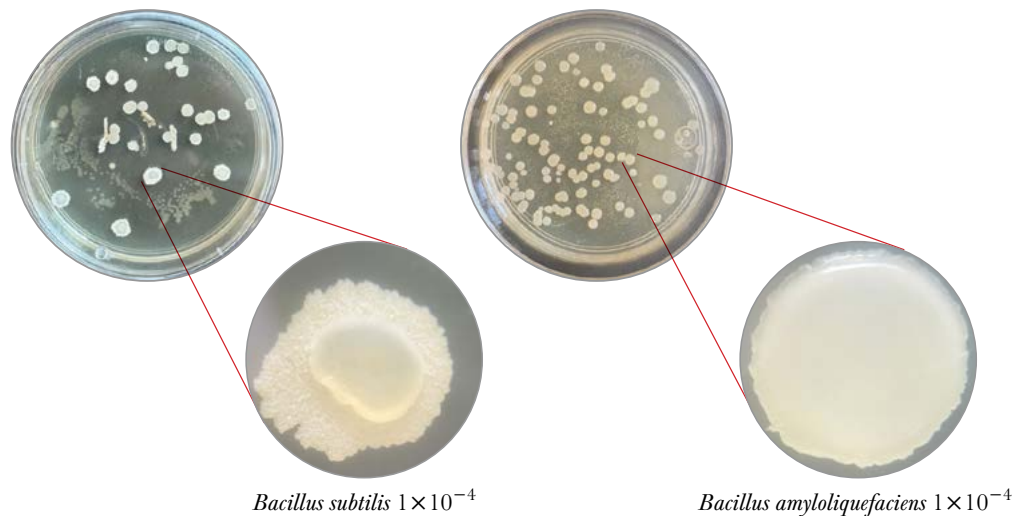


Figure 6. Colonies of *Bacillus* spp. extracted from the soil with application of bioactive products.

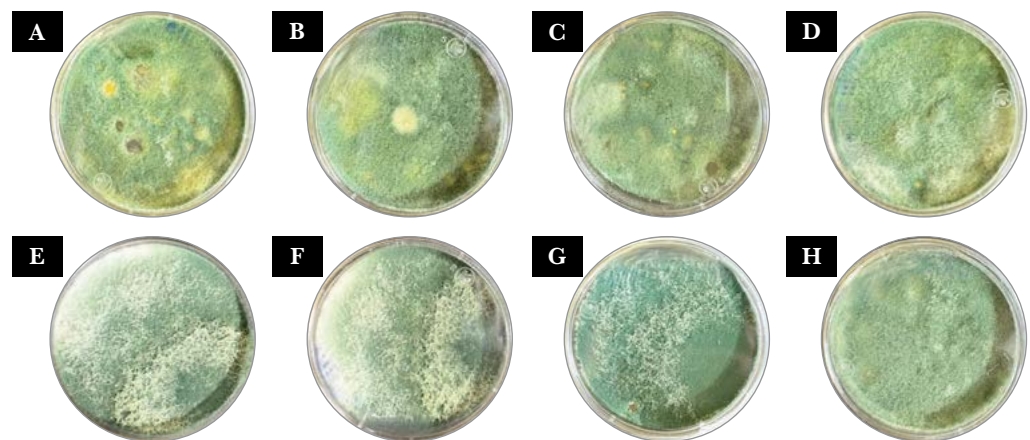


Figure 7. Colonies of *Trichoderma* spp. extracted from soil with application of bioactive products. A) *Trichoderma harzianum* + M.O. + plant. B) *Trichoderma harzianum* + plant. C) *Trichoderma harzianum* + M.O. D) *Trichoderma harzianum*. E) *Trichoderma harzianum* strain KRL-AG2 + M.O. + plant. F) *Trichoderma harzianum* strain KRL-AG2 + plant. G) *Trichoderma harzianum* strain KRL-AG2 + M.O. H) *Trichoderma harzianum* strain KRL-AG2.

in treatments with plants, ranging from 9.3×10^6 to 1.09×10^7 CFU g^{-1} and 10^7 CFU g^{-1} , respectively, and these values declined even further in the absence of plants. This phenomenon may be explained by the findings of Larena and Melgarejo (1996), who reported that *Bacillus* species exhibit low efficiency in soils under continuous lettuce production, as such soils commonly display a neutral to alkaline pH.

In contrast, *Trichoderma* strains showed remarkably stable concentrations (10^4 CFU g^{-1}) across all scenarios, thereby demonstrating their strong saprophytic capacity. This observation is consistent with the findings of Singh *et al.* (2024), suggesting a marked ability to adapt to the environment and exploit organic matter efficiently. *Trichoderma* exhibited superior establishment in the soil of each experimental unit, whereas the concentration of *Bacillus* species was the most adversely affected. This pattern may also be explained by the observations of Larena and Melgarejo (1996), who described the low efficiency of *Bacillus* species in soils where lettuce is continuously cultivated, due to the fact that such soils tend to exhibit a neutral to slightly acidic pH. The opposite appears to occur with *Trichoderma*, since this organism thrives within that pH range, which enables it to establish, develop, and exert a protective effect on the plant (Martínez *et al.*, 2013), a response that was not observed for the *Bacillus* species.

Parasitism of *Trichoderma*

With respect to the parasitism exerted by *Trichoderma* species, observations under light microscopy revealed that the two strains used in this assay were capable of penetrating, infecting, and coiling around the mycelium of *S. minor* (Figure 8). Together with their faster growth rate relative to *S. minor*, this prevented the development of both mycelium and sclerotia. The parasitism of *S. minor* sclerotia observed in this study has been extensively documented in the scientific literature by several authors (Arias *et al.*, 2007; Petkova *et al.*, 2024; Kishan *et al.*, 2017), who describe *Trichoderma* spp. as biological control agents with the capacity to induce mycoparasitism, a process in which *Trichoderma* spp. grows chemotropically toward the host, adheres to its hyphae, coils around them, and in some cases penetrates them (Carsolio *et al.*, 1999). In other words, it can attack both the sclerotia and the mycelium of the pathogen (Chitrampalam *et al.*, 2008).

In light of the foregoing, it is evident that *Trichoderma* exerts a direct effect on the biology of the fungus, since once it infects the pathogen and consumes its cellular contents, structural damage occurs that prevents proper germination and reproduction. When incorporated into an integrated disease management strategy, *Trichoderma* may act on the sclerotia and reduce inoculum levels in the field, particularly when combined with appropriate cultural practices such as the removal of diseased plants. Future research should consider the pH factor in order to more precisely determine the mycoparasitic activity of these organisms and their functional effect on the pathogen.

Trichoderma harzianum (strains KRL-AG2 and harzianum) demonstrated the capacity to act as an effective biological control agent against *Sclerotinia minor* in lettuce, primarily through mycoparasitism, by reducing sclerotial viability and limiting disease progression under the conditions evaluated. Although no significant differences were observed among treatments for the fresh weight of lettuce, the statistical analysis revealed significant

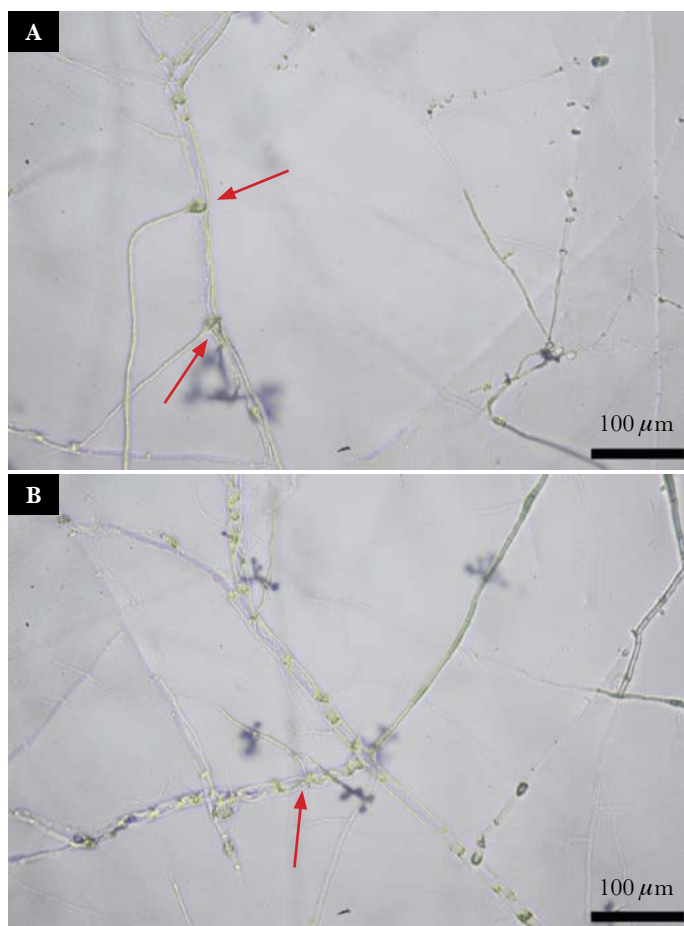


Figure 8. Parasitism of *Trichoderma* strains on *S. minor* mycelium. A) Mycelium penetration. B) Mycelium coiling.

differences between the evaluated treatments and the absolute control for this variable, indicating disease mitigation at varying levels depending on the product applied. From a biological standpoint, the treatments that showed the greatest efficacy in terms of disease incidence reduction and suppression of the biological capacities of *S. minor* were those based on *T. harzianum* and *T. harzianum* strain KRL-AG2, whereas the *Bacillus*-based treatments and the plant extract exhibited lower effectiveness.

It was also determined that the presence of the host plant is essential for the germination of *Sclerotinia minor* sclerotia, and that the incorporation of organic matter did not exert a statistically significant effect on disease incidence. Nevertheless, from a biological perspective, greater pathogen aggressiveness was observed in those experimental units amended with organic matter, which also appeared to exert a positive effect on both the pathogen and the biological control organisms within the *S. minor*-*Lactuca sativa* L. epidemiological system.

Based on the methodology employed and the results obtained, it is recommended that *Trichoderma* applications be carried out after crop transplantation and before the first senescent leaves collapse, at 7-day intervals, with a maximum of four drench applications.

For future research, it is important to evaluate soil factors such as pH, since this variable influences both pathogen behavior and the performance of biological control agents. Likewise, the study of organic matter within this epidemiological system is of considerable importance, as it will help clarify its role in the different microbial interactions involved and its impact on disease development.

CONCLUSIONS

These findings support the use of *Trichoderma* as an environmentally sound tool that directly affects the germination capacity of sclerotia, reduces the inoculum present in the field, and lowers disease incidence over time. In addition, these products are relatively low in cost, which may facilitate their adoption by a greater number of producers, particularly small- and medium-scale growers, thereby strengthening their integrated disease management strategies.

ACKNOWLEDGMENTS

The authors express their gratitude to the Ministry of Science, Technology, Humanities, and Innovation for funding this research, as well as to the Colegio de Postgraduados and the Universidad Autónoma Chapingo for providing access to laboratory facilities, materials, and academic guidance from their faculty members.

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Value chain analysis of cheese production in the municipality of Aculco (State of Mexico) Mexico

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ABSTRACT

Objective: to analyze the value chain of cheese production at the municipality scale, through a mixed approach combining the general guide, for evaluating value chains development, with the specific approach from a business perspective.

Design/Methodology/Approach: the integration of both approaches allowed for a systemic analysis incorporating social and business dimensions. Main social actors, relationships and bottlenecks—congested points such as operations, specialized work force, or transport—along the chain were identified. Those processes that generate value and determine competitiveness in the local context were highlighted.

Results: the value chain of cheese production in Aculco shows highly artisanal specialization, strong cultural roots, and significant contribution to local development. However, through the SWOT analysis, we found this production chain faces limitations in the articulation among producers, scarce innovation, and vulnerability to external economic and political factors. The mixed approach in this study conformed a methodological reference which can be applied to other agrifood systems to identify and strengthen organizational and business management capacities.

Limitations/Implications of the study: the analysis was limited to a specific municipality (Aculco, State of Mexico, Mexico) and results must be interpreted within that territorial scope. However, as a methodological reference it is applicable to other production chains, in localities with similar characteristics.

Findings/Conclusions: the value chain of cheese production in Aculco has both economic and cultural values, which make it a suitable option for the development of the local population. It is necessary to promote territorial integration and encourage sustainable practices that allow producers to foresee strategic scenarios in order to be adapted to changes in the environment.

Keywords: food security, productive integration, territorial development, SWOT analysis, mixed approach for value chains.

Citation: González-Hernández, V., Martínez-Castañeda, F. E., Zarco-González, M. M., Vilchis-Granados, G. B., Callejas-Juárez, N., Herrera-Haro, J. G., & Rogers-Montoya, N. A. (2026). Value chain analysis of cheese production in the municipality of Aculco (State of Mexico) Mexico. *Agro Productividad*. <https://doi.org/10.32854/z6z47m82>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: January 12, 2026.

Accepted: March 17, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 191-199.

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INTRODUCTION

Milk production has been widely studied from several technical, social and economic approaches. Those include production systems, feeding, genetic improvement, animal



health, public policies, product quality, and marketing mechanisms, as well as their role for food security. However, the analysis of production linkage and the consolidation of the value chain have received less attention. In Mexico, the Economic Commission for Latin America and the Caribbean-ECLAC identified that dairy sector is dominated by micro, small, and medium-sized family enterprises, where an increase in the added value generates multiplier effects throughout the production chain (De Groot, 2018).

The United Nations Food and Agriculture Organization-FAO (2024) defines the dairy production chain as an integrated system of interdependent activities. These activities fulfill economic and social functions, hold a key role in the management of health risks and in the transition to sustainable systems. In the dairy sector, competitiveness depends on a holistic view of the production chain and the efficient use of resources, rather than on isolated capital investments (Cravero & Montagna, 2020).

In Mexico, the dairy sector contributes about 17% of the value of livestock production and 6% of the GDP of the food industry according to Mexico's National chamber of milk industry owners-CANILEC (2024). Industrial cheese production reached 632 895 tons (Megagrams, Mg) in 2023 according to data from Mexico's National Institute of statistics and geography (INEGI, 2024). In the State of Mexico, annual consumption *per capita* is 117 kg, supplied mainly by the Aculco-Polotitlán-Jilotepec basin, which generates 23% of state production. Aculco, the head municipality, is paramount with an annual production of 142 616 Mg of cheese at a 9.79% average growth between 2006 and 2023, according to the Department of Agriculture in the State of Mexico (SECAMPO, 2024).

From a localized Agri-Food Systems approach, territorial links integrate production, processing and marketing, although small producers tend to operate in a fragmented way, limiting the generation of collective value (Macías, 2019).

In this context, the objective was to analyze the value chain of cheese production at the municipality scale, through a mixed approach combining the general guide, for evaluating value chains development, with the specific approach from a business perspective.

MATERIALS AND METHODS

Data collection

The information was collected during January to July 2024 through the panel technique that was applied to milk producers, cheese makers, vendors and tourism service providers in the municipality of Aculco, State of Mexico. This made it possible to obtain information on the productive, commercial and service interactions, as components of the value chain. The subsequent analysis in this study was done with the General Guide for the Development of Value Chains (Nutz & Sievers, 2016), complemented with the value chain methodology (Porter, 1985) from a business perspective. Both methodologies share a systemic and strategic approach, which made it possible to comprehensively address the structure, interactions and dynamics of the value chain of cheese production in the municipality of Aculco (State of Mexico), Mexico.

Then, the SWOT analysis of the local production and marketing system of milk and cheese in the municipality was developed to evaluate production development in regard to internal strengths and weaknesses, along with external opportunities and threats. Finally,

the information obtained from the mixed approach was integrated to discuss and make the analysis specific from the business perspective.

RESULTS AND DISCUSSION

The cheese production sector was identified as a strategic axis of local development, as its capacities became visible, to articulate economic activities, generate employment and strengthen the territorial identity in the municipality of Aculco.

In the production chain, we first identified input suppliers of livestock feed and veterinary services, which are essential for productive and health sustainability. Milk producers are the generating nucleus of the raw material; while the intermediate link —represented by the boteros, the large volume transporters— articulates the collection, transport and distribution to the transformation units. From this point, the chain bifurcates into formal cheese production, with sanitary controls and standardized processes; and informal production, based on artisanal practices and minimum technified operation.

Both flows require transformation, packaging processes and packaging materials to ensure product quality. Finally, commercial intermediaries link production with markets and the final consumer. The chain has a heterogeneous and multi-sectoral structure, where processes of value addition and risk transfer are concentrated. Value chain operation is supported by an institutional component that includes Government agencies, support programs, as well as research institutions and technical advisors that facilitate the transfer of knowledge and innovation. We present the SWOT analysis (Strengths, Weaknesses, Opportunities, and Threats) of the local milk and cheese production chain, as well as the marketing system in the municipality of Aculco (Table 1).

Table 1. SWOT analysis of the local production and marketing system of milk and cheese in Aculco (State of Mexico) Mexico.

Strengths	Opportunities
<ul style="list-style-type: none"> • Territorial recognition of the municipality as a specialized producer of milk and cheese. • Designation of “Magical Town”, a Mexican socio-cultural label which reinforces cultural and gastronomic identity promoting tourism. • Availability of government programs aimed at strengthening agrifood production and tourism. • Presence of local and regional suppliers that foster productive linkage and territorial integration. 	<ul style="list-style-type: none"> • Consolidation of a consistent brand image with regional and state-wide projection. • Comprehensive implementation of food safety and traceability measures throughout the value chain. • Adoption of technological and organizational innovation to enhance efficiency and competitiveness. • Certification on official quality and proven safety of products, that strengthen consumer confidence. • Growth of tourism-related business units through the diversification of value-added products.
Weaknesses	Threats
<ul style="list-style-type: none"> • Lack of a consistent product offer and a consolidated collective brand. • Absence of administrative control and accounting records within production units. • Lack of systematic monitoring of milk quality and overall quality of the final product. • Reduction of public resources allocated to production support and infrastructure. • Insufficiency in the quantity of technical workers hired and trained in management, safety and marketing. • Lack of a defined consumer profile and scarce strategies on territorial-segmented marketing. 	<ul style="list-style-type: none"> • Climate change effects, including erratic rainfall and decreased forage productivity. • Reduction of subsidies for fertilizers, seeds and livestock inputs. • Price fluctuation of essential inputs for production. • Deficient tourist signage and insufficient access and service infrastructure. • Absence of clearly defined market niches. • Risk of a decrease in product quality, undermining the customer-quality relationship.

The cheese value chain in Aculco (Figure 1) represents the functional and regulatory scheme of the local production system, integrating processes, actors, institutions and regulations at four interrelated levels. The first level describes the production sequence, from cattle breeding and milking to the production, distribution, marketing of cheese and their eventual exports, so reflecting a local agrifood system oriented to internal and external markets.

The second level corresponds to support institutions, such as the Aculco City Council, Mexico’s Secretariat of Agriculture (SADER), Mexico’s Secretariat for Tourism (SECTUR), and a Magical Town denomination Promotion Committee. All of which provide training, technical assistance, and commercial promotion to producers, and micro-scale, small-scale or medium business, called in Mexico “MiPyMES” (Vilchis-Granados *et al.*, 2024). The central level integrates the production chain, with differences between formal and informal production. In the latter, family labor and seasonality influence the stability of the system. At the baseline level are the official standards that regulate quality and safety, to guarantee the traceability and competitiveness of the local production system.

In the analysis of the cheese value chain, two types of producers were identified: small producers and semi-industrial producers. Small-scale producers, both milk and cheese, produce with low levels of technified operation and standardization; have limited administrative and financial knowledge; and depend mainly on family labor. These conditions, together with the persistence of traditional practices, restrict productivity and affect product quality. One of the main challenges is to ensure the quality of the milk from milking, since producers deliver it to the boteros without applying quality and safety standards. Then, the latter operate with frequent interruptions in the cold chain during transport.

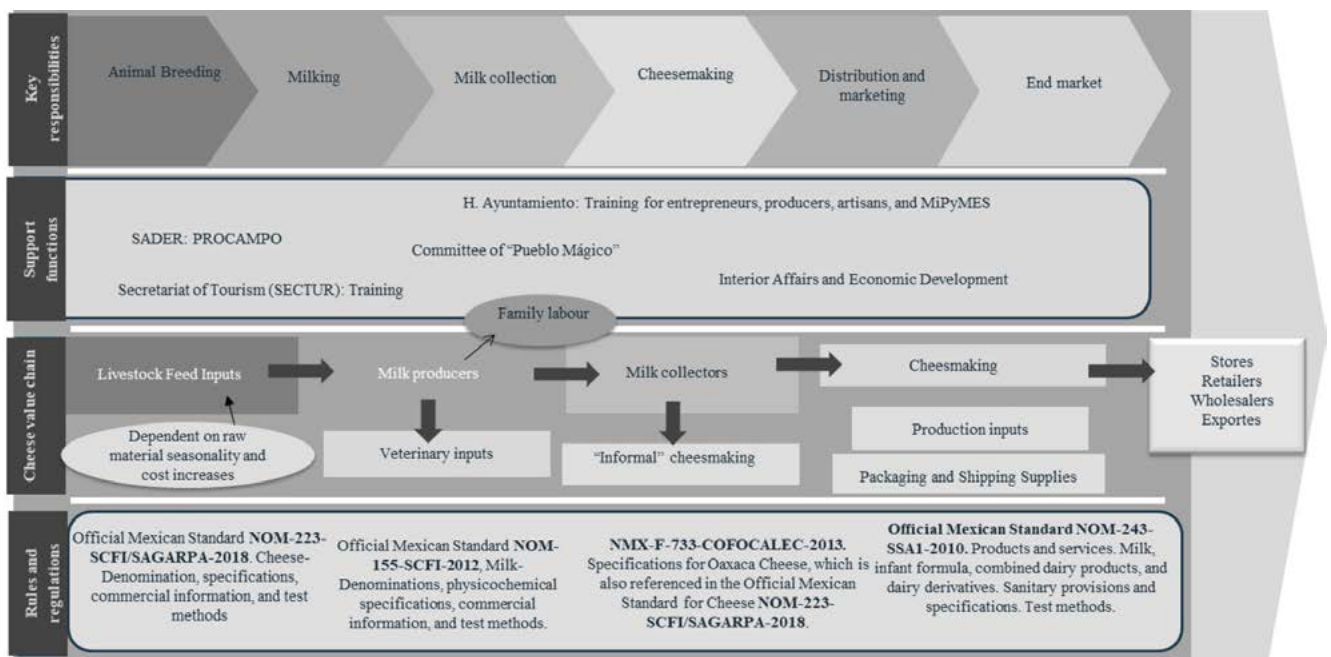


Figure 1. Functional structure and institutional framework of the cheese value chain in Aculco (State of Mexico) Mexico.

Semi-industrial producers have incorporated improvements in sanitary control and production processes; however, in some cases they resort to the use of non-dairy additives to increase the yield of the cheese. Although there are efforts for continuous improvement, technical and management limitations persist, especially affecting exporters, who face greater demands for product quality and traceability. Poor knowledge in Administration management limits access to credits, innovation and training in key areas such as genetics, health and quality. Likewise, the absence of nutritional labels and origin denomination, associated with the requirements of wholesalers, generates market asymmetries and hinders the construction of a territorial identity, which is in turn aggravated by the weak coordination among social actors.

Support actions from the municipality have been punctual and limited in scope. Among those, the Aculco Cheese Festivals are outstanding, since they generated local income increases and tourism promotion. Some producers have created micro-business with a presence on digital social networks; however, the lack of commercial planning reduces their consolidation. At the same time, agrifood tourism initiatives, such as those promoted by “Xplora Aculco”, link cheese production with tourist activities, favoring economic diversification and the territorial visibility of the dairy cheese system.

According to Porter (1985), the value chain aims to optimize production efficiency, increase profitability, improve quality, foster innovation and promote product differentiation. In the case of the Aculco dairy cheese system, the production structure presents significant limitations in the articulation of social actors, and a weak functional integration between the different elements of the chain, which restricts the generation of added value and the competitiveness of the system.

The milk value chain in Aculco is characterized by a marked segmentation between primary production and cheese processing. In the initial stage, small-scale producers predominate, who produce with low levels of technified operation, limited infrastructure for cooling milk and limited administration management capacities. The marketing of milk mainly occurs through local intermediaries, without formal quality control mechanisms or traceability, which prevents the establishment of stable links with processors and limits the possibilities of differentiation in the market. On the other hand, artisanal and semi-industrial processors constitute the link with the greatest potential for value addition. However, this development is restricted by the lack of coordination with primary producers, the lack of common quality standards, and weaknesses in trade promotion strategies. This disconnection between primary and support activities reflects a fragmented performance of the production system.

From the analytical perspective of Crespo *et al.* (2014) and Hidalgo-Milpa *et al.* (2016), the value chain shows a system with a low level of technified operation, plus fragmented economics dynamic between producers, collectors, and processors (García-Villegas *et al.*, 2021). The business infrastructure is mostly composite of productive units located in domestic spaces, without formal administration or financial records, which limits planning, control of costs, and strategic decision-making. Human resources management is mainly based on family or informal labor, without systematic training processes for building technical and administrative capacities. In the technological aspect, equipment is reduced to basic

stainless-steel containers and stations, without incorporating productive innovations that improve efficiency or safety. Likewise, the supply of forage inputs, particularly during the dry season, revealed limited planning and a high dependence on external resources.

Analysis of primary activities reveals structural weaknesses in internal and external logistics. The availability of milk depends on the small number of producers, which limits the volume and quality of the input, while marketing channels are concentrated in local and regional markets, without formal distribution, positioning or loyalty strategies. Marketing actions are incipient and lack systematic records of sales and consumer feedback, making continuous improvement difficult.

Overall, the cheese value chain in Aculco operates in a fragmented manner and with little coordination between actors. Although productive units have the potential to promote local development, the lack of standardization, administrative controls, and differentiation strategies restricts their insertion in broader markets. As Durán-Rojas (2020) stated, greater articulation between actors is key to improving competitiveness. However, in contexts such as Aculco, this integration is limited by the lack of an associative culture that allows taking advantage of synergies, economies of scale, and strengthening the sustainability of the production system.

The intervention model for the development of value chains (Figure 2) proposes a comprehensive scheme aimed at strengthening the technical, organizational and commercial capacities of small cheese producers in the municipality of Aculco. This model emphasizes the need to implement comprehensive strategies to overcome structural deficiencies and enhance the competitiveness of the production system (Oddone & Padilla, 2017). Through its implementation, output, results, and impact stages, the model seeks to optimize inputs and productive activities in order to generate synergies that strengthen the profitability and sustainability of the system. Training, access to markets, and financing were considered as strategic axes, these understood as enabling conditions for effective inclusion in the value chain. The approach is based on the methodology of value chain analysis and responds to the main challenges identified in the previous diagnosis. Among those are the low technified operation, the weak articulation among social actors, the limited administration management and the scarce differentiation of products (Porter, 1985).

In accordance with ECLAC (CEPAL, 2014), it is recognized that small-scale producers have a high potential for the generation of employment and the economic revitalization of rural areas. This is particularly relevant in the agro-industrial transformation of milk, which can generate between four and 17 jobs for every 100 L processed, depending on the level of technified operation and productive organization. However, in order to this potential be translated into tangible benefits, it is essential to consolidate a structured action plan that allows us to move towards a more efficient, competitive and sustainable production model.

The model articulates four strategic components: inputs, activities, outputs, and results, aimed at generating a final impact on the economic and social well-being of the producing communities. In the Inputs component, the need to guarantee the quality of the raw material is raised through the preparation of budgets for the acquisition of balanced feed,



Figure 2. Intervention model for the value chain of cheese production in Aculco (State of Mexico) Mexico. Photographs: Vianney González-Hernández.

technical training, collaboration with support institutions, and the improvement of facilities and equipment. Likewise, the importance of adequate supplies, and technical expertise in animal health and disease management is emphasized. Since these are elements that contribute to increasing productivity and ensuring the safety of the final product.

The Activities component focuses on applied research and milk production under quality standards, as well as on the production of cheeses differentiated by flavor and texture. This section seeks to optimize production and management processes, promoting a more efficient use of resources and greater coordination between the agents of the chain. The technified operation of processes, accompanied by the continuous training of human resources, is conceived as a central axis to increase the competitiveness of the system.

In the Products component, the model proposes the establishment of analysis tools for the monitoring of the value chain, the strengthening of work force training, and the consolidation of products with greater added value. Differentiation by quality is considered a key element to position local production in regional and national markets. As well as to reinforce a collective image that associates Aculco cheese with high standards of authenticity and quality.

Finally, the Results component translates into tangible improvements in customer satisfaction, the competitiveness of the sector, and the financial performance of small agribusinesses. We considered here the reduction of unfair intermediation practices, the implementation of support policies, and the promotion of regulatory changes favorable to local producers. These results constitute the basis for generating a positive impact on

the rural economy, which would be reflected in better-paid jobs, increased family income, poverty reduction, and strengthening of territorial identity. This improvement would be based on product quality and customer loyalty.

Overall, the proposed intervention model represents a systemic strategy that integrates the technical, economic and social aspects of the cheese value chain in Aculco. The implementation would allow progressing towards a sustainable territorial development model, based on cooperation among producers, institutions, and commercial agents, where quality, innovation and collective organization can be consolidated as the main drivers of competitiveness and resilience of the local agrifood sector.

CONCLUSIONS

The analysis of the cheese value chain in Aculco (State of Mexico) Mexico made it evident that, despite economic, cultural and territorial importance, the production system operates under conditions of organizational fragmentation, low technified operation, and limited coordination among social actors. These characteristics restrict the generation of added value, competitiveness, and sustainability of the local agrifood system.

The strengthening of the organizational, technical and management capacities of small producers, together with the promotion of mechanisms of articulation and territorial cooperation, is essential to move towards a more efficient and resilient production model. In this sense, the value chain approach is consolidated as a strategic tool to guide comprehensive interventions that contribute to the sustainable territorial development of the dairy cheese sector in the municipality of Aculco.

ACKNOWLEDGMENTS

To the cheese producers of the municipality of Aculco, State of Mexico for their active participation. To the Autonomous University of the State of Mexico, through the UAEMéx 7209/2022CIB research project. To the Secretariat of Science, Humanities, Technology, and Innovation – SECIHTI, for the scholarship awarded for the doctoral studies of Vianey González Hernández.

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Nutritional Diagnosis in Oil Palm Using UAV-Based Multispectral Imagery: A Global Bibliometric Analysis, 2005-2024

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ABSTRACT

This study examines trends in research productivity, thematic evolution, and knowledge gaps regarding the use of unmanned aerial vehicles (UAVs) equipped with multispectral sensors for nutrient diagnosis in oil palm plantations. A bibliometric analysis was conducted on publications indexed in Web of Science and Scopus over the 2005-2024 period, following four phases: 1) a qualitative exploratory review of 50 scientific articles to identify search terms; 2) a comprehensive systematic search using 83 search equations, without restrictions on language, document type, or research area; 3) temporal delimitation through frequency analysis; and 4) cross-validation through thematic relevance mapping. The extracted data included title, authors, keywords, abstract, publication year, citations, and country.

The bibliometric analysis reveals a substantial knowledge gap in the application of multispectral UAV technology for estimating nutrient concentrations in oil palm. Despite the exponential growth of UAV research during 2017-2024 and plant nutrition studies during 2005-2024, the intersection of these fields in oil palm remains markedly underdeveloped, with only five publications identified. Research is concentrated in Malaysia (30%), consistent with its status as the world's leading producer. This gap constitutes both a challenge and a promising opportunity for future research, particularly in the development of standardized protocols for non-destructive nutrient diagnosis that could help address production stagnation in plantations.

Keywords: bibliometric, UAV, Oil palm, nutrient.

Citation: Velázquez-de la Cruz, J. A., Rincón-Ramírez, J. A., Aceves-Navarro, L. A., Rodríguez-Ortiz, G., Obrador-Olan, J. J., Juárez-López, J. F. (2026). Nutritional Diagnosis in Oil Palm Using UAV-Based Multispectral Imagery: A Global Bibliometric Analysis, 2005-2024. *Agro Productividad*. <https://doi.org/10.32854/1j9pc56>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: January 23, 2026.

Accepted: April 18, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 201-211.

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INTRODUCTION

Agricultural crop productivity is multifactorial, with nutritional status representing one of the most decisive factors in the expression of the genetic potential of crops (Marschner and Marschner, 2012). This factor accounts for up to 60% of yield variation when elements such as water and solar radiation are not limiting (Fageria *et al.*, 2011). This premise is

grounded in the Law of the Minimum established by Justus von Liebig in 1840, which states that plant growth and development are constrained by the scarcest nutrient, even when all other nutrients are present at optimal levels (Van Der Ploeg *et al.*, 1999). Therefore, timely nutritional diagnosis constitutes a strategic component of agronomic management, particularly in long-cycle perennial crops, where deficiencies may affect yield up to 24 months later (Rhebergen *et al.*, 2019).

Historically, the diagnosis of crop nutritional status has relied on destructive plant tissue sampling for laboratory analysis. Because this approach represents only a minute fraction of the total biomass, it introduces estimation errors that become magnified in large-scale plantations. Consequently, fertilizers are often applied uniformly across spatially variable fields, resulting simultaneously in overfertilized and nutrient-deficient zones, with adverse economic and environmental consequences (Robertson *et al.*, 2013).

In the specific case of oil palm, these limitations of conventional diagnosis partially explain the lower productive efficiency observed in cultivated plantations. Although this crop can attain yields of 10 tons of crude palm oil per hectare per year under optimal conditions (Barcelos *et al.*, 2015; Corley and Tinker, 2016), the global average remains stagnant at $2.8 \text{ t ha}^{-1} \text{ year}^{-1}$. This yield gap has driven global expansion of cultivated area at an average rate of one million hectares per year (Food and Agriculture Organization of the United Nations [FAO], 2024).

In response to the inherent limitations of destructive leaf sampling, remote sensing using unmanned aerial vehicles (UAVs) equipped with multispectral sensors has emerged as a technological alternative for non-destructive nutritional diagnosis. UAVs provide spatial information at centimetric resolution and temporal flexibility, attributes that are particularly relevant in tropical agricultural systems, where plantation scale, soil heterogeneity, and climatic conditions hinder conventional monitoring based on point sampling (Matese *et al.*, 2015).

In tropical contexts, this capability becomes especially important because perennial crops such as oil palm exhibit dense, stratified canopies, continuous phenological cycles, and nutritional variability that demand diagnostic tools with high spatial resolution. Likewise, these systems require temporal frequencies capable of adapting to the narrow operational windows imposed by persistent cloud cover.

Recent scientific literature documents successful applications of multispectral UAVs for nutritional diagnosis in diverse crops, including nitrogen in rice (*Oryza sativa* L.) (Yang *et al.*, 2025), phosphorus and potassium in apple (*Malus domestica* Borkh.) (Sun *et al.*, 2023), and multiple nutrients in maize (*Zea mays* L.) (Cheng *et al.*, 2024; Han *et al.*, 2022). Nevertheless, most of these advances have been developed in temperate agroecosystems with low-stature annual crops, which limits their direct extrapolation to tropical perennial plantations.

Despite the exponential growth of this field, no comprehensive bibliometric analysis has yet systematically synthesized trends in scientific productivity and the critical knowledge gaps that constrain technological transfer toward large-scale commercial adoption in the tropics. Previous reviews, such as those by Mulla (2013) and Khanal *et al.* (2020), focused on satellite remote sensing and general UAV applications in agriculture; however, none has consolidated, through bibliometric analysis, the specific body of knowledge concerning

nutritional diagnosis with multispectral sensors in tropical perennial crops. This lack of synthesis is further exacerbated by the difficulty of determining precisely when UAVs began to be applied to agricultural nutritional assessment. Such uncertainty complicates the temporal delimitation of a field that has evolved in a fragmented manner across disciplines such as tropical agronomy, remote sensing, and data science.

Given the need to consolidate the dispersed scientific knowledge on the use of UAVs equipped with multispectral sensors for agricultural nutritional diagnosis, and considering the absence of a precise record of their origin in agriculture, particularly in oil palm, it is methodologically necessary to adopt a longitudinal approach. This perspective will make it possible to capture not only the emergence of these technologies, but also the evolution of the nutritional monitoring methods that preceded them. Within this framework, the following research question arises: What are the trends in scientific productivity, thematic evolution, consolidated methodological protocols, and critical knowledge gaps in the use of UAVs equipped with multispectral sensors for nutritional diagnosis in agricultural crops? This bibliometric analysis will enable the systematic identification of the knowledge gaps that should be addressed through future research.

MATERIALS AND METHODS

An exploratory-descriptive bibliometric design was employed to examine the scientific literature using the Web of Science (WoS) and Scopus databases, both widely recognized for their exploratory capacity and rigorous scientific indexing (Singh *et al.*, 2021). The study was structured into five phases.

In phase one, the search terms were identified through an exploratory analysis of 50 scientific articles related to nutritional diagnosis in oil palm, agricultural remote sensing, and UAV applications. From each article, author keywords and frequently occurring terms in titles and abstracts were extracted. These terms were subsequently grouped into categories. In phase two, a comprehensive stepwise search strategy was designed at three levels: (i) simple searches by individual term, (ii) binary combinations across categories, and (iii) the complete integration of the three groups. This combinatorial approach generated 83 search equations (11 simple, 36 binary, and 36 triple), adapted to the syntax of each database. No restrictions were applied regarding language, document type, or subject area in order to maximize comprehensiveness. The following data were extracted from each record: title, authors, author keywords, full abstract, publication year, citation count, and country. In phase three, temporal distribution was analyzed through annual frequency series and interannual growth rates, with the aim of identifying inflection points associated with technological milestones and defining the optimal temporal range for detailed analysis. The selection criterion was established as the interval encompassing at least 75% of the accumulated productivity. Likewise, citation count was used as an impact indicator to identify seminal publications. In phase four, thematic filtering was applied to the publications included within the previously delimited optimal temporal range, based on three simultaneous inclusion criteria: (i) the study specifically addressed oil palm, (ii) its primary focus was nutritional diagnosis or deficiency identification, and (iii) it employed multispectral imagery acquired through UAVs. Similarly, studies on other crops without

direct comparison to oil palm, investigations lacking a nutritional component, studies based exclusively on satellite imagery, duplicate records, and documents without an available abstract were excluded. In phase five, descriptive analysis and keyword co-occurrence network analysis were conducted using VOSviewer v. 1.6.20 (van Eck & Waltman, 2010), establishing a minimum threshold of two occurrences.

RESULTS AND DISCUSSION

Critical Elements of the Research

The analysis made it possible to identify 11 terms associated with the conceptual axes of the study. These terms were classified into three thematic categories, namely: A) target crop, B) nutritional diagnosis, and C) UAV-based remote sensing (Table 1). These terms constituted the foundation of the investigation and will hereafter be referred to as critical elements.

Thematic Context

The comparative analysis between the WoS and Scopus databases revealed substantial differences in the volume of indexed publications. WoS registered a total of 979,773 records, exceeding Scopus (880,641 articles) by 11%. Nevertheless, both databases exhibited exponential growth in the number of publications over the analyzed period. The distribution across thematic categories revealed a marked asymmetry in scientific output (Table 1). Category B (nutritional aspects) accounted for 78% of the total publications, with 766,634 articles in Scopus and 589,702 in WoS. In contrast, Category A (crop) represented less than 1%. This seemingly anomalous disproportion is interpreted as a consequence of the broad semantic scope of the term “nutrient.”

Likewise, within each category, a concentration around specific terms was observed, which functioned as thematic cores. Term A1 (oil palm) accounted for 84% of the publications within Category A, whereas B2 (nutrient) represented virtually the entirety of Category B. In turn, within Category C, the term UAV accounted for 37% of the publications in that category (Table 1).

The combination of two categories makes it possible to identify consolidated lines of research. The A1-B2 intersection yielded 904 publications in Scopus and 970 in WoS, representing the most robust research line focused on crop nutrition. The second significant intersection occurred between B2-C1, with 383 and 321 publications, respectively,

Table 1. Search terms grouped into three thematic categories.

C –UAV-Based Remote Sensing	B-Nutritional Diagnosis	A-Crop	
UAV	Nutrient	Oil palm	1
Drone	Nutrient	African oil palm	2
Dron		<i>Elaeis guineensis</i>	3
RPA			4
Unnamed vehicle aerial			5
RPAS			6

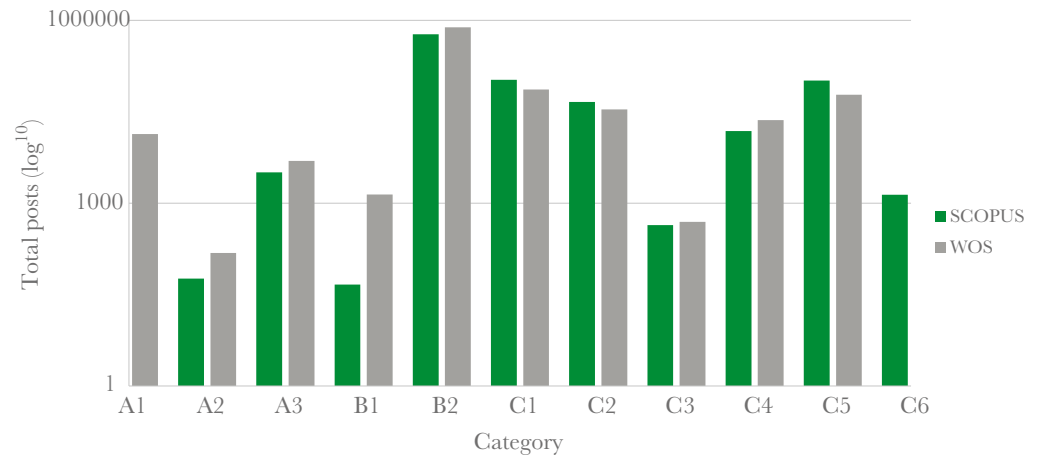


Figure 1. Number of publications of the critical elements on a logarithmic scale. Category B stands out prominently in both databases.

indicating steady growth in this line of research. In turn, the B2-C5 combination also showed notable relevance, with 376 articles in Scopus and 259 in WoS, suggesting that the term UAV appears more frequently in the literature than its full designation (Figure 2).

The intersection of the three categories (Figure 3) reveals an extremely limited, yet meaningful, landscape. The principal line, A1-B2-C1, accounts for five articles in total, whereas A1-B2-C5 yielded only three articles, and the A3-B2-C5 intersection resulted in merely three publications (Figure 3). This extreme scarcity of studies simultaneously integrating crop-related, nutritional, and technological dimensions represents less than 0.001% of the total volume analyzed.

Commercial Development of oil palm and scientific research

The establishment of oil palm as a commercial plantation crop can be situated within the period from 1907 to 1950. During this interval, oil palm evolved from a system of

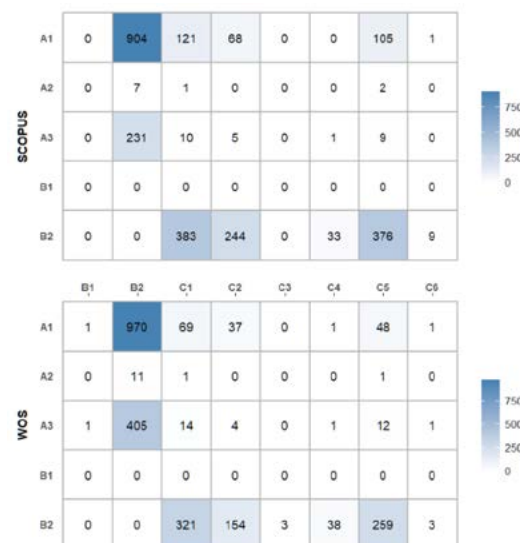


Figure 2. Diagram of the binary combination of categories.

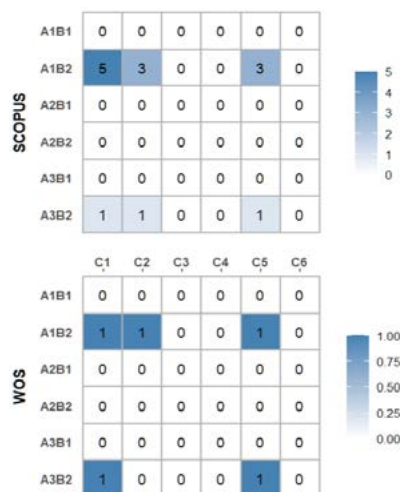


Figure 3. Diagram of the intersection of the three categories.

wild harvesting in Nigeria (1907) (Sarbah, 1909) to organized plantation systems, first in Indonesia and the Democratic Republic of the Congo (1911), followed by Malaysia in 1917 (Din, 2019). This expansion was substantial and became firmly consolidated during the 1940s, when Nigeria, Indonesia, the Democratic Republic of the Congo, and Malaysia led global production. However, intensification under monoculture created favorable conditions for the emergence of phytosanitary problems, as reflected in the entomological nature of the earliest scientific publications. Among these were the description of nematodes by Yampolsky (1924), the incidence of the fungus *Fusarium oxysporum* in African plantations (Wardlaw, 1946), the beetle *Cephalolia elaeidis* described by Maulik (1920), and the moth *Pimelephila ghesquierei* reported by Tams (1930). The predominance of pest-related studies indicates that biological challenges during the early decades of commercial cultivation largely determined the initial research priorities. Consequently, studies on plant nutrition gained relevance toward the end of the 1940s with the work of Chapman and Gray (1949). During this period, the development of the Beckman DU spectrophotometer by Arnold Beckman proved decisive for the systematic nutritional monitoring of commercial plantations, as it significantly reduced analysis time. Research was concentrated in three principal geographic centers —Malaysia, the United Kingdom, and the United States— which collectively produced 29 publications, a figure that contrasts sharply with the rapid pace of commercial plantation expansion.

Nevertheless, the most accelerated period of oil palm establishment occurred from 1950 onward, when the crop expanded to 43 territories, covering an estimated 3.4 million hectares worldwide (Food and Agriculture Organization of the United Nations [FAO], 2024), with Nigeria, Malaysia, the Democratic Republic of the Congo, and Guinea as the main producers. Once plantations were established, the field entered a transition toward a period of research maturity, evidenced by the diversity of topics aimed at intensifying production (Table 2). These included foundational studies on oil palm growth (Corley *et al.*, 1971), germination protocols (Rees, 1962), optimization of planting densities (Corley, 1973), pathological characterization of economically important species (Turner, 1965;

Sankaran, 1970), and analyses of fruit yield (Hardon *et al.*, 1969). Other studies addressed nutritional aspects (Munsell *et al.*, 1950) and the digestibility of palm oil for human consumption (Iwatsuru and Nakamura, 1950).

More specifically, research on crop nutrition increased substantially in two stages. In the 1950s, macronutrients were of primary importance, with particular emphasis on potassium. Subsequently, in the 1970s, attention shifted toward the identification of boron as a critical micronutrient. Scientific output was led by Malaysia, the Netherlands, Nigeria, the United Kingdom, the Congo, Scotland, and the United States.

The definitive consolidation of oil palm research took place between 1975 and 2000, with a total of 970 publications, averaging 39 articles per year. During this period, research emphasized the diverse applications of oil palm residues, including the use of natural fibers for composite materials (Sreekala *et al.*, 1997; Wollerdorfer and Bader, 1998), wood utilization (Zaini *et al.*, 1996), food applications and effects on human health (Torstensen *et al.*, 2000), as well as charcoal production from residues (Guo and Lua, 2000). In parallel, mathematical models were developed to describe growth (Goudriaan and Monteith, 1990) and pollination processes (Syed, 1979).

In contrast, research on nutrition declined during this period. The countries generating the highest scientific output were Malaysia, the United Kingdom, France, Nigeria, the United States, England, and Côte d’Ivoire. By that time, the estimated global oil palm area had reached 10.3 million hectares distributed across 44 territories, among which Malaysia, Nigeria, and Indonesia stood out prominently.

Finally, the most significant period recorded an average of 589 articles per year and accounted for more than 75% of total scientific output. This stage began specifically in 2005, showing a direct correlation between the number of articles and harvested area, which suggests that research output was driven by the expansion of cultivated land. The comparative analysis further showed that Scopus contained 7% more articles than WoS,

Table 2. Literature frequency by critical element, elements A1, B2, C1 represent the highest frequency.

	Rank		A1	A2	A3	B1	B2	C1	C2	C3	C4	C5	C6	TOTAL
	SCOPUS	2000	2025	15.90	0.05	2.91	0.04	502.82	105.04	44.72	0.33	12.04	102.18	1.34
1975		2000	0.75	0.00	0.25	0.00	79.28	0.42	0.75	0.10	2.89	0.32	0.04	84.80
1950		1975	0.12	0.00	0.03	0.00	7.12	0.00	0.19	0.00	0.35	0.00	0.00	7.81
1925		1950	0.02	0.00	0.00	0.00	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.44
1900		1925	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.07
Total		20 166			589 748		270 727							
WOS	Rank		A1	A2	A3	B1	B2	C1	C2	C3	C4	C5	C6	TOTAL
	2000	2025	12.46	0.15	4.16	1.37	63.60	73.21	33.31	0.31	17.18	60.14	1.18	267.07
	1975	2000	0.99	0.00	0.67	0.00	113.89	0.30	0.99	164.00	5.62	0.00	0.04	286.50
	1950	1975	0.10	0.00	0.10	0.00	13.90	0.00	0.16	0.00	0.36	0.00	0.00	14.62
	1925	1950	0.01	0.00	0.00	0.00	2.76	0.00	0.00	0.00	0.00	0.00	0.00	2.77
	1900	1925	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.14
Total		18 663			768 016		193 094							

*Thousands of items. The descriptions are related to Table 1.

The exhaustive search conducted across the two databases revealed significant disparities in the retrieval of specialized information, as the specific search equation UAV nutrient oil palm yielded only one article in WoS, compared with five documents in Scopus. This quantitative difference suggests variations in the indexing algorithms and thematic coverage of each platform, particularly in research integrating multiple technological disciplines, which is consistent with the findings of Mongeon and Paul-Hus (2016) regarding asymmetries in coverage across bibliographic databases. The five documents identified in Scopus comprise three scientific articles, one conference paper, and one conference review, suggesting that this research line remains in an emergent stage and is still undergoing methodological consolidation, where scientific output has not yet reached the critical mass characteristic of mature disciplines (Donthu *et al.*, 2021). Consequently, the simultaneous consultation of multiple databases constitutes an indispensable strategy to ensure the comprehensiveness of reviews in interdisciplinary fields with incipient scientific production (MacDonald *et al.*, 2024).

The results reveal high variability despite the use of spectral imagery acquired by remotely piloted aircraft systems (RPAS). This variability may be explained by the application of different data analysis techniques; moreover, it should be considered that this technology is relatively recent in comparison with agronomic concepts that have been established for more than a century. Uktoro *et al.* (2024), through image classification, obtained a very low coefficient of determination ($R^2=0.413$) for the estimation of nitrogen, phosphorus, and potassium. Santoro (2024) reported correlation coefficients of 0.81 for nitrogen and phosphorus, and 0.90 for potassium, magnesium, and calcium, using vegetation indices; however, boron estimation showed important limitations, with an R^2 of 0.26. Likewise, Amirruddin *et al.* (2022) achieved an R^2 of 0.77 for nitrogen estimation by implementing synthetic minority oversampling and machine learning.

CONCLUSION

Therefore, it is concluded that the technological convergence analyzed is currently in a transitional stage, moving from academic theory toward technical validation under field conditions. It is therefore imperative to promote research aimed at standardizing the use of multispectral sensors in perennial crops, thereby enabling the closure of the gap between the data acquisition capacity of UAVs and the precise physiological interpretation of the nutritional status of oil palm.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Secretariat of Science, Humanities, Technology, and Innovation (SECIHTI) for the support provided through the 2023-2 national scholarship program for graduate studies, as well as the Colegio de Postgraduados, Tabasco Campus.






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Feeding strategies in lactating hair sheep: Effect of bypass fat on the resumption of postpartum ovarian activity

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ABSTRACT

Objective: To evaluate the effect of bypass fat during the postpartum period in Pelibuey ewes synchronized with an intravaginal progesterone-releasing device (CIDR).

Design/methodology/approach: Seventy-six Pelibuey sheep suckling were used during the postpartum period, with an average body condition score of 3. The ewes were randomly assigned to one of four treatments: T1 = cs (continuous suckling; n = 19), T2 = CS (controlled suckling; n = 19), T3 = cs + bypass fat (ac + FAT; n = 19), and T4 = CS + bypass fat (AC + FAT; n = 19). The experimental design was completely randomized with a 2 × 2 factorial arrangement. There were two factors: factor A was suckling, with two respective levels: 1, continuous (c); 2, controlled (C); while factor B was bypass fat supplementation, grouped into two levels: 1, no bypass fat in the diet; 2, bypass fat in the diet. The SAS statistical package (2009) was used for data analysis, performing logistic regression tests (PROC LOGISTIC), Poisson distribution (PROC GENMOD), and survival analysis (PROC LIFEREG) according to each variable.

Results: bypass fat and type of lactation had no effect on the variables of estrus manifestation, time to estrus, return to estrus, pregnancy rate, and birth rate ($p > 0.05$). However, statistical differences ($p < 0.05$) were found in the variables of embryonic loss ($p = 0.0005$), prolificacy ($p = 0.04$), and fertility ($p = 0.03$).

Limitations on study/implications: The results were obtained with a limited number of sheep in each of the treatments.

Findings/conclusions: The interaction of controlled lactation and supplementation with bypass fat is an efficient and cost-effective management strategy that improves reproductive efficiency during the postpartum period by reducing the interval between calving and first ovulation.

Keywords: bypass fat, suckling, postpartum.

Citation: Robles-Morales, A., Hernández-Mendo, O., Sánchez-Del Real, C., Cortez-Romero, C., & Gallegos-Sánchez, J. (2026). Feeding strategies in lactating hair sheep: Effect of bypass fat on the resumption of postpartum ovarian activity. *Agro Productividad*. <https://doi.org/10.32854/5ndgsb83>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: February 9, 2026.

Accepted: April 23, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 213-221.

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INTRODUCTION

The nutritional status of animals directly influences reproductive activity (Rodríguez *et al.*, 2021), as it can stimulate early onset of puberty, promote cyclicity, and increase ovulation



rates (Halac, 2016). In any animal production system, diets must ensure the inclusion of essential nutrients such as energy, proteins, vitamins, and minerals, as these are necessary for various vital functions in each individual (Campos, 2015). At the physiological level, changes in body condition affect gonadotropin secretion (LH/FSH; Galina *et al.*, 2021).

Reproductive activity is associated with energy availability, which depends on the integration between energy metabolism and the hypothalamic-pituitary-ovarian axis (Alomar, 2012). Forage-based diets implemented in different production systems are often deficient and do not meet nutritional requirements, which is why complementary energy sources have been added (Leal-Isidro, 2020).

As an energy source and due to its structural characteristics, bypass fat is not degraded in the rumen and can be utilised in the small intestine. It is therefore widely used in ruminant diets, as it benefits energy balance, reduces metabolic problems, stimulates milk production and, in terms of reproductive parameters, promotes follicular development, ovulation rate and embryo implantation. In addition, there have been cases in which it increases the conception rate (Osorio-Marín, 2021). Therefore, the objective of this study was to evaluate the effect of including bypass fat to the diet of Pelibuey ewes synchronized with a CIDR during the postpartum period, assessing the following reproductive variables (estrus manifestation, time to estrus, return to estrus, pregnancy rate, embryonic/fetal loss, lambing rate, prolificacy, and fecundity).

The objective of this study was to evaluate the effect in the diet of Pelibuey sheep during the postpartum period to measure the recovery of ovarian activity (oestrus manifestation, oestrus duration, return to oestrus, pregnancy rate, embryonic/foetal loss, lambing rate, prolificacy and fertility).

MATERIALS AND METHODS

Study site

Animal management was conducted in accordance with animal welfare regulations, in line with protocol COBIAN/020/25 and the Regulations for the Use and Care of Animals for Research at the Colegio de Postgraduados.

The experimental period took place from March to November 2024 at the Sheep and Goat Reproduction Laboratory (LaROCa) at the Montecillo Campus of the Colegio de Postgraduados, located in the municipality of Texcoco in the State of Mexico (19° 27' 51" N, 98° 54' 34" W, at an altitude of 2,250 m above sea level).

The study included 76 lactating hair sheep during the postpartum period, with an average weight of 55 ± 8 kg and an average body condition score of 3.0 on a scale of 1 to 5, where 1 indicates a very thin ewe and 5 a very fat ewe. The sheep were randomly assigned to one of four treatments at the time of lambing. A completely randomized experimental design with a 2×2 factorial arrangement was used, where the study factors were: A: type of suckling, grouped into two levels; 1, continuous suckling (c; the lambs remained with their mothers 24 hours a day); 2, controlled suckling (C; the lambs were nursed in two 30-minute periods per day, the first at 8:00 a.m. and the second at 4:00 p.m.; after suckling, the lambs were separated from their mothers) and B) bypass fat supplementation in the diet, with two levels: 1) without bypass fat and 2) with bypass fat, resulting in four

treatments: T1=continuous suckling (cs), T2=controlled suckling (CS), T3=cs with bypass fat (cs+FAT), and T4=CS with bypass fat (CS+FAT), with 19 sheep per treatment.

Before the experiment began, the facilities were thoroughly inspected, and a preventive sanitation protocol was implemented. The ewes were dewormed and had their hooves trimmed. They also received a single 1.90 mg kg⁻¹ dose of vitamin concentrates (Vigantol[®], Elanco) to prevent deficiencies in vitamins A, D and E.

At birth, the lambs received care, including navel disinfection and the application of a healing, disinfectant aerosol spray (Aluspray[®], Vétoquinol). They also received an intramuscular injection of 0.02 mg kg⁻¹ of Vigantol[®] and 0.3 mL of selenium (Mu-Se[®], Intervet), as well as an oral dose of Nutri-Drench[®] to strengthen their immune systems.

The ewes received a total mixed ration balanced according to NRC requirements (2007; Tables 3 and 4) and had free access to water prior to the initiation of the synchronization protocol. In treatments supplemented with bypass fat, a modified diet containing 2.5% bypass fat was offered for nine days, starting simultaneously with the synchronization protocol (Tables 1 and 2). The protocol began at 45 days postpartum with the insertion of an intravaginal device (CIDR; 0.3 g progesterone). Seven days later, 1.5 mL of PGF₂α (Lutalyse[®]) and 300 IU of eCG (Novormon[®]) were administered intramuscularly. Two days later, the CIDR was removed and estrus detection began every four hours for 72 consecutive hours using teaser males fitted with aprons to prevent mating.

Pregnancy diagnosis was performed transrectally at 37 and 50 days after mating using a portable ultrasound device (UMS900 Neo) with a ventral transducer (Figure 1).

The variables evaluated were estrus expression, time to estrus, return to estrus, embryonic/fetal loss, pregnancy rate, lambing rate, prolificacy, and fecundity. Data was analyzed using SAS statistical software version 9.0. Binomial variables (estrus expression, return to estrus, pregnancy, and lambing) were analyzed using logistic regression (PROC LOGISTIC), while numerical variables (prolificacy and fecundity) were adjusted to a Poisson distribution using the GENMOD procedure (PROC GENMOD), applying the

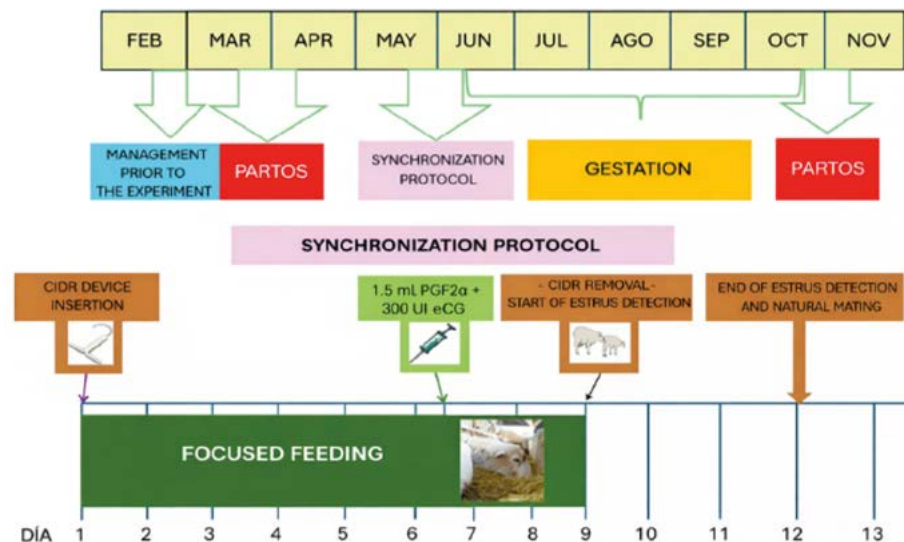


Figure 1. Flow diagram showing the experimental timeline.

Table 1. Diet offered to animals during the experimental period, adjusting the amount of bypass fat (FAT) for cs+FAT and CS+FAT treatments.

Ingredient	Fat-supplemented	Non-fat-supplemented
Ground maize	25.20	34.00
Oat straw	23.00	17.30
Chopped alfalfa hay	20.00	20.00
Wheat bran	10.00	10.00
Molasses	7.00	7.00
Soybean meal	5.60	5.50
Corn gluten meal	4.10	3.50
Protected vegetable fat	2.50	0.00
Mineral-vitamin premix (sheep)	2.00	2.00
Sodium chloride	0.50	0.50
Calcium carbonate	0.10	0.20

Table 2. Nutritional composition of diets with and without bypass fat (FAT) offered during the synchronization protocol as a feeding strategy.

Nutrient	Treatments	
	With FAT	Without FAT
DM (%)	88.35	87.75
ME (Mcal)	2.50	2.50
CP (%)	14.04	14.02
RDP (%)	9.00	9.00
UDP (%)	5.04	5.02
E.E. (%)	5.68	3.12
CF(%)	17.18	15.04
Ash (%)	8.37	8.13

Table 3. Ingredients of the general diet offered to ewes prior to the synchronization protocol and focused nutrition.

Ingredient	Amount (%)
Oat hay	50.0
Alfalfa hay	35.0
Commercial concentrate (Reproductive sheep: Purina®)	7.0
Molasses	5.0
Mineral premix (Oveja plus)	2.0
Total	100.0

Bootstrap resampling method to improve confidence interval estimation. Time to estrus was analyzed using parametric survival models (PROC LIFEREG), and survival curves were generated using PROC LIFETEST for graphical comparison among treatments. Means were compared using Tukeys test ($p \leq 0.05$).

Table 4. Nutritional composition of the general diet offered to ewes prior to the synchronization protocol and focused nutrition.

Nutrient	Amount (BTO)
Moisture (H), %	13.81
Dry Matter (DM)	86.19
Ash (C), %	9.29
Organic Matter (OM), %	76.90
Crude Protein (CP), %	11.79
Ether Extract (EE), %	2.73
Crude Fiber (CF), %	16.90
Nitrogen Free Extract (NFE), %	45.48
Total, %	100

RESULTS AND DISCUSSION

After data analysis using the respective statistical methods, results for estrus expression, time to estrus, return to estrus, pregnancy rate, embryonic/fetal losses, lambing rate, prolificacy, and fecundity are presented (Tables 5 and 6).

Estrus expression

No differences were found for estrus expression ($p=0.31$). However, in the CS+FAT treatment, 100% of the ewes expressed estrus. Similar results were reported by Ali (2017) and Urviola *et al.* (2020), who observed 100% estrus expression when synchronization protocols using fluorogestone acetate (8-12 days with 500 IU of eCG) combined with bypass fat supplementation were applied. Bypass fat improves steroid hormone synthesis, particularly estradiol, which is essential for estrus expression. Likewise, the progestogen exerted the expected effect (Morales-Terán *et al.*, 2010). Additionally, controlled suckling directly accelerates the resumption of ovarian activity by temporarily inhibiting suckling stimulus, favoring hormonal recovery of the reproductive axis and increasing pulsatile GnRH secretion (Delgadillo *et al.*, 2008).

Table 5. Results for estrus expression, time to estrus, return to estrus, and pregnancy rate.

Treatment	n	Estrus expression n (%)	Time to estrus (h)	Return to estrus n (%)	Pregnancy rate n (%)
cs	19	16 (84.21)	22.95	3 (15.78)	11 (57.89)
CS	19	18 (94.73)	23.98	4 (21.05)	11 (57.89)
cs+FAT	19	17 (89.47)	24.22	2 (10.52)	13 (68.42)
CS+FAT	19	19 (100.00)	22.40	3 (15.78)	16 (84.21)
Total	76	70 (92.10)	22.40±2.08	12 (15.79)	51 (67.10)

Different letters within rows indicate differences among treatments ($P<0.05$).

cs=continuous suckling, CS=controlled suckling, cs+FAT=continuous suckling+bypass fat, CS+FAT=controlled suckling+bypass fat.

Table 6. Results for lambing, embryonic/fetal losses, prolificacy, and fecundity.

Treatment	N	Lambing n (%)	Embryonic/fetal losses n (%)	Prolificacy	Fecundity
cs	19	11 (57.89)	2 (10.52)	15/11 (1.35)	15/19 (0.78)
CS	19	12 (63.15)	2 (10.52)	19/12 (1.58)	19/19 (1.0)
cs+FAT	19	13 (68.42)	2 (10.52)	24/13 (1.84)	24/19 (1.26)
CS+FAT	19	16 (84.21)	0 (0)	29/16 (1.81)	29/19 (1.52)
Total	76	52 (68.42)	6 (7.89)	87/52 (1.67)	87/76 (1.14)

Different letters within rows indicate differences among treatments ($P < 0.05$).

cs=continuous suckling, CS=controlled suckling, cs+FAT=continuous suckling+bypass fat, CS+FAT=controlled suckling+bypass fat.

Time to estrus

No differences were detected among treatments for time to estrus ($p=0.98$). These results may be attributed to the metabolic status of the ewes, as bypass fat provides high energy availability, favoring follicular maturation and a faster response to eCG stimulation (Zarazaga *et al.*, 2010; Abecia *et al.*, 2012). Moreover, the results suggest that all females were under adequate physiological and environmental conditions, which allowed similar estrus onset times (22.40 ± 2.07 h).

Return to estrus

No differences were observed for return to estrus ($p=0.85$); however, the cs+FAT treatment showed a lower percentage of ewes returning to estrus (10.52%). This indicates that bypass fat inclusion reduces the risk of embryonic loss, as energy imbalances can affect oocyte quality and lead to embryo loss (Meza-Herrera *et al.*, 2011).

Pregnancy rate

No significant differences were observed in pregnancy rate; however, ewes in the CS+FAT treatment showed the highest pregnancy rate (84.21%). Adequate energy availability during the breeding period favors the maintenance of early pregnancy (Jiménez-Pérez *et al.*, 2012). Controlled suckling facilitates ovarian activity recovery by reducing the negative effect of estradiol on pulsatile GnRH secretion. The combination of controlled suckling and bypass fat supplementation resulted in higher pregnancy rates (Vargas and Jaramillo, 2013).

Lambing rate

The average lambing rate was 68.42%, with no differences among treatments ($p=0.33$). Ewes subjected to controlled suckling with bypass fat supplementation numerically showed the highest lambing rate (84.21%). These results may be explained by improved progesterone secretion from the corpus luteum due to fat supplementation, which enhances steroid synthesis by increasing cholesterol availability, a progesterone precursor essential for pregnancy maintenance (Rahbar *et al.*, 2014). Polyunsaturated fatty acids, particularly omega-3 and omega-6, regulate uterine prostaglandin production

and improve maternal-embryonic communication, reducing embryonic/fetal losses (Hidalgo *et al.*, 2020).

Embryonic/fetal losses

Significant differences were observed in embryonic/fetal losses ($p=0.0005$). The CS+FAT treatment showed no losses, indicating that appropriate nutritional and reproductive management ensures fetal survival. Lipids enhance *corpus luteum* function (Hughes *et al.*, 2019). Additionally, bypass fat contains polyunsaturated fatty acids (n^{-6} and n^{-3}), which act as immunomodulatory and anti-inflammatory agents by modifying endometrial cell membrane composition, reducing $PGF_2\alpha$ synthesis, and increasing PGE release (Cheng *et al.*, 2004). Lipid supplementation also alters the expression of genes associated with implantation (IGF1, VEGFA, SPP1, and LIF), facilitating angiogenesis and blastocyst adhesion, thereby reducing embryonic loss (Contreras-Solís *et al.*, 2025).

Prolificacy

Prolificacy differed among treatments ($p=0.04$), with higher values in cs+FAT and CS+FAT treatments (1.81 and 1.84, respectively). These results suggest that suckling management improves physiological response (Akbarinejad and Cushman, 2024) and that bypass fat reduces the negative effects of lactation on reproduction (Toalombo Vargas *et al.*, 2022). Controlled suckling partially improves prolificacy by restoring the hypothalamic-pituitary-ovarian axis (Calik-Ksepka *et al.*, 2022). The combined use of nutritional strategies and suckling management increased prolificacy, indicating its usefulness for improving reproductive efficiency.

Fecundity

Differences were found for fecundity ($p=0.03$), with higher fecundity indices observed in the CS+FAT treatment. Controlled suckling reduces suckling stimulus frequency, allowing ovarian activity recovery and restoration of pulsatile GnRH/LH secretion. Bypass fat supplementation provides energy and cholesterol, a precursor for progesterone and estradiol synthesis. These results contrast with those reported by Muñoz-García *et al.* (2015) possibly due to the inclusion of bypass fat, which may have increased steroid hormone secretion.

CONCLUSIONS

Including bypass fat in the diet and controlled suckling in synchronized hair sheep during the postpartum period reduces embryonic and fetal losses and improves prolificacy and fertility. Including bypass fat and controlled suckling in the diet of synchronized hair sheep during the postpartum period reduces embryonic and fetal losses and improves prolificacy and fertility.

ACKNOWLEDGEMENTS

To the Colegio de Postgraduados, to the Sheep and Goat Reproduction Laboratory (LaROCa) and the LGAC-Technological Innovation and Food Safety in Livestock for funding the project.

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Soil quality and rehabilitation of tepetates using board-ditches

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ABSTRACT

Objective: Physical and chemical properties, as well as microbial respiration, were evaluated during the rainy and dry seasons in a vegetation-free *tepetate* treated with board-ditches and in a relic forest soil, in order to determine soil quality.

Design/methodology/approach: The study areas were: vegetation-free *tepetate*, *tepetate* with board-ditches, and relic forest soil. Within 625 m² of each area, a systematic sampling scheme was established to collect samples during the rainy and dry seasons. Data were analyzed under a randomized block experimental design with a 3×2 factorial arrangement. A Principal Component Analysis (PCA) was performed to determine the degree of association between soil properties and soil types by season.

Results: In tepetates treated with board-ditches, bulk density and electrical conductivity decreased, and a neutral pH was observed, with these effects being more evident during the rainy season. Cation exchange capacity, phosphorus, and inorganic nitrogen increased across all three soil types, particularly in the rainy season. PCA explained 75% of the total variation, accounted for by PC1 and PC2. The biplot graph showed that relic forest soil was associated with organic matter and calcium in both seasons, whereas *tepetate* with board-ditches, in both dry and rainy periods, was associated with the physical component.

Limitations of the study/implications: Physical and chemical properties in rehabilitated *tepetates* may take several years to exhibit significant changes compared to non-intervened sites.

Findings/conclusions: Three years after the establishment of the board-ditches, and together with the presence of herbaceous vegetation and grasses, a decrease in bulk density was observed during the rainy season, favoring increased porosity. Electrical conductivity also decreased, and the neutral pH indicates a reduction in soluble salts. PCA revealed that forest soil, in both seasons, is associated with organic matter and calcium; whereas tepetate with board-ditches, during the dry season, is associated with bulk density and sand content, and during the rainy season with particle density and silt, indicating that soil quality remains low.

Keywords: Soil degradation, soil conservation practices, physicochemical properties, volcanic soil.

Citation: Velasco-Sánchez, A. R., García-Gallegos, E., Cristóbal-Acevedo, D., & Hernández-Acosta, E. (2026). Soil quality and rehabilitation of tepetates using board-ditches. *Agro Productividad*. <https://doi.org/10.32854/g54s7t79>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: February 9, 2026.

Accepted: April 23, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 223-236.

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INTRODUCTION

Soil quality is fundamental to the functioning of terrestrial ecosystems, as it determines the soil's capacity to sustain essential ecological functions and provide ecosystem services

such as biodiversity conservation, biological productivity, water regulation, and nutrient cycling (FAO, 2023). However, the loss of vegetation cover caused by deforestation and forest fires has led to the formation of forest relics and extensive areas of degraded soil, such as *tepetates*, which have largely lost the functions associated with adequate soil quality (SEMARNAT, 2018).

Tepetates are characterized by low porosity, low organic matter content, and high compaction, which hinder root development and water infiltration (Velázquez-Rodríguez *et al.*, 2022). Their rehabilitation is therefore a challenge; however, various actions have been implemented, such as tillage, land leveling, terrace construction, contour furrows, crop rotation, and the incorporation of organic matter and fertilizers, all aimed at improving soil quality (Comisión Nacional Forestal, 2023). Nevertheless, one of the soil conservation practices that has proven successful in their rehabilitation is the use of board-ditches, which enhance moisture retention and thereby promote the establishment of native vegetation. Although they may induce changes in floristic composition, they also help stabilize and protect the bunds from erosion (Reyes Carrillo *et al.*, 2019; García Gallegos *et al.*, 2023). Doria Treviño *et al.* (2022) report that three years after establishing board-ditches as a soil conservation practice, increased water infiltration and higher organic matter content were observed, driven by the establishment of aboveground and root biomass of annual species, thereby improving soil quality. The latter is determined through the analysis of its physical, chemical, and biological properties (Quisimalin *et al.*, 2024), which is essential for assessing its potential for agricultural or forestry use.

In the state of Tlaxcala, 93.7% of the land area is recognized as being affected by erosion to varying degrees, with one of the main causes being soil erosion and the intensive use of soil resources (Alvarado Cardona *et al.*, 2007). Particularly on San Gregorio Hill, within the community of San Diego Metepec, Tlaxcala, significant ecosystem degradation is observed, mainly due to the loss of native vegetation and anthropogenic activities, resulting in the depletion of this resource and the exposure of *tepetates* (García Zepeda & López Corral, 2012). Therefore, alternatives have been sought, such as the construction of board-ditches as soil conservation practices to restore ecosystem functioning in the area. García Gallegos *et al.* (2023) analyzed *tepetate* soils with board-ditches at different sites in the state of Tlaxcala through their physical, chemical, and biological properties, finding that five years after the establishment of these conservation practices, there was a positive impact on organic matter content and bulk density. However, at another site where board-ditches had been established for more than 40 years, no significant changes were observed, with the type of established vegetation being a determining factor.

Based on the above, the following objectives were established: 1) to evaluate, during the rainy and dry seasons, the soil quality of relic forest soil, vegetation-free *tepetate*, and *tepetate* rehabilitated with board-ditches, through the determination of their physical and chemical properties, as well as microbial respiration; and 2) to relate soil variables by area and season using Principal Component Analysis in order to identify the most relevant indicators of soil quality.

The hypothesis proposed is that relic forest soil exhibits more favorable soil properties, whereas *tepetate* with board-ditches will show significant recovery in comparison to vegetation-free *tepetate*. This difference is expected to be more evident during the rainy season due to increased moisture, vegetation establishment, higher organic matter content, and enhanced microbial activity.

MATERIALS AND METHODS

Study area

The study area is located within communal land on San Gregorio Hill, in the community of San Diego Metepec, south of the municipality of Tlaxcala. This site is important for the region and was originally characterized by a valuable forest ecosystem dominated by *Pinus*, *Quercus*, *Juniperus*, and *Cupressus*. However, decades of degradation driven by accelerated erosion, loss of vegetation cover, and land-use change have significantly compromised its capacity to provide ecosystem services. The site is located at 19° 16' 47.443" N and 98° 15' 25.574" W, at an altitude of 2,340 m above sea level. The dominant soil order is Cambisol, a young and weakly developed soil with evident changes in structure, color, and clay content. Land use is primarily rainfed agriculture. Mean annual precipitation ranges from 700 to 800 mm, the climate is temperate subhumid, and the mean annual temperature is 14 °C (INEGI, 2010).

Within the communal area, three sites were selected, each with specific conditions: 1) a *tepetate* area with complete absence of vegetation and the presence of surface stoniness, covering 2,070.02 m²; 2) an area of 1,895.98 m² where, as part of the *tepetate* rehabilitation strategy, board-ditches were established in October 2021. These structures measured 2.64 m in length, 0.68 m in width, and 0.32 m in depth, providing a rainwater capture volume of 0.58 m³ per season. A total of 28 board-ditches were constructed in this area. On each board, *Pinus leiophylla* and *Cupressus lusitanica* were planted, benefiting from increased moisture retention; at the time of sampling, survival exceeded 85%. In addition, herbaceous plants and grasses were observed on the boards and between ditches, predominantly from the families Asteraceae and Poaceae; and (3) a relic forest soil area covering 39,194.70 m², dominated by *Pinus*, *Cupressus*, and *Juniperus*, as well as plant species from the families Asteraceae, Cactaceae, Lamiaceae, Pteridaceae, and Rubiaceae.

Sampling and sample preparation

A systematic sampling approach, according to Schweizer (2011), was used in each study area to collect samples during both the rainy season (October 2024) and the dry season (February 2025). Based on the slope conditions of each area, 100 m² quadrats were established, covering a total surface area of 625 m². Within each quadrat, three simple random samples were collected at a depth of 0-20 cm. Subsequently, five composite samples per quadrat were obtained using the quartering method. In the conservation structures, *tepetate* soil samples were collected directly from the boards. According to the NOM-021-SEMARNAT-2000 (Semarnat, 2002), each sample was air-dried at room temperature in the shade on Kraft paper and subsequently sieved through a 2 mm mesh to obtain a homogeneous particle size.

Soil analyses

Bulk density, particle density, texture, porosity, pH, electrical conductivity, organic matter, calcium, magnesium, potassium, sodium, cation exchange capacity, extractable phosphorus, and inorganic nitrogen were determined for each sample, following the procedures specified in the NOM-021-SEMARNAT-2000 (Semarnat, 2002).

Microbial respiration was determined through closed-system incubation (CO₂) according to Paolini Gómez (2018). Soil samples were placed in 250 mL glass jars at 60% moisture content and incubated for 24 h at 22±2 °C. The released CO₂ was trapped using 0.2 N NaOH. At the end of the incubation period, CO₂ was precipitated with 2% BaCl₂ and titrated with 0.2 N HCl using phenolphthalein as an indicator. Microbial respiration was expressed as mg C-CO₂ kg⁻¹ soil day⁻¹ and calculated using Equation 1.

$$mg\ C - CO_2 = (Vb - Vm) \times 6 \times NHCl$$

where: *Vb*=mL of HCl used for the blank, *Vm*=mL of HCl used for the soil sample, 6=equivalent weight of carbon, NHCl=normality of HCl.

Data analysis

The data were analyzed under a randomized block experimental design with a 3×2 factorial arrangement, where the first factor was soil type (vegetation-free *tepetate*, *tepetate* with board-ditches, and relic forest soil) and the second factor was season (rainy and dry). A total of six treatments with five blocks (replicates) were evaluated. Data were subjected to the Shapiro-Wilk normality test (p>0.05), followed by analysis of variance (ANOVA) and mean comparison using the John Tukey test (p<0.05). The statistical linear model used to analyze the data is presented in Equation 2.

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + C_k + \varepsilon_{ijk}$$

where: *Y_{ijk}*=response variable, *μ*=overall mean, *A_i*=effect of the site factor, *B_j*=effect of the season factor, (*AB*)_{*ij*}=site x season interaction, *C_k*=block effect and *ε_{ijk}*=experimental error.

Regarding microbial respiration data, the normality assumption was not met; therefore, the nonparametric Kruskal-Wallis test (p<0.05) was used. Subsequently, the Dunn test (p<0.05) was applied to identify which specific group pairs showed significant differences.

Finally, Pearson correlation and Principal Component Analysis (PCA) were performed to determine the degree of association between soil properties and sites by season. Prior to analysis, the data for each variable were standardized to ensure that all variables contributed equally regardless of their units. All analyses were performed using the free 2020 version of the statistical software InfoStat (Di Rienzo *et al.*, 2020).

RESULTS

Soil properties

The analysis of variance showed that the soil type factor was significantly different ($p < 0.05$) for most variables, except for silt and potassium. Significant differences were observed for the season factor in clay, sand, magnesium, phosphorus, and nitrogen. The interaction between factors was significant ($p < 0.05$) for magnesium and nitrogen (Table 1).

Among the physical properties, particle density showed the highest value in vegetation-free tepetate during the rainy season (2.62 g cm^{-3}), with significant differences ($p < 0.05$) (Table 2). In contrast, the lowest values corresponded to relic forest soil during the dry season (2.38 g cm^{-3}) compared to vegetation-free *tepetate* and *tepetate* with board-ditches in both seasons. On the other hand, bulk density was significantly higher ($p < 0.05$) in vegetation-free *tepetate* during the rainy season and in *tepetate* with board-ditches in both seasons. According to the NOM-021-SEMARNAT-2000 (Semarnat, 2002), bulk density values for volcanic soils should be lower than 1 g cm^{-3} , which was only observed in relic forest soil during the rainy season. Regarding porosity percentage, significantly higher values ($p < 0.05$) were recorded in relic forest soil during the same season compared to vegetation-free *tepetate* and *tepetate* with board-ditches.

In both vegetation-free *tepetate* and *tepetate* with board-ditches, the sand fraction was predominant, with significant differences ($p < 0.05$) observed during the dry season compared to the rainy season. Regarding clay percentage, statistically significant differences

Table 1. Significance values of the physical and chemical properties in the three soil types during the rainy and dry seasons.

Parameter	Factor A Soil Type	Factor B Period	Interaction A×B
PD (g cm^{-3})	0.0001	0.0294	0.7127
BD (g cm^{-3})	0.0008	0.3608	0.1714
Porosity (%)	0.0065	0.0665	0.0768
Clay (%)	0.0001	0.0001	0.9994
Silt (%)	0.3322	0.3657	0.3582
Sand (%)	0.0388	0.0003	0.3067
pH	0.0001	0.0890	0.4030
EC (dS m^{-1})	0.0001	0.6504	0.8416
OM (%)	0.0003	0.8336	0.6999
CEC [$\text{Cmo}(+) \text{ kg}^{-1}$]	0.0009	0.0649	0.3055
Ca (mg kg^{-1})	0.0001	0.6008	0.5860
Mg (mg kg^{-1})	0.0005	0.0247	0.0093
K (mg kg^{-1})	0.1564	0.2924	0.6201
Na (mg kg^{-1})	0.0001	0.4278	0.7288
P (mg kg^{-1})	0.0001	0.0001	0.5916
Inorg. N (mg kg^{-1})	0.0001	0.0001	0.0001

Table 2. Physical and chemical properties in the three study sites during the rainy and dry seasons.

Parameter	Board ditch <i>Tepetate</i>		Vegetation-free <i>Tepetate</i>		Relic forest soil	
	Rainy	Dry	Rainy	Dry	Rainy	Dry
PD (g cm ⁻³)	2.57 ab	2.49 abc	2.62 a	2.59 ab	2.45 bc	2.38 c
BD (g cm ⁻³)	1.21 a	1.26 a	1.21 a	1.15 ab	0.97 b	1.09 ab
Porosity (%)	53.14 ab	49.31 b	53.76 ab	55.63 ab	60.42 a	54.3 ab
Clay (%)	12.00 ab	6.95 bc	10.07 bc	4.95 c	17.11 a	12.08 ab
Silt (%)	20.36 a	18.00 a	23.49 a	19.6 a	17.85 a	19.6 a
Sand (%)	67.64 ab	75.05 a	66.44 b	75.45 a	65.04 b	68.32 ab
pH	6.73 a	6.48 ab	5.82 c	5.81 c	6.37 ab	6.25 b
EC (dS m ⁻¹)	0.02 b	0.04 b	0.27 a	0.26 a	0.07 b	0.11 ab
OM (%)	1.28 b	1.30 b	0.69 b	1.52 b	8.34 a	6.72 ab
CEC [Cmol(+) kg ⁻¹]	10.01 b	7.78 b	11.68 ab	11.51 ab	18.52 a	13.37 ab
Ca (mg kg ⁻¹)	1232 b	1254.63 b	1234.18 b	1336.15 b	3285.52 a	2677.1 ab
Mg (mg kg ⁻¹)	1702.01 a	1075.87 b	1852.92 a	1933.25 a	1771.35 a	1681.84 a
K (mg kg ⁻¹)	452 a	386.4 a	509.87 a	358.40 a	562.40 a	568.40 a
Na (mg kg ⁻¹)	83.6 a	66.8 ab	61.87 abc	58.8 abc	20.40 bc	19.20 c
P (mg kg ⁻¹)	19.84 b	5.63 c	20.44 b	7.03 c	25.68 a	10.17 c
Inorg. N (mg kg ⁻¹)	0.78 b	0.13 c	2.50 a	0.07 c	0.60 bc	0.35 bc

Different letters within each row indicate significant differences (Tukey, $p < 0.05$). PD=Particle density, BD=Bulk density, EC=Electrical conductivity, OM=Organic matter, CEC=Cation exchange capacity.

($p < 0.05$) were found, with the highest values recorded in relic forest soil during the rainy season. Silt percentage was significant in the three soil types analyzed and in both seasons. Regarding pH, values were classified as moderately acidic (5.1-6.5), according to the NOM-021-SEMARNAT-2000 (Semarnat, 2002), in all soil types analyzed, except for tepetate with board-ditches during the rainy season, where a significantly higher value ($p < 0.05$) classified as neutral (6.6-7.3) was recorded. Electrical conductivity showed significant values ($p < 0.05$) in vegetation-free *tepetate* during both seasons, whereas lower values were observed in tepetate with board-ditches and relic forest soil in both periods. Mexican regulations indicate that values < 1 dS m⁻¹ reflect the absence of effects associated with salt accumulation.

Organic matter in relic forest soil showed a significant value ($p < 0.05$) during the rainy season. According to the NOM-021-SEMARNAT-2000 (Semarnat, 2002), values between 6.1 and 10.9% are classified as medium concentration. In contrast, organic matter content in *tepetate* with board-ditches and vegetation-free *tepetate* was classified as very low ($< 4\%$).

Similarly, relic forest soil during the rainy season showed the highest cation exchange capacity, with significant differences ($p < 0.05$), classified as medium [15-25 cmol(+) kg⁻¹]. In the dry season, cation exchange capacity was classified as low [5-15 cmol(+) kg⁻¹] in relic forest soil, vegetation-free *tepetate*, and *tepetate* with board-ditches.

Regarding nutrient content, calcium showed a significantly higher value ($p < 0.05$) in relic forest soil during the rainy season, being classified as rich (2500-5000 mg kg⁻¹),

whereas in both *tepetate* conditions the concentrations were classified as medium (1000-2500 mg kg⁻¹).

As for magnesium, concentrations in the three soil types and both seasons were classified as very rich (>120 mg kg⁻¹). This nutrient showed significant values ($p < 0.05$) in relic forest soil and vegetation-free *tepetate* during both seasons, except that the significance of *tepetate* with board-ditches was only observed during the dry season.

Potassium was significantly higher ($p < 0.05$) in the three soil types and during both seasons. Concentrations were classified as very rich (>320 mg kg⁻¹), according to the classification proposed by Vázquez (1997).

Regarding sodium, *tepetate* with board-ditches showed a significant value ($p < 0.05$) during the rainy season compared to the dry season, vegetation-free *tepetate*, and relic forest soil. Phosphorus content in relic forest soil during the rainy season was significantly higher ($p < 0.05$). According to the NOM-021-SEMARNAT-2000 (Semarnat, 2002), the value is classified as medium (15-30 mg kg⁻¹), as was also observed in vegetation-free *tepetate* and *tepetate* with board-ditches during the same season. Finally, inorganic nitrogen content was classified as very low (0-10 mg kg⁻¹) in all cases according to Mexican regulations. However, vegetation-free *tepetate* showed a significant value ($p < 0.05$) during the rainy season compared to *tepetate* with board-ditches and relic forest soil in both seasons.

Microbial respiration, determined through C-CO₂ content, did not show statistically significant differences according to the medians ($p < 0.05$). However, the highest values were observed in vegetation-free *tepetate* during the rainy season, followed by relic forest soil and *tepetate* with board-ditches, whereas during the dry season an increase was observed in relic forest soil (Table 3).

Relationship among soil properties

Correlation analysis showed that bulk density was the variable with the highest number of significant and negative interactions, particularly with cation exchange capacity ($r = -0.97$), porosity ($r = -0.92$), organic matter ($r = -0.92$), and calcium ($r = -0.94$). Porosity was positively correlated with cation exchange capacity ($r = 0.95$). Sand content showed a significant negative correlation with phosphorus content ($r = -0.90$). Soil pH was negatively correlated with electrical conductivity ($r = -0.98$). Organic matter was negatively correlated with sodium content ($r = -0.93$), which in turn was also negatively correlated with calcium ($r = -0.93$).

Table 3. Median values of microbial respiration in the three study sites by season.

Sites	Period	Microbial respiration (mg C-CO ₂ kg ⁻¹ soil day ⁻¹)
Board dith Tepetate	Rainy	7.3
Vegetation-free Tepetate		14.6
Relic forest soil		11
Board dith Tepetate	Dry	11
Vegetation-free Tepetate		11
Relic forest soil		14.6

According to the Principal Component Analysis (PCA), the different variables were grouped into three components, all with eigenvalues >1 , which together explained 75% of the total system variability. PC1 accounted for 47% of the variability, whereas PC2 explained 27% (Table 4).

PC1 was explained by the positive loadings of calcium, organic matter, cation exchange capacity, and clay content, indicating that higher PC1 values are associated with increased nutrient availability and clay content. In contrast, the negative loading of bulk density indicates an inverse relationship. This component was associated with the organic and physical fractions of the soil. PC2 was explained by the positive loadings of silt, magnesium, inorganic nitrogen, electrical conductivity, and particle density, as well as by the negative loading of microbial respiration. This indicates that as this component increases, microbial respiration decreases, highlighting the importance of the physical and nutrient related components in relation to biological activity.

The biplot graph shows the variables associated with each soil type by season. In the case of relic forest soil during the rainy and dry seasons, similar characteristics were shared in terms of organic matter and calcium content. In contrast, during the dry season, tepetate rehabilitated with board-ditches was directly associated with bulk density and

Table 4. Proportion of variance explained by each principal component.

Component	Value	% Total variance	% Cumulative variance
1	8.07	0.47	0.47
2	4.61	0.27	0.75
Loadings			
Parameter	CP1	CP2	
Particle density	-0.21	0.34	
Bulk density	-0.33	0.01	
Porosity	0.29	0.15	
Clay	0.31	0.01	
Silt	-0.09	0.39	
Sand	-0.26	-0.18	
pH	0.05	-0.28	
Electrical conductivity	-0.07	0.34	
Organic matter	0.33	-0.14	
Calcium	0.34	-0.12	
Magnesium	0.13	0.37	
Potassium	0.30	0.05	
Sodium	-0.29	0.10	
Cation exchange capacity	0.33	0.08	
Phosphorus	0.22	0.23	
Inorganic nitrogen	-0.001	0.36	
Microbial respiration	-0.07	-0.32	

sand percentage, whereas during the rainy season it showed an association with particle density and silt content (Figure 1).

Soil properties

Volcanic soils exhibit physical characteristics that promote adequate structural stability and considerable resistance to degradation. However, these characteristics are affected by anthropogenic activities, as is the case for soil particle and bulk density. The particle density values obtained in both relic forest soil and *tepetates* were lower than the 2.65 g cm^{-3} established by the FAO (2025), indicating the absence of highly dense materials (Tolimir *et al.*, 2020). On the other hand, bulk density values are associated with high organic matter content, mainly in relic forest soil during both seasons (Semarnat, 2002). Pérez Hernández *et al.* (2023) reported that in conserved temperate soils with pine-oak forest for 50 years, bulk density was 0.32 g cm^{-3} , whereas in recently disturbed soils, where all vegetation had been removed, an increase in bulk density (0.62 g cm^{-3}) was observed. Therefore, this property may increase in the short term if soil conservation practices are not implemented.

Bulk density values in the *tepetates* under study were lower than those reported by Gama-Castro *et al.* (2007), who found values ranging from 1.70 to 1.96 g cm^{-3} for hardened materials. This influenced porosity, which according to Kaurichev (1984), cited by Jaramillo (2002), is classified as satisfactory (50–55%) in the soil types analyzed, except for *tepetate* with board-ditches during the dry season. On the other hand, in disturbed soils of Tlaxcala, García Gallegos and Hernández Acosta (2024) reported porosity values ranging from 42.6 to 44.4%. Therefore, it can be inferred that three years after establishing conservation practices for *tepetate* rehabilitation, a slight decrease in bulk density has occurred due to vegetation establishment during the rainy season. Regarding particle proportions, sand percentage was predominant in both *tepetates* and relic forest soil. Feifel *et al.* (2024) mention that soils dominated by the sand fraction tend to exhibit increased water retention in surface layers; however, this also implies greater evaporation and reduced water availability, thereby limiting vegetation growth.

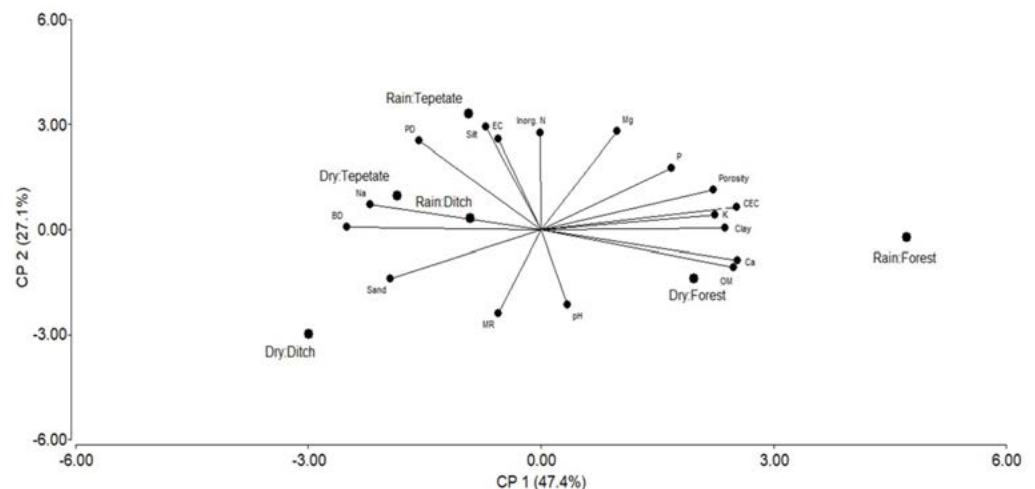


Figure 1. Biplot graph of soil properties in relic forest soil, vegetation-free tepetate, and tepetate rehabilitated with board-ditches on San Gregorio Hill, Metepec, Tlaxcala.

According to the chemical properties, the pH values obtained indicated favorable conditions for nutrient availability, as reported by Estrada-Herrera *et al.* (2017), who found pH values ranging from 5.7 to 7.2 in relic forest and degraded soils, respectively. Acidity is associated with organic matter decomposition and the release of organic acids (Hong *et al.*, 2019), whereas an increase in pH during the rainy season reflects a buffering effect associated with soil moisture (Manirakiza *et al.*, 2025). Electrical conductivity values in vegetation-free tepetate during both seasons agree with those reported by Navarro-Garza *et al.* (2004) for tepetates in eastern Tlaxcala (0.29 dS m^{-1}), considered low, with even lower values observed in *tepetate* with board-ditches and in the relic forest soil analyzed in this study.

Regarding organic matter content, values in the *tepetates* were higher than those reported by Munive-Martínez *et al.* (2018), who recorded 0.30% organic matter in a *tepetate* from Atlangatepec, Tlaxcala. In contrast, the percentage of organic matter was higher in relic forest soil, which is characteristic of this type of soil. Cation exchange capacity was also higher in the same soil. Litter accumulation, the formation of organo-mineral complexes, and biological activity contribute to soils with greater exchange capacity and availability of exchangeable bases, as mentioned by Nsengimana *et al.* (2024).

The high concentrations of calcium, magnesium, and potassium in the three soil types are related to the volcanic parent material from which these soils originated, which is rich in feldspars and other calcium, magnesium, and potassium rich minerals. In particular, magnesium content increases due to the presence of ferromagnesian minerals or feldspars rich in this element (Rodríguez-Valdivia *et al.*, 2021).

Lower concentrations than those found in this study have been reported in other areas of the state of Tlaxcala. Munive-Martínez *et al.* (2018), in vegetation-free *tepetate*, reported potassium, calcium, and magnesium contents of 2.15 , 13.63 , and 7.90 mg kg^{-1} , respectively. García Gallegos *et al.* (2023), in *tepetates* with board-ditches established in 2012, reported $1,510 \text{ mg kg}^{-1}$ of calcium, 288 mg kg^{-1} of magnesium, and 156 mg kg^{-1} of potassium. However, sodium content reached $5,370.5 \text{ mg kg}^{-1}$, a value considerably higher than that obtained in the *tepetates* analyzed in this study.

In contrast, the high phosphorus values in relic forest soil may be attributed to the greater input of litter and plant residues, which release nutrients through decomposition, as mentioned by Lemanowicz (2018). In *tepetates*, García Gallegos and Hernández Acosta (2024) obtained an average phosphorus content of 37.2 mg kg^{-1} in degraded soils where *tepetate* outcrops occur at some sites within La Malinche National Park during the dry season, a value higher than the phosphorus concentrations recorded in the *tepetate* study sites during both seasons.

Regarding inorganic nitrogen content, values were low in both relic forest soil and tepetates. Febles-Díaz *et al.* (2023) indicate that this may be related to the geological origin of these soils, since *tepetates*, when non-rehabilitated, exhibit low availability of this element, resulting in poor plant growth and a lack of essential nutrients. Therefore, three years after the establishment of board-ditches, there is still no significant increase in phosphorus and nitrogen content, which is reflected in the biological component. García Gallegos *et al.* (2023) reported microbial respiration values of 23.3 and $23.7 \text{ mg C-CO}_2 \text{ kg}^{-1}$ soil for soils

rehabilitated with board-ditches established in 2012 and 2015, respectively, values higher than those observed in the three soil types and during both periods in the present study. For *tepetates*, Álvarez-Solís *et al.* (2000) mention that the incorporation of organic residues together with plant establishment promotes an increase in microbial activity, thereby contributing to the rehabilitation process of this substrate.

Relationship among soil properties

Bulk density was negatively correlated with porosity, organic matter, cation exchange capacity, and calcium content. This indicates that a decrease in bulk density promotes an increase in pore space and greater humification of organic matter, which in turn provides higher exchange capacity due to the presence of humus and promotes mineralization processes that enhance nutrient availability, ultimately favoring vegetation establishment (Barajas Guzmán *et al.*, 2020). On the other hand, García Gallegos and Hernández Acosta (2024) found that bulk density was the variable with the highest number of significant negative interactions, particularly with manganese ($r=0.85$), field capacity ($r=-0.85$), permanent wilting point ($r=-0.75$), and available water content ($r=-0.85$). This indicates that an increase in bulk density reduces plant-available water and, consequently, leads to greater soil compaction.

Principal Component Analysis (PCA) (Table 4) showed that PC1 and PC2 accounted for 75% of the total variation, and the biplot graph indicated that relic forest soil in both seasons exhibited a stronger association with organic matter and calcium (Figure 1). In contrast, during the dry season, sand content and bulk density were the variables associated with tepetate under conservation practices, suggesting a sandy texture and an increase in porosity (Table 2), whereas during the rainy season, tepetate was associated with particle density and silt content, indicating that seasonality influences the rehabilitation process of tepetate. Rosero *et al.* (2019) reported that in forest, grassland, and silvopastoral system soils, PC1 explained 48.48% of the total variability and was determined by cation exchange capacity and calcium, whereas PC2 explained 21.69% through the sand and clay fractions, pH, and total N. This allowed the identification of soil properties that significantly influence soil quality. García Gallegos *et al.* (2023), with the establishment of board-ditches for *tepetate* rehabilitation, obtained that PC1, with 52.1%, showed the greatest total variability of the data and was explained by cation exchange capacity, calcium, organic carbon, and organic matter. PC2, with 22.4%, was determined by the sand and clay fractions, pH, and total N, which differs from what was obtained in the present study. Hence, soil variables depend on the specific conditions of each study site, the time since the establishment of conservation practices, and the type of vegetation present (García Gallegos *et al.*, 2023).

Reyes Carrillo *et al.* (2019) mention that the construction of board-ditches increases water capture during the rainy season and consequently promotes the establishment of herbaceous plants and grasses during the initial stages. This was observed three years after the rehabilitation of *tepetate* with board-ditches, which agrees with Doria Treviño *et al.* (2022), who mentioned that after the same number of years following the establishment of soil conservation practices, herbaceous cover was observed, favoring moisture retention and an increase in organic matter accumulation, as well as a decrease in bulk density, which

in turn increases porosity. This situation was also observed in the *tepetates* rehabilitated with board-ditches.

CONCLUSIONS

In the three soil types, an increase in cation exchange capacity, phosphorus, and inorganic nitrogen was observed during the rainy season. Particularly, three years after the establishment of board-ditches as a conservation practice for tepetate rehabilitation, the presence of herbaceous plants and grasses, a decrease in bulk density, neutral pH, and a reduction in electrical conductivity were observed, indicating an improvement in edaphic quality compared to vegetation-free *tepetate*, with this effect being more evident during the rainy season.

Principal Component Analysis showed that two components (PC1 and PC2) accounted for 75% of the total variation produced. Bulk density and sand content during the dry season were the variables influencing the rehabilitation process, whereas silt content and bulk density were associated with the rainy season. On the other hand, organic matter and calcium in relic forest soil showed no changes during either season.

ACKNOWLEDGEMENTS

The authors express their sincere appreciation to the civil association Tlalli Atoktli from San Diego Metepec for their valuable support in carrying out this research, as well as to SECIHTI for granting the scholarship that made it possible for Ana Rosario Velasco Sánchez to pursue her master's studies.

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Antibacterial, antioxidant, and GC-MS profiling of five Yucatán plant leaf extracts against *Listeria monocytogenes*, *Salmonella enteritidis*, and *Escherichia coli*

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ABSTRACT

Objective: To determine the antibacterial and antioxidant activity and to identify the volatile compounds in extracts from *Bixa orellana*, *Cnidioscolus aconitifolius*, *Mentha spicata*, *Piper auritum*, and *Lippia origanoides* from Yucatan.

Design/methodology/approach: Antibacterial activity was evaluated using the disk diffusion method; antioxidant activity was assessed through 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, and volatile compounds were identified by gas chromatography-mass spectrometry.

Results: In antibacterial assays, *L. origanoides* and *M. spicata* significantly inhibited *L. monocytogenes*; *B. orellana* was effective against *E. coli*, and *L. origanoides* extract showed significant inhibition of *S. enteritidis* at 40 mg/mL. In antioxidant activity, *B. orellana*, *L. origanoides*, and *M. spicata* showed the highest antioxidant capacity percentages (92.1-100%, $p < 0.05$) at 1 mg/mL. Chromatographic analysis tentatively identified the presence of compounds such as carvacrol, phytol, ishwarene, palmitic acid, and linolenic acid.

Limitations on study/implications: Although further MIC/MBC determinations and food-matrix validation are required, the high biological activity observed in *B. orellana*, *L. origanoides*, and *M. spicata*, suggests their potential as natural preservative candidates.

Findings/conclusions: The extracts with the highest antioxidant and antibacterial effects at 1 and 40 mg/mL, respectively, are *B. orellana*, *L. origanoides*, and *M. spicata*. GC-MS analyses tentatively identified the presence of bioactive compounds such as phytol, β -Cyclocitral, linolenic, and palmitic acid, carvacrol, thymol, guaiol acetate, epiglobulol and ishwarene. Results suggest that these three plant extracts represent promising natural alternatives for application in agro-food systems, specifically as potential antioxidant and selective antimicrobial agents..

Keywords: Pathogenic strains, Natural antibacterials, Natural additives, Bioactive compounds, Medicinal plants.

Citation: Olvera-Aguirre, G., Segura-Campos, M. R., Gómez-Hernández, E., Jiménez-Villeda, B. E., Piñeiro-Vázquez, Á. T., & Chay-Canul, A. J. (2026). Antibacterial, antioxidant, and GC-MS profiling of five Yucatán plant leaf extracts against *Listeria monocytogenes*, *Salmonella enteritidis*, and *Escherichia coli*. *Agro Productividad*. <https://doi.org/10.32854/zmxqpg66>

Academic Editor: Jorge Cadena Iniguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: March 3, 2026.

Accepted: April 29, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 237-248.

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INTRODUCTION

There is a rich biodiversity of plants, some of which additionally serve as food preservatives. Some of these plants can improve color and flavor of foods, and serve in the development of new agricultural products for their bioactivity (Uuh Narvaez & Segura Campos, 2020).

The bioactive compounds (BC) from these plants are advantageous for food preservation and disease prevention (Batiha *et al.*, 2021). Globally, foodborne illnesses affect 1 in 10 people. Around 31 pathogenic bacteria found in different foods cause approximately 420,000 deaths per year worldwide. Species of *Salmonella* sp., *Listeria* sp., and *E. coli* are the most dangerous pathogenic strains globally (Lee & Yoon, 2021). Consequently, plants to inhibit or eliminate these pathogens are constantly being analyzed.

Cnidocolus aconitifolius is a widely consumed plant in Nigeria and the Mesoamerican region. The BC in *C. aconitifolius* prevent cardiovascular disease when it is consumed in food or teas (Manzanilla Valdez & Segura Campos, 2021; Orji *et al.*, 2016). Additionally, *C. aconitifolius* extract is used as a nephroprotective and hepatoprotective in phytotherapy (Manzanilla Valdez & Segura Campos, 2021). Other plants, like *L. origanoides*, *P. auritum*, *B. orellana*, and *M. spicata*, are also used in many forms (extract, powder, essential oil) in cuisines all over the world and in traditional medicine (Bautista-Hernández *et al.*, 2021).

Extracts can be used to inhibit the growth of Gram (+) and Gram (–) strains, but, the natural habitat of the plants, such as geographical location and climatic conditions, will affect the quality of the extracts (Olvera-Aguirre *et al.*, 2020). This is why it is so important to repeat and document these studies in different geographical regions. Additionally, it is important to make a comparative profiling of these five species collected from Yucatán under a single extraction protocol; paired bioactivity + GC-MS volatiles. To that end, the objective of this study was to determine the antibacterial and antioxidant activity and identify the volatile compounds in extracts from *B. orellana*, *C. aconitifolius*, *M. spicata*, *P. auritum*, and *L. origanoides* from Yucatan.

MATERIALS AND METHODS

Plant matter and storage

The collected species (*Bixa orellana*, *Cnidocolus aconitifolius*, *Piper auritum*, and *Lippia origanoides*) were from established crop in agricultural research areas at “La Curva” research and production unit, located at 21.075058 N and –89.504310 W, in the municipality of Conkal, Yucatan, Mexico. The fifth plant species, *M. spicata*, was purchased fresh from a known established crop from a local farm in the municipality of Xcanatun, Yucatan, Mexico located at 21° 04' 24" N and 89° 37' 50" W.

Five hundred grams of fresh leaves were separated from the plants on the same day, washed by submerging them into tap water for one minute and then disinfected with 2 mL of commercial 0.08% ionized silver solution for each L of water for 15 minutes. The leaves were then dried at 40 °C for 72 h. After being completely dried, the leaves were then ground in a Cemotec™ 1090 Sample Mill (Thermo SCIENTIFIC, Galicia, Spain) to obtain a uniform particle and then finally pulverized in a Cyclotec™ 1093 Sample Mill

(Thermo SCIENTIFIC, Galicia, Spain). The powder obtained was then stored in dark airtight bags at $-20\text{ }^{\circ}\text{C}$ until extraction.

Extraction Process

Extractions of every plant sample were performed three times with independent biological replicates made from 5 different plants. A solid-liquid extraction (magnetic stirring, 2h) was carried out at a ratio of 1:20 (w/v, $25\text{ }^{\circ}\text{C}$) in water-ethanol (50%) (1:1 v/v), and roto evaporated (Rotavapor R-215, water bath B-49, vacuum pump V-700, and a vacuum controller V-850. Buchi[®], Switzerland) to remove the solvents (ethanol was removed at 77 millibars and 60 rpm; water was removed instantly adjusting pressure to 32 millibars and 180 rpm both at $50\text{ }^{\circ}\text{C}$). The remainder was frozen at $-20\text{ }^{\circ}\text{C}$ for 72 h. At the end of this period, the samples were lyophilized until they had a constant weight. The extracts' yield was calculated as dry matter. The solidified samples were ground and stored at $-20\text{ }^{\circ}\text{C}$ in dark airtight bags until analysis. The extraction yield was calculated with Equation 1.

$$\text{Yield}(\%) = \frac{\text{mass of dried extract}}{\text{mass of dried plant material}} \times 100 \quad (1)$$

Antioxidant Activity

The DPPH radical scavenging activity was determined by using the methodology described by Uuh Narváez *et al.* (2021) with slight modifications. First $175\text{ }\mu\text{L}$ of DPPH radical ($100\text{ }\mu\text{M}$ in methanol) and $25\text{ }\mu\text{L}$ (1 mg/mL) of the extract were used. These were then mixed and held in the dark for 30 minutes. Absorbance was measured at 517 nm. This was repeated four times. The DPPH radical scavenging activity was calculated as:

$$\text{DPPH radical scavenging activity}(\%) = \left[\frac{Ab - Ae}{Ab} \right] \times 100 \quad (2)$$

where: *Ab* is the absorbance of the DPPH radical without extract and *Ae* is the absorbance of the DPPH radical with extract.

The neutralization activity of $\text{ABTS}^{\bullet+}$ was repeated 4 times and carried out following the method proposed by Uuh Narváez *et al.* (2021) with some modifications. The $\text{ABTS}^{\bullet+}$ cation was released through the interaction of $\text{ABTS}^{\bullet+}$ (7 mM) with potassium persulfate (2.45 mM) for 16 h in the dark. The solution with the released $\text{ABTS}^{\bullet+}$ cation was adjusted to an absorbance of $0.7 (\pm 0.02)$ with ethanol at 734 nm. Subsequently, $10\text{ }\mu\text{L}$ of extract (1 mg/mL) was added to $195\text{ }\mu\text{L}$ of $\text{ABTS}^{\bullet+}$ radical solution and the absorbance was measured after 6 minutes at 734 nm. The results were calculated using Equation 2.

Bacterial strains and culture media

The biotechnology and microbiology laboratory at the Yucatán Autonomous University culture collection supplied the bacterial strains: *Listeria monocytogenes* ATCC 51414, *Salmonella enteritidis* ATCC 13076, and *Escherichia coli* ATCC 10536. The strains

were maintained at $-20\text{ }^{\circ}\text{C}$ in BHI containing 40% of glycerol (v/v). Disk diffusion was performed on Muller Hinton agar purchased from BD Bioxon (Mexico).

Antibacterial activity

The extracts' antibacterial activity was assessed using the disk diffusion method on Mueller Hinton agar following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) with six repetitions per treatment. This was realized following the methodology of Madrazo-León *et al.* (2022).

Disk diffusion test

Bacterial suspensions, adjusted to a turbidity matching the 0.5 McFarland standard, were inoculated onto Mueller Hinton agar in 100 mm Petri dishes using sterile swabs. Sterile paper disks with a 6 mm diameter were then placed on the surface of the agar, each loaded with $10\text{ }\mu\text{L}$ of each extract at concentrations of 10, 20, and 40 mg/mL. Penicillin and gentamicin ($500\text{ }\mu\text{g/mL}$) were used as positive controls for Gram-positive (*L. monocytogenes*) and Gram-negative (*S. enteritidis* y *E. coli*) bacteria, respectively. Sodium nitrite (10 mg/mL) was included as an additional control, while distilled water served as the negative control and as a solvent for the extracts. The inoculated plates were incubated for 24 hours at $37\text{ }^{\circ}\text{C}$. After incubation, inhibition diameters zones were measured to assess bacterial susceptibility. Antibacterial activity was classified according to inhibition zone diameter as follows: no inhibition “–” (0 mm), inactive “+” (<10 mm), partially active “++” (10-13 mm), and active “+++” (>14 mm) (Madrazo-León *et al.*, 2022).

Bioactive compounds identification

GC-MS analysis

The secondary metabolites of the extracts were performed with the gas chromatography technique coupled to mass spectrometry described by Gómez-Gutiérrez *et al.* (2023).

Data analysis

For the study, a completely randomized design was used (Equation 3) (Cochran & Cox, 1965). Data were analyzed using SAS statistical software, version 9.0. An analysis of variance (ANOVA) was performed using the PROC ANOVA procedure, and mean comparisons were conducted using Tukey with significance was determined at $p\leq 0.05$.

$$Y_{ij} = \mu + T_i + e_{ij} \quad (3)$$

where: Y_{ij} (observed value of dependent variable) antioxidant and antibacterial activity of PE; μ overall mean; T_i (fix effect of the treatment) T1: *B. orellana*, T2: *L. origanoides*, T3: *M. spicata*, T4: *P. auritum*, T5: *C. aconitifolius*, and e_{ij} random error term.

RESULTS AND DISCUSSION

Extraction yield is a critical parameter in determining the efficiency and practicality of obtaining BC from plant materials. A higher yield is a useful indicator of extraction

efficiency and ensures better use of natural resources. In this study, a hydroalcoholic solution (ethanol 50:50 v/v) was selected to balance polarity and optimize the extraction of both hydrophilic and lipophilic compounds.

Rodrigues *et al.* (2020) evaluated three techniques for *C. aconitifolius*: Subcritical Water Extraction (SWE), Microwave Assisted Extraction (MAE), and Soxhlet, reporting yields of 35-39%, 9-18%, and 31-33% (wet basis), respectively. In the present study, the ethanol (50:50 v/v) extraction yielded 26.3% on a dry basis (Table 1), which is lower than SWE and Soxhlet, but higher than MAE. Yield differences may be attributed to factors such as solvent polarity, extraction time and temperature, technique used, and environmental conditions of the plant's origin (Olvera-Aguirre *et al.*, 2020).

Antioxidant Activity

The estimation of antioxidant activity using the DPPH method has been employed to evaluate the presence of BC in plant leaves. The DPPH radical undergoes a color change from purple to yellow upon interaction with BC. Similarly, the ABTS^{•+} assay assesses the reduction in the intensity of the radical's color due to BC (Zeb, 2021).

Antioxidant activity using the DPPH method (Table 1) revealed that the *B. orellana* extract showed the highest antioxidant activity, inhibiting 100% of free radicals at a concentration of 1 mg/mL. The results, however, did not show statistically significant differences ($p > 0.05$) between *L. organoides*, *M. spicata* and *B. orellana*. These findings indicate that all three have a strong capacity to neutralize free radicals and be considered potential sources of antioxidants.

Pavlović *et al.* (2021) assessed *M. piperita* using the DPPH method with extracts obtained through 24 hours maceration in 70% methanol, 70% ethanol, and hot water, combined with ultrasound one hour before and after maceration. The highest scavenging activity was reported at 250 mg/mL, ranging from 61.27 (water), 92.80 (ethanol), and 93.30% (methanol), respectively. In contrast, the present study evaluated *M. spicata* at a dose of 40 mg/mL which achieved a DPPH inhibition of 97.3% (Table 1). Additionally, *B. orellana* and *L. organoides* also showed higher antioxidant activity compared to those reported by Pavlović *et al.* (2021), high scavenging activity was observed under the present extraction and assay conditions.

Table 1. Antioxidant activity and yield of leaves and extracts from 5 plant species collected in the Yucatán Peninsula.

Plant	Common name	Family	Extract yield (%)	DPPH (%)	ABTS ^{•+} (%)
<i>Cnidoscolus aconitifolius</i>	Chaya, Chaya spinach tree	Euphorbiaceae	26.3±0.9 ^a	40.2±2.7 ^c	80.7±1.3 ^{bc}
<i>Piper auritum</i>	Sacred pepper, Momo	Piperaceae	13.7±1.0 ^c	43.2±2.5 ^c	71.8±1.9 ^c
<i>Mentha spicata</i>	Yerbabuena, Spearmint	Lamiaceae	16.4±1.1 ^b	97.3±2.5 ^{ab}	92.1±0.2 ^a
<i>Lippia organoides</i>	Oregano	Verbenaceae	12.0±1.4 ^c	98.1±4.0 ^{ab}	92.5±0.3 ^a
<i>Bixa orellana</i>	Achiote, Annatto	Bixaceae	21.5±0.9 ^{ab}	100±1.9 ^a	91.6±0.4 ^a
P value	-	-	<0.001	<0.001	<0.001

% yield: dry matter base; values are expressed as mean ±: standard error; different superscripts indicate significant differences between plants (Tukey HSD, $p \leq 0.05$).

In the ABTS^{•+} assay, no significant differences were found between *L. origanoides*, *M. spicata*, and *B. orellana* (Table 1), with free radical scavenging ranging from 91.56% and 92.46% at 1 mg/mL. These results confirm the high antioxidant potential of these extracts and their effectiveness as radical neutralizers. Uuh-Narváez *et al.* (2021) reported 100% ABTS^{•+} inhibition in *B. orellana* seed extracts, supporting the notion that both seeds and leaves are valuable antioxidant sources and validating their long-standing use in traditional Mayan gastronomy.

Mentha spicata is widely recognized for its strong antioxidant capacity and ability to scavenge free radicals. Biswas *et al.* (2012) reported up to 76% ABTS^{•+} inhibition in hot water extracts of *M. spicata* leaves using a 1:20 solute-solvent ratio. In line with these findings, the present study confirms that *B. orellana*, *L. origanoides*, and *M. spicata* possess considerable antioxidant potential. Together, the results from both DPPH and ABTS^{•+} assays support their use as promising natural sources of antioxidant compounds. Additional studies will be required to evaluate the most effective dosages in biological systems and the nutritional applications of these extracts.

Antibacterial Activity

The antibacterial activity of the five PE was assessed by measuring the average inhibition zones (mm) against selected bacterial strains (Table 2). Although *B. orellana*, *L. origanoides* and *M. spicata* extracts exhibited some degree of antimicrobial activity, none reached the efficacy levels observed with the positive controls, gentamicin and ampicillin.

In the present study, the highest antimicrobial activity against *L. monocytogenes* was observed with *M. spicata* and *L. origanoides*. Additionally, *L. origanoides* exhibited the strongest effect against *S. enteritidis*, while *B. orellana* performed best against *E. coli*, all at a concentration of 40 mg/mL. These results suggest that these PE partially inhibit *L. monocytogenes*, *S. enteritidis*, and *E. coli* growth.

Table 2. Microbial inhibition zone in mm for *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enteritidis* with hydroalcoholic extracts.

Bacterial strain	C+	C-	SN	<i>B. orellana</i>	<i>C. aconitifolius</i>	<i>P. auritum</i>	<i>L. origanoides</i>	<i>M. spicata</i>
LM10	17.7±0.7 ^{Ab}	ND	ND	7.4±0.3 ^{Cc}	ND	ND	7.57±0.1 ^{Cc}	6.84±0.2 ^{Cb}
LM20	17.7±0.7 ^{Ab}	ND	ND	8.03±0.1 ^{BCb}	ND	ND	8.12±0.2 ^{BCb}	7.51±0.1 ^{Cb}
LM40	17.7±0.7 ^{Ab}	ND	ND	9.51±0.2 ^{Ba}	ND	ND	10.2±0.3 ^{Ba}	10.3±0.6 ^{Ba}
EC10	21.4±0.6 ^{Aa}	ND	ND	8.56±1.1 ^{Cc}	ND	ND	8.26±0.4 ^{BCb}	7.50±1.2 ^{Cb}
EC20	21.4±0.6 ^{Aa}	ND	ND	9.35±1.2 ^{BCb}	ND	ND	9.09±0.1 ^{Bb}	8.85±1.1 ^{BCb}
EC40	21.4±0.6 ^{Aa}	ND	ND	15.4±1.8 ^{Ba}	ND	ND	12.6±1.3 ^{Ba}	12.1±0.1 ^{Ba}
SE10	19.6±0.5 ^{Aab}	ND	ND	7.23±0.2 ^{Cc}	ND	ND	7.48±0.4 ^{Cb}	6.00±0 ^{Dc}
SE20	19.6±0.5 ^{Aab}	ND	ND	8.00±0.2 ^{Cc}	ND	ND	10.4±1.2 ^{BCb}	6.00±0 ^{Dc}
SE40	19.6±0.5 ^{Aab}	ND	ND	10.1±0.8 ^{BCb}	ND	ND	12.7±0.4 ^{Ba}	6.00±0 ^{Dc}
P value	<0.01	-	-	<0.001	-	-	<0.001	<0.001

LM: *Listeria monocytogenes*; EC: *Escherichia coli*; SE: *Salmonella enteritidis*; 10, 20, 40: indicates mg/mL; C+: 500 µg/mL Penicillin/Gentamicin; C-: distilled water; SN: sodium nitrite (10 mg/mL); ND: not detected; values are expressed as mean ± standard error. Uppercase letters indicate differences between treatments (columns). Lowercase letters indicate differences between doses of the same strain (rows) (Tukey HDS, p≤0.05).

Pinto *et al.* (2013), Click or tap here to enter text. evaluated the antibacterial activity of the *L. origanoides* methanol extract against antibiotic-resistant strains, including *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans* and *C. parapsilosis*, and found that at doses of 200 mg/mL it inhibited *S. aureus*, *C. albicans* and *C. parapsilosis* with a 12-13 and 11.7 and 7.1 mm inhibition zone, respectively. In the present study, inhibition zones ranged from 12.1 to 15.4 mm using 40 mg/mL, suggesting that the ethanol PE inhibited better *E. coli* strain, than the methanol one used in the Pinto *et al.* (2013) work. That results could be likely due to its BC: r-cymene, carvacrol, thymol, and g-terpinene.

Table 3 summarizes the qualitative classification of inhibition. At 40mg/mL, *B. orellana* was active against *E. coli* and partially active against *S. enteritidis*. *L. origanoides* was partially active against both Gram-positive (*L. monocytogenes*) and Gram-negative (*S. enteritidis* and *E. coli*). *M. spicata* was partially active against *E. coli* at 40 mg/mL.

Overall, *L. origanoides* presented the broadest antimicrobial spectrum, inhibiting both Gram-positive and Gram-negative bacteria. The antibacterial activity observed may be associated with the presence of bioactive compounds capable of disrupting bacterial cell membranes, increasing membrane permeability, causing ion leakage, and ultimately leading to cell death, as previously reported (Olvera-Aguirre *et al.*, 2023). These findings are consistent with previous studies. For example, Arumugam *et al.* (2010) reported inhibition zones of 9 and 21 mm against *E. coli* and *Salmonella typhi*, respectively, using ethanolic extracts of *M. spicata*, whereas aqueous fractions showed no antibacterial activity. This highlights the importance of extraction solvent and phytochemical composition in determining antimicrobial efficacy. Although none of the plant extracts at 40 mg/mL reached the inhibition levels of conventional antibiotics, the observed activity, particularly at higher concentration, inhibit moderately *E. coli* and *S. enteritidis*. That supports the idea of these plants used in traditional cuisine as selective natural antimicrobial agent for applications in food preservation.

Table 3. Degree of inhibition for *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enteritidis* growth produced by five hydroalcoholic extracts from plant species collected in the Yucatan Peninsula.

Bacterial strain	C+	C-	SN	<i>B. orellana</i>	<i>C. aconitifolius</i>	<i>P. auritum</i>	<i>L. origanoides</i>	<i>M. spicata</i>
LM10	+++	-	-	+	-	-	+	+
LM20	+++	-	-	+	-	-	+	+
LM40	+++	-	-	+	-	-	++	+
EC10	+++	-	-	+	-	-	+	+
EC20	+++	-	-	+	-	-	+	+
EC40	+++	-	-	+++	-	-	++	++
SE10	+++	-	-	+	-	-	+	+
SE20	+++	-	-	+	-	-	++	+
SE40	+++	-	-	++	-	-	++	+

LM: *Listeria monocytogenes*; EC: *Escherichia coli*; SE: *Salmonella enteritidis*; 10, 20, 40: indicates mg/mL; C+: 500 µg/mL Penicillin/Gentamicin; C-: distilled water; SN: sodium nitrite (10 mg/mL); (-): No inhibition zone (+): <10 mm, "inactive"; (++) : 10-13 mm "partially active"; (+++) : >14 mm "active".

Identification of volatile compounds

The volatile composition of the five plant extracts (Table 4) provides valuable insight into their biological activities and functional applications. Only compounds representing >5% of the total chromatographic area were considered major constituents. The identified compounds correspond mainly to fatty acids, diterpenes, phenolic monoterpenes, and oxygenated sesquiterpenes associated with antioxidant and antimicrobial activity.

Thymol, an isomer of carvacrol, is commonly used to enhance food quality and shelf life with no negative effects on human health (Rathod *et al.*, 2021). It exhibits

Table 4. GC-MS test shows the identification of volatile compounds in ethanolic extracts.

Compound (IUPAC / accepted name)	Common name	% Area (Observed)	Plant species	Reported bioactivity
5-Isopropyl-2-methylphenol	Carvacrol	9.8	<i>L. origanoides</i>	Antibacterial, antioxidant
2,6-Di-tert-butyl-4-hydroxyanisole				
Phenol, 2-methyl-5-(1-methylethyl)	—	9.8	<i>L. origanoides</i>	Related to carvacrol-like activity
(2E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	Phytol	10.9	<i>L. origanoides</i>	Antioxidant, anti-inflammatory
(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	α -Linolenic acid	8.9	<i>L. origanoides</i>	Antioxidant (fatty acid)
Hexadecanoic acid	Palmitic acid	8.1	<i>L. origanoides</i>	Antioxidant precursor
4,4-Dimethylpent-2-enal	—	7.7	<i>L. origanoides</i>	Limited reports
5-Methyl-2-(propan-2-yl)phenol	Thymol	†	<i>L. origanoides</i>	Antioxidant, antibacterial
2,6,6-Trimethylcyclohex-1-ene-1-carbaldehyde	β -Cyclocitral	19.0	<i>M. spicata</i>	Antioxidant
Ethyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	Ethyl linolenate	14.8	<i>M. spicata</i>	Antioxidant derivative
p-Menthane-1,2,3-triol	—	6.8	<i>M. spicata</i>	Limited reports
5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	—	5.7	<i>M. spicata</i>	Limited reports
Nepetalactone (4 α ,7 β ,7 α)	—	6.4	<i>M. spicata</i>	Insect bioactivity
(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	α -Linolenic acid	15.5	<i>M. spicata</i>	Antioxidant
Hexadecanoic acid	Palmitic acid	14.4	<i>M. spicata</i>	Antioxidant precursor
n-Hexadecanoic acid	Palmitic acid	18.8	<i>P. auritum</i>	Antibacterial precursor
(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	α -Linolenic acid	18.6	<i>P. auritum</i>	Antioxidant
(2E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	Phytol	14.3	<i>P. auritum</i>	Antioxidant
Ethyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	Ethyl linolenate	12.0	<i>P. auritum</i>	Antioxidant
Pellitorine	—	†	<i>P. auritum</i>	Antibacterial, insecticidal
(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	α -Linolenic acid	40.9	<i>C. aconitifolius</i>	Antioxidant
Hexadecanoic acid	Palmitic acid	25.1	<i>C. aconitifolius</i>	Antioxidant precursor
(2E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	Phytol	21.1	<i>C. aconitifolius</i>	Antioxidant
Ethyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	Ethyl linolenate	10.5	<i>C. aconitifolius</i>	Antioxidant
Guaiol acetate	—	29.0	<i>B. orellana</i>	Antibacterial, antioxidant
Epiglobulol	—	18.6	<i>B. orellana</i>	Antibacterial
(-)-Globulol	—	16.8	<i>B. orellana</i>	Antibacterial
Ishwarane	—	†	<i>B. orellana</i>	Limited reports
Geranyl- α -terpinene	—	†	<i>B. orellana</i>	Limited reports

LO: *Lippia origanoides*; MS: *Mentha spicata*; PA: *Piper auritum*; CA: *Cnidocolus aconitifolius*; BO: *Bixa orellana*; “—”: not founded; †: compound present below 5%.

antioxidant, anti-inflammatory, and antibacterial activities and is mainly found in *Thymus* and *Origanum* species (Escobar *et al.*, 2020; Kachur & Suntres, 2020). Thymol generally shows greater antioxidant potential than carvacrol, attributed to superior hydrogen-donating and singlet and triplet oxygen-quenching capabilities (Rathod *et al.*, 2021).

In this study, carvacrol was among the principal BC identified in *L. origanoides*. This phenolic monoterpene exert antibacterial activity against *E. coli*, *Salmonella*, and *Bacillus cereus* by disrupting and depolarizing the cytoplasmic membrane (Olvera-Aguirre *et al.*, 2023). Carvacrol was one of the strongest compounds in the extract, suggesting its potential application in food preservation.

Traditionally, *M. spicata* and *C. aconitifolius* are used in Mayan cuisine to prepare pastas and herbal teas. Uuh Narváez & Segura Campos (2020) reported compounds such as epicatechin, p-coumaric acid, caffeic acid, and chlorogenic acid in *C. aconitifolius*. Although different compounds were identified in this study, notably α -Linolenic acid, palmitic acid and phytol, compounds with antioxidant and antimicrobial properties (Murugan *et al.*, 2025).

In *M. spicata*, β -Cyclocitral, Ethyl linolenate, and nepetalactone ($4\alpha,7\beta,7\alpha$) (monoterpene from the Lamiaceae family), were identified. Nepetalactone act as insect repellent and also serve as attractant for cats and aphids (Gomes *et al.*, 2021). The other two compounds have strong bioactivity (Havaux, 2020).

Piper auritum contains BCs, like fatty acids, phytol, ethyl linolenate, and pellitorine, a pungent alkamide from the Piperaceae family. This compound has antibacterial (*Mycobacterium tuberculosis*, *K. aerogenes*) and insecticidal activity and is valued in food and medicine (Khan *et al.*, 2019). And it was founded in a small quantity that is not sufficient to has an effect on the pathogen strains evaluated.

Linolenic acid, found in *P. auritum* and *C. aconitifolius*, can negatively impact oxidative stability and flavor when heated repeatedly at concentrations >4%, resulting in the accumulation of polar oxidation products and a fishy flavor (Frankel, 2012). Phytol is a common acyclic diterpene found in all five plant species analyzed, and exhibits antibacterial, antioxidant and anti-inflammatory activity (Eksi *et al.*, 2020).

A saturated fatty acid (n-hexadecanoic acid) was detected in all plants except *B. orellana*. This compound function as natural antioxidant and preservative in food (Gómez-Sequeda *et al.*, 2020). Additionally inhibits phospholipase A2 and acts as an anti-inflammatory, antibacterial, and antifungal agent (Aparna *et al.*, 2012).

The last plant analyzed was *B. orellana*, which is known for its antibacterial activity because of the BC in its leaves (alkaloids, triterpenes, steroids, tannins, saponins, flavonoids, phenols, proteins, glycosides and sugars, fats, and oils) with known antibacterial activity. It holds cultural and culinary importance in Mayan traditions (Zarza-García *et al.*, 2021), primarily for its flavoring and coloring properties.

Sesquiterpenes identified in *B. orellana* include guaiol acetate, (-)-globulol, and epiglobulol. Globulol, the main antibacterial in *Eucalyptus globulus*, is effective against *Xanthomonas vesicatoria* and *Bacillus subtilis*. Epiglobulol has been found in *Ficus hispida*, *Myrcia falax*, and *Acmella oleracea* (Fauzi *et al.*, 2021), and shows antibacterial,

antifungal, and antioxidant activity. Ishwarane, a rare sesquiterpene found in this study, has also been identified in *Aristolochia indica* roots (Dos S. Junior *et al.*, 2013).

The BCs identified across the five species are primarily antioxidant and moderate antibacterial in nature, supporting their traditional use in food preservation and flavor enhancement. Characterizing and standardizing these plant extracts is essential to determine optimal dosages for applications in food and other industries.

Overall, fatty acids and phytol were common components across most species, whereas phenolic monoterpenes were distinctive in *L. origanoides* and sesquiterpenes in *B. orellana*. These compositional differences may explain variations in biological activity and support the potential application of these extracts as natural additives in agro-food systems. Further studies under practical conditions are recommended.

CONCLUSIONS

The extracts with the highest antioxidant and moderate antibacterial effects at 1 and 40 mg/mL, respectively, are *B. orellana*, *L. origanoides*, and *M. spicata*. GC-MS analyses tentatively identified the presence of bioactive compounds such as phytol, β -Cyclocitral, linolenic, and palmitic acid, carvacrol, thymol, guaiol acetate, epiglobulol and ishwarene. Results suggest that these three plant extracts represent promising natural alternatives for application in agro-food systems, specifically as potential antioxidant and selective antimicrobial agents.

ACKNOWLEDGEMENTS

To SECIHTI for financing the postgraduate studies of the first author, grant number: [929660] . To Malina Rauschenfels and Yusuf Albasri for providing language support.

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Indicator microorganisms for quality in pulque from three producing municipalities in the state of Mexico

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Citation: Palomares-Domínguez, J. C., Rojas-Puebla, I., Gutiérrez-Ibáñez, A. T., Ahumada-Cota, R. E., Bernal-Martínez, L.R., & Ocaña-de Jesús, R. L. (2026). Indicator microorganisms for quality in pulque from three producing municipalities in the state of Mexico. *Agro Productividad*. <https://doi.org/10.32854/ntw95k49>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: March 8, 2026.

Accepted: April 26, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 249-261.

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ABSTRACT

Objective: To evaluate the physicochemical parameters and microbiological quality of pulque produced in three municipalities in the State of Mexico: Temoaya, Jiquipilco, and Ocoyoacac.

Design/methodology/approach: The physicochemical parameters evaluated included pH, total soluble solids (°BX) and alcohol content.

In addition, microorganism's indicative of microbiological quality was quantified, including: aerobic mesophilic bacteria, fungi, yeasts, and coliforms, complemented by the presence of lactic acid bacteria. Presumptive identification and antimicrobial susceptibility testing were performed on coliform isolates.

Results: The physicochemical results were within the typical ranges for pulque, with pH values 3.30 to 4.02, total soluble solids 4.62 to 6.37 °Bx and alcohol content 4.42 to 4.87%. Microbiological analyses revealed the presence of aerobic mesophiles, lactic acid bacteria, and yeasts in all samples. Coliform bacteria were detected only 20% of the samples. Biochemical test allowed the presumptive identification of *Escherichia coli* and *Shigella flexneri*. Antimicrobial susceptibility revealed, resistance of these isolates to antibiotic such as ampicillin, carbenicillin, cephalothin, cefotaxime and nitrofurantoin.

Limitations/implications: The study was limited to specific localities, which may affect the generalizability on the results. Nevertheless, it highlights the need to strengthen hygienic practices and microbiological control.

Findings/conclusions: The microbiological variability observed among municipalities highlights the influence of production and commercialization conditions. The detection of antibiotic-resistant bacteria represents a potential risk to food safety.

Keywords: Fermentation, bacteria, yeast, analyses, food safety.



INTRODUCTION

Pulque is one of the oldest fermented beverages in Mesoamerica and has traditionally been associated with its consumption during pre-Hispanic ceremonies (Escalante *et al.*, 2016; SADER, 2023). Following colonization, its production gained economic relevance with the establishment of pulque-producing haciendas; however, its consumption declined at the beginning of the twentieth century due to the introduction of industrialized beverages and discrediting campaigns (Trejo *et al.*, 2020; SADER, 2023). In recent years, this beverage has experienced a resurgence driven by its cultural, nutritional, and artisanal value, as well as by its promotion through regional fairs that highlight the importance of its production and consumption (Rojas *et al.*, 2016; Pérez & Cardoso, 2020).

In Mexico, pulque production is concentrated in the states of Hidalgo, Tlaxcala, Puebla, and the State of Mexico, the latter ranking third nationwide with a production close to 26,000 L, mainly in the municipalities of Jiquipilco, Ocoyoacac, and Temoaya (SECAMPO, 2023; SIAP, 2024). Pulque is obtained through the fermentation of aguamiel, the sap of agave plants, which provides a favorable environment for the development of microorganisms such as lactic acid bacteria and yeasts, responsible for acidification, ethanol production, and sensory characteristics (Escalante *et al.*, 2016; Rocha *et al.*, 2020; Álvarez *et al.*, 2020; NOM-199-SCFI-2017; Ruíz *et al.*, 2023). Its production is an artisanal process that does not involve distillation and, depending on the region, employs different agave species (Enríquez *et al.*, 2017; Villarreal *et al.*, 2019). Aguamiel is obtained by scraping the cavity formed in the center of the agave stem, where initial microbial development occurs during its accumulation in the cajete of the maguey plant. Subsequently, aguamiel undergoes the fermentation process, which involves not only autochthonous microorganisms but also those incorporated throughout different handling stages, including collection, transportation, inoculum (semilla) preparation, product handling, and storage (Tamang *et al.*, 2016; Amazará & Quintero, 2022; Colón *et al.*, 2025).

The microbiological quality of pulque depends directly on agricultural crop management and hygienic production practices (Cervantes & Pedroza, 2007; Gómez *et al.*, 2011). However, the process is not exempt from the risk of contamination by spoilage or pathogenic microorganisms, which may affect the quality and safety of the product, particularly when antimicrobial-resistant microorganisms are involved (Gómez *et al.*, 2011; Escalante, 2016; Lappe *et al.*, 2023).

Among the factors associated with contamination are the exposure of agave plants to domestic and farm animals, inadequate hygiene during aguamiel extraction and handling, poor sanitary conditions in production and storage areas, and inadequate distribution practices (Natera, 2005; Rojas *et al.*, 2016).

In this context, the quantification of indicator microorganisms of quality, such as aerobic mesophiles, total coliforms, fecal coliforms, *Escherichia coli*, molds, and yeasts, is a key factor in evaluating and ensuring the microbiological quality of various fermented beverages, including pulque (Ramos *et al.*, 2014; NOM-210-SSA1-2014).

Therefore, the objective of this study was to evaluate the microbiological quality of pulque produced in three municipalities of the State of Mexico (Temoaya, Jiquipilco, and Ocoyoacac) through the quantification of aerobic mesophiles, lactic acid bacteria, total

coliforms, fecal coliforms, molds, and yeasts, with the aim of identifying microbiological parameters associated with this traditional beverage.

MATERIALS AND METHODS

Study Area

Pulque samples were obtained from points of sale in three producing municipalities of the State of Mexico: Temoaya (19° 24'-19° 35' N), Jiquipilco (19° 29'-19° 42' N), and Ocoyoacac (19° 12'-19° 18' N) (Figure 1) (INEGI, 2024).

Sampling

Physicochemical and microbiological evaluations were carried out at the Food Safety Laboratory of the Faculty of Agricultural Sciences at the Autonomous University of the State of Mexico. Sample collection was performed aseptically from products intended for sale. Duplicate samples of 100 mL of natural pulque were collected in sterile containers from five producers in each municipality. The samples were transported under refrigerated conditions (4 ± 2 °C) to the laboratory in accordance with the guidelines established by the Mexican Official Standard NOM-109-SSA1-1994.

Physicochemical Analyses

pH was measured directly in the samples using a previously calibrated potentiometer (Conductronic PC45). Total soluble solids (TSS) were determined by measuring the refractive index in degrees Brix (°Bx) using a Milwaukee MA871 digital refractometer. Alcohol content was determined in accordance with the Mexican Official Standard NOM-199-SCFI-2017.

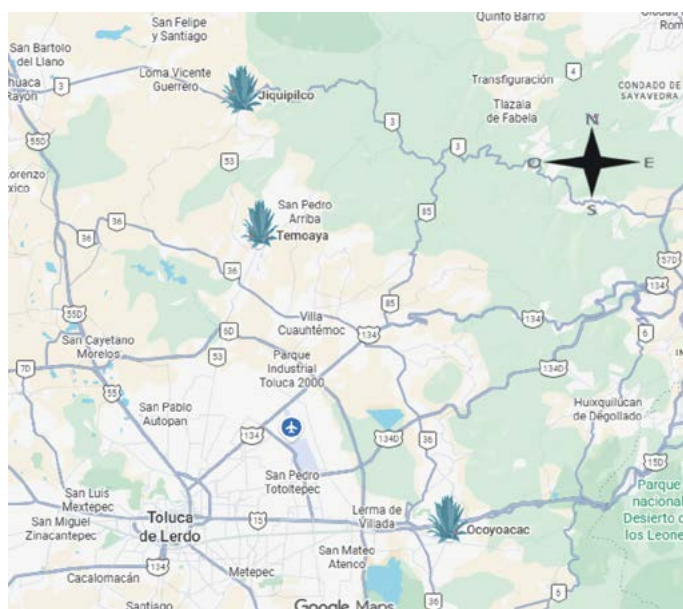


Figure 1. Geographic location of the municipalities of Jiquipilco, Temoaya and Ocoyoacac (Adapted from Google Maps, 2025).

Microbiological Analyses

Samples were analyzed through the preparation of serial dilutions (10^{-1} to 10^{-6}) using buffered peptone water (BD Difco). From these dilutions, viable counts of indicator microorganisms of microbiological quality were performed, including aerobic mesophiles according to NOM-092-SSA1-1994 and coliforms in accordance with the Mexican Official Standard NOM-210-SSA1-2014. The determination of lactic acid bacteria was carried out following the methodology established in NOM-110-SSA1-1994. Likewise, microbiological counts of molds and yeasts were performed according to the procedures described in NOM-111-SSA1-1994.

From the prepared dilutions, 100 μL aliquots were plated by surface spreading onto Petri dishes containing Standard Methods Agar (MCD LAB) for aerobic mesophiles; MRS agar (Millipore) for lactic acid bacteria; Violet Red Bile agar (MCD LAB) for coliforms; Potato Dextrose Agar (MCD LAB) for molds; and WL Nutrient Agar (MCD LAB) for yeasts associated with fermentation processes. Inoculated plates were incubated under the following conditions: aerobic mesophiles at 35 ± 2 °C for 24 h; lactic acid bacteria at 35 ± 2 °C for 5 days; total coliforms at 35 ± 2 °C for 24 h; molds at 25 ± 1 °C for 5 days; and yeasts at 30 °C for 48 h. After incubation, colony counts were performed for each microbial group evaluated, and results were expressed as Log CFU/mL.

The obtained results were analyzed using analysis of variance (ANOVA) for each microbial group evaluated. When statistically significant differences were detected ($p < 0.05$), Fisher's exact test was applied. Statistical analyses were performed using GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA).

Obtaining Pure Cultures and Phenotypic Identification

Once colony counts were completed, colonies were selected based on their colonial morphology according to the microbial group evaluated, with the aim of obtaining axenic cultures, performing the corresponding tests, and preserving the isolates in storage vials.

Isolated BAL were subjected to Gram staining and catalase testing, while yeasts were stained with cotton blue (López *et al.*, 2014).

From the coliform isolates obtained (60), Gram staining and subculturing on selective media, including MacConkey agar (MCD LAB), Eosin Methylene Blue agar (MCD LAB), and *Salmonella-Shigella* agar (BD Bioxon), were performed. These tests were complemented by the determination of Indole, Methyl Red, Voges-Proskauer, and Citrate (IMViC) reactivity according to the procedures established in NOM-210-SSA1-2014, as well as complementary tests for Motility, Indole, and Ornithine (MIO), Catalase, Triple Sugar Iron (TSI), and Urease (Table 3) (Winn & Koneman, 2008; NOM-210-SSA1-2014).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was evaluated using the disk diffusion method (Kirby & Baüer), following the protocol described by the Clinical and Laboratory Standards Institute (CLSI, 2018). Susceptibility to 12 antimicrobials (Oxoid, UK) was evaluated: Amikacin (KA) 30 μg , Ampicillin (AMP) 10 μg , Carbenicillin (CAR) 100 μg , Cephalothin (KF) 30 μg , Cefotaxime (CTX) 30 μg , Ceftriaxone (CRO) 30 μg , Chloramphenicol (CL)

30 μg , Gentamicin (GM) 10 μg , Netilmicin (NET) 30 μg , Nitrofurantoin (F) 300 μg , Pefloxacin (PEF) 5 μg , and Trimethoprim-sulfamethoxazole (STX) 1.5/23.75 μg . Results were interpreted according to the inhibition zone diameter criteria established by CLSI (2018) for each antibiotic and classified as susceptible (S), intermediate (I), or resistant (R).

RESULTS AND DISCUSSION

Physicochemical Analyses

The results obtained for the physicochemical parameters evaluated are presented in Table 1. The samples showed an acidic profile with values ranging from 3.30 to 4.02. Statistically significant differences were observed among municipalities ($p \leq 0.05$). The highest pH values were obtained in samples from Ocoyoacac, whereas the most acidic values were recorded in Jiquipilco; however, all results were within the ranges reported for traditional pulque.

Regarding total soluble solids ($^{\circ}\text{Bx}$), statistically significant differences were identified ($p < 0.05$). The municipality with the highest values was Ocoyoacac, followed by Temoaya and Jiquipilco.

With respect to alcohol content, the samples presented values ranging from 4.42 to 4.87%, with no statistically significant differences among municipalities.

Microbiological Analyses

Figure 2 shows the counts of microbiological quality indicators obtained, including aerobic mesophilic bacteria, lactic acid bacteria, and yeasts, which were present in 100% of the samples analyzed. In contrast, coliforms were detected in only 20% of the samples corresponding to two municipalities, while fungi did not develop in any of the samples.

Within the aerobic mesophile group (Figure 2a), the municipalities of Jiquipilco and Temoaya presented the highest counts, with no statistically significant differences between them ($p > 0.05$), whereas Ocoyoacac showed significantly lower values. Counts of lactic acid bacteria did not show statistically significant differences ($p > 0.05$), with similar counts observed among the three municipalities (Figure 2b).

In the case of the coliform group (Figure 2c), significant differences were observed among the municipalities evaluated ($p < 0.05$ -0.001). No coliform growth was detected in Ocoyoacac, whereas Jiquipilco and Temoaya showed detectable counts, with the latter presenting the highest values.

For yeasts, highly significant statistical differences were identified among the three municipalities ($p < 0.001$). Temoaya presented the highest values, followed by Jiquipilco, while Ocoyoacac showed the lowest counts (Figure 2d).

Table 1. Physicochemical Properties of Pulque.

Parameters	Municipalities		
	Ocoyoacac	Jiquipilco	Temoaya
pH	4.02 \pm 0.08	3.30 \pm 0.15	3.49 \pm 0.11
$^{\circ}\text{Bx}$	6.37 \pm 0.23	4.62 \pm 1.38	5.43 \pm 1.33
Alcohol (%)	4.87 \pm 0.38	4.42 \pm 0.25	4.76 \pm 0.40

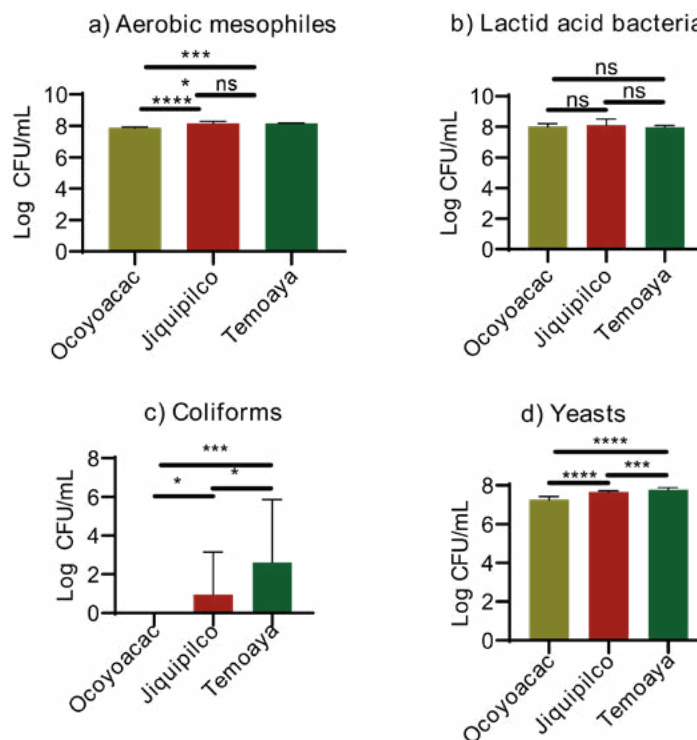


Figure 2. Counts of indicator microorganisms of microbiological quality in pulque. Fisher's exact test was performed: ns=not statistically significant ($p>0.05$), $*=p<0.05$, $**=p<0.001$, and $***=p<0.0001$.

Phenotypic Identification

The results of the biochemical identification tests performed on strains isolated from pulque are shown in Table 2. The tests carried out allowed the evaluation of metabolic characteristics, which are important for the presumptive identification of enteric bacteria. The obtained profiles were consistent with the reactions described in the literature for *Escherichia coli* and *Shigella flexneri*.

Table 2. Biochemical Identification Tests of Strains Isolated from Pulque.

Bacteria	Reaction										
	M	I	O	H ₂ O ₂	CS	RM	VP	U	TSI	GAS	H ₂ S
<i>Escherichia coli</i>	+	+	+	+	-	+	-	-	A/A	+	-
<i>Shigella flexneri</i>	-	+ (-)	+ (-)	+	-	+	-	-	K/A	-	-
*A.2	+	-	+	+	+	+	-	-	K/A	-	-
*A.4	+	-	+	+	+	+	-	-	K/A	-	-
*A.15	+	-	+	+	+	+	-	-	K/A	-	-
*M.22	+	+	+	+	-	+	-	-	A/A	+	-
*M.23	+	+	+	+	-	+	-	-	A/A	+	-
*M.25	+	+	+	+	-	+	-	-	A/A	+	-

* Isolates; M: Motility, I: Indole, O: Ornithine, H₂O₂: Catalase, CS: Simmons Citrate, MR: Methyl Red, VP: Voges-Proskauer, U: Urea, TSI: Triple Sugar Iron. K: Alkaline reaction (red color), A: Acid reaction (yellow color), Gas: Gas production, H₂S: Hydrogen sulfide production (black color).

Antimicrobial Susceptibility

The results obtained from the antimicrobial susceptibility tests of strains isolated from pulque, determined by the disk diffusion method, are shown in Table 3. The group of *E. coli* isolates showed 50% resistance to the antibiotics analyzed, 17% intermediate susceptibility, and 33% complete susceptibility. Resistance was mainly observed against ampicillin, carbenicillin, cephalothin, cefotaxime, nitrofurantoin, and trimethoprim-sulfamethoxazole, evidencing a multidrug resistance profile, particularly against β -lactam antibiotics.

In contrast, the strains identified as *Shigella* showed 50% susceptibility, 8% intermediate susceptibility, and 42% resistance. These results indicate greater overall susceptibility, although resistance to ampicillin, carbenicillin, cephalothin, cefotaxime, and nitrofurantoin was also observed.

The physicochemical and microbiological results obtained in the present study highlight the importance of pulque as a complex fermentative system whose physicochemical and microbiological quality is closely related to production conditions, autochthonous microbiota, fermentation conditions, and traditional production practices. This is consistent with other studies emphasizing the importance of the microbiological diversity of this beverage (SADER, 2019; Rodríguez *et al.*, 2021; Astudillo *et al.*, 2023).

The acidic pH observed in the samples complies with the standards established for traditional fermented beverages in NOM-199-SCFI-2017. Likewise, these values are consistent with the ranges reported for pulque by Álvarez *et al.* (2020) and Medina *et al.* (2023), who highlight that acidic pH occurs as a result of the production of organic acids, mainly lactic and acetic acids, which in some cases may inhibit pathogenic microorganisms (Cervantes & Pedroza, 2007; Medina *et al.*, 2023; Astudillo *et al.*, 2023).

Table 3. Antimicrobial Susceptibility of Strains Isolated from Pulque.

Antimicrobial	Inhibition (mm) <i>E. coli</i>	Interpretation	Inhibition (mm) <i>Shigella</i>	Interpretation
Amikacin	35	S	26	S
Ampicilin	0	R	0	R
Carbenicilin	0	R	0	R
Cephalothin	0	R	0	R
Cefotaxime	0	R	0	R
Ceftriaxone	20	I	30.2	S
Chloramphenicol	17.2	I	19.6	S
Gentamicin	27	S	21.6	S
Netilmicin	31	S	26.4	S
Nitrofurantoin	0	R	0	R
Pefloxacin	27	S	20.5	I
Trimethoprim Sulfamethoxazole	0	R	20.5	S

R: Resistant; I: Intermediate; S: Susceptible.

Regarding total soluble solids ($^{\circ}\text{Bx}$), variations were observed that could be related to the type of maguey, the characteristics of the semilla or initial inoculum used, and the fermentation time (Enrquez *et al.*, 2017; Medina *et al.*, 2023; Liborio *et al.*, 2024). Likewise, the decrease in $^{\circ}\text{Bx}$ values in samples that presented higher microbial counts could indicate greater sugar consumption and increased fermentative activity by lactic acid bacteria and yeasts (Escalante *et al.*, 2016; Prez & Cardoso, 2020).

The relationship between the presence of different microbial groups and the physicochemical parameters of pulque has been described as an important characteristic of fermentation-derived products, in which the microorganisms that develop play a fundamental role in the final quality of the product (Escalante *et al.*, 2016; Ruz *et al.*, 2023).

The results obtained for alcohol content indicate that the samples comply with the parameters established for fresh pulque in NOM-199-SCFI-2017, which establishes an alcoholic content range of 4% to 7.5% Alc. Vol. These values are consistent with other studies, such as those by Gmez *et al.* (2011) and Medina *et al.* (2023), who indicate that these concentrations depend on fermentable sugars and the population dynamics of the yeasts present.

The presence of aerobic mesophiles is associated with the natural fermentative process of pulque, with significant counts linked to the nature of the product, as occurs in other beverages obtained through fermentation such as tuba and tepache (De la Fuente *et al.*, 2015; Amazar & Quintero, 2022; Liborio *et al.*, 2024). It is important to note that the bacterial counts obtained in the present study were higher than those reported by Escalante *et al.* (2008) and Liborio *et al.* (2024), who evaluated the bacterial community of pulque produced in the state of Morelos and in San Agustn Tlaxiaca, Hidalgo, Mexico, respectively.

The predominant presence of lactic acid bacteria is consistent with studies reporting a greater abundance of this microbial group in pulque production compared with other evaluated microbial groups (Lappe *et al.*, 2023; Escalante *et al.*, 2016; Tamang *et al.*, 2016). Likewise, these studies highlight their role in product acidification, the development of sensory characteristics, and their potential probiotic properties (Escalante *et al.*, 2008; Ruz *et al.*, 2023; Giles *et al.*, 2024).

The yeast counts reported are consistent with Escalante *et al.* (2016) and Coln *et al.* (2025), who indicate that yeasts predominate during the intermediate and final stages of pulque fermentation. These microorganisms participate in the transformation of sugars present in aguamiel, thereby contributing to the production of ethanol, aromatic compounds, and secondary metabolites. In the same context, it has been reported that the metabolic activity of these microorganisms is related to changes in physicochemical parameters during the fermentation process (Chacn *et al.*, 2025).

In the present study, coliform bacteria were detected at low levels, which is consistent with other reports indicating that these bacteria, as they are not native to the fermentative process, may be introduced during the collection, storage, and commercialization of the product (Escalante *et al.*, 2008; Gmez *et al.*, 2011; lvarez *et al.*, 2020; Medina *et al.*, 2023; Liborio *et al.*, 2024). Likewise, several authors have reported that, as the

fermentation process progresses, factors such as changes in acidity, ethanol production, and microbial competition reduce the viability of these microorganisms (Gómez *et al.*, 2011; Jeon *et al.*, 2015).

The microbial communities present in pulque interact throughout the different stages of the production process, including crop development, collection, inoculation during fermentation, and transportation. Together, these microorganisms determine the microbiological quality and sensory characteristics of pulque (Álvarez *et al.*, 2020; Medina *et al.*, 2023).

On the other hand, coliform bacteria are not usually present in pulque, which may be attributed to its low pH and the metabolic activity of the microbial communities responsible for the fermentation process (Gómez *et al.*, 2011; Medina *et al.*, 2023). These factors create an adverse environment for enteropathogenic microorganisms, which may be inhibited by metabolites produced by bacteria and yeasts. Nevertheless, the introduction of undesirable microorganisms may affect the microbiological quality of the product, as they compete with beneficial microorganisms, alter the fermentation profile, and generate compounds with negative effects on the flavor, odor, and texture of the product (Escalante *et al.*, 2008; Jeon *et al.*, 2015). In this context, the detection of coliforms reinforces the need to improve hygiene practices during the production, handling, and commercialization of the product.

Phenotypic Identification

Biochemical tests showed profiles compatible with enterobacteria such as *Escherichia coli* and *Shigella flexneri*. These results are consistent with those reported by Cervantes and Pedroza (2007), who identified the presence of Gram-negative coccobacilli in their study and mentioned that the persistence of these microorganisms is associated with the lack of product standardization and inadequate hygiene practices during production. Similarly, Gómez *et al.* (2011) reported that *E. coli* O157:H7 may develop tolerance to acidic and alcoholic conditions in pulque, representing a potential health risk to consumers. In contrast, some studies suggest that the presence of pathogenic bacteria, particularly in aguamiel, is usually limited. An example is the work conducted by Gómez *et al.* (2011), who evaluated the fermentation process of aguamiel inoculated with five enteropathogenic strains (*Salmonella typhimurium*, *Escherichia coli*, *Shigella flexneri*, *Staphylococcus aureus*, and *Enterobacter aerogenes*) and observed that, under *in vitro* conditions, the development of these bacteria was inhibited. This inhibition has been attributed to the action of antimicrobial metabolites produced by lactic acid bacteria, which contribute to microbiological stability and reduce the risk of diseases associated with the consumption of this traditional beverage (Silva *et al.*, 2025). Conversely, Jeon *et al.* (2015) examined different fermented beverages and identified the survival of coliform bacteria. This highlights the importance of monitoring microbiological quality indicators together with traditional quality parameters.

Antimicrobial Susceptibility

The antimicrobial susceptibility profiles of strains isolated from pulque showed multidrug resistance in *E. coli* isolates and a lower proportion of resistance in *Shigella*.

This behavior is consistent with that reported by Oliveira *et al.* (2017), who, in their study on resistance to different antibiotics used to treat *E. coli* infections, highlighted the low effectiveness of ampicillin, amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole, nalidixic acid, and ciprofloxacin, with resistance rates higher than 50%. Sosa and Chapoñan (2022) also reported an evolutionary trend of resistance in *E. coli* to several antibiotics, including ampicillin, amoxicillin, cephalosporins, aminoglycosides (kanamycin), quinolones (ciprofloxacin), and sulfonamides (trimethoprim-sulfamethoxazole). Similarly, Duarte *et al.* (2021) identified comparable resistance patterns in *Shigella* spp., including resistance to trimethoprim-sulfamethoxazole, ciprofloxacin, and ampicillin.

The antimicrobial susceptibility patterns observed are of public health importance, since fermented foods may contain bacteria harboring resistance genes (Vinayamohan *et al.*, 2023). Likewise, several studies have indicated that antimicrobial-resistant bacteria may be transmitted from animals or plants to humans through agricultural production systems (Krahulcová *et al.*, 2022; Vinayamohan *et al.*, 2023; FAO, 2025).

The microbiological quality of pulque is determined by multiple factors, and currently there are no specific regulations in force regarding the safety of this beverage. This limits the establishment of standardized quality and safety parameters. Therefore, it is crucial to continue conducting studies of this type and to deepen microbiological analyses of pulque, evaluate production processes, and provide training to producers in order to generate information that may serve as a basis for establishing specific criteria.

CONCLUSIONS

The present study evaluated the physicochemical characteristics and microbiological quality of pulque produced in three municipalities of the State of Mexico: Temoaya, Jiquipilco, and Ocoyoacac. The results demonstrated that pulque complies with the physicochemical parameters established by Mexican regulations, as well as with the predominant presence of microorganisms characteristic of the fermentation process, such as lactic acid bacteria and yeasts. Nevertheless, the detection of coliforms with antibiotic resistance in some samples reveals potential deficiencies during collection, production, and commercialization processes. Therefore, it is necessary to implement good manufacturing and hygiene practices, as well as training programs directed toward producers and vendors. Further research addressing the microbiological diversity of pulque from the State of Mexico is recommended in order to strengthen its artisanal, cultural, and safety value as a traditional beverage.

ACKNOWLEDGMENTS

The authors express their gratitude to the master pulque producers from the municipalities of Temoaya, Jiquipilco, and Ocoyoacac for their collaboration. Their participation was fundamental for access to the samples and for understanding the context of artisanal pulque production. The authors also thank the Consejo Mexiquense de Ciencia y Tecnología (COMECyT) for the financial support provided through project COMECYT FICDTEM-2023-105.

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Perception of moronga consumption through food neophilia and food neophobia

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ABSTRACT

Objective: This study aims to analyze consumers' perceptions of moronga consumption through food neophilia and food neophobia.

Design/methodology/approach: An online questionnaire was administered to 276 consumers over 18 years of age. To analyze perceptions of moronga, the Free Word Association (FWA) technique was employed, and the results were organized into categories and dimensions. Likewise, the Food Neophobia Scale was applied to segment participants according to their level of neophilia or neophobia toward new foods. The data obtained were analyzed using the chi-square test. Finally, socioeconomic variables were incorporated, which were also evaluated with a chi-square test to identify perceptual differences between generations.

Results: Seven categories and twelve dimensions were obtained, revealing consumers' perceptions of moronga, the most representative being: gastronomic connections, dislike and mistrust, and animals. Although no significant differences were found between the groups, perceptions vary according to sociological generation. The results provided insights into perceptions of moronga and broadened the understanding of this food.

Limitations on study/implications: The study provides an empirical basis for informed decision-making regarding the marketing and promotion of traditional foods that may initially be rejected, highlighting the importance of considering generational differences in food perceptions.

Findings/conclusions: This study is original in its application of the theoretical framework of food neophilia/neophobia and the Free Word Association technique to a traditional food such as moronga, a topic rarely addressed in academic research. Its main value lies in revealing consumers' deep perceptions and segmenting them according to generational profiles, thereby offering an accurate diagnosis.

Keywords: Moronga, neophobia, neophilia, consumers, perception.

Citation: Ortega-González, C. I., Molina-Castillo, S., García-Rodea, L. F., González-Gutiérrez, B. (2026). Perception of moronga consumption through food neophilia and food neophobia. *Agro Productividad*. <https://doi.org/10.32854/05xry044>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: March 20, 2026.

Accepted: April 30, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 263-274.

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INTRODUCTION

In the context of globalization, contemporary food systems are characterized by increasing dietary homogenization driven by the food industry, which has displaced the consumption of numerous traditional and local foods. This phenomenon has eroded the



cultural and culinary value of these products, a process accelerated by factors such as intense competition with new ultra-processed foods, the lack of effective promotion of traditional alternatives, and the emergence of consumer-related psychological barriers, particularly food neophobia.

Food neophobia, defined as the rejection of or distrust toward new, unfamiliar, or infrequently consumed foods (Dovey *et al.*, 2008), functions as a perceptual filter influenced by learned eating practices and sensory expectations. This rejection has been documented in relation to a wide range of products, from wild edible mushrooms (Molina-Castillo *et al.*, 2022) and insects (Ramírez-Rivera *et al.*, 2025) to, most notably, offal and animal by-products (Bearth *et al.*, 2021).

A psychological construct closely related to food neophobia is food-related disgust sensitivity, understood as the tendency to experience aversion toward specific food-related cues (Egolf *et al.*, 2018). This factor constitutes one of the most powerful barriers to the consumption of certain foods, as products that are unpleasant in appearance, smell, or taste are unlikely to be accepted (Fischler, 1995; Harris, 2011). In this context, edible animal by-products, or offal, represent an emblematic case of this process of displacement and stigmatization (Bearth *et al.*, 2021). Historically, their consumption was associated with the need to make full use of available resources, minimize waste, and value the animal in its entirety, a practice deeply rooted in diverse cultures and social groups (Harris, 2011). However, in modern Western societies, these products have become progressively less popular and increasingly unfamiliar (Henchion *et al.*, 2016). Bearth *et al.* (2021) further confirmed that the willingness to consume such products is negatively associated with high levels of food neophobia and disgust sensitivity. Their findings also indicate that consumers perceive by-products from adult animals (beef cattle) more negatively than those from younger animals (veal), associating them with greater risks and stronger feelings of disgust, despite the fact that animal age is not necessarily related to food safety. Within this broad group of foods, moronga emerges as a paradigmatic product for study. This sausage embodies the tension between its value as a culturally rooted food and the rejection driven by negative associations and primary sensory aversions. This duality, in which a product is simultaneously perceived as a “delicacy” and as an “undesirable” food, has been widely examined in food anthropology (Harris, 2011). Such rejection can be understood through psychological mechanisms such as the “law of contagion” (Rozin *et al.*, 1987), whereby the essential properties associated with blood and viscera are perceived as being transferred to the food itself. This tension between tradition and modernity, as well as between the sacred and the taboo, makes moronga an ideal subject for analyzing the psychosocial factors that shape food consumption within a context of modern “gastro-anomie” (Fischler, 1995).

Therefore, the aim of this study was to analyze the reasons underlying the acceptance and rejection of moronga consumption among consumers, based on their sensory, emotional, and cultural perceptions. The study employed the Food Neophobia Scale as its main analytical framework while also considering the crucial role of disgust sensitivity and previous experiences. This research seeks to contribute to the understanding of the factors that determine the survival or decline of traditional foods within the modern food landscape.

MATERIALS AND METHODS

This exploratory study collected data through a questionnaire distributed via social media platforms (Espinoza-Ortega *et al.*, 2021). The instrument remained available for responses between February and April 2025. The questionnaire targeted Mexican individuals over 18 years of age with internet access. More than 70% of the Mexican population lives in urban areas and has access to the internet (INEGI, 2024). The questionnaire was divided into two sections. In the first section, the Free Word Association (FWA) technique was used to identify participants' perceptions of moronga. This qualitative and projective method employs an inducing term to elicit participants' immediate and spontaneous reactions (Guerrero *et al.*, 2010). The technique has proven to be effective and reliable for analyzing consumers' perceptions, attitudes, and opinions, which explains its widespread application in food-related research (Sánchez-Vega *et al.*, 2021; Rojas-Rivas *et al.*, 2022). Participants were asked to mention the first three words that came to mind when reading the word "moronga." In the second section, the Food Neophobia Scale proposed by Pliner and Hobden (1992) and adapted by Molina-Castillo *et al.* (2022) was used. This scale allows the analysis of consumers' willingness to accept or reject innovative, unfamiliar, or infrequently consumed foods (Pliner & Salvy, 2006). Responses were evaluated using a five-point Likert scale, where: 1=never; 2=almost never; 3=indifferent; 4=sometimes; and 5=always (Table 1).

Data analysis

The words obtained through the FWA technique were grouped into categories according to their similarity and synonymy, regardless of whether they had been mentioned by the same participant, and were subsequently regrouped into dimensions (Guerrero *et al.*, 2010; Rojas-Rivas *et al.*, 2019).

Once the scale had been completed, the scores for items 1, 4, 6, 9, and 10 were reverse-coded. Subsequently, the assigned values were summed to obtain a total score ranging from 10 to 50 points, following the procedure established by Pliner and Hobden (1992).

Table 1. Items of the Food Neophobia Scale.

Items
1. I am constantly trying new and different foods (I)
2. I am suspicious of foods that are unfamiliar to me
3. If I don't know a food, I reject it
4. I would like to try foods from different cultures (I)
5. Food from other cultures looks strange to eat
6. If I have the opportunity, I try new foods (I)
7. I am afraid of eating things I have never tried before
8. I'm very picky about the foods I eat
9. I am able to eat almost anything (I)
10. I would like to eat foods that are different from what I usually eat (I)

Source: Author's own elaboration based on Molina-Castillo *et al.* (2022) and Pliner and Hobden (1992).

Note: (I)=Inverted.

Likewise, quartiles were identified to classify consumers according to their level of food neophobia (Molina-Castillo *et al.*, 2025). The lower quartile included consumers with neophilic attitudes, the middle quartiles included consumers with intermediate attitudes, and the upper quartile included consumers with neophobic attitudes.

Once the consumer groups had been identified, the associations that each group established with moronga were analyzed based on the dimensions and categories derived from the FWA technique. The dimensions were analyzed according to neophilic, intermediate, and neophobic attitudes using the cell chi-square test, with the significance level set at 5% (Field, 2013). The results of the category analysis by consumer group were presented through correspondence analysis.

Subsequently, the chi-square test, Z-test, and Bonferroni method were used to identify statistically significant differences among the groups regarding socioeconomic characteristics, with the significance level established at 0.05. The information obtained was entered into an Excel database, and the statistical analyses were subsequently performed using SPSS version 24 and XLSTAT software.

RESULTS AND DISCUSSION

A total of 300 questionnaires were completed, of which 24 were excluded because the respondents were under 18 years of age. Therefore, the final sample consisted of 276 consumers aged between 18 and 67 years. Most participants were women (65.9%), belonged to Generation Z (59.4%), and had an undergraduate degree (67.8%).

Classification of neophilia and neophobia. The first stage of the study consisted of identifying the levels of food neophilia and food neophobia among participants. Consumers' responses regarding food acceptance or rejection were used to determine their position on the neophilia-neophobia scale, where the lowest score was 10 and the highest was 49.

Three groups of consumers were identified according to their levels of food neophobia. The first quartile (scores from 10 to 25) included neophilic consumers, representing 27.2% of the sample. The second quartile (scores from 26 to 32) comprised consumers with intermediate neophobia (50.7%), while the third quartile (scores from 33 to 48) included consumers with the highest levels of food neophobia (22.1%).

Free Word Association analysis

In the FWA task, a total of 728 words were mentioned, which were grouped according to synonymy, resulting in 12 categories linked to seven dimensions (Table 2). The most prominent dimensions were "Gastronomic Connections" (61.7%), "Dislike and Distrust" (13.0%), and "Animals" (9.5%) (Table 2).

The dimension "Gastronomic Connections" encompasses aspects such as culinary use, preparation methods, culinary identity, and ingredients, reflecting that consumers seek not only to satisfy a biological need, but also to establish a cultural and emotional connection with food (Brillat-Savarin, 1825).

The dimension "Dislike and Distrust" reveals consumers' aversion toward moronga, as well as their perception of it as an unfamiliar food, expressed through concepts such as disgusting, unpleasant, ugly, unknown, unusual, and strange. This rejection can be

Table 2. Associations Related to Moronga.

Dimensions	Categories	Main Words	Value (%)
Gastronomic connections			61.7
	Culinary use	Food, tacos, stew, rellena (mexican dish)	29.8
	Ingredients	Blood, tripe, meat, fat	25.0
	Methods of preparation	Sausage, cooking, frying	4.0
	Culinary Identity	Tradition, butchery, Mexico	2.9
Dislike and distrust			13.0
	Dislike	Disgusting, unpleasant, ugly	10.7
	Unknown	Unknown, unusual, curiosity, strange	2.3
Animals			9.5
	Links to animals	Pig, animal, sheep, offal	9.5
Sensory			4.7
	Color	Brown, black, red	3.3
	Flavor	Flavor, tasteless, insipid	1.4
Hedonic			4.7
	Taste	Tasty, flavorful, delicious	4.7
Others			4.3
	Others	Nothing, nickname	4.3
Blood			2.2
	Plasma	Clot, curd	2.2

explained through the theory of “culturally learned food aversions” (Rozin & Fallon, 1987), which proposes that individuals develop aversions toward certain foods primarily for symbolic and cultural reasons rather than for biological or sensory ones. Likewise, Hartmann and Siegrist (2018) highlight the impact of disgust and repulsion toward certain foods, ranging from basic human avoidance behaviors, such as food neophobia, to social norms in which certain foods, particularly those derived from blood or viscera, generate rejection for symbolic rather than sensory reasons (Fischler, 1988).

The dimension “Animals” refers to participants’ perceptions of moronga through concepts such as pig, animal, sheep, and offal. Vialles (1994) argues that the symbolic distance between the animal and the food is key to cultural acceptance. The use of terms such as viscera reflects what Douglas (1966) described as “matter out of place,” whereby certain animal parts generate rejection because they transgress cultural boundaries regarding what is considered appropriate for consumption.

The dimension “Sensory” refers to intrinsic attributes of moronga, such as flavor and color, with prominent associations including flavor, tasteless, and insipid. In this regard, Rozin and Fallon (1987) mention that some foods are rejected or accepted primarily because of their sensory characteristics, such as taste, smell, and appearance, as well as previous learning experiences and cultural contexts (Spence, 2017). According to Tan *et al.* (2016), flavor is an important predictor of food consumption; however, sensory liking alone is not sufficient for the acceptance of a novel food (Roininen *et al.*, 1999).

The dimension “Hedonic” includes pleasant associations expressed by consumers through concepts such as tasty, flavorful, and delicious. According to Hirschman and Holbrook (1982), hedonism is defined as the sensory, imaginative, and affective dimension that characterizes a person’s interaction with a product.

The dimension “Others” encompasses residual concepts such as nothing and nickname. Schmitt (2017) states that meanings vary according to cultural context, which may also be associated with foods.

The dimension “Blood” refers to plasma-related concepts such as clot and curd. According to Fiddes (2004), blood as food evokes religious and biological taboos, which influence its ambivalent perception.

Once the associations that participants had regarding moronga were identified, the associations of each group were analyzed according to their levels of food neophobia (Table 3).

It was observed that the Neophilic group primarily associated moronga with the dimension “Hedonic” and less frequently with the dimensions “Dislike and Distrust” and “Others.” The Intermediate group mainly associated it with the dimension “Others,” whereas the Neophobic group primarily linked moronga with the dimension “Dislike and Distrust.” These findings were corroborated through correspondence analysis (Figure 1), which showed that the Neophilic group was more closely associated with the category “Pleasantness” and less associated with “Others,” in contrast to the Intermediate group, whereas the Neophobic group showed greater proximity to the category “Disgust.”

Socioeconomic characteristics of the identified groups

The statistical analysis established that there were no significant differences in any of the socioeconomic characteristics among the identified groups (Table 4).

However, a greater presence of Generation Z consumers was observed among the Neophilic and Neophobic groups, whereas Millennials were more prevalent in the Intermediate group. In this regard, Rabadán and Bernabéu (2021) identified that levels of food neophobia tend to increase from middle age onward. Regarding gender, a slightly higher presence of women was observed in the Neophilic group. In this sense, several

Table 3. Cell Chi-Square Analysis of Dimensions by Consumer Group.

Dimension	Neophilics n=27.2%	Intermediate n=50.7%	Neophobic n=22.1
Gastronomic connections	64.8	63.0	55.1
Dislike and distrust	7.6 (-)	11.7	22.8 (+)
Animals	11.9	7.7	10.2
Sensory	3.8	5.4	4.2
Hedonic	8.6 (+)	3.7	1.8
Others	1.4 (-)	6.3 (+)	3.6
Blood	1.9	2.3	2.4

Note: (+) or (-) indicate that the observed values are higher or lower than those theoretically expected according to the Chi-square test per cell. Alpha value: 0.05.

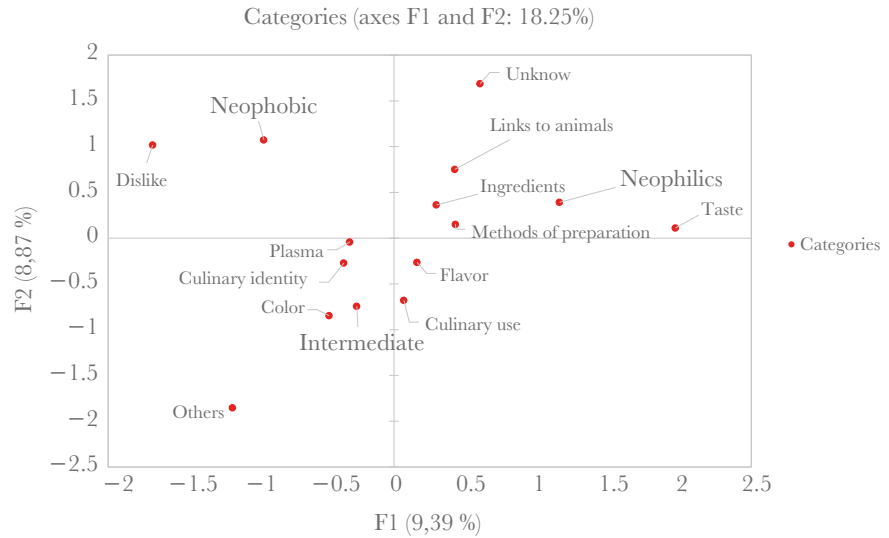


Figure 1. Correspondence Analysis of Groups and Categories.

Table 4. Socioeconomic Characteristics by Identified Group.

		Neophilics 27.2%	Intermediate 50.7%	Neophobic 22.1%	P
Gender					
	Male	32.0	35.0	34.4	.905
	Female	68.0	65.0	65.6	
Generation					
	Baby Boomer	1.3	1.4	0.0	.213
	Generation X	16.0	12.9	6.6	
	Millennial	17.3	31.4	29.5	
	Generation Z	65.3	54.3	63.9	
Education					
	Elementary school	1.3	1.4	0.0	.611
	Middle school	8.0	7.9	3.3	
	High school	16.0	21.4	13.1	
	College	70.7	62.9	75.4	
	Graduate school	4.0	6.4	8.2	
Marital status					
	Single	76.0	69.3	70.5	.686
	Married	24.0	28.6	27.9	
	Other	0.0	2.1	1.6	

Chi-square analysis: $p < 0.05$ and Z-test with Bonferroni correction.

studies have reported that socioeconomic characteristics such as gender and age influence levels of food neophobia (Flight *et al.*, 2003).

Schnettler *et al.* (2008) mention that, in the case of meat consumption in southern Chile, sociodemographic characteristics determine differences in both the frequency and type of

meat demanded by consumers. However, this perspective, which assigns a determining role to sociodemographic factors, remains debated in the literature. Some studies, such as that of Verbeke and Vackier (2004), support this view by identifying variables such as age and gender as significant predictors of consumption behavior, whereas other studies suggest that the explanatory power of these variables may be limited.

On the other hand, studies such as that of Barrena and Sánchez (2012) argue that sociodemographic factors, although relevant, frequently act as mediating variables, and that psychological aspects such as risk aversion and variety-seeking behavior, as well as lifestyles and environmental factors, possess stronger predictive power regarding food choice and food neophobia. Therefore, although there is consensus that sociodemographic variables exert an influence, their central and exclusive role has been questioned, pointing instead toward a more comprehensive and multifactorial explanatory model of consumer behavior.

Consumer groups and their perceptions of moronga

Neophilic consumers

This group was the second largest in the study and was composed mainly of individuals with university-level education. These consumers primarily associated moronga with hedonic aspects related to liking, highlighting concepts such as “tasty,” “flavorful,” and “delicious,” and to a lesser extent with the dimension “Dislike and Distrust.” For this group, consuming moronga does not represent a threat to their health.

The perceptions of this group are consistent with the literature linking higher educational levels to lower food neophobia (Flight *et al.*, 2003), as education is often associated with greater cultural curiosity and exposure to a wider diversity of foods. However, the results invite a deeper discussion. The predominance of hedonic associations suggests that risk perception is not a determining factor for neophilic consumers, partially contradicting models that portray neophilia simply as the absence of perceived danger.

Authors such as Pliner and Hobden (1992) propose that the willingness to try new foods is more closely linked to sensation seeking and anticipated hedonic experience than to a rational evaluation of risk. Therefore, this group does not ignore risk, but rather overrides it with the expectation of a positive sensory reward.

Furthermore, the overrepresentation of individuals with university-level education may also reflect greater cultural capital (Bourdieu, 1984), whereby the consumption of traditional or unusual foods becomes a form of social distinction and cultural appreciation, further enhancing the hedonic experience beyond taste itself.

Intermediate consumers

This group comprised the largest proportion of respondents and was mainly represented by women with university-level and high school education. The associations within this group tended toward the dimension “Others,” in which concepts such as nothing and nickname were mentioned. The predominant association with “nothing” or “nickname” suggests a profound cultural and cognitive disconnection from the product. For these respondents, moronga does not evoke a strong culinary, nutritional, or identity-related

meaning. The fact that it is perceived primarily as a “nickname” indicates that the term may persist in colloquial language while having completely lost its tangible referent and original gastronomic meaning.

The foregoing reflects a process of cultural erosion, in which a traditional food disappears from the practical and symbolic culinary horizon of a modern and educated segment of the population. The fact that this group is composed of individuals with higher education is particularly noteworthy. This distancing from local food traditions may exemplify how formal education and urbanization are correlated with a departure from certain culinary practices considered traditional or rural.

This finding contrasts with that observed in the other group studied, in which food neophobia (Tuorila *et al.*, 2001; Siegrist *et al.*, 2013) and active rejection associated with sensory characteristics such as blood (Bearth *et al.*, 2021; Llauger *et al.*, 2021) were the primary drivers of aversion. In this case, indifference predominates.

Neophobic consumers

This group, which represents the smallest proportion of respondents and is composed mainly of women, primarily associates moronga with disgust. Their perceptions are far removed from aspects such as culinary use or culinary identity. Furthermore, the presence of blood and raw meat is negatively associated with living animals and, therefore, generates rejection (Llauger *et al.*, 2021).

This disgust or rejection toward animal by-products, such as blood, is consistent with the findings reported by studies such as those of Bearth *et al.* (2021) and Llauger *et al.* (2021). This aversion may be partially explained by food neophobia, defined as the rejection of unfamiliar foods and associated with dietary and consumption choices (Tuorila *et al.*, 2001; Siegrist *et al.*, 2013).

The current context further reinforces this aversion. Edible animal by-products have become less popular and, consequently, more unfamiliar in the cuisines of Western societies; therefore, individuals with high levels of food neophobia may be less willing to engage with these animal-derived products (Henchion *et al.*, 2016). Although it has been demonstrated that the use of familiar carrier foods increases the acceptance of novel foods (Hartmann *et al.*, 2015), exceptions may occur when ingredient combinations are perceived as inappropriate (Stallberg-White & Pliner, 1999).

If presented separately, the discussion should focus on interpreting the results (without repeating them), considering their intrinsic characteristics, their consistency with the proposed hypothesis, and their similarities or differences in relation to findings from previously conducted studies. In all cases, the discussion should provide an explanation of the results beyond merely comparing them with similar studies.

CONCLUSIONS

This study made it possible to characterize consumers' perceptions toward foods derived from animal viscera and offal, using moronga as a case study. The results revealed the existence of three clearly differentiated consumer segments according to their level of food neophilia or food neophobia. The most prevalent profile corresponded to

intermediate consumers, who constituted the majority group and associated moronga with unrelated terms, reflecting a disconnection from the product. This group was followed by neophilic consumers, who associated moronga consumption with hedonic and pleasurable experiences. In contrast, the neophobic group, which represented the smallest proportion of respondents, exhibited a clear aversion and perception of disgust toward this product. It is noteworthy that no statistically significant differences were observed in the distribution of these groups according to sociodemographic variables such as age, gender, and educational level. This suggests that attitudes toward moronga and, by extension, toward meat by-products are more strongly influenced by psychological and cultural dimensions than by conventional demographic factors.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Consejo Mexiquense de Ciencia y Tecnología for funding the project CIKAS--FICDTEM-25-043.

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Market for non-traditional meats

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ABSTRACT

Objective: To determine the factors affecting the demand for non-traditional meats in the municipality of Iguala, Guerrero, Mexico.

Design/Methodology/Approach: A questionnaire was administered that included questions on sociodemographic and economic characteristics, as well as consumer tastes and preferences. A total of 511 surveys were conducted from February to July 2021 in the municipality of Iguala, Guerrero, Mexico. The information collected was used to perform a relative frequency analysis and to estimate a binary logistic regression model.

Results: The most consumed non-traditional meats were quail (26%), rabbit (23%), iguana (21%), deer (18%), and squab (12%). These meats were purchased mainly in local markets (45%), and their prices varied according to the place of purchase, time of year, and product presentation. The probability of consumption was determined primarily by price and gender. Consumption of these non-traditional meats was occasional and took place within a poorly developed regional market, although the products showed commercial potential.

Limitations of the Study/Implications: Further research is needed on the nutritional contributions of these meats to human health, as well as on their quality, in order to ensure public health.

Findings/Conclusions: The main limitations preventing these meats from achieving a greater market share are limited knowledge of their nutritional and health benefits, the lack of commercial dissemination strategies, and insufficient market availability to enable their integration into consumers' daily diets.

Keywords: non-traditional meats, demand, consumers.

Citation: Espinosa-Rodríguez, M., Antúñez-Ocampo, O. M., Sabino-López, J. E., Delgado-Núñez, E. J., Ortega-Acosta, S. A., & Palemón-Alberto, F. (2026). Market for non-traditional meats. *Agro Productividad*. <https://doi.org/10.32854/kz69xc65>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: January 15, 2026.

Accepted: April 14, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 275-283.

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INTRODUCTION

The human diet consists of plant-based foods and foods of animal origin, including meat, which is a source of nutrients and protein whose contributions vary by type. Meat consumption in Mexico is high but disproportionate. In 2022, the highest *per capita* meat consumption was chicken (35.9 kg), followed by pork (21.1 kg), beef (15.2 kg), and, to a lesser extent, goat (301 g) and sheep (531 g) (Panorama Agroalimentario, 2023). This increase in demand has led to meat imports from other countries to meet domestic consumption, which affects national producers, raises consumer prices because of higher transportation costs, and has negative effects on animal welfare and the environment (La Gra *et al.*, 2016).



In Mexico, the market for traditional meats (beef, chicken, and pork) is concentrated in a small number of vertically integrated firms that, in oligopolistic fashion, control most of the production chain. This structure has resulted from the growth and modernization of these firms under public policies characterized by minimal government intervention (UNCTAD, 2013). Likewise, meat production in Mexico is classified according to the technology used, the levels of vertical and horizontal integration, and the markets served into three production systems: technified, semi-technified, and backyard. These systems, in turn, affect production costs and, ultimately, consumer prices at the end of the marketing chain (Sánchez and Salazar, 2016).

On the other hand, population growth and rising incomes increase demand for foods with greater added value, including relatively unfamiliar products whose introduction into the market requires a strong commercial strategy that highlights their advantages and benefits (Brambila, 2011). According to Tomek and Kaiser (2014), demand for a product is determined by its price; the prices of substitute and complementary goods; population size; per capita income; promotion; and consumer tastes and preferences. These factors can alter the behavior of the meat sector and create opportunities for new types of meat within the agrifood system. In this regard, there are alternative meat sources from backyard and even wild species (*i.e.*, quail, squab, rabbit, iguana, and deer), which provide consumers with alternative sources of protein that may offer greater health benefits and lower environmental impact at more affordable prices (Arcos-García *et al.*, 2010; SADER, 2019).

In this context, quail (*Coturnix coturnix*) exhibits early maturity, high egg production, and favorable productive performance (Vásquez and Ballesteros, 2007). Another option is squab (*Columba livia*), which can be raised in small spaces and has market potential (Meluzzi and Milandri, 1988). Rabbit (*Oryctolagus cuniculus*) is also a species whose consumption has increased; its meat is low in cholesterol and fat, high in protein, easily incorporated into a healthy and balanced diet, and suitable for all population groups (SADER, 2019). Similarly, the green iguana (*Iguana L.*) is a source of protein and is associated with medicinal attributes (Arcos-García *et al.*, 2010). In turn, deer (*Odocoileus virginianus*) is the most important game species in Mexico and North America, and its production is considered suitable for local and regional agricultural systems (Villareal *et al.*, 2011).

Although a trend toward alternative species has begun to emerge, including rabbit meat (Jaramillo *et al.*, 2015), iguana (Granados *et al.*, 2019), deer (García-Flores *et al.*, 2021), ostrich (Aguilar and Medina, 2013), and quail (Shiroma, 2021), these meats are still undervalued because they are perceived as having lower nutritional value and quality than traditional meats (Polawska *et al.*, 2012). Therefore, the objective of this study was to determine the factors influencing the demand for non-traditional meats in the municipality of Iguala de la Independencia, Guerrero, Mexico.

MATERIALS AND METHODS

The study was conducted in the municipality of Iguala de la Independencia, located in the northern region of the state of Guerrero, Mexico, at the geographic coordinates of 18° 20' 41" N and 99° 32' 34" W, at an altitude of 720 m (Gobierno del Estado de Guerrero,

2015). The sample size was calculated using the simple random sampling formula for finite populations proposed by McDaniel and Gates (2016).

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

where: N =total population of the study universe: 154,173 (INEGI, 2020); n =sample size; p =estimated proportion of positive variability (50%); q =estimated proportion of negative variability (50%); d =permitted estimation error (5%); and Z =value corresponding to the Gaussian distribution at a 95% confidence level ($Z=1.96$).

Substituting the values, $n = \frac{(154173)(1.96)^2(0.5)(0.5)}{(0.05)^2(154173-1) + (1.96)^2(0.5)(0.5)} = 383$

The sample size was 383 individuals; however, 511 surveys were conducted from February to July 2021. Respondents were selected at random and included individuals over 18 years of age. The questionnaire included sociodemographic items in the first section and questions on tastes and preferences in the second. The information collected was analyzed using relative frequency analysis in Excel[®] 2010, and a regression model was estimated using SPSS (Statistical Package for the Social Sciences[®]), version 21. Because the dependent variable was dichotomous, a binary logistic regression model was used to determine the probability of occurrence of the event of interest. Model fit was assessed using the Hosmer-Lemeshow test. The impact of each independent variable on the dependent variable was evaluated using the p value and the Wald statistic, as indicated by Pérez (2004).

RESULTS AND DISCUSSION

Sociodemographic characteristics of the respondents

Sixty-five percent of the respondents were women and 35% were men, ranging in age from 20 to 50 years. Regarding occupation, 42% were homemakers, 36% were public servants, 15% owned their own businesses, and 7% worked in the private sector. In terms of educational level, 38% had completed university studies, 25% had completed upper secondary education, 21% had completed secondary school, 12% had completed primary school, and 4% reported other levels of education. With respect to monthly income, 83% of the respondents reported earnings between MXN 5,000 and 10,000; 11% reported between MXN 10,001 and 15,000; and 6% reported incomes above MXN 15,001.

Cáffaro *et al.* (2018) reported that the socioeconomic and demographic factors influencing preferences for the consumption of traditional meats are related primarily to age and, to a lesser extent, sex, as well as place of residence and employment status. Thus, consumers of these meats are mainly women between 20 and 50 years of age, with monthly incomes ranging from MXN 5,000 to 10,000, who prefer meats that are tasty, easy to prepare, nutritious, and beneficial to their families' health. This finding is consistent with

Fasone and Privitera (2002), who indicated that consumers of non-traditional meats are individuals between 41 and 50 years of age, mostly women, who focus on purchasing safe meats with high nutritional value and good flavor, preferably from farm animals; however, they tend to have medium to high income levels.

Purchasing behavior characteristics

Quail meat (26%) was the most frequently consumed, whereas squab meat was the least consumed (12%) (Figure 1a). The main attributes consumers value when purchasing these meats are good flavor (38%) and, to a much lesser extent, price (5%) (Figure 1b). However, the attributes valued in this type of meat vary by species, although they are all primarily associated with flavor, perceived health benefits, and ease of preparation (Table 1). Therefore, the commercial strategy should focus on highlighting these attributes.

In this regard, Barragán *et al.* (2018) indicated that the decisive factors for consumers at the time of meat purchase are meat color and place of purchase. Likewise, Joe *et al.* (2013) reported that the determining factor in meat purchase is color, as it is associated with freshness and nutritional value. Similarly, Enciso and Bukart (2017) noted that 37.2% of consumers include chicken meat in their diet because of its health benefits, 21.5% because of its flavor, 12.2% because of its ease of preparation, 7.5% because of its price, and 21.6% because of other characteristics. In the same study, beef consumption was reported to be based on flavor (48.6%), habit (12.8%), ease of preparation (11.9%), variety of preparation (8.2%), and other characteristics (18.5%). These same authors also indicated that the consumption of pork and fish is primarily based on attributes related to flavor (44% and 50%), health benefits (26% and 30%), and nutritional quality (12% and 16%), respectively.

Place of purchase is determined by consumers' income level. High-income consumers tend to purchase meat products in shopping centers, whereas middle-income consumers buy them from butcher shops in regional or municipal markets (Arana *et al.*, 2012). In this regard, these meats are mainly purchased in local public markets (45%) (Figure 2a).

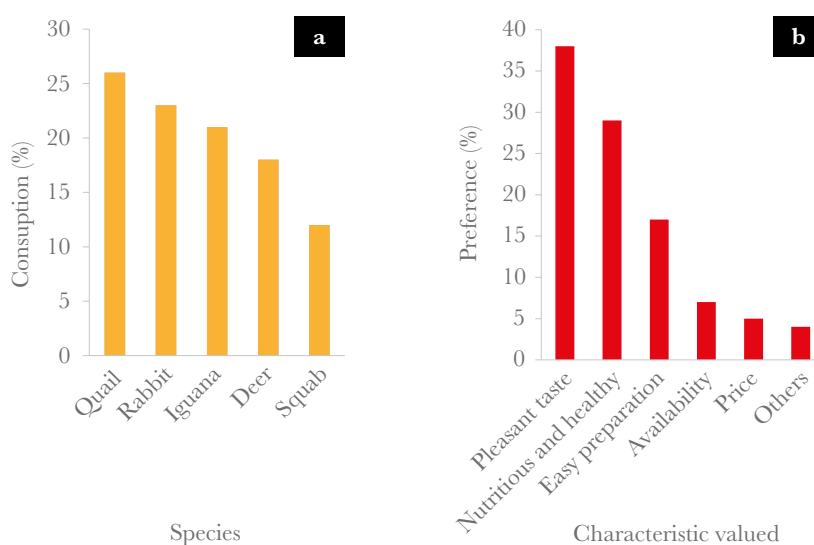


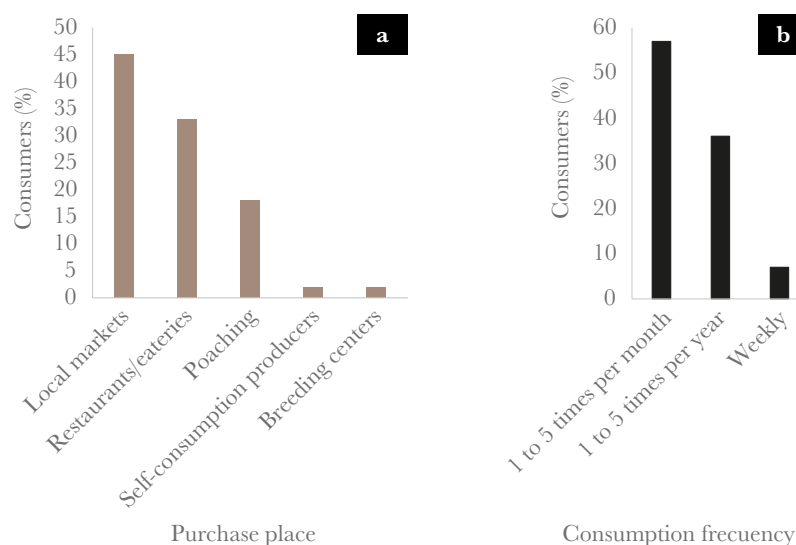
Figure 1. (a) Consumption preference; (b) Attributes valued in the purchase of non-traditional meats.

Table 1. Attributes valued in the purchase of non-traditional meats.

Species	Order of importance		
	1	2	3
Deer	Flavor	Nutrition and health	Ease of preparation
Squab	Flavor	Ease of preparation	Nutrition and health
Iguana	Flavor	Nutrition and health	Ease of preparation
Quail	Ease of preparation	Flavor	Nutrition and health
Rabbit	Flavor	Nutrition and health	Ease of preparation

These meat products come from farms and street vendors in communities near this municipality, mainly Cocula and Metlapa, as well as from small producers, organizations, and agricultural institutions located within the municipality itself. On the other hand, 18% of consumers reported obtaining these products through poaching, particularly in the case of deer and iguana meat. In this context, Joe *et al.* (2013) reported that the places where these types of meat are sold depend on consumer demands, as consumers seek safety and confidence that the product is harmless and complies with sanitary regulations. However, Enciso and Bukart (2017) indicated that the place of purchase varies according to species; for example, chicken is commonly purchased in traditional markets, whereas beef and pork purchases are more concentrated in supermarkets. Other authors have reported that most consumers purchase these meats from retail outlets (Cáffaro *et al.*, 2018).

With respect to consumption frequency, 57% of the respondents consume this type of meat one to five times per month (Figure 2b), which is consistent with Cáffaro *et al.* (2018), who reported that these meats are generally consumed once per month. These products are purchased by the kilogram (38%), as carcasses (32%), live (13%), and, to a lesser extent, by the piece (17%). In contrast, among traditional meats, chicken shows the

**Figure 2.** (a) Places of purchase; (b) Frequency of consumption of non-traditional meats.

highest level of consumption (47%), followed by beef (41%) and pork (12%), although the frequency of consumption is the same for all three types (three times per week) (Arana *et al.*, 2012). This pattern differs from the findings of Enciso and Bukart (2017), who reported that beef consumption (75.2%) exceeds that of chicken (63.7%), pork (56.5%), and fish (49.1%), all of which are consumed one to three times per week.

Prices vary according to species and form of purchase (Table 2). The results showed that squab, iguana, and quail meat are commonly purchased as carcasses, whereas rabbit and deer meat are more frequently purchased by the kilogram; deer meat is sometimes also purchased by the piece. Quail and squab meat are marketed in carcass packages; for example, 10 dressed quail cost MXN 220, 6 dressed quail cost MXN 135, and 4 dressed squabs cost MXN 215.

In this regard, Retana and Padilla (2018) reported that the price of deer meat in the subsistence market is around MXN 85 per piece, whereas in Wildlife Management Units (UMAs) it may reach MXN 220. In the same context, the price of iguana ranges from MXN 25 to 50 when sold live, depending on size; however, in the legal market, its price ranges from MXN 150 to 250 during periods of higher demand, such as Easter season (Orozco, 2019). This suggests that price is one of the most important factors determining demand, together with consumer income and the price of substitute goods (Cruz and Sánchez, 2014). In contrast, the main problem in the commercialization of deer and iguana is poaching, which causes the consumer market price to be lower than that of legal breeding operations. Price is therefore a determining factor in purchase decisions among low-income consumers (Arana *et al.*, 2012).

On the other hand, the reasons for not consuming this type of meat are as follows: (1) lack of legalization for the sale of meat, particularly in the case of deer and iguana; (2) failure to comply with certified quality standards, such as Federal Inspection Type (TIF), ISO standards, standards established by the Ministry of Agriculture and Rural Development, México Calidad Suprema, and other certifications; (3) lack of packaging with nutritional information; (4) limited knowledge of product traceability and production standardization; (5) insufficient dissemination, advertising, and promotion; (6) lack of year-round availability; (7) limited points of sale and distribution channels; (8) unaffordable prices, particularly in the case of deer meat; (9) limited awareness of the benefits to human health; (10) lack of assurance of compliance with sanitary regulations; and (11) limited knowledge of how to prepare dishes using these meats.

Table 2. Price of non-traditional meats in Iguala, Guerrero, México.

Specie	Carcasses	Kilogram	Live	Piece (\$)
	\$ MXN			
Deer	25-80	30-130	30-100	ND
Squab	ND	80-400	ND	50-900*
Iguana	35-80	25-100	20-30	ND
Quail	50-250	80-300	50-120	ND
Rabbit	70-130	50-120	80-150	ND

*Price variation according to the cut or body part of the animal. ND: Not detected. MXN: Mexican pesos.

Similar reasons were reported by Ruano (2011), who stated that the production and limited commercialization of non-traditional meats (*e.g.*, deer meat) are due to the lack of marketing plans, insufficient financial support for producers, inadequate management systems, limited or nonexistent producer training, lack of reforms to the legal and institutional framework governing the use of meat and breeding stock, and limited added value in the final product. In contrast, Arana *et al.* (2012) argued that meat consumption is related to food safety attributes valued by consumers, including meat handling at the point of sale, slaughter date, and the animal's disease history.

Logistic regression statistical model

According to the Hosmer-Lemeshow statistic, the estimated model produced a value of 0.72. Likewise, the estimators with values greater than zero (education, income, age, and gender) increased the probability of purchase (Table 3). In other words, for each additional year of education, the odds of purchase increased by 1.015 times, or 1.5%; for each one-unit increase in income, the odds of purchase increased by 1.082 times, or 8.2%; and for each additional year of age, the odds of purchase increased by 1.009 times, or 0.9%. In addition, the *p* values and the Wald statistic indicated that the factors with the greatest statistical significance for the probability of consumption were price (0.000) and gender (0.020).

Table 3. Parameter estimates obtained using the logistic procedure.

Parameter	Estimate	Standard error	Wald	p-value	Odds ratios
Intercept	20.016	20007.8	0.000	0.999	
Education	0.015	0.031	0.239	0.625	1.015
Income	0.079	0.219	0.128	0.720	1.082
Price *			115.076	0.000	
Price 1	-21.755	20007.8	0.000	0.999	0.000
Price 2	-19.060	20007.8	0.000	0.999	0.000
Price 3	-17.101	20007.8	0.000	0.999	0.000
Age	0.009	0.010	0.857	0.355	1.009
Gender	0.580	0.249	5.422	0.020	1.786

*Price 1: de \$1-100 MXN; Price 2: \$101-200 MXN; Price 3: \$201-300 MXN; MXN: Mexican pesos.

The statistical values are considered acceptable, in accordance with Pérez (2004). Likewise, the observed signs that explain the direction of each variable are consistent with economic theory, indicating that as education, income, and age increase, the probability of consumption also increases, whereas as the selling price increases, consumption decreases (Tomek and Kaiser, 2014).

CONCLUSIONS

The consumption of non-traditional meats in Iguala, Guerrero, is characterized as occasional, with a regional and underdeveloped market. This situation is due, on the one

hand, to the limited availability of these meats and, on the other, to the lack of commercial strategies that highlight their nutritional attributes and health benefits in order to increase consumption. Price and gender are the factors that most strongly determine the probability of consuming these non-traditional meats.

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Socio-productive characterization of peanut (*Arachis hypogaea* L.) producers in Chiapas, Mexico

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ABSTRACT

Objective: To characterize the production conditions, main constraints and willingness of small-scale peanut producers in Mérida, Cintalapa, Chiapas, regarding the potential utilization of peanut shells as a by-product.

Design/methodology/approach: A quantitative, non-experimental, cross-sectional study with descriptive and exploratory aims was conducted. Data were collected using a local census approach, including all active peanut producers identified in the community (n=14). Structured surveys were used to gather information, which was analyzed using descriptive statistics, Spearman's rank correlation, the Mann-Whitney U test and Fisher's exact test.

Results: Producers operated on a small scale, with a predominance of family-based learning and self-saved seed use. Drought, pests, and economic problems were the main constraints affecting the system. The cultivated area and farming experience were positively associated with production but negatively associated with yield. This indicates that production depends more on expanding the cultivated area than improving efficiency within it. Although most producers expressed an interest in marketing peanut shells, collective organization remained limited.

Research limitations/implications: The study focused on the 14 producers who make up the local peanut-producing population in the Mérida community. Despite its local scope, the findings provide valuable empirical evidence for understanding the conditions, constraints, and organizational restrictions of small-scale farming.

Findings/conclusions: Peanut production in Mérida is sustained by small-scale units characterised by family-based learning and the predominant use of self-saved seeds. Under this production logic, production volume depends more on expanding the area than improving yield. The system's efficiency and the potential for using peanut shells as a by-product are limited by the identified constraints in production and organization.

Keywords: family farming; crop yield; productive organization; by-product valorization; Chiapas.

Citation: Domínguez-Castañón, L.M., Guevara-Hernández, F., La O-Arias, M.A., Cadena-Iñiguez, P., Gómez Padilla, E.J. (2026). Caracterización socioproductiva de productores de cacahuate (*Arachis hypogaea* L.) en Chiapas, México. *Agro Productividad*. <https://doi.org/10.32854/jzrwft31>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: January 23, 2026.

Accepted: April 29, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 285-296.

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INTRODUCTION

Peanut (*Arachis hypogaea* L.) cultivation constitutes a substantial component of Mexico's agricultural sector, encompassing both commercial and subsistence farming

methodologies. The nation cultivates peanuts across roughly 47,500 hectares, generating over 70,000 tonnes, with an average yield of approximately 1.75 tonnes per hectare (SIAP, 2024). This underscores the crop's significance within diverse agricultural contexts. In Chiapas, peanuts continue to be a vital agricultural commodity in numerous locales, serving as a critical income source for rural agriculturalists.

Cintalapa de Figueroa is one such municipality where peanut production is integral to local economic strategies (SIAP, 2024; Estudillo Pérez, 2021).

In small-scale farming contexts, peanut cultivation is an economic activity sustained by local knowledge, family-based learning, and inherited management strategies. In this region, peanuts are mainly cultivated by family farming units as part of broader crop diversification and peasant household reproduction arrangements, often alongside other regionally important crops such as maize.

Conversely, peanut cultivation is subject to a range of limitations, encompassing drought conditions, pest infestations, inadequate resource availability, inefficiencies in post-harvest handling, and a lack of cohesive organizational structures. These challenges can impede the efficacy of the crop and restrict opportunities for diversification and value enhancement within production entities (Liverpool-Tasie *et al.*, 2020; Fierros & Ávila-Foucat, 2017; Mouratiadou *et al.*, 2024).

Beyond the edible part of the peanut plant, its cultivation produces by-products, particularly peanut shells, whose potential uses have been somewhat overlooked locally. Recent studies indicate that these materials have practical and technological applications in fields such as manufacturing, environmental protection, and agriculture (Capanoglu *et al.*, 2022).

Peanut shells have been proposed for various applications, including the creation of biochar to improve soil, the production of adsorbent materials, and other uses that are beneficial in terms of productivity, the environment, or agro-industry (Liu *et al.*, 2019; Toledo-Jaldin *et al.*, 2023; Gerber & Roberts, 2024; Sawargaonkar *et al.*, 2024).

These alternatives suggest that peanut shells could be seen as more than just agricultural waste, but also as by-products with potential for value creation.

Despite the productive relevance of peanut cultivation and the potential uses reported for peanut shells, local empirical evidence remains limited regarding the conditions under which small-scale producers operate, the constraints affecting their production systems, and the organizational context that could support the use of this by-product. In small-scale rural territories, the potential use of peanut shells depends not only on the availability of technical alternatives but also on production conditions, producers' knowledge, management practices, and organizational capacity. Therefore, before proposing strategies for the valorization of peanut shells, it is necessary to understand the production context, the main constraints faced by farmers, and their willingness to consider possible uses for this by-product.

Therefore, this study aimed to describe the production methods, main challenges, and willingness of small-scale peanut farmers in Mérida, Cintalapa de Figueroa, Chiapas, to use peanut shells.

MATERIALS AND METHODS

Study design

The study adopted a quantitative, non-experimental, cross-sectional design with descriptive and exploratory objectives. Nonparametric bivariate analyses were employed to identify correlations among productive, technical and organizational variables. The study also had an applied focus, seeking to generate empirical evidence on peanut production conditions and the willingness of small-scale family farmers to use peanut shells. The analysis was based on information obtained directly from active producers in the community via a census survey conducted once during the 2023-24 agricultural cycle.

Study area and study population

The study was conducted in the community of Mérida in the municipality of Cintalapa in the state of Chiapas in Mexico (see Figure 1). This rural area is characterized by peanut cultivation, which is an important activity for small-scale producers, primarily under traditional management schemes and on relatively small plots. The area's agroecological and socioeconomic conditions make it pertinent to analyze production constraints, management practices, and the potential use of agricultural by-products.

The reference population consisted of all active peanut producers identified in the community during the study period. A total of 14 producers were recorded; therefore, data collection covered the entire available study population.

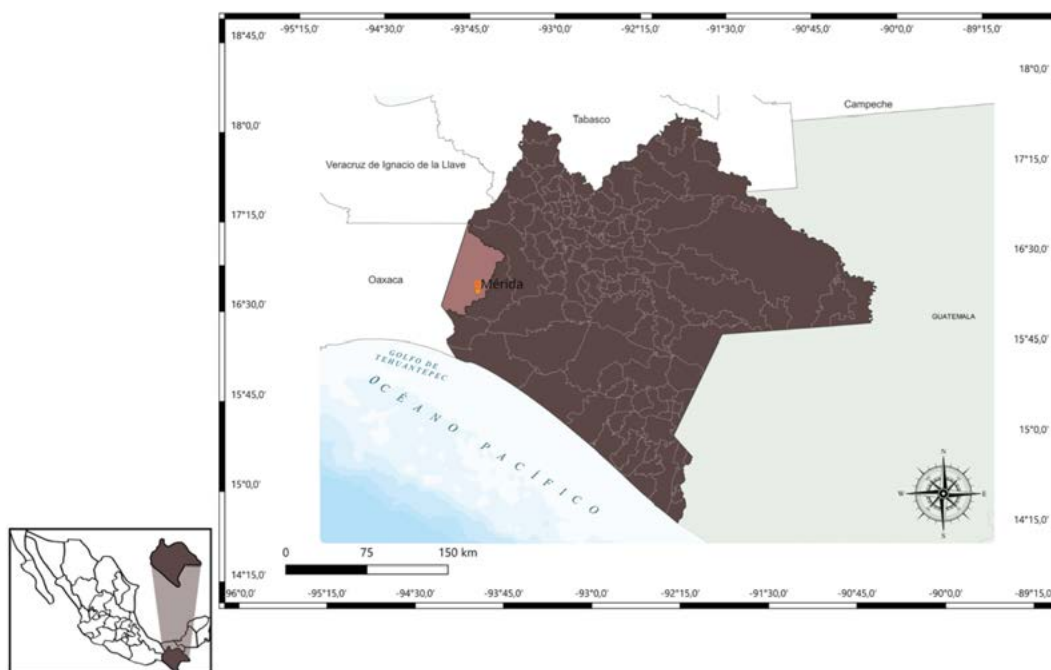


Figure 1. Location of Mérida community, Cintalapa municipality, Chiapas, Mexico. Source: Elaborated by the authors based on INEGI (2024).

Variables, survey instrument, and data collection

Information was obtained through a structured survey administered directly to the producers. The instrument included questions on productive characteristics, management practices, system constraints, and aspects associated with knowledge and the potential use of peanut shells. Responses were subsequently organized into a producer-level data matrix. Variables were grouped into four dimensions: productive, technical and management, system constraints, and organizational and utilization-related (Table 1). This organization made it possible to structure the analysis consistently with the study objective and to facilitate interpretation of the results.

The productive dimension included farming experience, expressed in years; cultivated area (ha); production (t); and yield, calculated as the ratio between production and cultivated area ($t\ ha^{-1}$). The technical and management dimension comprised variables associated with the use of organic fertilizer, pesticide use, fertilizer use, product grading, training, and type of storage. The system constraints dimension included the presence of pests, drought, economic problems, labor shortage, and a derived variable termed total number of problems, obtained by summing the main reported constraints. Finally, the organizational and utilization-related dimension included variables related to the source of learning, seed source, satisfaction with the activity, nutritional knowledge of the crop, knowledge of peanut shell uses, interest in marketing peanut shells, and attempts to form a cooperative.

To facilitate the analysis and reduce category dispersion in a small dataset, some categorical variables were recoded into more synthetic groups. Learning sources were grouped into two categories: family-based and non-family-based. Nutritional knowledge was reorganized into low and high knowledge. Satisfaction was grouped into low and high. Storage was regrouped into warehouse and household storage. These recodings allowed for clearer description and more consistent analytical organization.

Data were collected through direct contact with producers during the study period, and the information was reviewed and cleaned before statistical analysis. The final data matrix consisted of 14 observations and the variables selected for the study.

Table 1. Analytical dimensions and variables.

Dimension	Variables
Productive	Farming experience, cultivated area, production, and yield
Technical and management	Use of organic fertilizer, pesticide use, fertilizer use, product grading, training, and storage
System constraints	Presence of pests, drought, economic problems, labor shortage, and total number of problems
Organizational and utilization-related	Learning source, seed source, satisfaction, nutritional knowledge, knowledge of peanut shell uses, interest in marketing peanut shells, and cooperative attempt

Note. The productive dimension included continuous quantitative variables; in the remaining dimensions, dichotomous categorical variables predominated. Learning source, nutritional knowledge, satisfaction, and storage were recoded into synthetic groups.

Statistical analysis

Descriptive statistics were calculated for quantitative variables using measures of central tendency and dispersion, whereas absolute frequencies and percentages were obtained for categorical and binary variables. These analyses were used to characterize the producers' productive, technical, and organizational profile.

Associations among quantitative variables were explored using Spearman's rank correlation coefficient, which is appropriate for small samples and for data that do not require strict normality assumptions (Conover, 1999). The relationships examined included cultivated area with production, cultivated area with yield, farming experience with production, farming experience with yield, and total number of problems with yield.

To compare yield between two groups defined by dichotomous management practices, the Mann-Whitney U test was used, given the small sample size and the nonparametric nature of the data. For each comparison, the number of observations per group (n), median yield, the U statistic, and the significance level (p) were reported (Conover, 1999).

The associations between binary categorical variables were assessed using Fisher's exact test, which is appropriate for contingency tables with small samples and low expected frequencies (Agresti, 2002). Specifically, the association between pest presence and pesticide use was analyzed. Other variables related to peanut shell utilization and productive organization were analyzed descriptively because of the marked imbalance in response distribution.

RESULTS AND DISCUSSION

Productive profile of farmers

Peanut producers in the community of Mérida had an average of 24.64 years of farming experience, with a median of 21.00 years and a range from 5.00 to 60.00 years, reflecting the coexistence of both relatively recent and long-established farming experience among producers (Table 2).

These results indicate that peanut cultivation is part of a locally rooted productive tradition, although the degree of accumulated experience varies among farmers. Cultivated area had a mean of 1.76 ha and a median of 1.65 ha, with values ranging from 1.00 to 3.40 ha, confirming that the system operates through small-scale production units. Consistent with this, average production was 1.70 t per farmer, with a median of 1.66 t and a range

Table 2. Descriptive statistics for quantitative variables recorded in peanut farmers from Mérida, Cintalapa, Chiapas, Mexico.

Variable	Mean	Median	Standard deviation	Min-max
Farming experience (years)	24.64	21.00	15.79	5.00-60.00
Cultivated area (ha)	1.76	1.65	0.64	1.00-3.40
Production (t)	1.70	1.66	0.37	1.10-2.33
Yield ($t\ ha^{-1}$)	1.00	1.03	0.13	0.69-1.18
Total number of problems	2.29	2.00	0.91	1.00-4.00

Min-max = minimum-maximum.

of 1.10 to 2.33 t, indicating a limited productive base consistent with peasant farming schemes.

Average yield was 1.00 t ha⁻¹, with a median of 1.03 t ha⁻¹ and relatively limited variation among farmers (0.69-1.18 t ha⁻¹), indicating that, despite differences in experience and plot size, performance per unit area remained within a relatively homogeneous range. Finally, the total number of problems had a mean of 2.29 and a median of 2.00, with values ranging from 1.00 to 4.00, showing that farmers face recurrent constraints, although with variable intensity.

Socio-productive, organizational, and knowledge-related characteristics

From a socio-productive perspective, learning of the farming activity showed a clear predominance of the family component, as 78.6% of the farmers reported having acquired this knowledge within the household, whereas 21.4% reported a non-family source of learning. Consistent with this pattern, 92.9% used self-saved seed, evidencing marked autonomy in seed supply and low dependence on external sources (Table 3).

This pattern is consistent with what has been documented for other peasant farming systems in the Frailesca region, where productive reproduction relies on the family-based management of knowledge and the persistence of local materials. It is also consistent with findings on informal seed systems among small-scale producers, in which the selection, conservation, and use of self-saved seed form part of a management logic grounded in local experience (Hlatshwayo *et al.*, 2021).

In contrast, nutritional knowledge of the crop was predominantly low, as 78.6% of the farmers fell into this category, indicating that the productive relevance of peanut does not

Table 3. Frequencies and percentages of socio-productive, organizational, and knowledge-related variables in peanut farmers.

Variable	Category	sample (n)	Value (%)
Learning source	Family-based	11	78.6
	Non-family-based	3	21.4
Seed source	Self-saved	13	92.9
	Purchased	1	7.1
Nutritional knowledge	Low	11	78.6
	High	3	21.4
Satisfaction with the activity	High	12	85.7
	Low	2	14.3
Storage	Household	7	50.0
	Warehouse	7	50.0
Knowledge of peanut shell uses	Yes	11	78.6
	No	3	21.4
Interest in marketing peanut shells	Yes	13	92.9
	No	1	7.1
Attempt to form a cooperative	Yes	1	7.1
	No	13	92.9

necessarily translate into broad appropriation of its food-related attributes or its valorization potential. Likewise, 85.7% reported a high level of satisfaction with the activity, suggesting that, even in small-scale production units, the crop retains practical value and continuity within local productive experience.

Regarding postharvest conditions and by-product use, storage was evenly distributed between household storage and warehouse storage (50.0% in each case). In addition, 78.6% of the farmers reported knowledge of possible uses of peanut shells, and 92.9% expressed interest in marketing them. However, only 7.1% indicated having attempted to organize into a cooperative.

These results indicate a willingness to use the by-product, although this possibility rests on a limited organizational base, a situation consistent with what has been described in groundnut value chains, where weak articulation among producers constrains value addition and collective marketing (Posey *et al.*, 2024).

Management practices and production constraints

Beyond socio-productive and organizational traits, crop dynamics are also expressed through the management practices used and the constraints that farmers face during the production cycle. For this reason, a set of technical and constraint-related variables was examined to identify the main factors shaping the practical functioning of the peanut production system (Figure 2).

In terms of management, pesticide use was the most frequent practice, reported by 78.6% of the farmers, followed by product grading and training, both at 64.3%. In contrast, the use of organic fertilizer (35.7%) and synthetic fertilizers (28.6%) was less common. This pattern suggests a management approach oriented more toward phytosanitary control and activities related to product conditioning or commercial improvement than toward strengthening the nutritional status of the crop.

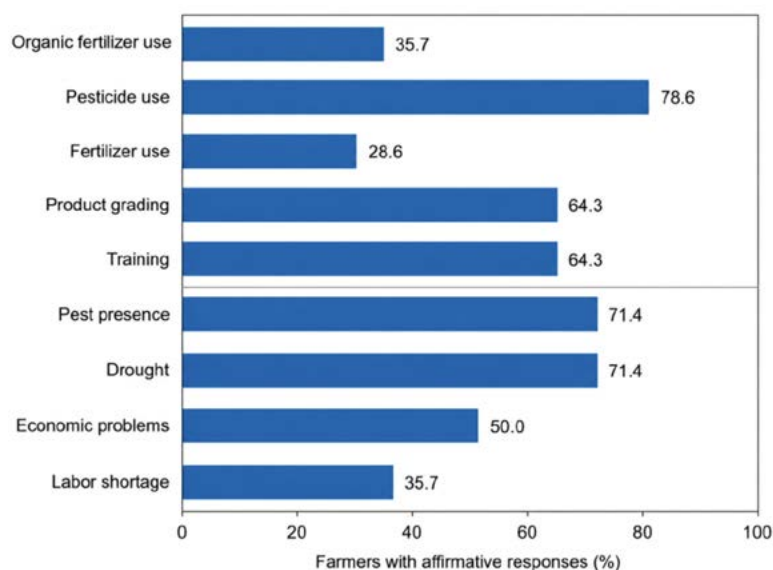


Figure 2. Technical variables and production constraints for peanut farmers in Mérida, Cintalapa, Chiapas.

Regarding constraints, pest presence and drought emerged as the main problems, both reported by 71.4% of the farmers. These were followed by economic problems, mentioned by 50.0%, and, to a lesser extent, labor shortage, reported by 35.7%. These results show that peanut production develops under both biophysical and socioeconomic pressures, in which water stress and phytosanitary problems constitute the main constraints affecting the system.

The prevalence of pests and drought, coupled with the limited use of both organic and synthetic fertilizers, aligns with findings concerning small-scale peanut production systems. High input costs, water scarcity, and phytosanitary issues are often identified as significant constraints on productivity (Sinare *et al.*, 2021; Gelaye *et al.*, 2024). Within this framework, the frequent application of pesticides in Mérida appears to prioritize the immediate resolution of phytosanitary concerns, as opposed to integrated crop management. This approach diverges from strategies that advocate for more comprehensive methods, such as integrated pest management and climate stress adaptation (Gelaye *et al.*, 2024).

In addition, the relatively high presence of product grading and training, together with the intermediate weight of economic problems and the lower incidence of labor shortage, suggests that the system faces not only agronomic constraints but also commercial, technical, and operational tensions. This pattern indicates that peanut management in the community is oriented more toward responding to immediate crop pressures than toward consolidating long-term, integrated strategies for nutrition, prevention, and productive strengthening.

Associations between structural variables and productive performance

Associations between cultivated area, farming experience, and problem pressure and the main productive indicators were explored (Table 4). Cultivated area was strongly and positively associated with production ($r_s=0.960$; $p<0.001$), but negatively associated with yield ($r_s=-0.796$; $p=0.001$). Farming experience showed the same pattern, with a positive association with production ($r_s=0.820$; $p<0.001$) and a negative association with yield ($r_s=-0.605$; $p=0.022$). In contrast, the total number of reported problems was not significantly associated with yield ($r_s=0.208$; $p=0.475$).

These results suggest that neither cultivated area nor accumulated farming experience, by themselves, guarantee better relative crop performance. This pattern suggests that some

Table 4. Association between structural variables, experience-related variables, and problem pressure variables and productive indicators.

Association evaluated	Spearman's rank correlation coefficient (r)	Significance level (p)
Cultivated area - Production	0.960	<0.001
Cultivated area - Yield	-0.796	0.001
Farming experience - Production	0.820	<0.001
Farming experience - Yield	-0.605	0.022
Total number of problems - Yield	0.208	0.475

farmers have responded to production constraints by expanding cultivated area rather than by improving productivity per unit area.

Accumulated farming experience does not appear to translate automatically into innovation or more effective practices. This observation aligns with existing research on peasant farming systems in the Frailesca region. These systems maintain productivity through intricate sociotechnical pathways. These pathways are influenced by constrained financial resources, the integration of local farming methods with elements of agricultural modernization, and the continued use of locally sourced or self-saved materials. This reflects the regional approach to sustaining production (Fonseca-Flores *et al.*, 2025).

The absence of a substantial correlation between the aggregate number of reported issues and yield further implies that the simple acknowledgment of limitations does not adequately account for productive output. Conversely, yield seems to be contingent upon a more intricate interplay of structural, technical, and contextual elements. In this sense, the observed pattern indicates that in Mérida, peanut farming operates on a small scale. Therefore, increases in total production depend more on expanding the area used for farming than on improving the number of peanuts produced per unit of land.

According to the 2024 SIAP municipal agricultural statistics, Cintalapa de Figueroa led Chiapas in both harvested area, with 2,061 hectares, and peanut production, yielding 3,544.92 tons. However, its average yield (1.72 t ha^{-1}) was lower than that recorded in municipalities with a smaller production scale, such as Frontera Comalapa (2.88 t ha^{-1}), Chicomuselo (2.81 t ha^{-1}), and La Trinitaria (2.74 t ha^{-1}). This contrast suggests that the leading productive position of Cintalapa de Figueroa is supported mainly by the extent of cultivated area rather than by greater efficiency per unit area.

The results of the present study are consistent with this pattern. Cultivated area was positively associated with production but negatively associated with yield, indicating a logic of extensive rather than intensive growth. In the community analyzed, production is also sustained largely by family-based learning, the predominant use of self-saved seed, and inherited management practices, all within a context of weak organizational articulation and limited technical intensification. These elements suggest that the relatively low productivity seen in Cintalapa de Figueroa is not just a local issue, but rather a specific example of a larger trend in peanut farming throughout Chiapas.

From this perspective, peanut should not be understood as an isolated crop. Instead, it should be viewed as part of a regional strategy for diversifying production and adapting to changing conditions. This understanding aligns with existing research on other peasant agroecosystems in central Chiapas. In these systems, peanuts are part of rotation, association, and spatial distribution practices. These practices are determined by seasonal changes, local conditions, and the management choices of farming families (Pizaña Vidal *et al.*, 2026).

It also agrees with recent studies in the region that show differentiated forms of agroecosystem adaptation and management in response to temporality and unequal resource availability (Gómez-Padilla *et al.*, 2026).

This pattern may also be interpreted through the concept of the yield gap (Yg), which recognizes that observed field productivity is conditioned not only by crop potential, but

also by biophysical, technical, and socioeconomic limitations (van Ittersum *et al.*, 2013). In small-scale peanut systems, constraints such as drought, pests, diseases, and high fertilizer costs limit productivity and shape adaptive responses under resource-restricted conditions (Sinare *et al.*, 2021). From a systemic perspective, this is consistent with a dynamic of limited relative efficiency and with an organizational base that remains too weak to support value-added uses of by-products and collective responses to system-level problems.

Yield differences according to management practices

Yield differences were also evaluated across groups of farmers according to specific management practices (Table 5).

The comparisons did not reveal significant yield differences associated with organic fertilizer use, training, or fertilizer use. Although some groups showed slight differences in median yield, these did not reach statistical significance, suggesting that, in the sample analyzed, the presence of these practices alone was not sufficient to explain variation in yield. This finding reinforces the idea that yield responds to a more complex interaction of structural, technical, and contextual factors.

Descriptively, although most farmers reported having received training, the non-use of inputs such as organic and synthetic fertilizers also predominated. This pattern suggests a gap between access to training and the effective adoption of management practices within the production system.

This does not imply that technical management lacks significance in peanut production; instead, the practices documented within the analyzed sample proved inadequate to account for yield disparities. Biological alternatives, including indigenous arbuscular mycorrhizal fungi and vermicompost leachate, have been shown to enhance yield and crop quality in field settings during peanut cultivation (Sánchez-Roque *et al.*, 2022). Consequently, the impact of management is contingent upon the specific practices employed and their effective incorporation into the overall system.

The results indicate that peanut production in Mérida, Cintalapa de Figueroa, is sustained by small-scale units in which family-based learning, inherited practices, and limited productive intensification converge. Although the crop retains local economic importance and producers expressed willingness to consider the potential use of peanut shells, weak organizational articulation and the production constraints identified limit the consolidation of value-added alternatives.

Table 5. Comparison of yield by management practices using the Mann-Whitney U test.

Grouping variable	Group	Farmers (n)	Median yield (t ha ⁻¹)	test statistic (U)	significance level (p)
Organic fertilizer use	Yes	5	1.03	25.0	0.79
	No	9	1.06		
Training	Yes	9	1.03	30.0	0.35
	No	5	1.07		
Fertilizer use	Yes	4	0.99	22.0	0.83
	No	10	1.03		

Accordingly, the prospects for by-product valorization depend not only on technical feasibility, but also on the socio-productive conditions under which the system currently operates. On this basis, future research should examine more closely the barriers to cooperative organization, the adoption of agroecological or management practices aimed at improving yield, and the local mechanisms that could support improvement processes consistent with the productive and ecological logic of the territory.

CONCLUSION

Peanut production in Mérida, Cintalapa de Figueroa, Chiapas, is sustained by small-scale farming units characterized by family-based learning, the predominant use of self-saved seed, and the continuity of local agricultural knowledge. Cultivated area and farming experience were positively associated with production but negatively associated with yield, indicating that output depends more on area expansion than on improvements in efficiency per unit area, which reflects an extensive production pattern. The productive and organizational constraints identified limit both collective action among farmers and the potential use of peanut shells as a value-added by-product. Under these conditions, the prospects for peanut shell valorization depend not only on its technical feasibility but also on the socio-productive context and local organizational capacity in which the system operates.

ACKNOWLEDGMENTS

The authors thank the Benemérita Universidad Autónoma de Chiapas for the institutional support provided, as well as the Digital Technological Agency of the State of Chiapas (ADITECH) for the support given to the first two authors through the State System of Researchers, 2024 call.

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Fog water capture in temperate forests

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ABSTRACT

Objective: To develop a procedure for estimating fog water capture in temperate forests for three genera *Pinus*, *Abies* and *Quercus*, in locations lacking specialized instrumentation.

Design/Methodology/Approach: Three one hectare plots were established in temperate forest. Seventy-five rain gauges, with a precision of 0.1 mm, were randomly distributed across each plot. To analyze of fog water collection, precipitation events were recorded daily during the rainy season (June-September). From these data, events were selected only if at least one rain gauge showed no precipitation while a minimum volume of water was recorded in the remaining gauges.

Results: The *Abies* forest captured a total of 4.7 mm of fog water, followed by *Quercus* 2.89 mm, and *Pinus* 1.12 mm.

Study Limitations/Implications: The evaluation was to the rainy season, with data recorded on a daily basis. The accessibility challenge was overcome.

Findings/Conclusions: The proposed procedure provides reliable results, enabling the assessment of fog water contribution as an initial strategy when specialized instrumentation is unavailable.

Keywords: Fir, highland Pine, Oak, horizontal precipitation.

Citation: Flores-Ayala, E., Buendía-Rodríguez, E., Flores-García, A., Pineda-Ojeda, T., Gutiérrez-García, J. V., Islas-Gutiérrez, F., Pérez-Miranda, R., & Carrillo-Anzures, F. (2026). Fog water capture in temperate forests. *Agro Productividad*. <https://doi.org/10.32854/1qmpwc11>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: January 6, 2026.

Accepted: April 29, 2026.

Published on-line: June 26, 2026.

Agro Productividad, 19(5). May. 2026. pp: 297-305.

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INTRODUCTION

Globally, water scarcity is one of the most important challenges for human development. Currently, 2.4 billion people live in countries affected by water stress. In addition, there has been an increase in the frequency and intensity of extreme weather events, including droughts and floods, which further exacerbates ecosystem degradation (UN, 2023), particularly forest ecosystems. In Latin America and the Caribbean, 34% of the world's total freshwater resources are available. However, despite this high value (28,000 m³ per inhabitant per year) and an average annual precipitation of 1,600 mm, some regions experience water stress due to differences in precipitation patterns and water demand associated with economic activities (FAO, 2025).

In Mexico, hydrological basins must meet the high water demand of the population. Daily *per capita* water consumption is estimated at 366 liters (UN, 2025), which requires careful resource management beginning with rainwater capture in the country's forests and tropical ecosystems. In this regard, the National Water Commission (CONAGUA) and the Ministry of Environment and Natural Resources (SEMARNAT) aim to mitigate water supply limitations in the country. Both institutions work jointly under the National Water Program (PNH) 2024-2030 (CONAGUA, 2024) and the future Water Agenda 2030, promoting irrigation technification in agriculture and rainwater harvesting as key strategies for long-term water security.

Rainfall interception (I) is the capacity of the tree canopy to store or retain rainwater within its structure (leaves, branches, and stem) during and after a precipitation event. This amount can represent up to one quarter of total precipitation (Navar, 2020). Interception values (I) have mainly focused on comparing throughfall (Pd) with precipitation recorded outside the forest in experimental plots, which represents total or incident precipitation (Pi). However, an additional and scarcely evaluated fraction of water may originate from fog or horizontal precipitation captured by vegetation, which is rarely differentiated from water derived from Pi (Kavianpour *et al.*, 2025).

Fog consists of tiny suspended water droplets whose small size prevents their falling velocity from exceeding the upward force of air, thereby avoiding precipitation (Villegas *et al.*, 2008). When fog is transported by wind, droplets collide with vegetation surfaces, where they coalesce into larger droplets that eventually fall to the ground. This process, known as fog interception, constitutes an important water source for many ecosystems (Prada *et al.*, 2009). These fluxes can be quantitatively relevant for the local water balance (Tognetti, 2015), especially during dry seasons or along coastal altitudinal gradients (Weathers *et al.*, 2020). According to Baguskas (2016), fog promotes seedling survival and reduces mortality during summer droughts, with direct implications for natural regeneration and restoration success. Furthermore, authors such as Berry *et al.* (2015) report that fog influences tree physiology by increasing leaf water potential and reducing transpiration. Some epiphytes, mosses, and understory species strongly depend on this additional water input in the form of fog (Liu *et al.*, 2021), supporting biodiversity and microclimate regulation (Berrones, 2022).

Fog is not only a source of water; it also provides nutrients (N, P) and atmospheric pollutants (Jofre-Meléndez *et al.*, 2015) that influence soil and water fertility and chemistry, with consequences for species composition and biogeochemical processes, making it an important ecological vector (Weathers *et al.*, 2020). According to Zhan *et al.* (2020), fog interception can result in measurable contributions to soil moisture, which may vary considerably due to topography, forest edge effects, and canopy structure (Harr, 1982; Ewing *et al.*, 2009), as well as vegetation cover, which in turn depends on the type of silvicultural treatment applied (Monárrez-González, 2023). Fog interception is also influenced by fascicle size, with smaller fascicles being more efficient (Went, 1955; Praskievicz and Sigdel, 2023). Therefore, fog water capture is considered a viable technology to address water scarcity; however, its success depends on both technological and socioeconomic factors, including financing and maintenance (Sowmya *et al.*, 2025).

Dunne and Leopold (1978) point out that estimating I captured in forests in the form of fog remains a methodological challenge, particularly considering that this fraction is relatively small compared with Pi. In interception studies, conventional rain gauges are typically installed in open fields; therefore, they rarely capture fog drip water generated under the forest canopy (Vogelmann *et al.*, 1968; Kavianpour *et al.*, 2025). Consequently, fog interception is often quantified using indirect methods (Schmid *et al.*, 2011). Among the methods employed are mesh collectors and modeling techniques (Imteaz *et al.*, 2011). However, in both cases, the potential contribution of fog water captured by trees is being evaluated (Ritter *et al.*, 2008; Regalado and Ritter, 2010). Other approaches include the Standard Fog Collector and the Large Fog Collector, structurally improved designs such as the CloudFisher and the Warka Water Tower, as well as laboratory-scale innovations using biomimetic wettability-patterned surfaces, electrospun nanofibers, and Janus membranes (Sowmya *et al.*, 2025).

Fog precipitation represents a significant potential in forest ecosystems, as forests play a crucial role in improving water availability, particularly during dry periods. Integrating fog water inputs into regional water resource management and forest conservation strategies is essential to ensure the sustainability of these valuable ecosystems. Therefore, the objective of this study is to apply a procedure to identify fog water capture and estimate its contribution to the water balance in temperate forests dominated by the genera *Pinus*, *Abies*, and *Quercus* within a hydrologically important forested watershed.

MATERIALS AND METHODS

Study Area

This study was conducted within the Texcoco River watershed, located in the eastern part of the Valley of Mexico basin in the municipality of Texcoco, within the ejidal forest area of San Pablo Ixayoc (Figure 1). The watershed topography is composed of three well-defined zones: a lowland zone extending from the watershed gauging station to the 2,300 m a.s.l. contour line; a hilly zone between the 2,300 m and 2,500 m a.s.l. contour lines; and a mountainous zone, where this study was carried out, ranging from 2,500 m to over 4,000 m a.s.l., which marks the upper boundary of the watershed and covers an area of 14.74 km².

The climate of the area is classified as Cb'(w2) temperate subhumid to semi-cold, with a mean temperature ranging from 5 °C to 12 °C. Average monthly precipitation ranges from 381.4 mm to 868 mm, with July being the wettest month. The mean annual temperature is 10.95 °C; January is the coldest month and May is the warmest. Regarding soil types, chromic Vertisols dominate, characterized by their high clay content. Vegetation is distributed in well-defined zones dominated by *Pinus* in the upper part, *Abies* in the middle zone, and *Quercus* in the lower zone (Carrillo, 2015).

The study was carried out using infrastructure installed under the research project “Rainfall Interception Study for Silvicultural Management Purposes” of the National Research Program on Sustainable Forest Management of the National Institute for Forestry, Agricultural and Livestock Research (INIFAP). Three representative sites were selected

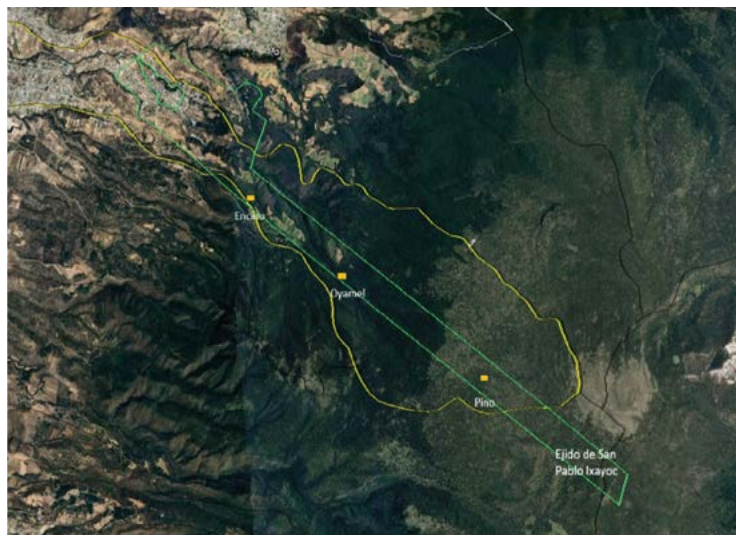


Figure 1. Location of the plots within the Texcoco River watershed in the ejidal forest area of San Pablo Ixayoc, Texcoco.

along the altitudinal gradient and forest types. In 2009, the first site was established in a pure *Abies religiosa* (Kunth) Schlttdl. & Cham. forest at an altitude of 3,000 m a.s.l. In 2010, the second plot was established in an oak forest dominated by *Quercus rugosa* Née at an altitude of 2,850 m a.s.l., and in 2011, a third site was established in a pure *Pinus hartwegii* Lindl. forest at an altitude of 3,650 m a.s.l. (Figure 2).

The general characteristics regarding the location and year of establishment of the study areas are presented in Table 1.



Figure 2. (a) *Abies religiosa* (Kunth) Schlttdl. & Cham. forest, (b) *Quercus* spp. forest, and (c) *Pinus hartwegii* Lindl. forest.

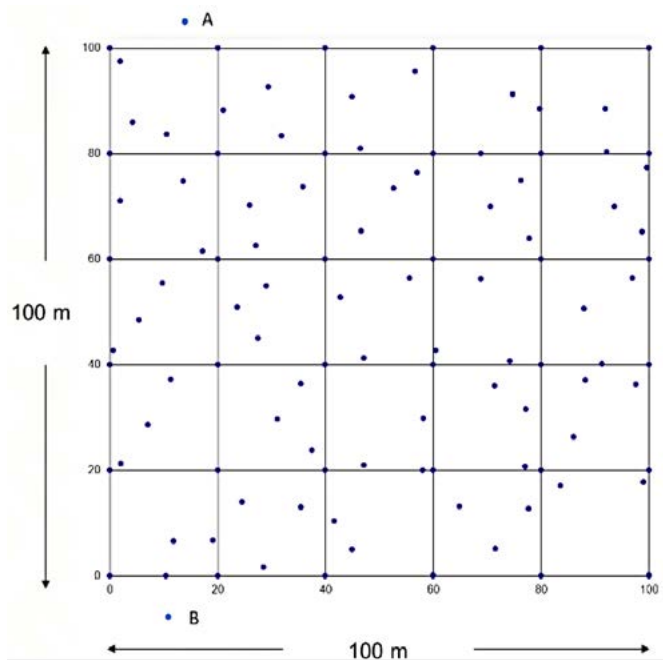
Table 1. Location of the plots within the ejido of San Pablo Ixayoc, Texcoco, State of Mexico.

Parcela	Coordenadas	Nombre del paraje/ altitud	Año
Oyamel: <i>Abies religiosa</i> (Kunt) Schlt. & Cham.	19° 26' 16.92" 98° 45' 40.81"	Atlapulco: 3,000 m	2009
Encino: <i>Quercus rugosa</i> Née <i>Quercus deserticola</i> Trel. <i>Quercus laurina</i> Humb. & Bonpl.	19° 27' 09.11" 98° 46' 19.23"	Tlatzala: 2,900 m	2010
Pino: <i>Pinus hartwegii</i> Lindl.	19° 25' 09.85" 98° 44' 15.51"	Cañada Honda: 3,650 m	2011

Experimental Plots

A detailed inventory of the dominant vegetation was conducted. Data collection included the following variables: species, diameter at breast height (DBH), clear bole height, total height, and spatial location using coordinates (x, y) within each plot. Botanical samples of all tree species were collected for identification at the Forest Herbarium of the Forestry Division of the Autonomous University of Chapingo.

Three plots of one hectare each (100 m × 100 m) were established. In each plot, 75 True Chek[®] direct-reading rain gauges were installed, with a capacity of 150 mm and a precision of 0.1 mm, randomly distributed under the tree canopy, along with two additional rain gauges placed in open conditions (A and B, outside the plot) (Figure 3). The rain gauges were mounted on supports at a height of 0.90 m above ground level to prevent splash effects.

**Figure 3.** Plot layout and distribution of rain gauges.

Daily precipitation measurements were conducted, and the amount of rainfall intercepted by the canopy was estimated as the difference between total precipitation measured under open conditions and the overall average obtained from the water volume recorded in rain gauges located under the canopy. To measure water captured from fog, the database generated from rainfall interception studies was used. To quantify fog water capture volumes, events were selected and analyzed in which at least one rain gauge showed no precipitation record and only a minimal water volume was recorded in the remaining rain gauges, which was attributed to the presence of fog. The rationale behind this procedure is that conventional rain gauges are not suitable for directly recording fog water. However, trees act as condensation elements that enable fog to be transformed into water. When tree foliage becomes saturated with condensed water, it drips and precipitates to the ground, where it is collected by the rain gauges.

Finally, the study considered only those events in which at least 10 rain gauges were empty for each studied species. For each selected event, it was verified that no previous precipitation event had occurred to avoid residual drip effects. This was proposed as a conservative criterion aimed at minimizing potential sources of error and allowing a more robust interpretation of horizontal precipitation capture. The working premise was that rain gauges showing any recorded water volume reflected fog drip resulting from fog particles intercepted by vegetation, which coalesce on canopy surfaces and, due to gravity, drip to the ground and/or are captured by the measuring equipment (Holder and Bruijnzeel, 2004).

Data analysis

Information previously generated from the rainfall interception study was grouped by genus, analyzing precipitation data for each year and study period. The data were analyzed by rainfall period, constructing a database of rainfall events and considering events in which at least one rain gauge recorded no water volume.

RESULTS AND DISCUSSION

A total of 74 rainfall events were recorded, with 27 events showing at least one empty rain gauge in *Abies religiosa*; 63 total rainfall events with 16 events showing at least one empty rain gauge in *Quercus* spp.; and 72 total rainfall events with 12 events showing at least one empty rain gauge in *Pinus hartwegii* (Table 2).

Table 2. Database by studied genus, analyzed rainfall period, and number of analyzed events.

Especie	Total rainfall events	Rainfall events	Fog events	Period analyzed
<i>Abies religiosa</i> (Kunt) Schltdl. & Cham	74	66	8	May 22 to September 30
<i>Quercus</i> spp.	63	56	7	May 27 to October 30
<i>Pinus hartwegii</i> Lindl.	72	68	4	June 24 to September 30

The analyzed results show that the *Abies* forest captured a total of 4.7 mm of fog-derived water, *Quercus* 2.89 mm, and *Pinus* 1.12 mm during the study periods, equivalent to 47.0, 28.9, and 11.2 m³ ha⁻¹, respectively. According to Palán and Křeček (2018), fragmented montane forests recorded an average fog drip of 81 mm year⁻¹, equivalent to 7% of the annual gross precipitation. Although our data do not allow a direct annual comparison due to shorter observation periods and the exploratory nature of this study, the event-based estimates obtained (0.28-0.59 mm) are lower in magnitude; however, the comparison remains valuable, as even low fog inputs can represent a relevant fraction of the water balance. Berrones *et al.* (2022), using higher-precision equipment, reported fog capture values per event between 0.1 and 0.2 mm, while Praskievicz and Sigdel (2023) reported average total values per event ranging from 0.24 to 0.69 mm, which are very similar to those found in this study. This suggests that despite the use of an indirect and conservative method, the magnitude of fog water capture inferred from the rain gauge network is consistent with values reported in the literature.

Table 3 summarizes the volumes of fog water captured in *Abies*, *Quercus* spp., and *Pinus* forests, considering only events in which 10 or more rain gauges remained without precipitation records.

In all cases, these values represent additional water inputs that, although smaller than vertical precipitation, are concentrated within a specific subset of events and forest types. During the development of this study, one of the main difficulties was the daily data collection, as measurements had to be conducted during the rainy season, particularly in the *Pinus hartwegii* forest, which is located at the highest elevation and on the steepest terrain. This limitation was partially compensated by the type of rain gauges used, which allowed for rapid and easy data recording.

Table 3. Total and average amounts of water capture per event from fog.

Forest	TNE	TFWM	AWE (mm)
<i>Abies religiosa</i> (Kunt) Schlttdl. & Cham.	8	4.70	0.59
<i>Quercus</i> spp.	7	2.89	0.41
<i>Pinus hartwegii</i> Lindl.	4	1.12	0.28

TNE=Total number of events classified as fog (events with ≥ 10 rain gauges without records).

TFWM=Total amount of fog water measured according to TNE (mm).

AWE=Average amount of fog water per event (mm).

CONCLUSIONS

This study documents and applies an exploratory procedure to infer fog water capture from a network of direct-reading rain gauges originally installed to evaluate rainfall interception in temperate forests of *Abies*, *Quercus*, and *Pinus*. The spatial criterion (events with ≥ 10 rain gauges without precipitation records and absence of prior rainfall) allowed the identification of a subset of fog events and the estimation of their minimum contribution to the water balance. The results should be interpreted as minimum estimates, conditioned by the indirect nature of the method and the absence of micrometeorological measurements and fog collectors. Nevertheless, the reanalysis of rainfall interception databases emerges

as a viable alternative to constrain the expected range of fog water capture in hydrological-forest watersheds and to guide future studies integrating rain gauge networks, fog collectors, and continuous atmospheric records.

Developing and validating practical methodologies for measuring fog water capture is essential to strengthen forest management, as it may serve as a basis for designing silvicultural treatments that conserve or enhance canopy structure, species composition, and the functional integrity of forest ecosystems. Furthermore, these results highlight a potential analytical approach and represent an opportunity to deepen the understanding of this process. Fog water capture in temperate forests represents a hydrological process of high ecological relevance whose understanding remains limited in Mexico, mainly due to the scarcity of local research and the lack of standardized, accessible, and field-adapted methodologies.

ACKNOWLEDGEMENTS

The authors acknowledge the National Institute for Forestry, Agricultural and Livestock Research (INIFAP) for the financial support provided and express their special thanks to the ejido authorities of the San Pablo Ixayoc community for the support provided during fieldwork.

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