

AGRO PRODUCTIVIDAD

From Garden to Table:
Viola spp.,
 Ornamental and
 Edible Flowers

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The effect of rootstock on the anatomy of the *Phaseolus vulgaris* L. scion: graft union, stomatal density, stomatal index, and leaf thickness

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ABSTRACT

Objective: To evaluate the effect of the *Phaseolus acutifolius* A. Gray rootstock on the establishment of the *Phaseolus vulgaris* L. scion, analyzing the anatomy of the graft union, stomatal density, stomatal index, and leaf thickness.

Design/Methodology/Approach: A completely randomized experimental design was used. The experimental unit consisted of one plant per pot. Data were analyzed using Tukey's Mean Comparison Test ($p \leq 0.05$). Some segments of the graft union were fixed in formaldehyde-acetic acid-alcohol (FAA), embedded in paraffin, and stained with safranin and fast green, while others were analyzed fresh. Fragments of the central leaflet, fixed in 3% glutaraldehyde and processed for observation under a Scanning Electron Microscope, were used to describe leaf anatomy.

Results: Five stages were identified during the formation of the graft union: development of the necrotic layer, callus proliferation, vascular cambium differentiation, vascular tissue restoration, and restoration of the epidermis. Grafted plants recorded increases of 20%, 24.7%, and 66% in stomatal density, stomatal index (on the underside of leaves), and in mesophyll thickness, respectively.

Study Limitations/Implications: The *Phaseolus acutifolius* rootstock was used in the anatomical evaluation of the *Phaseolus vulgaris* scion under irrigation.

Findings/Conclusions: The anatomical compatibility between rootstock and scion was confirmed, allowing the development of new plants with foliar micromorphological characteristics that modify their physiological behavior.

Keywords: common bean, heterograft, graft union anatomy, leaf anatomy, stomata.

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INTRODUCTION

Grafting is a vegetative propagation technique that physically joins organs from different plants (heterograft) or from the same plant (homograft) into a single plant (Loupit *et al.*, 2023). The upper section of the grafted plant is known as scion, while the lower



section is called rootstock. The plant that produces the scion is commercially productive and resistant or tolerant to foliar diseases. Meanwhile, the rootstock serves as the root of the grafted plant and usually has no economic value; however, it contains genes that include characteristics such as greater vigor and resistance or tolerance to soil limitations (Bohra *et al.*, 2022). The scion-rootstock interaction results in anatomical changes that impact the characteristics of the grafted plant and improve scion yield, nutrient and water uptake, and vigor (Sharma & Zheng, 2019). These changes are different depending on the rootstocks (Tedesco *et al.*, 2020). The grafted plant should maintain the characteristics and benefits of both plants (Basto-Pool *et al.*, 2021).

Grafting has been used for centuries with perennial plants —mainly fruit trees, forest trees, and ornamental trees. Since the beginning of the 20th century, it has also been practiced on vegetables, such as Cucurbitaceae and Solanaceae (Gautier *et al.*, 2019). However, research has not achieved the same advances regarding the genus *Phaseolus*.

In Latin America and North Africa, bean (*P. vulgaris* L.) is the most consumed species of the genus *Phaseolus*, as a result of its carbohydrate, protein, vitamin, and mineral content (Abdelhafez *et al.*, 2021). This legume is mainly produced in dryland; however, droughts decrease bean yield depending on the intensity, duration, and phenological stage in which the event takes place (Domínguez *et al.*, 2019). *P. vulgaris* cv. ‘Pinto Saltillo’ has an indeterminate bushy growth habit (type II) (CIAT, 1982). Its yield reaches 2.3 kg ha⁻¹. This legume was chosen because its shelf life is longer than the shelf life of other cultivars. Its testa slowly darkens under storing conditions. It contains 22.1% protein and has a short cooking time (Sánchez, 2001). The brown tepary bean (*P. acutifolius* A. Gray) used as a rootstock has an indeterminate prostrate growth habit (type III) (CIAT, 1982). It has a deep and vigorous root system, which enables it to absorb groundwater and adapt to low-rainfall regions (Jimenez-Galindo *et al.*, 2018).

In order to obtain cultivars tolerant to drought stress, plant breeding programs focus on the generation of inter- and intraspecific crosses between cultivars of the genus *Phaseolus*. The tepary bean (*P. acutifolius*) stands out for its high tolerance to drought and extreme temperatures. However, its percentage of introgression with *P. vulgaris* is low; consequently, numerous selection and production cycles are required to obtain tolerant cultivars.

These limitations could be addressed in the short term through the production of grafted bean plants, which could increase crop tolerance to water stress (Basto-Pool *et al.*, 2021).

The level of adaptation to drought stress can be assessed by measuring various anatomical parameters of leaves, including the thickness of the upper and lower epidermis, total leaf thickness, the thickness of the palisade and spongy parenchyma tissues, the size of the vascular bundle, the size and density of stomata, and other structural attributes that are directly linked to drought resistance (Taratima *et al.*, 2020; Sun *et al.*, 2022). Likewise, determining the affinity of the cultivar to be grafted is fundamental to avoid compatibility problems and epidermal modifications of the leaves of the grafted plants. This element is important because the gas exchange of photosynthesis takes place in these organs. Photosynthesis is a chemical process through which photoassimilates are translocated to the plant organs that requires them. When stomatal density increases in the leaves of

the grafting, the rate of CO₂ assimilation, transpiration, and stomatal conductance also increases (Ayala-Arreola *et al.*, 2010). Therefore, the objective of this research was to evaluate the effect of the brown tepary (*P. acutifolius*) rootstock on the establishment of the *P. vulgaris*, cv. 'Pinto Saltillo' scion, evaluating the anatomy of the graft union, stomatal density, stomatal index, and leaf thickness.

MATERIALS AND METHODS

Plant material and experimental design

The study was established in a tunnel-type greenhouse at the Colegio de Postgraduados (COLPOS) - Campus Montecillo, Texcoco, State of Mexico (19° 27' 40" N, 98° 54' 19" W, and 2,353 m.a.s.l.). Brown tepary bean plants (*P. acutifolius*) and *P. vulgaris* cv. 'Pinto Saltillo' were used as rootstocks and scions, respectively. Seeds from both species were individually sown in 120 mL propagation tubes with peat moss as substrate. The method proposed by Lee *et al.* (2010) was used to make cleft grafts when the first compound leaf (V3 stage) of the plants appeared (CIAT, 1982). Fifteen days after grafting (15 DAG), the plants were transplanted into 2 L plastic pots with tezontle (≤ 5 mm particle diameter). Irrigation was carried out for 90 d with 100% Steiner nutrient solution (Steiner, 1961). Treatments consisted of 12 repetitions of grafted and non-grafted beans, distributed into a completely randomized experimental design. The experimental unit was one plant per pot.

Anatomy of the graft union

Four 5×5 mm stem fragments from grafted plants were taken 1 cm below and 1 cm above the graft union; subsequently, they were fixed in formaldehyde-acetic acid-alcohol (FAA) for 48 h under vacuum. They were then washed with deionized water (three 15-minute rinses each) and dehydrated in an ethanol series: 50%, 60%, 70%, 80%, 96%, and 100% (two changes), for 4 h each. Afterwards, they were placed in an ethanol:xylene transition medium, using 3:1, 1:1, and 1:3 ratios, followed by two xylene changes (100%), for 4 h each.

The samples were infiltrated in McCormick Scientific™ Paraplast Plus paraffin (Supplier: Leica Biosystems) in an oven at 60 °C, in two changes: first in paraffin with an intermediate solvent (xylene) for 4 h and the second in pure paraffin for 24 h. An American Optical Company™ rotary microtome (USA) was used to cut 7-10 μm-thickness serial sections (cross-section and longitudinal). Chrome alum adhesive [1% gelatin, 0.1% phenol, and 0.05% chrome alum (KCr(SO₄)₂ 12(H₂O)) in water] was used to adhere the sections to slides. After 12 h on a hot plate (60 °C), sections were deparaffinized with xylene (100%; three changes of 3 min each) and hydrated in an ethanol series (100%, 96%, 70%, and 50%; 3 min each). The sections on the slides were stained with safranin [0.05%; Safranin O (C.I. 50240) for microscopy, Merck] in a saline solution (3% NaCl in water) for 2 h. Subsequently, the sections were washed twice with deionized water for 3 min and dehydrated in an ethanol series (50%, 70%, 90%, and 100%) for 2 min each. Afterwards, the sections were stained with 0.12% Fast Green FCF (C.I. 42053 for microscopy, Merck) in 95% ethanol for 1 min. The dye excess was removed with 96% and 100% ethanol,

followed by three xylene changes (100%), for 5 min each. The slides were mounted in synthetic resin and dried on a hot plate at 60 °C.

Additionally, 1 cm fragments of fresh stem were cross-sectioned with a hand-held microtome. In order to prevent dehydration, the 100- μ m thick sections were retrieved with a fine paintbrush and placed in 100×20 mm Petri dishes, with distilled water, at laboratory temperature. Afterwards, they were placed on a slide with a drop of water and covered with coverslips. The anatomy of the graft union was documented with a ZEISS SteREO Discovery V20 optical microscope and a Canon SD digital camera.

Stomatal density, stomatal index, and leaf thickness

Initially, 5×5 mm sections were obtained from the central leaflets of trifoliate leaves—which are located in the middle stratum of grafted and non-grafted *P. vulgaris* plants. Afterwards, they were fixed in glutaraldehyde solution [3% glutaraldehyde in Sorensen's phosphate buffer (0.1 M, pH 7.2) for 24 h under vacuum]. Subsequently, the same buffer was used for two rinses of 10 min each. The samples were post-fixed with 2% osmium tetroxide in water for 2 h, followed by two rinses with deionized water for 30 min each. Subsequently, they were dehydrated for 40 min each, in 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% ethanol series (two changes). The samples were dried at 31 °C with a Tousimis™ Samdri-780A critical-point dryer and coated with a thin layer of 80:20 gold:palladium, using a metal ionizer (Fine Coat Ion Sputter JFC-1100). A JEOL JSM-6390 scanning electron microscope was operated at 10 kV for observations.

The ImageJ (v. 1.51k, NIH, USA) software was used to establish stomatal density, stomatal index, and leaf thickness. Stomata and epidermal cells were counted on the adaxial (upper) surface and abaxial (lower) surface of four central leaflets, with eight repetitions, respectively. Mesophyll thickness (including palisade parenchyma and spongy parenchyma) was determined in three central leaflets, with four repetitions, using the midrib as a reference.

Using the number of stomata and the number of epidermal cells, stomatal density (SD) and stomatal index (SI) were estimated according to the following expressions:

$$SD = \frac{\text{number of stomata}}{\text{mm}^2}$$

$$SI = \left(\frac{SD}{SD + ECD} \right) \times 100$$

where *SD*=stomatal density, *SI*=stomatal index, and *ECD*=epidermal cell density (Willmer & Fricker, 1996).

Statistical analysis

SAS (SAS, 2002) was used to subject the stomatal density, stomatal index, and leaf thickness data to an analysis of variance and a mean comparison test (Tukey: $p \leq 0.05$).

RESULTS AND DISCUSSION

Anatomy of the graft union

Five stages of the graft union formation were identified: 1) development of a necrotic layer, 2) callus proliferation in the graft interface, 3) differentiation of a new vascular cambium, 4) restoration of new vascular tissue, and 5) restoration of the continuity of the epidermis in the graft union. These stages have been described for various plant species and graft types. The difference lies in the time required to complete each stage, as it takes less time for herbaceous plants than for woody plants (Melnyk, 2017; Wang *et al.*, 2017).

No cambial activity was recorded during the first hours after the physical union of the rootstock and the scion. However, after 24 h, a necrotic layer (Figure 1a) —characterized by intensely stained cells (Figures 1c-d)— was observed on the rootstock and scion. This layer is the initial response to the damage caused by the cut. It progressively breaks down, facilitating the formation of a common cell wall (Tamilselvi & Pugalendhi, 2017; Adhikari *et al.*, 2022).

The polysaccharides accumulation —mainly pectin secreted by cells in the graft interface— plays a crucial role in the initial physical union of the plants, because these polysaccharides are adherents (Sala *et al.*, 2019). However, initial adhesion on the graft surface does not guarantee a successful graft union: the functional vascular connection between the scion and rootstock must be established during subsequent stages (Velasco-Alvarado *et al.*, 2017; Martínez *et al.*, 2020).

The first evidence of cell division was the development of the callus (5 DAG), which formed a bridge at the scion-rootstock interface (Figures 1b and d). Subsequently, differentiation of the new vascular cambium and the restoration of new vascular tissue became evident (Figure 1e). The continuity of the epidermis at the scion-rootstock union (15 DAG) indicated the complete establishment of the union (Figure 1f).

This process matched the descriptions of Rasool *et al.* (2020) and Tedesco *et al.* (2023), who identified the connection of vascular tissues as the key event for a successful graft.

Auxins seem to play a critical role in vascular differentiation in the graft union. Authors such as Nanda and Melnyk (2018) and Wang *et al.* (2017) highlighted that polar auxin transport —temporarily interrupted by the cut— accumulates at the base of the rootstock and influences cell growth and vascular tissue differentiation, especially in the graft union (Habibi *et al.*, 2022).

Stomatal density, stomatal index, and leaf thickness

The leaflets of grafted and non-grafted plants displayed dorsiventral characteristics, with a uniseriate adaxial (upper surface) and abaxial (lower surface) epidermis of irregularly shaped cells and wavy cell walls. Paracytic stomata were found on both leaf surfaces. They had a pair of subsidiary cells parallel to the ostiole, covered by a striated cuticle. The results of this study confirm the amphistomatic nature of bean leaves. Two main types of trichomes were identified: a unicellular, hooked trichome with an enlarged base and a multicellular trichome with an enlarged, glandular distal end. Both were located on the primary vein, at the branching point of the veins, and on both leaflet blades (Figure 2 a-d). The type of trichomes found in bean plants is consistent with those found by Jiménez

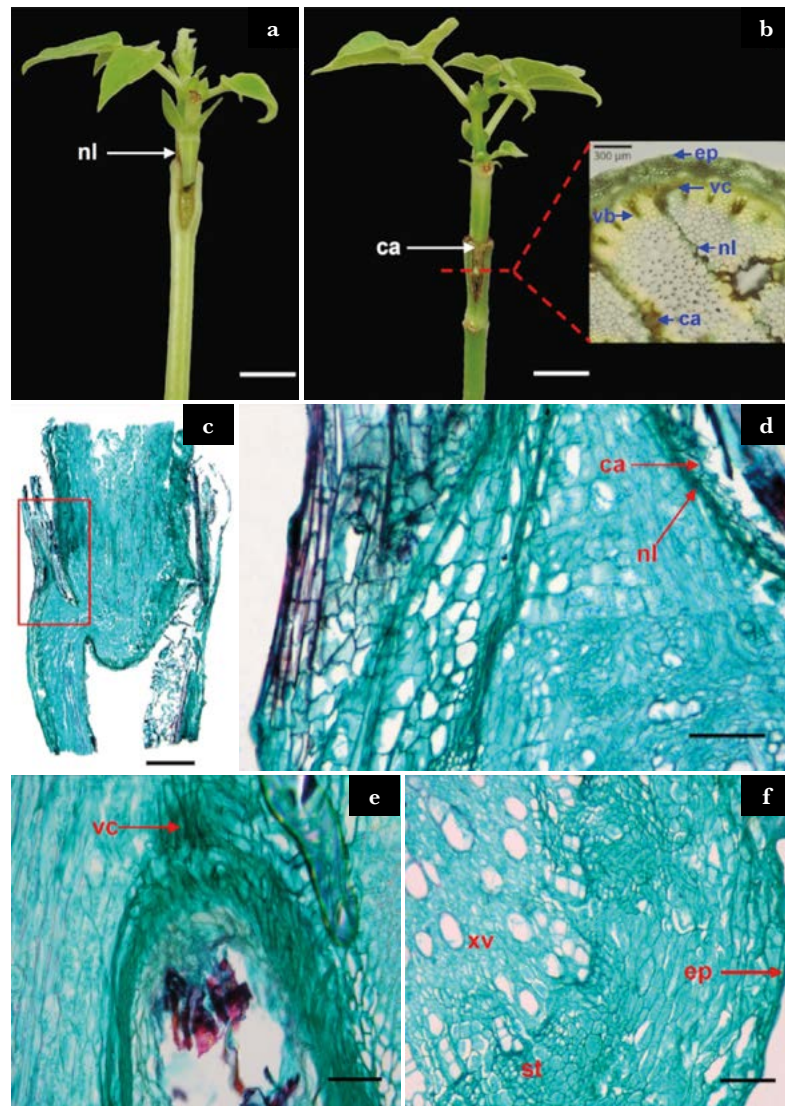


Figure 1. Formation of the *Phaseolus vulgaris* L. (scion)-*P. acutifolius* A. Gray (rootstock) heterograft union. a) Union showing the necrotic layer (24 h); b) Callus development in the graft interface and cross-section of the union (5 DAG); c), d), e) Longitudinal sections, and f) cross-section of the graft union stained with safranin-fast green; d) Close-up of the union shown in the red box in c); e) Differentiation of vascular cambium (15 DAG); f) Continuity of the epidermis (ep) in the scion-rootstock union (15 DAG). Callus (ca), vascular cambium (vc), necrotic layer (nl), vascular bundle (vb), xylem vessel (xv), and sieve tube (st). Bar: 1 cm (a, b), 1 mm (c), 50 μm (d), 100 μm (e), and 50 μm (f).

et al. (2012) in the adaxial and abaxial epidermis, including unicellular trichomes and multicellular trichomes, both with an enlarged base.

The main vascular bundle has developed in the midrib; the less prominent veins laterally arranged form the vascular bundle system of the reticulate venation (Figure 3a, b).

In grafted and non-grafted *Phaseolus* plants, the stomatal density (SD) and stomatal index (SI) of the upper surface did not record significant differences (Tukey, $p \leq 0.05$). However, the SD and SI of the underside of leaves of grafted plants were 20% and 24.7% higher than in non-grafted plants (Table 1).

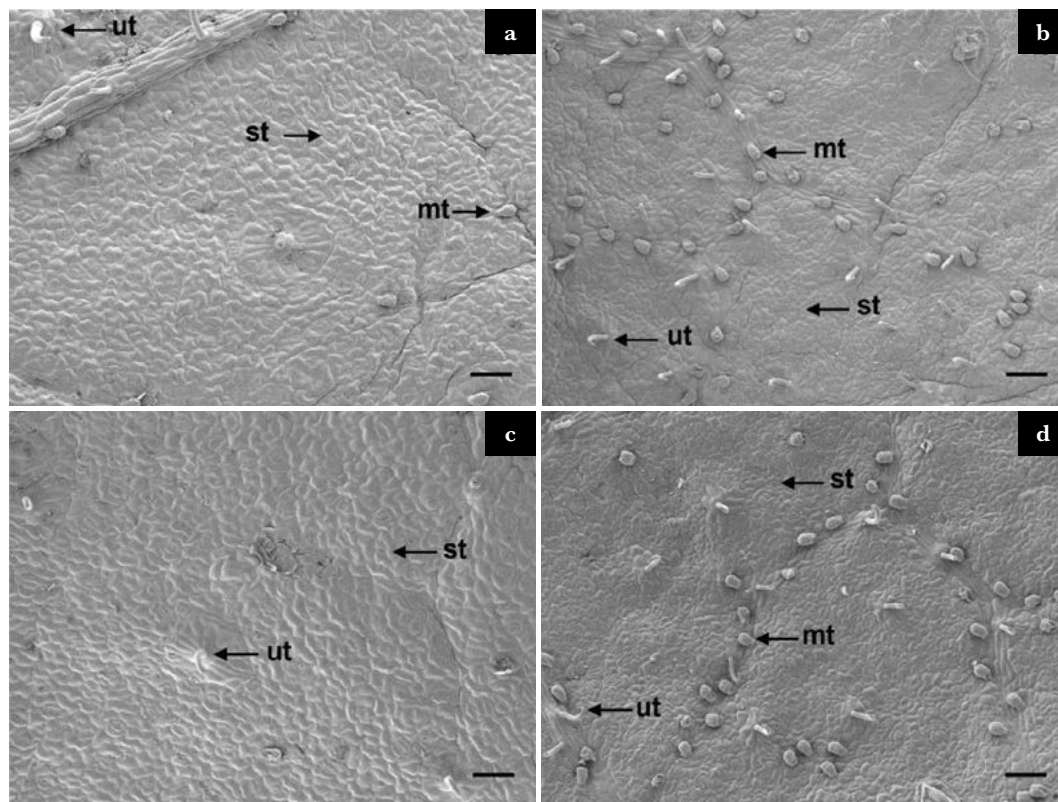


Figure 2. Leaf epidermis. Non-grafted *Phaseolus vulgaris* L. plant, upper surface (a) and lower surface (b). Heterograft *P. vulgaris* L. (scion)-*P. acutifolius* A. Gray (rootstock), upper surface (c) and lower surface (d), observed in a JEOL JSM-6390 microscope. Stoma (st), unicellular trichome (ut), multicellular trichome (mt). Bar: 100 μ m.

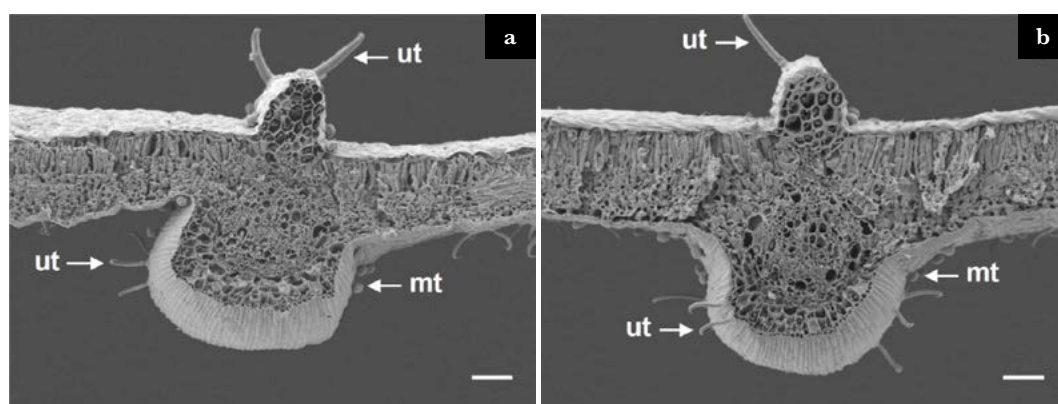


Figure 3. Cross-sections of the midrib of the central leaflet. a) Non-grafted *Phaseolus vulgaris* L. plant. b) Heterograft of *P. vulgaris* L. (scion)-*P. acutifolius* A. Gray. (rootstock) observed in the JEOL JSM-6390 microscope. Unicellular trichome (ut), multicellular trichome (mt). Bar: 100 μ m.

These results matched the findings of Camposeco-Montejo *et al.* (2018), who reported similar increases in grafted bell pepper plants, associated with higher rates of CO₂ assimilation, transpiration, and stomatal conductance, as well as efficient water use and stress tolerance (Hernández *et al.*, 2021). However, the results were different from those

Table 1. Effect of *Phaseolus acutifolius* A. Gray (rootstock) on the stomatal density and stomatal index of *P. vulgaris* (scion).

| | Stomatal density (SD) (stomata/mm ²) | | Stomatal index (SI) (%) | |
|-------------|--|----------------|-------------------------|--------------|
| | Upper surface | Underside | Upper surface | Underside |
| Grafted | 89.06±9.83 a | 279.96±26.63 a | 16.31±0.90 a | 22.33±1.02 a |
| Non-grafted | 107.32±10.96 a | 233.24±22.00 b | 17.09±1.47 a | 17.90±1.74 b |

Data are means ± SE. Different letters in the columns indicate statistical differences (Tukey, $p \leq 0.05$), $n=8$.

recorded by Peralta-Manjarrez *et al.* (2016) for grafted cucumber (*Cucumis sativus* L.) plants: the upper and lower surfaces of leaves recorded a reduction in SD and SI.

Stomatal density in bean leaves of both grafted and non-grafted plants fell within the range described for angiosperms: 100-300 stomata/mm² (Esau, 1977). This situation suggests that bean leaves follow general patterns of stomatal development, similar to other angiosperm species. Specific variations could be the result of species-specific genetic information and the environmental conditions in which the plants develop. Plants adjust their stomatal density under water stress (Bishnoi *et al.*, 2022). Low stomatal density in *Triticum aestivum* L. is associated with higher yield under water stress (Li *et al.*, 2017), while, in *P. vulgaris*, SD increases under drought conditions (Polania *et al.*, 2020). Apple trees (*Malus domestica* Borkh.) grafted onto dwarfing rootstocks recorded a lower SD than apple trees grafted onto vigorous rootstocks (Zhou *et al.*, 2020).

The leaf mesophyll was different between palisade parenchyma near the upper surface and spongy parenchyma near the lower surface (Figure 4).

The interaction of the scion with the rootstock increased palisade parenchyma and spongy parenchyma ratios by 70.5% and 61.7%, respectively (Table 2). This increase resulted in a 66% thicker mesophyll.

Ayala-Arreola *et al.* (2010) reported an increase of SD, ECD, and palisade parenchyma thickness in grafted avocado (*Persea americana* Mill.) plants; their results are similar to those

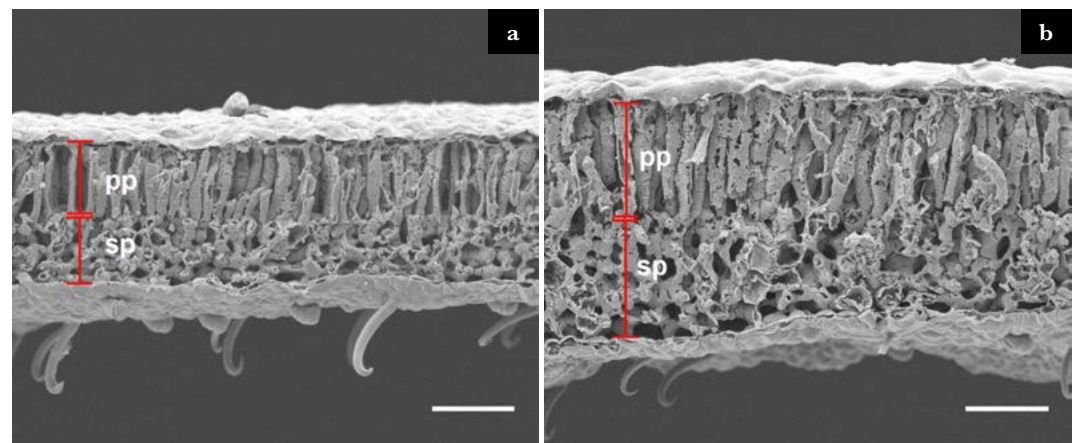


Figure 4. Cross-sections of the central leaflet. a) Non-grafted plant of *Phaseolus vulgaris* L. and b) Heterograft of *P. vulgaris* L. (scion)-*P. acutifolius* A. Gray (rootstock) observed in a JEOL JSM-6390 microscope. Palisade parenchyma (pp), spongy parenchyma (sp). Bar: 100 μ m.

Table 2. Effect of *Phaseolus acutifolius* A. Gray (rootstock) on the palisade parenchyma, spongy parenchyma, and mesophyll thickness of *P. vulgaris* L. (scion).

| | Palisade parenchyma (μm) | Spongy parenchyma (μm) | Mesophyll thickness (μm) |
|-------------|--|--|--|
| Grafted | 151.36 \pm 5.36 a | 147.53 \pm 5.83 a | 298.89 \pm 9.01 a |
| Non-grafted | 88.76 \pm 1.11 b | 91.23 \pm 2.78 b | 179.98 \pm 3.20 b |

Data are means \pm SE. Different letters in the columns indicate statistical differences (Tukey, $p \leq 0.05$), $n=8$.

recorded in this study. However, Zhou *et al.* (2020) reported different results in apple trees grafted onto dwarfing rootstocks, where the palisade parenchyma:spongy parenchyma ratio decreased.

Grafting can have similar effects in different plant species and promote anatomical modifications that potentially improve the efficiency of the physiological processes of plants. The increase in mesophyll thickness associated with a higher SD could improve gas exchange and photosynthetic capacity, favoring the growth and productivity of grafted plants. These results highlight the importance of grafting to adapt to adverse conditions and to optimize key anatomical characteristics for the physiological efficiency of plants.

CONCLUSIONS

The union between the scion and the rootstock formed a strong and functional connection that facilitated the continuous flow of nutrients and water in the grafted bean plants. The scion-rootstock interaction increased the thickness of the mesophyll, as well as the SD and SI on the underside of the leaves, resulting in the modification of gas exchange and transpiration and photosynthetic rates.

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Organic substrates improve the quality of *Swietenia mahagoni* (L.) Jacq. under nursery conditions

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ABSTRACT

Objective: To determine the best substrates that favor the quality of mahogany seedlings under nursery conditions.

Design/methodology/approach: The variables evaluated were plant height, stem diameter, aerial and root dry weight, main root length, slenderness, and Dickson quality index. The treatments were: cocoa husk (Ch), coconut fiber (Cf) and pine sawdust (Ps) and volumetric mixtures of cocoa husk, coconut fiber and pine sawdust, established under a completely randomized design with eight treatments.

Results: The results of the analysis of variance showed statistically significant differences ($p < 0.05$) for all the variables analyzed. The species showed a more favorable morphological response in the Ch60 (Ch-60% + Cf-20% + Ps-20%) and Ch50 (Ch-50% + Cf-30% + Ps-20%) mixtures. Furthermore, the discriminant analysis allowed the differentiation of the substrates from the linear combination of the variables, where the height and aerial dry weight were the greatest contributors.

Limitations on study/implications: The study was conducted in a nursery, where conditions (irrigation, temperature, pest protection) are ideal. In the field, results may vary due to factors such as water stress, weed competition, or pathogen attacks. Therefore, it opens avenues for studying substrate combinations, mycorrhizal inoculation, or adaptation to post-transplant stress conditions.

Findings/conclusions: It is concluded that the treatments composed of the highest percentage of cocoa husk were the best substrates, so their use is recommended for the production of mahogany in containers.

Keywords: sawdust, cocoa husk, coconut fiber, morphology, nursery.

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INTRODUCTION

The use of containers in seedling production has proven to be highly effective. Physiologically, it promotes root formation, since their use fosters the development of an abundant root system and functional absorbent hairs capable of branching and producing a denser system; these hairs remain attached to the growing medium, which facilitates taking root, especially when removing the plants from the containers (Cobas *et al.*, 2020).

For nursery production, it is important to choose a substrate with chemical and physical characteristics that provide sufficient nutrients and physical support for good seedling growth (Svartz and Raimondo, 2022). These characteristics include slightly acidic pH, fertility, freedom from pests and diseases, in addition to presenting values in total porosity of 70%, aeration porosity of 10%, and water retention porosity of 55% (Rasool *et al.*, 2024).

According to López *et al.* (2018), the type of substrate used in nurseries is one of the factors that influence the quality and production cost of a plant; therefore, it is essential to look for options that reduce costs and guarantee plant quality. In Cuba, forest soil is the most frequently used substrate in forest nurseries that employ the traditional system (Falcón *et al.*, 2019). However, organic residues such as coconut fiber, cocoa husks, and sawdust can be used in the formulation of substrates and/or mixtures, for the production of different native species in containers.

Mahogany is one of the species selected for the enrichment of secondary forests (Álvarez, 2017), taking into account that it has characteristics such as being: facultative heliophilous, stabilizing, colonizing of different successional stages, tolerant to competition, and with a broad ecology that colonizes both primary plant formations (from which they originate) and secondary ones (Ricardo *et al.*, 2016).

Due to its multiple uses, this species is highly valued for its wood, but considering the slow growth of the species and overexploitation in many of its natural distribution areas, it has been included in Appendix II of the Convention on National Trade of Endangered Species of Wild Fauna and Flora (*Convención sobre Comercio Nacional de Especies Amenazadas de Fauna y Flora Silvestres*, CITES), making it necessary to undertake reforestation and enrichment programs with this valuable species (Labrador *et al.*, 2017).

For the reasons stated above, and due to the importance of the species for multiple uses and because it is under threat, this study was carried out with the objective of determining the best substrates that favor the morphological attributes of *S. mahagoni* seedlings under nursery conditions, for their incorporation into reforestation plans.

MATERIALS AND METHODS

The research was carried out in a nursery under a green mesh shade net with 50% shade (Figure 1A), at the facilities of the Center for the Study of Agricultural, Livestock and Forestry Technologies (Centro de Estudio de Tecnologías Agropecuarias y Forestales, CETAF), belonging to the University of Guantánamo (UG), located at 20° 12' 21" latitude North and 75° 13' 37" longitude West at 87 meters above sea level.

The climatic characteristics of the region reflected in the climograph, with a 10-year data series (Figure 1B) are marked by three dry stages: January-April, June-July, and November-December. Rainfall was slightly above 100 mm in the months of May and October, while in August and September it was lower, according to information from the Cuban Meteorological Institute (INSMET, 2023).

Mahogany seeds with a germination capacity of 92% were used, obtained from ripe fruits collected from the mass located in the municipality of Jamaica, belonging to the Guantánamo Agroforestry Company (20° 11' 44" N, 75° 08' 38" W). Sowing was carried

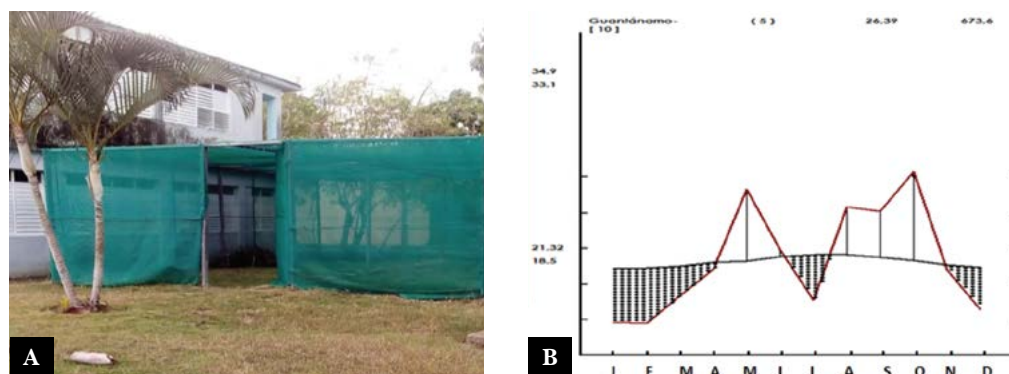


Figure 1. Growing house (A) and climatic conditions (B).

out in plastic containers with a volume of 200 cm³, using different volumetric mixtures of decomposed cocoa husk (*Theobroma cacao* L.), coconut fiber (*Cocos nucifera* L.), and pine sawdust (*Pinus cubensis*). The composition of the substrates, as well as the treatments used, are shown in Table 1.

Likewise, Table 2 shows the chemical-physical composition of each of the substrates that were analyzed in the soil laboratory of the UBCT Guantánamo. The methodology described by Ansorena (1994) was used to determine the physical properties: apparent density (AD), true density (TD), total porosity (TP) and moisture retention (MR). Chemical analyses were performed based on Cuban standards (CS) for this type of analysis (CS-XX 2009), where the percentage of organic matter (OM), potassium (K), sodium (Na), phosphorus (P), calcium (Ca), nitrogen (N), pH and electrical conductivity (EC) content were determined.

Experimental design

The experiment consisted of eight treatments defined by different concentrations of cocoa husk, coconut fiber, pine sawdust, and volumetric mixtures between them (Table 1). Four replicates were used for each treatment, with 30 plants each, for a total of 960 plants in the experiment. A completely randomized design was used for treatment distribution.

Table 1. Composition of the substrates used in the experiment.

| Substrate | Abbreviation | Treatment | Composition (%) |
|--|--------------|-----------|-----------------|
| Cocoa husks | Cc | Cc | 100 |
| Coconut fiber | Fc | Fc | 100 |
| Pine sawdust | As | As | 100 |
| Cocoa husks+coconut fiber+pine sawdust | Cc+Fc+As | Cc60 | 60+20+20 |
| | Cc+Fc+As | Cc50 | 50+30+20 |
| | Cc+Fc+As | Cc40 | 40+40+20 |
| | Cc+Fc+As | Cc30 | 30+50+20 |
| | Cc+Fc+As | Cc20 | 20+60+20 |

Table 2. Chemical and physical characteristics of the substrates used in the experiment.

| | Treatments | | | | | | | |
|--------------------------|------------|-------|-------|-------|-------|-------|-------|-------|
| | Cc | Fc | As | Cc60 | Cc50 | Cc40 | Cc30 | Cc20 |
| pH | 5,90 | 5,49 | 5,76 | 6,20 | 6,31 | 6,57 | 6,66 | 6,70 |
| OM (%) | 89,18 | 71,73 | 50,41 | 77,20 | 73,78 | 68,96 | 66,66 | 66,02 |
| N (%) | 1,87 | 1,43 | 0,52 | 1,86 | 1,82 | 1,54 | 1,33 | 1,40 |
| P (%) | 1,45 | 0,11 | 0,093 | 1,43 | 1,42 | 1,42 | 1,36 | 0,10 |
| K ⁺ (%) | 1,81 | 1,39 | 1,18 | 1,79 | 1,74 | 1,55 | 1,59 | 1,19 |
| Ca ²⁺ (%) | 0,69 | 0,45 | 0,54 | 0,68 | 0,68 | 0,46 | 0,68 | 0,56 |
| Na ⁺ (%) | 0,09 | 0,06 | 0,03 | 0,08 | 0,07 | 0,05 | 0,04 | 0,05 |
| EC (dS m ⁻¹) | 0,65 | 3,54 | 3,27 | 3,13 | 2,61 | 2,68 | 3,08 | 2,27 |
| AD (g mL ⁻¹) | 0,28 | 0,19 | 0,54 | 0,38 | 0,37 | 0,42 | 0,33 | 0,31 |
| RD (g mL ⁻¹) | 1,53 | 1,48 | 2,05 | 1,67 | 1,70 | 1,72 | 1,73 | 1,68 |
| TP (%) | 84,97 | 55,45 | 70,26 | 82,02 | 81,87 | 77,60 | 78,40 | 76,16 |
| HR (%) | 62,60 | 78,09 | 50,88 | 65,49 | 64,41 | 66,33 | 71,54 | 70,76 |

Percentage of organic matter (OM), potassium content (K), sodium (Na), phosphorus (P), calcium (Ca), nitrogen (N), hydrogen potential (pH) and electrical conductivity (EC), apparent density (AD), true density (RD), total porosity (TP) and moisture retention (HR).

Attributes evaluated and sampling procedure

After four months in the nursery, the height of 50 plants, located in the center of each tray (usable plot) per treatment avoiding the edge effect, was measured. This measurement was taken from the root collar to the tip of the apical bud, every 15 days, using a 0.1 mm precision graduated ruler.

A vernier caliper was used to measure stem diameter, with a precision of 0.02 mm. This measurement was made every 15 days.

The aerial and root dry weights were determined after the samples were placed in a forced-air circulation oven (BINDER) for 72 hours, at a temperature of 70 °C until constant mass was reached. The determination was performed on a Sartorius CPA324S balance with a margin of error of 0.1 mg.

The length of the main root was measured from the neck to the apex, using a 0.1 mm precision ruler. The fine and thick roots were also counted.

Based on the above attributes, the following relationships and indices were evaluated:

- Aerial part to root part ratio (ADW/RDW): estimated as the quotient between the dry weight of the aerial part and the dry weight of the root in grams.
- Slenderness Index (H/Drc): calculated with the quotient between the height in centimeters and the diameter of the stem at the root neck in millimeters.
- Dickson Quality Index (DQI): was determined using equation (1):

$$DQI = \frac{\text{Total dry weight (g)}}{\frac{\text{Height (cm)}}{\text{Diameter (mm)}} + \frac{\text{Dry aerial weight (g)}}{\text{Root dry weight (g)}}}$$

Statistical analysis

For the comparison of morphological attributes and indices between substrates, an analysis of variance was performed and when there was a significant difference ($p \leq 0.05$), the means were compared according to Duncan's Multiple Range Test ($p \leq 0.05$), since the data were adjusted to a normal distribution, verified by the Shapiro-Wilks Test.

Discriminant analysis was used to analyze the position of each substrate defined by the first two classification functions, and to identify the morphological attributes that most contributed to their differentiation. The substrates were considered as groups *a priori*. Data processing was performed using the SPSS statistical package, version 23 for Windows.

RESULTS AND DISCUSSION

Morphological attributes and indices evaluated

Table 3 shows the means of the different morphological attributes and indices, where significant differences were observed between the substrates used, except for substrates Ch60 and Ch50, which had the best values for all the attributes evaluated. These values may be influenced by a correct or abundant supply of nutrients from the substrates, so these combinations were more favorable for plant development and growth.

Similar results were obtained by Aguirre *et al.* (2018), who found that the use of organic substrates with adequate physical and chemical characteristics favored the growth and development of the species *Azadirachta indica* A. Juss. and *Ceiba pentandra* (L.) grown in a nursery.

Like height, diameter showed the best values in substrates Ch60 and Ch50, with no significant differences between them, but with differences from the rest of the substrates. These increases may be related to the concentrations of nitrogen, phosphorus, and potassium present in these substrates (Table 2). In this regard, Da Ros *et al.* (2015) propose that the presence of nitrogen in the initial phase of seedling production increases the growth rate of stem mass due to cell expansion, owing to the function of proteins in the development of cell walls and the cytoskeleton.

Table 3. Mean values and standard error of morphological attributes and indices.

| Substrate | A (cm) | DT (mm) | PSA (g) | PSR (g) | LRP (cm) | A/DT | PSA/PSR | ICD |
|----------------|--------------------|-------------------|-------------------|-------------------|---------------------|-------------------|-------------------|-------------------|
| Cc | 18,89 ^c | 3,87 ^d | 0,62 ^b | 0,43 ^c | 17,15 ^d | 4,88 ^b | 1,47 ^c | 0,16 ^c |
| Fc | 14,81 ^g | 3,16 ^f | 0,50 ^d | 0,30 ^e | 15,15 ^g | 4,68 ^c | 1,68 ^b | 0,12 ^e |
| As | 15,65 ^f | 3,15 ^f | 0,34 ^e | 0,22 ^f | 15,64 ^f | 4,96 ^a | 1,47 ^c | 0,09 ^f |
| Cc60 | 25,10 ^a | 5,91 ^a | 0,75 ^a | 0,53 ^a | 19,17 ^a | 4,34 ^e | 1,42 ^d | 0,21 ^a |
| Cc50 | 24,20 ^a | 5,56 ^a | 0,74 ^a | 0,52 ^a | 18,25 ^{ab} | 4,26 ^f | 1,41 ^d | 0,22 ^a |
| Cc40 | 20,38 ^b | 4,10 ^b | 0,64 ^b | 0,47 ^b | 17,50 ^{bc} | 4,47 ^d | 1,34 ^d | 0,18 ^b |
| Cc30 | 18,28 ^d | 3,98 ^c | 0,58 ^c | 0,29 ^e | 17,16 ^d | 4,60 ^c | 2,01 ^a | 0,13 ^e |
| Cc20 | 16,78 ^e | 3,75 ^c | 0,57 ^c | 0,35 ^d | 16,50 ^c | 4,68 ^c | 1,63 ^c | 0,14 ^d |
| Standard error | 0,174* | 0,048* | 0,006* | 0,005* | 0,065* | 0,012* | 0,011* | 0,002* |

A: height; Dcr: root collar diameter; PSA: aerial dry weight; PSR: root dry weight; LRP: main root length; A/Dcr=Slenderness Index; PSA/PSR= ratio of aerial parts to root parts; ICD=Dickson quality index.

Other authors report that optimal nitrogen concentrations in the substrate influence seedling quality (Valkinir *et al.*, 2017), and have documented rapid development of large, dark green stems and leaves containing large amounts of chlorophyll which absorb relatively high amounts of light and produce large amounts of carbohydrates that are used in the formation of absorbent stem, leaf, and root cells.

These results coincide with those obtained by Ricárdez *et al.* (2020), when they stated that the greatest cocoa growth was observed in the presence of optimal nitrogen and phosphorus values. In addition, these same authors observed that the growth rate of aerial biomass, and therefore the area available to intercept radiant energy, is strongly dependent on the availability of nutrients.

Substrates Ch60 and Ch50 promoted the best root and aerial growth (Table 3), which is associated with the improvement in the physical and chemical properties of these mixtures; that is, an adequate relationship between air, water and availability of nutrients favors the increase in biomass accumulation rates. These variables are relevant attributes for the performance and survival of plants in the field and are complementary to height and diameter to better describe the morphological quality of individuals produced in containers (Márquez and Martínez, 2022).

When analyzing the values obtained in the Slenderness index (H/Drc), it is observed that the plants with the best performance were those grown in the Ch60 and Ch50 substrates, because they had a lower value, which is related to greater robustness and vigor in the stem, that is, better quality.

These results agree with those obtained by Venancio *et al.* (2022), who point out that the lower the value of this index, the greater the resistance of plants to the action of stress factors such as wind and competition with weeds, which indicates greater mechanical resistance during planting operations; and that on the one hand, the total development of the plant is large and, at the same time, the aerial and root fractions are balanced.

Similar responses were reached by Falcón *et al.* (2022), who highlight among the relationships of the morphological parameters used to evaluate the quality of seedlings of tree species, the slenderness index, which constitutes one of the most important attributes to estimate the growth of seedlings after establishment in the field and relates the resistance of the plant with its photosynthetic capacity.

Regarding the ADW/RDW ratio (Table 2), substrates Ch60, Ch50, and Ch40 recorded the best values, that is, the lowest averages; this indicated high quality, due to a good balance between the biomass of the aerial part and the root (Falcón *et al.*, 2021), as well as greater chances of success when planted in sites with low rainfall.

The results obtained agree with Rueda *et al.* (2014), who stated that to obtain quality seedlings, the ADW/RDW ratio should be less than 2.0, resulting in a greater capacity to overcome the critical rooting period. In the experiment, this ratio was found to be both above and below the recommended range, being lower in substrates Ch60, Ch50, and Ch40, which demonstrates the capacity of the seedlings obtained in these substrates to adapt to stressful environments.

Regarding the Dickson quality index (DQI), the highest means were obtained in substrates Ch60 and Ch50 with 0.21 and 0.22, respectively (Table 3), indicating that the

plants produced in these substrates are within quality parameters and suitable for planting, since their DQI was ≥ 0.2 (Falcón *et al.*, 2019). According to Villar *et al.* (2003), the DQI is a fairly solid index that expresses, in a single value, the quality of the plants.

Venancio *et al.* (2022) agree with the aforementioned and also express that the DQI is a good indicator, since it weighs important characteristics for the evaluation of plant quality and considers the vigor and balance of mass distribution in the plant.

In this study, the best results were obtained with substrates Ch60 (60% cocoa husk + 20% coconut fiber + 20% sawdust) and Ch50 (50% cocoa husk + 30% coconut fiber + 20% sawdust). In this sense, the study demonstrates that the addition of organic compounds to the substrates provided better conditions for the growth of mahogany plants by having a good balance between the availability of nutrients and the physical conditions, mainly aeration and water retention.

Discriminant analysis to select the best substrates

The two discriminant functions used for the classification (Table 4) explained 99.5% of the variation between substrates. According to the standardized coefficients, the most important variable in discriminating between substrates in the first function was height, while in the second function the most important discriminating variable was aerial dry weight (Table 4). These variables can be proposed as predictors for substrate selection.

Table 4. Results of the discriminant analysis, standardized coefficients of each variable, and centroids of the substrates in the two discriminant functions.

| | Function | |
|------------------------|---|--------|
| | 1 | 2 |
| Eigenvalues | 438,863 | 3,683 |
| % variation | 98,7 | 0,8 |
| % cumulative variation | 98,7 | 99,5 |
| Attributes | Standardized coefficients of discriminant functions | |
| Height | 0,801 | 0,226 |
| Diameter | 0,531 | -0,667 |
| Root dry weight | 0,157 | 0,259 |
| Aerial dry weight | 0,110 | 0,852 |
| Total dry weight | -0,112 | 0,053 |
| Substrates | Centroids | |
| Cc | -3,702 | 3,434 |
| Fc | -25,641 | -0,381 |
| As | -22,712 | -1,216 |
| Cc60 | 29,028 | -0,363 |
| Cc50 | 35,083 | -1,306 |
| Cc40 | 5,083 | 2,575 |
| Cc30 | -6,634 | -2,642 |
| Cc20 | -10,505 | -2,800 |

Alonso *et al.* (2015) obtained similar results when they used discriminant analysis to differentiate the substrates, which also facilitated the recognition of the height variable as the one with the greatest contribution to the discrimination.

Figure 2 represents the multivariate observations in the discriminant space made up by the two functions, where the separation of the substrates into three well-defined groups is evident. On the left of the graph there are the Cf and Ps substrates, while substrates Ch60 and Ch50 were located at the opposite end, and the remaining substrates (Ch, Ch40, C30, and Ch20) remained in the center.

The attributes height and aerial dry weight were those that had the greatest weight in the distribution of the groups (Table 3), with substrates Ch60 and Ch50 being the ones where seedlings showed the greatest growth and development, while the Cf and Ps substrates (individual components) were the ones with the lowest values in these attributes.

According to Valenzuela (2019), substrates should be made with more than one organic material that promotes an adequate balance of nutrients, with the aim of promoting plant growth and development. This aspect could have influenced the better results obtained with substrates Ch60 and Ch50, made with three organic materials.

Based on the discriminant analysis, it was established that the best treatments were always associated with substrates Ch60 and Ch50, and therefore, these mixtures favored the morphophysiological attributes of mahogany plants. This result is similar to those obtained by Alonso *et al.* (2015), who used discriminant analysis as a tool to select the best substrates studied in the nursery.

Frequently, statistical analyses used for the evaluation and selection of substrates based on morphological variables consist of simple variance analysis (Reyes *et al.*, 2018; Domínguez-Liévano and Espinosa-Zaragoza, 2021). In many cases, the variables evaluated do not clearly indicate the differentiation between them and the selection due to limitations of the analysis method. Therefore, the discriminant analysis statistical method as an interpretation tool may be a useful option in these cases.

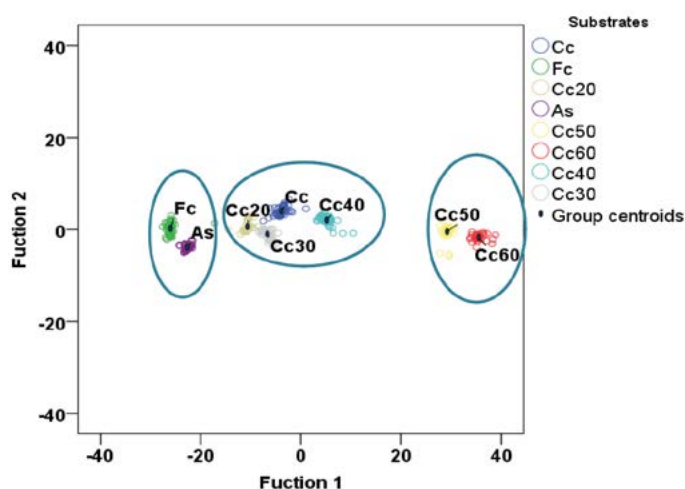


Figure 2. Scatter plot of the substrates in the two discriminant functions.

CONCLUSIONS

The best morphological attributes were obtained in the mixtures of cocoa husk, coconut fiber, and sawdust in proportions 60:20:20 and 50:30:20 (Ch60 and Ch50).

The use of organic substrates made from local materials proves to be a viable alternative to implement in the production of mahogany forest plants.

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Seed germination and biomass of cabbage (*Brassica oleracea* var. *capitata*) seedlings in conventional agriculture (ConvAg) and cold-water agriculture (ColdAg)

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ABSTRACT

Objective: To evaluate the effects of cold-water agriculture (ColdAg) vs. conventional agriculture (ConvAg) conditions on seed germination and fresh and dry biomass accumulation of cabbage (*Brassica oleracea* var. *capitata* L.) seedlings.

Design/methodology/approach: The effects on germination parameters and fresh and dry biomass accumulation of cabbage (*Brassica oleracea* var. *capitata* L.) seedlings were evaluated under the following treatments: (1) cold-water agriculture conditions (ColdAg: 35 °C temperature inside the growth chamber, 14.8 °C water flow temperature, 85% relative humidity) and (2) conventional agriculture (ConvAg: 25 °C temperature inside the growth chamber, 20 °C irrigation water temperature, 70% relative humidity). In both cases, the light intensity was $334 \mu\text{mol m}^{-2} \text{s}^{-1}$. Each condition had three replicates, and the experimental unit was a pot with commercial substrate in which 10 seeds were sown. The experiment was conducted in controlled growth chambers, and the treatments were distributed in these completely at random. The germination data obtained were used to estimate the variables daily total germination (DTG), median germination time (T_{50}), germination percentage (GP), coefficient of velocity of germination (CVG), and germination index (GI). In addition, the weight of the fresh and dry biomass of the seedlings was determined. With the results obtained, variance analysis and Tukey mean comparison tests were performed ($p \leq 0.05$).

Results: Seed germination in ConvAg occurred 2 d earlier than in ColdAg. Likewise, daily total germination (DTG) was higher in ConvAg. However, fresh and dry seedling biomass was higher in ColdAg, exceeding the ConvAg treatment by 66.3 and 146.7%, respectively.

Limitations of the study/implications: This technology requires sufficient space and specialized technical and scientific equipment, as well as the availability of cold water throughout the crop cycle.

Findings/conclusions: ColdAg technology allowed good germination of cabbage seeds and greater production of fresh and dry seedling biomass.

Keywords: Brassicaceae, cabbage, ColdAg, ConvAg, germination, biomass.

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INTRODUCTION

Global food security faces serious challenges related to the impacts of global climate change on agriculture and the increasing demand for food by the ever-growing population. This makes it necessary to develop new technologies that allow food production under limiting conditions (FAO, 2017), which in turn are energy efficient and environmentally sustainable in the long term (Rabbi *et al.*, 2019; WMO, 2023; García-Huante *et al.*, 2024; Xiao *et al.*, 2024). These include cold-water agriculture (ColdAg) or conditioning for soil cooling in areas under controlled warm climate conditions, where thermal stress is generated in the roots of the crop, obtaining plants with better development and growth (Graamans *et al.*, 2018). This technology was developed from a marine renewable energy system known as Ocean Thermal Energy Conversion (OTEC), through which cold deep seawater and warm surface water are pumped to produce electrical energy, and the volume of ocean water can be used to generate temperate zone crops in tropical areas (García-Huante *et al.*, 2024). Under these conditions, higher yields per unit area have been reported, as well as greater efficiency in water use, compared to crops under conventional agriculture (ConvAg) conditions. This technology is being implemented in some Asian countries with densely populated areas and where the consumption of cabbage, lettuce, tomato, and radish is high (Ghosh and Ganguly, 2017). In this study, cabbage was used as a model crop, a species that can adapt to a wide range of environmental conditions. It is a vitamin C-rich species with high demand in the fresh market, as it has an optimal shelf life for long-distance shipments and high yield. The objective of the study was to compare the effects of conventional agriculture (ConvAg) and cold-water agriculture (ColdAg) conditions on germination variables and fresh and dry biomass production.

MATERIALS AND METHODS

Biological material and experiment setup

Cabbage (*Brassica oleracea* var. *capitata* L.) cv. Royal Vantage (Sakata Seed America, Morgan Hill, CA, USA) seeds were used. This research was conducted under the conditions described in Table 1. Under both experimental conditions, the light intensity was $334 \mu\text{mol m}^{-2} \text{s}^{-1}$.

For each treatment, three 300 mL plastic pots (height 7.5 cm, base 7 cm, mouth 10 cm) were used. The pots were filled with the commercial substrate. The physical and chemical properties of the substrate are described in Table 2.

Table 1. Experimental conditions to test the effects of cold-water agriculture (ColdAg) on germination and early growth of cabbage (*Brassica oleracea* var. *capitata*) compared to conventional agriculture (ConvAg).

| Treatment | Temperature | Relative humidity (%) | Total seeds per pots | Total seeds |
|-----------|---|-----------------------|----------------------|-------------|
| ColdAg | 35 °C in germination chamber/Cold water flow at 14.8 °C | 85 | 10 | 30 |
| ConvAg | 25 °C in germination chamber/Irrigation water at 20 °C | 70 | 10 | 30 |

Table 2. Chemical and physical properties of the commercial substrate used to test the effects of conventional agriculture (ConvAg) and cold-water agriculture (ColdAg) on seed germination and early seedling growth of cabbage (*Brassica oleracea* var. *capitata*).

| pH | Electrical conductivity (dS m^{-1}) | Bulk density (g cm^{-3}) | | Ca | K | Mg | Na |
|------------------------|---|--|--------|---|-----------------|-------------------------|-------|
| | | | | (cmol ₍₊₎ kg ⁻¹) | | | |
| 6.0 | 0.402 | 0.658 | | 18.264 | 9.278 | 12.807 | 3.015 |
| Cu | Fe | Mn | Zn | Field capacity | | Permanent wilting point | |
| (mg kg ⁻¹) | | | | (% gravimetric water content) | | | |
| 1.595 | 30.525 | 64.568 | 34.024 | 73 | | 55 | |
| Porosity (%) | | | | | | | |
| Total | | | Airing | | Water retention | | |
| 68 | | | 22 | | 46 | | |
| Unavailable water | | Easily available water | | Reserve water | | Hardly available water | |
| (%) | | | | | | | |
| 10 | | 6 | | 7 | | 32 | |

Thirty seeds per treatment were sown in three pots (10 seeds per pot, experimental unit) at a depth of 0.5 cm in a horizontal arrangement.

For ColdAg conditions, a 0.9 mm diameter plastic hose was passed horizontally at the middle height of each of the three pots through which cold distilled water (14.8 °C) was constantly recirculated in a closed-circuit system. The cold water was moved by means of a water chiller (S&A CW-3000, Guangzhou, China), which integrates an internal hydrostatic pump, a metal coil and a fan, in order to cool the substrate placed in the pots (Figure 1, upper image). The experimental units of the ColdAg treatment were placed in the upper part of a growth chamber (Shellab[®] LI15, Cornelius, OR, USA), on a plastic tray. The incubation conditions were: day and night temperatures of 35 and 30 °C, respectively; 12 h of light; relative humidity of 85%; and light intensity of 334 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

For ConvAg (Figure 1, bottom image), a second growth chamber was used where the experimental units were placed. The conditions inside the chamber were: day and night temperatures of 25 and 20 °C, respectively; 12 h of light; relative humidity of 70%; and light intensity of 334 $\mu\text{mol m}^{-2} \text{s}^{-1}$. In this treatment, there was no cold-water flow to lower the temperature of the substrate in the root zone.

In both treatments (ColdAg and ConvAg), each pot was initially watered with 100 mL of tap water, then the substrate was watered until reaching field capacity twice a day (at 08:00 h and at 16:00 h).

The experiment lasted 23 d from sowing the seeds to harvesting the seedlings.

Germination

Germinated seeds were recorded every 24 h for 16 d. The following variables were estimated from these data: median germination time (T_{50}), germination percentage (GP), daily total germination (DTG), coefficient of velocity of germination (CVG), and germination index (GI), according to the methodologies described by González-Zertuche and Orozco-Segovia (1996) and Kader (2005) (Table 3).

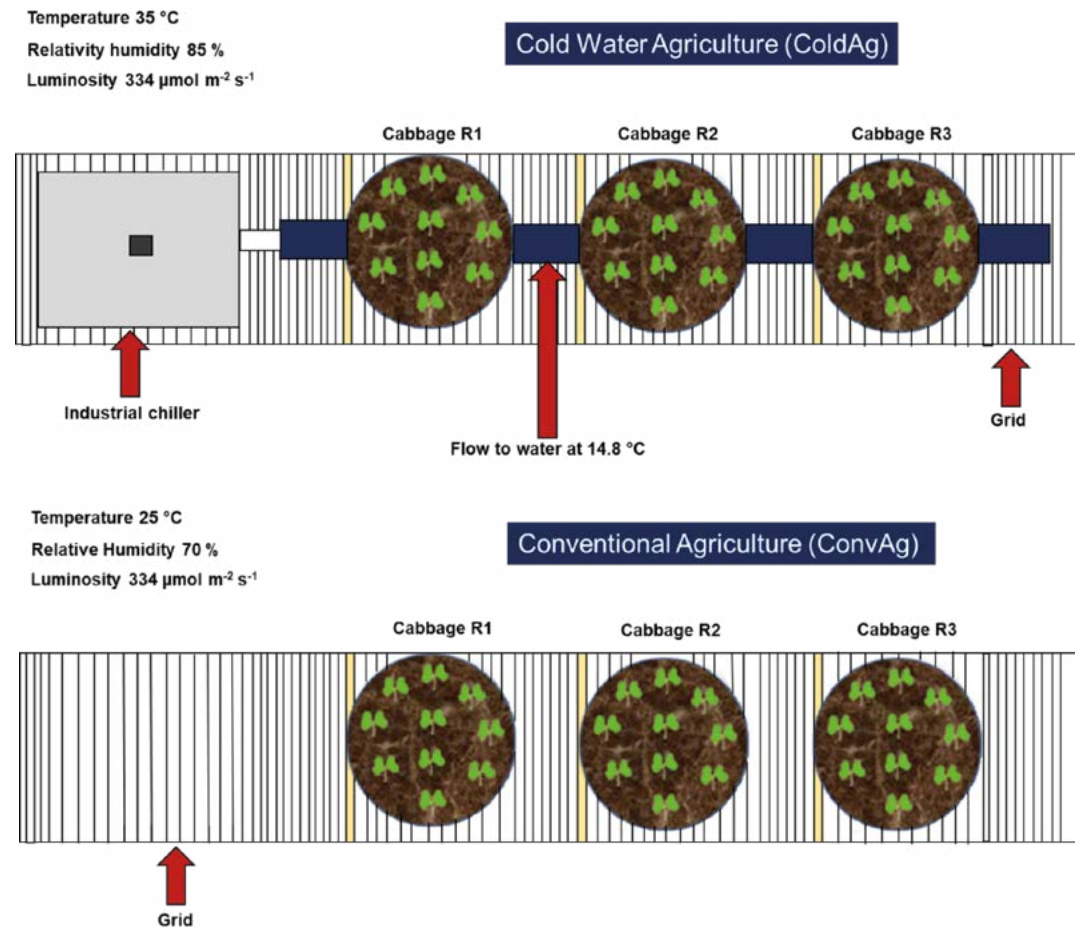


Figure 1. Schematic representation of seeds and plant distribution of cabbage (*Brassica oleracea* var. *capitata*) in the growth chamber to test the effects of cold agriculture (ColdAg, upper image) in comparison with conventional agriculture (ConvAg, bottom image).

Table 3. Germination parameters, formulas and description of variables considered to evaluate the effects of cold-water agriculture (ColdAg) on the germination of cabbage (*Brassica oleracea* var. *capitata*) seeds compared to conventional agriculture (ConvAg).

| Parameter | Formula | Description |
|--|--|--|
| Median germination time (T ₅₀) | <i>Time in days in which 50% germination is obtained</i> | The value of the average germination time implies the number of days necessary to have 50% of the sown seeds germinated. |
| Germination percentage (GP) | $GP = \text{No. of seeds germinated in a seed lot} \times 100$ | The higher the GP value, the greater the germination of a seed population. |
| Daily total germination (DTG) | $DTG = \text{No. of seeds germinated daily}$ | It is the value of germinated seeds per day until the end of the germination process. |
| Coefficient of velocity of germination (CVG) | $CVG = \frac{\text{Total of seeds germinated}}{\sum (N1 + N2 / 2 + \dots + Nn / n)}$ | The CVG gives an indication of the rapidity of germination. It increases when the number of germinated seeds increases and the time required for germination decreases. Theoretically, the highest CVG possible is 100. This would occur if all seeds germinated on the first day. |
| Germination index (GI) | $GI(\%) = RSG - RGR / 100$ | Indicate the relation of the relative seed germination (RSG) and the relative growth of radicle (RGR). |

Fresh and dry biomass

At 23 d after sowing, the seedlings were harvested. The weight of the fresh biomass was obtained from each replicate, and after drying in a forced air oven at 72 °C for 72 h, the weight of the dry biomass was obtained. In both cases, an analytical scale was used (Adventurer Pro AV213C[®], Ohaus; Parsippany, NJ, USA).

Statistical analysis

A variance analysis was performed on the data obtained and the means were compared with the Tukey test with a significance value of 95%. For these analyses, the statistical software SAS version 9.4 was used.

RESULTS AND DISCUSSION

Germination parameters

Table 4 shows the results obtained in the germination variables. The variables median germination time, germination percentage, coefficient of velocity of germination, and germination index were not different between the evaluated treatments.

In the variable daily total germination (DTG), differences were recorded between treatments, observing that the ConvAg treatment exceeded the ColdAg by 128.9% (Table 4). The optimal temperatures for germination of cabbage seeds range between 15 and 25 °C; these temperatures coincide with those of the ConvAg treatment (20-25 °C). With temperatures equal to or greater than 30 °C, germination can be accelerated; however, these conditions are detrimental to the initial development of the seedling (Červenski *et al.*, 2022). In this study, it was observed that thermal stress due to soil cooling and heat from the environment (ColdAg) delays the germination process and therefore prolongs it, which can impact the total germination percentage. It is important to highlight that, although the germination percentage was not statistically different between treatments, it is 4% higher in ColdAg than in ConvAg. This may be due to the fact that soil cooling causes sufficient moisture in the substrate or soil for the germination process to take place without adverse effects (Červenski *et al.*, 2022).

Biomass weights

The treatments significantly affected the fresh and dry biomass production of the seedlings that were harvested 23 d after sowing (Figure 2).

Table 4. Seed germination indicators of cabbage (*Brassica oleracea* var. *capitata* L.) under cold-water agriculture (ColdAg) and conventional agriculture (ConvAg) conditions.

| Treatment | DTG | T ₅₀ (days) | GP | CVG | GI |
|-----------|-------------|------------------------|-----------|--------------|-------------|
| ColdAg | 1.87±0.49 b | 5 | 66±1.04 a | 16.61±1.04 a | 6.17±0.35 a |
| ConvAg | 4.28±0.64 a | 5 | 62±0.55 a | 19.42±0.77 a | 5.40±0.26 a |

Means ± SE with a different letter in each row indicate that there are statistical differences. Letters in each row with different letters are statistically different (Tukey, $p \leq 0.05$), $n=3$. DTG: Daily total germination; T₅₀: Median germination time; GP: Germination percentage, CVG: Coefficient of velocity of germination; GI: Germination index.

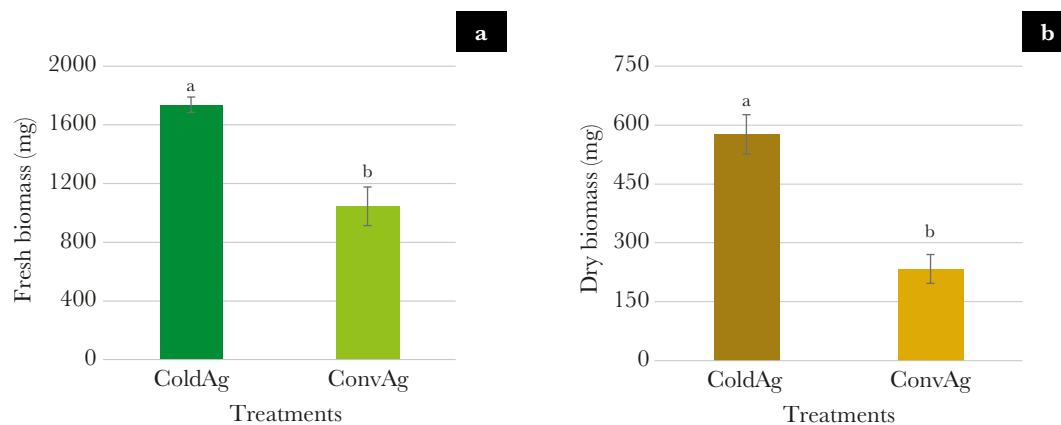


Figure 2. Weight of fresh (a) and dry (b) biomass of cabbage (*Brassica oleracea* var. *capitata* L.) seedlings per experimental unit, under cold-water agriculture (ColdAg) and conventional agriculture (ConvAg) conditions. Means \pm SE with different letters in each subfigure indicate that there are statistical differences (Tukey, $p \leq 0.05$), $n=3$.

The fresh seedling biomass in the ColdAg treatment exceeded the ConvAg treatment by approximately 66.3% (Figure 2a), while the dry seedling biomass of the ColdAg treatment was 149.7% higher than that of ConvAg (Figure 2b). Soil cooling generates condensation of water vapor existing between the soil pores and allows sufficient humidity for the roots to absorb and transport water, generating greater seedling development (Craven and Weaver, 1998; Sabri *et al.*, 2018; Sha'Arani *et al.*, 2021).

When high ambient temperatures are present (*i.e.*, 35-37 °C), the germination percentage for cabbage seeds decreases by more than 50% (Elson *et al.*, 1992). However, if high ambient and low soil temperatures are combined, better conditions for germination can be achieved, since relative humidity can increase by 20% and thus sufficient soil moisture can be achieved (Červenski *et al.*, 2022).

In the present experiment, the soil was cooled to 14.8 °C and the relative humidity was 85% inside the growth chamber, which increased the production of fresh and dry biomass of seedlings. Cool soil temperature allows for better growth and development of cabbage seedlings, especially at temperatures between 13 and 21 °C (Červenski *et al.*, 2022). It has been demonstrated that periods with a warm shoot but cold root system, especially at high elevations in the morning hours, may allow relatively high photosynthetic activity despite cold soil (Göbel *et al.*, 2019). Nevertheless, the physiological mechanisms which allow plant species to maintain a relatively high root water uptake at a low soil temperature are not yet fully understood and deserve further investigation.

CONCLUSIONS

Although the cold-water agriculture system (ColdAg) can prolong the germination period of cabbage seeds, it presents better results in the production of fresh and dry biomass of seedlings compared to seedlings grown under conventional agriculture (ConvAg) conditions.

In future studies it will be necessary to research the physiological, biochemical, and molecular processes that ColdAg triggers in the plant, and that can explain in greater detail the productive responses observed.

ColdAg technology represents a potential alternative to guarantee food security for a growing global population, in production conditions increasingly affected by global climate change.

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Amaranth (*Amaranthus* spp.) value chain in Mexico

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ABSTRACT

Objective: To analyze the value chain of amaranth (*Amaranthus* spp.) in Mexico, considering the actors, their relationships and functions, as well as the factors influencing its performance.

Design/methodology/approach: The value chain map was obtained through targeted surveys and working panels with various stakeholders.

Results: The results highlight that small producers face greater difficulties, such as the lack of adequate technology. Additionally, a disconnection between producers, researchers, and the government was evidenced.

Limitations on study/implications: Obstacles in processing and marketing were identified, with relationships being purely commercial with collectors, as well as limited commercial promotion. Despite this, opportunities are perceived to improve marketing, especially at the national level, through awareness campaigns and marketing strategies. The importance of association among producers and institutional support to overcome challenges is emphasized.

Findings/conclusions: The study concludes with the need for a robust public policy to promote the consumption of amaranth, highlighting its nutritional and cultural properties. The study offers insight into the amaranth value chain in Mexico, highlighting its problems and proposing solutions for its strengthening.

Keywords: Production, Collection, Processing, Marketing, Consumption.

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INTRODUCTION

Amaranth (*Amaranthus* spp.) is a high-yield and versatile seed capable of thriving under adverse conditions. Its high nutritional value, both in quantity and quality, highlights it as a healthy food source for rural communities and as a viable crop alternative to address issues of malnutrition, public health, and climate change that affect society. In the international context, it has gained prominence in recent years due to its nutritional importance. Annually, approximately 201,000 t of the cereal are imported worldwide (Fierro *et al.*, 2020). The United States has emerged as the main importer of amaranth from Mexico, driven by its growing demand for organic, healthy, and nutritious products (Agri Food and Fisheries Information Service [SIAP], 2023).



At the national level, amaranth is primarily found in rainfed cultivation regions characterized by low levels of mechanization, family labor, and predominantly conventional systems. The cultivated area ranges from average plots of 0.5 to 3 ha to producers managing up to 200 ha, reflecting the diversity of scales and production methods in the sector (Ayala *et al.*, 2014). By 2022, the leading states in the production of this grain in Mexico, in terms of volume, were Puebla, Tlaxcala, and the State of Mexico, mainly concentrated in the central region of the country. Although Puebla is the main producer state in terms of volume, with 2,857.52 t, Tlaxcala surpasses it in production value (SIAP-Agri-Food Information System for Consultation [SIACON], 2022).

Contrary to its importance, the amaranth value chain in Mexico faces significant challenges due to the lack of resources and financing in the rural communities where it is cultivated (De la O *et al.*, 2012; Ayala *et al.*, 2012).

The lack of integration and organization among producers, combined with competition from more mechanizable crops and low selling prices, has resulted in reduced incomes for producers, amounting to only \$7,075.56 per ha (Ayala *et al.*, 2014). The creation of alliances and better management of distribution and marketing could enhance the competitiveness of the chain (Peña *et al.*, 2008). Identifying and addressing these limitations is crucial for improving producers' incomes and overall competitiveness. The objective of this study was to analyze the amaranth value chain, considering the actors, their relationships and functions, as well as the factors affecting the chain's competitiveness, to establish alternatives that can help overcome the limitations and improve producers' incomes.

MATERIALES Y METODOS

The value chain refers to how a set of actors interacts with a product to increase its value throughout the different links (Acosta, 2006).

Variables

Innovación. To determine the degree of influence of various factors, performance, and innovation were analyzed as indicators of the growth in amaranth production. The Venezian and Gamble (1969) equation, modified by Contreras (1999), was used (Equation 1), and data were taken from SIAP-SIACON (2022).

$$P_t = Y_0(A_t - A_0) + A_0(Y_t - Y_0) + (A_t - A_0)(Y_t - Y_0) \quad \text{Equation 1}$$

Where: P_t = Total increase in production for the analysis period; $Y_0(A_t - A_0)$ = Quantifies the contribution of the area; $A_0(Y_t - Y_0)$ = Quantifies the contribution of yield; $(A_t - A_0)(Y_t - Y_0)$ = Quantifies the combined effect of area and yield; A_0 = Initial average harvested area (1982=435 ha); A_t = Final average harvested area (2022=3,211.45 ha); Y_0 = Initial national average yield (1982=0.51 t ha⁻¹); Y_t = Final average yield (2022=1.78 t ha⁻¹).

To assess whether growth has been intensive or extensive, one can determine the proportion of each factor in the total increase in production (growth from 1982 to

2022=100%). Extensive growth involves an increase in harvested area, while intensive growth is related to an increase in yield. Additionally, combined growth implies an increase in both area and yield equally (Zarazúa *et al.*, 2009).

Value chain

Working panels

To characterize the amaranth value chain, producer panels were employed using the Delphi technique, aiming to obtain reliable and consensus-based responses from a group of experts (Franco *et al.*, 2018). These panels included representatives from Hidalgo, Tlaxcala, State of Mexico, Puebla, Morelos, and Mexico City.

In the first working session, participants received guidance on the fundamentals of value chain methodology and established initial guidelines for the analysis of the chain and competitiveness. A dialogue was initiated with experts in each panel to gather information related to production, technical parameters, and marketing systems for a baseline year. Feedback was received and strategies and recommendations were formulated. Validation of the results was conducted through a consensus process with the original panelists to verify the integrity and accuracy of the information. This process is crucial to ensure the validity and representativeness of the data obtained (Zavala *et al.*, 2012). The Amaranth value chain was modeled based on information provided by experts in two panels.

Attendees included input suppliers, producers from different regions, processors, academic and governmental institutions, NGOs, as well as stakeholders interested in the subject matter.

Field surveys

Surveys in the aforementioned states involved 96 producers, 8 processors, 12 experts, 6 traders, and 40 consumers. The surveys, consisting of qualitative questions, were transformed into quantitative data for detailed analysis. A group of producers was convened through non-probabilistic sampling (Pimienta, 2000). Efforts were made to ensure that producers had similar production systems and technological levels, as well as knowledge and information on technical parameters and production costs. The activity took place between June and December 2023.

Conceptual map of the amaranth value chain

To obtain the conceptual map of the amaranth value chain, different levels were analyzed, including actors and their roles, horizontal relationships, the domestic market, critical support services, technical assistance, quality management, logistics, and storage.

RESULTADOS Y DISCUSIÓN

National production of amaranth in the national context

The growth of amaranth production is determined by the combined interaction between the increase in planted area and the yield improvement of the crop by 64% (Factor Decomposition, Table 1). This implies an increase in both area and yield in a combined manner, suggesting that innovation adoption in amaranth is not high. The crop grows primarily based on an expanded cultivation area.

Table 1. Growth of amaranth production in the national context.

| Item | Surface | Yield | Surface–yield interaction | Total |
|----------------|---------|-------|---------------------------|-------|
| Value obtained | 1416 | 552 | 3526 | 5494 |
| Value (%) | 26 | 10 | 64 | 100 |

Author's elaboration with data from SIAP-SIACON, 2023.

According to Ayala *et al.* (2016) productivity increase is dependent on the adoption of technological innovations, which are recommended based on the characteristics of production zones (Estrada *et al.*, 2006). Muñoz *et al.* (2007) state that the adoption of technologies involves multiple factors, with one of the most critical being the involvement of trained extensionists who are knowledgeable about innovations. The low yield growth (Table 2) reflects the limited adoption of technologies.

Between 2000 and 2021, the yield per ha increased at an annual average growth rate (CAGR) of 1.94%, while production increased at 2.21%. The lower productivity increase compared to production is attributed to the low adoption of technological packages, as corroborated by fieldwork. The lack of continuous training and inadequate technical guidance also contribute to this stagnation.

Value chain and involved actors

Amaranth value chain map in Mexico

The amaranth value chain map (Figure 1) consists of six links. It includes input and information suppliers, followed by primary production, collectors, processors, marketing, and consumers. On the far right, there are stakeholders; however, some of these (the dotted ones) are not yet formally integrated.

Relationships among producers

Ayala *et al.* (2014) mentioned that in 2014, the links amongst producers were weak. However, due to their own initiative, they are currently organized into small informal groups. These associations are composed of proactive producers seeking to improve their production conditions through continuous learning. 21% of producers have organized themselves into groups driven by initiatives such as the field schools of the Secretariat of Welfare, which have been present since 2021 in Tlaxcala, Puebla, and Hidalgo, and will continue until 2024 in the State of Mexico.

While mechanization has facilitated land preparation and agricultural tasks, there are still some producers (24%) who use traditional methods such as the yoke. The National Institute of Forestry, Agricultural, and Livestock Research (INIFAP) has designed seeders and harvesters to optimize primary production, but access to these is not universal. Thus,

Table 2. Growth of amaranth production in the national context.

| Concept | Harvested area (%) | Production (%) | Yield (%) |
|---------|--------------------|----------------|-----------|
| CAGR | 1.04 | 2.21 | 1.94 |

Author's elaboration with data from SIAP-SIACON, 2023.

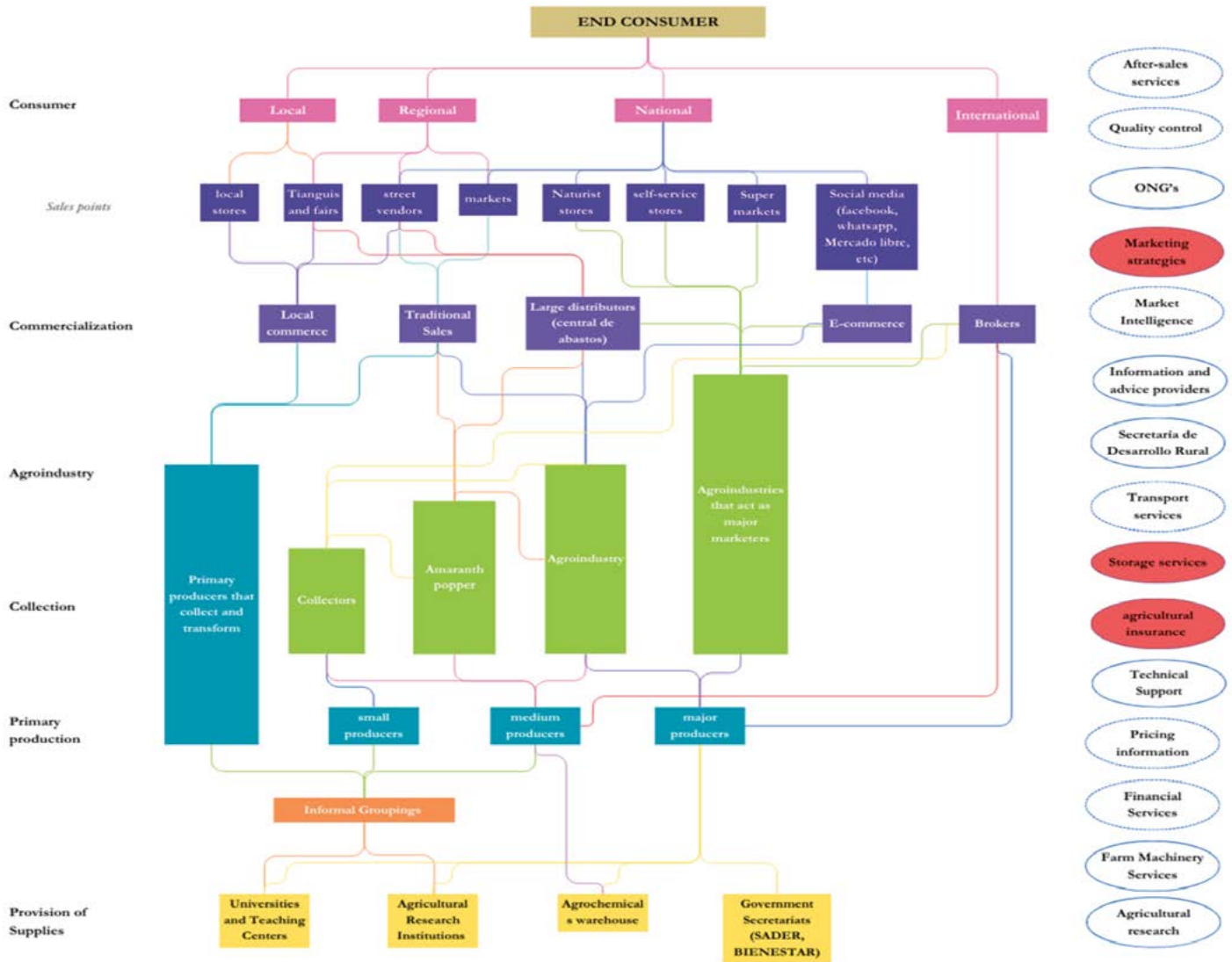


Figure 1. Linkages and relationships in the amaranth value chain in Mexico.

large producers with conditioned machinery rent it to their peers. Harvesting remains a challenge due to the high demand for labor (an average of 49 workdays) and the difficulty in finding workers due to low wages.

The field study also revealed that 89% of the producers do not own more than 10 ha. These small producers supply raw materials to the agro-industry. In contrast, producers with more than 10 ha sell their production directly to the processing industry. It is important to note that the percentage of producers involved in processing is minimal. Only 13% of those with up to 10 ha and 9% of those with more than 10 ha process their production or have small agro-industries. This represents a total of 23% of the surveyed producers. This is considered a low percentage, indicating an opportunity for more producers to integrate into this link (Figure 2).

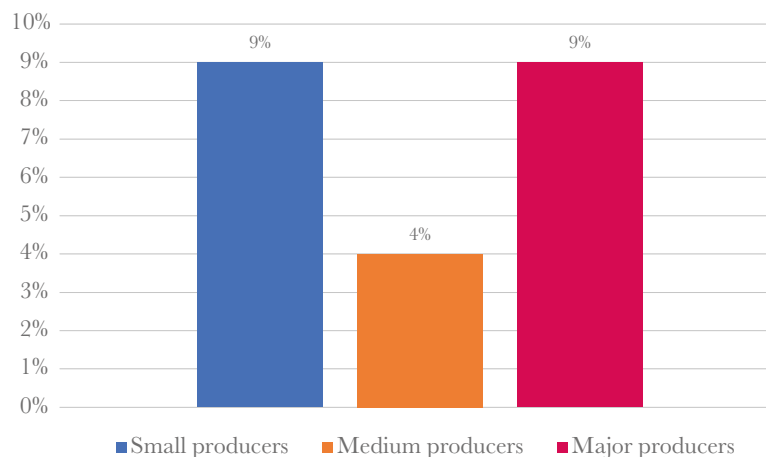


Figure 2. Classification of producers participating in processing.

Identified issue: Weaknesses were detected in the linkage between producers, researchers, and the government, along with deficient technical assistance and indiscriminate use of agrochemicals in production, mainly due to a lack of knowledge about technological packages. Climate changes also affect the crop and drive the search for more profitable alternatives, such as sorghum, which has displaced amaranth cultivation in Morelos. Inadequate agricultural practices and the lack of agricultural insurance or financial resources exacerbate the situation.

Collection

The commercialization of amaranth is characterized by a regional market (86%), although it has also managed to enter the national and international markets. The latter is to a lesser extent (5.26%), concentrated in Puebla and the State of Mexico. Some local collectors act as intermediaries and large collectors, who set the price and quality of the grain, often without knowledge of current regulations or process standardization.

Identified issue and alternatives: The collectors establish horizontal links with their peers, limiting the participation of producers in the value chain. It is necessary to develop effective marketing strategies that ensure proper quality management. To achieve this, efforts are focused on diversifying markets and establishing collection organizations that strengthen the negotiating position of producers.

Transformation

The transformation of amaranth is characterized by diverse relationships between processors and producers, supporting large-scale producers and selling to major industries. Institutions such as the College of Postgraduates (COLPOS) and the Meritorious Autonomous University of Puebla (BUAP) have promoted innovation and the development of technologies that facilitate amaranth processing. In Puebla, producer organizations like Amaranteo, Productores del Volcán Popocatepetl de Amaranto, Tochialegría, and Delice stand out for their efforts in amaranth transformation. Opportunities are identified to foster collaboration, promote research, support organizations, and diversify product offerings.

Identified issue: Existing equipment for grain popping is inadequate and affects the quality of the grain, thereby limiting its nutritional value. Additionally, most of the transformed products (90%) have high sugar content, highlighting the need for further research to develop amaranth-based foods with enhanced nutritional value.

Quality

In Mexico, the regulatory framework for amaranth intended for human consumption is established through two standards: Mexican Standard NMX-FF-114-SCFI-2009, which defines the quality specifications of the grain in terms of its physical, physicochemical, and microbiological characteristics, and Standard NMX-116-SCFI-2010, which complements the former by establishing necessary precautions to prevent contamination of popped grain during threshing, bagging, and storage stages (Secretaria de Economia [SE], 2009; 2010). Despite the existence of these standards, the study revealed that only 52% of producers establish criteria to ensure grain quality (Figure 3). These criteria are based on physical characteristics such as the presence of ferrous material, foreign matter, metallic particles, or black grain; physicochemical parameters such as moisture, ether extract, ashes, crude fiber, density, peroxide index, and heavy metals; and microbiological specifications including presence of molds and yeasts, aerobic mesophiles, total coliforms, *Salmonella* spp., *Staphylococcus aureus*, and aflatoxins (SE, 2009; 2010).

For processors, 74% are aware of the quality and safety requirements that must be met regarding raw materials and inputs, while 52% establish quality and safety criteria for their amaranth suppliers (Figure 4).

In NMX-FF-116-SCFI-2010, popped amaranth grain is classified into three quality grades: Category I, II, and III (Table 3), depending on its physical and physicochemical characteristics (SE, 2010).

Producers who establish quality criteria classify it into these three categories. Meanwhile, processors mentioned that the popped grain they use for their processes falls within categories III and II (Figure 5).

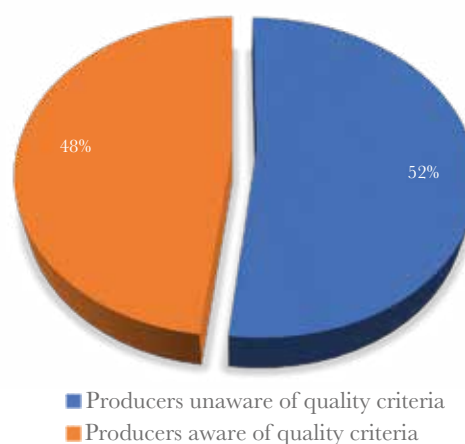


Figure 3. Awareness of Quality Criteria Compliance (Establishes quality criteria according to NMX-FF-116-SCFI-2010).

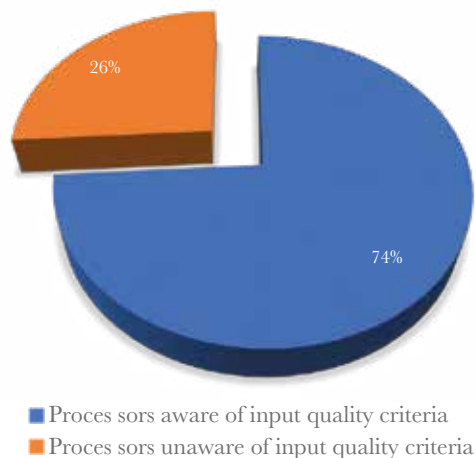


Figure 4. Knowledge of quality criteria that inputs must meet.

Table 3. Classification of grain according to NMX-FF-116-SCFI-2010.

| Criteria | Category I | Category II | Category III |
|---|------------|----------------|--------------|
| Grain popping (% of retention in 16mm screen) | 100 - 96 | 95,9 - 90 | Less than 90 |
| Presence of ferrous material (%) | <0,05 | 0,051 - 0,20 | |
| Foreign matter | See 6.6 | | |
| Metalic particles | Absent | | |
| Black grain (%) | ≤0,5 | | |
| Brunt grain | Absent | Not applicable | |

NMX-FF-116-SCFI-2010. (2010)

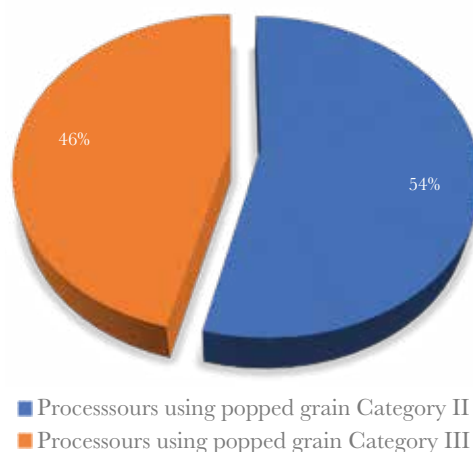


Figure 5. Classification of grain according to NMX-FF-116-SCFI-2010.

Although Mexican standards establish guidelines for the quality of amaranth, their non-mandatory nature and the lack of quinquennial reviews create gaps in regulation. The study reveals variations in the quality and safety of amaranth-based products, especially in the artisanal sector, highlighting the need for more robust regulation. Surveys and work

sessions indicate that 62% of the stakeholders involved in the value chain perceive low to moderate compliance with quality standards, indicating a lack of standardization in grain processing processes.

Commercialization

There are industries dedicated to large-scale marketing of processed amaranth such as Grupo San Miguel de Proyectos Agropecuarios, and Biogramin, among others. The Federal Government, through the National System for Integral Family Development (DIF) and Mexican Food Security (SEGALMEX), also provides another option for trade.

Identified issue: Small-scale marketing of amaranth represents one of the weakest points in the chain. Eighty percent of the production is controlled by intermediaries who unilaterally set prices, limiting market alternatives for small producers. In addition to this, there is a lack of policies regulating the traceability of products derived from amaranth, which fosters informal trade, especially in products like “alegría.”

Uses and Forms of Consumption

Despite the various applications of amaranth, its traditional use is primarily focused on making ‘alegría’, a sweet treat combining popped grains with honey, sugar, or piloncillo, among other ingredients. According to fieldwork conducted, 74% of the producers process amaranth into flour, sweets, and dietary supplements, while baking; and the production of healthy foods are 22% (Figure 6).

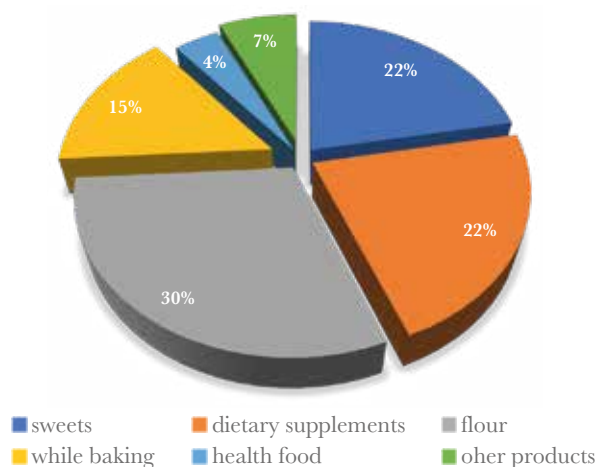


Figure 6. Types of products processed.

Table 4. Grains *per capita* consumption (kg) 2020-2023.

| Year | 2020 | 2021 | 2022 | 2023 |
|----------|---------|---------|---------|---------|
| Amaranth | 0.043 | 0.043 | 0.047 | 0.044 |
| Corn | 335.800 | 331.900 | 346.400 | 335.200 |
| Rice | 9.000 | 10.100 | 9.100 | 10.100 |
| Beans | 7.700 | 9.000 | 11.000 | 7.600 |

SADER-SIAP (2023).

Amaranth can be transformed into various products, satisfying different consumer needs and tastes. From traditional sweets like “alegrías,” “palanquetas,” “obleas,” and “mazapanes,” to granola, whole grain flours, extruded foods (snacks), oils, baby porridge, dietary supplements, bread products (bread and cookies), tamale flours, and nutritional foods. According to fieldwork studies, processors agree that, in addition to traditional “alegrías,” the most popular sweets in the market are simple or chocolate-combined bars, “palanquetas,” and cookies. Therefore, their productive activity focuses on these products.

Amaranth ranks 32nd in the basic basket and is categorized as a “treat” rather than recognized as a food, grouping it with other products like peanuts (Secretaría de Agricultura y Desarrollo Rural [SADER], 2023). In Mexico, its consumption is relatively low, with official figures indicating an average of 44.25 grams per capita consumption during the period from 2020 to 2023 (Table 4).

In the same period, the average per capita consumption was 330 kilograms for corn, 9.5 kg for rice, and 8.8 kg for beans (SADER-SIAP, 2023).

There is an absence of a state policy promoting amaranth consumption as a food, despite its importance for marginalized rural communities or children with nutritional issues. According to Espitia *et al.* (2021), the limitations of low amaranth consumption depend on political will where its consumption has not been popularized. It is noteworthy that only 34% of the interviewed producers consume it, and of these only 1% of their production is used for consumption.

Consumer Characterization

Consumer Profile

According to the field study, consumers of amaranth range in age from 20 to 65 years old, with an equal distribution between men and women. 76.2% of amaranth consumers have a bachelor’s degree level of education. In terms of occupation, 69% are employees, followed by students (14.3%) and homemakers (9.5%). Most consumers fall within an income range of 1 to 3 daily minimum wages.

Consumption habits

Consumption of amaranth varies among consumers. The majority (31%) consume it monthly or weekly (19%), while a smaller percentage (15%) consume it daily or annually. The main forms of consuming amaranth are through “alegrías” (a traditional sweet made with popped amaranth), cookies, and churros. However, it is also consumed in flour, baked goods, cereals, and dietary supplements, although to a lesser extent.

Regarding taste, 72.5% of consumers find it pleasant. However, a significant percentage is unaware of the nutritional value (45%) and properties of amaranth (55%). Despite this, it is believed that if consumers were informed about these attributes, they would be willing to consume or try amaranth more frequently. The willingness to purchase amaranth is high, as most consumers (50%) believe that if someone were to promote the qualities of amaranth, they would consider buying this product more often.

Challenges and opportunities

Education and research institutions

Within the value chain, various actors are involved at the same level such as input suppliers, education, and research institutions. In this regard, the crucial role played by research institutions such as INIFAP, COLPOS, and some universities stands out. These institutions not only conduct research but also provide advisory services to producers to help them adopt best practices in their crops. They have also developed improved varieties, with some seeds being cultivated by the producers. In the National Catalog of Plant Varieties of the National Seed Inspection and Certification Service (Snics, in Spanish), there are 14 registered varieties. However, there are still some varieties pending registration (it is estimated that there are 18 varieties in total).

Government support (since 2022)

There are government programs that provide support to amaranth producers, such as the “Production for Well-being Program”, which provides economic support to small and medium-sized ejido producers. The Ministry of Welfare offers guidance for an agroecological transition to producers. Since 2023, the “Fertilizers for Well-being” program has been implemented, which provides direct support with fertilizers. However, some producers (21%) also receive guidance for an agroecological transition, which contradicts the policy of fertilizer supply.

Although there are various current government supports, there are no specific supports for amaranth producers nor technological packages adapted to each region. Field schools are places where knowledge and experiences on agroecological production techniques are exchanged. Additionally, the Technical Accompaniment Strategy (EAT in Spanish) implements productive activities aimed at transitioning to glyphosate-free and transgenic seed-free production.

Vertical relationships and linkages

In the supply chain of input providers, there are informal traders who sell their products without adding value to the chain. Agrochemical stores only supply fertilizers, insecticides, and herbicides without access issues, maintaining solely a commercial relationship. Research institutions play a crucial role in technology generation, although they duplicate efforts and do not collaborate effectively. Government institutions provide training and participate in the process, benefiting producers. Companies offering credit, financing, or agricultural insurance are scarce. Buyers determine prices and maintain purely commercial relationships, except for Company San Miguel, which has a serious social commitment and seeks to improve the quality of life of its members (Ayala *et al.*, 2017).

Horizontal linkages

A culture of associativity among producers has been identified, allowing them to enhance their negotiation skills and modify power relations. The producers have established informal links that provide them with productive benefits. At the industrial level, there are horizontal linkages mainly among large producers. In the states of Morelos, Tlaxcala, and

Mexico City, stakeholders are represented in a planning committee of the Product System. On the other hand, in Puebla, integration into the National Product System is sought. According to surveys, there is low member participation in decision-making and a low level of cooperation among stakeholders. Only 24% of producers are formally organized, while the rest are informally grouped or participate in producer groups. Organization is crucial for generating economies of scale and facilitating negotiations and power relations in the value chain. Lack of organization can limit value generation in the chain.

Critical support services

Financial services

There is a lack of financing and agricultural insurance for production processes from primary production to agribusiness. Such support is necessary to improve technology and infrastructure in the agribusiness sector. Only 11% of respondents have access to credit due to high capital costs.

Technical assistance services

Technical assistance is also limited. In 2022-2023, only 21% of producers had the opportunity to participate in field schools.

Quality management services

Few key stakeholders are aware of Mexican standards for amaranth, and they are generally not used or referenced for compliance by producers, collectors, and marketers. In contrast, 74% of processors are aware of the quality and safety requirements for raw materials and inputs, and 52% establish quality and safety criteria for their amaranth suppliers. However, this is not the case for artisanal agribusinesses.

Following the modifications introduced in the Mexican Official Standard NOM-051-SCFI/SSA1-2010 in 2020, processors noted a decrease in the commercialization of their products, particularly those with high levels of sugars, sodium, and fats. Prior to these regulations, products made from amaranth were often considered healthy, but the presence of warning labels led consumers to reconsider their purchases. While consumption of these products initially decreased upon implementation of the regulation, once adapted to these changes, consumption stabilized without further growth. These Mexican standards, which are not mandatory, are not updated or reviewed on a five-year basis.

There is evidence of low compliance with quality and safety standards for many amaranth-based products, especially those produced at the artisanal level, highlighting the need for regulation. Stakeholders in the chain are aware of the low to medium compliance with quality standards.

Commercial intelligence

Changes in state governments have reduced market opportunities for the industries. In previous years, contracts were established with government institutions. There is an absence of policies that regulate the traceability of products derived from amaranth. Another issue is trademark registration, which for many has been a process fraught with

obstacles that hinder completion due to lack of knowledge or interest. Additionally, there is a lack of marketing strategies.

Social relationships influence the discovery of new marketing networks, which have been proven to lead to export opportunities. It is proposed to generate specialized content distributed through commonly used communication channels such as blogs and social media. Collaborations between recognized brands for the development of quality products should be pursued. Adopting and promoting a cause such as fair trade and consumption of domestic products is recommended. The introduction of the product and its derivatives by producer organizations into commercial chains should also be considered.

All the above considerations are based on the premise that the marketing link is not regulated. There is limited commercial promotion of amaranth-derived products in international markets. According to Ayala *et al.* (2014), this link is one of the most vulnerable in the chain.

Logistics and storage

Regarding logistics and storage services, no actors were identified as providing these services, which remains the same situation as in 2014 (Ayala *et al.*, 2017).

CONCLUSIONES







Amaranth production in Mexico faces several challenges and opportunities. Despite growth in production and crop yield, driven mainly by an increase in cultivated area, there is a low adoption of technological innovations that could further improve productivity. The lack of continuous training and technical assistance limits yield growth. Moreover, there is a disconnect between producers, researchers, and the government, hindering access to necessary resources and knowledge. The Amaranth value chain exhibits unequal distribution of benefits, with smaller producers primarily selling on a local level and having limited access to processing and commercialization opportunities. Lack of financing, adequate technical assistance, and quality management services exacerbate the challenges. However, there are opportunities to improve commercialization, especially in the domestic market, through awareness campaigns about the nutritional benefits of amaranth and targeted marketing strategies. Associativity among producers and support from research institutions and the government are key to overcoming obstacles and strengthening the amaranth value chain in Mexico. A public policy is needed to address the lack of consumer culture, as not all of the population is familiar with amaranth. This policy should promote a culture of quality consumption, highlighting its nutritional properties and cultural identity as a food.

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Evaluation of the use of artificial bait based on jackfruit (*Artocarpus heterophyllus*) meal as lure for freshwater crustaceans: a preliminary study

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ABSTRACT

Objective: To evaluate the effectiveness of an artificial bait made from jackfruit flour (*Artocarpus heterophyllus*) in capturing freshwater crustaceans by pot fishing was evaluated, comparing it with a traditional coconut paste bait.

Design/methodology/approach: Experimental trials were conducted with *Cherax quadricarinatus*, *Macrobrachium tenellum*, and *Procambarus clarkii* in controlled ponds.

Results: For *M. tenellum*, the jackfruit flour bait was more effective than the coconut paste bait, showing significant differences. For *C. quadricarinatus* and *P. clarkii*, no statistical differences were found in the number of organisms captured between the two baits.

Findings/conclusions: These findings suggest that jackfruit flour could be a viable and sustainable alternative bait in crustacean fisheries, reducing the dependence on animal-based baits. Further research under natural conditions is recommended to optimize its formulation and evaluate its impact on species selectivity.

Keywords: Fisheries, attractors, jackfruit flour, nasa traps.

INTRODUCTION

Fishing freshwater crustaceans represents an economic and food activity of great importance in various regions of the world, including México (Buhaya-Lora & Ramírez-Partida, 2013). Catch efficiency in this type of fishery depends largely on the type of bait used, which must be attractive to the target species, economically viable and environmentally sustainable (Karunanithi *et al.*, 2018). Traditionally, various types of natural and artificial baits have been used, including coconut paste, which has proven to be

effective due to its high oil content and its ability to attract crustaceans by releasing volatile compounds into the water (García Hernández *et al.*, 2015). The use of coconut fruit (*Cocos nucifera*) as bait in fisheries has been the subject of study and innovation in various regions. Research has shown that coconut paste, a by-product of oil extraction, can be used to create effective artificial baits for catching aquatic organisms using traps. This application not only revalorizes a low-cost by-product, but also offers a sustainable alternative to fresh baits, allowing their use throughout the year and improving fishing efficiency (Nolasco-Soria, 2014). In addition, in some fishing practices it has been observed that the use of coconut pieces can attract certain species, although the effectiveness of this method may vary depending on the region and the target species. For example, conversation among fishermen mention the use of “coconut” as bait, possibly referring to parts of the fruit, although the exact preparation and presentation is not specified (personal communication with fishermen from the Cajón de Peñas dam). Pacho *et al.* (2021) reported the use of roasted coconut to release its aroma before placing it in traps wrapped in nets in a fishery for different *Macrobrachium* species.

The results showed that traps with low-value fish were the most effective, especially the plastic ones. Roasted coconut, although it attracted some shrimp, had a lower catch rate compared to other baits, such as low-value fish and octopus. In the search for new alternatives, the fruit of the tropical tree *Artocarpus heterophyllus* commonly known as yaca, jaca, nanca, mangea or panapén has been identified. Jackfruit meal possesses several nutritional properties that make it viable as bait in freshwater crustacean fisheries (Ngurthankhumi *et al.*, 2024). Its high carbohydrate content, mainly starches and natural sugars, acts as a source of energy and attractant for aquatic organisms; its protein supply favors the release of nitrogenous compounds into the water, which can stimulate the response of crustaceans (Clerici & Carvalho-Silva, 2011). Although in lower proportion than other baits such as coconut paste, jackfruit meal contains lipids and essential oils that favor the dispersion of aromatic compounds in the water, while its volatile and phenolic compounds generate a characteristic aroma that could enhance its attractiveness and effectiveness in the capture of crustaceans (Cruz-Cansino *et al.*, 2021). Finally, its high dietary fiber content gives it structural stability, allowing greater durability in water and reducing its premature degradation, making it a potentially efficient and sustainable option for use in pot fishing (Clerici & Carvalho-Silva, 2011).

The objective of this study is to evaluate the use of an artificial bait based on jackfruit meal in the capture of freshwater crustaceans and to compare it with a traditional bait based on coconut paste. The effectiveness of both baits will be analyzed in terms of the quantity and type of crustaceans captured. With this, we seek to determine the viability of using jackfruit meal as a sustainable and economic alternative for freshwater fishing, thus promoting the integral use of this fruit and the diversification of inputs in the fishing activity.

MATERIALS AND METHODS

The study was carried out at the Laboratorio de Calidad de agua y Acuicultura experimental (LACUIC) of the Universidad de Guadalajara, located at the Centro

Universitario de la Costa in Puerto Vallarta, Jalisco, México. Six fruits of *A. heterophyllus* with an average weight of 4.18 ± 0.5 kg were used, from which only the pulp was extracted, discarding the seeds and peels. The extracted pulp was subjected to a drying process in a convection oven (NOVATECH[®]) at 60 °C for 48 h. Afterwards, the pulp was ground to a fine powder using a grain mill (Hamilton Beach 80335r). For the preparation of the artificial bait, jackfruit flour was used to which binders were added (gelatin and Pegabind[®]) (Table 1), with the objective of obtaining a gelatin that was poured into a metal tray to form a layer approximately 1.0 cm thick. Following the same methodology, a control bait was prepared based on coconut paste (*Cocos nucifera*), obtained from the company DUSTRY GROUP[®] according to the patent proposed by Nolasco-Soria *et al.* (2014) (MX2014015122).

For the evaluation of the baits, three plastic ponds (Rotoplas[®]) with a capacity of 1,700 L each were used. In each pond, considered an experimental unit (EU), 22 Australian lobsters (*Cherax quadricarinatus*), 96 crayfish (*Macrobrachium tenellum*) and 13 American red crayfish (*Procambarus clarkii*), all from the laboratory stock, were introduced. Prior to the start of the experiment, the organisms were subjected to a 24 h fasting period to standardize their hunger level and avoid biases in the response to the bait. Subsequently, one nase trap was set per experimental unit. The traps had a rectangular wire structure covered with mesh and dimensions of 50 cm long, 23 cm wide and 23 cm high, with two lateral entrances to facilitate the entry of the organisms. The baits were prepared in the form of squares of 50 g each and placed inside the traps. To minimize location bias, the traps were randomly distributed within the ponds. The traps remained submerged for 24 hours. At the end of this period, they were carefully removed to avoid loss of organisms, and the total number of captures of each species was counted. Finally, to determine significant differences in the predilection for each type of bait, the data obtained were analyzed using a χ^2 contingency table for two independent samples. The assumptions of the test were verified and statistical corrections were applied when necessary, establishing a significance level of $\alpha=0.05$.

RESULTS AND DISCUSSION

Trap fishing is one of the most widely used techniques worldwide, and its success depends largely on the efficiency of the bait used, either of natural or artificial origin, depending

Table 1. Composition and function of ingredients in the preparation of artificial bait for catching freshwater crustaceans.

| Ingredients | Quantity (g/kg) | Function | Description |
|-------------------------|---------------------------|---------------------------------|--|
| Jackfruit/Coconut Flour | 400 | Attractant molecules | Contains carbohydrates, proteins, and volatile compounds that can attract crustaceans. |
| Gelatin | 50 | Protein gelling agent | Protein that helps give consistency to the mixture. |
| Pegabind | 50 | Synthetic binding agent | Improves the cohesion of the bait in the water, preventing rapid dissolution. |
| Agua* | q. s. (≈ 1 L) | 1 L to make the gelling mixture | Allows the formation of a gel matrix. |

(*) q. s. = amount sufficient to complete.

on the target species (Masilan & Neethiselvan 2018). Traditionally, fishermen have used species such as squid (*Dosidicus gigas*), mackerel (*Scomber japonicus*), mullet (*Mugil cephalus*) and skipjack (*Katsuwonus pelamis*) as bait, however, these species are of high nutritional value and should be prioritized for human consumption (FAO, 2022).

In addition, the cost of bait increases considerably when these resources are scarce due to seasonality, which forces fishermen to travel long distances in their search, increasing fuel consumption and operating costs (Harnish & Willison, 2008; Spoor *et al.*, 2021). Faced with this problem, the fishing industry has begun to employ more sustainable alternatives, such as fishery remains (heads and guts of fish, bones, shrimp remains, among others), as well as processed fish silage that is placed in nets to gradually release leachates and attract target organisms (Dellinger *et al.*, 2016). Also, artificial baits with various characteristics in terms of texture, shape, size and attractant composition have now been developed. Some mimic small fish that act as natural prey, while others are formulated as stabilized pastes with chemical additives, combining ingredients such as fish, squid and mollusks to enhance their effectiveness (Ollis *et al.*, 2004).

In the present study, jackfruit flour was used as an attractant due to its characteristic odor when ripe (Cervantes-Robles, 2018), combined with a gelling agent that allowed the manufacture of the artificial bait. The results obtained showed differences in the effectiveness of the bait depending on the species captured. In the case of *C. quadricarinatus*, out of a total of 66 organisms used in the test, no significant statistical differences were found in the captures depending on the type of bait. The coconut paste bait resulted in the capture of 18 specimens (27% effectiveness), while the jackfruit flour bait allowed the capture of 9 organisms (14% effectiveness). For *M. tenellum*, there were statistical differences in the number of captures between the two baits. Of the 288 organisms in the ponds, the jackfruit meal bait captured 71 specimens (25% effectiveness), while the coconut paste bait captured 56 specimens (19% effectiveness), indicating a greater preference for the bait based on jackfruit meal in this species. As for *P. clarkii*, out of a total of 40 organisms in the test, no significant statistical differences were found between the baits. With the bait made from jackfruit, 25 organisms were captured (62% effectiveness), while with the coconut paste bait, 22 organisms were captured (55% effectiveness) (Table 2).

These results reinforce what was mentioned by Masilan y Neethiselvan (2018), who emphasize that the key to success in pot fishing lies in the type of bait used. Waddington y Meeuwing (2009) mention that approximately 8.8 million pots are used in the Western lobster (*Panulirus cygnus*) fishery in Western Australia, which implies the use of 14,000 tons of bait per year. This massive addition of organic material to marine ecosystems has

Table 2. Results of catches of various organisms in traps baited with coconut paste and jackfruit meal.

| Species | Organisms used (n) | Catches with coconut paste (n, %) | Catches with jackfruit meal (n, %) |
|----------------------------------|--------------------|-----------------------------------|------------------------------------|
| <i>Cherax quadricarinatus</i> | 66 | 18 (27%) | 9 (14%) |
| <i>Macrobrachium tenellum</i> ** | 288 | 56 (19%) | 71 (25%) ** |
| <i>Procambarus clarkii</i> | 40 | 22 (55%) | 25 (62%) |

Significant differences were detected in *Macrobrachium tenellum* by χ^2 ($p < 0.05$).

been shown to affect their functioning globally, so the development of more efficient and sustainable baits would contribute to both ecosystem conservation and fishery maintenance.

The bait used in our study is of vegetable origin, made with biodegradable products that, if consumed by the organisms, would not represent a negative impact, since they provide proteins and minerals. However, there are few studies that evaluate the use of vegetable baits in crustacean fishing. An example is the work Nolasco-Soria *et al.* (2014), who developed a bait based on hydrolyzed coconut paste for crustacean and freshwater fish fishing, obtaining good results in the capture of both species and managing to consolidate a patent. On the other hand (Ghazilou *et al.*, 2016) compared the use of baits of animal origin (fish remains) and vegetable origin (a combination of raw dough with turmeric), using video recordings in the natural environment to analyze their effect as attractants in fish communities. Their results showed that the animal baits attracted a greater diversity of organisms compared to the plant baits, which only attracted herbivorous species. This suggests that the type of bait directly influences the trophic composition of the organisms captured. Based on our results, it is important to continue research on the effect of plant baits in natural conditions, in order to develop better practices that optimize catches, minimize production costs and reduce the negative environmental impact associated with the use of conventional baits.

CONCLUSIONS

The present study evaluated the effectiveness of an artificial bait made from jackfruit meal in fishing for freshwater crustaceans and its comparison with a traditional coconut paste bait. The results showed that, although there were no significant differences in the capture of Australian lobster and American red crab, in the case of crayfish, the jackfruit meal bait was more effective than the coconut bait. These findings highlight the viability of the use of plant-based baits as a sustainable alternative in pot fishing, reducing dependence on animal-based baits and reducing the environmental impact of fishing activities. In addition, the implementation of biodegradable and low-cost ingredients, such as jackfruit, could represent an accessible and sustainable option for fishermen. Given that there are few studies on the use of plant-based baits for catching crustaceans, it is recommended that research continues under natural conditions to evaluate their effectiveness on a larger scale. It is also necessary to optimize the bait formulation and analyze its impact on the selectivity of target species. This will allow us to advance towards the development of more efficient and ecologically responsible fishing strategies.

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Market Research on the Consumer Preference of Biofertilizers in Mexico

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ABSTRACT

Objective: To understand the preferences of agricultural and fruit producers regarding the use of mineral fertilizers, organic fertilizers, or biofertilizers.

Design/Methodology/Approach: The consumer behavior theory was complemented with the ABC model of attitude: affective, behavioral, and cognitive. The tastes, preferences, and attitudes of producers were recorded regarding the use of different types of fertilizers. For this purpose, structured questionnaires, focus meetings, and direct observation were applied to a total sample of 100 producers.

Results: The analysis recorded negative signs in the years_p (years as producer) and years_c (years with the same crop) coefficients, showing that a greater number of years in both variables resulted in a higher probability of a null preference for the application of biofertilizers; however, farmers with 0 + to 20 years of experience who sow certain cereals are more likely to apply biofertilizers. For their part, producers of perennial crops (*e.g.*, fruit trees) did not show any preference for biofertilizers.

Study Limitations/Implications: External factors (*e.g.*, the local availability of biofertilizers, institutional support, or economic incentives) which could influence the decision of more experienced producers or producers who work with perennial crops to adopt biofertilizers were not taken into account.

Findings/Conclusions: Producer experience and their likelihood to use biofertilizers had an inverse relationship, suggesting that deeply-rooted traditional practices are a barrier to the adoption of sustainable technologies.

Keywords: Biofertilizers, market research, tastes, and preferences.

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INTRODUCTION

Biofertilizers have achieved international acceptance, given their multiple benefits to agriculture. Although they have been successfully applied in many developed countries, several factors have limited their use and approval by the agricultural industry of developing countries (Grageda-Cabrera *et al.*, 2012). In the last decades, several researches have been carried out in Mexico focused on their development, innovation, and validation (Chávez-Díaz *et al.*, 2020). These bio-inputs have improved soil fertility, optimized nutrient absorption, and reduced the environmental impact of synthetic fertilizers (Moreno Reséndez *et al.*, 2018).

The development and application of biofertilizers is an effective option to partially or fully replace mineral fertilizers (Grageda-Cabrera *et al.*, 2012). However, despite their advantages, their adoption in the global market has been limited and they only account for 5% of the world fertilizer market (Timmusk *et al.*, 2017). In Mexico, biofertilizers are currently produced by small companies and educational and research institutions. The Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) has also been involved in the process, with the support of federal and state governments (Infante-Jiménez *et al.*, 2020). During the presidency of Ernesto Zedillo Ponce de León (1994-2000), the first microbial biofertilizers were distributed in the Mexican countryside, through the Alianza para el Campo program. This initiative was implemented by INIFAP, during the 1999 spring-summer (SS), 1999-2000 autumn-winter (AW), and 2000 SS agricultural cycles (Aguirre-Medina *et al.*, 2009). The National Biofertilizer Program (PNB) was created in 1998 to exploit beneficial microorganisms that improve plant nutrition. Its main purposes were: 1) to expand research and technology validation into various crops of interest in several of the country's agroecological regions; 2) to promote and spread the use of biofertilizers; and 3) to train technician and civil servants of the Mexican agricultural sector (Garza *et al.*, 2003).

Within this context, the aim of this program was to promote and spread the use of biofertilizers, which at the time were little known and hardly used by Mexican farmers. An experimentation and training process focused on demonstrating their efficiency and facilitating their adoption was implemented to benefit a greater number of producers. This was a key strategy, because many farmers evaluated biofertilizers based on their short-, medium-, and long-term cost-benefit ratio and final users remained skeptical (Cruz-Cárdenas *et al.*, 2021). On 2011, upon the request of the Ministry of Agriculture, Livestock, Rural Development, Fisheries, and Food (SAGARPA, currently SADER, the Ministry of Agriculture and Rural Development), INIFAP started a research and technology transfer project focused on the use of biofertilizers and organic fertilizers in agriculture. The aim of this project was to provide information about the biofertilizers available in the market, to evaluate their impact on crop yield, and to promote the reduction of both the use of synthetic fertilizers and production costs.

Doubtlessly, a successful transference depends on the adoption of the technology. At the time, it was an even harder task, because biofertilizers faced a strong competition from mineral fertilizers. In this context, adoption is defined as the decision of producers to use (or refrain from using) a given technology, taking into consideration such factors as the price of the product, innovation, market availability, and the obstacles for the adoption of the said innovation (Sagastume *et al.*, 2006). Therefore, the objective of this research was to determine the preferences of agricultural and fruit producers —whether they preferred mineral, organic, or biological fertilizers. The research assumed that the answers of the producers would be biased, given their lack of information about biofertilizers. To rectify this situation, the research team provided an exhaustive explanation about the product, its function, its application, and its contribution to healthy environment and soils. The purpose was to determine their receptiveness to this “new product” and the arguments which backed their decision.

MATERIALS AND METHODS

Research Design

Exploratory research was developed in 2011, based on macroeconomic theory (specifically, consumer behavior). This approach is focused on the tastes and preferences of the consumer, determining, along with other aspects, what products the consumer would be willing to purchase (Nicholson, 1997). Consumer decisions were analyzed based on their own preferences. Likewise, consumer attitudes were analyzed in face of certain market events: 1) affective component; 2) behavioral component; and 3) cognitive component (Kinnear, 2003).

The research included both desk and field work. Field techniques (focal meetings, direct observation, and questionnaires) were defined and designed during the desk work stage. Likewise, the strategies that would be used to apply each technique and the methodology that would be used for the analysis of the collected data were defined. During the field work stage, the questionnaires were tested and the three techniques were applied.

Two types of non-probabilistic statistical sampling were chosen for the application of the questionnaires: purposive (Kerlinger, 1975; Otzen and Manterola, 2017) and volunteer (Pimentel Lastra, 2000). Purposive samplings were used with producers who had already cooperated with INIFAP, while volunteer sampling was applied with producers who were present when the plot was established and who voluntarily agreed to participate. The total sample was obtained from the application of questionnaires to 100 producers (Table 1). The personal characteristics and experience of the 47 cooperating producers were similar to those describe for the remaining 53. Consequently, all the questionnaires (100) were subjected to data analysis, increasing the statistical certainty of the results.

During the focal meetings, the research team and the producers discussed the traditional fertilization methods (mineral fertilizers), their effects on plants, and their consequences for the soil and the water table, as well as the application and benefits of biofertilizers. The producers who attended the focal meetings were part of the 100-producer sample.

The direct observation consisted of several stages. Once the plot had been established and after both guest and cooperating producers had attended a talk about biofertilizers, a visit to an agricultural product shop was simulated. Since they usually receive guidance and advise from the shop clerk, they were asked to pretend that the clerk could not provide them any information about the available biofertilizers. Therefore, they would have to make the decision on their own. All the biofertilizers used in the plots were placed at the same level and at the same distance from the producers, who were then asked to make their choice. This was mostly an individual exercise, to prevent producers from influencing each other's responses.

In this hypothetical situation, observation was focused on the following questions: 1) does the producer read the product formula; 2) does the producer read the instructions; 3) does the producer ask for the price; and 4) does the producer buy the product.

A descriptive analysis of the study population was based on the information collected. Subsequently, a discriminant analysis was applied to identify the variables that explain the preference of the producers (chemical fertilizers, organic fertilizers, or biofertilizers). An analysis of contingency tables was then used to examine the statistical relationship between

the variables, with the aim of explaining the choice of biofertilizers during the shopping simulation. Finally, relative frequencies were analyzed to identify the reasons that backed up the choice of biofertilizer.

The commercial biofertilizers evaluated in this study were defined and chosen based on the guidelines of SAGARPA. The inclusion of the said products was a response to the request made by several biofertilizer manufacturers, who wanted to subject their products to a technical evaluation. Once they were approved by the Ministry, their products could be included in different agricultural support programs.

RESULTS AND DISCUSSION

The average age of the overall sample was 51 years (mode: 60 years). Ninety-one percent of the participants were men. When they were asked if they knew how to read or write, 45% of the interviewees answered the question (93% of the said percentage replied “yes”). The study assumed that agricultural producers have an empirical understanding of fertilizers, their mixtures, application, and impact on crop development. To include this variable on the analysis, the following elements were taken into account: 1) their years of experience as agricultural producers (years_p); and 2) the period they had worked with the crop (years_c) to which the biofertilizer was applied. This information would increase the veracity of their observations about crop development, as well as their choice and satisfaction with the biofertilizers used. Nine of the producers interviewed grew perennial crops, while the rest used annual species (mainly maize). Agricultural production experience ranged from 2 to 66 years, with a mean and mode of 30 years. The time which the producers had worked with the crop to which the biofertilizer had been applied ranged from 2 to 66 years (mean: 26 years).

The focal meetings took place in four maize-producing areas: Tlahuac, Federal District (currently Mexico City); San Agustín Tlaxcala, Hidalgo; the municipality of Loma Alta, Chapa de Mota, State of Mexico, and the municipality of Hecelchakan, Campeche. The meeting in the Federal District included a cooperating producer—who did not conclude his agricultural engineering studies and had owned an agrochemical product shop for several years—, two agricultural engineers, and three guest producers.

The meeting in Hidalgo recorded the highest number of participants: 30 producers (40+ years old), who sow native maize in strictly rainfed plots. Meanwhile, in addition to the INIFAP technicians and researchers, ten producers attended the meeting at Lomas Altas. The producers were young (average age: 30 years) and had worked on agriculture for a few years, which increased their receptiveness. In Hecelchakan, Campeche, four producers—a father (65 years old) and his sons (34, 32, and 27 years old)— who only applied mineral fertilizers to maize participated in the meeting.

Demonstration plots were established with cooperating producers who had been previously chosen by INIFAP. These plots were supervised by a team of experts on biofertilizers from the said institute. The furrows were divided into control and treatments. The mineral fertilization formula that the cooperating producers normally used was applied to the control. In the treatment furrows, 50% of the mineral fertilizers were replaced with biofertilizers (one per each two furrows). Table 1 shows the plots that were included in this study.

Table 1. Demonstration plots, crops, biofertilizers, state, and number of cooperating producers.

| Federal entity | Cultivation | Number of producers | Commercial biofertilizers* |
|------------------|---------------|---------------------|---|
| Aguascalientes | Corn | 1 | Micorriza INIFAP [®] , BiofertiBuap Zea [®] + BiofosfoBuap [®] , Bio-Radix [®] + Spectrum Mico [®] + Nutripro [®] Forte + Nutripro [®] Xtra Alga |
| Campeche | Rice | 1 | Micorriza INIFAP [®] , BiofertiBuap [®] , AlgaEnzims [®] |
| | Corn | 2 | Micorriza INIFAP [®] , Bacteriano2709 [®] , FerbiliQ [®] , BiofertiBuap Zea [®] + BiofosfoBuap [®] |
| Chiapas | Corn | 3 | Micorriza INIFAP [®] , Bio-Radix [®] + Spectrum Mico [®] , FerbiliQ [®] |
| | Bean | 5 | Micorriza INIFAP [®] , BiofertiBuap Leg [®] + BiofosfoBuap [®] , Endospor [®] |
| | Sugarcane | 1 | Micorriza INIFAP [®] , Bio-Radix [®] + Spectrum Mico [®] y FerbiliQ [®] |
| Ciudad de México | Corn | 1 | Micorriza INIFAP [®] , FerbiliQ [®] , BiofertiBuap Zea [®] |
| Estado de México | Corn | 2 | FerbiliQ [®] , Micorriza INIFAP [®] , Bacteriano2709 [®] , BiofertiBuap Zea [®] + BiofosfoBuap [®] |
| Guanajuato | Bell pepper | 1 | Micorriza INIFAP [®] , BiofertiBuap [®] , Bio-Radix [®] + Spectrum Mico [®] , Nutripro [®] Forte + Nutripro [®] Xtra Alga |
| | Corn | 4 | Bacteriano2709 [®] , Micorriza INIFAP [®] , BiofosfoBuap [®] + BiofertiBuap Zea [®] , Bioradix + Spectrum Mico [®] |
| | Bean | 1 | Bacteriano2709 [®] , Micorriza INIFAP [®] , BiofosfoBuap [®] + BiofertiBuap Leg [®] , Bioradix + Spectrum Mico [®] |
| | Sorghum | 2 | Micorriza INIFAP [®] , BiofosfoBuap [®] + BiofertiBuap Sorghum [®] , Bio-Radix [®] + Spectrum Mico [®] |
| Guerrero | Corn | 2 | Micorriza INIFAP [®] , BiofertiBuap Zea [®] + BiofosfoBuap [®] , FerbiliQ [®] |
| Hidalgo | Orange | 1 | Micorriza INIFAP [®] , BiofertiBuap [®] , FerbiliQ [®] , Bacteriano2709 [®] |
| | Barley | 2 | Micorriza INIFAP [®] , Bacteriano2709 [®] , BiofertiBuap Hord [®] , AzoN [®] + Mycorfos [®] |
| | Corn | 2 | BiofertiBuap Zea [®] , Micorriza INIFAP [®] , Bacteriano2709 [®] , Endospor [®] |
| Morelos | Poinsettia | 1 | Micorriza INIFAP [®] , Bio-Radix [®] + Spectrum Mico [®] + Nutripro [®] Forte + Nutripro [®] Xtra Alga, BiofertiBuap [®] |
| | Tomato | 1 | Micorriza INIFAP [®] , BiofertiBuap [®] , Bio-Radix [®] + Spectrum Mico [®] + Nutripro [®] Forte + Nutripro [®] Xtra Alga |
| Nuevo León | Sorghum | 1 | Micorriza INIFAP [®] , FerbiliQ [®] , Bio-Radix [®] + Spectrum Mico [®] , BiofosfoBuap [®] , Bacteriano2709 [®] |
| | Grasses | 1 | Micorriza INIFAP [®] , FerbiliQ [®] , Fosfonat [®] , Bacteriano2709 [®] |
| | Citrus fruits | 1 | Micorriza INIFAP [®] , FerbiliQ [®] , Endospor [®] , Bacteriano2709 INI [®] , Bio-Radix [®] + Spectrum Mico [®] |
| Oaxaca | Corn | 6 | Micorriza INIFAP [®] , BiofertiBuap Zea [®] + BiofosfoBuap [®] , FerbiliQ [®] |
| Tlaxcala | Corn | 1 | AzoN [®] + Mycorfos [®] , Micorriza INIFAP [®] Micorriza INIFAP [®] , Bacteriano2709 [®] |
| | Barley | 1 | Micorriza INIFAP [®] , Bacteriano2709 [®] , BiofertiBuap Zea [®] , BiofosfoBuap [®] , Bio-Radix [®] + Spectrum Mico [®] |
| Veracruz | Corn | 1 | Micorriza INIFAP [®] , BiofertiBuap Zea [®] , Spectrum MicoBac [®] y FerbiliQ [®] |
| Yucatán | Corn | 1 | Micorriza INIFAP [®] , BiofertiBuap Zea [®] , FerbiliQ [®] , Bacteriano2709 [®] . |
| | Orange | 1 | Micorriza INIFAP [®] , BiofertiBuap [®] , AlgaEnzims [®] |
| TOTAL | | 47 | |

*Commercial biofertilizers: AlgaEnzims[®] (PalauBioquim, S.A. de C.V.); Micorriza INIFAP[®] (INIFAP); BiofertiBuap[®] (Benemérita Universidad Autónoma de Puebla); FerbiliQ[®] (Biosustenta S.A. de C.V.); Bacteriano2709[®] (INIFAP); BiofertiBuap Zea[®] (Benemérita Universidad Autónoma de Puebla); BiofosfoBuap[®] (Benemérita Universidad Autónoma de Puebla); Nutripro[®] Xtra Alga (Promotora Técnica Industrial, S.A. de C.V.); Nutripro[®] Forte (Promotora Técnica Industrial, S.A. de C.V.); Spectrum Mico[®] (Promotora Técnica Industrial, S.A. de C.V.); Bio-Radix[®] (Promotora Técnica Industrial, S.A. de C.V.); BiofertiBuap Leg[®] (Benemérita Universidad Autónoma de Puebla); AzoN[®] (BioFertilizar, S.A.S.); Mycorfos[®] (BioFertilizar, S.A.S.); Endospor[®] (Koppert); Fosfonat[®] (Tecnologías Naturales Internacional, S.A. de C.V.); BiofertiBuap Hord[®] (Benemérita Universidad Autónoma de Puebla); BiofertiBuap Sorghum[®] (Benemérita Universidad Autónoma de Puebla).

The producers expressed several concerns during the establishment of the plots. They were particularly insistent on the compatibility of biofertilizers with hybrid and native maize seeds, as well as their effectiveness with barley and wheat. Other concerns include the doses that must be applied according to the crop and number of seeds, as well as the mineral fertilizers that must be applied along with biofertilizers.

Producer preferences: chemical fertilizers, organic fertilizers, or biofertilizers

Determining consumer preference is fundamental, as it opens market opportunities and has a significant economic impact (Ramírez León, 2013). Their choices are supported by the experience they have acquired throughout their work. They clearly prefer mineral fertilizers, given their availability, ease of application, and quick and clear effect on crop yield. Additionally, handling experience and customs decisively influence their choice.

Therefore, biofertilizers face a daunting challenge, despite the argument in their favor that, unlike mineral fertilizers, they provide benefits to the soil and the environment (Chávez-Díaz *et al.*, 2020). Although mineral fertilizers increase the productivity of the crops during the first years of application, they are not as ideal as they are believed to be: they also have harmful effects, both on human health and the environment (Calderón *et al.*, 2019). Fertilization is not only expensive, but most producers make an inefficient use of these products (Ávila Marioni *et al.*, 2011).

Producers backed up their choice of mineral fertilizers as follows: 1) they are accessible; 2) they guarantee a safe and quick yield; 3) producers have experience handling the product; 4) they provide clear results in a short time; 5) they are widely available; 6) organic fertilizers are scarce; 7) less chemical fertilizers must be applied compared with organic fertilizers; 8) they are easy to apply, provide quick results, and are readily available in the market; 9) they are easier to apply with machinery; 10) they improve crop development and increase leaf area, height, and stem width; 11) their use is customary; and 12) the producers are unaware of other methods.

In the case of biofertilizers, the producers mentioned the following reasons: 1) they guarantee yield, without stimulating undergrowth (unlike organic fertilizers); 2) they remain effective, even under water excess conditions (unlike organic fertilizers, which can cause yellow leaves); 3) they are easier to apply and transport than organic fertilizers; 4) they are cheaper and do not depend on importations; 5) they do not harm the environment (unlike chemical fertilizers, which additionally can harm the humans who consume the harvest); 6) they reduce production costs; 7) they contribute to the retention of soil moisture and they are harmless and unexpensive; 8) they are efficient and are easily applied and transported (smaller volumes are required); 9) they are not toxic; 10) they reduce the use of chemical fertilizers; 11) they break down easily; and 12) they innovate agricultural handling.

Finally, the reasons to choose organic fertilizers included: 1) they improve yield; 2) they are a healthier and less expensive option; 3) manure is an excellent natural fertilizer; 4) they do not pollute the environment; 5) they do not involve a health risk for the persons who apply them; 6) they guarantee the safety of cattle that eat the feed prepared from the harvest; 7) they do not impact native plants; 8) unlike biofertilizers, they require less care

and are more resistant to heat and sun; 9) they have better results than chemical fertilizers; 10) they are less sensitive to weather conditions than biofertilizers; 11) the manure from the producer's cattle can be used as fertilizer; 12) they are rich in essential nutrients for the soil and the crops; 13) they have long-term beneficial effects; and 14) they improve soil texture and structure.

Biofertilizers were first used in Mexico in 1970 (Armenta-Bojórquez, 1986; 1990, cited in Armenta-Bojórquez *et al.*, 2010). Several biofertilizer brands were available on the market by 2011, when the federal government —through SAGARPA, supported by INIFAP— renewed their promotion throughout the nation. Since 2021, the Producción para el Bienestar program and the Estrategia de Acompañamiento Técnico (EAT) have trained producers in agroecological and sustainable practices (SADER, 2024), including the use of biofertilizers (<http://bioinsumos-agricultura.mx>). Consequently, the use of biofertilizers is now better known, as well as their benefits to human health and the soil.

A discriminant analysis determined producer preferences, using “preference for the use of biofertilizers” as a dichotomous variable (0=does not prefer them; 1=prefers them). The explanatory variables were treated as numerical variables and included: age of the producer; years of experience in farming; and years during which the crop on which the biofertilizer was applied was sown. All the producers who expressed their preference for biofertilizers sow annual crops, while no producers of perennial crops preferred this type of fertilizer (Table 2).

Based on this information, the discriminant analysis clearly identified two groups: those who prefer biofertilizers and those who do not (Table 3).

Almost 60% of the producers who declared that they do not prefer biofertilizers (41 of 69) and 72% of those who do (18 of 25) are properly classified. Table 4 shows the discriminant function of each group. The coefficients are very similar (particularly the age coefficient).

The discriminant function minimizes the likelihood of an error in the classification of the individuals of each group. The aim of generating each group is to establish a lineal relation between the study variables. The negative signs of the years_p (years as producer) and years_c (years with the crop) show that, the longer that producers have worked with

Table 2. States and crops of the statistically-chosen producers who replied affirmatively to the use of biofertilizers.

| Federal entity | Crops | | | | | | | | | Total |
|------------------|-------|--------|-----------------|------|------|--------------------------|---------|-------|----------------------------|-------|
| | Oat | Barley | Pepper and Corn | Bean | Corn | Bell pepper ¹ | Sorghum | Wheat | They did not declare crops | |
| Chiapas | | | | 1 | | | | | | 1 |
| Estado de México | 1 | | | 1 | | | | | | 2 |
| Guanajuato | | | | | | 2 | | | | 2 |
| Nuevo León | | | | | | | 1 | 1 | | 2 |
| Tlaxcala | | 1 | | | | | | | | 1 |
| Veracruz | | | 1 | | 9 | | 4 | | 1 | 15 |
| Yucatán | | | | | 1 | | | | | 1 |
| Total | 1 | 1 | 1 | 2 | 10 | 2 | 5 | 1 | 1 | 25 |

¹ Pepper is a perennial crop that is managed as an annual crop.

Table 3. Summary of the discriminant function.

| True bio | Classified | | Total |
|----------|------------|--------|--------|
| | 0 | 1 | |
| 0 | 41 | 28 | 69 |
| | 59.42 | 40.58 | 100.00 |
| 1 | 7 | 18 | 25 |
| | 28.00 | 72.00 | 100.00 |
| Total | 48 | 46 | 94 |
| | 51.06 | 48.94 | 100.00 |
| Priors | 0.5000 | 0.5000 | |

Table 4. Discriminant function.

| bio | 0 | 1 |
|----------|-----------|-----------|
| age | .555558 | .551945 |
| years_p | -.135597 | -.179427 |
| years_c | -.075680 | -.057740 |
| gender | 11.012400 | 11.292030 |
| constant | -16.37098 | -15.63704 |
| Priors | .5 | .5 |

a given crop and the longer their experience (years), the likelihood that they will have a negative reaction to biofertilizer is greater (negative signs).

Choice of biofertilizer that producers would buy

The most frequent reasons to choose a biofertilizer were: 1) the material, design, and information in the package; 2) ease of application; and 3) its components. In total, 63 reasons were given (Table 5). In this regard, the reason with the highest relative frequency was “the material, design, and information in the package” (12%, Micorriza INIFAP®), followed by “because it’s easily applied” (FerbiliQ®) and “for its components” (BUAP biofertilizers), which accounted for 14% of all the reasons (7% each).

Table 5. Factors that determined the choice of biofertilizers.

| Biofertilizer/Reasons | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Frequency |
|---|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Material, design, and labeling of the packaging | 8 0.12 | 1 0.01 | 6 0.09 | 1 0.01 | 0 | 5 0.07 | 4 0.06 | 0 | 1 0.01 | 0 | 26 |
| Because it is easy to apply | 3 0.04 | 1 0.01 | 4 0.06 | 0 | 0 | 5 0.07 | 3 0.04 | 0 | 0 | 0 | 16 |
| Due to its components | 1 0.01 | 0 | 5 0.07 | 0 | 1 0.01 | 3 0.04 | 1 | 1 0.01 | 0 | 1 0.01 | 13 |
| Because it is liquid/powder | 2 0.03 | 1 0.01 | 1 0.01 | 0 | 0 | 4 0.06 | 0 | 0 | 0 | 0 | 8 |
| Total | 14 | 3 | 16 | 1 | 1 | 17 | 8 | 1 | 1 | 1 | 63 |

1: Micorriza INIFAP®. 2: Bacteriano2709®. 3: BiofertiBuap. 4: Endospor®. 5: AlgaEnzims®. 6: FerbiliQ®. 7: Bio-Radix®. 8: Spectrum Mico®. 9: BactoCrop. 10: AzoN® and Mycorfos®.

During the simulation exercise (the agricultural input shop), the producers were highly puzzled by the lack of verbal information about the product from the shop clerk. Some participants said that they would not buy anything, until the shop technician suggested a product. In those cases, the producers did not check the product or looked at it only briefly. Other producers paid more attention to the packages, read the information, and mainly asked about the doses for different crops. When they were told that the clerk could not provide any information, they hesitantly made their choice. Except in a few cases, the producers did not check for an expiration date.

CONCLUSIONS

The aim of this exploratory study was to identify relevant aspects and problems that can guide future research lines in this area. Since this study was based on a non-probabilistic sample, the results can only be applied to the 100 participating producers. Statistical analysis revealed that more experienced agricultural producers are less likely to apply biofertilizers to their crops.

This phenomenon could be explained by the following hypothesis: deep-rooted practices or knowledge acquired throughout the years are difficult to change. Consequently, during the transference activities, experts must insist on the need for agricultural and fruit-growing activities that involve soil restoration, that do not pollute the water and the environment, and that do not harm consumer health. Biofertilizers are a good ally in the struggle to dismantle the entrenched belief that economic growth involves environmental degradation. During the simulated shopping for biofertilizers, the producers were mainly interested in acquiring a product that does not damage the soil.

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Husk tomato (*Physalis ixocarpa* Brot.) response to inoculation with beneficial microorganisms

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ABSTRACT

Objective: To evaluate the response and to determine the yield of husk tomato (*Physalis ixocarpa* Brot.) seedlings to inoculation, using different beneficial microorganisms and methods.

Design/Methodology/Approach: The experiment was set up under a randomized complete block design, with ten repetitions per treatment. Three biofertilizers were individually evaluated: *Rhizophagus intraradices*, *Azospirillum* sp., and *Pseudomonas fluorescens*. Different inoculation methods were evaluated for each biofertilizer. The experiment included a control treatment without inoculation. The inoculation methods were: 1) application to the substrate, 2) application to the seed, and 3) irrigation of the plant nursery.

Results: The results showed significant differences between inoculation methods. Seed inoculation recorded the best growth and yield response. The treatments inoculated with *R. intraradices* obtained the highest yield (74%). Regarding bacteria, *Azospirillum* sp. recorded the best yield (21%), followed by *Pseudomonas fluorescens* (18%).

Study Limitations/Implications: The mycorrhizal inoculation of seedlings before transplanting may have favored its performance, hindering a fair comparison of the treatments.

Findings/Conclusions: The most effective strategy was the seed inoculation method, because it significantly enhanced both crop growth and yield. Treatments inoculated with the *R. intraradices* arbuscular mycorrhizal fungus achieved the highest husk tomato yields, highlighting the potential of this symbiosis to increase crop productivity.

Keywords: *Physalis ixocarpa*, development, inoculation, microorganisms.

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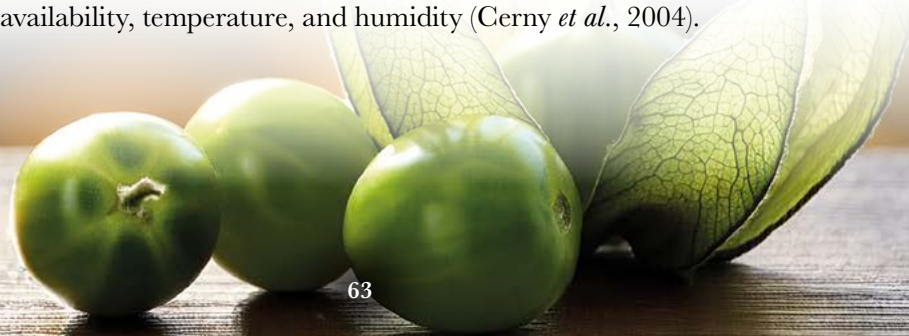
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INTRODUCTION

Currently, the production and acquisition of vegetable seedlings has experienced a significant growth (Yancha Villacis *et al.*, 2023), driven by the multiple benefits they provide. For example, seedlings grown in nurseries have significant advantages over traditional direct seeding (Lazcano-Bello *et al.*, 2021), Nursery seedlings are more precocious and uniform than seedlings directly grown in the soil. Furthermore, their development can be precisely regulated through the control of such factors as light, irrigation, nutrient availability, temperature, and humidity (Cerny *et al.*, 2004).



In this context, the objective is to produce high-quality seedlings with remarkable morphological and physiological characteristics, such as height, stem diameter, root length (Araméndiz-Tatis *et al.*, 2013; Souri and Sooraki, 2019), number of leaves, and leaf area (Vidigal *et al.*, 2011). In addition, these seedlings must be free of pests and diseases, guaranteeing a better field establishment and optimal crop development. Consequently, applying biofertilizers is as a key solution for the strengthening of plant resistance to environmental stress and the optimization of their growth, development, and yield (Bustamante *et al.*, 2018). In addition, biofertilizers boost productivity and their use encourages a more sustainable agriculture, reducing the dependence on chemical fertilizers, minimizing environmental impact (Castro-Landín *et al.*, 2025), and promoting more efficient and resilient production systems.

Some of the microorganisms commonly used as biofertilizers include arbuscular mycorrhizal fungi (AMF). Gottshall *et al.* (2017) and other authors have studied the role of these microorganisms and their symbiosis in the mineral nutrition of plants, reporting a higher significant growth, development, and yield improve in mycorrhizal plants compared with control. This benefit is provided by the AMF ability to increase nutrient and water acquisition, through specialized structures that form in the rhizosphere, as a result of the root colonization carried out by the fungus (Leigh *et al.*, 2009). On the one hand, growth-promoting bacteria of the genus *Azospirillum* can fix significant amounts of atmospheric nitrogen and transfer it to the host plant (Aguirre *et al.*, 2018). Not only does yield increase, but nitrogen fertilization requirements can be reduced (Leite *et al.*, 2019). In addition, these bacteria provide other benefits (Parra, 2001), including the production of phytohormones (auxins, gibberellins, and cytokinins), the solubilization of phosphate, and the increase of water stress resistance (Cohen *et al.*, 2015; Fukami *et al.*, 2018; Oliveira *et al.*, 2018). On the other hand, bacteria of the genus *Pseudomonas* have a remarkable ability to solubilize phosphates (Otieno *et al.*, 2015), to induce root nodulation, and to fix nitrogen (Ogata and Zuñiga, 2008).

In addition, their inoculation can activate plant resistance mechanisms against various stress factors (Regeiro *et al.*, 2017) and provide protection against phytopathogenic fungi (Alvaréz-García *et al.*, 2020).

The inoculation of seedlings with these microorganisms can be integrated into the nursery production process, optimizing resources and reducing costs. Since seedling production in nurseries already requires significant labor, a simultaneous inoculation with these microorganisms represents a viable strategy to increase the efficiency of the procedure (Angulo-Castro *et al.*, 2018). Therefore, the objective of this study was to evaluate the response and yield of husk tomato (*Physalis ixocarpa* Brot.) seedlings to inoculation, using different beneficial microorganisms and methods.

MATERIALS AND METHODS

Overall experimental conditions

The experiments were carried out at the Santa Lucía de Prías facilities, Campo Experimental Valle de México (CEVAMEX), of the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP), located in the municipality of Texcoco,

State of Mexico (19° 26' 43.2" N, 98° 54' 16" W, 2,264 m.a.s.l.), during the 2012-2013 autumn-winter cycle. Husk tomato (*Physalis ixocarpa* Brot. variety Diamante) seeds were sown in 200-cavities Styrofoam trays, which were previously disinfected with a 5% chlorine solution and afterwards rinsed with water. Subsequently, the cavities were filled with the Sunshine Mix #3[®] peat moss commercial substrate.

Effect of inoculants at the nursery stage

An experiment was established for each microorganism evaluated. A commercial bioinoculant based on a dose of 500 g ha⁻¹ of *Rhizophagus intraradices* (Schenck & Sm.) Walker & Schüßler arbuscular mycorrhizal fungus was used, with a concentration of 40 spores per gram (INIFAP^{MR}, Experimental Field CEVAMEX, INIFAP). The experiment was conducted under a randomized complete block design, with ten repetitions per treatment. Three biofertilizers were individually evaluated: *Rhizophagus intraradices*, *Azospirillum* sp., and *Pseudomonas fluorescens*. Different inoculation methods were used for the evaluation of each biofertilizer. The experiment also included a control treatment without inoculation. The following mycorrhizal treatments were applied:

- T1 = Application to the substrate (10 g of mycorrhiza per liter of substrate);
- T2 = Application to the seed (2.4 g of mycorrhiza per 100 g of seed);
- T3 = Irrigation of plant nursery (50 mL of a solution prepared with 100 g of mycorrhiza in 2 L of water per tray); and
- T4 = Control without inoculation.

The *Azospirillum* sp. strain came from the INIFAP germplasm bank and had a colony forming units [CFUs] concentration of 1×10⁸ per gram of substrate. The following treatments were used to evaluate *Azospirillum* sp.:

- T1 = Application to the seed (1.5 kg of inoculant per 59 kg of seed);
- T2 = Application to the substrate (54 g of inoculant per 659 g of substrate); and
- T3 = Control without inoculation.

The *Pseudomonas fluorescens* strain 7027 came from INIFAP and had a concentration of 1×10⁸ CFUs/g of substrate. The following three treatments were used to evaluate *Pseudomonas fluorescens*:

- T1 = Application to the seed (1.5 kg of inoculant per 59 kg of seed);
- T2 = Application to the substrate (54 g of inoculant per 659 g of substrate); and
- T3 = Control without inoculation.

Each assay was individually analyzed, maintaining the same experimental conditions and randomized complete block design, in order to ensure comparability and to reduce experimental variability.

Evaluated variables

Data were recorded until the seedlings had four fully developed leaves. This stage took place at 35, 41, and 40 days after sowing (DAS) for *R. intraradices*, *Azospirillum* sp., and *P. fluorescens*, respectively. The variables evaluated included germination percentage, plant height (PH), number of leaves (NL); root length (RL); stem diameter (SD); shoot fresh weight (SFW); shoot dry weight (SDW); root fresh weight (RFW); and root dry weight (RDW). Germination percentage was determined counting the number of seedlings that emerged at DAS. Meanwhile, plant height (PH) was measured from the base of the stem to the apex of the last leaf using a graduated ruler. In addition, root ratio (RR% = root dry weight/total plant dry weight) and R:S ratio (RDW/SDW) were calculated. The total biomass was dried in a Binder forced-air oven, at 70 °C for 72 h. Afterwards, it was weighed on a Sartorius CPA324 analytical balance. Colonization percentage was estimated in four plants at 35 days post-inoculation (DPI), washing the roots under running water and staining them, based on the technique described by Phillips and Hayman (1970). The method described by McGonigle *et al.* (1990) was used to make permanent preparations with the stained roots, in order to observe mycelium, spores, vesicles, and coils structures under the microscope.

Field experimental conditions

The experiment was established in the Santa Lucía de Prías facilities, Campo Experimental Valle de México (CEVAMEX). The soil had a clay loam texture, with a 6.99 pH and 2.15% Mo. In addition, it contained 13.0, 36.86, and 574 mg/kg of N, P, and K, respectively. A randomized complete block design, with ten repetitions per treatment was used in the experiment. The *Azospirillum* sp. and *P. fluorescens* treatments that were previously evaluated in the nursery were transplanted. However, an additional treatment (T5) was included for *R. intraradices*. This inoculation treatment consisted of a pre-transplanting irrigation of 70 g of mycorrhiza, diluted in 1 L of water per tray.

The plots used in the experiment had four 6-m-long furrows. The distance between furrows was 1.2 m and the distance between plants was 30 cm. Before transplanting, Confidor (2 mL/500 mL of water per 1,000 plants) was sprayed on the root ball of the seedlings. All the crops received the recommended 80-20-20 NPK chemical fertilization dose (Güemes-Guillen *et al.*, 2001). The variables evaluated at harvest included fruit yield and size classification. The latter variable was based on the following equatorial diameter parameters: small (3-4.5 cm), medium (4.5-5.5 cm), and large (5.5-6.5 cm). A SARTORIUS™ TS 1352Q37 precision scale was used to determine fruit weight per plant. Subsequently, these results were used to estimate total fruit yield (TFY, t ha⁻¹), which was expressed in kg plant⁻¹. The first fruit cutting was made 87 days after transplanting, followed by a second fruit cutting 56 days after the first cutting.

Statistical analysis

The effect of treatments was statistically analyzed using two-way analysis of variance (ANOVA) with Statistical Analysis System software (SAS v. 6.1, Institute, 1999). When the ANOVA result showed significant difference among treatments for each parameter,

the least significant difference (LSD) test at 5% probability level was applied for means separation.

RESULTS AND DISCUSSION

Plant nursery

Seed germination (99%) was recorded for all treatments three days after sowing. Table 1 shows the effect of inoculation using different microorganisms. Growth variables recorded significant differences ($p \leq 0.05$) in the statistical analysis. Depending on the application method, mycorrhizal inoculation (*R. intraradices*) showed significant differences ($p \leq 0.05$) in the height of husk tomato seedlings.

Seed inoculation recorded a 34% increase compared with non-inoculated plants (9.18 cm), reaching a height of 12.35 cm. In addition, this method registered the highest colonization percentage (57.5%) of all the treatments (Table 1). Angulo *et al.* (2018) obtained similar results in the evaluation of bell pepper and jalapeño pepper growth parameters. The treatments inoculated with beneficial microorganisms favored taller plants in both cultivars. The *G. cubense* treatment applied to bell peppers obtained taller plants ($p \leq 0.05$) than other treatments. Likewise, the plant growth-promoting bacteria (PGPB) treatment applied to jalapeño peppers resulted in taller plants.

No significant differences were recorded between the RL, SD, SDW, and RDW variables (Table 1). The inoculation method had no effect on the aerial dry biomass at 35 DAS. Velasco *et al.* (2001) reported a dry weight increase in *Physalis* plants inoculated with

Table 1. Growth variable means of husk tomato seedlings grown in a nursery.

| Treatment | NL | PH (cm) | SD (mm) | SFW (g) | SDW (g) | RFW (g) | RDW (g) | COL (%) |
|--|--------|----------|---------|-----------|----------|-----------|----------|---------|
| <i>Rhizophagus intraradices</i> | | | | | | | | |
| T1 Substrate | 6.0 a | 10.37 bc | 1.5 a | 0.6898 ab | 0.0707 a | 0.9758 a | 0.0301 a | 55.8 b |
| T2 Seed | 5.9 a | 12.35 a | 1.7 a | 0.7322 a | 0.0733 a | 0.9437 a | 0.0445 a | 57.5 a |
| T3 Irrigation | 5.8 ab | 10.72 b | 1.5 a | 0.6829 ab | 0.0749 a | 0.8414 ab | 0.0281 a | 55.8 b |
| T4 Without inoculation | 5.3 b | 9.18 c | 1.7 a | 0.5878 b | 0.0606 a | 0.9036 ab | 0.0264 a | 26.6 c |
| CV | 7.4 | 9.8 | 35.2 | 16.2 | 19.8 | 41.5 | 93.4 | 0 |
| <i>Azospirillum sp.</i> | | | | | | | | |
| T1 Substrate | 6.1 a | 9.61 a | 0.99 a | 0.4208 a | 0.0600 a | 0.5963 a | 0.0307 a | NA |
| T2 Seed | 5.9 a | 10.0 a | 1.10 a | 0.4411 a | 0.0680 a | 0.4893 a | 0.0311 a | NA |
| T3 Without inoculation | 5.7 a | 8.35 b | 0.99 a | 0.3638 a | 0.0561 a | 0.4876 a | 0.0191 b | NA |
| CV | 6.3 | 10.8 | 24.5 | 18.4 | 23.5 | 38.8 | 32.5 | NA |
| <i>Pseudomonas fluorescens</i> | | | | | | | | |
| T1 Substrate | 4.7 b | 8.08 a | 1.18 a | 0.3737 a | 0.0575ab | 0.7570 a | 0.0382 a | NA |
| T2 Seed | 4.8 b | 7.17 a | 0.90 a | 0.3017 b | 0.0442 b | 0.6222 a | 0.0251 b | NA |
| T3 Without inoculation | 5.5 a | 8.38 a | 1.25 a | 0.3974 a | 0.0629 a | 0.7110 a | 0.0275 b | NA |
| CV | 8.8 | 15.1 | 41.8 | 15.8 | 21.5 | 27.1 | 25.6 | NA |

NL: Number of leaves, PH: Plant height, SD: Stem diameter, SFW: Shoot fresh weight, SDW: Shoot dry weight, RFW: Root fresh weight, RDW: Root dry weight, COL: Colonization. N/A: Not applicable. Means with different letters are statistically different at $p \leq 0.05$. CV=coefficient of variation.

G. intraradices, after 60 DAS; however, they pointed out that no effects were registered at 40 DAS. Peil and Gálvez (2005) mentioned that, in the case of tomato, the percentage of root dry matter varies between 17% and 20% during the initial stage. In this study all treatments recorded greater root ratios (Table 1).

The highest root ratio (RR) (30.5%) was achieved by seed inoculated with mycorrhiza. Meanwhile, *Azospirillum* sp. and *P. fluorescens* obtained a bacteria RR of 62% and 52%, respectively (Table 2). Bacteria showed a different development than mycorrhiza. On the one hand, *Azospirillum* sp. recorded significant differences between inoculation methods; however, seed inoculation recorded the greatest plant height (PH) (10 cm), reaching a higher increase (19%) than the rest of the treatments. On the other hand, *P. fluorescens* recorded the lowest height values of all the microorganisms: 7.87 cm in average (16% increase). These data were similar to the results of Villaseñor-Tulais *et al.* (2023), who recorded a 14.64% increase in the height of husk tomato plants, inoculated with *Pseudomonas fluorescens* strain UM270 at the moment of the transplanting. Meanwhile, the *Azospirillum* sp. inoculation recorded the highest effect, producing more leaves (NL). This phenomenon can be explained by the phytohormones produced by *Azospirillum*, which induce cell division and expansion, stem and root elongation, activation of bud growth, branch development, regulation of leaf senescence, and chlorophyll production (Wong *et al.*, 2015). However, the *P. fluorescens* inoculation only showed significant differences in NL, SDW, and RDW.

Yield

Fruit size and total yield (TY) showed significant differences ($p \leq 0.05$) between *R. intraradices* inoculation methods in each of the two cuttings (Table 3). In contrast, no

Table 2. Root behavior means per treatment in husk tomato seedlings.

| Treatment | RL (cm) | RR (%) | R:S |
|--|---------|---------|----------|
| <i>Rhizophagus intraradices</i> | | | |
| T1 Substrate | 9.17 a | 29.6 a | 0.422 a |
| T2 Seed | 9.04 a | 30.5 a | 0.621 a |
| T3 Irrigation | 9.56 a | 26.6 a | 0.373 a |
| T4 Without inoculation | 9.41 a | 29.8 a | 0.439 a |
| CV | 20.9 | 37.65 | 95.0 |
| <i>Azospirillum</i> sp. | | | |
| T1 Substrate | 9.17 a | 31.3 ab | 0.462 ab |
| T2 Seed | 12.35 a | 33.8 a | 0.518 a |
| T3 Without inoculation | 10.72 b | 24.8 b | 0.344 b |
| CV | 19.7 | 21.7 | 26.9 |
| <i>Pseudomonas fluorescens</i> | | | |
| T1 Substrate | 9.61 a | 36.9 a | 0.599 a |
| T2 Seed | 10.0 a | 39.9 a | 0.666 a |
| T3 Without inoculation | 8.35 b | 30.6 b | 0.448 b |
| CV | 18.4 | 14.6 | 22.8 |

RL: root length; RR: root ratio; R:S: root dry weight/shoot dry weight ratio. Means with different letters are statistically different at $p \leq 0.05$. CV=coefficient of variation.

Table 3. Husk tomato yield (t ha^{-1}) per cutting depending on the inoculation methods with beneficial microorganisms.

| Treatment | SFY | MFY | LFY | SFY | MFY | LFY | TY |
|---------------------------------------|---------------|---------|---------|----------------|---------|--------|---------|
| | First cutting | | | Second cutting | | | |
| <i>Rhizopagus intraradices</i> | | | | | | | |
| T1 Substrate | 0.53 a | 4.63 b | 1.65 b | 2.63 a | 0.70 b | 0.00 a | 10.7 b |
| T2 Seed | 1.47 a | 6.00 ab | 3.00 ab | 3.33 a | 0.76 ab | 0.23 a | 16.5 ab |
| T3 Irrigation | 1.41 a | 8.23 a | 2.10 ab | 4.16 a | 1.66 a | 0.30 a | 18.7 a |
| T4 Without inoculation | 1.45 a | 4.16 b | 1.62 b | 5.83 a | 1.13 ab | 0.33 a | 14.6 ab |
| T5 Pre-transplanting | 0.53 a | 7.01 ab | 4.3 a | 4.30 a | 0.86 ab | 0.16 a | 17.3 ab |
| DMS | 1.08 | 3.50 | 2.30 | 3.56 | 0.92 | 0.37 | 2.49 |
| <i>Azospirillum sp.</i> | | | | | | | |
| T1 Seed | 11.44 a | 8.61 a | 2.52 a | 1.80 a | 0.47 a | 0.11 a | 21.0 a |
| T2 Substrate | 7.88 a | 7.67 ab | 1.43 ab | 1.58 a | 0.53 a | 0.02 a | 17.3 a |
| T3 Without inoculation | 5.02 a | 4.23 b | 1.34 b | 2.98 a | 0.62 a | 0.19 a | 17.3 a |
| DMS | 7.11 | 3.67 | 1.12 | 2.53 | 0.50 | 0.58 | 2.01 |
| <i>Pseudomonas fluorescens</i> | | | | | | | |
| T1 Seed | 7.73 a | 7.57 ab | 3.89 a | 1.90 a | 0.86 a | 0.00 a | 21.6 a |
| T2 Substrate | 7.69 a | 4.38 b | 2.03 a | 2.69 a | 1.01 a | 0.10 a | 18.6 a |
| T3 Without inoculation | 5.14 a | 8.9 a | 3.05 a | 2.17 a | 0.54 a | 0.00 a | 22.0 a |
| DMS | 5.70 | 4.50 | 2.08 | 2.18 | 1.02 | 0.16 | 1.79 |

SFY: small fruit yield; MFY: medium-sized fruit yield; LFY: large fruit yield; TY: total yield. Rotten fruits were removed to determine fruit yield. LSD: least significant difference. Means with different letters are statistically different at $p \leq 0.05$.

significant differences were observed with *P. fluorescens*. The *R. intraradices* inoculation by irrigation recorded the highest yield (18.7 t ha^{-1}), followed by seed inoculation (16.5 t ha^{-1}). The production of medium-sized fruits predominated during the first cutting, while, small-sized fruits made the greatest contribution to yield increase during the second cutting. The bacterium *Azospirillum sp.* showed a similar behavior; however, small fruits predominated in both cuttings with the *P. fluorescens* inoculation. The highest yield was obtained by treatments inoculated with *R. intraradices* (74% increase). In the case of bacteria, the best yield was obtained by *Azospirillum sp.* (21%), followed by *P. fluorescens* (18%).

These promising results prove the potential of these microorganisms associated with this crop. Other authors have reported excellent results in husk tomato cultivation, combining chemical fertilizers with organic amendments. Aguiñaga-Bravo *et al.* (2020) reported that —compared with the exclusive use of chemical fertilization— the combination of chemical fertilizers and organic amendments in husk tomato cultivation increases yield by up to 55.5%. Rivadeneyra-Manzanilla *et al.* (2024) found that the application of vermicompost increased yield by 44.61%, compared with the absolute control treatment. According to Álvarez-Sánchez *et al.* (2021), biofertilizers, organic fertilizers, biostimulants, and bioregulators of plant growth can improve yield. These results match the findings of this study.

CONCLUSIONS

The seed inoculation method was the most effective option, because it promoted a greater growth and yield than the other evaluated methods. Treatments inoculated with the *Rhizophagus intraradices* arbuscular mycorrhizal fungus recorded the highest yields. *Azospirillum* sp. was the bacteria strain that produced the highest yield. The inoculation with mycorrhiza increased fruit production. In addition, it produced a higher ratio of medium-sized fruits. Meanwhile, *Pseudomonas fluorescens* and *Azospirillum* sp. favored the production of small- and medium-sized fruits, consequently contributing to a more uniform and balanced harvest.

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Native rhizobia and their role in the nodulation, growth, and development of *Leucaena leucocephala* (Lam.) de Wit seedlings

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ABSTRACT

Objective: To evaluate the effect of native rhizobial strains on nodulation, growth, dry biomass production, total nitrogen (N) and carbon (C) content in plant tissue, as well as nitrogenase activity in seedlings of *Leucaena leucocephala*.

Design/Methodology/Approach: The study followed a completely randomized design. Seeds of *L. leucocephala* were germinated in an inert substrate, and six treatments were evaluated, corresponding to native rhizobial strains and an uninoculated control. Plants were harvested at 45, 75, and 105 days, and the following parameters were measured: (i) total number of nodules, (ii) plant height (root and shoot), (iii) dry plant weight, (iv) carbon and nitrogen content in dry biomass, and (v) nitrogenase enzyme activity.

Results: Treatments with strains 40, 41-2, and 46 showed the highest number of nodules at 75 days. At 105 days, plants inoculated with strain 74 had a significantly higher average dry biomass ($p < 0.001$). The average nitrogen content was significantly higher ($p < 0.0001$) in treatments with strains 26, 34b, 46, and in control. No significant differences were observed in carbon content among treatments. Nitrogenase activity was confirmed in all inoculated treatments but was absent in control.

Limitations/Implications: The results obtained with native *Rhizobium* sp. strains highlight their potential to enhance biological nitrogen fixation in *Leucaena leucocephala*, which could contribute to the development of more sustainable agroforestry systems, especially in nitrogen-deficient soils.

Findings/Conclusions: This study highlights the potential of certain native rhizobial strains for inoculating *L. leucocephala*, enhancing its growth and development. However, further research under field conditions is needed to confirm these findings.

Keywords: Biological nitrogen fixation, Fodder biomass, Native rhizobia strains, Nitrogenase, Sustainable livestock.

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INTRODUCTION

Food demand is expected to increase anywhere (between 35-56%) by 2050, while crop yields are growing too slowly (or even decreasing), making it increasingly difficult to meet this projected demand (Valin *et al.*, 2013; van Dijk *et al.*, 2021). In addition, tropical soils in many regions are deficient in nitrogen (N), and this deficiency reduces the productivity of

crops, pastures, and livestock, further exacerbating food production challenges (Soumare *et al.*, 2020; Udvardi *et al.*, 2021). One solution used so far has been N fertilization, which is necessary to maintain or increase the nitrogen reserves in soil and sustain agricultural productivity. However, its excessive use has led to negative environmental impacts, such as eutrophication of water bodies, volatilization of compounds into the atmosphere, and changes in soil through alterations in organic matter content, microbial life, and acidity (Khan and Mohammad, 2014; Pan *et al.*, 2016; Singh, 2018).

In this context, it is important to consider sustainable strategies for soil fertility management. One alternative is the use of symbiotic nitrogen fixation by legumes, which fixes atmospheric N to soil (Vanlauwe *et al.*, 2019). Legumes can enhance agroecosystem productivity by forming associations with soil bacteria, as biological nitrogen fixation has been reported to range from 16.0 to 389 kg ha⁻¹, depending on the region and legume species (Pereyra *et al.*, 2015; Kebede, 2021).

A promising example of nitrogen-fixing legume is *Leucaena leucocephala*, a widely distributed shrub in the tropics. It revitalizes monoculture-based animal production systems into competitive silvopastoral systems, offering an affordable protein source for livestock due to its nitrogen-fixing ability (Shelton and Dalzell, 2007; Murgueitio *et al.*, 2014). However, the effectiveness of nodulation in *Leucaena* spp. depends on the association with specific rhizobial strains, highlighting the need for systematic inoculation with selected strains (mostly of the genus *Rhizobium*) to maximize symbiotic efficiency (Wong *et al.*, 1989; Turk and Keyser, 1992; Bala and Giller, 2001).

Incorporating shrub legumes like *Leucaena* sp. can significantly enhance soil fertility due to their nitrogen-fixing ability in symbiosis with rhizobia bacteria, enhancing soil fertility with rates surpassing 250-500 kg N per ha annually in tropical regions (Casanova-Lugo *et al.*, 2014). Beyond soil benefits (such as increase of soil organic C and soil total N), legumes offer significant nutritional value, with *L. leucocephala*-grass pastures in Australia yielding more beef and profits than grass-only systems (Bueno y Camargo, 2015; Hopkins *et al.*, 2019); however, the successful introduction of legumes into new areas depends on the presence of compatible rhizobia in the soil (Clúa *et al.*, 2018).

In this sense, there is growing interest in using rhizobia as inoculants to enhance crop productivity. For legumes with specific symbiotic requirements, such as *Leucaena* sp., identifying and isolating effective rhizobial strains can be beneficial. Sourcing rhizobia from the legume's native region (such as Mexico) offers promising potential, given the rich diversity of strains found there, including some not reported elsewhere (Martínez-Romero and Caballero-Mellado, 1996).

Moreover, apart from facilitating nodulation and nitrogen fixation, certain rhizobia strains produce growth-promoting compounds, including phytohormones like indole acetic acid (IAA) and gibberellins; given IAA's role in plant functions, its production by rhizobia is noteworthy (Lugtenberg and Kamilova, 2009; Maithani *et al.*, 2023).

It has been reported that legumes often show greater compatibility with specific rhizobial strains; similarly, *L. leucocephala* exhibits selective associations, with nodulation occurring primarily when inoculated with its own species-specific rhizobia (Wong *et al.*, 1989; Turk and Keyser, 1992). This highlights the importance of identifying compatible

rhizobial strains for *L. leucocephala* to enhance symbiotic efficiency in regions where it is being introduced or promoted. Such is the case of *L. leucocephala* cv. Cunningham, which has been promoted in silvopastoral systems due to its high nutritional value (276.8 g crude protein per kg of dry matter) (Cuartas *et al.*, 2015). The aim was to evaluate the effect of native rhizobial strains on nodulation, growth, dry biomass production, total nitrogen (N) and carbon (C) content in plant tissue, as well as nitrogenase activity in seedlings of *Leucaena leucocephala*.

MATERIALS AND METHODS

Experiment conditions: The experiment was conducted under semi-controlled conditions in a greenhouse at the Faculty of Veterinary Medicine and Zootechnics of the Universidad Autónoma de Yucatán (Mérida, México). The substrate employed was limestone rock powder, sterilized beforehand in an oven at 120 °C for 24 h to eliminate any present bacteria or fungi. Following sterilization, the substrate was placed into clean 5 L plastic bags.

L. leucocephala seeds were previously disinfected and scarified (with hot water at 80 °C for 2 min.), in each bag, three seeds were sown. After a week of post-germination, thinning was conducted to retain only one seedling per bag. Finally, irrigation was performed up to the field capacity of the substrate using the following nitrogen-free nutrient solution: 279 K₂SO₄; 493 MgSO₄·7H₂O; 0.23 KH₂PO₄; 145 K₂HPO₄; 371 CaCl₂·2H₂O; 1.43 H₃BO₃; 1.02 MnSO₄; 0.22 ZnSO₄; 0.08 CuSO₄; 0.05 Na₂MoO₄; 0.10 CoCl₂·4H₂O; 16.70 FeCl₃·6H₂O (quantities are in mg L⁻¹).

Rhizobia isolation, inoculant preparation and inoculation: Six previously biochemical and molecular characterized native rhizobium strains were selected (Tzec-Gamboa *et al.*, 2020), chosen for their production of specific growth-promoting compounds (Table 1). Rhizobia strains were isolated from two localities of Yucatán, Cauce (21° 01' 13.2" N; 89° 42' 29.7" W) and Motul (21° 04' 07.2" N; 89° 16' 45.7" W).

The inoculum preparation of the rhizobial isolates was performed in 125 mL Erlenmeyer flasks with 50 mL of yeast extract-Mannitol (ELM) medium. The flasks were incubated at 30 °C under continuous stirring at 120 rpm until the exponential phase of each of the inoculum was reached. Cell growth was standardized with medium turbidity at a given optical density, relating it to a standard viable count curve, adjusting it to 10⁷-10⁹ cells mL⁻¹.

The cultivated rhizobia cells served as inoculum for *L. leucocephala* cv. Cunningham seeds. Prior to sowing, 1 mL of the desired inoculum in saline solution (10⁷-10⁹ cells mL⁻¹) was applied per seed (9 ml per bag). A week after sowing, a second inoculation of 3 mL of the inoculant was administered per bag.

Experimental design: Six native rhizobia strains (treatments) (Table 1) and one control (non-inoculated) were evaluated using a completely randomized design, with 15 experimental units (replicates) per treatment [7 treatments × 15 replicas = 105 *L. leucocephala* plants]. Three destructive samplings were conducted (at 45, 75, and 105 days after inoculation). For each sampling, five plants from each treatment were randomly selected.

Table 1. Native rhizobia strains used as inoculants in this study, including main characteristics and the site where obtained.

| Rhizobia Strain | Genus | GPC produced | Site | DT (h ⁻¹) |
|-----------------|--------------------------|--------------|--------|-----------------------|
| 26 | <i>Sinorhizobium</i> sp. | IAA | Caucel | 4.3 |
| 34b | <i>Sinorhizobium</i> sp. | IAA, SID | Caucel | 3.5 |
| 40 | <i>Sinorhizobium</i> sp. | IAA | Caucel | 4.5 |
| 41(2) | <i>Ralstonia</i> sp. | IAA, SID | Caucel | 4 |
| 46 | <i>Sinorhizobium</i> sp. | IAA | Caucel | 3.5 |
| 74 | <i>Rhizobium</i> sp. | IAA | Motul | 4.0 |

Note: GPC=Growth promoting compound; IAA=Indoleacetic Acid; SID=Siderophores; DT=Duplication time.

Evaluation of agronomic variables: For sampling and subsequent measurement of agronomic variables, each selected bag was placed on a sterile plastic tray. The bag was then laterally cut open, and the substrate was carefully removed to avoid damaging the roots of the plants. Once all the substrate was removed, the following measurements were conducted: 1) Nodule count per plant; 2) Plant height (from the base of the stem to the apex), the roots were cleaned, using a sterile scalpel, the above-ground and root portions were separated and measured using a ruler; and 3) Dry biomass, both segments of the plant were placed in paper bags and dried in an oven at 60 °C until a constant weight was achieved, and then weighed.

Laboratory analysis: For the determination of total Carbon and Nitrogen in both the root and aerial parts of the plants; after drying, they were ground; and then, an elemental analysis was conducted using a Carbon/Nitrogen element analyzer (FLASH 2000[®] Series Organic Elemental Analyzers from Thermo Scientific).

Atmospheric nitrogen fixation was verified by the activity of the nitrogenase with the technique of reduction of acetylene to ethylene described by Peoples *et al.* (2009). The technique consisted of sectioning the nodules with a little of the root of each plant, the excess soil was removed and placed in vials with serological caps on the lid, 10% v/v of the air contained in the vial was removed, and the same amount of high purity acetylene was injected in an amount equivalent to 10% of the total volume of the vial, it was injected into the hermetically sealed container. The roots with the nodules were incubated at room temperature for one hour.

After incubation, two samples were taken from each container with 1 ml syringes, which were analyzed on a Hewlett Packard 5890 (HP; Palo Alto, CA, USA) gas chromatograph equipped with a flame ionization detector (FID) and a column HP-PLOT/Q column (50 m × 0.23 mm internal diameter). Helium was used as carrier gas (1 ml min⁻¹), hydrogen (45 ml min⁻¹), nitrogen (10 ml min⁻¹), and air (450 ml min⁻¹). The run temperatures were injector 110 °C, oven 60 °C and detector 160 °C. Acetylene and ethylene were used as reference standards (Hardy *et al.*, 1973; Vessey, 1994).

Statistical analysis: Data from the experiment were first subjected to test of normality and homogeneity of variance for each variable and then to analysis of variance (ANOVA),

using a significance level of $P < 0.05$. Treatment means were compared using Tukey HSD significance test (SAS for Windows).

RESULTS AND DISCUSSION

Nodulation

Nodules were observed in all treatments; however, in some treatments, nodules could not be identified at the first sampling (45 days). Nodulation at 45 days was scarce and was only observed in the treatments inoculated with strains (40, 46 and 74). The other inoculated treatments with strains 26, 41-2, 34b and 74 showed formation of nodules after 75 days, while control plants that were not inoculated did not develop nodules (Table 2). The nodules were oval, brown in color and of variable dimensions between 2-3 mm in diameter (Figure 1).

The treatments inoculated with strains 40, 41-2 and 46, had the greatest number of nodules in the shortest time (75 days), with an average of 15.3, 9.6 and 16.6 nodules per plant respectively, at 105 days a decrease was observed in the nodulation of these treatments. In the other treatments, the number of nodules increased gradually reaching a maximum average at 105 days, strain 26 had the highest average value with 17.6 nodules per plant at 105 days and was also the highest average among all treatments (Table 2).

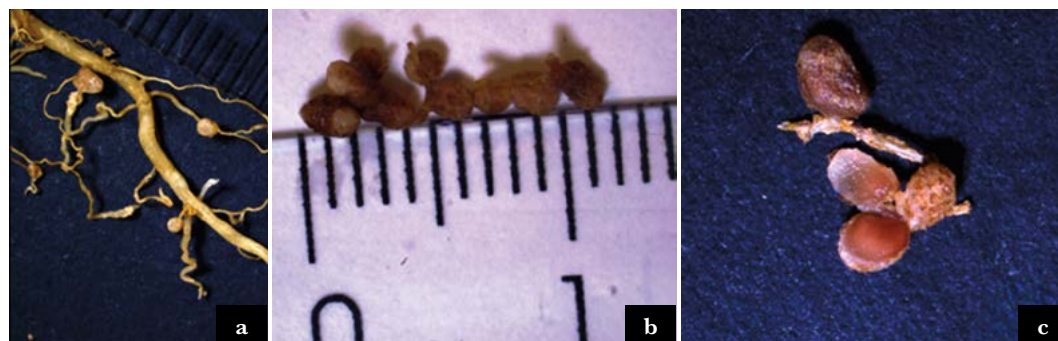


Figure 1. Main characteristics of the nodules found during the development of the experiment. (a) Distribution of nodules in the root system of plants, (b) Measurement of nodules (c) Active nodules, a longitudinal section of the nodule is shown, and the reddish coloration of the interior is observed, as an indicator of the presence of the enzyme nitrogenase.

Table 2. Average number of nodules obtained in *Leucaena leucocephala* plants on different growth days.

| Rhizobia Strain | Nodule number (average per plant) | | |
|-----------------|-----------------------------------|---------|----------|
| | 45 days | 75 days | 105 days |
| 26 | 0 | 5 | 18 |
| 34b | 0 | 3 | 10 |
| 40 | 2 | 15 | 6 |
| 41-2 | 0 | 10 | 10 |
| 46 | 4 | 17 | 11 |
| 74 | 4 | 6 | 16 |
| Control | 0 | 0 | 0 |

Biomass

Plants inoculated with strain 26 had the highest average dry biomass ($0.11 \pm 0.03 \text{ g plant}^{-1}$) at 45 days, this was significantly different from the other treatments. The average dry biomass of the other treatments was all very similar. However, treatments inoculated with strains 46 and 34b had the lowest dry biomass (Table 3).

At 75 days, the plants inoculated with strain 74 had the highest average dry biomass, the difference was significant compared to other treatments ($0.45 \pm 0.24 \text{ g plant}^{-1}$). The treatment inoculated with strain 26 had the lowest average dry biomass. At 105 days, plants inoculated with strain 74 had a significantly higher average dry biomass. The other treatments had similar dry biomass between them, no significant difference was observed. At 45 days, strain 26 had the highest average dry biomass despite not having developed nodules. However, at 75 days this strain 26 was the treatment with lowest dry biomass.

Dry biomass of the control, at 45 and 75 days, was the same as the treatments inoculated with strains 41-2 and 40. At 75 days, it was the same as the treatments inoculated with strains 40, 46, and 34b. At 105 days the dry biomass was equal to the treatments except for the treatment inoculated with the 74 strain that was greater with significant differences over all the treatments including the control.

Plant growth

Plant growth was assessed by measuring the length of roots and shoots (Figure 2). At 45 days, the plants with the highest growth were those inoculated with the 74 and 41-2 strains, the plants with the lowest growth were those inoculated with the strains 40, strains 34b and 46 had a similar growth characteristic (without significant differences) (Table 4).

At 75 days, the plants inoculated with the 74 strains were the ones with the longest shoots, these were significantly different ($P < 0.01$), the plants inoculated with the strains 26, 34b and 46 had shorter shoots, similar to the control. At 105 days, plants inoculated with strains 74, 40 and 34b were the ones with the highest growth and these differences were significant. The plants that were not inoculated (control) had similar length shoots to those of the treatments during the first two samplings (45 and 75 days), but at 105 days the growth of the control plants was significantly reduced compared to the other treatments.

Table 3. Dry biomass (average g plant^{-1} of DM \pm Standart error) of *L. leucocephala* plants on different growth days.

| Rhizobia Strain | 45 days | 75 days | 105 days |
|-----------------|--------------------------|--------------------------|-------------------------|
| 26 | $0.11 \pm 0.03\text{a}$ | $0.21 \pm 0.03\text{c}$ | $0.28 \pm 0.08\text{b}$ |
| 34b | $0.04 \pm 0.09\text{e}$ | $0.33 \pm 0.09\text{b}$ | $0.33 \pm 0.09\text{b}$ |
| 40 | $0.07 \pm 0.01\text{bc}$ | $0.29 \pm 0.07\text{b}$ | $0.29 \pm 0.06\text{b}$ |
| 41-2 | $0.08 \pm 0.02\text{bc}$ | $0.26 \pm 0.09\text{bc}$ | $0.26 \pm 0.09\text{b}$ |
| 46 | $0.05 \pm 0.01\text{de}$ | $0.30 \pm 0.07\text{b}$ | $0.30 \pm 0.07\text{b}$ |
| 74 | $0.07 \pm 0.02\text{cd}$ | $0.45 \pm 0.24\text{a}$ | $0.45 \pm 0.23\text{a}$ |
| Control | $0.08 \pm 0.02\text{b}$ | $0.31 \pm 0.09\text{b}$ | $0.31 \pm 0.09\text{b}$ |

Note: DM=Dry matter. Values with the same letter are not significantly different, $P < 0.01$).



Figure 2. Measuring plants during the experiment. (a) *L. leucocephala* plants at 105 days, (b) *Leucaena* shoot (c) *Leucaena* root.

Table 4. Plants height (cm) of *L. leucocephala* plants on different growth days.

| Rhizobia Strain | 45 days | 75 days | 105 days |
|-----------------|--------------------------|-------------|--------------|
| | Above-ground height (cm) | | |
| 26 | 17.70±1.07ab | 19.29±0.67a | 20.03±6.34ab |
| 34b | 14.94±0.57b | 19.34±0.85a | 18.56±5.87ab |
| 40 | 17.41±0.63 ab | 20.81±0.79a | 19.14±6.05ab |
| 41-2 | 19.43±1.49a | 19.91±1.05a | 17.94±5.67b |
| 46 | 17.46±0.86 ab | 18.46±0.84a | 16.37±5.18ab |
| 74 | 18.30±0.96 ab | 18.96±0.79a | 22.12±6.99a |
| Control | 18.42±1.02 ab | 17.32±0.76a | 18.22±5.76ab |
| | Root length (cm) | | |
| 26 | 6.73±0.27a | 13.09±1.76a | 21.27±2.31a |
| 34b | 5.97±0.57a | 14.98±1.40a | 23.92±3.28a |
| 40 | 5.64±0.41a | 14.92±6.45a | 22.70±3.37a |
| 41-2 | 6.32±0.63a | 23.44±2.53a | 19.89±2.97a |
| 46 | 4.97±0.69a | 17.64±2.81a | 15.12±2.97a |
| 74 | 7.88±2.04a | 18.44±2.45a | 23.53±4.54a |
| Control | 7.88±2.04a | 11.99±1.62a | 7.94±0.89b |

Regarding root length, at 45 and 75 days, no significant differences were observed among any of the treatments, including the control. However, by 105 days, all strains exhibited significant differences compared to the control. Strains 34b and 74 showed the most substantial root development, though without significant differences between them or with any of the other strains (Table 4).

Nitrogen and carbon content

At 45 days, the plants inoculated with strain 34 had the highest average total nitrogen content, which was significantly different to all other treatments and the treatment with the lowest average nitrogen content was inoculated with strain 40 (Table 5). However, at the

Table 5. Nitrogen content (%) in *L. leucocephala* plants on different growth days.

| Rhizobia Strain | 45 days | 75 days | 105 days |
|-----------------|------------|------------|------------|
| 26 | 5.3±0.01c | 3.1±0.13d | 3.6±0.00a |
| 34b | 5.8±0.11a | 3.9±0.05b | 3.8±0.15a |
| 40 | 4.5±0.01e | 3.2±0.18d | 2.9±0.45c |
| 41-2 | 4.7±0.12d | 3.1±0.07d | 3.2±0.17bc |
| 46 | 5.4±0.03bc | 3.8±0.07c | 3.9±0.06a |
| 74 | 4.7±0.09d | 3.9±0.11bc | 3.5±0.09ab |
| Control | 5.5±0.15b | 4.6±0.00a | 3.8±0.09a |

next sampling opportunity at 75 days a decrease in nitrogen content in plants was observed in all treatments. The control treatment maintained a higher nitrogen content with respect to the other treatments and this was significantly higher than other treatments.

At 105 days, the treatments inoculated with strains 26, 34b, and 46 together with the control, are those that stand out in their nitrogen content. However, it is noteworthy that in the course of time, in most treatments, a decrease in their nitrogen content was observed. However, the nitrogen content of the plants inoculated with strains 46, 26 and 41-2, which showed a decrease in N content between the first and second samples, were the only treatments that were able to increase their nitrogen content at 105 days. The carbon content was similar between the treatments and remained so until the end of the experiment (day 105), there were no significant differences (Table 6).

Nitrogenase activity

Nitrogen fixation by rhizobia was confirmed by the reduction of acetylene to ethylene by the enzyme nitrogenase present in the nodules. The activity of the nitrogenase enzyme was confirmed in all treatments except the control (Figure 3). However, response varied widely among treatments. The nodules of the strain 26 recorded the highest enzyme nitrogenase activity, as well as the highest concentration of ethylene (8214 $\mu\text{M C}_2\text{H}_4 \text{ mg}^{-1} \text{ nodules h}^{-1}$). Compared with these plants inoculated with strains 41-2 and 74 had the lowest values of enzyme activity with 989 and 581 $\mu\text{M C}_2\text{H}_4 \text{ mg}^{-1} \text{ nodules h}^{-1}$, respectively. The uninoculated treatment did not develop nodules, so it was not included in this analysis.

Table 6. Carbon content (%) in *L. leucocephala* plants on different growth days.

| Rhizobia Strain | 45 days | 75 days | 105 days |
|-----------------|------------|------------|------------|
| 26 | 39.38±0.11 | 37.90±0.56 | 38.79±0.22 |
| 34b | 39.42±0.16 | 38.02±0.16 | 37.41±0.46 |
| 40 | 38.36±0.08 | 37.95±0.90 | 40.32±7.05 |
| 41-2 | 38.96±0.45 | 38.20±0.26 | 37.65±0.53 |
| 46 | 39.82±0.37 | 39.15±0.10 | 38.13±0.25 |
| 74 | 39.49±0.16 | 38.96±0.34 | 38.03±0.60 |
| Control | 39.26±0.06 | 38.21±0.18 | 38.53±0.22 |

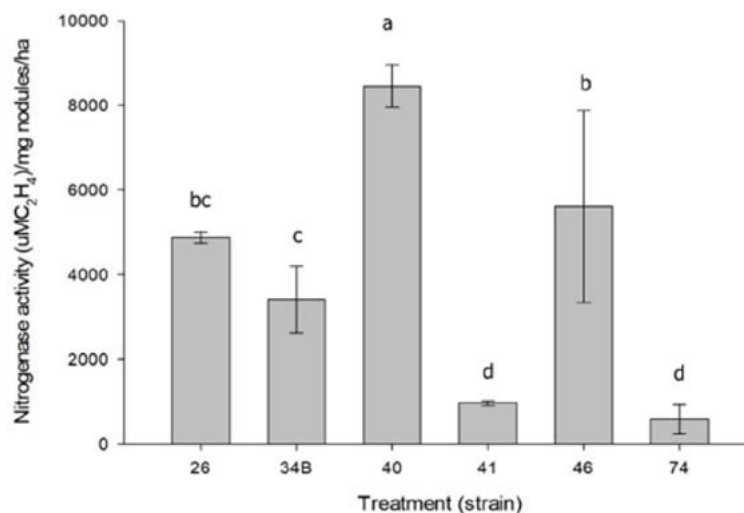


Figure 3. Nitrogenase enzyme activity in active nodules of *Leucaena leucocephala* seedlings inoculated with different native strains of rhizobia. Note: Standard error is presented in parentheses.

Generally, the average number of nodules per plant increased with the age of the plant, with a maximum of 18 and 16 nodules per plant from strains 26 and 74, respectively at 105 days. These findings are similar with those of Crespo-Flores *et al.* (2022), who discovered more nodulation in rhizobia-inoculated plants, although nodulation was observed in all treatments, there was variability among the strains, including the control (unlike in the present study, where the control showed no nodules). This might be caused by various factors, including the specificity between the host plant and the species/strain of rhizobia as well as the natural genetic variation of both (Borges, 2006; Duran *et al.*, 2013; Menezes *et al.*, 2017; Torres-Gutiérrez *et al.*, 2017).

In the present study, most treatments (26, 34b, 41-2, and the control) showed no nodulation at 45 days; similarly, Bueno and Camargo (2015) reported no nodulation during the first seven weeks (49 days), highlighting the effectiveness of the strains that did manage to induce nodulation (40, 46, and 74). Additionally, in the same study the nodulation peak was reached at 18 weeks (126 days), with an average of 25 nodules per plant, while in this study it was reach at 105 days; although the study ended at 105 days, a decrease in the number of nodules could not be observed in all treatments, which could mean that in some of the treatments the peak could have been reached days later.

Three of the treatments evaluated established effective symbiosis by day 45 through the formation of nodules. However, by day 105, some strains showed a decrease in nodule numbers, which is likely due to nodule senescence. This decline is likely due to nodule senescence, although pinpointing the exact causes, whether genetic factors of the strains or abiotic factors (*v.g.* drought or darkness) (Zhou *et al.*, 2021) is challenging at this stage.

Although the treatments inoculated with strains 26 and 34 did not develop nodules at 45 days, they excelled in total dry biomass and nitrogen content; while no correlation was observed between nodulation, dry biomass and nitrogen content at 45 days, this could be explained that during the first days of development, the seedlings take the necessary

nutrients for the development of the plantlets from the seeds (Salisbury and Ross 1992; Sánchez *et al.*, 2011). Another explanation is that the strains used are producers of growth promoting compounds such as IAA; since in a previous study we reported that these strains produce these compounds (Tzec-Gamboa *et al.*, 2020), and it has been shown that the effect of multiple hormones produced by rhizobia strains affects not only nodulation, but also other processes such as germination and plant growth (Santillana *et al.*, 2005).

Strains 40 and 46, belonging to the genus *Sinorhizobium* had the highest average number of nodules at 75 days, they showed high dry biomass production and plant height. Symbiosis with *Sinorhizobium*, was particularly effective, since all strains tested were able to nodulate at 105 days, as did strain 74 (*Rhizobium*), *Sinorhizobium* is a genus of bacteria, which has been reported as nodulant of *L. leucocephala*, however, not all strains belonging to this genus are able to do it (Wang *et al.*, 2002). Regarding *Rhizobium*, several studies highlight it as a common nodulating genus for *L. leucocephala*; however, it is important to emphasize that this symbiosis exhibits a high degree of specificity, even within the *Rhizobium* genus itself (Trinick, 1968; Moawad and Bohlool, 1984; Chen *et al.*, 2021; Ríos-Ruiz *et al.*, 2024).

Furthermore, treatment with strain 74 showed the highest dry biomass and best growth at 75 days. However, nodulation peaked at 105 days (16 nodules), with nodulation showing a positive correlation with time.

Perhaps the compounds produced by the rhizobia during plant establishment initially aid plants in more efficiently utilizing nutrients, rather than promoting nodule development. At 75 days, apart from the treatment inoculated with strain 74, all other treatments had a similar response, and no correlation was observed between nodulation and dry biomass.

As for nitrogen content, there were no significant differences between most of the strains and the control (105 days). However, in previous studies, it has been suggested that plant growth is independent of nodule growth, since although the N content in plants decreases over time, the total amino acid content does not change (Pereyra *et al.*, 2015). Similarly, Singleton and Tavares (1986) found a similar behavior where the nitrogen content in shoots was lower compared to the roots, attributing it to the concentration of the *Rhizobium* population.

Values for nitrogenase activity are shown in Figure 3, the amount of nitrogen fixed in this experiment compared favorably to previously published values, although it was not anywhere near as high as the values of $22,069 \mu\text{M C}_2\text{H}_4 \text{ mg nodules}^{-1} \text{ H}^{-1}$ reported in works performed in peanut inoculated with *Bradyrhizobium* (which is another promising nitrogen fixing genus) and a series of combinations of PGPR's (Badawi *et al.*, 2011). The selected inoculants showed a positive effect on growth, nodulation and nitrogen fixation. Plants inoculated with strain 26, which had the highest activity of the enzyme at 105 days, was the only treatment that increased plant growth by 16%, compared to the growth it showed at 75 days.

Although only root sections containing the nodules were used in the trial, the values obtained at 105 days were higher than those reported by Anthraper and Dubois (2003) in *L. Leucocephala* when using the complete root system, where levels of $10 \text{ Methylene plant}^{-1} \text{ H}^{-1}$, were obtained. However, observed a 100% increase in enzyme activity after 147 days, due to an increase in the number of nodules.

CONCLUSIONS

All the treatments showed nodulation, suggesting that the strains are able to form a symbiosis with *L. leucocephala*. But among the strains, the plant response and nitrogenase activity varied, indicating that some strains have more potential for nitrogen fixation as well as for help in seedling development and growth. For their quantity of nodules generated and nitrogenase activity, for instance, strains 26 and 74 stood out; generally showing better performance in dry biomass generation and shoot growth. These results suggest that this interaction might foster positive effects on the establishment of *L. leucocephala* in low nitrogen soils. To evaluate the effectiveness of the strains, it is imperative to verify the observed effects under field conditions. Furthermore, treatment inoculated with strain 74 showed the highest dry biomass and best growth at 75 days. However, nodulation peaked at 105 days, with nodulation showing a positive correlation with time.

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Impact of the precipitation on yield and chemical composition of oat (*Avena sativa* L.) hay forage grown in the northwestern Chihuahua, Mexico

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ABSTRACT

Objective: Assess yield and quality of oat (*Avena sativa* L.) hay growing under five rainfall scenarios: optimal, 503 mm (R-101%); middle, 277 mm (R-55%) and 260 mm (R-52%); critical, 180 mm (R-36%) and 140 mm (R-30%) of precipitation in northwestern of Chihuahua, Mexico.

Design/Methodology/Approach: The data corresponds to a previous study of oat varieties planted under rainfed conditions and harvested at dough grain maturity stage. The data analyzed employed the analysis of variance. The variables dry matter yield (DMY), total digestible nutrients yield (TDNY), relative feed value (RFV), crude protein yield (CPY), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were studied.

Results: Yield and chemical content differed significantly ($P < 0.001$) by the effect of rainfall scenario. The DMY and TDNY decreased as much as 43.5 and 41.6% in oats grown under critical rainfall scenario respect to an optimal rainfall scenario ($P < 0.001$). Likewise, NDF, ADF and ADL contents decreased ($P < 0.001$) by 5, 10 and 13%, respectively. The CP content varied due to the rainfall scenario ($P < 0.001$), but it was not correlated with this factor ($r = 0.218$, $P = 0.069$).

Study Limitations/Implications: In the state of Chihuahua, oats are grown under rainfed conditions; however, the increasingly prolonged periods of drought make it necessary to develop and evaluate new drought-resistant oat varieties.

Findings/Conclusions: In this region, oats grown under rainfall scenarios of up to 52% of the optimal (500 mm) yield acceptable amounts of dry matter with sufficient nutritive value for feeding cattle.

Keywords: cereals, climate change, digestibility, drought, fodder, precipitation, rainfed.

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INTRODUCTION

Whole plant oat (*Avena sativa* L.) hay is an excellent source of forage used in livestock feed in many regions of the world. In Mexico, oats are mainly grown under rainfed conditions (96%) and, of the total area established, 93.5% is for fodder production (SIACON, 2023).

The state of Chihuahua, Mexico, ranks first in cultivating this grass, whose contributions in 2022 were 31.2% of the national total (SIACON, 2023). In the State, 18.5% of the total area used for crops is used to grow fodder oats; and of the main fodder crops, the area sown with oats accounted for 48.8% (INEGI, 2023). Oats are mainly grown in the northwestern of the State under rainfed conditions (96%). In this region, oats are traditionally harvested for fodder at the physiological maturity stage. The oat hay produced is the main source of forage used in the feeding of dairy and beef cattle in the region (Avila *et al.*, 2006; Villazon *et al.*, 2017).

In this region of the State, climate change is affecting agricultural production (Villazon *et al.*, 2017). Changed precipitation conditions is one of the major climate factors affecting crop production (Neenu *et al.*, 2013). The scarcity and irregular distribution of the rainfall have an impact on the yield and quality of the oat crop (Kim *et al.*, 2005; Kim *et al.*, 2006). Consequently, variation rainfall scenarios can have an impact on oat forage yield and quality. In a research, Kim *et al.* (2005) found significant differences between locations on yield and forage quality of rye. They attributed higher DM and TDN yields to higher precipitation, 70 and 44.5% more, respectively. Also, Mut *et al.* (2018) observed significant differences in oat yield, with a range of 6.67 to 12.27 t ha⁻¹ of hay. Under three rainfall scenarios: normal, 30% decrease in rainfall and 30% increase in rainfall, Lai *et al.* (2022) found that with normal rainfall DM yield values of soybean, oat, and vetch were 3,617, 5,284 and, 2,631 kg ha⁻¹ but increased 26.2, 13.2 and 26.4%, respectively, when rainfall was increased 30%. In a subsequent publication, Lai *et al.* (2024) concluded that the forage and wheat grain production are strongly related to precipitation and increasing precipitation significantly enhanced production.

For local farmers in Chihuahua, growing rainfed oats is the last alternative when weather conditions make it impossible to plant corn in April or beans in June. Growth and production of this cereal are directly related to temperature and rainfall (Amado *et al.*, 2000; Jurado *et al.*, 2016). In this region, for oat forage production, rainfall above 500 mm is considered favourable; intermediate rainfall, 300 mm; and critical rainfall below 200 mm (Salmeron *et al.*, 2003; Jurado *et al.*, 2016). However, there is little evidence of the impact of precipitation on oat hay production and quality. Therefore, the objective was to know the productive behaviour and nutritional quality traits of oat hay by effect of rainfall scenarios, grown in the northwestern of the state of Chihuahua, Mexico.

MATERIALS AND METHODS

Experimental locations

The data used for this study corresponds to a previous study in which seven oat varieties were sown under two planting systems at five localities in northwestern Chihuahua state (in the Baja Babicora, the main oat-producing area in Mexico). The localities were: Teseachi-Namiquipa (28° 53' N, 107° 25' W), Santo Tomas-Guerrero (28° 33' N, 107° 30' W), Choqueque-Cusihuiachi (28° 14' N, 106° 50' W), Santa Ana-Guerrero (28° 33' N, 107° 30' W) and Lazaro Cardenas-Cuauhtemoc (28° 25' N, 106° 52' W). Sowing dates at each location were: Jul-28, Jul-23, Aug-02, Jul-25 and Aug-02, 2005, respectively.

Experimental design

Hay yield and chemical composition data corresponding to the seven oat varieties sown under two planting systems, made a total of fourteen observations. Those oat varieties were growing under five rainfall scenarios (according to locality): 503 mm (R-101%) which corresponds to 500 mm of precipitation in the growing of the oat crop, considered as optimal or favourable; 277 mm (R-55%) and 260 mm (R-52%), considered as middle; 180 mm (R-36%) and 140 mm (R-30%), considered critical for this region of the state of Chihuahua (Salmeron *et al.*, 2003; Jurado *et al.*, 2016). All oats were harvested at dough grain stage (78 ± 4 d). The sowing density was 100 kg ha^{-1} of seed, and the fertilization rate was 30-40-00 kg ha^{-1} of N-P-K. The data were distributed in an experimental arrangement completely randomized design, where oat varieties were the replications.

Dry matter and haymaking process

Dry matter yield (DMY) per hectare was determined by throwing a one-meter square five times. All oat plants within the square were cut and weighed, then the material was left in the plot for ten days to simulate the haymaking process. Drying was completed in an oven at $60 \text{ }^\circ\text{C}$ for 24 h to calculate the dry matter yield per hectare. The samples were then ground to 1 mm in a Wiley[®] mill (Arthur H. Tomas, Philadelphia, PA, USA).

Variables studied

Total dry matter (method # 930.15), ash (method # 942.05) and crude protein (CP, method # 990.03) were determined in the samples according to AOAC (1995) procedures. In addition, the cell wall fractions neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined. NDF analysis was carried out using Na_2SO_3 and α -amylase. ADL analysis was performed in a beaker by immersing the samples in a 72% H_2SO_4 solution. These fractions were determined sequentially on the Ankom200[®] fibre analyser using Ankom F57[®] bags, following the procedures proposed by the company (Ankom Technology, Fairport, NY, USA). Additionally, the relative feed value (RFV) of the forage samples was calculated using the following simplified equation:

$$RFV = (88.9 - (0.779 \times FDA) \times (120 / NDF)) / 1.29$$

(Cherney and Parson, 2020), as well as total digestible nutrients (TDN), by:

$$TDN = 88.9 - (0.79 \times ADF)$$

(Holland *et al.*, 1990). The TDN and CP results were multiplied by the DMY per hectare to obtain the TDN and CP yield per hectare.

Statistical analysis

Data were analysed with the GLM procedure using the SAS statistical software package (SAS, 2006) employed the analysis of variance (ANOVA). Each variable was

analysed separately, in a univariate way. Means were compared using the least significant difference (LSD, $P \leq 0.05$) test. Pearson's correlation coefficients (r) analysis was also carried out among the variables studied, considering as important those that were significant at $P \leq 0.05$.

The following statistical model was used:

$$Y_{ij} = \mu + R_i + E_{ij}$$

Where Y_{ij} = response variable measured in the j -th repetition that received the i -th treatment, μ = overall mean, R_i = treatment effect i , E_{ij} = random error.

RESULTS AND DISCUSSION

Dry matter and nutrient yield variables

DMY and TDNY showed a rainfall effect. The highest DMY and TDNY were found in the optimal rainfall (R-101%), followed by the scenario R-55%. The lowest yields were found at R-36% and R-30% rainfall scenarios (Figure 1), this corresponds to 43.5% and 41.6% less DMY and TDNY with respect to the optimal rainfall scenario (R-101%), respectively.

Crude protein yield (CPY) showed a quadratic trend with respect to rainfall scenario ($P < 0.0001$). CPY was highest at R-101%, followed by R-55% and R-30% rainfall scenarios (543, 363 and 319 kg ha^{-1} , respectively). The lowest CPY was at R-52% scenario, a reduction of 58.2% with respect to the optimal rainfall scenario (Figure 2). For relative feed value (RFV), the highest values occurred at critical rainfall scenarios (128 and 129), and the lowest values were at the optimal and middle (117, 111 and 113), for R-36%, R-30%, R-101%, R-55% and R-52%, respectively (Figure 2).

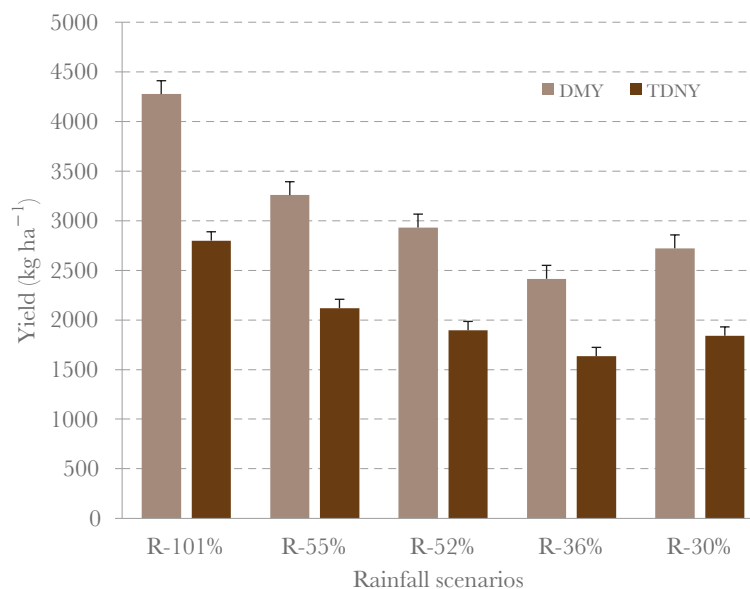


Figure 1. Dry matter yield (DMY) and total digestible nutrients yield (TDNY) of oats hay growing under different rainfall scenarios in the northwestern of Chihuahua, Mexico.

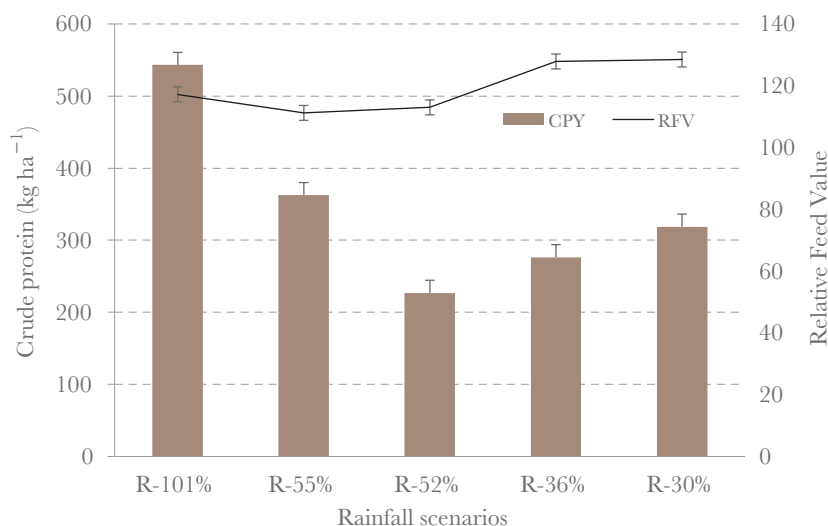


Figure 2. Crude protein yield (CPY) and relative feed value (RFV) of oats hay growing under different rainfall scenarios in the northwestern of Chihuahua, Mexico.

Chemical composition variables

Crude protein (CP) content of oat hay was highest ($P < 0.001$) at R-101%, followed by R-30%, and middle at R-55% and R-36%, and lowest at R-52%. The most important statistical difference was among R-101% and R-52% rainfall scenarios, with a decrease of 38.4% less CP at R-52% compared to R-101% (Table 1).

Respect to neutral detergent fiber (NDF) and acid detergent fiber (ADF) content, the lowest values were at rainfall critical scenarios (R-36% and R-30%), and the highest values were at optimal and middle scenarios (R-101%, R-55% and R-52%), with a difference of 5.1 and 3.9 units between the highest and the lowest value for NDF and ADF, respectively (Table 1). The lignin (ADL) content showed a similar trend to NDF and ADF, with the lowest values at R-36% and R-30% and the highest values at R-101%, R-55% and R-52% (Table 1). It is interesting to note the low values of NDF, ADF and ADL in oat hay at R-36%

Table 1. Chemical composition of oats hay growing under different rainfall scenarios in the northwestern of Chihuahua, Mexico.

| Rainfall scenarios | Chemical composition (% of DM) | | | |
|--------------------|--------------------------------|--------------------|-------------------|--------------------|
| | CP | NDF | ADF | ADL |
| R-101% | 12.7 ^a | 52.3 ^b | 29.7 ^a | 3.01 ^a |
| R-55% | 11.2 ^b | 54.8 ^a | 30.2 ^a | 2.69 ^{bc} |
| R-52% | 7.8 ^c | 53.9 ^{ab} | 30.6 ^a | 2.81 ^b |
| R-36% | 11.5 ^b | 49.7 ^c | 26.7 ^b | 2.39 ^d |
| R-30% | 11.8 ^{ab} | 49.5 ^c | 26.8 ^b | 2.61 ^c |
| SEM | 0.4 | 0.8 | 0.5 | 0.10 |

SEM = standard error of the mean, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin. Rainfall scenarios: R-101% (503 mm, optimum), R-55% (277 mm), R-52% (260 mm), R-36% (180 mm), R-30% (149 mm). ^{a,b,c,d} Means with different letters within columns are different ($P < 0.05$).

and R-30%, which also had low forage yields and high RFV values, may be a consequence of lower biomass production in plants, requiring less fiber (cell walls) to support the biomass weight, as Jehangir *et al.* (2013) reported similar behaviour with oats.

The correlation analysis showed a significant positive correlation between the rainfall scenario and the yield (DMY, TDNY, CPY) and chemical composition (NDF, ADF, ADL) variables, and a negative correlation with the RFV variable, while the CP content showed no effect (Table 2).

Dry matter and nutrient yield variables

Dry matter yield and nutrients per harvested area are main indicators to assess the productivity of a forage crop (Ojeda *et al.*, 2018; Lai *et al.*, 2022; Lai *et al.*, 2024). TDN is the nutrients available to livestock and is related to the concentration of ADF present in the forage: as ADF increases there is a decrease in TDN (Mut *et al.*, 2015).

Like others (Ramos *et al.*, 2011; Lai *et al.*, 2022), in this study precipitation significantly impacted DMY and TDNY per harvested area (Figure 1). In this regard Kim *et al.* (2005; 2006) observed a significant effect of locality on DMY, but they attributed higher DM, CP and TDN yields to air temperature rather than precipitation. We found that the higher yield was due more to precipitation since it was more variable than temperature. The exception was at R-52%, which, compared to R-30%, had lower precipitation, but similar DMY and TDNY (Figure 1). It is possible that factors other than rainfall influenced this result in these localities, as has been suggested by others (Kim *et al.*, 2006; Mut *et al.*, 2018). It is widely documented that the absence of rainfall induces slow or null plants growth (Ramos *et al.*, 2011). In the study by Ramos *et al.* (2011), more and better rainfall distribution was observed in 2006 compared to 2007; consequently, forage production was much higher in the first year (5,000 and 1,500 kg ha⁻¹ of DM, respectively); however, they also attributed this result to the delayed sowing date and a period of low temperatures in 2007. Similarly, Lai *et al.* (2024), in four growing seasons and three rainfall scenarios (normal, -30% and +30%), observed a significant impact of precipitation on the forage yield of oats harvested at flowering stage. The oat biomass ranged from 3.66 t ha⁻¹ in -30% scenario to 7.66 t ha⁻¹ in +30% scenario. In 2017-2018

Table 2. Correlation coefficients between quality components of oat hay and rainfall scenarios.

| Quality components | Rainfall scenario | DMY | TDNY | RFV | CPY | CP | NDF | ADF |
|--------------------|-------------------|----------|----------|-----------|----------|-----------|----------|----------|
| DMY | 0.769*** | | | | | | | |
| TDNY | 0.749*** | 0.994*** | | | | | | |
| RFV | -0.335** | -0.330** | -0.235** | | | | | |
| CPY | 0.729*** | 0.846*** | 0.869*** | -0.051 | | | | |
| CP | 0.218 | 0.142 | 0.191 | 0.400*** | 0.636*** | | | |
| NDF | 0.265* | 0.282* | 0.187 | -0.990*** | 0.026 | -0.373** | | |
| ADF | 0.417*** | 0.375*** | 0.278* | -0.976*** | 0.060 | -0.442*** | 0.948*** | |
| ADL | 0.563*** | 0.522*** | 0.466*** | -0.585*** | 0.364** | -0.087 | 0.550*** | 0.667*** |

*Significant at $P \leq 0.05$, **Significant at $P \leq 0.01$, ***Significant at $P \leq 0.001$.

growing season, with a normal rainfall of 339 mm, dry 238 mm (−30%) and excessive wet of 441 mm (+30%), the biomass of oat in +30% scenario was increased by 16.6% compared to the normal scenario. They also found that the forage oat production shown to be strongly related to period precipitation, with a correlation coefficient $R^2=0.80$ ($P<0.001$).

Cereal forage yield depends on the influence of genetic (species, variety, or genotype), climatic (temperature, water availability, rainfall distribution, daylight hours, growing season), agronomic (fertilisation type, tillage, planting system, plant density), soil (fertility, structure, texture) factors present in a region or locality (Shah *et al.*, 2015; Lucio *et al.*, 2022). The presence, degree of influence and interaction of these factors in the crops growing season impacts forage yield and quality (Espitia-Rangel *et al.*, 2012). For example, under rainfed conditions, the amount and distribution of precipitation has a strong impact on oat yield (Sanchez *et al.*, 2014). Consequently, significant variations in yield are expected, so forage yield is a function of rainfall (Table 2). Oat producers in northwestern Chihuahua agree drought is the main problem limiting oat yield production in the region (Avila *et al.*, 2006).

In the northwestern state of Chihuahua, under good rainfed conditions, forage yields of oats harvested at physiological maturity (~92 d) exceed 5000 kg ha⁻¹ of DM (Salmeron *et al.*, 2003; Avila *et al.*, 2006), while in poor to bad rainfed conditions yields range from 1000 to 3000 kg ha⁻¹, with an average of 2800 kg ha⁻¹ for the three rainfall scenarios (Avila *et al.*, 2006). In the High Valleys of Mexico, in oats harvesting at dough grain stage and growing under good rainfed conditions, yields ranged from 9223 to 19454 kg ha⁻¹ of DM (Espitia-Rangel *et al.*, 2012). In Zacatecas, Sánchez *et al.* (2014) observed yields of 2712 to 4764 kg ha⁻¹ of DM and 46.2 to 56.4% TDN in oats harvesting at milky-dough stage. In other latitudes of the world, yields of 3312 to 6826 kg of DM and 2050 to 4283 kg ha⁻¹ of TDN, at heading stage (Kim *et al.*, 2006); 6530 to 7760 kg ha⁻¹ DM, at 50% flowering stage (Jehangir *et al.*, 2013); and, 1470 to 5484 kg ha⁻¹ DM, at milky stage (Ramos *et al.*, 2011) are reported. This wide variation in oat forage DMY and TDN influenced by variation in days to cutting (Mendoza-Pedroza *et al.*, 2021) and by environment-genotype interaction (Ramos *et al.*, 2011; Espitia-Rangel *et al.*, 2012; Jehangir *et al.*, 2013; Mut *et al.*, 2018) mainly. On the other hand, under irrigated conditions, yields of 5355, 5800 and 8720 kg ha⁻¹ DM were reported in Mexico (Sosa-Montes *et al.*, 2020; Lucio *et al.*, 2022; Solano *et al.*, 2022, respectively).

Regarding CPY, Kim *et al.* (2006) reported a CPY of 1022 to 799 kg ha⁻¹ at 393 and 52 mm of rainfall when oats were harvested between the milk to dough grain stages. In Mexico, Sanchez *et al.* (2014) reported a range of 315 to 544 kg ha⁻¹, based on the variety. Mendoza-Pedroza *et al.* (2021) obtained a range of 380 to 600 kg ha⁻¹ as a function of days to cutting, with the highest CPY at 75 d and then decreasing as days to cutting increased. In both studies the CPY values are alike to those obtained in this study, particularly at R-101%. We found that the CP content in oat hay trend to be low at dough grain stage, the inverse occurs with the DMY; therefore, the higher CPY observed is largely attributed to the higher DM production, which in turn depends on precipitation.

The RFV is an index that integrates NDF and ADF values, and it is used to predict the intake and energy value of a forage and is derived from the intake and DM digestibility of the forage (Mut *et al.*, 2015). In agreement with Horrocks and Vallentine (1999) forages with RFV values above 151, between 150-125, 124-103, 102-87, 86-75 and below 75 are considered as first, premium, good, standard, poor and bad, respectively. According to this classification, oats growing at R-36% and R-30% scenarios reached premium quality, while most were good (Figure 2). Kim *et al.* (2006) reported values from 119 and 141 at low (141 and 52 mm) and 96 and 89 at high (235 and 393 mm) precipitation. Also, Mut *et al.* (2018) reported values from 96 to 105 in oats established in three locations and growing under rainfed conditions.

Chemical composition variables

The CP content changed largely by rainfall scenario effect (Table 1). In the study by Kim *et al.* (2006), showed a significant effect of precipitation on CP content; in general, CP content in oat forage decreased with higher precipitation and increased with lower precipitation. We found a similar trend from the R-52% rainfall scenario (Figure 2). It could be that the rainfall above ~250 mm increases the CP content because of major regrowth of the plant, and below 250 mm the increase of CP is due to dilution effect of the fiber components. Mut *et al.* (2018) presents significant differences between locations from 8.6 to 11.9% of CP in oat hay harvested at the milk to dough grain stages; however, it is likely that this variation in CP content is due to the other factors since rainfall behaviour showed little variation between localities and years. In Mexico, Mendoza-Pedroza *et al.* (2021) reported 14.7% at 75 d at cutting. Finally, Sanchez *et al.* (2014) observed similar values ($P > 0.05$) among the oat varieties they evaluated, ranging from 11.3 to 13.2%.

Cell walls fractions NDF, ADF and ADL showed a similar trend. With favourable rainfall scenarios (R-101%, R-55%, R-52%) these cell walls components were higher, while in less favourable rainfall scenarios (R-36%, R-30%) these tended to decrease (Table 1). As mentioned above, this could be a result of lower biomass production in plants, consequence of a lower precipitation, leading to a lower deposition of these structural carbohydrates required to support the biomass weight. In addition, a fiber dilution effect has been reported due to the onset of starch filling of the kernel and the amount of grain produced by the plant at this maturity stage (Khorasani *et al.*, 2007), an event that starts with the grain development stage (Rosser *et al.*, 2013; Zhang *et al.*, 2023); moreover, it coincides with a gradual decrease in the leaf-stem ratio and an increase in the panicle-leaf-stem ratio (Khorasani *et al.*, 1997). All this leads to a change in the proportion of structural and non-structural carbohydrates on the whole oat plant.

A study found that the NDF and ADF contents in oat during the spring season (higher rainfall) were significantly higher compared to during the autumn season (lower rainfall), ranged from 62.4 to 46.1% for NDF and from 37.4 to 24.9% for ADF, respectively (Kim *et al.*, 2006). On the other hand, Mut *et al.* (2018) reported 53.0 to 63.0% for NDF and 31.5 to 39.4% for ADF at three locations. In Mexico, Sanchez *et al.* (2014) and Mendoza-Pedroza *et al.* (2021) reported similar values for NDF (51.3 to 61.1% and 51.1 to 59.6%, respectively) and for ADF (34.9 to 42.7% and 29.9 to 34.9%, respectively) in oats cut between the

milk-dough grain stages. The above values are close to those observed in this study. For ADL, Mendoza-Pedroza *et al.* (2021) obtained 1.8-1.9% in oats harvested at 75 and 90 d, respectively. In the study by Mendoza-Pedroza *et al.* (2021), there was an increase in fiber content with increasing days to cutting.

The values of NDF, ADF, and ADL found in this study are close to those reported by Ramos *et al.* (2011) (54.0, 29.2 and 2.9%, respectively) in oat harvested at milky stage. In the other hand, in the Ramos *et al.* (2011) study, as well as in the study by Mendoza-Pedroza *et al.* (2021), the cell walls fractions increased in the forage as maturity advanced.

Oat forage quality is a function of dry matter yield per harvested area and its chemical composition (protein, soluble carbohydrates, and fiber). The stage of maturity at cutting is a factor that has a strong impact on quality (Liu and Mahmood, 2015). At early growing stages, oat hay is favoured for high protein and soluble carbohydrate content and low fiber content, but low dry matter production. The opposite occurs when oats are cut at later stages of maturity. As a result, it has been proposed that for optimum digestible nutrient production, oats should be harvested at the milk to dough grain stages; however, it has been observed that after the dough grain stage, digestible nutrient production can be significantly higher (Zhang *et al.*, 2023), as was the case in this study when harvesting at dough stage compared to other trials where oats were harvested at an earlier maturity stage (Kaur and Goyal, 2017; Favre *et al.*, 2019; Kilicalp and Turk, 2023). This implies that, under good rainfall conditions, fertilization, and adequate tillage technique, in northwestern Chihuahua oats can be harvested between the dough grain and physiological maturity stages in order to obtain the maximum yield of digestible nutrients.

The results of correlation analysis (Table 2) showed that DMY and TDNY are strongly correlated with the amount of rainfall during the growing season of oat. Likewise, cell wall fractions (NDF, ADF, ADL) were correlated with amount of rainfall. The RFV variable showed a negative correlation with the amount of rainfall; that is, the more rainfall RFV tends to decrease, this is expected since RFV is dependent on the variables ADF and NDF. On the other hand, CP content showed no correlation with rainfall but was negatively correlated with NDF and ADF fractions. These results confirm that when these fiber fractions increase, CP content decreases, and the correlation between CP and ADL tends to be low or null.

Finally, even when oats were grown under a middle rainfall scenario of R-52%, DMY (2931 kg ha⁻¹) was higher than the average of 2800 kg ha⁻¹ recorded for this region of the state (Avila *et al.*, 2006). Likewise, under this scenario de CP, NDF, ADF and ADL were 7.8, 53.9, 30.6 and 2.81%, which means acceptable digestible nutrient yields and oat forage quality.

CONCLUSIONS

In the northwestern state of Chihuahua, under rainfed conditions, oat hay yield and quality are severely affected by rainfall scenarios. The dry matter and total digestible nutrients yields decrease as much as 43.5 and 41.6% in oats grown under critical rainfall scenario with respect to an optimal rainfall scenario. Likewise, NDF, ADF and ADL contents decreased by 5, 10 and 13%, respectively. The CP content varied due to the

rainfall scenario, but it was not correlated with this factor. Furthermore, it was confirmed that a decrease in CP content implies an increase in NDF and ADF contents. In this region, oats grown under rainfall scenarios of up to 52-55% of the optimum (500 mm), yield acceptable amounts of dry matter with sufficient nutritive value for feed cattle, as observed in this study.

CONFLICT OF INTEREST

All the authors declare that they have no conflicts of interest.

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From a hierarchical organizational culture to a clan culture: the case of the Colegio de Postgraduados, Veracruz Campus

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ABSTRACT

Objective: To identify the dominant and desired organizational cultures within the academic community.

Design/methodology/approach: The Organizational Culture Assessment Instrument (OCAI) questionnaire was administered to 28 academics using a pragmatic methodology based on systems theory.

Results: The findings reveal a predominantly hierarchical culture, although there is a strong aspiration toward a clan-type culture.

Limitations and implications of the study: The results suggest that fostering a clan-type organizational culture within hierarchical institutions can enhance commitment, cohesion, and organizational effectiveness, though it requires flexible leadership and well-structured change strategies.

Conclusions: It is concluded that a transition toward more collaborative organizational structures could strengthen institutional identity, cohesion, and effectiveness. Further research on organizational resilience and dynamic capabilities is recommended.

Keywords: behavior, organization, administrative structure

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INTRODUCTION

An organization whether for-profit or non-profit, formal or informal, centralized or decentralized is composed of individuals who interact within a dynamic system shaped and guided by leaders, structures, routines, rules, and norms of its environment (Schein, 1988). Together, these elements form a society structured by diverse functional systems. These functional systems emerge through an organizational culture, which, according to Pedraza *et al.* (2015), comprises significant internal and external experiences that individuals in an organization have encountered and the strategies they have implemented to adapt to it.

Recent studies indicate that public educational institutions face tensions between traditional hierarchical models and more horizontal, participatory management schemes, such as those promoted by a clan-type culture. These tensions affect coordination, the effectiveness of collegial bodies, and the perceived legitimacy of decision-making processes. Examples of this are found in studies by Cancho & Huamán (2020), Lysytsia & Druker (2021), Parveen (2021), Bernatová & Kuklišová (2024), and Oliveira & Alves (2024).

In this context, it becomes particularly relevant to assess what type of organizational culture predominates at the Colegio de Postgraduados, Campus Veracruz (CPVer), and what model its members aspire to especially in an environment that prioritizes collaborative academic work, the generation of relevant knowledge, and the training of high-level human resources. This research is grounded in General Systems Theory, viewing CPVer as a complex and dynamically coupled organization, whose components interact non-linearly.

It also adopts a pragmatic and instrumentalist epistemological approach, recognizing that the knowledge generated should serve to transform institutional reality. For the analysis, the study employs the Competing Values Framework (CVF), developed by Cameron and Quinn (1999), using the Organizational Culture Assessment Instrument (OCAI), which identifies four types of organizational culture: clan, adhocracy, market, and hierarchy. This model has proven effective in diagnosing cultural alignment in educational institutions and in proposing change strategies. Thus, the aim of this study is to analyze the organizational culture of the Colegio de Postgraduados, Campus Veracruz, in order to identify the predominant cultural type, the one desired by its academic community, and to assess whether current institutional actions support the achievement of the expected outcomes.

MATERIALS AND METHODS

This exploratory study was conducted to gather information that would help understand the organizational environment of the Colegio de Postgraduados, Veracruz Campus (CPVer). As a case study, it allowed for field research, using surveys as the primary research strategy, administered to the actors directly involved in the phenomenon under investigation, who served as the main source of information. The research was carried out in four phases. The first involved a case study at CPVer through the application of the Organizational Culture Assessment Instrument (OCAI) to academic staff. The purpose of this instrument is to diagnose the dominant organizational culture by identifying four organizational subcultures: clan, hierarchy, adhocracy, and market. The OCAI comprises six questions, each with four answer options. Participants assign scores to each option by distributing 100 points among the four responses, giving the highest score to the option that best reflects the current practice at CPVer.

According to Hernández, Fernández, and Baptista (2014), the minimum sample size recommended for case studies ranges from six to ten participants. In this study, the instrument was administered to all 28 academic staff members at CPVer, qualifying as a convenience sample.

The OCAI instrument is divided into two parts: the first assesses the current organizational culture, and the second evaluates the desired organizational culture. Each item in the questionnaire corresponds to a specific cultural type: item A represents the clan culture, item B the adhocracy culture, item C the market culture, and item D the hierarchical culture.

The dimensions assessed by the OCAI are as follows:

1. **Dominant characteristics:** describe what the organization is like.
2. **Organizational leadership:** evaluates how leadership is exercised and transmitted within the organization.
3. **Personnel management:** analyzes how employees are treated and the overall work environment.
4. **Organizational unity:** examines internal cohesion and the mechanisms that foster interpersonal bonds.
5. **Strategic emphasis:** identifies how institutional goals are achieved.

6. **Criteria of success:** defines the standards by which success in employee development is judged, estimated, or recognized.

Given the nature of the convenience sample, a pilot study was conducted to refine aspects related to the instrument's administration. This pilot was applied to administrative and unionized personnel at CPVer and concluded with a feedback session regarding the content of the instrument, including suggestions for improving instructions or clarifying any ambiguous questions.

Subsequently, the OCAI was formally administered to the key actors in this research.

The second phase involved entering the data into the OCAI database and calculating Cronbach's Alpha for validation purposes. The third phase focused on producing summaries and radial graphs that illustrated the preferred means for achieving organizational goals. The fourth and final phase consisted of analyzing the results

RESULTS AND DISCUSSION

Every organizational structure is designed by establishing hierarchies, procedures, responsibilities, among other elements, and emphasizing decision-making processes where communication and routines are central axes making organizational culture a key component.

Organizational culture functions as a tool within any administrative structure, enabling the understanding of organizational functioning through factors such as values, beliefs, climate, norms, symbols, and philosophy. The Cronbach's Alpha coefficient was calculated at 0.897. The results show that the greatest gaps are found between the Clan and Hierarchical organizational cultures, indicating a pronounced need for a Clan culture rather than the currently prevailing Hierarchical one (Table 1). Notably, the Clan culture is currently the least practiced. Regarding the desired culture, the Adhocracy type ranks second in preference.

Additionally, radial graphs depicting the results of the organizational culture assessment are presented. These illustrate both the current perception and the projected perception five years into the future (Figure 1), highlighting the type of organizational culture deemed necessary by the academic community at CPVer.

Organizational culture has a significant impact on the effectiveness of CPVer. It must be considered as the guiding framework for addressing and resolving challenges inherent to the institution's academic population, as it conveys identity, strengthens loyalty, and

Table 1. Results of the current and required organizational culture.

| Current culture | | Required culture | | Cultural gap | |
|-----------------|-------|------------------|-------|--------------|--------|
| Clan | 14.33 | Clan | 31.83 | Clan | 17.50 |
| Adhocracy | 20.21 | Adhocracy | 31.32 | Adhocracy | 11.11 |
| Market | 29.08 | Market | 19.06 | Market | -10.03 |
| Hierarchy | 40.42 | Hierarchy | 19.70 | Hierarchy | -20.72 |

Source: Own elaboration.

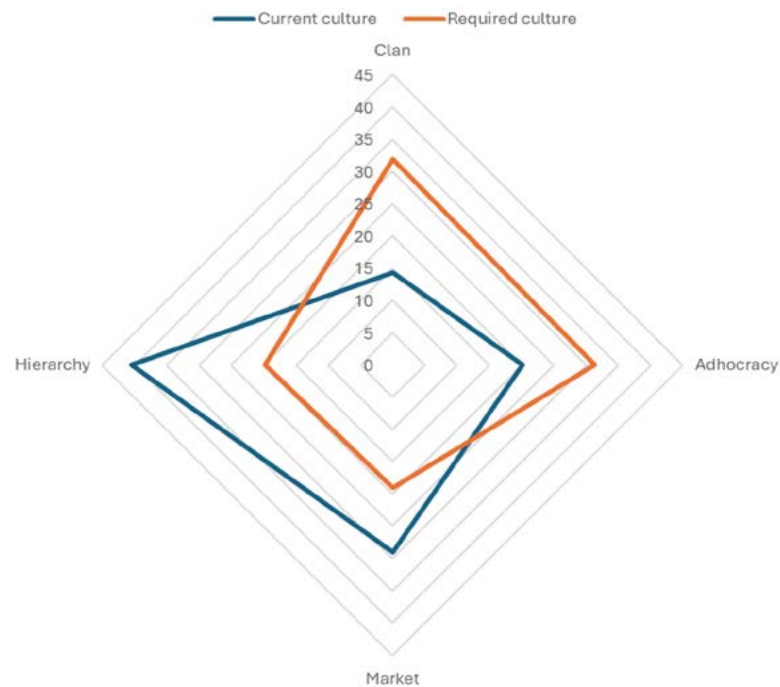


Figure 1. Results of current organizational culture.

influences decision-making. Culture acts as the cohesive force, serving as a bridge between organizational behavior and the strategies implemented within the institution (Robbins, 1999; Smircich, 1983). Moreover, a strong organizational culture enhances effectiveness and increases the commitment of academic collaborators and leaders (Parveen & Tripathi, 2021).

According to Schein, organizational culture can be analyzed through three levels: artifacts, espoused and enacted values, and underlying assumptions. The first two represent manifestations, though not the culture itself. Relating Schein's (1988) framework to Smircich's (1983) perspective, studying organizational culture as a variable is limited to these manifestations. However, through the use of questionnaires and observation, it becomes possible to reveal the basic assumptions that constitute organizational culture.

The use of models to evaluate organizational culture was proposed by Cameron & Quinn (1999) through the Competing Values Framework (CVF), which enabled a diagnostic of the cultural type using the Organizational Culture Assessment Instrument (OCAI). This model is based on the principle that organizational culture is defined by two dimensions: degree of freedom and orientation.

It is important to note that all four cultural types are present within CPVer's academic community, aligning with the assertions of Schein (1988) and Sánchez *et al.* (2006), who argue that organizations develop a dominant culture over time, adapting it in response to environmental challenges and changes.

Studying organizational culture, in line with Robbins (1999) and Smircich (1983), allows one to describe how problems are addressed and also serves as a mechanism to reduce issues inherent to CPVer's academic population. Culture conveys identity,

reinforces loyalty, influences decision-making, and acts as a cohesive element a bridge between structure and strategy.

The application of the OCAI instrument enabled a diagnosis and analysis of the dynamics and impact of academic relationships, providing a valid approach to the complexity of organizational culture within CPVer. The results show the coexistence of all four cultural types, with Hierarchical culture as the dominant one unsurprisingly, given CPVer's status as a public research institution characterized by bureaucratic structures. It exhibits strong internal orientation due to its need for control and stability. This reflects how the external environment significantly influences CPVer, as it depends on governmental policies and the National Center for Humanities, Sciences, and Technologies. Internally, its function through Collegiate Bodies relies on effective administrative leadership and alignment with the current Institutional Plan.

Additionally, as a higher education institution, hierarchical culture is common due to:

- **Historical heritage:** Educational organizations traditionally operate under hierarchical structures dating back centuries in some cases.
- **Traditional academic model:** Faculty members occupy positions of authority.
- **Departmentalization:** The College and by extension CPVer operates through its own collegiate bodies, establishing a clear hierarchy in decision-making and administration.
- **Emphasis on research and teaching:** Authority and recognition within the institution are linked to research and teaching, reinforcing hierarchical status.
- **Institutional conservatism:** CPVer is inherently traditional and conservative, which contributes to slower adoption of structural changes.

In descending order, the second most prevalent cultural type is Adhocracy, characterized by dynamism, entrepreneurial spirit, and a creative environment. These traits are inherent to the academic population engaged in specialized research in agricultural, forestry, livestock, and aquaculture sciences. The presence of innovative, curious, persistent, and competent researchers plays a crucial role in the advancement of knowledge on tropical agroecosystems and in the training of human resources. Support from leadership for research development is essential.

The third cultural type, Market, is marked by result-oriented leadership and a focus on performance. The Colegio de Postgraduados is evaluated by the Ministry of Agriculture and Rural Development (AGRICULTURA) and, at the campus level, by the External Evaluation Committee. Their evaluation criteria align with the Strategic Plan, and these assessments serve as indicators for the institution's sustainability.

The evaluation of academic productivity is conducted by the Campus Academic Committee, as detailed in institutional regulations. It is essential to meet the minimum productivity requirements established for each researcher's category, in addition to the specific goals set by each Subdirectorate. Therefore, success is defined in terms of achieving those indicators, reaffirming an outward orientation while requiring internal control and stability.

Finally, the highest-rated cultural type is the Clan culture, in which the organization is perceived as a friendly place to work and where individuals share a strong sense of community. This perception aligns with the researcher's observations of the academic population, as various subgroups exist, and team cohesion is often fractured. However, it is emphasized that despite internal divisions at Campus Veracruz, when collective interests or evaluations are at stake, all academic staff actively participate, demonstrating consensus, engagement, and loyalty.

At this point, it is important to highlight a case study by Bernatová & Kuklišová (2024) involving Slovak universities, which demonstrated that leadership positively influences organizational culture most notably, mediated by organizational trust. The Campus Academic Committee, as the collegiate body that supports Campus leadership in decisions regarding core academic functions, should not limit its role to monitoring and enforcing regulations. Instead, it should channel its efforts toward achieving the Campus's performance indicators, which involves promoting organizational trust.

In this context, trust stems from CPVer's adherence to formal processes and procedures. It has been identified that formal rules and institutional policies are what hold the academic population together. The Academic Committee is perceived as the organizer concerned with effective operations through high levels of control. Consequently, the academic community expresses a desire for a more familiar environment, one that fosters teamwork and commitment.

The need for a Clan culture is not exclusive to CPVer. Other case studies have also revealed a predominance of Hierarchical culture in universities, alongside a desire for a Clan-type culture (Mebariki & Barka, 2021; Paredes & Paredes, 2021; Rahman, Partiwi & Theopilus, 2021; Tikson *et al.*, 2023). This cultural gap may hinder the implementation of organizational changes.

Promoting a Clan culture would bring greater flexibility and innovation through an internal orientation. However, such a transformation is unlikely to occur within five years, as suggested in the instrument, due to the academic population's resistance to change. Nevertheless, the integration of new faculty members may support this transition, aiming for improved coordination and a cohesive campus guided by clear norms to maintain stability, control, and effective functioning of core areas. In this scenario, the Academic Committee would need to implement strategies to improve fluidity in endorsing, accepting, and recommending processes under its purview. In other words, leadership capacities must align with fostering a collaborative environment (Rahman, Partiwi & Theopilus, 2021), a transition that is essential for implementing effective management strategies.

The academic population also identifies Adhocracy and Market cultures as preferred alternatives to Hierarchical culture. This may stem from a desire for greater recognition of creativity or a need for institutional support to implement change. These cultures are more externally oriented and differentiated, where external factors influence adaptation, and such cultural shifts may be temporary.

The largest negative gap appears in the Hierarchical culture, indicating that the environment is perceived as excessively strict, and the current enforcement of regulations is seen as overly rigid. This may be attributed to the fact that most faculty members have

an average tenure of about 30 years, during which regulations have become increasingly strict in tandem with changes in government and policy implementation. As a result, the Academic Committee is sometimes perceived as a “punisher.” However, thanks to the Committee’s actions, Campus Veracruz has benefited and holds one of the highest average ratings across the three core areas when compared to all other campuses of the Colegio de Postgraduados.

At this point, it is worth noting alignment with the arguments of Hartnell, Ou, Kinicki, Choi & Karam (2019), specifically regarding:

- a. Hierarchical culture will have a stronger positive relationship with an organization’s exploitation strategy than Clan, Adhocracy, or Market cultures.
- b. Clan, Adhocracy, and Market cultures will have a stronger positive relationship with an organization’s organic structure than Hierarchical culture.

Moreover, both the evidence from the aforementioned study and the results of this research indicate that leadership, strategy, and structure function as integration mechanisms that create and reinforce an organization’s culture. Sánchez-Quintanar and García-Cue (2013) analyzed the organizational climate at the Colegio de Postgraduados, Montecillo Campus (CPMon), highlighting that the institution’s organizational capacities and limitations are closely tied to those of the academic staff. They found that organizational behaviors at CP are governed by formal principles, concepts, and official definitions signaling a hierarchical organizational culture. However, a significant limitation among CPMon’s academic staff lies in two behaviors: on one hand, the assimilation of responsibility-avoidant behaviors and a tendency to express aggression diffusely, leading to frustration; on the other, the presence of a complex authority structure that has contributed to a form of Emotional Depression Syndrome within the academic community.

In this context, and considering the experience observed in CPMon’s organizational climate, it becomes essential that CPVer avoids such depressive dynamics within its academic community and fosters harmony to ensure the effective development of its core activities. Thus, it is necessary to implement a Clan culture as the organizational model to enable the academic body to achieve the expected success for CPVer.

The CPVer academic staff’s call for a Clan culture stems from a desire to dismantle deeply institutionalized routines and access new learning systems. This model focuses on the development and coordination of organizational members through teamwork, empowerment, and communication to increase flexibility and adaptability contrasting with a hierarchical culture that emphasizes process efficiency.

This suggests that attempts to change an organization’s culture by merely providing resources and expecting individuals to change their values and accepted routines are likely to be ineffective. Leaders must instead be encouraged to adopt a systems perspective and initiate cultural transformation by addressing the factors that support organizational culture.

While there is a need for values that support flexibility, the current culture hierarchical is primarily focused on process efficiency. It is geared toward stability, order, and control,

behaviors closely linked to resource and technology optimization through formalized routines that reduce ambiguity and enhance performance. However, these very traits are what CPVer's academic staff criticize, as internal controls and rigid structures support an exploitation strategy (Hartnell, Ou, Kinicki, Choi & Karam, 2019).

Leadership must also be considered. Although the institutional structure promotes decentralization through Collegiate Bodies enabling open communication channels and improving adaptability in response to environmental changes there is a gap in fostering flexibility. This is due to the institutional need to implement existing policies and achieve organizational goals, prompting the Academic Committee at CPVer to adopt a directive and dominant leadership style. This style is marked by a focus on clear performance indicators, strict adherence to rules, and the achievement of consistent, efficient results. Furthermore, the organizational structure explicitly defines the responsibilities of both the academic staff and the Academic Committee, with documented roles and processes for regulatory compliance, yielding consistently effective outcomes.

Nonetheless, Clan culture aligns with motivational leadership, where goals are achieved collaboratively, and relationships are built on mutual trust something CPVer academics have expressed they need. They indicate a desire for greater involvement in decision-making, citing a decline in collaboration and open communication that has been exacerbated by the hierarchical culture.

These findings suggest that CPVer's effectiveness and efficiency are not solely tied to organizational culture but are also influenced by other elements. The coexistence of the four cultural types explains the variations observed in the performance of a loosely coupled system. This study underscores the importance of identifying both the current and needed cultural configurations within such a system, while raising awareness about its inherent tensions. At the same time, the Collegiate Body known as the Academic Committee must promote both stability and change, efficiency and effectiveness, innovation and collaboration, as well as competition, to achieve institutional objectives and goals.

The results provide a framework for proposing organizational change at the Colegio de Postgraduados, recognizing that leadership, strategy, and structure are mechanisms that shape and reinforce organizational culture. Given the organization's nature as a loosely coupled system, meaningful cultural change must address these elements. So far, attempts at cultural change have largely failed, often ignored or becoming sources of internal conflict (Schein, 1998). Therefore, attention must be given to academics, incentives, control mechanisms, and the organizational structure itself, as these factors influence behavior and, in turn, drive changes in organizational culture (Hrebiniak, 2013). Accordingly, the Academic Committee at CPVer must adopt a systemic perspective and drive cultural transformation through the foundational elements of organizational culture, in alignment with upper management and institutional leadership.

CONCLUSIONS

Although it was identified that all four types of organizational culture (Clan, Adhocracy, Market, and Hierarchy) coexist at CPVer with the Hierarchical culture being dominant due to historical legacy, traditional academic models, departmentalization, emphasis on

research and teaching, and institutional conservatism some educational institutions are actively working to promote more collaborative, inclusive, and participatory cultures. These institutions recognize the benefits of fostering creativity, innovation, and diversity of thought in the academic environment, a desire also acknowledged by CPVer's academic staff. Their aspiration is to transition toward a Clan-type organizational culture one that is friendly, fosters interpersonal relationships, and addresses the current lack of cohesion caused by the existence of many subgroups within the institution. Ultimately, a Clan culture would enable CPVer's Collegiate Bodies to be seen as facilitators of the institution's mission. However, such a transition is unlikely to occur immediately. It must be acknowledged that the refinement of organizational culture is a continuous process. While gradual, this change is key to the institution's success. New adaptations must align with the goals of each core area and the indicators of the Colegio de Postgraduados, ensuring that strategies are implemented to achieve organizational effectiveness. It is also essential that, at the central level, organizational culture is considered in the design or modification of the institutional structure. Although the need for change is recognized, the nature of the institution requires further research to uncover the practical consequences of such change. These efforts must consider the effects of multiple cultural dimensions and the structural elements of the Colegio de Postgraduados. It is recommended that studies be conducted on the development of dynamic capabilities, resistance to change, and organizational resilience studies that should encompass not only the achievements, learning, and adaptations of academic staff but also those of students and administrative personnel. These efforts will provide greater clarity on the integrated functioning of the elements that make up this loosely coupled system.

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Valorization of rambutan (*Nephelium lappaceum* L.) peel extract for the green synthesis of silver nanoparticles

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ABSTRACT

Objective: To evaluate the use of rambutan peel extract as a reducing agent for the synthesis of Ag nanoparticles.

Design/methodology/approach: The extract of rambutan peels was prepared by adding 5 g of finely powdered dried peels to 100 mL of distilled water, and stirring the mixture for 15 min at 75 °C. The filtered extract was then used as a reducing agent for a 10 mM AgNO₃ solution. The effect of extract volume, reaction time and temperature were evaluated.

Results: Low amounts of extract and increased temperature promote a more effective and complete formation of AgNPs, and their band gap energy was estimated experimentally to be 2.75 eV.

Limitations on study/implications: Although UV-Vis spectroscopy confirmed the synthesis of AgNPs, additional characterization techniques should be employed in future studies.

Findings/conclusions: The use of rambutan peel extract is an effective biotechnological alternative for the green synthesis of AgNPs.

Keywords: Green synthesis, metal nanoparticles, photocatalyst, silver, sustainability.

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INTRODUCTION

Rambutan (*Nephelium lappaceum* L.) is a tropical fruit belonging to the Sapindaceae family. This fruit is native to Southeast Asia and its name derives from the Malay term “rambut,” which means “hair,” in reference to the long projections that cover its peel (Hernández-Hernández *et al.*, 2019) (Figure 1). Rambutan shares similarities with other tropical fruits such as lychee (*Litchi chinensis*) and its cultivation has expanded in tropical regions of Latin America, Africa and the Caribbean, where it has adapted favorably.



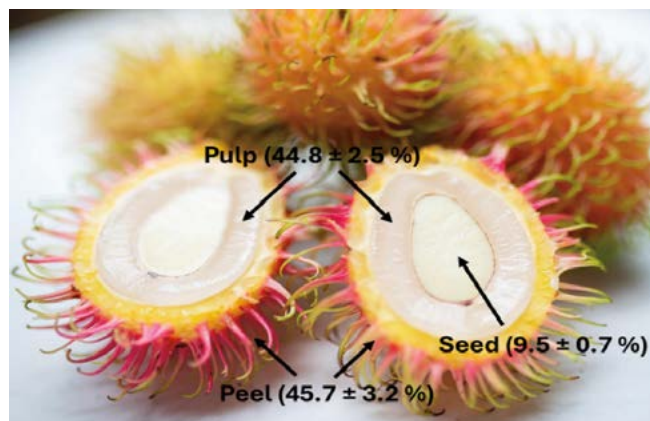


Figure 1. Rambutan fruit and percentage by weight of its constituents according to Solís-Fuentes *et al.*, (2010) (image used under a Creative Commons license).

In Mexico, rambutan is cultivated in the states of Chiapas, Oaxaca, Tabasco, Guerrero, Colima, San Luis Potosí, Nayarit and Michoacán. Among them, Chiapas is the largest producer of rambutan in Mexico, where more than 90% of the crops are concentrated with an annual production of 12,200 tons (CONAGUA, 2023). In addition, the cultivation of this exotic fruit has been consolidated as an economic alternative for producers in various tropical regions, contributing to agricultural diversification and the local economy (Hernández-Hernández *et al.*, 2019). However, few research has been done on the use of rambutan peel for biotechnological applications and use of its bioactive components, which could have applications in the food, pharmaceutical and cosmetic industries. Therefore, this work is focused on the valorization of rambutan peel extract for biogenic synthesis of silver nanoparticles (AgNPs), which have wide medical, biomedical and cosmetic applications, as well as an important use in the food, textile, and electronics industries, highlighting the environmental applications for water purification due to their excellent antibacterial and antiseptic properties.

In recent years, the green synthesis of AgNPs has emerged as an innovative and environmentally friendly alternative to conventional physical and chemical methods, which often involve the use of toxic substances and high energy consumption. This approach utilizes natural extracts from plants, microorganisms, or agro-industrial products as reducing and stabilizing agents, facilitating the conversion of silver ions (Ag⁺) into metallic nanoparticles. Furthermore, the use of agro-industrial residues, such as rambutan peels, contributes to the circular economy by valorizing by-products that would otherwise be discarded. Therefore, the green synthesis of AgNPs using aqueous rambutan peel extract not only represents a biotechnological advancement, but also a viable and sustainable strategy to address contemporary technological and environmental challenges.

Therefore, the main objective of this work was to evaluate the use of rambutan peel extract as a reducing agent for the synthesis of AgNPs, the volume of aqueous peel extract, the reaction time, and temperature of synthesis were the main factors evaluated for the effective synthesis of AgNPs.

MATERIALS AND METHODS

Materials

Rambutan peel was selected as subject of study since it is one of the principal agro-industrial residues in the Southern of México. For this purpose, fresh rambutan fruits were acquired at the local market in the Soconusco Region, Chiapas, which were transported to the laboratory and immediately processed. On the other hand, AgNO_3 (purity $\geq 99.0\%$, Meyer) was used as source of Ag^+ ions and precursor of Ag nanoparticles, while distilled water (Meyer) was used to prepare the extract of rambutan peels as well as the appropriate solutions of AgNO_3 . To adjust the pH, a solution 10% m/v of NaOH (purity $\geq 99.0\%$, Meyer) was used.

Preparation of the rambutan peel extract

Rambutan peels were separated manually and washed several times with tap water followed by two washes with distilled water at room temperature to remove dust and impurities. Washed peels were dried at 60 °C overnight and then ground to a fine powder (particle size $< 1\text{mm}$). The extract of rambutan peels was prepared adding 5 g of dry powder to 100 mL of distilled water and heated at 75 °C for 15 min with vigorous stirring. The extract was cooled to room temperature, vacuum filtered to remove the solid particles (cellulose filter, 30 μm pore size) and stored at 4 °C until used (7 days as maximum).

Synthesis of Ag nanoparticles

The aqueous extract of rambutan peels was used as a reducing agent to produce Ag nanoparticles in colloidal suspension. For this purpose, several volumes of extract (1, 3, and 5 mL) were added dropwise to 50 mL of 10 mM AgNO_3 solution, then pH was adjusted to 11 and the resulting solution was stirred at 75 °C for different durations (1, 2, 3, and 4 hours). During the reaction, a visible color change was observed (from pale yellow to dark brown) indicating the formation of AgNPs. The synthesized AgNPs were stored in a sealed glass vial and protected from light at 4 °C.

Characterization

UV-Vis spectroscopy is considered a suitable technique to characterize metal nanoparticles, and this technique was used as the first step in the verification of the AgNPs since this analysis is fast, economical, and easily applicable. The UV-Vis spectra of the prepared AgNPs were recorded using a Genesys 10S spectrophotometer (Thermo Scientific) in the range of 200-800 nm. Before analysis, each solution of AgNPs was diluted for proper analysis as follows: 1 mL of prepared samples was transferred to 50 mL volumetric flask and diluted with distilled water.

RESULTS AND DISCUSSION

In this study, AgNPs were successfully synthesized by a green method using the aqueous extract of rambutan peels, which was confirmed by UV-Vis spectroscopy. The UV-Vis spectra of the prepared AgNPs are shown in Figure 2, where the maximum absorption is observed between 350 and 450 nm, corresponding to the typical absorption region of

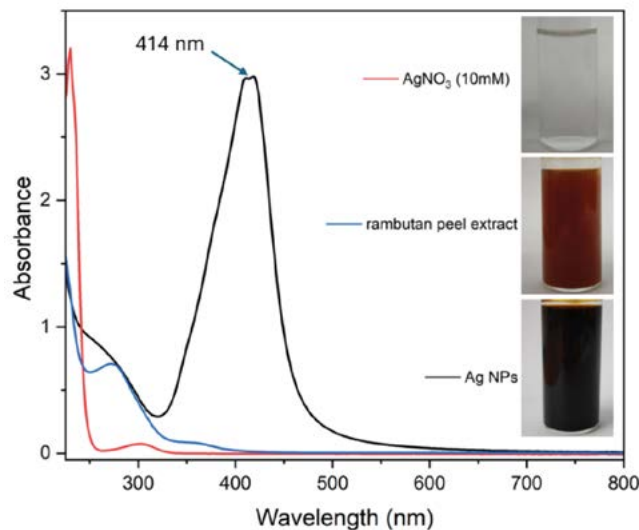
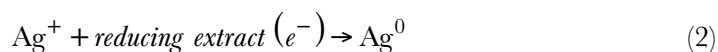


Figure 2. UV-Vis spectra of the synthesized AgNPs (black line), AgNO_3 10mM (red line), and rambutan peel extract (blue line).

AgNPs (Lestari *et al.*, 2018). Also, both UV-Vis spectra of the rambutan peel extract and the solution of AgNO_3 (10 mM) were included in the same figure for comparative purposes. Figure 2 shows that the maximum absorption of AgNPs is located at 414 nm, this peak in the visible and near-ultraviolet spectrum is associated with the surface plasmon resonance (SPR) on AgNPs, a common phenomenon in metallic nanoparticles where electrons in the surface layer are excited by photons, generating collective oscillations of free electrons that travel parallel to the surface and can give rise to reactive oxygen species (ROS). Additionally, according to the literature, the formation of AgNPs was indicated by the color change from straw yellow to dark brown, as result of the reduction and stabilization of Ag^+ ions (Dua *et al.*, 2023; Kumar *et al.*, 2015), which is depicted in the inset of Figure 2.

In previous studies, it has been reported that biomolecules/phytochemicals such as anthocyanins, ellagitannins, ellagic acid, corilagin, geraniin, syringic acid, *p*-coumaric acid, and phenolic compounds contained in the extract of rambutan peel could play a key role as reducing agent of Ag^+ ions, mainly by interaction with -OH and -CHO groups, which are electron donors and also responsible for the stabilization of the AgNPs by the interacting with the oxidized forms (-C=O, -COOH) (Kumar *et al.*, 2015). Thus, the biomolecules present in the aqueous extract of rambutan peels are responsible for the reduction of Ag^+ ions to Ag^0 in a single step:



The effect of the extract volume, reaction time and temperature for the synthesis of AgNPs were evaluated. Figure 3a shows the UV-Vis spectra of AgNPs prepared adding 1, 3, and 5 mL of rambutan peel extract, and it was observed that the increase of volume

extract (5 mL) produce a broad and asymmetrical peak from 325 to 520, indicating the formation of Ag particles with a broad interval of size and non-uniform shape, which is consistent with those reported in other studies for AgNPs synthesis (Lestari *et al.*, 2018). Furthermore, a red shift is observed (blue line) from 414 to 424 nm, which is also associated with the increase of size of AgNPs. According to Mie theory the absorption bandwidth and peak position depends on the size of the AgNPs, as the diameter of the particles increases (agglomeration or aggregation), the polarization of them with light is not homogeneous, which is expressed in the shifts and broadening signals (Sharma *et al.*, 2016). On the contrary, prepared samples with 1 and 3 mL of extract exhibited the highest intensity on the absorbance peak at 414 nm, indicating a more complete transformation of the Ag^+ ions into AgNPs. Based on these results, 1 mL of rambutan peel extract was used as the appropriate volume for the synthesis of AgNPs. These observations underscore the critical role of the extract concentration for the green preparation of AgNPs, where low amounts of the reducing agent facilitate a more effective and complete formation of AgNPs.

In addition, the reaction time is a critical factor influencing the formation, stability, and morphology of AgNPs during green synthesis, as mentioned for (Ghaffari-Moghaddam *et al.*, 2014; Huq *et al.*, 2022). In this study, the synthesis of AgNPs using rambutan peel extract was monitored over various reaction times (1, 2, 3, and 4 hours) under constant temperature (75 °C) and stirring conditions. It is worth mentioning that the color change of the reaction mixture from pale yellow to dark brown occurred immediately after pH adjustment, indicating the reduction of Ag^+ ions and the formation of AgNPs. This visual observation was supported by UV-Vis spectroscopy, which showed the characteristic SPR peak around 414 nm (Figure 3b). The data suggest that nucleation, growth and stabilization of AgNPs occurred predominantly within the first two hours of reaction. No substantial increase in absorbance was observed after this time, indicating that Ag^+ ions reduction had reached saturation. Extended reaction times beyond this point (two hours) did not enhance nanoparticle yield but could potentially lead to agglomeration or changes in size distribution. Therefore, in this work, two hours was selected as the appropriate reaction time for the green synthesis of AgNPs.

On other hand, the effect of the temperature for the synthesis of AgNPs also was evaluated at two levels: at room temperature (24 °C) and 75 °C (to minimize water evaporation). Figure 3c shows the results of these experiments and it is observed that the increase of temperature promotes a complete and more effective formation of AgNPs, which is agree to those reported by (Dua *et al.*, 2023) who synthesized AgNPs using green extract, and indicates that temperature between 60-80 °C contributes to obtain nanoparticles with sizes <100 nm. In this work, the particle size of the prepared AgNPs was calculated from the UV-Vis spectra using Mie theory, which is a suitable approximation to describing how particles scatter light, depending on their size and optical properties, through the following equation:

$$2R = \frac{\lambda_{\max}^2 V_f}{\pi c \omega} \quad (3)$$

Where R is the radius of the particle, λ_{\max}^2 is the wavelength at maximum intensity of the SPR, V_f is the velocity of the electron at Fermi levels ($1.4 \times 10^6 \text{ ms}^{-1}$ for Ag), c is the velocity of light, and $\Delta\lambda$ is the FWHM of the SPR signal (full width at half maximum) (Pragatheeswaran *et al.*, 2010). The size of the AgNPs was determined in the range between 3.7 and 4.5 nm under the following conditions: 50 mL of 10mM AgNO_3 + 1 mL of rambutan peel extract, pH 11, and 2 hours of synthesis at 75 °C with magnetic stirring. Which agree with those reported in other studies where size of the synthesized AgNPs were experimentally measured by transmission electronic microscopy (TEM) ranging between 3.2 and 7.8 nm (Gharibshahi *et al.*, 2017).

Among the several applications of the AgNPs, the use for water and food disinfection is the most outstanding. Even, AgNPs has been evaluated as photocatalyst for water decontamination under sunlight (Dua *et al.*, 2023). For this reason, the optical band gap (E_g) of the prepared AgNPs was estimated experimentally from the UV-Vis spectra through the Tauc's equation:

$$(\alpha h\nu)^n = A(h\nu - E_g) \quad (4)$$

where α is the absorption coefficient, h is Planck constant, ν is frequency, A is proportionality constant, E_g is the optical band gap, and n is Tauc exponent ($n=2$ for indirect allowed transition). Practically, this method includes plotting $(\alpha h\nu)^n$ vs. $h\nu$, and extrapolating the

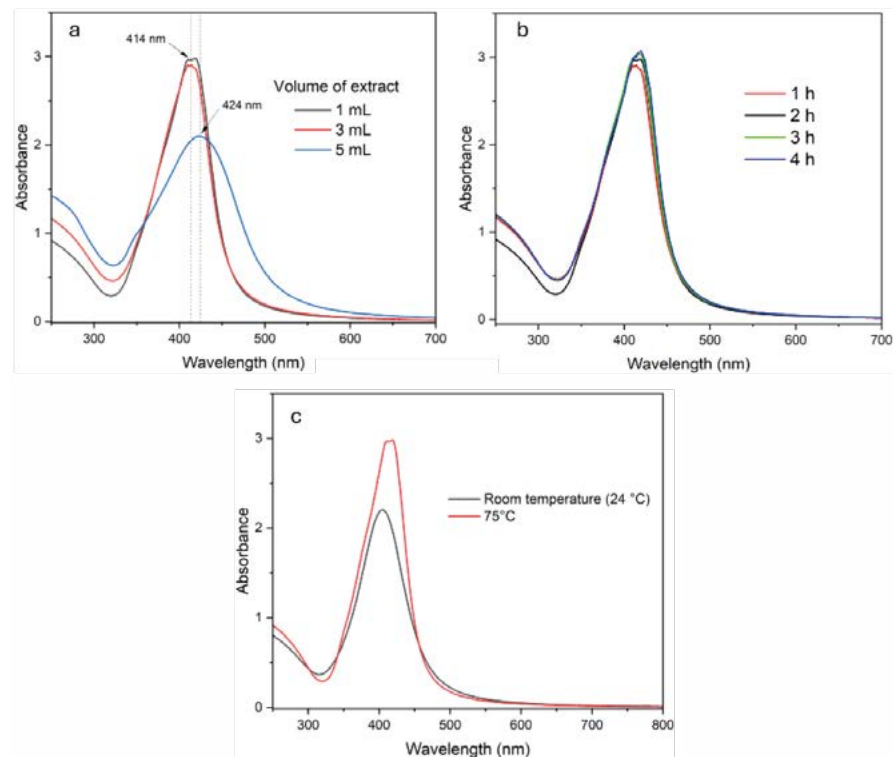


Figure 3. a) UV-Vis spectra of AgNPs prepared with different extract volumes, b) UV-Vis spectra of AgNPs prepared at different reaction times, c) effect of temperature for the synthesis of AgNPs.

linear range beyond the absorption edge yielding the value of the E_g on the abscissa axis, as shown in Figure 4.

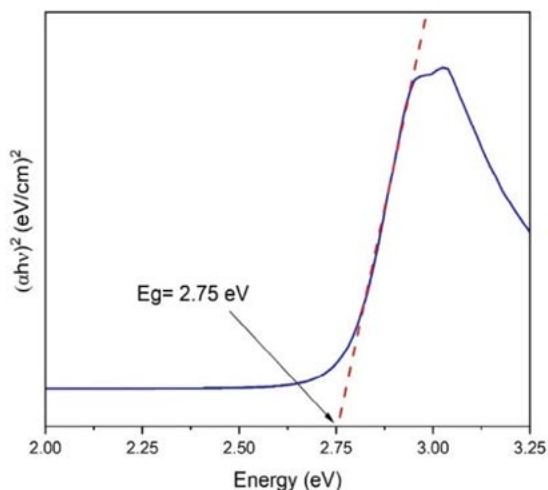


Figure 4. Estimated band gap of AgNPs using Tauc plot from UV-Vis spectra.

In this work, 2.75 eV ($\lambda = 450.8$ nm) was experimentally estimated as the optical E_g of AgNPs, which is according to those reported by (Arumai Selvan *et al.*, 2018) who prepared AgNPs using several vegetable extracts, obtaining E_g values between 2.75 and 2.77 eV. In a few words, E_g can be explained as the minimum energy a photon must have to excite an electron to high energy bands, which can promote charge separation and transfer, ideal for photocatalytic systems. In the case of Ag nanoparticles, they primarily exhibit a metallic band structure, and their optical properties are related to plasmonic behavior and interband transitions, where the plasmonic absorption often dominates the optical spectra of AgNPs. However, in photocatalytic applications for water decontamination, it has been widely reported that electrons in the 4d states of AgNPs are excited to high-energy 5sp bands under UV-Visible radiation. Thus, the excited electrons can reduce the dissolved oxygen molecules to form superoxide radicals, while photoinduced holes left in the inner d bands can abstract the electrons of the polluting molecules (Varghese Alex *et al.*, 2020). Both superoxide radicals and photo-holes are the agents responsible for the degradation of the pollutants, giving rise to an interesting and promising alternative for environmental applications using AgNPs under a UV-Visible light source such as natural solar light.

CONCLUSIONS

The use of rambutan peel extract is an effective alternative for green synthesis of Ag nanoparticles, which contributes to the valorization of agro-industrial residues. The production of AgNPs was confirmed by UV-Vis spectroscopy as the first step, but further studies should employ other characterization techniques such as DRX, SEM-EDX, and FTIR spectroscopy. Based on the results, extract volume and temperature are crucial parameters for the preparation of AgNPs, highlighting that low amounts of the

reducing agent and increased temperature facilitate more effective and complete AgNPs formation.

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Acceptance, purchase intent and preference of semi-industrial chocolate

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ABSTRACT

Objective: To determine consumer liking for three types of semi-industrial chocolate and identify the critical attributes for acceptability, purchase intent, and preference among local consumers.

Design/methodology/approach: The affective testing method was conducted with 60 regular consumers in the region, who evaluated acceptability on appearance, color, aroma, flavor, aftertaste, and texture using a nine-point hedonic scale. Data were analyzed using analysis of variance. Acceptance and purchase intent were measured using a binomial scale (yes/no), and preference was measured using a three-point ordinal scale.

Results: Analysis of variance revealed that consumers liking was significantly different among the three types of chocolate, with overall liking scores ranging from 6.18 to 8.10 on the hedonic scale. The sample with the highest liking was milk chocolate, and the lowest was dark chocolate. Flavor and aftertaste were the key attributes for overall acceptability and purchase intent. Preference analysis indicated that milk chocolate was the most preferred.

Limitations on study/implications: The findings are limited to local consumers in the region and therefore cannot be nationally generalized.

Findings/conclusions: This study highlighted that the most important sensory attributes for local consumers when choosing chocolate products were flavor, aftertaste, and texture.

Keywords: consumer, chocolate, preference, cocoa. liking.

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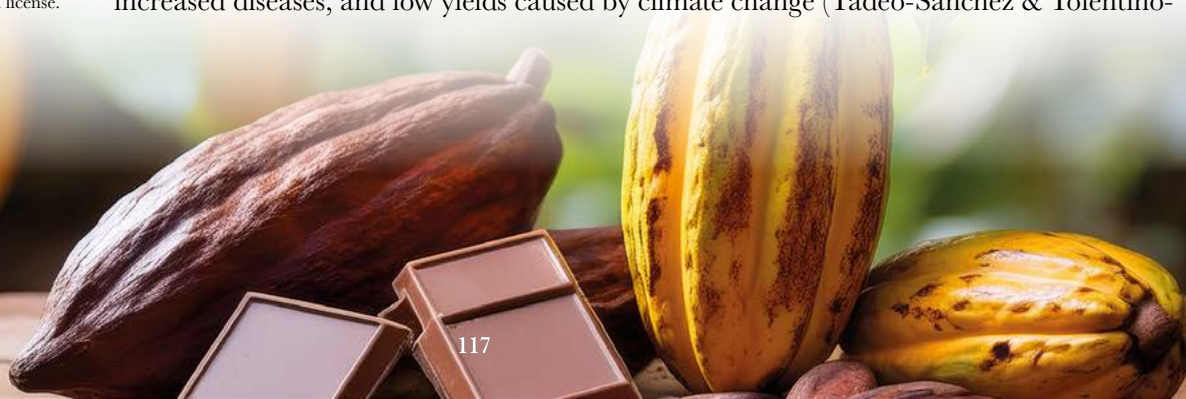
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INTRODUCTION

The global chocolate market is anticipated to reach \$145.17 billion in retail sales by 2026 (Toma & Săseanu, 2020). In Mexico cacao (*Theobroma cacao* L.) holds significant cultural and economic importance, particularly in the southeast region, where it is considered part of the regional heritage (Camacho-Gómez, 2019). However, cocoa production has been undermined by the multiple problems in cacao cultivation, such as insecurity, pod theft, increased diseases, and low yields caused by climate change (Tadeo-Sánchez & Tolentino-



Martínez, 2020). As a result, many cacao producers are moving towards small chocolate manufacture aiming to increase their participation in the value chain, even if they face financial and technical challenges for product development (Martínez-Salvador & Martínez-Salvador, 2020). Understanding consumer preferences is essential for producers involved in adding value to cacao as it helps them choose formulations that are more likely to succeed in the market (Jaramillo, 2016).

Modern technology such as motor mills to grind cocoa beans have replaced the use of labor. This and other innovations have scaled up chocolate production to an industrial level (Fins, Somarriba & Quesada, 2013). Integrating chocolate processing along with cacao production opens an opportunity for a more profitable business for small producing farms. This would improve traceability, agroecological management, and production of healthier dark chocolates (Charry, Torres, & Narjes, 2023).

The wide variety and production of fine, aromatic chocolates have created diverse market niches that demand attention. Humans have an evolutionary preference for sweetness and an aversion to bitterness. Food acceptance or rejection is largely influenced by sensory factors, primarily taste, followed by smell and appearance (Contreras, 1995). Research on food consumption and acceptability has relied on studies involving consumers as well as on studies conducted by manufacturers to meet customer needs (Costell, 2001). The objective of the study was to assess consumer liking for three types of commercial chocolate, identify key attributes influencing acceptability and purchase intent based on consumer perception, and determine preferences.

MATERIALS AND METHODS

Location of the study area

The research was conducted at Campus Tabasco of the Colegio de Postgraduados, located at Huimanguillo, Tabasco, coordinates: 17° 58' 34" N, 93° 23' 11" W. Huimanguillo belongs to the Chontalpa region of Tabasco. It is the second largest region in the state, with a land area of 8,407.74 Km² (García-Sánchez, 2014; Ayuso, 2023). Agriculture is among the main economic activities, with cacao cultivation accounting for 48,436 hectares, and 9,975 families depend on this crop (Córdova-Lázaro *et al.*, 2018).

Sample size determination

The sample size was calculated using a simple random sampling design, considering a binomial categorical variable, and a total population of 152 individuals. In the study, random tests were conducted with 60 consumers from the Tabasco Campus, which represents 40%. According to the Universidad Popular Autónoma Estado de Puebla (2014), for affective testing, the minimum recommended sample size is 30 untrained consumers.

Chocolate samples

Three chocolate samples of each of the available types on the market were prepared: milk chocolate, semisweet chocolate and bitter chocolate (Table 1). The samples were prepared in the chocolate school at Colegio de Postgraduados, Campus Tabasco, México.

Table 1. Formulations for each type of chocolate.

| Ingredients | Type of chocolate | | |
|-------------------------|-------------------|-------------|------|
| | Milk | Semi-bitter | Dark |
| Cocoa paste | 30% | 50% | 70% |
| Brown sugar | 28% | 30% | 20% |
| Cocoa butter | 20% | 20% | 10% |
| Whole milk powder | 20% | - | - |
| Natural vanilla extract | 2% | - | - |

Cacao was purchased from a local producer at “Hacienda La Ceiba”, located at 18° 10' 12.95" N and 93° 37' 17.39" W. The beans were roasted in batches (5 Kg each) for approximately 30 min using a 5 Kg roaster (MICRON, México). The grain was dehulled using mechanical equipment (model CACDES003, Citlali, México), and the cocoa nibs were then ground in a pulverizer mill (Pulvex 200, Pulvex, México). Finally, the cocoa paste was refined in a 5 Kg-capacity refiner operating at 1,400 rpm for 8 h (SANTHA 20 LBS, SANTHA, USA). During this process, all ingredients were mixed according to the formulation and then molded into pieces of approximately 8-10 g. The pieces were placed in a freezing display case (TEM-150, TORREY, Mexico) to crystallize at 4-5 °C. The samples were wrapped in wax paper, sealed in small glass jars, and stored refrigerated at 4 °C until they were evaluated by consumers. Each sample was assigned a random three-digit code, generated using the free R Studio software.

Questionnaire and consumer study (techniques)

Participants (n=60) were asked to complete a questionnaire written in Spanish comprising three sections: 1) demographic information, including name, sex, age, place of birth, monthly income, chocolate consumption frequency, consumption preparation, preferred type of chocolate and the main sensory characteristic that most affects its acceptability. 2) liking was measured for each attribute (appearance, color, smell, flavor, aftertaste and texture when chewing (UPAEP, 2014) using a 9-point scale (1=I dislike it very much, 5=I neither like it nor dislike it, 9=I like it very much). Consumers were asked whether the sample was acceptable (yes/no), as well as their purchase intent (yes/no). 3) preference was measured asking consumers to order the samples from 1 to 3, where “1” was the most preferred and “3” the least preferred sample (Ramírez, 2012; Cárdenas-Mazón *et al.*, 2018).

The laboratory was equipped with air conditioners, white lights and cabins to avoid bias in the experiment. Within each cubicle, the participants were randomly presented with the three coded samples, the questionnaire, a napkin and a pen to respond. A brief introduction to sensory evaluation and the senses was given, as well as the handling of samples for evaluation. Each consumer received three samples in a completely random order. In this study each treatment was evaluated 60 times. Water was provided for palate cleansing in between trials (Díaz, Pinoargote & Castillo, 2012).

Consumer profile

The age of most of the participants was between 18 and 54 years old (95%) distributed in 55% women and 45% men. The majority of consumers were originally from Tabasco (88.3%). The majority regularly consume chocolate (55%) while the remaining 45% indicated that they consume it occasionally. Regarding consumption preparation, the majority indicated that they prefer to consume it solid (65%). Regarding the type of chocolate, the majority preferred milk chocolate 58.3%, whereas 33.3% preferred semisweet chocolate and the remaining 8.4% preferred bitter or dark chocolate. Finally, before tasting the samples, participants indicated that flavor (48.3%), aroma (16.7%) and appearance (11.7%) were the main attributes of chocolate that affect its overall acceptability.

Statistical analysis

Frequency tables were constructed from the demographic data. Analysis of variance (ANOVA) was used to identify significant differences in liking among the three sample treatments. Multiple logistic regression was used to find the key attributes for overall acceptance and purchase intent. The K-fold Cross-validation technique (no pre-processing, resampling=10 fold) was used to validate the logistics models. A Friedman's test and the analogue of the least significant difference were used to identify which of the three treatments was the most preferred. Finally, principal component analysis (PCA) was used to reduce dimensions and construct a biplot of product-attributes to observe the correlation among attributes and multivariate product similarities. Data were analyzed with R software version 4.0 using an $\alpha=0.05$.

RESULTS AND DISCUSSION

Differences between treatments

ANOVA results indicated a significant difference in liking based on sensory attributes among the three types of chocolate (Table 2).

According to the averages for liking on the hedonic scale by attribute, milk chocolate scored 7.6-8.10, being the treatment with the highest scores. Dark chocolate had the lowest scores overall, ranging from 6.18-7.83. However, it is worth mentioning that liking for all attributes by treatment was higher than the midpoint of the scale. Ramírez-Navas *et al.* (2014) analyzed manjar blanco, a typical Colombian dessert, using a nine-point hedonic scale, evaluating color, smell, flavor, and texture in four treatments (A, B, C, D). They found that treatment D obtained the best scores for smell (6.07 ± 1.86), flavor (6.79 ± 2.01),

Table 2. Chocolate liking across sensory attributes.

| Treatments | Appearance | Color | Aroma | Flavor | Aftertaste | Texture |
|-----------------------|------------|------------|------------|--------------|--------------|-------------|
| Milk chocolate | 7.85±1.47a | 8.00±1.12a | 7.87±1.27a | 8.10±1.08a | 7.62±1.58a | 7.76±1.48a |
| Semi-bitter chocolate | 7.63±1.13a | 7.63±1.28a | 7.43±1.38a | 8.10±1.08a | 6.72±1.88b | 7.35±1.44ab |
| Dark chocolate | 7.70±1.23a | 7.83±1.14a | 7.70±3.69a | 6.32±2.00b | 6.18±2.06b | 6.82±1.95b |
| p-value | 0.64 | 0.237 | 0.607 | 2.56e-07 *** | 0.000161 *** | 0.00787 ** |

*Mean values \pm standard deviation on the hedonic scale (n=60). Means with the same letter in the same column are not significantly different according to the Tukey test ($\alpha=0.05$).

and texture (6.53 ± 2.06), whereas the color of sample C was the most pleasant (6.72 ± 1.96). They conclude that brand D was superior to the other brands in terms of consumer liking because it had a soft, smooth texture on the inside and a sandy and cracked texture on the surface. Regarding the results of the hedonic scale of dark chocolate, this seemed to be a little less pleasing, with a score of 6.32 different from the other treatments that obtained a value of 8.10. Similarly, Díaz, Pinoargote and Castillo (2012), they found significant differences among treatments compared to their white chocolate in terms of aroma, flavor, astringency, and acidity among other attributes. Their results indicated a higher score for their treatments with enzymes after roasting at 140 °C, which enhanced flavor and improved acidity, as well as reduced astringency.

In terms of aftertaste, milk chocolate stood out as it obtained a liking score of 7.62, higher than semi-sweet chocolate with 6.72 and dark chocolate with 6.18. Finally, in terms of texture, it was found that both milk chocolate and semi-sweet chocolate obtained similar texture scores between 7.35 and 7.76 points respectively, whereas dark chocolate obtained a lower score (6.82) although similar to the semi-sweet treatment. Pieracci *et al.* (2021), pointed out that texture and aftertaste are negatively affected by factors such as fat level, particle size and effects of poor chocolate tempering such as “sugar bloom” or “fat bloom”, the latter, for example, is the migration of fat to the surface, dragging cocoa butter crystallizing on the surface, which generates a bad appearance for the consumer. The same with sugar, only this effect is produced by humidity (Beckett, 2019 p.21).

Critical sensory attributes for acceptability and purchase intent

Metrics for the acceptability logistic model (ROC=0.92; sensitivity=0.61; specificity=0.94; and AIC=103.29) indicated the strong ability of the model to discriminate between acceptability and rejection. Table 3 shows the results from the multiple logistic regression analysis. Based on the p-values for the attribute coefficients it was found that the critical attributes impacting overall acceptability (independently of the formulation) were flavor ($p=0.0475$) and aftertaste ($p=0.0247$).

Despite consumers initially responded (prior to trying the chocolate samples) the most important sensory attributes affecting overall acceptability were flavor, aroma and

Table 3. Key attributes for overall acceptability according to multiple logistic regression analysis.

| Attribute | Coefficient | Pr(> z) |
|------------|-------------|-----------|
| Appearance | -0.2526 | 0.3512 |
| Color | -0.3132 | 0.3552 |
| Aroma | 0.3140 | 0.1675 |
| Flavor | 0.5244 | 0.0475 * |
| Aftertaste | 0.6324 | 0.0247 * |
| Texture | 0.1537 | 0.4839 |

* Pr(> |z|) values that indicate the significance of the coefficient in the multiple logistic regression and allow identifying the critical attributes for product acceptability.

appearance, results from logistic regression indicated that taste perception provided more information regarding consumer liking or overall acceptability. The most significant sensory attributes were flavor, aftertaste, and texture perceived in the mouth. Indiarito *et al.* (2025) in their study with different vegan milk chocolates, they found no significant differences in color, appearance, and texture, but they did find differences in aftertaste, flavor, brightness, and aroma. Richter and Lannes (2007) found that a dietary chocolate formulation similar to the commercial ones, obtained a higher score in flavor and texture, with a significant difference to the control. In their study, consumers valued the smooth flavor, the texture of the filling and a pleasant sweet flavor. Pérez-Obrador *et al.*, (2025) evaluated consumer preferences for three types of chocolate, where the sweet milk treatment was preferred for its sweetness, smoothness and cultural evocations as well as pleasant sensations. However, there is a growing acceptance of dark chocolate among adults who associate it with higher quality and health benefits (Córdova *et al.*, 2023).

Eating is an act that involves different dimensions, such as cultural, biological, economic, and psychological. Therefore, the act of choosing or accepting a food is usually influenced by consumer attitudes, acquired habits, previous experiences with food, appetite, mood, and many other factors that affect food choice (Téllez, 2019). Flavor and aftertaste are essential attributes in determining food acceptance, as they relate to the senses of taste and touch within the oral cavity. These sensory experiences begin to develop in the last weeks of gestation, continue when consuming certain foods during infancy and further develop food acceptance criteria (González & Reyes, 2023).

Purchase intent followed a similar pattern as acceptability. Metrics for the logistic model (ROC=0.91; sensitivity=0.68; specificity=0.93; and AIC=124.17) for purchase intent indicated the strong ability of the model to discriminate purchase intent. Table 4 shows the key attributes for purchase intent for all treatments: flavor ($p=0.00294$) and aftertaste ($p=0.00930$).

These attributes can be focused on developing chocolates that producers, small micro-entrepreneurs, or other interested parties can exploit by generating attractive products that ensure a place in the product range offered, in an attempt to offer differentiated products of sensory quality to the local or national market.

Table 4. Key attributes for purchase intent according to multiple logistic regression analysis.

| Attribute | Coefficient | Pr(> z) |
|------------|-------------|-----------|
| Appearance | -0.0839 | 0.7305 |
| Color | 0.2040 | 0.4764 |
| Aroma | 0.0954 | 0.2661 |
| Flavor | 0.6907 | 0.0029 ** |
| Aftertaste | 0.6098 | 0.0093 ** |
| Texture | 0.1090 | 0.5763 |

* Pr(> |z|) values that indicate the significance of the coefficient in the multiple logistic regression and allow identifying the critical attributes for the purchase intent of the product.

In Figure 1, the milk chocolate treatment was oriented towards the positive side of variable axes demonstrating higher values in liking (according to the hedonic scale).

Regarding variable correlation, there were two correlated variable groups: 1) aroma, appearance and color, and 2) flavor, aftertaste and texture.

The first attribute subgroup is associated with organs based on visual or external phenomena, whereas the second variable subgroup is based on mechanical stimuli in the corresponding organs (Braun, 1997). Among the three treatments, the profile of milk chocolate was sweeter, less acidic, light brown, and reduced in shine, in contrast to dark chocolates, which have a more intense, bitter flavor, greater shine, and a dark brown color (Bartkiene *et al.*, 2021; Pieracci *et al.*, 2021). The treatments for dark and semi-sweet chocolate were similar between them but differed from the milk treatment. Milk chocolate was the most accepted among the three treatments. This is likely due to the innate preferences for sweet taste, which is formed by the consumption of breast milk during the first stages of infancy (González & Reyes, 2023). As Dip (2019) pointed out, eating behavior is closely related to perceived flavor.

Preference

The result from the Friedman test indicated that the preference differed ($p < 0.0003$) among chocolate types. The post-hoc test carried out with the analogue of the least significant difference based on the comparison of rank sums for each treatment indicated that the most preferred chocolate was milk chocolate, followed by semi-sweet chocolate and finally bitter chocolate (Table 5).

Consumers tend to consume food according to their tastes and needs as well as their preference for food. It is understandable that with the increasing per capita consumption of sugary foods, the palates of those accustomed to these foods tend to prefer them (Kamil

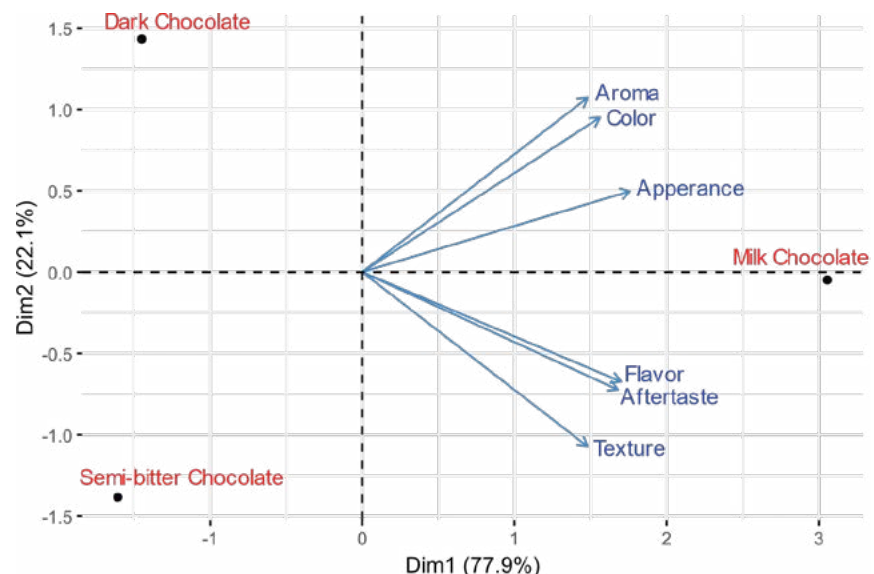


Figure 1. Biplot involving chocolate treatments and sensory attributes.

Table 5. Differences in preference of chocolate samples.

| Treatments | Rank sum | Letter grouping |
|-----------------------|----------|-----------------|
| Dark chocolate | 150 | a |
| Semi-bitter chocolate | 120 | b |
| Milk chocolate | 90 | c |
| LSD | 18.94 | |

*LSD=Least significant difference.

& Wilson, 2021). Consumers tend to consume foods according to their tastes and needs (Brambila, 2006); just as their (emotional) preference for foods leads to many nuances that can be explored from different perspectives and disciplines (Vélez & García, 2003).

CONCLUSIONS

Consumer satisfaction with chocolates in Tabasco was influenced by taste and touch: flavor, aftertaste, and texture were the critical sensory attributes. Consumer preference for chocolates tended toward sweet chocolate flavors, with milk chocolate being the most preferred. This is due to the fact that, on a sensory level, dark chocolate offers a more intense profile, with bitter, astringent flavors that tend to be rejected by most palates or those accustomed to sweet tastes. Regarding overall acceptability and purchase intent, flavor was the most determining attribute, followed by aftertaste. These attributes are detectable in the mouth and are the most important factors in determining whether we accept and purchase such products.

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Distribution and importance of insects and mites associated with jackfruit (*Artocarpus heterophyllus* Lam)

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ABSTRACT

Objective: Due to the recent introduction and cultivation of jackfruit in Mexico, agronomic information regarding its associated pests remains limited. To enhance the agronomic understanding of this crop particularly concerning the insects and mites associated with it this work presents a comprehensive and updated review of phytophagous species across all regions where jackfruit (*Artocarpus heterophyllus*) is cultivated.

The review draws upon technical reports, scientific articles, databases, full texts from ScienceDirect and Scopus, as well as other specialized catalogs. A total of 81 insect species and one mite species have been identified in association with jackfruit cultivation worldwide. Among these, 48.3% cause damage to foliage, 26.3% infest shoots, 14.4% feed on the fruit, and 11.0% affect the branches.

The highest pest prevalence is reported in Asian countries such as India, the Philippines, and Bangladesh, where the primary pest is the shoot and fruit borer *Deaphania caesalis*, infesting approximately 27% of the fruits in Bangladesh and between 30% to 40% of plants in India. In contrast, in Mexico, the predominant pest is the branch borer *Neoptychodes trilineatus*, which affects up to 84.7% of jackfruit trees.

Keywords: Pests, tropical fruits, distribution.

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INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* Lam.), native to India, was introduced to Nayarit, Mexico, around 1985. This fruit tree is extensively cultivated in India, Malaysia, the Philippines, and Bangladesh (Rajkumar *et al.*, 2018). In Mexico, its cultivation expanded from 15 hectares with a production of 1 ton in 1993 to 1,754.2 hectares and 36,717.38 tons in 2022, reaching a market value of 455.31 million pesos (SIAP, 2023). The majority of production is concentrated in Nayarit, although it is also cultivated in Jalisco, Michoacán, Colima, Hidalgo, and Veracruz, primarily for export to the United States. Agronomic information on pests associated with jackfruit remains scarce. In Mexico, the identity, biology, feeding habits, distribution, and management of these pests are largely unknown (Hernández-Fuentes *et al.*, 2019). Although previous studies exist (Rodríguez-Palomera *et al.*, 2017; Hernández-Fuentes *et al.*, 2018-2022; Velázquez-Monreal *et al.*, 2021; Arias-



Corpus *et al.*, 2022), Khan *et al.* (2021) report over 250 insect species and eight mite species associated with jackfruit in other cultivating countries, with no specific data available for Mexico. This study provides a comprehensive review of insect and mite species associated with jackfruit, focusing exclusively on species confirmed through observations of adult insect emergence, visible field damage, and taxonomic verification by specialists.

MATERIALS AND METHODS

An exhaustive review was conducted using technical reports and various databases, including ResearchGate, Academia.edu, CABI Invasive Species Compendium, Crop Protection Compendium, Plantwise Knowledge Bank, as well as search engines such as Google Scholar and ScienceDirect. The names and taxonomic classifications of the reported species were cross-referenced with the Global Biodiversity Information Facility (GBIF) and the Catalogue of Life databases. Reports lacking species-level identification or failing to confirm phytophagous associations were excluded, as arthropods may be present on plant structures in non-phytophagous stages, such as resting or mating (Hernández-Fuentes *et al.*, 2022). The biological status as a pest, the specific plant structures damaged, and the geographic distribution of each species were documented. Priority was given to compiling pest lists from other countries before incorporating those identified in Mexico's primary producing states: Nayarit, Colima, and Jalisco.

RESULTS AND DISCUSSION

A total of 81 insect species and one phytophagous mite species have been recorded in association with jackfruit (*Artocarpus heterophyllus*). The most represented orders are Hemiptera (43 species) and Coleoptera (18 species), while Orthoptera, Hymenoptera, and Acari each account for only one species. Most reports originate from Asian countries particularly India, the Philippines, and Bangladesh where jackfruit is extensively cultivated and exhibits greater genetic diversity. Rajkumari *et al.* (2014) reported 35 insect pest species in India, while Rajkumari *et al.* (2018) documented 38 species, and Kallekkattil *et al.* (2020) identified 51 species through direct sampling. Of the total species, 48.3% cause damage to foliage, followed by those infesting shoots (26.3%), fruits (14.4%), and branches (11.0%). Regarding mites, only *Tetranychus pacificus* has been reported, as documented by Hernández-Fuentes *et al.* (2019). Other mite species have been recorded on related plants such as *Paratetranychus biharensis* on *Artocarpus altilis* (Flechtmann *et al.*, 1999) and *T. ludeni* on *A. incisa* (Gutierrez & Schicha, 1983) but they are not directly associated with jackfruit. Among the pests, branch and fruit borers are considered the most damaging (Tandon, 1998). *Diaphania caesalis* infests an average of 27% of fruits in Bangladesh (Khan & Islam, 2004) and 30-40% of jackfruit plants in India (Soumya *et al.*, 2015). In Mexico, the primary pest is the branch borer *Neoptychodes trilineatus*, which causes significant damage by feeding on shoots and the skins of developing fruits (Hernández-Fuentes *et al.*, 2022). An average incidence of two larvae per kilogram of dry wood was recorded, with 84.7% of trees examined showing signs of infestation. The red spider mite *T. pacificus* is also a major pest, causing visible foliar damage that can lead to premature leaf drop (Hernández-Fuentes *et al.*, 2019).

Table 1. Insect and mite species associated with jackfruit *Artocarpus heterophyllus* Lam.

| Order | Family | Species | Damage ¹ | Country | Reference |
|---------------------------------------|-----------------------------|--|---------------------|--------------------------|---|
| Lepidoptera | Crambidae | <i>Diaphania</i> (= <i>Glyphodes</i>) <i>caesalis</i> Walker | a, b | India, Bangladesh, China | Butani (1978), Kham e Islam (2004), Soumya <i>et al.</i> (2015), Patel <i>et al.</i> (2016), Saha <i>et al.</i> (2017), Rajkumar <i>et al.</i> (2018), Ngangom y Bandyopadhyay (2018), Singh <i>et al.</i> (2018) Kallekkattil <i>et al.</i> (2019, 2020), Wang (2020), Hiremath <i>et al.</i> (2022) |
| | | <i>Glyphodes</i> (= <i>Diaphania</i>) <i>bivitalis</i> Guenée | c | India | Butani (1978), Kallekkattil <i>et al.</i> (2020) |
| | | <i>Conogethes punctiferalis</i> Guenée | a, e | India | Kallekkattil <i>et al.</i> (2020) |
| | Arctiidae | <i>Amata passalis</i> Fabricius | c | India | Kallekkattil <i>et al.</i> (2020) |
| | | <i>Eilema antica</i> Walker | c | India | Kallekkattil <i>et al.</i> (2020) |
| | Lymantridae | <i>Olene mendosa</i> Hübner | c | India | Kallekkattil <i>et al.</i> (2020) |
| | | <i>Perina nuda</i> Fabricius | c | India | Butani (1978) |
| | Bombycidae | <i>Trilocha varians</i> Walker | c | Philippines | Navasero <i>et al.</i> (2013) |
| | Metarbelidae | <i>Inderbela</i> (= <i>Inderbela</i>) <i>tetraonis</i> Moore | c | India | |
| Coleoptera | Attelabidae | <i>Apoderus tranquebaricus</i> J.C.Fabricius | c | India | Kallekkattil <i>et al.</i> (2020) |
| | Cerambycidae | <i>Glenea multiguttata</i> Guérin-Méneville | c | India | Kallekkattil <i>et al.</i> (2020) |
| | | <i>Epepeotes luscus</i> Fabricius | d | India | Butani (1978) |
| | | <i>Sthenias grisator</i> Fabricius | d | India | Butani (1978) |
| | | <i>Apriona germari</i> (= <i>germarii</i>) Hope | d | India | Butani (1978) |
| | | <i>Olenecamptus bilobus</i> Fabricius | c | India | Kallekkattil <i>et al.</i> (2020) |
| | | <i>Batocera rufomaculata</i> Degeer | d | India, Bangladesh | Butani (1978), Ahmed <i>et al.</i> (2013), Ngangom y Bandyopadhyay (2018), Kallekkattil <i>et al.</i> (2020) |
| | | <i>B. rubus</i> L. | d | India | Butani (1978) |
| | | <i>Ptychodes trilineatus</i> L. (= <i>Neoptychodes trilineatus</i>) | d | USA | Horton (1917) |
| | Curculionidae | <i>Myllocerus undecimpustulatus</i> Faust | c | India | Kallekkattil <i>et al.</i> (2020) |
| | | <i>M. discolor</i> Boheman | c | India | Kallekkattil <i>et al.</i> (2020) |
| | | <i>M. dorsatus</i> Fabricius | c | India | Kallekkattil <i>et al.</i> (2020) |
| | | <i>M. viridanus</i> Fabricius | c | India | [16]Kallekkattil <i>et al.</i> (2020) |
| | | <i>Ochyromera artocarpi</i> Marshall | a, e | India | Butani (1978), Ngangom y Bandyopadhyay (2018), Kallekkattil <i>et al.</i> (2020, 2021) |
| | | <i>Onychocnemis careyae</i> Marshall | c | India | Butani (1978) |
| <i>Teluropus ballardi</i> Marshall | | c | India | Butani (1978) | |
| <i>Platypus indicus</i> H. Strohmeyer | | d | India | Butani (1978) | |
| Hemiptera | Plataspidae (“Plataspidae”) | <i>Coptosoma siamicum</i> Walker | b, c | India | Kallekkattil <i>et al.</i> (2020) |
| | Aphididae | <i>Greenidea artocarpi</i> Westwood | b, c | India | Butani (1978), Kallekkattil <i>et al.</i> (2020) |

Table 1. Continues...

| Order | Family | Species | Damage ¹ | Country | Reference |
|--|--|---|---------------------|-----------------------------------|--|
| Hemiptera | Aphididae | <i>Toxoptera odinae</i> Van der Goot | b, c | India | Kallekkattil <i>et al.</i> (2020) |
| | | <i>T. aurantii</i> Boyer de Fonscolombe | c | India | Butani (1978) |
| | Aprophoridae | <i>Clovia lineaticollis</i> De Motschulsky | b, c | India | Kallekkattil <i>et al.</i> (2020) |
| | Aleurodidae | <i>Aleurotrachelus caeruleus</i> Singh | c | India | Butani (1978) |
| | | <i>Pealius schimae</i> Takahashi | c | India | Butani (1978) |
| | | <i>Aleurodicus rugioperculatus</i> Martin | c | Sri Lanka | Dilrukshika <i>et al.</i> (2023) |
| | Cicadellidae | <i>Apheliona indica</i> Dworakowska & Singh Sohi | b, c | India | Kallekkattil <i>et al.</i> (2020) |
| | | <i>Kolla ceylonica</i> Melichar | b, c | India | Kallekkattil <i>et al.</i> (2020) |
| | Coccidae | <i>Ceroplastes floridensis</i> Comstock | b, c | India | Kallekkattil <i>et al.</i> (2020) |
| | | <i>Ceroplastes rubens</i> Maskell | b, c | India | Butani (1978) |
| | | <i>Chloropulvinaria psidii</i> Maskell | b, c | India | Butani (1978) |
| | | <i>Coccus acutessimus</i> (= <i>C. acutessimus</i> Fernald) Green | b, c | India | Butani (1978) |
| | Diaspididae | <i>Pinnaspis aspidistrae</i> Signoret | c | India | Butani (1978), Kallekkattil <i>et al.</i> (2020) |
| | | <i>Hemiberlesia lataniae</i> Signoret | a, b, c | India | Butani (1978) |
| | | <i>Parlaspis papillosa</i> Green | c | India | Butani (1978) |
| | | <i>Semelaspidus artocarpi</i> Green | c | India | Butani (1978) |
| | Eurybrachidae | <i>Eurybrachis tomentosa</i> Fabricius | b | India | Kallekkattil <i>et al.</i> (2020) |
| | Margarodidae | <i>Drosicha mangiferae</i> Stebbing | b, d | India | Butani (1978), Kallekkattil <i>et al.</i> (2020) |
| | | <i>D. stebbingi</i> Stebbing | b, d | India | Butani (1978) |
| | | <i>Icerya aegyptiaca</i> Douglas | b, c | India | Butani (1978), Kallekkattil <i>et al.</i> (2020) |
| | | <i>I. seychellarum</i> Westwood | b, c | India | Kallekkattil <i>et al.</i> (2020) |
| | Membracidae | <i>Leptocentrus scutellus</i> (scutellatus) Distant | c | India | Kallekkattil <i>et al.</i> (2020) |
| | | <i>Otinotus oneratus</i> Walker | b | India | Butani (1978) |
| | Pentatomidae | <i>Eupaleopada cocinna</i> Westwood | b | India | Kallekkattil <i>et al.</i> (2020) |
| | Pseudococcidae | <i>Coccidohystrix insolita</i> Green | c, d | India | Kallekkattil <i>et al.</i> (2020) |
| | | <i>Ferrisia virgata</i> Cockerell | a, b, c | India | Butani (1978), Kallekkattil <i>et al.</i> (2020) |
| | | <i>Nippaecoccus viridis</i> Newstead | a, b, c | India | Butani (1978), Kallekkattil <i>et al.</i> (2020) |
| <i>Paracoccus marginatus</i> Williams & Granara de Willink | | a,b,c | India | Kallekkattil <i>et al.</i> (2020) | |
| <i>Planococcus citri</i> Risso | | a, b, c | India | Kallekkattil <i>et al.</i> (2020) | |
| Tingidae | <i>Cystechila delineata</i> Distant | c | India | Kallekkattil <i>et al.</i> (2020) | |
| | <i>Stephanitis charieis</i> Drake & Mohanasundarum | c | India | Butani (1978) | |
| | <i>Alloiothucha artocarpi</i> Horváth | c | Laos | Souma <i>et al.</i> (2022) | |
| Cercopidae | <i>Cosmoscarta relata</i> Distant | b, c | India | Butani (1978) | |
| | <i>Clovia lineaticollis</i> Melichar | b, c | India | Butani (1978) | |

Table 1. Continues...

| Order | Family | Species | Damage ¹ | Country | Reference |
|--------------|---------------|--|---------------------|------------------------|---------------------------------------|
| Thysanoptera | Thripidae | <i>Jakthrips ignacimuthui</i> Bhatti & Ranganath | b, c | India | Kallekkattil <i>et al.</i> (2020) |
| | | <i>Pseudodendrothrips dwivarna</i> Ramakrishna & Margabandhu | b, c | India | Butani (1978) |
| Orthoptera | Tettigoniidae | <i>Onomarchus uninotatus</i> Serville | c | India | Sreeja <i>et al.</i> (2023) |
| Diptera | Tephritidae | <i>Bactrocera umbrosa</i> Fabricius | a | Indonesia, Philippines | Ubaub y Rosales (2014), Manwan (2017) |
| | | <i>Drosophila melanogaster</i> Meigen | a | Indonesia | Manwan (2017) |
| | | <i>Dacus dorsalis</i> Hendel | a | India | Butani (1978) |
| | Cecidomyiidae | <i>Camptomyia artocarpi</i> Nayar | a | India | Butani (1978) |
| | | <i>Rubsaamenia artocarpi</i> Nayar | a | India | Butani (1978) |
| | Therevidae | <i>Ptecticus rufus</i> Doleschall | a | India | Butani (1978) |
| | | <i>P. australis</i> Schiner | a | India | Butani (1978) |

1: a=fruits, b=sprouts, c=foliage, d=branches y e=flowers.

Table 2. Insect and mite species associated with jackfruit *Artocarpus heterophyllus* Lam. in Mexico.

| Order | Family | Species | Damage ² | Country | Reference |
|-------------|--|--|---------------------|---|---|
| Coleoptera | Cerambycidae | <i>Neoptychodes trilineatus</i> L. (= <i>Ptychodes trilineatus</i>) | d | Mexico | Hernández-Fuentes <i>et al.</i> (2020, 2021, 2022), Horton (1917) |
| Hemiptera | Diaspididae | <i>Pinnaspis strachani</i> Cooley | a,b,c,d | Mexico | Hernandez-Fuentes <i>et al.</i> (2021), Arias-Corpuz <i>et al.</i> (2021) |
| | | <i>Pseudischnaspis bowreyi</i> Cockerell | c | Mexico | Arias-Corpuz <i>et al.</i> (2021) |
| | Coccidae | <i>Milviscutulus mangiferae</i> Green | a,b,c,d | Mexico | Hernández-Fuentes <i>et al.</i> (2021), Arias-Corpuz <i>et al.</i> (2021) |
| | Pseudococcidae | <i>Ferrisia virgata</i> Cockerell | b, c | | |
| | | <i>Maconellicoccus hirsutus</i> Green | b,c | Mexico | Hernández-Fuentes <i>et al.</i> (2021) |
| | Coreidae | <i>Piezogaster odiosus</i> Stal. | c | Mexico | Hernández-Fuentes <i>et al.</i> (2018, 2021) |
| Aphididae | <i>Toxoptera aurantii</i> Boyer de Fonscolombe | c | Mexico | Rodríguez-Palomera <i>et al.</i> (2017) | |
| Hymenoptera | Formicidae | <i>Atta cephalotes</i> L. | c | Mexico | Hernández-Fuentes <i>et al.</i> (2021) |
| Acari | Tetranychidae | <i>Tetranychus pacificus</i> McGregor | c | Mexico | Hernández-Fuentes <i>et al.</i> (2019, 2021) |

2:a=fruits, b=sprouts, c=foliage, d=branches y e=flowers.

CONCLUSIONS

A total of 42 references meeting the established methodological criteria were reviewed, with the majority of reports originating from Asian countries such as India, the Philippines, and Bangladesh, where jackfruit is widely cultivated. Among the identified species, 48.3% damage the foliage, followed by those infesting shoots (26.3%), fruits (14.4%), and branches (11.0%). Borer insects affecting branches, shoots, and fruits are responsible for the most severe damage. Notably, *Diaphania caesalis* infests an average of 27% of fruits and 30% to 40% of jackfruit plants, while *Neoptychodes trilineatus* can infest up to 84.7% of trees. Additionally,

the red spider mite *Tetranychus pacificus* causes premature defoliation, affecting up to 100% of the foliage. In Mexico, pest reports are relatively recent. Given the short cultivation history of jackfruit in the country, it is likely that some pest species are still in the process of dispersal and establishment within the main production areas.

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Biofactories as a strategy to promote sustainable agricultural development

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ABSTRACT

Objective: Explain the factors that most significantly affect the innovation of biofertilizers produced and marketed by biofactories in the agricultural sector of Michoacán.

Design/methodology/approach: The methodology involved structural equation modeling using partial least squares with SmartPLS4 software, a bibliometric study and the logical framework methodology.

Results: Sustainability, productivity and profitability are the key factors that influence the innovation of biofertilizers produced and marketed by biofactories in the agricultural sector of Michoacán. The three constructs explain 78.5% of the variance in Innovation ($R^2=0.785$).

Limitations on study/implications: There are other constructs that can be incorporated into the variables. Each species of agricultural crop needs certain microorganisms as a bioinoculant, so the biofertilizer products market produces new types every year.

Findings/conclusions: For agricultural producers, the search for new markets, higher profits, higher sales, reduction in production costs, and sustainable fertilization alternatives significantly impact the use of biofertilizers. The factors that drive farmers to use bioinoculants are the search for a differentiated organic product for export. The use of biofertilizers has shown growth compared to synthetic ones, between 5 and 10%. In the structural model, Profitability represents 60% of innovation in the agricultural sector of Michoacán, Sustainability 19.2% and Productivity 18.8%. Mexico is innovative in the use of bioinoculants since every year it uses new species of microorganisms in its preparation.

Keywords: Biofactories, innovation, biofertilizers, sustainability.

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INTRODUCTION

Mexico ranks as the 11th largest agricultural producer globally, driven by an export surge facilitated through trade liberalization and the signing of 12 free trade agreements with more than 44 countries (SIAP-SAGARPA, 2018). The fruit sector holds strategic importance, with berries (strawberries, blackberries, blueberries, and raspberries) ranking third after beer and avocado, contributing 10.6% to the total export value (SIAP-SAGARPA, 2018). However, the country's agri-food activity is often predatory, causing extensive pollution of soils, groundwater, rivers, oceans, and the atmosphere. Agriculture accounts for approximately 15% of Mexico's Gross Domestic Product when factoring in environmental degradation and resource depletion (Morales, 2014). For instance, cultivating one hectare of corn typically requires an investment of 4,000 pesos in chemical fertilizers, compared to just 400 pesos for biofertilizers. Incorporating biofertilizers into corn production could generate an additional income of 2,000 pesos per hectare, benefiting nearly 400,000 small-scale producers (Andrade, 2018).

Nitrogen fertilizers Mexico's most consumed and produced have become increasingly costly, with prices rising by 500% in the first decade of the 21st century (Secretaría de Economía, 2012). Their efficiency remains low: crops utilize less than 20% of applied

nitrogen and phosphate fertilizers. The remaining unused portion contributes to significant environmental damage, contaminating soil and water and releasing nitrous oxide, a potent greenhouse gas that exacerbates climate change. Mexico has played a pivotal role in nitrogen fixation research, laying the foundation for the development of biofertilizers. In 1980, the Nitrogen Fixation Research Center (now the Genomic Sciences Center) was established at the National Autonomous University of Mexico, becoming a global pioneer in the field (Morales, 2014). By 2018, biofertilizers were being used on over four million hectares in Mexico. These inputs not only yield economic benefits but also enable access to organic markets, resulting in higher prices and improved sanitary conditions for crops. The National Institute of Forestry, Agricultural, and Livestock Research (INIFAP) conducted experimental trials on high-yield corn, revealing that nitrogen fertilizer use can be reduced by 30% without compromising, and in some cases improving, crop yields (Guzmán, 2018). In 2020, the state of Michoacán tripled its budget for the Sustainable Agriculture Program to 45 million pesos, aiming to incorporate 20,000 hectares into this initiative. According to Rubén Medina Niño, head of the Secretariat of Rural and Agri-Food Development (SEDRUA), the program began in 2019 with three crops over 6,000 hectares. Encouraged by yield increases of 40-80%, the program expanded to cover 12 crops. Innovations under the Sustainable Agriculture Program target a range of products including corn, strawberries, fruit trees (*e.g.*, guava, mango, grapefruit), hibiscus, lentils, and rice. These innovations have successfully lowered production costs by replacing agrochemicals with biofertilizers, fostering healthier crops, and promoting soil conservation and regeneration. Currently, there are 1,129,000 hectares under organic production over 5% of Mexico's total agricultural area with coffee, avocado, safflower, and corn occupying the most land. The primary organic exports are avocado, banana, and coffee (SIAP-SAGARPA, 2018). To further advance agroecological innovation, the Ministry of Agriculture and Rural Development (SADER) has prioritized the replacement of chemical fertilizers with bioinoculants. Governor Alfredo Ramírez Bedolla reported that, through the AGROSANO program, 22,000 tons of biofertilizer were distributed to farmers. Cuauhtémoc Ramírez Romero, head of SADER, described the program as part of a broader agroecological transition aimed at increasing profitability, improving soil fertility and yields, and reducing production costs (SADER, 2022). In 2022, a bioinput production network was established across 113 municipalities in Michoacán, with plans for expansion to decrease costs, protect land, prevent pollution, and reduce foreign dependency (Mendoza, 2022). Biofertilizers are broadly defined as natural organic materials, products of biological recycling, or human-made preparations that enhance soil fertility and productivity, either partially or entirely replacing chemical fertilizers (Vela *et al.*, 2018). A more specific academic definition refers to biofertilizers as products containing microorganisms that, when inoculated into the soil, form associations or symbioses with plants, improving their nutrition and offering protective benefits (Vessey, 2003). Meetings and surveys conducted with avocado and blackberry producers in Ziracuaretiro, Los Reyes, and Peribán, as well as biofactory representatives, helped identify the key variables of this study. Innovation serves as the dependent variable, while sustainability, productivity, and profitability are considered independent variables. International research including studies in the United States, Spain, Italy, China, Cuba,

Brazil, and Mexico has addressed the role of biofactories in agriculture, particularly their environmental implications and relevance to global sustainability efforts. Agrochemicals, though commonly used to increase profitability, have severely detrimental effects on soil health, aquifers, air quality, and human health. Communities relying on chemical inputs face serious health risks due to hazardous waste. In Michoacán, about 90% of farmers use agrochemicals, and approximately 50 million empty containers are improperly discarded each year in ravines, waterways, landfills, and along roadsides (SAGARPA, 2015). Synthetic fertilizer prices continue to climb, with only 20% of the product effectively utilized by crops. Nonetheless, opportunities lie in the expanding demand for organic products in both domestic and international markets. This underscores the importance of continued research on biofertilizers. The primary aim of this study is to identify the key factors that influence the innovation of biofertilizers produced and marketed by biofactories in the agricultural sector of Michoacán. The central hypothesis posits that sustainability, productivity, and profitability are the principal drivers of innovation in this context.

MATERIALS AND METHODS

Based on the 129 questionnaires completed during multiple field visits to producers in Los Reyes and Ziracuaretiro, Michoacán, a data matrix was constructed incorporating 29 indicators. These indicators were organized into three exogenous latent variables and one endogenous latent variable innovation. Structural Equation Modeling using Partial Least Squares (PLS-SEM) was conducted with the SmartPLS4 software to carry out the analysis.

A 5-point Likert scale was employed as the measurement instrument, where a score of 5 indicated complete agreement with a statement and 1 indicated complete disagreement. The Likert-type coding allowed numerical values to be assigned to the responses, facilitating data processing and ensuring symmetrical distribution around a central response category for each item. PLS-SEM does not assume any specific data distribution, making it suitable for exploratory research with complex models and small to medium sample sizes. In this study, respondents evaluated each item using the Likert scale, where higher scores reflected stronger agreement with the corresponding statements. In the constructed data matrix, each row represented an individual respondent, and each column corresponded to a specific questionnaire item. For example, the first row included the complete response set from respondent 1. All analyses were conducted using SmartPLS4. According to Cohen (1992), a minimum of 54 observations is sufficient to detect R^2 values around 0.25, assuming a significance level of 5% and statistical power of 80%. A t-value of 1.96 corresponds to a p-value of 0.05. In formative measurement models, p-values must be below 0.05 to confirm that the external weights are statistically significant at the 5% level.

RESULTS AND DISCUSSION

The following results were obtained using Partial Least Squares Structural Equation Modeling (PLS-SEM). Drawing on data from 129 completed questionnaires collected during multiple visits to producers in Los Reyes and Ziracuaretiro, Michoacán, a data matrix was constructed containing 29 indicators. These indicators were categorized under three exogenous latent variables and one endogenous latent variable innovation. The

statistical analysis was conducted using the SmartPLS4 software to model and assess the structural relationships among the variables in this study.

In the nomogram above, the blue circles on the left represent the exogenous latent variables: productivity (Prod_), sustainability (Sust_), and profitability (Prof_). The blue circle on the right represents the endogenous latent variable, or dependent variable: innovation (Innv_). The yellow rectangles connected to each construct denote the observed indicators or items. The lines linking indicators to constructs, as well as constructs to one another, represent the hypothesized relationships within the model. The numerical values between indicators and their respective constructs indicate the outer weights, reflecting the contribution of each item to its construct. Items with outer weights greater than 0.10 are considered statistically significant. The values between the latent variables represent Cronbach's alpha coefficients, which assess the internal consistency and reliability of the constructs and the overall model. This is a reflective measurement model, as indicated by the direction of the path lines extending from the constructs to the indicators. The model demonstrates a reliability level of 88.4%, based on the constructs and indicators included. These indicators were derived from responses to the survey administered to agricultural producers.

The following image illustrates the structural relationships among the latent variables. The exogenous latent variable Profitability exhibits the strongest influence on the endogenous variable Innovation, with a path coefficient of 0.574 and a total of six associated items. This is followed by Productivity (0.199), which includes eight items, and Sustainability

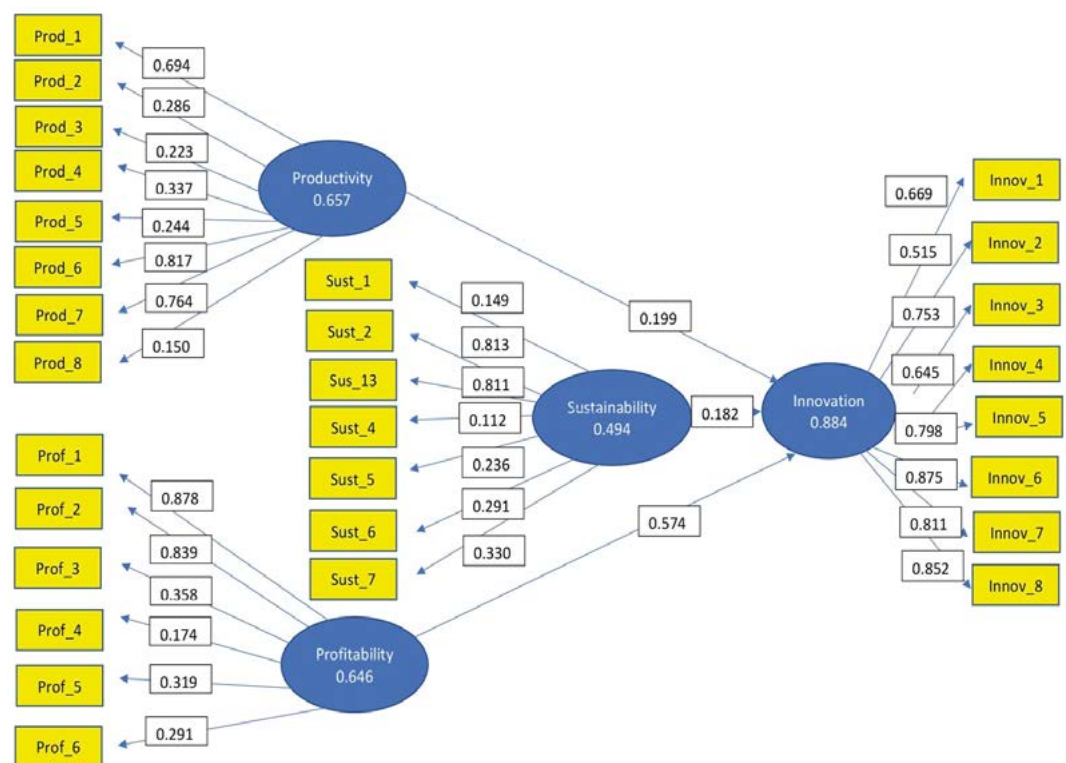


Figure 1. Structural Model with the Endogenous Variable Innovation as the Dependent Variable.

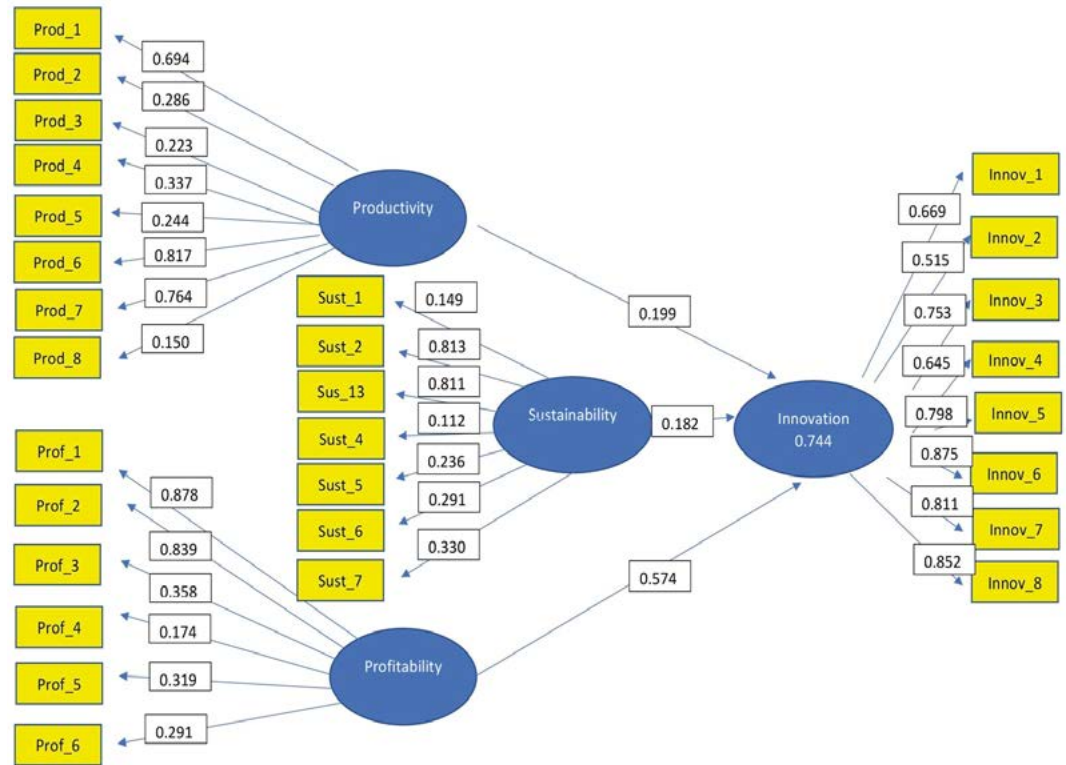


Figure 2. Initial structural model with complete survey indicators.

(0.182), represented by seven items. Collectively, these three constructs account for 74.4% of the variance in Innovation ($R^2=0.744$). These results were obtained prior to refining the measurement model by eliminating items with insufficient contribution. Specifically, indicators with outer weights below 0.10 and external loadings below 0.40 were excluded. Additionally, indicators demonstrating high collinearity often signaled by negative path coefficients were also removed. The final model considered both the relative and partial contributions of each indicator to ensure optimal predictive validity.

Among the exogenous latent variables, Profitability contributes most significantly to the endogenous latent variable Innovation, accounting for 60% of its predictive value. The most influential item within the Profitability construct is item 1 (0.638), which relates to profit gains, as producers seek greater profitability through the use of biofertilizers. Item 2 (0.445) addresses fair pricing, a motivation cited by producers in meetings, who emphasized the potential for higher returns through the marketing of organic products, especially in export markets. Item 5 (0.162) pertains to reduced production costs. Conversely, items 3, 4, and 6 representing sales, biofertilizer pricing, and government support via agricultural programs were discarded due to low external weights and loadings.

For the Sustainability variable, the most significant indicators, as highlighted by both producers and the SmartPLS4 analysis, include sust_2 and sust_3, which reflect the perceived positive environmental impacts of biofertilizers and their role in enhancing soil fertility. Indicators with low predictive power were removed from the model, including those related to groundwater contamination reduction, job creation, perceived health

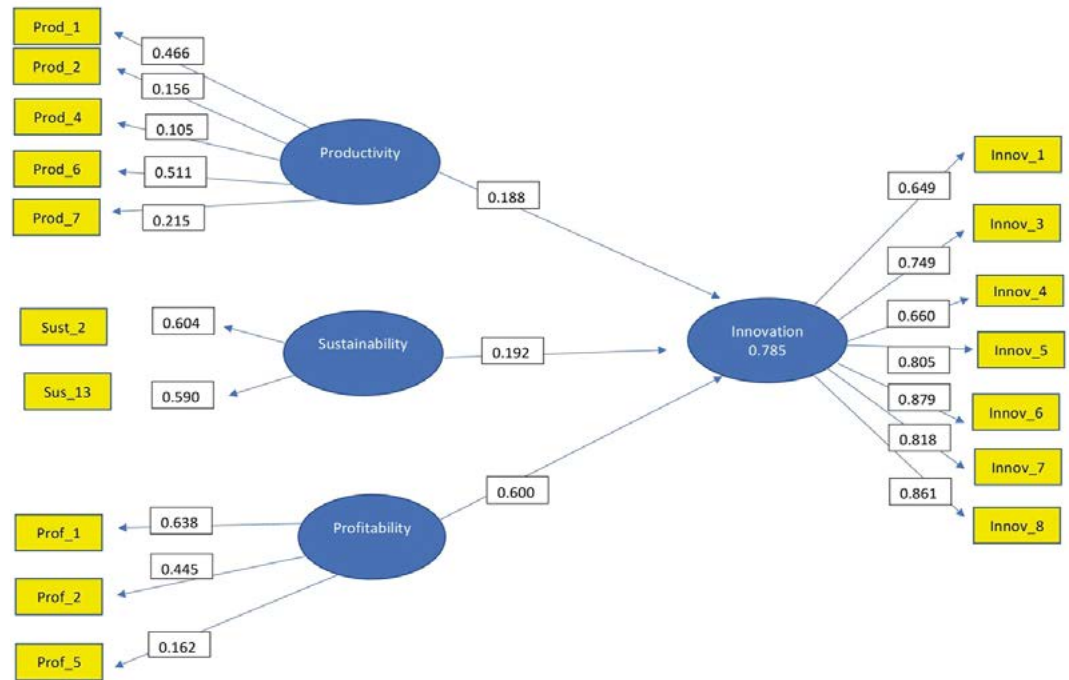


Figure 3. Structural model of innovation-biofertilizers.

benefits, crop adaptability, and issues of packaging and storage. Within the Productivity construct, the most significant items are prod_6 (0.511), which addresses increased productivity through improved soil fertility; prod_1 (0.466), which reflects the belief that biofertilizers enhance yields; and prod_7 (0.215), which indicates a motivation to transition from synthetic fertilizers to bioinoculants. Additionally, prod_2 underscores the advantage of reduced waste generation, as biofertilizers are perceived to be more efficiently utilized compared to conventional fertilizers, which have a utilization rate of only about 20%. Prod_4 highlights the role of government support programs; however, producers noted the lack of effective assistance in the region. The state-level biofactory initiative has failed to provide adequate training, and the few biofertilizers distributed have been largely ineffective due to the absence of viable microorganisms. In a formative-reflective model, a minimum R^2 of 0.50 is required to establish convergent validity. The final model achieved an R^2 of 0.785, indicating strong explanatory power. Together, the exogenous variables Profitability, Sustainability, and Productivity explain 78.5% of the variance in Innovation related to biofertilizer use in the agricultural sector of Michoacán. The remaining 21.5% is attributed to other factors not captured within this model, either due to exclusion during the refinement process or because they were not originally considered. The nomogram demonstrates that Cronbach's alpha increased slightly from 0.884 to 0.889 after removing less significant indicators those with outer weights below 0.10 or low external loadings. Indicators with a weight above 0.10 are considered significant based on their relative contribution, while external loadings reflect their absolute contribution. Although the increase in reliability is marginal, the refinement enhances the structural model's robustness and compliance with statistical thresholds.

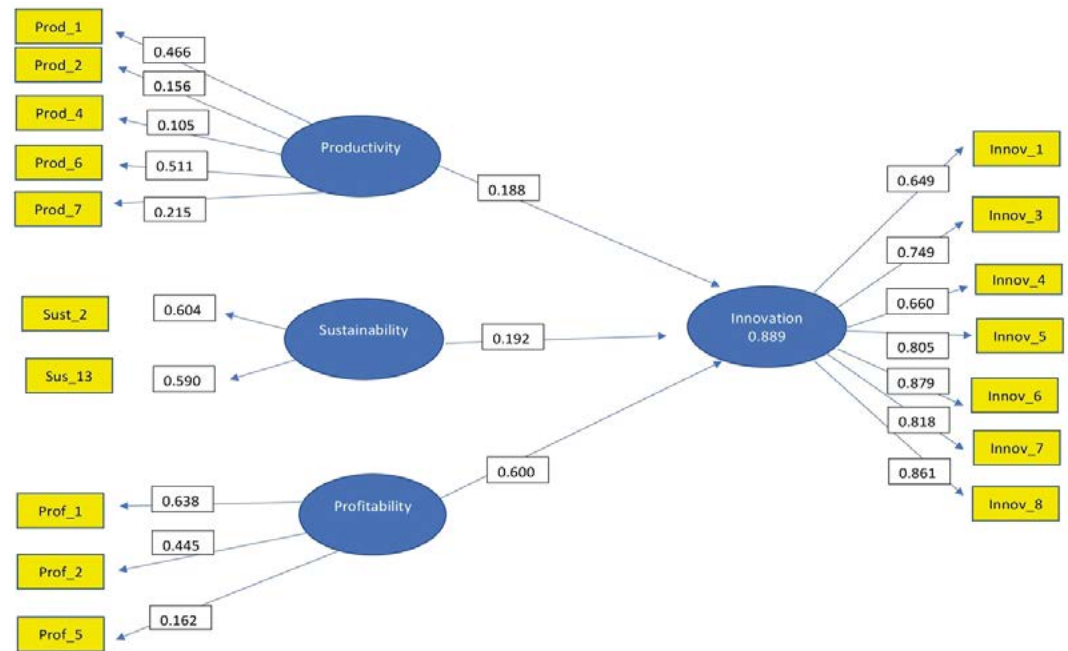


Figure 4. Cronbach's alpha in the Structural Modeling of Biofertilizer Innovation.

Collinearity does not reach critical levels in any of the formatively measured constructs, indicating that multicollinearity is not a concern in estimating the PLS nomogram of the extended Innovation model. The figure presents the external weights for each indicator contributing to the model's constructs, all of which are statistically significant, with values exceeding the 0.10 threshold. The lowest observed weight is 0.105, associated with the Productivity construct, suggesting that for producers, the drive to achieve higher productivity is positively associated with biofertilizer use. The highest external weight, 0.638, corresponds to the Profitability construct, underscoring its substantial importance for producers. This weight reflects the motivation to increase profitability through reduced production costs and the opportunity to market a differentiated product. Producers are incentivized to use biofertilizers to access organic export markets, where higher prices perceived as fair can be obtained. Within the Innovation construct, the most significant indicators are *innv_6* (0.879) and *innv_8* (0.861), making them the strongest contributors to the endogenous variable in the structural model. For the exogenous variables, the most significant indicators include *prod_6* (0.842), *rent_1* and *rent_2* (0.837), *sust_2* (0.841), and *sust_3* (0.833). To assess the statistical significance and relevance of these external weights, a bootstrapping procedure will be performed using the Bias-Corrected and Accelerated (BCA) bootstrap method. This analysis will be conducted with a significance level of 0.05, using 5,000 bootstrap samples and a two-tailed test to ensure robust and reliable results.

In the formative measurement model, p-values below 0.05 confirm the statistical significance of the external weights at the 5% level. For agricultural producers in Michoacán, key drivers influencing the use of biofertilizers include the search for new markets, higher profits, increased sales, reduced production costs, the adoption of sustainable fertilization alternatives, the emergence of new biofertilizer brands, and effective knowledge transfer.

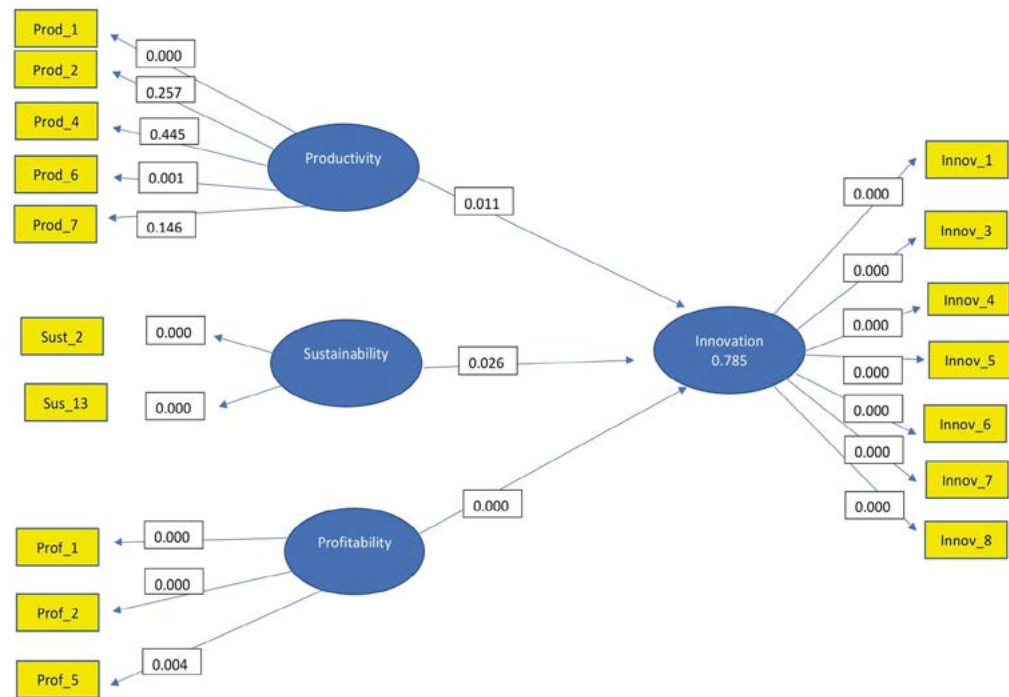


Figure 5. Booststrapping P-values.

The latent variables Profitability, Sustainability, and Productivity exhibit external weights of 0.011, 0.026, and 0.000, respectively each statistically significant in determining Innovation in the context of biofertilizer use in Michoacán's agricultural sector. These values confirm that the construct measures are both reliable and valid. Furthermore, the p-values for the relationships between the exogenous and endogenous variables are below 0.05, and the t-statistics exceed 1.96, establishing significance at the 5% level. In the importance-performance map, constructs located in the upper-right quadrant those with both high importance and high performance warrant strategic focus. Notably, the exogenous variable Profitability occupies this quadrant, highlighting its potential to drive performance improvement. Therefore, actions aimed at promoting innovation in the use, marketing, and production of biofertilizers should prioritize the indicators under this construct. Specifically, indicators Rent_1 and Rent_2 from the Profitability construct show the highest levels of importance and performance. These findings suggest that strategic efforts should target these indicators to maximize their impact on innovation in the agricultural sector. Profitability emerges as the most influential variable on Innovation, driven by producers' motivation to access premium organic export markets, secure higher prices, and reduce production costs through more efficient and less expensive alternatives to chemical fertilizers. While Sustainability is also a relevant factor, it ranks below Profitability in impact. The indicators contributing most to Sustainability relate to the enhancement of soil fertility. Although producers recognize the harmful effects of agrochemicals on human health and ecosystems, this awareness is not as strong a motivator. This is reflected in the modest shift toward biofertilizer use, which currently represents only 5% to 10% of total fertilization. Productivity remains a significant driver as well, with producers observing

that biofertilizers restore soil fertility and improve nutrient absorption by crops, thereby increasing yield potential. The pursuit of higher profitability through more productive crops continues to be their primary incentive. Regarding reliability, Cronbach's alpha a conservative measure assuming equal reliability across all indicators supports the model's internal consistency. However, composite reliability, which accounts for the varying external loadings of indicators, offers a more nuanced assessment. Reliability values between 0.60 and 0.70 are considered acceptable, while those between 0.70 and 0.90 are satisfactory. Values exceeding 0.90 may indicate redundancy and are generally discouraged. In this study, all four constructs fall within acceptable or satisfactory reliability thresholds, with especially strong reliability observed in the Innovation and Productivity constructs.

CONCLUSIONS

Based on the hypothesis of this research, sustainability, productivity, and profitability are the key factors influencing biofertilizer innovation produced and marketed by biofactories in the agricultural sector of Michoacán, Mexico. This hypothesis is quantitatively supported by the SmartPLS4 analysis. Within the structural model and its indicators, profitability accounts for 60% of the variance in innovation, while sustainability and productivity account for 19.2% and 18.8%, respectively. Biofertilizers are primarily applied in organic crops intended for international markets, with the United States as the principal destination, followed by Canada, Japan, and European countries such as Germany. The significance of sustainability and productivity in fostering biofertilizer innovation is thus confirmed. Producers recognize the environmental benefits of biofertilizer use, particularly in improving soil fertility, and their non-toxic effects on human health, consumers, field workers, and local ecosystems. Moreover, biofertilizers generate less waste, as they are absorbed more efficiently by crops compared to conventional fertilizers. In the productivity construct, the most impactful indicator is the improvement of soil fertility. Nutrient-enriched soils not only increase yield but also facilitate more effective nutrient absorption by crops. However, a major credibility issue emerged when state-distributed biofertilizers in 2022 were found ineffective due to the absence of viable microorganisms, a core component of biofertilizer efficacy. From a profitability perspective, producers are motivated by the lower cost of biofertilizers relative to chemical alternatives, their greater absorption efficiency, and their potential to reduce long-term production expenses. Unlike synthetic fertilizers, which require larger quantities with each cycle, biofertilizers gradually enrich soil health, enabling recovery in degraded soils within approximately five years. A key incentive is the ability to access organic export markets, which offer premium prices perceived as fair compensation. Several types of innovation are being implemented within agricultural systems, including diversification into export crops such as horticultural and grains, adoption of new and hybrid varieties, use of grafts and patented seeds, reforestation, agro-industrial transformation, application of biotechnology, fertigation, precision agriculture, drones, improved input management, good agricultural practices, agroclimatic monitoring, cold storage, smart machinery, automated packaging, GPS-guided harvesters, and integrated pest and disease management involving cultural, genetic, biological, and

chemical strategies, as well as financial diversification, digitization of administrative systems, flexible labor hiring methods, agricultural machinery rentals, contract farming, direct sales, targeting seasonal market windows, promotional campaigns, upgraded packaging and presentation, global certification, and digital marketing through websites and social media platforms, along with efforts to access government support. One of the main economic incentives for adopting innovations that promote sustainability is the price premium in organic markets, particularly foreign markets. In Mexico, the United States continues to be the main importer of agro-exported goods. Biofertilizers represent a sustainable agricultural input, offering multiple benefits: they enhance productivity, generate profits, and do not harm human health, biodiversity, or agricultural soils. Moreover, they help prevent environmental contamination, especially in water bodies and the atmosphere. They have also become a source of employment through biofactories established in Michoacán, creating jobs in production, distribution, and training-related activities. Their efficacy has been demonstrated in increasing agricultural soil productivity by approximately 40% through enhanced fertility. This contributes to cost reductions, strengthens both national and export-oriented organic markets, and supports premium pricing for sustainable products. As such, biofertilizers represent an innovative bioproduct with a transformative impact across all dimensions of sustainability. They have revitalized the production of traditional staple crops such as corn, beans, rice, coffee, and cocoa, and are also being applied to commercially valuable crops like sugarcane, bananas, limes, berries, and avocados, driving innovation across the agricultural sector.

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Egg production of Creole hens fed with live larvae of the Black Soldier Fly (*Hermetia illucens* L.)

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ABSTRACT

Objective: To evaluate the consumption of live larvae of *Hermetia illucens* L. and a complement (corn, wheat bran, vitamins and minerals) in the productive response of Creole hens compared to a diet based on corn, soybean meal, vitamins and minerals.

Methodology: 44 Creole hens were used to evaluate 2 experimental diets: a diet based on corn-soybean and a diet with live larvae and a complement. The larvae used were produced in a rearing chamber with controlled temperature and relative humidity. To evaluate productive behavior, the following variables were measured during eighth weeks: feed intake (g bird⁻¹ week⁻¹), egg production (kg bird⁻¹ week⁻¹), feed conversion (kg kg⁻¹), laying percentage (%), average egg weight (g), egg mass (g) and mortality. The design was completely randomized, with repeated measurements over time using the MIXED procedure of SAS 9.0. The means were compared with Tukey's test ($\alpha=0.05$).

Results: Significant differences ($P\leq 0.05$) were observed in feed intake and feed conversion, with the remaining means ($P>0.05$) there were no differences. The average values of each variable were not different ($P>0.05$).

Limitations of the study: The use of fly larvae implies a previous proximal analysis to know the nutritional contribution, this will depend on the type of substratum that is given to the larvae.

Conclusions: Live larvae of Black Soldier Fly with a supplement based on corn, wheat bran, vitamins and minerals can replace soybean meal in a diet for Creole hens.

Keywords: Soybean meal substitution, live fly larvae, Creole birds.

INTRODUCTION

Commercial or intensive poultry farming heavily relies on the production of cereals and oilseeds, primarily corn and soybean meal, whose amino acid profile offers a



balance that closely matches the nutritional requirements of poultry. However, Mexico is not self-sufficient in the production of these inputs, and to meet national consumption, \$8.18 billion are allocated to the importation of corn and soybean (USDA, 2023). The production of soybean and corn requires large land areas and significant amounts of water, which negatively affects ecosystems by causing deforestation of jungles and forests, changes in land use, and climate alterations. The increase in the demand for poultry products has generated the need to explore alternative sources of energy and protein with high nutritional value, in order to reduce the use of grains and oilseeds in animal feed. In this context, insects such as the larvae of the Black Soldier Fly (*Hermetia illucens* L.) represent a viable alternative to partially meet the nutritional needs of poultry farming. These larvae possess a high fat content (30%) and protein content (40%), which could meet the nutritional requirements of native hens. Additionally, they have a short life cycle, transform organic waste into high-value protein sources, and can feed on various by-products from the agri-food industry (Star *et al.*, 2020). Insect production requires significantly less land and water than grain production (Tahamtani *et al.*, 2021), which can contribute to the sustainable development of national poultry farming. The aim of this research was to evaluate the productive response of native hens fed a conventional diet based on corn and soybean meal, in comparison with an alternative diet based on live Black Soldier Fly larvae supplemented with a feed additive.

MATERIALS AND METHODS

Location

The study was conducted between spring and summer of 2024 at the experimental poultry farm of the Colegio de Postgraduados, Montecillo Campus, Texcoco, State of Mexico, located at coordinates 19° 27' 38" N and 98° 54' 10" W, at an altitude of 2,250 meters above sea level. The climate is temperate sub-humid, with summer rains, an average temperature of 14.6 °C, and an annual accumulated precipitation of 558.5 mm (García, 2004).

Animals and housing

The study was reviewed and approved by the Ethics Committee of the Colegio de Postgraduados (COBIAN/017/23; COLPOS, 2016). A total of 44 native hens (Creole hens) aged 44 weeks were used to evaluate two experimental diets: one diet formulated with corn, soybean meal, vitamins, and minerals (corn-soy diet), and another diet containing live larvae plus a supplement made from corn, wheat bran, vitamins, and minerals (larvae diet). The birds were randomly assigned, with 22 individuals per diet, for a period of eight weeks (each hen was considered an experimental unit). Prior to the trial, the birds underwent a two-week adaptation period to the experimental diets. The hens were housed individually in cages (45 cm × 30 cm × 23 cm) inside a shed with natural light and ventilation, regulated by side curtains. The photoperiod was gradually adjusted to reach 16L:8D. Temperature (20-22 °C) and relative humidity (47.1-64.3%) were recorded daily using a temperature and humidity sensor (Data Logger Humidity/Temperature, Model RHT10).

Black soldier fly larvae production

To establish the Black Soldier Fly population, live larvae were obtained from a supplier in Toluca, State of Mexico. The adult flies produced were exposed to sunlight daily (8-10 hours) to stimulate mating. The cage contained an attractant (moist poultry litter) and wooden boards (15 cm × 5 cm × 2 cm) secured with rubber bands to facilitate oviposition. Eggs were collected and placed in Petri dishes for 3-4 days; upon hatching, the larvae were transferred to plastic trays (33.5 cm × 27 cm × 7.5 cm) with an initial mixture of chicken feed (100 g), wheat bran (50 g), and water (200-400 mL). After five days, the mixture was increased to: chicken feed (300 g), wheat bran (150 g), water (300 mL), and vegetable (tomato, cucumber) and fruit (mango, apple, banana, grape) waste. The larvae were reared in a climate-controlled chamber (28-32 °C) with relative humidity (65-75%). In this study, fifth or sixth instar larvae were used, before the color change, to minimize chitin content (Bejaei & Cheng, 2020).

Diets

The diets were offered as follows: the corn-soy-based diet, with vitamins and minerals, was administered at a rate of 110 g of feed per bird per day; the larvae diet consisted of 72 g of live larvae per bird per day, plus 82 g of a supplement made with corn, wheat bran, vitamins, and minerals, provided *ad libitum* (Table 1).

Chemical analysis of the diets

The corn-soy diet, the larvae as offered, and the supplement were analyzed (AOAC, 2005) in the Animal Nutrition Laboratories of the Universidad Autónoma Chapingo and the Colegio de Postgraduados (Table 2).

Variables

To evaluate productive performance, the following variables were determined: feed intake ($\text{g bird}^{-1} \text{ week}^{-1}$), egg production ($\text{kg bird}^{-1} \text{ week}^{-1}$), feed conversion ratio (kg kg^{-1}), laying rate (%), average egg weight (g), egg mass (g) and mortality.

Statistical analysis

Productive variable data were analyzed under a completely randomized design with repeated measures over time, using the MIXED procedure of SAS software version 9.0 (SAS, 2002). Means were compared using Tukey's test ($\alpha=0.05$).

RESULTS AND DISCUSSION

Productive variables

Significant differences ($P \leq 0.05$) were observed only in the variables feed intake and feed conversion ratio; no differences were found in the remaining variables ($P > 0.05$) (Table 3). Birds fed with larvae consumed significantly less feed (80.91, 67.73, 110.3, 81.11, and 99.94 g) ($P \leq 0.05$) compared to the corn-soy-based diet during weeks 3, 4, 5, 6, and 8. Additionally, in week 2, the feed conversion ratio was lower ($P \leq 0.05$) with the larvae-based diet (0.50 kg of feed per kg of egg). Regarding the average values of each variable, no significant differences were observed ($P > 0.05$).

Table 1. Composition (%) and calculated analysis of the corn-soy diet and the diet with larvae plus supplement, as-fed basis.

| Ingredients | Corn-soybean diet | Diet with larvae* |
|------------------------------------|-------------------|-------------------|
| Yellow corn (7.5% CP) | 65.08 | 41.72 |
| Soybean paste (46.5% CP) | 23.93 | 0.00 |
| Wheat bran (15% CP) | 0.00 | 26.49 |
| Fine calcium carbonate | 4.39 | 4.13 |
| Coarse calcium carbonate | 4.39 | 4.13 |
| Dicalcium phosphate (21% Ca/18% P) | 1.28 | 0.53 |
| Common salt | 0.35 | 0.24 |
| Soybean oil | 0.23 | 0.00 |
| *Premix of vitamins and minerals | 0.20 | 0.19 |
| Sodium bicarbonate | 0.15 | 0.00 |
| Live BSF larvae (32.56% DM) | 0.00 | 22.51 |
| Calculated analysis (%) | | |
| ME (kcal/kg) | 2650 | 2650 |
| CP | 16.00 | 16.00 |
| Calcium | 3.750 | 3.750 |
| Total phosphorus | 0.611 | 0.616 |
| Available phosphorus | 0.350 | 0.321 |
| Lysine | 0.842 | 0.723 |
| Methionine | 0.255 | 0.287 |
| Methionine + cystine | 0.530 | 0.504 |
| Tryptophan | 0.182 | 0.218 |
| Threonine | 0.608 | 0.567 |

*The sum of the composition of live larvae and the supplement is almost similar to that of the control diet. BSF: black soldier fly. DM: dry matter. *Premix of vitamins and minerals, Contribution per kg of feed: vitamin A, 12000 IU; vitamin D₃, 5000 IU; vitamin E, 85 IU; vitamin K₃, 3 mg; vitamin B₁, 3 mg; vitamin B₂, 8 mg; vitamin B₃, 62 mg; vitamin B₆, 4 mg; vitamin B₁₂, 0.017 mg; pantothenic acid, 14.5 mg; folic acid, 1.8 mg; biotin, 0.2 mg; Zn, 100 mg; Mn, 120 mg; I, 1.25 mg; Se, 0.3 mg; Cu, 15 mg; Fe, 80 mg.

Table 2. Nutritional composition determined from corn-soybean diet and diet with larvae plus a supplement, on a dry basis (%).

| Variable (%) | Corn-soybean diet | *BSFL | Complement |
|---------------|-------------------|-------|------------|
| Dry matter | 93.56 | 32.56 | 93.58 |
| Moisture | 6.44 | 67.44 | 6.42 |
| Crude protein | 16.73 | 38.49 | 9.11 |
| Ether extract | 1.56 | 35.48 | 2.10 |
| Ash | 13.43 | 4.13 | 15.53 |

*BSFL: Black Soldier Fly Larvae. Dry matter and moisture are reported as fed basis. Crude protein, ether extract, and ash are reported as dry basis. Corn-soybean diet: 110 g bird⁻¹ day⁻¹, live larvae 72 g bird⁻¹ day⁻¹ plus a complement 86 g bird⁻¹ day⁻¹. Amino acid content of BSFL expressed as percentage of crude protein: lysine, 5.243; methionine, 1.784; threonine, 3.624; tryptophan, 1.538; arginine, 4.725; cystine, 0.897; methionine + cystine, 2.466; isoleucine, 3.805; leucine, 6.986; valine, 5.272; histidine, 2.713; phenylalanine, 3.510; glycine, 5.058; serine, 3.617; proline, 4.928; and alanine, 5.747 (EVONIK Operations GmbH).

Table 3. Productive behavior of Creole hens fed with a corn-soy diet and a diet with live Black Soldier Fly larvae plus a supplement.

| Diet | Week | | | | | | | | Average |
|---|--------|-------------------|---------------------|---------------------|---------------------|---------------------|--------|---------------------|---------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | |
| Feed consumption (g bird⁻¹ week⁻¹) | | | | | | | | | |
| Corn-soybean | 517.15 | 507.32 | 535.25 ^a | 587.63 ^a | 626.48 ^a | 621.44 ^a | 538.01 | 511.23 ^a | 527.62 |
| Larvae | 482.13 | 491.66 | 454.34 ^b | 519.90 ^b | 516.18 ^b | 540.33 ^b | 504.97 | 411.29 ^b | 518.16 |
| SE | 36.24 | 35.72 | 33.81 | 33.93 | 35.22 | 35.11 | 37.09 | 38.17 | 17.47 |
| Egg produced (kg) | | | | | | | | | |
| Corn-soybean | 0.2696 | 0.2626 | 0.2250 | 0.2987 | 0.2205 | 0.2270 | 0.2218 | 0.2359 | 0.24 |
| Larvae | 0.2650 | 0.2526 | 0.1927 | 0.2175 | 0.2183 | 0.2078 | 0.1781 | 0.1978 | 0.22 |
| SE | 0.01 | 0.02 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 |
| Feed conversion (kg kg⁻¹) | | | | | | | | | |
| Corn-soybean | 2.59 | 2.46 ^a | 2.94 | 2.87 | 3.32 | 3.32 | 3.67 | 3.36 | 3.00 |
| Larvae | 2.02 | 1.96 ^b | 2.78 | 2.87 | 3.08 | 2.98 | 2.93 | 3.36 | 2.89 |
| SE | 0.25 | 0.26 | 0.26 | 0.25 | 0.26 | 0.25 | 0.26 | 0.27 | 0.20 |
| Posture (%) | | | | | | | | | |
| Corn-soybean | 62.14 | 70.05 | 61.11 | 69.17 | 61.34 | 60.14 | 59.82 | 55.70 | 58.76 |
| Larvae | 70.76 | 69.64 | 59.27 | 58.63 | 59.98 | 56.39 | 50.33 | 50.84 | 59.78 |
| SE | 3.45 | 3.60 | 3.55 | 3.55 | 3.59 | 3.54 | 3.60 | 3.55 | 3.71 |
| Average egg weight (g) | | | | | | | | | |
| Corn-soybean | 53.20 | 53.37 | 50.37 | 52.61 | 52.47 | 54.00 | 54.30 | 54.49 | 51.97 |
| Larvae | 52.14 | 46.12 | 46.40 | 52.32 | 52.43 | 52.91 | 51.28 | 53.56 | 52.00 |
| SE | 1.08 | 1.12 | 1.12 | 1.13 | 1.13 | 1.12 | 1.14 | 1.16 | 0.94 |
| Egg mass (g) | | | | | | | | | |
| Corn-soybean | 37.85 | 37.52 | 32.15 | 36.54 | 31.80 | 32.42 | 31.68 | 30.33 | 31.00 |
| Larvae | 32.96 | 33.95 | 27.52 | 31.08 | 31.46 | 29.69 | 25.44 | 28.26 | 31.62 |
| SE | 1.86 | 1.92 | 1.90 | 1.90 | 1.92 | 1.90 | 1.92 | 1.92 | 2.02 |

^{ab} Means with different superscript letters in each column within each variable indicate significant differences ($P \leq 0.05$). SE: Standard error.

Feed intake, during five of the eight weeks of the study, showed differences in favor of the larvae-based diet; however, the overall average was not significant. The data from this study differ from those reported by Star *et al.* (2020) in Dekalb White hens aged 67 to 78 weeks, where cumulative feed intake was lower with the diet containing 12 g of larvae and various protein sources (123 g bird⁻¹ day⁻¹), compared to the control diet (133 g bird⁻¹ day⁻¹) reported by those authors. These results align with those observed in weeks 46, 47, 48, 49, and 51 of age in the present study, with 72 g of live larvae bird⁻¹ day⁻¹ plus the supplement that did not include soybean meal. Feed conversion ratio in week 2 was higher by 500 g of feed per kg of egg produced for the birds fed the control diet, indicating a better performance of the birds fed the larvae-based diet (2.02 g g⁻¹). Star *et al.* (2020) reported similar values (2.391 g g⁻¹) in Dekalb White hens aged 67 to 78 weeks fed a live larvae diet. However, Bellezza *et al.* (2024) reported a similar feed conversion ratio between a wheat-soybean-sunflower diet (3.30 g g⁻¹) and the live larvae diet (3.40 g g⁻¹) in native hens aged

44 to 52 weeks. These results contradict the findings of this study, in which differences were observed (corn-soy diet: 2.46 g g^{-1} ; live larvae diet: 1.96 g g^{-1}) at 45 weeks of age.

A possible explanation for this variability is that the nutritional requirements, in terms of protein and energy, of native hens may be lower than those of commercial layers, as they have not undergone selection for increased egg production. Therefore, egg production can be maintained using Black Soldier Fly larvae as a substitute for conventional ingredients such as soybean meal.

CONCLUSION

Live Black Soldier Fly larvae, supplemented with a mixture of corn, wheat bran, vitamins, and minerals, can replace soybean meal in diets for native laying hens. Further research is recommended, along with the development of a small-scale, low-cost larvae production model that benefits small-scale rural producers.

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Identification and characterization of small dairy producers from San Miguel Coatlinchán and their relationship with their socioeconomic structure

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ABSTRACT

Objective: To identify the total number of small dairy producers and to characterize their socioeconomic structure, land ownership status, production system, and the effects of urban growth.

Design/Methodology/Approach: Mixed-method research with non-probability sampling was applied through a survey to 32 dairy producers, based on the registry of ejidos of San Miguel Coatlinchán.

Results: The total number of small dairy producers, their spatial distribution, and the problems generated by urban growth were identified, as well as prospects for the survival of the production.

Study Limitations/Implications: Internal and external factors (*e.g.*, urban growth) that condition primary activities must be identified.

Findings/Conclusions: The lack of government support for training activities and technical advice, along with the abandonment of producer organizations, limits the capacity of the producers to boost their activity, to produce dairy by-products, and to improve their income.

Keywords: small milk farmers, socioeconomic structure, urban growth.

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INTRODUCTION

Small-scale dairy production is one of the most common activities carried out by rural families throughout Mexico. Small production units work with 3 to 30 cows and ≤ 4.5 ha to feed their livestock (SADER 2022; Hernández *et al.*, 2013; and Hemme *et al.*, 2007). According to FAO (2010), small-scale dairy producers should be a priority in public policies, because they generate income and employment, thus alleviating poverty and vulnerability. Likewise, they guarantee food security in rural areas.

In their 2024 livestock inventory, SIAP (Agri-Food and Fisheries Information Service) estimated a dairy production of 13.333 billion liters of milk per year —58% of which is



produced by small producers who have up to 100 milking cows. However, this percentage does not differentiate between types of production systems. Mexican milk production systems have been identified and characterized according to their productive, economic, agroecological and social importance as follows: specialized intensive system (SIS), family-backyard system (FBS), and dual-purpose system (DPS) (Avilés, 2024). Gallegos (2023) considered various aspects to differentiate production systems, including the type of livestock, technology applied, geographic location, type of feed, and the characterization of national production systems. SIS, FBS, and DPS account for 70%, 21%, and 9% of the national production, respectively (Robledo, 2018).

The aim of this study was to identify the total number of small dairy producers in the town of San Miguel Coatlinchán. A survey was conducted to determine socioeconomic data, including age, educational level, production systems, local market ties, and land ownership.

Dairy producers in Coatlinchán do not establish their pens following a specific location order. Producers usually set up pens in their homes, which allows them to take care of them and sell their products directly to the population. Nowadays, these pens have been absorbed by urban dynamics and growth, generating new relationships of coexistence between producers and residents and leading to conflictive relationships that did not previously exist. This situation has modified productive and organizational forms. Nevertheless, rural processes and identities have not been lost.

MATERIALS AND METHODS

Location and characteristics of the study area

The town of San Miguel Coatlinchán is located within the southern perimeter of the municipality of Texcoco in the State of Mexico, near the old Lake Texcoco, on the bridge

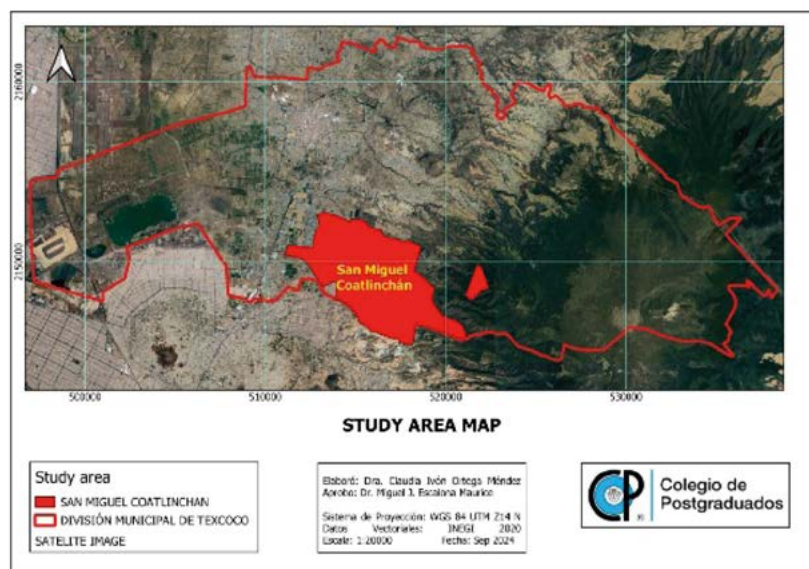


Figure 1. Location of the study area. Figure developed by the authors based on vector data from INEGI (2020).

path to Mexico City. According to the 2020 census conducted by Mexico's National Institute of Statistics and Geography (INEGI), the town has a total population of 13,705 inhabitants, out of which 6,700 are men and 7,005 are women, making it the second most populated town in the municipality.

The samples were selected based on the *ejidatario* registry, which was made up of a universal set of 295 *ejidatarios* (*ejido* landowners) using non-probability sampling (Hernández *et al.*, 2018). The reliability level was 95%.

$$n = \frac{npq}{(N-1)D + pq}$$

Where n = sample size, N = population size, p = probability of success = 0.5, $q = 1 - p$, $D = (B/z)^2$, B = maximum acceptable error = 0.10, Z = 90% confidence interval = 1.64.

Only 10 surveys were applied to the sample value ($n = 55$), because the rest of the *ejidatarios* has another activity.

Direct fieldwork enabled the identification of 22 additional small-scale producers, bringing the total to 33 completed surveys. No other producers in the entire area engaged in this activity. To validate the survey topics, an exploratory work was conducted with key stakeholders to define the main topics. Fieldwork was carried out from March 11 to May 20, 2024, and producers and their families were surveyed directly at the milking sites.

RESULTS AND DISCUSSION

A total universal set of local small milk producers allowed the research team to determine the overall situation of the dairy activity, therefore obtaining a concentrate of an updated registry, which included the following information: total number of cows, estimated production, production management strategies, and, above all, socioeconomic characteristics based on which each producer can be evaluated.

Demographics

Demographic data can help to determine the statistical characteristics of the target population. In this stage, identification data was collected from small dairy producers, including: age, sex, marital status, highest level of education, and number of children. Out of the 32 small dairy producers, 30 were men and 2 were women. According to the following age graph, 53% had between 41 and 59 years old.

According to Table 1, 12.5% of all the producers did not finish primary school, 28.125% finished primary school, 50% finished secondary school, and 9.375% have an incomplete bachelor's degree.

Typification of dairy producers

Small producers who belong to the second or third generation in dairy production families have dedicated practically their entire lives to this activity, which has always been their main occupation. They have been immersed in dairy production, because their

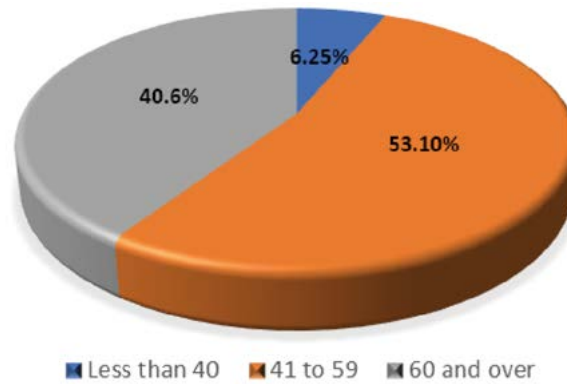


Figure 2. Age of the dairy producers.

Table 1. Educational level of the dairy producers.

| Level of education | Frequency | Value (%) |
|---------------------------|-----------|-----------|
| Unfinished primary school | 4 | 12.5 |
| Primary school completed | 9 | 28.125 |
| Secondary | 16 | 50 |
| Unfinished degree | 3 | 9.375 |
| Total | 32 | 100 |

father and/or grandfather were already engaged in this labor and they were expected to participate in their family business (agriculture and livestock).

For all producers and their ancestors, dairy activity has been their main source of income. They do not work —nor have they ever worked— in any government department and, therefore, if they are older than 60, they do not have a pension.

In this sense, 59.375% of small dairy producers would prefer if their children did not continue with this activity. They believe that it is a very arduous activity that does not only prevent them from spending time with their family, it also involves high expenses and low income. However, their main reason is that they want their children to study, become professionals, and work in places where they are provided with medical services, social security, and a fixed salary. Therefore, they support their studies and, whenever possible, they send them to private schools to achieve higher education.

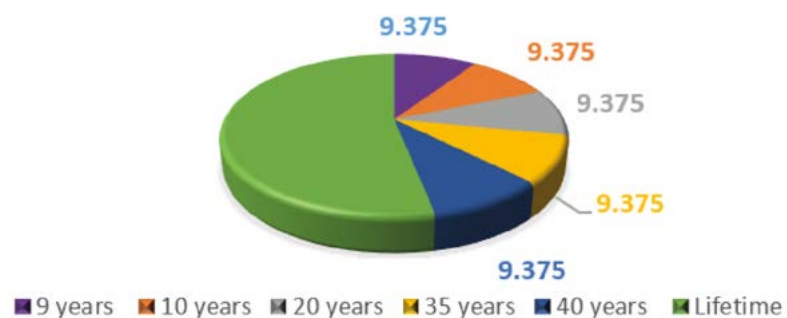


Figure 3. Years of dairy activity.

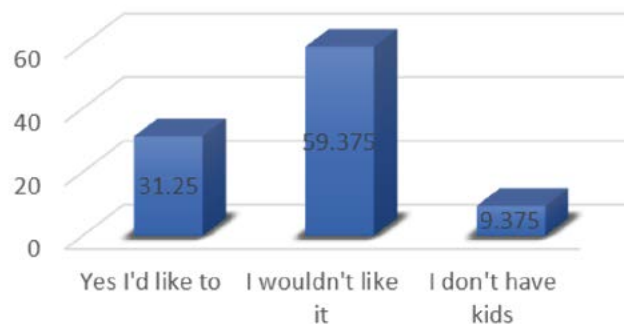


Figure 4. Percentage of producers who would like their children to continue or not with dairy production.

The following table identifies some variables that could prevent dairy producers from carrying out this activity.

Dairy production

Seven producers owned 1 to 5 milking cows, while eleven had between 6 and 10 cows, twelve had between 11 and 15 cows, one had 30 cows, and another had 45 cows. All producers owned some Holstein cows, 9.4% owned only one Jersey or Canadienne cow, and 6.25% owned only one Holstein×Swiss cross.

The participating farmers mentioned that, before 2018, they received technical assistance from the Mexican Ministry of Agricultural Development (SEDAGRO, currently SADER) for their milking, vaccination, deworming, medication, and/or insemination activities. However, when the left-wing government that refers to itself as the “Fourth Transformation [of Mexico]” came to power, that support was no longer available. Producers report that students and workers from COLPOS - Montecillo and the Universidad Autónoma de Chapingo (UACH) sometimes visit them and give them advice or suggestions on the management and health of their livestock.

The producers feed their livestock mainly with pasture, corn silage and stover, alfalfa (hay and green), agro-industrial by-products (bran, corn gluten feed, brewer grains), supplements (minerals, vitamins, additives), concentrates (corn, soybean, wheat, oats,

Table 2. Causes that dairy producers consider will prevent them from continuing with the activity.

| Cause | Frequency | % |
|---|-----------|-------|
| Age | 6 | 18.75 |
| Age and not being able to cover the expenses | 14 | 43.75 |
| Age, not having space for my livestock and not being able to cover the expenses | 3 | 9.375 |
| Not having land for agriculture and not being able to cover the expenses | 3 | 9.375 |
| Not being able to cover the expenses | 3 | 9.375 |
| None | 3 | 9.375 |
| Total | 32 | 100 |

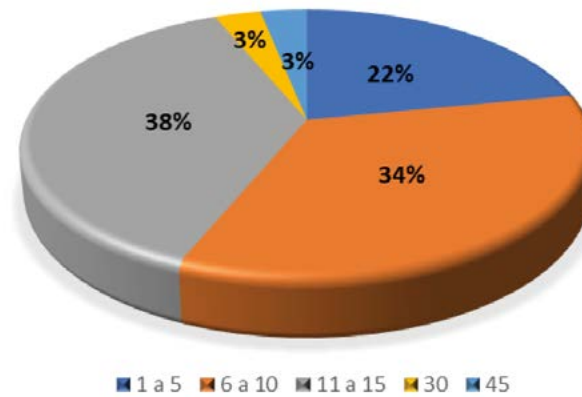


Figure 5. Percentage of dairy producers per number of milking cows.

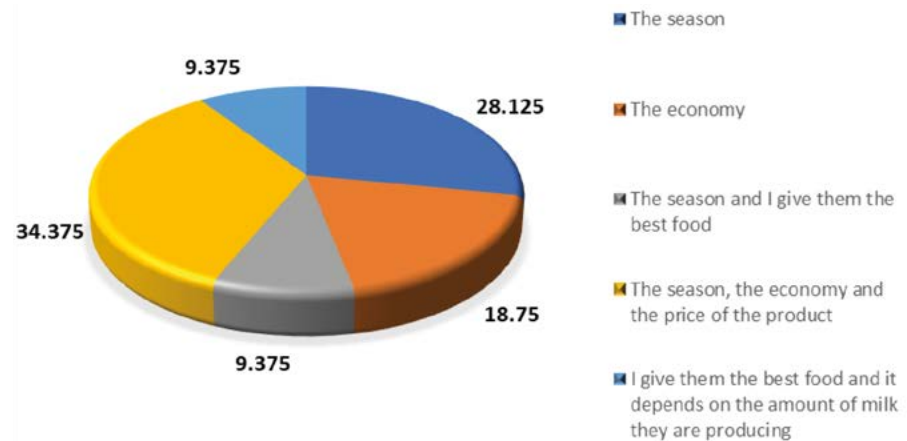


Figure 6. Type of feed for dairy cattle.

bread, ground straw), salt blocks, silage made from other forages, concentrated feeds, and ground bakery waste with bran and other fodder grains. This feed depends mainly on the performance of the harvest in a given season, the price of the products, and their savings.

Producers pointed out that, in a good season, cows produce 15 to 32 liters of milk per day, while, in a bad season, they only produce 2 to 15 liters of milk per day. These figures are used to estimate production indicators. Technique-wise, 90.375% of the small producers milk by hand and only 9.375% have a milking machine. The milking supplies they use include 20-L plastic containers where the milk is stored during the milking process, water to wash the cows’ udders, disinfectants, and the post-milking teat sealant.

Cows that permanently stop producing milk are sold at a loss to local or outsider butchers. The price depends on the age of the cow and ranges from MXN\$2,000 to MXN\$12,000 pesos. Male calves are sold for MXN\$3,000 pesos or they are kept for fattening and then sold at MXN\$17,000 to MXN\$33,000 pesos. Any profits are used to buy food or medicine for the rest of the livestock or for family emergencies. “Male calves are our piggy bank,” the producers say.

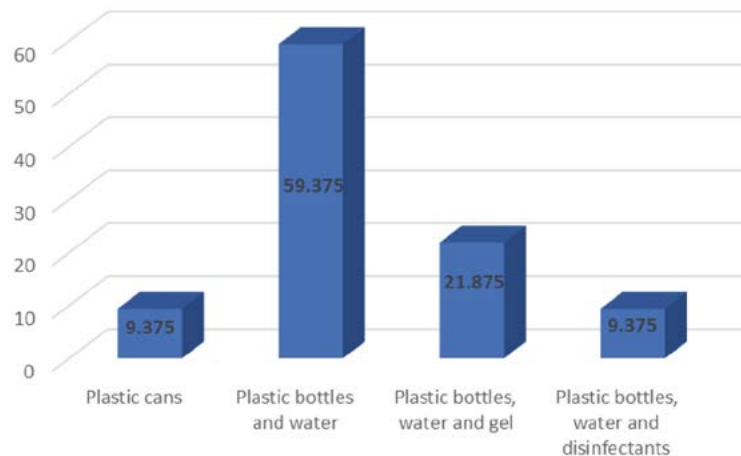


Figure 7. Milking supplies.

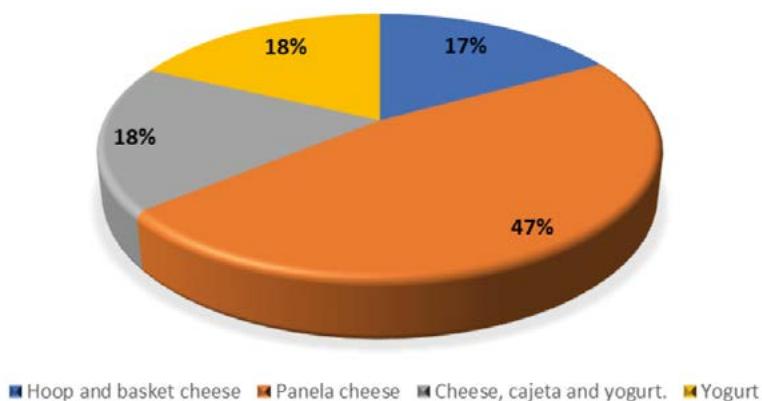


Figure 8. Percentage of small producers who manufacture some type of dairy by-product.



Figure 9. Pen infrastructure.

Milk commercialization

Producers do not hire personnel for the handling of dairy cattle or related production activities: 46.8% of the producers are directly in charge of these tasks, while 53.2% involve their families. Children milk the cows, sweep the pens, and feed the cattle, wives sweep the pens, and daughters-in-law and brothers help to harvest and milk.

The price of milk depends on to whom and where it is sold. The highest price ranges from MXN\$12 pesos to MXN\$16 pesos per liter. Milk is sold door-to-door in the same locality. They can also sell it at a local milk collection center, where they are paid MXN\$8 pesos per liter. Outsider or local buyers who process dairy by-products to make cheese, yogurt, etc., arrive at this place to collect milk from several producers and pay MXN\$9 pesos per liter. The average price of a liter of fresh milk is MXN\$10.50 pesos, but the price in rural areas ranges from MXN\$8 to MXN\$9 pesos (SIAP, 2023).

More than half (53.1%) of producers make some kind of dairy by-product. *Panela* cheese is produced by 47% of them, while *cajeta* (caramel sauce), yogurt, *queso de aro* (a type of fresh cheese), and *canasto* cheese are each manufactured by 18% of producers. They learned to make these by-products through courses offered by SEDAGRO or through training provided by UACH. These by-products are sold to local families and kept for personal consumption. Their sale is mainly the responsibility of the wives of the producers.

More than half (53.1%) of the producers would like to be trained in how to make some dairy by-products, while less than half (46.9%) are not interested, do not have the time, or have already tried and consider that the products and supplies are very expensive and they cannot afford to buy them.

Pen infrastructure

Based on the visits and surveys, the infrastructure is of low-medium quality. Producers typically lack the capital to maintain pens in optimal conditions for dairy production management. Good livestock practices must be implemented to ensure food safety, livestock management, livestock feeding, and waste management. The purpose of these measures is to manufacture dairy products in the best conditions possible and to reduce the risks posed by physical, chemical, or microbiological agents that diminish milk quality (SENASICA, 2019). Table 3 shows the surface area that producers allocate for their pens. A minimum of 50 m² is required for 5 cows. Sixty-eight percent of producers have cement pens, covered by corrugated metal sheets. Their walls are made of concrete blocks and some have rustic cement floors. Most producers (78%) have their own pens and the rest rent them. All pens contain drinking troughs, a roof, and a fence (at least made of wooden sticks). Consequently, this infrastructure is classified as semi-rustic.

Land ownership

Regarding the number of plots owned by dairy producers, 28% have one plot, 34% have two plots, and 38% have three plots. In terms of ownership, 33.3% are private property and 66.7% are *ejidos*.

According to the national agrarian distribution, to own land in Mexico, people need to comply with the following requirements: to be Mexican; to be an adult or of any age if they are in charge of a family or they inherited an *ejido*; to have lived at least a year in the vicinity of the corresponding *ejido* (except when they inherited the *ejido*); or to meet the internal requirements established by each *ejido* (L.A., 2024). Land ownership is proven with the agrarian rights certificate and the smallholding certificate.

Table 3. Pen infrastructure.

| Characteristic | Data | Producers (%) |
|-----------------|---|---------------|
| Surface | 50-100 m ² | 34.375 |
| | 101-200 m ² | 18.75 |
| | 201-400 m ² | 18.75 |
| | 401-600 m ² | 18.75 |
| | 800 m ² | 9.375 |
| Structure | Built | 68.75 |
| | Semi-rustic | 31.25 |
| Facilities | Own | 78.125 |
| | Loaned | 21.875 |
| Content | Drinking fountains, Feeding troughs, roof. | 18.75 |
| | Perimeter fence, drinking fountains, feeders, roof. | 81.25 |
| Condition | Good | 46.875 |
| | Regular | 43.75 |
| | Bad | 9.375 |
| Antique (years) | 3-15 | 21.872 |
| | 16-30 | 40.625 |
| | 31-45 | 28.125 |
| | 46-60 | 9.375 |

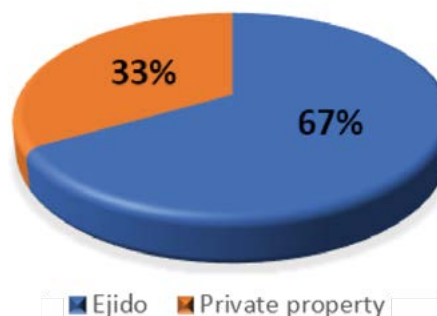


Figure 10. Type of land ownership system.

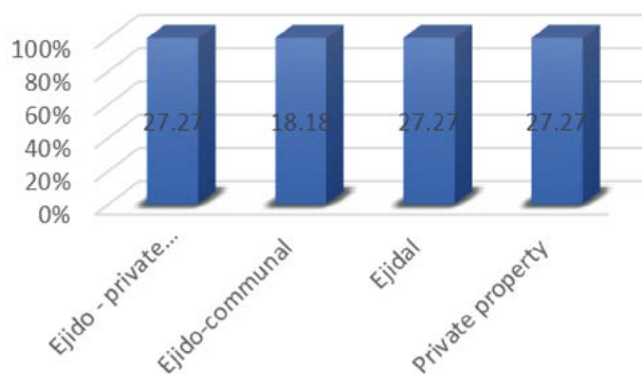


Figure 11. Producer land ownership system.

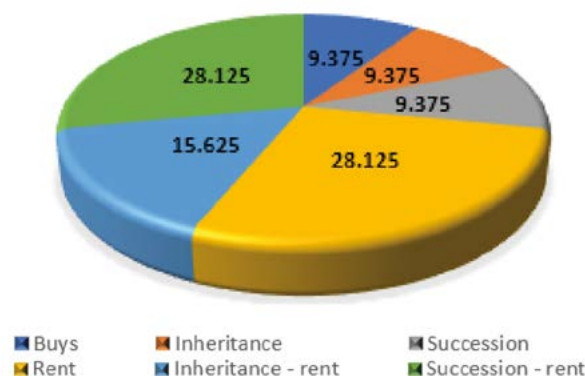


Figure 12. Origin of the ownership of the production plots.

The land is used as a small agricultural property, where cattle sheds are established and the animals are allowed to graze. The crops are managed under a rainfed system, while the water for the livestock is extracted from wells (drinking water). The producers have acquired the land through one of the following options: purchase, inheritance, succession, and rental.

Agriculture

The main crops of the last agricultural cycle are corn, forage corn, beans, oats, wheat, vegetables, barley, and tomatoes, which are primarily grown for livestock and family consumption.

Under previous governments, dairy producers received some level of support for the fields (*e.g.*, fertilizers or seeds) through direct support programs for agricultural areas such as PROCAMPO or “Producción para el Bienestar” and for the livestock (*e.g.*, new acquisitions, technical assistance, or insemination) from SEDAGRO. The left-wing government that came into power in late 2018 terminated the limited subsidies or support programs available for agricultural producers. Now they only have access to the pension for senior citizens, a program that is not aimed at farming, much less at livestock, and which only benefits small dairy producers who meet the required age.

CONCLUSIONS

Dairy production is a family activity that has been passed on from generation to generation within the town; however, the sons and daughters of small producers believe that the physical and economic costs of continuing with this activity are too high and that it does not generate a “decent” income that would allow them to have a better quality of life. Instead, they look for a more secure income, based on non-agricultural activities. The current government has clearly abandoned small dairy producers to their fate. The lack of an internal organization shows a total lack of interest, which hinders the creation of a local market that allows them to sell milk or other dairy by-products. This situation has a negative impact on the family economy. Advice and government programs would be necessary to strengthen the capacities of the producers. However, they report that with the

price of MXN\$10.50 per liter of milk established by the SIAP, they would achieve balance and their income would improve.

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Regulatory protection of the production and marketing of honey and its derivatives in Mexico

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ABSTRACT

Objective: To analyze state and protection regulations in the production and marketing processes of honey in Mexico.

Design/methodology/approach: The dogmatic legal method was used to analyze existing beekeeping laws in the states with the highest honey production in Mexico, along with secondary regulations and public policies related to the production and marketing of honey and its derivatives.

Results: The laws issued are lacking and insufficient to ensure the protection of the marketing of honey and its derivatives.

Limitations of the study/implications: It was not possible to analyze the protection of all types of honey due to the absence of specific regulations. Production records are subjective and imprecise, resulting in the exclusion of many producers; consequently, support for production is inadequate. The lack of regulation and specific legal frameworks threatens the livelihoods of rural communities where honey is produced.

Findings/Conclusions: It is necessary to enact a federal law to regulate the production and marketing processes of honey and its derivatives, incorporating schemes that value the products, types of honey, and traditional knowledge of indigenous peoples and producers.

Keywords: legal framework, agri-food heritage, sustainability, SDGs.

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INTRODUCTION

Bees provide multiple essential ecosystem services, such as biodiversity conservation, ecological stability, and pollination. They also produce apicultural products like honey and its derivatives, which have a significant impact on the economic well-being of rural communities [1-3]. Mexican honey is highly valued in international markets due to its unique organoleptic and sensory properties [4], a result of the country's rich diversity of nectar-producing flowers [5]. Notable examples include the creamy honey from the Central Highlands, citrus blossom honeys (*Citrus sinensis*) from Veracruz and Tamaulipas,

campanita honey (*Ipomoea triloba*) typical of Oaxaca, Puebla, and Guerrero, and aromatic mangrove honeys (*Rhizophora mangle*) from the Yucatán Peninsula. This floral diversity has given Mexican honey a competitive advantage, positioning the country as the ninth largest producer and sixth largest exporter globally [6]. According to data from the Secretariat of Agriculture and Rural Development (SADER) [6], by the end of 2023, Mexico reached a production of 58,033.18 tons of honey, generating an estimated income of \$2,522,534,377.00 MXN, reflecting the economic significance of this activity within the national livestock sector [7]. The National Atlas of Bees and Apicultural Products [8] highlights that beekeeping in Mexico holds high social and economic value, being considered a viable alternative for fostering development in vulnerable rural communities [9-10]. This activity not only contributes to job and income generation but also serves as an important source of foreign exchange for the country [5]. Furthermore, apicultural production represents a sustainable way of life, as it relies on non-invasive ecosystem management without generating significant greenhouse gas emissions [11]. Thus, it aligns with the principles of Sustainable Development Goal (SDG) 12: “Responsible Consumption and Production,” contributing to the achievement of the 2030 Agenda [12]. The aim of this study was to analyze the Mexican legal framework related to the production and commercialization of honey nationwide, with the aim of identifying existing legal mechanisms for the protection, regulation, and strengthening of this activity and its derivatives.

MATERIALS AND METHODS

Territorial delimitation

For the regulatory analysis, the states with the highest contribution to national honey production were considered, based on consolidated data from the end of 2023 [7]. The nine selected states were: Yucatán (16.28% of national production), Chiapas (10.15%), Jalisco (10.00%), Veracruz (9.03%), Oaxaca (8.26%), Campeche (7.94%), Quintana Roo (4.70%), Puebla (4.11%), and Guerrero (3.64%). From this selection, those states with current apiculture legislation were identified: Yucatán [13], Campeche [14], Jalisco [15], Veracruz [16], Quintana Roo [17], and Guerrero [18]. Once the corresponding state laws were collected, an analysis was conducted. Given that this was an exploratory, non-experimental study, the dogmatic legal method was used, based on the theory of positive law, also referred to as objective law [19]. This approach focuses on the systematic analysis of legal norms to interpret their content, understand the legislator’s intent, and propose solutions to concrete legal issues not only through normative description but also by proposing interpretative alternatives [20,21]. The information was organized into double-entry tables. First, data on the states with current apiculture laws were systematized. Second, a search and review were conducted for complementary regulations corresponding to those state laws. This search revealed that only Yucatán [22] and Campeche [23] have complementary regulatory provisions. A comparative analysis of the identified legal frameworks was then carried out, highlighting commonalities between existing laws and regulations. Special attention was given to innovative provisions offering explicit protection mechanisms for apiculture production. Additionally, federal-level regulatory instruments applicable to the production and marketing of honey were reviewed. These included the current Mexican Official

Standards (NOMs), the National Atlas of Bees and Apicultural Products [8], the National Strategy for the Conservation and Sustainable Use of Pollinators (ENCUSP) developed by SADER and SEMARNAT [24], the Manual of Good Livestock Practices in Honey Production [25], and the Manual of Best Practices in Honey Handling and Packaging [26]. Finally, a content analysis of the applicable regulations was conducted to identify the relevant legal elements affecting the processes of production, handling, transformation, and commercialization of honey and its derivatives.

RESULTS AND DISCUSSION

Six states have beekeeping laws that regulate the general aspects of production processes: Yucatán, Campeche, Jalisco, Veracruz, Quintana Roo, and Guerrero. In contrast, Chiapas, Oaxaca, and Puebla do not have specific beekeeping laws.

In Chiapas, production is regulated under the Law for the Promotion and Health of Livestock, which addresses the protection of livestock activities, control of livestock registries, promotion of organic production, and genetic improvement of bees. It also includes procedures for engaging in organic beekeeping. Oaxaca regulates production through the Livestock Law, which includes provisions related to the establishment of apiaries and their appropriate distances, the obligations of beekeepers, use and protection of beekeeping areas, and pollination services. The law also outlines the responsibilities of the Secretariat regarding honey production and marketing. Puebla regulates beekeeping through the State Livestock Law, emphasizing the establishment of apiaries and prioritizing training, transformation, and innovation in the sector. It promotes support mechanisms for the implementation and coordination of production chains (Table 1).

A review of the beekeeping laws in the six states with specific legislation (Yucatán, Campeche, Jalisco, Veracruz, Quintana Roo, and Guerrero) revealed several common

Table 1. Laws that regulate the honey production process in Chiapas, Oaxaca and Puebla.

| Main topics addressed in state laws | | | |
|-------------------------------------|---|---|--|
| | Chiapas | Oaxaca | Puebla |
| Organization of beekeeping | Regulate, protect and promote livestock activity. Promote the sustainable development of production, health, safety, quality, classification, control of the mobilization and marketing. Control of the National Livestock Register. Organic production. Genetic improvement of the bee and control of Africanization | Genetic improvement of queen bees. Promotion of markets for livestock products in international markets, providing price details within the different markets. General topics of apiary installation and pollination services. Prohibition of insecticides or pesticides. | Establishment of apiaries; rules for family apiaries. Promotion of productive chains, which serve as a boost to livestock activity. Promotion of training, research and technology transfer among beekeepers in the state, promoting the participation of livestock organizations, educational institutions and those who carry out beekeeping activities. |
| Beekeeping production articles | 19 articles | 24 articles | 08 articles |

Source: Own elaboration based on the livestock laws of the States of Chiapas, Oaxaca and Puebla.

elements. Shared aspects include the regulation of exploitation methods, commercialization, and development of beekeeping products; guidelines for the establishment of apiaries; promotion of bee genetic improvement; frameworks for organizing producers; specific rules for the commercialization and certification of honey and its derivatives; certification of collection centers; and the promotion of specialized research centers in apiculture, among other key points. Each state law also includes specific elements that enhance the valorization of honey. Yucatán focuses on ensuring product safety, establishing advisory mechanisms for beekeepers, and mandating the relevant Secretariat to promote beekeeping, local consumption, and organic honey certification. Campeche prioritizes market access and the creation of a Research Center aimed at training specialized technicians to strengthen technology transfer to producers. Jalisco highlights the central role of beekeeping organizations in bolstering the rural economy, promoting technological exchange as a tool to increase production. Veracruz supports apiculture research and development through the establishment of research centers and ensures that extraction and packaging facilities comply with Official Mexican Standards. Quintana Roo promotes the valorization of apiculture products by strengthening marketing channels, certifying collection centers, products, and by-products, and providing ongoing beekeeper training. It also incorporates Mayan meliponiculture principles as cultural and economic heritage. Guerrero, through its Law No. 393 for Beekeeping Promotion, assigns the state's Secretariat of Economy the execution of programs targeting agro-industrial development, including honey collection, processing, and marketing. It also promotes producer organization under a business-oriented vision, encourages strategic alliances, and proposes financial tools to support the self-management of beekeeping organizations, emphasizing product quality and safety as well as its positioning in local, national, and international markets. Among the six states mentioned, only Yucatán and Campeche have regulations that complement their beekeeping laws (Table 2). Both include provisions regarding the procedures and requirements for the establishment of apiaries and the registration of beekeepers into recognized organizations. Specifically, Yucatán's regulation mandates the competent Secretariat to oversee laboratories that issue official certifications and manage transit permits for honey and its derivatives. However, it omits critical aspects such as product safety, technical assistance to beekeepers, and the promotion of honey consumption. Meanwhile, Campeche's beekeeping regulation outlines strategies to enhance access to

Table 2. Provisions of the regulations of the beekeeping laws of the states of Yucatán and Campeche, Mexico.

| Regulations of beekeeping laws | |
|--|---|
| Yucatan | Campeche |
| Corresponding procedures to be able to install apiaries. | Procedures necessary for the installation of apiaries. |
| The Secretariat will register the laboratories that are dedicated to the certification of bee products in the State. | Requirements to be a member of beekeeping organizations |
| Requirements to be a member of beekeeping organizations. | |
| Processing transit guides to market honey and byproducts. | |

Source: Own elaboration based on the livestock laws of the States of Chiapas, Oaxaca and Puebla.

local, national, and international markets, as well as the creation of the aforementioned Apiculture Research Center.

Although each state has implemented strategies tailored to its specific needs, these efforts remain insufficient due to the lack of clear and territorially grounded regulations. The dogmatic legal analysis conducted was based on the following standards: NOM-004-SAG/GAN-2018, NOM-145-SCFI-2001, NOM-001-ZOO-1994, and NOM-002-SAG/GAN-2016.

From another perspective, the analysis of public policies issued by the Federal Government helped identify key documents such as the Manual of Good Livestock Practices in Honey Production, which establishes guidelines to reduce contamination risks throughout the various stages of production, aiming to obtain a safe, high-quality product. This manual also lays the technical foundations for organic beekeeping. Likewise, the Manual of Good Practices in the Handling and Packaging of Honey outlines requirements for handling and packaging honey, including facility design standards, production stages, traceability, organic honey certification norms, and commercialization criteria. However, both manuals are mainly oriented toward large-scale packaging plants and do not address the specific needs of small-scale producers. On the other hand, the National Strategy for the Conservation and Sustainable Use of Pollinators (ENCUSP) aims to preserve honey-producing bees for the benefit of society, ecosystems, and biodiversity. This strategy is based on the guiding principles of the 2019-2024 National Development Plan, particularly the principle of “Leave no one behind, leave no one out,” with emphasis on respecting Indigenous peoples, their customs, their right to self-determination, and the preservation of their territories. This vision promotes the strengthening of a legal and regulatory framework that supports the protection of pollinators as an essential component of ecosystem services. Finally, the National Atlas of Bees and Beekeeping Products serves as a relevant information source, addressing key issues for those engaged in honey and beekeeping product production. It is a vital reference tool for strengthening national apiculture.

CONCLUSIONS

Beekeeping in Mexico holds high ecological, social, and economic value, yet faces significant regulatory barriers. Although some states have beekeeping laws and there are related official standards (NOMs), there is no federal law that comprehensively regulates bee protection, honey production, certification, or commercialization of honey and its byproducts. The current framework is limited, declarative, and lacks operational capacity. It fails to adequately address products such as beeswax, propolis, or royal jelly, missing their potential in key sectors like pharmaceuticals and cosmetics. Furthermore, it overlooks native bees such as *Melipona*, whose honey possesses unique characteristics and cultural significance, especially in Mayan communities.

Regarding current public policies, these are mainly oriented toward industrial-scale production, excluding small-scale beekeepers who sell through informal channels without quality control, traceability, or safety standards, thereby posing health risks and economic losses. For these reasons, it is essential to strengthen the legal framework to include the various types of honey, recognize traditional knowledge, and promote more

equitable and sustainable value chains. A comprehensive and inclusive regulation would help improve rural incomes and contribute to the achievement of several Sustainable Development Goals.

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Cost-benefit of implementing a vaccination and deworming program in dual-purpose production units in Veracruz tropics

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ABSTRACT

Objective: To evaluate the cost-benefit of implementing a Vaccination and Deworming Program (PdVyD) in commercial farms in the central region of Veracruz, Mexico.

Design/Methodology/Approach: Based on a survey, information on livestock health problems and practices was collected from eight farms located in six municipalities in this region.

Results: No farmer has an adequate program for controlling livestock diseases, so it would be useful to rely on a PdVyD. Cattle deaths were recorded in all farms. Average cost per head in Mexican pesos (MXN/head) of medicines was grouped in four categories: A) Vaccines for reproductive diseases, \$49.85; B) Bovine rabies vaccine, \$22.27; C) Clostridiasis vaccines, \$22.63; and D) Dewormers, \$21.22. The total cost of a PdVyD for a medium size farm (70 head) was \$8,655 a year; in this, the Vaccination Program (PV, groups A, B, and C) accounts for \$6,633 (76.6%) a year, and the Deworming Program (PD) (group D) for \$2,022 (23.4%) a year. For a small size farm (25 head), the total cost of PdVyD was \$3,128 a year, broken down into \$2369 (75.7%) for PV, and \$759 (24.3%) for PD.

Study Limitations/Implications: The analysis is focused on the last year of operations and only considers eight production units.

Findings/Conclusions: Practices for preventing diseases among the participants lacks regularity. The cost of a PdVyD is lower than livestock losses, even without considering losses in productive efficiency due to diseases. The implementation of a PdVyD requires to improve farms management and promotion among producers by the technicians.

Key words: animal health, bovine cattle, planning, prevention, profitability.

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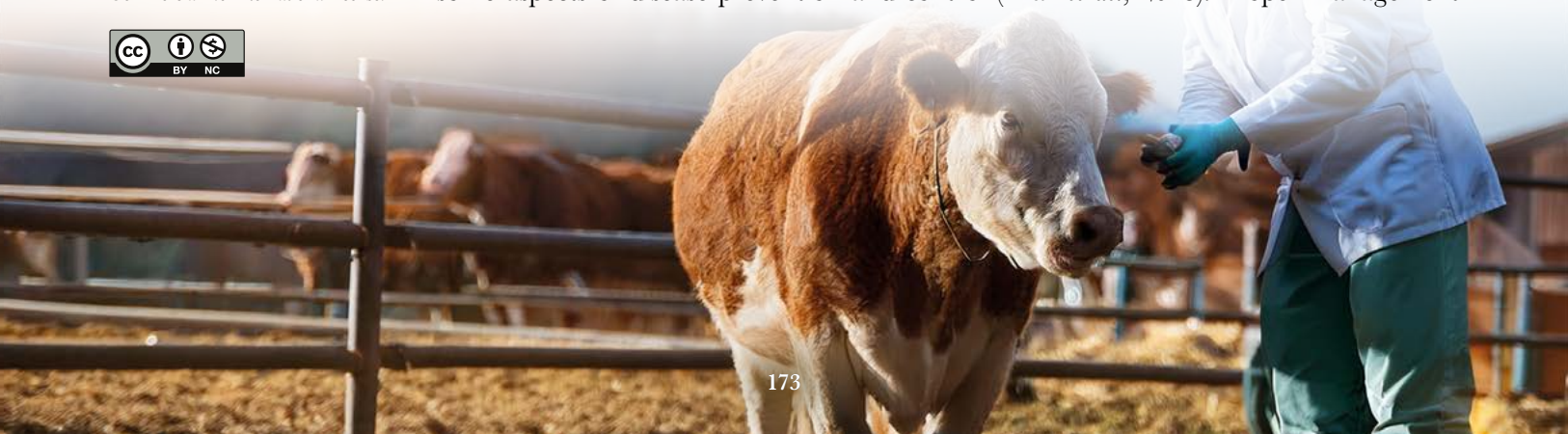
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INTRODUCTION

Among the main limitations of bovine production in the tropics are health-related problems, due to ignorance (or poor knowledge) of both producers and technicians in some aspects of disease prevention and control (Díaz *et al.*, 2018). Proper management



should include disease control in pregnant cows, heifers, bulls, and calves. In all stages and conditions, it is essential to implement a preventive medicine management plan. Certainly, mass-vaccination campaigns have lowered the incidence of diseases but wider eradication and prevention campaigns are required (Younger, 2017). In this context, deworming and vaccination against high incidence and/or very aggressive diseases plays a fundamental role. These actions must be carried out through a calendar that considers the periodic application of biologicals for those diseases that require it, considering the time of year and the composition of the herd (Abad *et al.*, 2016).

Kappes *et al.*, 2023 points out to the existence of a gap in knowledge related to the global burdens of animal diseases and their impacts on safe and efficient production systems. There are studies that estimate the annual cost of preventive measures and the annual cost of some diseases, such as brucellosis in cattle in Colorado (Salman *et al.*, 1990) and in Chile and Argentina (Gallacher, 2010); in turn, Solórzano Thompson *et al.* (2020) calculated the social economic benefit of disease prevention in animals and their effect on public health in Costa Rica. In Mexico, previous studies on benefit-cost analysis of animal health programs are scarce (Xolalpa *et al.*, 1993; Guerrero *et al.*, 1995; Xolalpa *et al.*, 2004; Hernández-Hernández *et al.*, 2013) and completely absent regarding vaccination and deworming programs for dual-purpose livestock. Consequently, as Rodríguez-Vivas *et al.* (2017) mention, when talking on livestock health problems, the first challenge is precisely how to estimate the economic loss in productivity due to this cause, particularly at the herd-level. Due to the aforementioned, this document evaluates the cost of implementing a vaccination and deworming program in commercial farms in the central region of Veracruz, Mexico.

MATERIAL Y METHODS

Location

The field work stage of this study was carried out from January to June 2023 in the Sotavento region, which is located on the coastal plain of the central region of the state of Veracruz Mexico (17° 03', 22° 27' N and 93° 36', 98° 36' W).

Study design

Eight cooperating farmers residing in Manlio Fabio Altamirano, Jamapa, Soledad de Doblado, Camarón de Tejada, Zentla, and Comapa were identified, all raising cattle in a dual-purpose system. Farms were classified according to the methodology proposed by Trejo and Floriuk (2010), namely small (<60 cows), medium (61-200 cows), and large (>200 cows). A survey on social and economic aspects of the farm was applied to them, focused on the costs of animal health (vaccines and dewormers), type of livestock feeding, reproduction management, level of integration/development, and livestock inventory. In addition to herd size, the number of livestock deaths was recorded, and indirectly the cost of a vaccination and deworming program (PdVyD) was estimated. A second survey was applied to 10 suppliers of veterinary products and services, and to providers of animal production services, to know the commercial price for the different products used, and thus to carry out the cost analysis of the proposal for a PdVyD program.

Cabinet phase

The information collected was captured in an electronic Microsoft Excel™ spreadsheet and analyzed using descriptive statistics. A qualitative analysis of the responses to livestock management practices in each farm was developed, especially in relation to vaccination and deworming practices, as well as the number of livestock deaths.

PdVyD costs computation

Based on each drug cost, the cost for each group was calculated, taking into account the doses and the number of times per year in which vaccines and dewormers are administered. First, the unit cost of the dose was calculated according to each drug. Vaccines against reproductive diseases are made from active or modified virus, and applied according to the reproductive stage of the females, following the recommendation of the laboratories. The other vaccines are administered at identical doses, regardless of the size and age of the animal, therefore, the cost is identical in all stages. In the case of dewormers, the dose depends on the weight of the animal. Three sizes of animals were considered: calf = 40 kg, yearling = 200 kg, and adult = 500 kg. To estimate the cost of the PdVyD for a small and medium farm, the average values of calf, yearling, and cow were taken as a basis. Two examples of farms were selected. A medium farm with a 70 heads herd (farm 7 in Soledad de Doblado) and a small one, with 25 heads (farm 3 in Camarón de Tejada). In order not to underestimate the cost per program, in each case the average commercial value was assigned for each production stage. The PdV cost was obtained by multiplying the number of heads in each category by the corresponding unit cost per head of each program. The sum of both programs resulted in the total cost of the PdVyD by type of producer.

RESULTS AND DISCUSSION

Farms management

The sample size is small, but this is a consequence of the fact that it is not common in the region to routinely implement a disease control program. A high proportion of producers carry out sanitary practices very sporadically, without programming. In the farms under study, crosses of *Bos taurus* cattle (Swiss, Holstein, and Angus) with *Bos indicus* (Indubrasil, Brahman, Gyr) in different proportions are the most common view, as well as *Bos indicus* of various breeds. Two of the farms are medium-sized (61 to 200 heads) and the rest are small (≤ 60 heads). All of them use Bull's Front (*Paspalum notatum*), Pangola (*Digitaria decumbens*), Insurgente (*Brachiaria brizantha*) and Paral (*Brachiaria mutica*) for grazing. Only one farm utilizes electric fencing and the rest resorts to extensive grazing with fixed fences. They all provide food supplement during the dry season and have their own breeding stock. In seven farms they use bulls, only in one case does artificial insemination and bull is employed. Most hire technical assistance service for each event that occurs, and only one uses veterinary services periodically. The farms place their products in domestic markets, but only part of the production of one of them is exported.

Animal health status

Livestock deaths occurred in all farms, with this problem concentrating on calves and some cows, being the main cause of infectious diseases. In all farms, cattle are vaccinated and dewormed, although not based on a PdVyD; even though all participants see the advantage of having a defined vaccination and deworming program. In this sense, Luna *et al.* (2007), in a study in Costa Rica, found that endoparasiticides are frequently used inappropriately, since producers usually select the drugs based on their own criteria and without a veterinarian's advice. Table 1 presents drug categories considered in the Vaccination and Deworming program (PdVyD) grouped according to the disease to be prevented. The first group include vaccines that protect against: infectious bovine rhinotracheitis (IBR), bovine viral diarrhoea virus (BVD) (Types 1 and 2), parainfluenza virus 3 (PI3), bovine respiratory syncytial virus (BRSV), and some strains of *Leptospira* spp. Bovine rabies vaccines are highly effective (Yakobson *et al.*, 2015), and even short-term antibody response produce titers over one year later, although antibody response to rabies vaccination was influenced by vaccine brand (Gilbert *et al.*, 2015). Group C includes biologics used for the prevention of symptomatic charcoal, malignant edema, infectious necrotic hepatitis, enterotoxemias, myositis, pasteurellosis, *Mannheimia haemolytica* and *Histophilus somni* infections. The dewormers used basically belong to three chemical families: imidazothiazoles, benzimidazoles, and macrocyclic lactones (Riviere and Papich, 2018)

Cost of the vaccination and deworming program

Table 2 shows the costs by type of medication for vaccinating and deworming. The products shown are only an example, and in no way represent an endorsement for any particular brand or product. The estimated prices are the prices of the products offered to the consumer in veterinary pharmacies in the study area.

Based only on PdVyD averages, the vaccine for reproductive health represented the largest investment; the other two vaccines groups had very similar values (Table 3). In this regard, Gudiño (2019) mentions that reproductive diseases receive the least attention, when the opposite should be the case, since without cows in optimal conditions the long-term viability of livestock farming is put at risk. The vaccination program was 4.46 times more expensive than the deworming program, since the average cost per vaccinated animal was \$94.75 MXN (81.75%) *versus* \$21.22 MXN (18.25%) per dewormed animal.

Table 1. Products in the Vaccination and Deworming Program (PdVyD) of farmers in central Veracruz, Mexico grouped according to type of products used.

| Category | Description | Group |
|----------|--|-------|
| 1 | Vaccines for diseases affecting reproduction | A |
| 2 | Vaccine against bovine rabies | B |
| 3 | Vaccines for Clostridium diseases in cattle | C |
| 4 | Dewormers for bovine cattle | D |

Source: Table developed by authors based on information provided by farmers.

Table 2. Unit costs of products considered for bovine cattle PdVyD in the central region of Veracruz, Mexico. Amounts in Mexican pesos (MXN).

| Group | Product | Id | Calf \$MX | Yearling \$MX | Cow \$MX |
|-------|------------------------------|----|-----------|---------------|----------|
| A | Bovilis vista once SQ | 1a | 47.92 | 47.92 | 47.92 |
| | Bovilis vista 5 l5 SQ | 1b | 44.50 | 44.50 | 44.50 |
| | Bovi-Shield Gold FP5L5 | 1c | 47.40 | 47.40 | 47.40 |
| | Bovimmune Protector L5 | 1d | 59.40 | 59.40 | 59.40 |
| | Average cost | | 49.85 | 49.85 | 49.85 |
| B | Vacuna Biozoo | 2a | 27.93 | 27.93 | 27.93 |
| | Derrisan Sanfer | 2b | 18.93 | 18.93 | 18.93 |
| | Vac-Rabat-Bovis_Virbac | 2c | 19.94 | 19.94 | 19.94 |
| | Average cost | | 22.27 | 22.27 | 22.27 |
| C | Biovac 11v_Biozoo | 3a | 19.36 | 19.36 | 19.36 |
| | Once vías_Gortie | 3b | 28.95 | 28.95 | 28.95 |
| | Adbac_Adler | 3c | 19.94 | 19.94 | 19.94 |
| | Average cost | | 22.63 | 22.63 | 22.63 |
| D | Cydetin_Zoetis | 4a | 3.01 | 15.04 | 37.60 |
| | Valbazen 10%_Zoetis | 4b | 7.40 | 37.00 | 92.50 |
| | Virbamec_Virbac | 4c | 1.27 | 6.36 | 15.90 |
| | L-vermizol vit_Aranda | 4d | 3.33 | 16.63 | 41.58 |
| | Iverfull-Macrovit ADE_Aranda | 4e | 2.20 | 11.01 | 27.53 |
| | Average cost | | 3.44 | 17.21 | 43.02 |

Source: Table developed by authors based on information provided by farmers.

Table 3. Average cost by product group and cattle size. Amounts in Mexican pesos (\$MXN).

| Item | Average Cost, \$MXN | Calf, \$MXN | Yearling, \$MXN | Cow, \$MXN |
|---------------|---------------------|-------------|-----------------|------------|
| Reproduction | 49.85 | 49.85 | 49.85 | 49.85 |
| Bovine rabies | 22.27 | 22.27 | 22.27 | 22.27 |
| 11 ways | 22.63 | 22.63 | 22.63 | 22.63 |
| Dewormers | 21.22 | 3.44 | 17.21 | 43.02 |
| | | 98.19 | 111.96 | 137.77 |

Source: Table developed by authors based on information provided by farmers.

Table 4 presents the unit cost per head of both programs for farms of different sizes. Erb (1988) points out the antagonism between the interests of the veterinarian and those of the livestock farmer. The veterinarian provides important data for economic decision-making, such as information on the probability of disease, its costs, and available control programs, and can help structure a formal cost-benefit analyses; in contrast, the livestock farmer has a certain degree of risk aversion and a variable willingness to sacrifice profits in favor of personal satisfaction. The conflict between these decisions influences the decisions of which products to use and at what stages the vaccines will be administered.

Table 4. Average unit cost of Vaccination and Deworming Programs for a medium and small cattle farm. Amounts in \$MXN.

| Cattle stage | Heads | Unit cost per head | PdV | Unit cost per head | PdD |
|-------------------------|-------|--------------------|----------|--------------------|----------|
| Medium size farm | | | | | |
| Bulls | 2 | 94.75 | 189.50 | 43.02 | 86.04 |
| Steers | 0 | 94.75 | 0.00 | 17.21 | 0.00 |
| Bull calf | 10 | 94.75 | 947.50 | 3.44 | 34.40 |
| Female calf | 15 | 94.75 | 1,421.75 | 3.44 | 51.60 |
| Yearlings | 11 | 94.75 | 1042.25 | 43.02 | 473.22 |
| Heifers | 0 | 94.75 | 0.00 | 43.02 | 0.00 |
| Cows | 32 | 94.75 | 3,032.00 | 43.02 | 1,376.64 |
| Total | 70 | | 6,633.00 | | 2,021.90 |
| Grand Total | | | | | 8,654.90 |
| Small size farm | | | | | |
| Bulls | 0 | 94.75 | 0.00 | 43.02 | 0.00 |
| Steers | 0 | 94.75 | 0.00 | 17.21 | 0.00 |
| Bull calf | 5 | 94.75 | 473.75 | 3.44 | 17.20 |
| Female calf | 3 | 94.75 | 284.25 | 3.44 | 10.32 |
| Yearlings | 0 | 94.75 | 0.00 | 43.02 | 0.00 |
| Heifers | 2 | 94.75 | 189.50 | 43.02 | 86.04 |
| Cows | 15 | 94.75 | 1,421.25 | 43.02 | 645.30 |
| Total | 25 | | 2368.75 | | 758.86 |
| Grand Total | | | | | 3,127.61 |

Source: Table developed by authors based on information provided by farmers.

In a small-scale farm, the total cost does not exceed \$3,500 MXN a year, so this investment may be considered as good since the PdVyD implementation prevents the death of more than a single head. In the case of the medium-size farm, the annual investment of \$8,500 MXN is comparable to selling a 180 kg calf. Of course, some considerations must be made regarding the aforementioned budgets. For instance, product prices may vary in a period of time as short as a month or less, and this may significantly modify the figures shown.

Vaccination and deworming schedule

Any vaccination and deworming schedule is an administrative tool to prevent and minimize the impact of the most common diseases, which is why it constitutes more of an investment than an expense. For developing a preventive calendar, Quiroz and Rodríguez (2012) propose including a series of basic measures that must be present in all farms in a region, as well as others that basically depend on the production system and the health history of each farm. Successful disease prevention is strongly associated with good management of quality food and water sources for livestock (Cruz and Romero, 2018). There are many health activities that can be carried out throughout a year. A schedule helps to organize and to prepare the yearly activities to serve as a base for future planning,

and also helps to remind the manager to perform the required tasks at specific times of the year and serve as a record. Basic yearly management activities and timing depend upon each particular farm goals, but the time in which management steps must be completed is important for cattle health and efficiency to ensure that all needed tasks are accomplished and avoid unfavored weather conditions that may induce illness and problems (Prewitt, 2015; Rodricks and Singh, 2024). Table 5 presents a proposed vaccination and deworming schedule that is recommended for cattle in the central region of the Veracruz tropics.

Table 5. Proposed vaccination and deworming schedule for cattle in the central region of the state of Veracruz, Mexico.

| Activities | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|-------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Deworming, adults | | | | | X | | | | X | | | |
| Deworming, calves | | | | | X | | | | X | | | |
| Deworming, yearlings | X | | | | X | | | | X | | | |
| Vaccination, 11 ways | | X | X | | | | | | | X | | |
| Vaccination, IBR, DVB Leptospirosis | | | | | | | | | | X | | |
| Bovine rabies vaccination, adults | | | | | X | | | | | | | |
| Bovine rabies vaccination, calves | X | | | | X | | | | | X | | |

Source: Table developed by authors based on information provided by farmers.

CONCLUSIONS

This study identified areas of opportunity related to the design and operation of a vaccination and deworming program and the demonstration of the benefit of estimating its cost and derived benefits. Producers are willing to carry out a vaccination and deworming programs in their farms, but they do not develop it due to lack of good planning and advice. The loss due to death of animals or delay in fertility is huge compared to the economic benefit of using dewormers and vaccines in a timely manner. The proposal of this program requires improvements in the comprehensive management of resources in the farm, and the ability of technicians to gain trust and convince cattle farmers.

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Bio-Inspired Optimization of Convolutional Neural Networks for Enhanced Maize Disease Detection in Precision Agriculture

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ABSTRACT

Objective: This study develops an automated, image-based system for early detection of four key maize diseases: *Puccinia sorghi*, *Cochliobolus carbonum*, *Bipolaris maydis*, and *Exserohilum turcicum* using Convolutional Neural Network (CNN) optimized through bio-inspired algorithms.

Design/methodology/approach: A dataset of 17,280 high-resolution images across six disease stages was preprocessed and used to train CNNs. Two metaheuristic algorithms, Spider Monkey Optimization (SMO) and Squirrel Search Algorithm (SSA), were applied to optimize weights and hyperparameters. An 80/20 training-validation split was used, and performance was assessed with standard classification metrics.

Results: The SMO-optimized CNN outperformed SSA, achieving 95.14% accuracy versus 89.74%. SMO also yielded better precision, recall, and F1-scores, showing strong performance even in distinguishing visually similar symptoms.

Study Limitations/Implications: SMO's computational demands may limit its usability in low-resource settings. Some classification confusion persisted, highlighting the need for improved feature extraction and broader datasets for increased generalization.

Findings/conclusions: CNNs optimized with SMO provide a robust tool for maize disease diagnosis, reducing analysis time and enabling more precise crop management. Future work will explore hybrid optimization methods to enhance scalability and real-time application in precision agriculture.

Keywords: Convolutional Neural Networks, Maize Disease Detection, Intelligent Precision Agriculture.

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INTRODUCTION

Maize (*Zea mays* L.) is a staple crop with significant global economic and nutritional relevance, particularly in regions where it underpins food security. Despite its importance, maize production is increasingly undermined by plant diseases, notably Common Rust (*Puccinia sorghi*), Gray Leaf Spot (*Cochliobolus carbonum*), Asphalt Spot (*Bipolaris maydis*), and Northern Corn Leaf Blight (*Exserohilum turcicum*). These pathogens disrupt photosynthetic processes and compromise overall plant health, often leading to considerable yield reductions (Pereira *et al.*, 2019; Sharma *et al.*, 2017).

Conventional approaches to disease diagnosis in the field, such as visual scouting by experts, are laborious, time-intensive, and subject to subjective error. Their effectiveness is further constrained in large-scale or resource-limited agricultural systems (Haug *et al.*, 2020). In recent years, advances in artificial intelligence (AI) and computer vision have paved the way for automated, image-based plant disease diagnostics. Among these, Convolutional Neural Networks (CNNs) have emerged as a particularly effective framework for high-accuracy classification tasks in agriculture (LeCun *et al.*, 2015; Goodfellow *et al.*, 2016).

CNNs can extract layered visual features directly from raw images, eliminating the need for manual feature engineering. Their capacity to capture subtle disease symptoms in complex agricultural scenes makes them well-suited for crop diagnostics (Krizhevsky *et al.*, 2012). However, CNN performance is highly sensitive to hyperparameter tuning and weight optimization, processes that are computationally demanding and prone to local minima.

To enhance training efficacy, bio-inspired optimization algorithms —such as Spider Monkey Optimization (SMO) and Squirrel Search Algorithm (SSA)— have been integrated into CNN frameworks. These algorithms replicate animal foraging behavior to more effectively navigate high-dimensional solution spaces, thereby improving convergence and predictive accuracy (Ali *et al.*, 2019; Mirjalili *et al.*, 2014).

This study presents a CNN architecture optimized with SMO and SSA to detect four major maize diseases. A dataset of 17,280 annotated images spanning multiple disease stages was employed. The central aim is to evaluate the efficacy of these optimization methods in enhancing CNN-based diagnostics and supporting the broader goals of precision agriculture.

MATERIALES Y METHODS

The method section of this study is based on Computer Vision, a field of AI that enables machines to interpret and understand visual data, similar to human vision. This technology automates tasks like image classification and object detection. In agriculture, it helps detect and classify crop diseases. This study uses a Computer Vision System to analyze maize leaf images and identify diseases. The system involves several steps: image acquisition with high-resolution cameras, preprocessing for noise reduction, feature extraction using CNNs, and classification to detect diseases. Post-processing refines the results, providing actionable recommendations, such as applying fungicides to prevent disease spread (Haug *et al.*, 2020).

Computer vision is a field within computer science and artificial intelligence that enables machines to interpret and analyze visual data, similar to how humans use their eyes and brain. Through specialized algorithms and models, computers can extract meaningful information from images and videos. This technology is widely applied in industries such as robotics, healthcare, and automotive systems, supporting tasks like image classification, object detection, and facial recognition (Szeliski, 2010).

Computer Vision System

A Computer Vision System (also referred to as an Artificial Vision System) is designed to replicate the human ability to perceive, interpret, and analyze visual data from the world,

enabling machines to make decisions based on visual input. These systems are increasingly used for a wide range of tasks, from image classification to object detection and facial recognition (Szeliski, 2010). The components of a computer vision system typically include the following stages:

Image Acquisition

The first step in a computer vision system is the acquisition of visual data, typically through cameras or other imaging devices. The quality and type of devices used depend on the specific application. These devices can range from high-resolution digital cameras and infrared cameras to specialized sensors. The images captured in this stage serve as the raw data for subsequent processing and analysis (Gonzalez & Woods, 2008).

Preprocessing

Preprocessing prepares images for analysis by enhancing quality and consistency. It includes:

- **Noise Reduction:** Filters like Gaussian blur remove visual artifacts that may obscure key features (Jain, 1989).
- **Normalization:** Adjusts brightness and contrast to standardize images under varying lighting conditions.
- **Resizing and Cropping:** Focuses on the region of interest (ROI) and reduces computational cost.
- **Data Augmentation:** Expands the dataset using rotations, flips, scaling, and color adjustments to improve model robustness (Perez & Wang, 2017).

Building Model

Feature Extraction

Once the image is preprocessed, the next step is featuring extraction. In traditional computer vision, the system identifies relevant visual patterns. While traditional methods relied on manual selection of features (edges, textures, shapes), deep learning—particularly Convolutional Neural Networks (CNNs)—now automates this process. CNNs detect simple elements in early layers and learn more complex, disease-specific patterns in deeper layers (LeCun *et al.*, 2015).

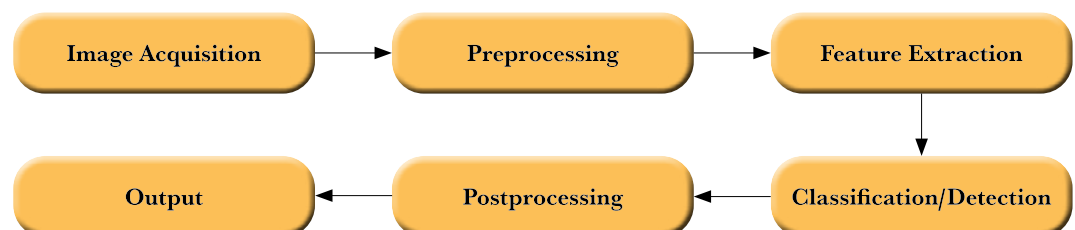


Figure 1. Diagram of Computer Vision System.

Optimization

Classification

After feature extraction, the system moves to the classification or detection phase, where the primary goal is to assign a label to the image or identify specific objects within it. Therefore, the system categorizes the image or identifies specific objects within it:

- Image Classification assigns a disease label to an image (*e.g.*, Common Rust or Gray Leaf Spot).
- Object Detection identifies and localizes diseased areas using bounding boxes.

Advanced systems apply semantic or instance segmentation, which classify pixels individually for more detailed analysis (He *et al.*, 2016; Long *et al.*, 2015).

Post-Processing

Post-processing refines model outputs by:

- Eliminating irrelevant or false detections.
- Smoothing segmentation boundaries to improve visual clarity and result accuracy (Ronneberger *et al.*, 2015).

These steps ensure the outputs are both precise and interpretable.

Output

The final stage generates actionable insights. In agriculture, this might include detecting Northern Corn Leaf Blight and recommending a fungicide. These outputs support real-time decision-making, helping farmers respond promptly and effectively to disease outbreaks (Haug *et al.*, 2020).

Metrics

Evaluating the performance of the computer vision system is critical to ensuring its accuracy in detecting and classifying maize diseases. To achieve this, several key evaluation metrics were employed, including accuracy, precision, recall, F1-score, and a confusion matrix. These metrics provide a comprehensive understanding of the model's classification performance.

Precision

The proportion of true positive predictions among all predicted positives, measuring the model's ability to avoid false positives.

$$Precision = \frac{True\ Positives}{True\ Positives + False\ Positives} \quad (1)$$

Recall

The proportion of true positive predictions among all actual positives, assessing the model's ability to detect all instances of a specific disease.

$$\text{Precision} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}} \quad (2)$$

F1-Score

The harmonic mean of precision and recall, providing a balanced measure of the model's performance.

$$F1 - \text{Score} = 2 \times \frac{\text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}} \quad (3)$$

Proposal Method

Convolutional Neuronal Networks

Convolutional Neural Networks (CNNs) are a specialized class of deep learning models designed to process structured grid data, such as images. Widely used in visual recognition tasks, CNNs excel in image classification, object detection, and semantic segmentation due to their ability to automatically learn hierarchical features from image data (LeCun, Bengio, & Hinton, 2015). In agriculture, CNNs are particularly effective for automating disease identification in crops like maize. By analyzing high-resolution images of leaves, these models can detect and classify plant diseases with high accuracy. Unlike traditional methods that require manual feature engineering, CNNs learn directly from raw pixel data through multiple layers that extract and refine visual patterns (Goodfellow *et al.*, 2016).

CNNs consist of several key layers that perform specific functions:

Step 1. Convolutional Layer

The convolutional layer is the core component of a CNN. It applies filters (also called kernels) that slide across the image to detect local features like edges, textures, and shapes. Early layers capture basic visual elements, while deeper layers learn more complex patterns, including object parts and disease-specific markers.

Step 2. Activation Function

After each convolution operation, an activation function like ReLU (Rectified Linear Unit) introduces non-linearity, enabling the network to model complex patterns. ReLU is commonly used because it accelerates training and helps prevent the vanishing gradient problem (Nair & Hinton, 2010).

Step 3. Pooling Layer

Pooling layers follow convolutional layers and reduce the image's spatial dimensions, lowering computational cost and minimizing overfitting. The most common method,

max pooling, selects the highest value in each region, preserving key information from the feature map.

Step 4. Fully Connected Layer

After several convolutional and pooling layers, the network typically flattens the feature maps into a one-dimensional vector. This vector is passed through one or more fully connected layers, where each neuron is connected to all the neurons in the previous layer. The fully connected layer allows the model to make the final classification based on the extracted features.

Step 5. Softmax Activation

The CNN's output layer commonly uses a softmax activation function for multi-class classification. It assigns probabilities to each class—such as Common Rust or Gray Leaf Spot—allowing the model to determine the most likely maize disease (Krizhevsky *et al.*, 2012).

Spider Monkey Optimization

Spider Monkey Optimization is a bio-inspired algorithm that simulates the cooperative foraging behavior of spider monkeys. As part of the swarm intelligence family, SMO effectively balances exploration (searching new areas) and exploitation (refining promising solutions), making it well-suited for solving complex optimization problems. In SMO, each monkey represents a potential solution, and the group collectively moves toward better options based on shared knowledge and individual experience. The algorithm follows main phases:

- Initialization: A population of spider monkeys is randomly generated.
- Fitness Evaluation: Each solution is assessed using an objective function.
- Position Update: Individuals adjust their positions relative to the leader.
- Leader-Follower Mechanism: Monkeys are guided by the best performer while maintaining exploratory behavior.
- Termination Condition: The process stops when a set number of iterations or a satisfactory solution is reached.

In Spider Monkey Optimization, each monkey's position $X_i = (x_{i1}, x_{i2}, \dots, x_{in})$ represents a potential solution within the search space, where n is the number of decision variables. The fitness function evaluates how well a given position solves the problem.

Monkeys adjust their positions based on the best-known solution X_{best} , using the update formula:

$$X_i^{new} = X_i + r \cdot (X_{best} - X_i) + \varepsilon \quad (4)$$

where: X_i is the current position of the i -th spider monkey. X_{best} is the position of the best solution found so far (the leader). r is a random factor between 0 and 1 that controls the extent to which the monkey moves towards the leader and explores new regions.

This leader-follower dynamic guides the population toward optimal regions while allowing for exploration. Followers move toward the leader but retain randomness in their search, helping the algorithm avoid local minima and discover better solutions over time (Ali *et al.*, 2019).

Optimization of CNN Parameters

In Convolutional Neural Networks, Spider Monkey Optimization is used to fine-tune both weights and hyperparameters. Each monkey's position represents a candidate parameter set, and its performance is evaluated through a loss function $L(W)$, typically categorical cross-entropy:

$$L(W) = \sum_{i=1}^N y_i \log(p_i) \quad (5)$$

where: N is the number of samples in the dataset. y_i is the true label of the i -th sample. p_i is the predicted probability for class y_i from the CNN output.

The fitness function $f(x) = L(W)$ determines how well each parameter set performs. SMO minimizes this loss by exploring the solution space, balancing exploration and exploitation to improve classification accuracy. Despite its computational demands, SMO is highly effective for optimizing CNNs in complex tasks like maize disease detection, making it a valuable tool for precision agriculture and other applied AI fields.

Squirrel Search Algorithm

The Squirrel Search Algorithm is a bio-inspired optimization technique based on the foraging behavior of squirrels. It has proven effective in solving complex problems in fields such as machine learning and artificial intelligence (Mirjalili *et al.*, 2014). In this study, SSA is applied to optimize the parameters of a Convolutional Neural Network for accurate classification of maize diseases, including common rust, gray leaf spot, asphalt spot, and leaf blight. SSA operates in two key phases: exploration, where squirrels search broadly for food (solutions), and exploitation, where they focus on the most promising regions. Each squirrel's position $X_i = (x_{i1}, x_{i2}, \dots, x_{in})$ represents a candidate solution in a d - dimensional space. The position is updated:

$$x_i(t+1) = x_i(t) + \beta(X_{best}(t) - x_i(t)) + \alpha \cdot r(t) \quad (6)$$

where: $x_i(t)$ is the position of the squirrel at time t . $X_{best}(t)$ is the best position found by the squirrels up to time t . β is the step size toward the best solution. $\alpha \cdot r(t)$ is a random vector drawn from a uniform distribution between $[-1, 1]$, which introduces stochasticity in the search process (Mirjalili *et al.*, 2014).

Integration of SSA with Convolutional Neural Networks

Integrating the Squirrel Search Algorithm with Convolutional Neural Networks aims to optimize both weights and hyperparameters to improve classification performance in maize disease detection.

In this approach, each squirrel's position represents a unique set of CNN hyperparameters—such as learning rate, number of layers, filter size, and batch size. SSA explores the search space to identify combinations that minimize classification error or maximize accuracy on a validation dataset.

By applying SSA, the CNN achieves improved accuracy in identifying diseases like common rust, gray leaf spot, asphalt spot, and leaf blight. This nature-inspired strategy enhances the CNN's ability to generalize and adapt, offering a valuable method for optimizing deep learning models in agricultural applications.

Maize

Maize (*Zea mays*) is one of the world's most important staple crops, providing essential nutrients and energy. However, its productivity is frequently threatened by foliar diseases such as Common Rust, Gray Leaf Spot, Asphalt Spot, and Northern Corn Leaf Blight, which reduce photosynthetic efficiency and crop yield if not managed promptly.

RESULTS AND DISCUSSION

The integration of computer vision systems in agriculture marks a crucial advancement in crop health monitoring. As global food demand grows and plant diseases become more prevalent, there is an urgent need for accurate, efficient, and scalable diagnostic tools. Traditional methods—based on manual inspection and expert judgment—are often slow, error-prone, and impractical for large-scale farming.

This study aimed to develop an AI-based system using Convolutional Neural Networks to automatically classify four major maize diseases: *Puccinia sorghi* (Common Rust), *Cochliobolus carbonum* (Gray Leaf Spot), *Bipolaris maydis* (Asphalt Spot), and *Exserohilum turcicum* (Northern Corn Leaf Blight). The following section details the development process of the computer vision model designed for disease detection.

Image acquisition (Data Collection)

The initial step in developing a computer vision system for maize disease detection involves assembling a comprehensive and diverse dataset of high-resolution maize leaf images. In this study, a total of 17,280 images were collected, with 4,320 images representing each of the four target diseases: *Puccinia sorghi* (Common Rust), *Cochliobolus carbonum* (Gray Leaf Spot), *Bipolaris maydis* (Asphalt Spot), and *Exserohilum turcicum* (Northern Corn Leaf Blight). Each disease was documented across six distinct stages of progression, ranging from early infection to advanced symptoms, enabling the model to learn variations in visual patterns over time.

Images were captured using a 12-megapixel wide-angle camera (f/1.6 aperture) equipped with optical image stabilization, ensuring sharp, high-fidelity captures under diverse field conditions. Photographs were taken under controlled lighting and from multiple leaf

orientations to enhance the model's robustness and generalization capability. To ensure broad visual coverage, images were acquired from six different regions of the plant, capturing varied textures, angles, and symptom presentations. Additionally, the dataset was balanced to include both healthy and infected leaves, providing a solid foundation for training a Convolutional Neural Network (CNN) capable of accurate and scalable disease classification in maize.

Preprocessing

Following image collection, preprocessing is a critical step to prepare data for analysis by CNN. The objective is to standardize the data set, reduce noise, and introduce variability to enhance model robustness. In this study, a structured preprocessing pipeline was implemented, incorporating normalization, resizing, and data augmentation to ensure consistency and improve learning performance.

Normalization

To standardize the pixel intensity values, all images were normalized by scaling the values from their original range of 0-255 to a range of 0-1. The normalization was performed using the following formula:

$$Normalized = \frac{Pixel\ Value}{255} \quad (7)$$

Resizing

For consistency and compatibility with the CNN input requirements, All images were resized to 480×480 pixels to standardize dimensions and meet CNN input requirements while preserving visual detail.

Rotations

Images were rotated by 0°, 30°, 60°, 90°, 120°, 150°, 180°, 210°, 240°, 270°, 300° and 330°. This step increases the robustness of the model in recognizing disease patterns from different angles.

Zoom

Images were zoomed in (120%) and out (80%) to simulate distance variability between the camera and the leaf.

Random Cropping

Random subregions were extracted to vary disease positioning, improving model generalization.

Brightness and Contrast Adjustments

Brightness was scaled by 1.2 and 0.8 to reflect different lighting scenarios in the field.

Perspective Transformations

Slight corner distortions simulated camera angle changes, helping the model adapt to different viewpoints.

Flipping

Horizontal and vertical flips added variation in leaf orientation.

Noise Addition

Gaussian noise was introduced to mimic real-world imperfections, enhancing the model's resilience to noisy data.

Feature Extraction Using CNN (Building Model)

The core of the maize disease detection system is a Convolutional Neural Network (CNN), which automatically extracts hierarchical features from raw images. Unlike traditional models requiring manual feature engineering, CNNs learn complex spatial patterns critical for accurate classification.

The architecture consists of multiple layers, each contributing to the feature extraction process. Table 1 summarizes the structure of the network.

To ensure optimal accuracy and efficiency, several hyperparameters were fine-tuned during model development. These include filter size, which determines how much spatial information is captured, and stride, which controls how the kernel moves across the image. Kernel dimensions define the receptive field of each convolutional layer.

By carefully adjusting these hyperparameters based on validation performance, the CNN achieves a balance between classification accuracy and computational cost. This enables practical deployment of the model in real-world maize disease monitoring applications.

Model Training

Once the CNN architecture is established, the next step is training the model on the labeled dataset. The CNN is trained using supervised learning, where the model is provided

Table 1. CNN Architecture Layer by Layer Summary.

| Layer | Output Dimensions |
|-----------------------|-------------------|
| Input Layer | 480×480×3 |
| Conv2D+ReLU | 480×480×16 |
| Max Pooling | 240×240×16 |
| Conv2D+ReLU | 240×240×32 |
| Max Pooling | 120×120×32 |
| Conv2D+ReLU | 120×120×64 |
| Max Pooling | 160×60×64 |
| Flattening | 230,400 |
| Fully Connected Layer | 4 |

with images labeled with the corresponding disease type (or “healthy” for non-diseased leaves). The model learns to map input images to their correct labels by minimizing the loss function during training.

Data Split

The dataset included 17,280 images evenly distributed across four maize diseases: *Puccinia sorghi*, *Cochliobolus carbonum*, *Bipolaris maydis*, and *Exserohilum turcicum*. To ensure reliable model performance, the data was divided into 80% for training (13,824 images) and 20% for validation (3,456 images).

The training set was used to optimize the CNN’s weights, while the validation set provided an unbiased measure of generalization. This strategy helps the model learn disease features effectively while minimizing the risk of overfitting to the training data.

Loss Function

Given the multi-class nature of this classification problem, categorical cross-entropy was selected as the loss function. This function quantifies the discrepancy between the predicted probability distribution and the true label distribution, ensuring the model effectively learns to differentiate between classes. The mathematical definition is provided in Equation 4.

Optimizer

To enhance the performance of the CNN from the outset, three key optimization strategies were employed: Squirrel Search Algorithm and Spider Monkey Optimization.

Weight Initialization

Squirrel Search Algorithm draws inspiration from squirrel foraging behavior to efficiently explore the solution space. It initializes candidate weights and hyperparameters, refining them iteratively based on the categorical cross-entropy loss from the validation set. Spider Monkey Optimization simulates social foraging to fine-tune hyperparameters and weight configurations. By enhancing both global and local search, SMO helps the model converge toward an optimal setup for maize disease classification.

Hyperparameter Tuning

In addition to optimizing the weights, the SSA and SMO played crucial roles in tuning key hyperparameters of the CNN. These algorithms explored various configurations of:

- **Learning Rate:** The Squirrel Search Algorithm and Spider Monkey Optimization were essential in fine-tuning the learning rate, dynamically exploring configurations to balance convergence speed and training stability. During the optimization phase, learning rates were adjusted to prevent divergence and promote efficient loss minimization.
- **Dropout Rate:** To reduce overfitting, SSA and SMO were used to optimize the dropout rate, determining the proportion of neurons deactivated during training.

By evaluating various configurations in the fully connected layers, these algorithms improved the model's generalization through stochastic regularization, balancing overfitting prevention with learning capacity.

- **Number of Filters:** The SSA and SMO were used to fine-tune the number of filters in each convolutional layer, balancing feature extraction and computational efficiency. By dynamically adjusting filter counts, the algorithms tailored model complexity to the dataset, enhancing representation while reducing unnecessary processing.

The SSA and SMO achieve this by simulating the behavior of animals foraging for resources, enabling both exploration of diverse hyperparameter configurations and exploitation of promising solutions.

The performance of the proposed computer vision system for maize disease classification was evaluated across different training epochs, comparing two configurations: CNN's+SMO (Spider Monkey Optimization) and CNN's+SSA (Spider Subspace Algorithm). The results, as shown in Table 2, include training and validation loss functions and accuracy metrics.

Table 2 indicates that the CNN combined with Spider Monkey Optimization (SMO) consistently outperformed the CNN with Squirrel Search Algorithm (SSA) across all epochs, achieving higher accuracy and lower loss during both the training and validation phases. These results suggest that SMO significantly enhances the training and optimization of the CNN, rendering it more effective for the task of maize disease classification.

The metrics were computed for each class to assess the model's capability to handle imbalanced datasets, where certain diseases are underrepresented. Table 3 provides a comparison of the classification performance of two bio-inspired optimization algorithms, Spider Monkey Optimization and Squirrel Search Algorithm, implemented in a Convolutional Neural Network for maize disease classification. The evaluation focuses on four key metrics: Precision, Recall, F1-Score, and Accuracy, measured across different training epochs (10, 20, 30, and 40).

Table 2. Summary Results.

| Training Epochs | Proposed | Loss Function | | Accuracy | |
|-----------------|-----------|---------------|------------|----------|------------|
| | | Training | Validation | Training | Validation |
| 10 | CNN's+SMO | 1.2541 | 1.8752 | 0.8904 | 0.8754 |
| | CNN's+SSA | 1.4587 | 1.7691 | 0.8879 | 0.8014 |
| 20 | CNN's+SMO | 0.7587 | 0.9872 | 0.9106 | 0.9047 |
| | CNN's+SSA | 0.9458 | 1.0567 | 0.9054 | 0.8381 |
| 30 | CNN's+SMO | 0.5891 | 0.7655 | 0.9358 | 0.9213 |
| | CNN's+SSA | 0.7841 | 0.9871 | 0.9245 | 0.8712 |
| 40 | CNN's+SMO | 0.3248 | 0.4218 | 0.9758 | 0.9514 |
| | CNN's+SSA | 0.6241 | 0.7861 | 0.9442 | 0.8974 |

Table 3. Classification Metrics for CNN with SMO and SSA Across Epochs.

| Epochs | Proposed | Precision | Recall | F1-Score | Accuracy |
|--------|-----------|-----------|--------|----------|----------|
| 10 | CNN's+SMO | 0.80 | 0.79 | 0.79 | 0.8754 |
| | CNN's+SSA | 0.77 | 0.74 | 0.75 | 0.8014 |
| 20 | CNN's+SMO | 0.84 | 0.82 | 0.83 | 0.9047 |
| | CNN's+SSA | 0.79 | 0.77 | 0.78 | 0.8381 |
| 30 | CNN's+SMO | 0.88 | 0.87 | 0.87 | 0.9213 |
| | CNN's+SSA | 0.83 | 0.8 | 0.81 | 0.8712 |
| 40 | CNN's+SMO | 0.92 | 0.91 | 0.91 | 0.9514 |
| | CNN's+SSA | 0.88 | 0.85 | 0.86 | 0.8974 |

The comparison of performance between SMO and SSA highlights SMO's superior optimization capabilities across all metrics and epochs. As training progresses, both methods exhibit improvements, suggesting that the model benefits from extended training.

Epoch-Wise Comparison

Epoch 10:

SMO achieved a Precision of 0.80, Recall of 0.79, F1-Score of 0.79, and an Accuracy of 87.54%, outperforming SSA, which recorded a Precision of 0.77, Recall of 0.74, F1-Score of 0.75, and Accuracy of 80.14%. At this early stage, SMO demonstrates a clear advantage, with a 7.4% higher accuracy, indicating faster convergence and better initial learning.

Epoch 40:

SMO reached its best performance with a Precision of 0.92, Recall of 0.91, F1-Score of 0.91, and an Accuracy of 95.14%. In contrast, SSA achieved 0.88, 0.85, 0.86, and 89.74%. SMO's 5.4% higher accuracy, combined with better balance across all metrics, underscores its overall superiority in optimizing the CNN for maize disease classification.

The spider monkey optimization consistently demonstrates superior performance, particularly during extended training periods, likely due to its enhanced global and local search capabilities. Although less effective than SMO, the squirrel search algorithm produces competitive results in the early stages of training, suggesting its viability as an alternative when computational resources or time are limited. Both optimization methods exhibit steady improvements with increasing epochs, highlighting the critical importance of adequate training duration in achieving high classification accuracy. By epoch 40, SMO achieves near-perfect accuracy 95.14%, underscoring its suitability for real-world applications requiring high reliability and precision. In contrast, SSA reaches a peak accuracy of 89.74%, indicating that further fine-tuning or additional training epochs could potentially reduce the performance gap. For high-stakes scenarios, such as precision agriculture, where accuracy and dependability are crucial, SMO emerges as the preferred optimization technique. Nonetheless, SSA remains a valuable option in situations where computational efficiency is prioritized over incremental improvements in accuracy.

The results presented in Table 3 confirm the superiority of SMO in optimizing Convolutional Neural Networks for maize disease classification, particularly in achieving higher precision, recall, and F1-scores across all epochs. While slightly less effective, SSA remains a viable alternative for scenarios where faster convergence and computational efficiency are critical. Its potential for further improvement underscores the importance of continued research in this area.

The confusion matrix provides a detailed analysis of the model’s classification performance across four maize disease categories: Asphalt Spot, Gray Leaf Spot, Common Rust, and Leaf Blight. Each cell represents the number of instances for a given true class (rows) versus the predicted class (columns). The diagonal values, representing true positives, reveal the model’s high accuracy, with 411 correctly classified instances for Asphalt Spot, 406 for Gray Leaf Spot, 431 for Common Rust, and 415 for Leaf Blight. These results underscore the model’s strong performance and reliability in maize disease classification. This high level of accuracy was achieved using a Convolutional Neural Network optimized with SMO and trained over 40 epochs, showcasing its effectiveness as a robust tool for precision agriculture applications. The corresponding confusion matrix is shown in Figure 2 below.

The off-diagonal values in the confusion matrix represent misclassifications, providing further insight into the model’s performance across the four maize disease categories. For Asphalt Spot, 19 instances were misclassified as Common Rust, and 2 as Leaf Blight. Gray Leaf Spot was misclassified as Asphalt Spot in 8 cases, and 9 instances were misclassified as both Common Rust and Leaf Blight. Common Rust had 1 instance misclassified as Asphalt Spot, while Leaf Blight was misclassified as Asphalt Spot and Gray Leaf Spot in 9 and 3 cases, respectively. The most significant misclassification

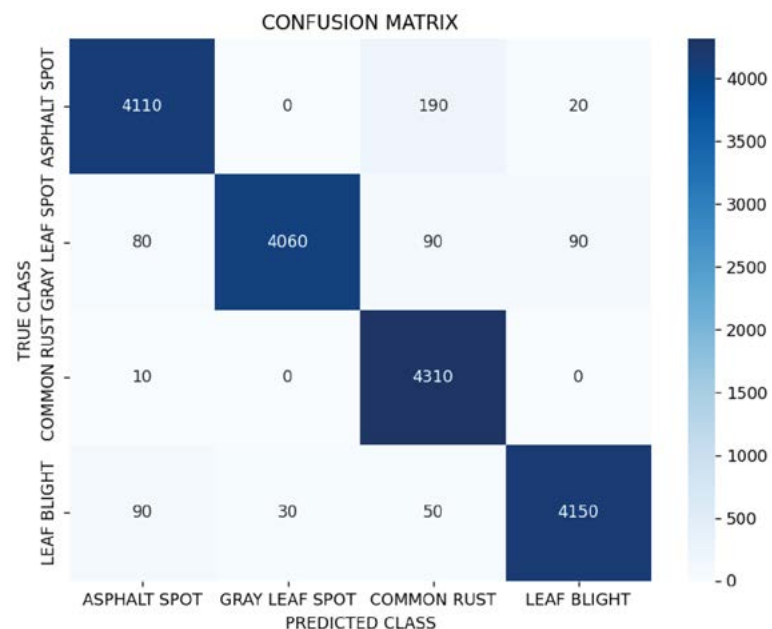


Figure 2. Confusion Matrix of CNN’s+SMO for 40 Epochs.

occurred between Asphalt Spot and Common Rust (19 instances), suggesting potential feature overlap between these diseases. Despite these errors, the majority of predictions align with the diagonal, indicating high classification accuracy across all categories. Notably, symmetric misclassification was observed between Gray Leaf Spot and Leaf Blight (9 instances each), highlighting potential overlap in visual features such as texture or color. These findings, as illustrated in Figure 2, suggest areas for further improvement, such as refining feature extraction or incorporating additional data to better distinguish between similar disease patterns.

CONCLUSION

The implementation of convolutional neural networks for the automated identification of four major maize diseases—Common Rust, Gray Leaf Spot, Asphalt Spot, and Northern Corn Leaf Blight—demonstrated high classification accuracy and robustness. Using Spider Monkey Optimization to optimize the CNN, the model achieved a precision of 92%, a recall of 91%, and an F1-score of 91% after 40 training epochs, with an overall accuracy of 95.14%. These results highlight the advantages of integrating bio-inspired optimization algorithms with deep learning techniques, resulting in improved feature extraction and classification capabilities. The model's ability to minimize misclassifications, particularly between Asphalt Spot and Common Rust (19 cases), underscores its robustness in distinguishing visually similar categories. This optimized approach provides a well-supported and reliable framework for automating disease detection, enabling timely and accurate interventions in precision agriculture.

Furthermore, the study emphasizes the importance of balanced datasets and sufficient training epochs, which contributed to the model's solid performance and dependability. By automating the disease identification process, the proposed system can significantly reduce diagnostic time and enhance crop yield management, potentially saving farmers up to 30% in crop loss costs annually. Future research should focus on further refining the CNN architecture and exploring hybrid optimization techniques to address remaining misclassification challenges and improve the system's scalability for large-scale agricultural applications.








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Characterization Techniques for Optical, Structural, Morphological and Biodegradation Properties of Food Films

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ABSTRACT

Objective: To address the main analytical methods used to characterize edible films in their optical, structural, morphological and biodegradation properties.

Design/methodology/approach: It addresses how these techniques contribute to the development of edible films with suitable characteristics according to the specific conditions required by the food. Key methods such as UV-Vis spectrophotometry, colorimetry, optical microscopy, Raman spectroscopy, infrared spectroscopy and scanning electron microscopy are discussed, showing their role in the evaluation of film properties such as transparency, chemical structure, surface morphology and biodegradability.

Findings/conclusions: The findings provided by these characterization techniques are essential for progress in the development of sustainable food packaging that enhances food safety, improves shelf life, and reduces environmental impact.

Keywords: edible films, optical properties, structural properties, morphological properties, biodegradability.

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INTRODUCTION

There is increasing interest in developing edible food films due to the need to replace the use of conventional plastics used as primary food packaging with sustainable alternatives such as biodegradable polymers. These materials are generally made from polysaccharides, proteins and lipids and in addition to serving as a first semi-permeable barrier between the food and its environment, they possess functional properties that contribute to food preservation. Among the various characteristics of edible films that can be evaluated are optical, structural and morphological properties, which are essential to determine the effectiveness of the films in protecting and preserving the food (Olawade *et al.*, 2024).

A comprehensive evaluation of the quality and applicability of edible films requires optical properties such as transparency, color stability, and light absorption. Conventional analytical techniques, including UV-Vis spectrophotometry and colorimetry, offer valuable insights into the films' capacity to impede harmful UV radiation and preserve their aesthetic appeal over time. Furthermore, optical microscopy is a fundamental tool for assessing surface uniformity and the distribution of components within the film matrix (Simona *et al.*, 2021; Nosal *et al.*, 2005).

In addition to optical properties, the structural composition of edible films is essential for understanding their functional behavior. Techniques such as Raman and infrared spectroscopy facilitate the identification of molecular interactions, component compatibility, and chemical stability. These analyses have been instrumental in optimizing film formulations, thereby enhancing their protective and mechanical properties (Hsu *et al.*, 2005; Sucheta *et al.*, 2019; Mohammadi *et al.*, 2018).

The performance of edible films is influenced by morphological properties, which affect parameters such as permeability, mechanical strength, and biodegradation rate. Consequently, advanced imaging techniques, including scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM), have emerged as instrumental tools for conducting detailed examinations of surface textures, porosities, and overall microstructures. These techniques facilitate a more profound comprehension of the interactions between film components and their impact on food preservation (Farhan and Hani, 2017).

Meanwhile, biodegradation properties are important for evaluating the environmental impact of edible films. A comprehensive understanding of the factors influencing their decomposition, such as polymer structure and environmental conditions, is imperative for the development of packaging solutions that are aligned with sustainability objectives (Agustina and Sasongko, 2023; Jaramillo *et al.*, 2016).

The present review article explores a variety of techniques for optical, structural, morphological, and biodegradation analyses employed for the purpose of characterizing edible films. The article highlights their importance in ensuring the quality, functionality, and environmental sustainability of these materials.

Optical properties

UV-Vis Spectrophotometry

UV-Vis spectrophotometry is an analytical method that quantifies light absorption in the ultraviolet (UV) and visible regions of the electromagnetic spectrum. This method is extensively employed in the assessment of edible films because of its capacity to analyze the optical and functional characteristics of materials. In a particular study, the transparency and UV protection effectiveness of edible films composed of carrageenan and orange essential oil were evaluated through the utilization of UV-Vis spectrophotometry (Simona *et al.*, 2021). The findings indicated that the amalgamation of these components led to a decrease in transmittance across both UV and visible wavelengths, thereby suggesting the potential of these films to function as UV shields (Simona *et al.*, 2021). In a separate study, the addition of calcium alginate and tea polyphenols to films was found to enhance their antioxidant properties, as evidenced by a decrease in optical transmittance (Biao *et al.*, 2019).

A UV-Vis spectrophotometer is employed to quantify the degree of absorption of the dried film subsequent to the preparation of a film-forming solution and the formation of the film through techniques such as casting. A study on edible films composed of potato starch, chitosan, and aloe vera gel employed UV-Vis spectrophotometry to investigate changes in the chemical structure and molecular configuration of the film components after exposure

to UV radiation (Bajer, 2020). This technique was also employed to assess the UV barrier qualities of starch films altered through photochemical processes, revealing that exposure to UV-A and UV-C radiation enhanced the films' UV protection capabilities (Shahabi-Ghahfarrokhi *et al.*, 2019).

UV-Vis spectrophotometry is a valuable technique for studying edible films because it provides complete information about the optical and functional properties of materials, which is necessary for better food packaging. A study on casein phosphopeptides in gelatin films demonstrated that these substances reduced the transmission of UV light. This effect was attributed to the enhanced antioxidant and antibiotic properties of the films (Khedri *et al.*, 2021). Furthermore, UV-Vis spectrophotometry evaluates the protective efficacy of films against UV rays, thereby enhancing food safety and prolonging shelf life (Dash *et al.*, 2019).

Colorimetry

Quantifying color in edible films is fundamental to evaluating their visual and commercial potential. The CIELab color system, a widely accepted method for color measurement; is composed of three components: L* (lightness), a* (green to red scale), and b* (blue to yellow scale). This system is frequently utilized for this assessment (Ahmad *et al.*, 2019; Bhatia *et al.*, 2022a). This technique enables precise representation of color, which is of high significance because consumers can easily detect small differences that influence their preferences. Furthermore, in the context of the evolution of transparent edible films, the colorimetric analysis can serve as a reliable indicator of the presence of specific components or alterations in the composition of the film, which may arise from storage conditions or environmental factors such as light and humidity (Zhelyazkov *et al.*, 2021; Arrieta *et al.*, 2014).

This analysis is of particular pertinence in the context of color stability investigations, as it facilitates the observation of alterations over the course of storage duration or under varying temperature exposures. Additionally, it enables the assessment of the impact of specific ingredients on the hue and brightness of the film (Luchese *et al.*, 2018; Homez *et al.*, 2018). The significance of these measurements is that color serves as a primary sensory attribute for the acceptability of a product and may also be indicative of film quality and safety. Color variations may signify lipid component oxidation, alterations in water activity, or interactions among substances (Yildirim-Yalcin *et al.*, 2021); specifically, the coloration of protein and polysaccharide-based edible films can be influenced by ultraviolet light exposure, pH levels, or temperature; hence, diligent monitoring is crucial to maintain a stable, high-quality product (Zhai *et al.*, 2018; Homez *et al.*, 2018).

Optical Microscopy

The optical microscope is a fundamental instrument employed in the analysis of food films that facilitates the observation and description of the surface and shape of these materials. The underlying principle of this method involves the utilization of visible light to illuminate the sample thereby creating a comprehensive representation of its surface characteristics. In the context of analyzing food films, the optical microscope is employed

to assess parameters such as uniformity, the presence of flaws, and the distribution of the film's components. In a study of hologram markers in biopolymer films, optical imaging in reflection mode was employed to examine the surface shape and determine the fabrication process of these markers (Podshivalov *et al.*, 2020). This approach ensures that edible films possess a structure that is suitable for their intended use as food packaging materials.

In order to obtain measurements by means of optical microscopy, it is necessary to create a sample of the edible film and subsequently position it beneath the microscope. A light source is employed to illuminate the sample, and the image is observed through the use of mirrors that magnify it. Scanning electron microscopy (SEM) has been employed in conjunction with optical microscopy in numerous studies, including those examining calcium alginate sheets infused with tea polyphenols. This approach yielded comprehensive images of the films' surface and interior architecture (Biao *et al.*, 2019). These metrics facilitate the assessment of film quality and utilization by examining parameters such as thickness, density, and uniformity, among other factors.

The optical microscope is a very useful tool for the analysis of edible films because it provides a comprehensive insight into their structural and surface properties. This profound understanding is vital to the development and improvement of food films that exhibit specific characteristics, such as strength, water vapor permeability, and transparency. For instance, studies employing optical imaging and other characterization methods on chitosan and whey protein films have demonstrated that the incorporation of chitosan enhances the films' strength and reduces their water vapor permeability (Tavares *et al.*, 2020); these alterations are of particular importance for food packaging applications, where edible films must keep food safe and last longer.

Structural Properties

Raman Spectroscopy

Raman spectroscopy is a photonic technique that utilizes monochromatic laser radiation directed at a sample. The scattered light exhibits characteristic frequency changes (Raman bands), which provide chemical and structural information about the material being analyzed (Soler Barrera *et al.*, 2013). Raman spectroscopy is a valuable technique for investigating the composition and structure of edible films thereby verifying their quality and safety. This technique's capability to identify chemical and biological impurities and evaluate the authenticity of components is relevant for ensuring the safety of edible films for human consumption (Neng *et al.*, 2020; Huang *et al.*, 2020). This approach has been shown to detect and quantify particular components, including lipids, proteins and other additives (Du *et al.*, 2019; Jiang *et al.*, 2020); this method does not require sample preparation, thus making it well-suited for real-time and online applications in the food sector (Hu *et al.*, 2019).

Infrared Spectroscopy

Alternatively, infrared spectroscopy is a rapid and simple way to detect polymeric materials using group frequencies and characteristic patterns in the spectral "fingerprint". The underlying principle of this method is the manner in which molecules absorb infrared

light; a process that generates vibrations within the chemical bonds. This technique allows for the characterization and study of hydrogen bonds and the identification of specific interactions (Pastor *et al.*, 2003); when polymers are compatible, molecular interactions occur, which makes infrared spectroscopy especially useful for identifying polymer compatibility in polymeric material blends, particularly Fourier transform infrared spectroscopy (FTIR) (M'Bareck *et al.*, 2009).

In a study, the Fourier-transform infrared (FTIR) spectroscopy technique was employed to analyze edible films composed of sodium alginate and casein infused with orange oil. This method enabled the detection of the distinctive peaks of the film components thereby facilitating the assessment of the films' compatibility and structural qualities (Bhatia *et al.*, 2022a). FTIR has been employed to examine edible films composed of gelatin and casein, demonstrating the impact of gallic acid on the films' structure and thermal stability (Bhatia *et al.*, 2022b).

Infrared spectroscopy is imperative for the analysis of edible films as it provides exhaustive insights into their chemical composition and molecular interactions. This is indispensable for the development of edible films with improved properties for specific food applications. Additionally, the capacity of infrared spectroscopy to facilitate rapid and non-destructive evaluation renders it a valuable technology for quality control and research on edible films (Li *et al.*, 2020).

Morphological Properties

The structure of polymers (morphology) can be determined through Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Atomic Force Microscopy (AFM), among other techniques. While SEM generates images in a range of 5 to 10 nm, electron microscopy can achieve magnifications of over 200,000 times (Seymour and Carraher, 1995).

Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) is a technique used to evaluate the surface microstructure of edible polymeric materials. This analysis provides both qualitative and quantitative insights into the organization of the material's components at microscopic detail; its organization and topographical study are done by generating high-resolution, three-dimensional images within a wide range of magnifications (Granada-Restrepo *et al.*, 2014). The images are generated by emitting secondary electrons from the sample when it is hit with a high-energy electron beam. The emitted electrons are detected and produce an electrical signal, which creates the image on a cathode ray tube screen (Vijayalakshmi *et al.*, 2024).

The importance of understanding morphology lies in determining changes in the structural properties of the films. It also helps assess characteristics such as porosity, permeability, flexibility, and resistance (De Paula Herrmann *et al.*, 2004; Giosafatto *et al.*, 2014). Morphology can indicate whether there is good compatibility between the compounds and plasticizers, showing a smooth surface when compatibility exists. Likewise, a compact structure in the cross-section suggests a developed network, while a porous film

allows deeper incorporation of the active compound, enabling its prolonged release (Nur Hanani, 2018).

In materials science, SEM is used for the morphological characterization of different materials, studies of adhesion in polymer matrices, fracture analysis, failure analysis, and the morphology of polymer particles, among other applications.

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is a technique that allows for the determination of morphological characteristics, structures (via electron diffraction), the construction of cell images (via high-resolution transmission electron microscopy, HRTEM), as well as the chemical composition of a sample through X-ray fluorescence by energy dispersive X-ray (EDXRF) (Ercius *et al.*, 2020).

In TEM microscopy, a beam of electrons with uniform current density is irradiated onto the sample. The image is generated on a fluorescent screen through the projection of the electron intensity distribution behind the sample, passed through a system of three to eight lenses (Reimer and Kohl, 2008). Depending on the study's goal and the material, information can be collected from individual particles, specific areas, or aggregates. The image can be recorded by directly exposing a photographic emulsion, by exposure to a vacuum plate, or even by using a camera with a coupled charge device (Reimer and Kohl, 2008).

Atomic Force Microscopy (AFM)

Atomic Force Microscopy (AFM) was developed in 1986 and collects image information through variations in the magnitude of the interaction between the probe and the material's surface being analyzed (Yang *et al.*, 2007). This type of microscopy allows for surface analysis through mechanical scanning using atomic forces, hence the name (Kawai *et al.*, 2014). AFM can be useful in analyzing different types of materials.

Regarding edible polymers (PCs), AFM provides qualitative and quantitative information at the nanoscale for analyzing the morphology of their surfaces, also known as topographic analysis (Zuo *et al.*, 2019). For example, AFM helps understand the interactions between proteins and surfactants in a polymeric composition, among other processes and mechanisms of their molecular interactions (often Van Der Waals forces) (García *et al.*, 2018).

Other applications of AFM in the food area include the qualitative analysis of the molecular structure of important food compounds, especially in the formation of macromolecule networks such as proteins and carbohydrates, and the study of their intermolecular interactions. Moreover, AFM allows the study of the topology (subtle physical properties) of food surfaces, such as roughness, homogeneity, morphology, and fractal analysis (García *et al.*, 2018).

Biodegradation Properties

Biodegradability is defined as the ability of a material to decompose through the enzymatic action of microorganisms, measured under specific storage periods and

conditions using standardized methods (López *et al.*, 2010). There are two types of biodegradations: aerobic and anaerobic. The former occurs in the presence of oxygen, while the latter takes place in its absence. In aerobic biodegradation, the waste products generated are biomass, carbon dioxide, water, and inorganic compounds; in anaerobic degradation, biomass, methane, intermediate metabolites, and inorganic compounds are produced (Kyrikou and Briassoulis, 2007). Complete biodegradation implies the destruction of the macromolecular support, resulting in the corresponding by-products (Rubio-Anaya and Guerrero-Beltrán, 2012).

Biodegradation involves various processes, such as the loss of mechanical strength (modifications to surface characteristics), degradation by microorganisms or enzymes, as well as the breaking of the main chain, and subsequent reduction in the molecular weight of the polymers.

Although edible films are considered biodegradable because they are based on natural biopolymers, their degradation rate depends on their molecular links, with a slower degradation observed in films formed through covalent bonds (Li and Chen, 2000). This biodegradation speed is determined by several environmental factors such as soil pH, humidity, temperature, partial oxygen pressure, microbial flora composition, and the presence of other nutrients (Lee, 1996; Kyrikou and Briassoulis, 2007), as well as intrinsic polymer parameters like surface area, monomeric composition, degree of crystallinity or amorphous regions, and molecular weight. It has been reported that higher crystallinity implies a slower biodegradation rate (Nishida and Tokiwa, 1995; Spyros and Kimmich, 1997), and polymers with lower molecular weight degrade more quickly than those with higher molecular weight (Jendrossek *et al.*, 1996).

There are various methods to characterize the biodegradation of a polymer. It can be done by measuring weight loss, changes in tensile strength, or in its physical and chemical properties, according to modifications in the molecular weight distribution or dimensional changes, as well as determining carbon dioxide production or bacterial activity in the soil (Singh and Sharma, 2008).

Research challenges in polymers are focused on developing films with natural biopolymers for making bags or packaging materials that have the shortest biodegradation time based on their intended use (Kean and Thanou, 2010).

Critical analysis of characterization techniques

Ultraviolet-visible (UV-Vis) spectrophotometry, colorimetry, and optical microscopy are essential non-destructive techniques for the optical and structural characterization of materials. These techniques facilitate the analysis of absorption, color, and surface morphology. However, it should be noted that UV-Vis spectrophotometry is restricted to the assessment of transparent substances, and optical microscopy has poor resolution. Raman Spectroscopy and Infrared Spectroscopy both offer detailed insights into the chemical composition and functional groups of materials; however, Raman spectroscopy may demonstrate diminished sensitivity to certain molecules, while infrared spectroscopy necessitates careful sample preparation to avoid interference. Biodegradation analysis is

crucial for evaluating environmental impact; nevertheless, testing may be prolonged due to high variability in behavior under uncontrolled conditions.

In essence, structural analysis employing scanning electron microscopy (SEM) and transmission electron microscopy (TEM) yields images at the nanoscale level, thereby facilitating comprehensive insights into the morphology and phase distribution within films. However, it is necessary to note that these methods are costly and require meticulous sample preparation. While Atomic Force Microscopy (AFM) is effective for assessing nanometric topography, it is less adept at analyzing interior morphology. Each of these techniques has specific applications and limitations, and their combination may offer a more comprehensive approach.

The development and application of new technologies in postharvest preservation present various challenges that must be addressed to ensure their effectiveness and feasibility at an industrial level. A primary challenge pertains to scalability and cost, as many of these techniques have demonstrated efficacy in laboratory studies but may necessitate substantial investments in infrastructure for large-scale implementation. Additionally, compatibility with existing industrial processes poses a significant obstacle, as the integration of new technologies into established production lines without compromising operational efficiency is intricate and necessitates technical and logistical adjustments.

Another fundamental challenge is compliance with food safety regulations and standards, which vary by region and may impose restrictions on the use of certain materials or methods. This issue is inextricably linked to environmental sustainability, as certain techniques may generate by-products or require substantial energy consumption, potentially impeding their adoption in industries seeking to minimize their ecological footprint. Furthermore, market and consumer acceptance plays a pivotal role, as public perceptions regarding the utilization of innovative food technologies can sway demand and commercial adoption.

CONCLUSIONS

The characterization techniques examined in this review are instrumental in evaluating the optical, structural, and biodegradation properties of edible films and coatings. These methods yield critical insights into optical transparency, structural integrity, molecular interactions, and degradation behavior, thereby contributing to the optimization of food packaging materials. UV-Vis spectrophotometry and colorimetry facilitate precise measurement of light absorption and color properties, which are required to evaluate film transparency and UV protection. Optical microscopy and SEM provide detailed morphological analysis, revealing surface uniformity, porosity, and structural integrity. TEM and AFM offer nanoscale insights into molecular organization and film composition. Spectroscopic techniques, including Raman and infrared spectroscopy, enhance our understanding of chemical interactions, polymer compatibility, and functional group modifications within the film matrix. Furthermore, biodegradability assessments are crucial for determining the environmental impact of these materials, which is imperative for the development of sustainable packaging solutions. However, these techniques are not without limitations, including the complexity of instrumentation, the time-intensive

of sample preparation, and the requirement of specialized expertise. Variability in methodological approaches may also lead to inconsistencies in data interpretation across different studies. To that end, future advancements in this field should prioritize the development of more standardized, rapid, and non-destructive analytical techniques that can be applied efficiently in industrial settings.

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Analysis of sustainability indicators of the sugarcane agroindustry in Veracruz, Mexico

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ABSTRACT

Objective: To analyze the sustainability indicators of the sugarcane agroindustry in Veracruz, Mexico. **Design/Methodology/Approach:** The performance of seven sugar mills was analyzed with data from ten harvests (from the 2013/2014 harvest to the 2022/2023 harvest). Performance was evaluated on the basis of 18 sustainability indicators (12 indicators for the field and 6 indicators for the factory). The data were evaluated with an analysis of variance, while the representation of the indicators was determined with the Principal Component Analysis (PCA). Results: The APPS (percentage of the area planted with a double furrow system), STBC (percentage of the area managed with biological control), and PGHA (percentage of the area harvested green) indicators allowed the research team to differentiate the performance between the evaluated sugar mills. The PCA showed that the San Cristóbal, San Miguelito, Central Progreso and La Gloria sugar mills recorded the highest percentages in the indicators of integrated pest management, application of organic fertilizers, and sugar cane yield.

Findings/Conclusions: El Modelo and La Providencia sugar mills have implemented strategies to promote sustainable practices, enabling them to improve field yield and factory efficiency. Finally, La Providencia, Pánuco, Central Progreso, and La Gloria sugar mills recorded the best compliance with Mexican official standards.

Keywords: Sustainability indicators, performance, sugar cane industry.

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INTRODUCTION

Starting with the 2010/2011 sugarcane harvest, the Comité Nacional para el Desarrollo Sustentable de la Caña de Azúcar (CONADESUCA) established a system of sustainability indicators for the sugarcane agroindustry in Mexico. This system was created to compile and organize the sustainability actions taken by the Comités de Producción y Calidad Cañera (CPCCC). A set of 18 indicators and indices are used for the annual measurement, evaluation, and communication about the sustainability in the sector. The system is an



essential tool for monitoring the progress and impact of sustainability initiatives over time, allowing a comprehensive view of the progress and challenges faced during the implementation of sustainable practices in the Mexican sugarcane sector. In recent years, there have been radical changes in the environment, due to the production of atmospheric carbon dioxide (CO₂) caused by anthropogenic activities. Climate change affects crop growth and yield (Ray *et al.*, 2015), impacting food production. According to Linnenluecke *et al.* (2020), various changes have impacted crops between 1964 and 2012. These crops include sugarcane, whose remarkable production in tropical and subtropical countries makes it a highly important product (Wiedenfeld, 2000). Sugarcane plays a major role in the production of sugar, bioethanol, and other products. In Mexico, 15 states and 267 municipalities produce sugarcane. The 2023 production reached 5,224,248 t. The field yield ranges from 60 to 70 t ha⁻¹ in rainfed systems and up to 100 t ha⁻¹ in irrigated systems. The State of Veracruz is the main producer of sugarcane in the country, producing approximately 40% of the country's sugarcane. Currently, research is mainly focused on forecasting sugarcane production through simulations for specific regions or areas (Everingham *et al.*, 2015a; Linnenluecke *et al.*, 2018 and 2020; Días *et al.*, 2020). However, analyzing the potential changes of sustainability indicators in both field and factory is fundamental to determining the current sustainability of the sugarcane agroindustry of Veracruz. Therefore, the objective of this research was to analyze the sustainability indicators of the sugarcane agroindustry in the State of Veracruz.

MATERIALS AND METHODS

The study is based on documentary research that explores, analyzes, and retrospectively reviews sustainability indicators, in both the field and the factory of the sugarcane agroindustry. Nine sugar mills from Veracruz were used as a sample to evaluate their performance over a ten-harvest period (from the 2013/2014 harvest to the 2022/2023 harvest). The following sugar mills were selected: Central La Providencia, San Miguelito, El Modelo, La Gloria, Central Progreso, Pánuco, and San Cristóbal. The 18 sustainability indicators proposed by CONADESUCA were analyzed. These indicators are divided into 12 field indicators and 6 factory indicators.

RESULTS AND DISCUSSION

Table 2 shows the probability values for the field and factory indicators of the sugar mills. The APPS (percentage of the area planted with the double furrow system), STBC (percentage of the area managed with biological control), and PGHA (percentage of the area harvested green) indicators allowed the differentiation of the performance between sugar mills. According to Índice de Sustentabilidad Global de los Ingenios en México (Cavazos, 2018)—which measures the overall sustainability of Mexican sugar mills—the sustainability indicators—and the inclusion of innovation as a sustainability strategy—have a differentiating effect on the means of the groups.

The representation of the indicators is included in Figure 1. San Cristóbal, San Miguelito, Central Progreso, and La Gloria recorded higher percentages in the following indicators: integrated pest management, application of organic fertilizers, and sugarcane

Table 1. Sustainability indicators per subsector.

| SUBSECTOR | Identifier | Indicator |
|-----------|------------|--|
| Field | APCT | Percentage of the area planted with conservation tillage |
| | APPS | Percentage of the area planted with the pineapple system |
| | APTI | Percentage of the area planted with technical irrigation |
| | FATR | Percentage of fertilized area based on technical recommendations |
| | SAOF | Percentage of surface with application of organic fertilizers |
| | ABIO | Percentage of surface with biofertilizer application |
| | AIPM | Percentage of surface area served with integrated pest management |
| | STBC | Percentage of surface treated with biological control |
| | SCLP | Percentage of surface area served by the Campo Limpio Program |
| | SCVE | Percentage of green harvested area |
| | ASYI | Average sugarcane yield (t/ha) |
| | PCTN | Production cost per ton (\$/t) |
| Factory | FAEF | Factory efficiency (%) |
| | KABE/KARBE | KABE/KARBE relationship |
| | SGSB | Percentage of steam generated with sugarcane bagasse |
| | EESB | Percentage of electrical energy cogenerated from sugarcane bagasse |
| | FCUC | Percentage of filter cake used for composting |
| | CREG | Compliance with Regulations |

Table 2. Probability values for field and factory indicators of the sugarcane agroindustry in Veracruz.

| Indicator | p-value | Indicator | p-value |
|-----------|---------|------------------------------|---------|
| APCT | ns | PCTN | ns |
| APPS | 0.006 | FAEF | ns |
| APTI | ns | KABE/KARBE | ns |
| FATR | ns | SGSB | ns |
| SAOF | ns | EESB | ns |
| ABIO | ns | FCUC | ns |
| AIPM | ns | CREG (NOM-002-SEMARNAT) | ns |
| STBC | 0.014 | CREG (NOM-085-SEMARNAT) | ns |
| SCLP | ns | CREG (NOM-001-SEMARNAT-2021) | ns |
| PGHA | 0.048 | CREG(NOM-081-SEMARNAT-1994) | ns |
| ASYI | ns | | |

APCT=Percentage of the area planted with conservation tillage; APPS=Percentage of the area planted with the pineapple system; APTI=Percentage of the area planted with technical irrigation; FATR=Percentage of the area fertilized based on technical recommendation; SAOF=Percentage of area with organic fertilizers applied; ABIO=Percentage of area with biofertilizers applied; AIPM=Percentage of area served with integrated pest management; STBC=Percentage of area served with biological control; SCLP=Percentage of area served with the Campo Limpio Program; PGHA=Percentage of area harvested green; ASYI=Average sugarcane yield (t/ha); PCTN=Production cost per ton (\$/t); EFA=Factory efficiency (%); KABE/KARBE=KABE / KARBE ratio; SGSB=Percentage of steam generated with sugarcane bagasse; EESB=Percentage of electrical energy cogenerated from sugarcane bagasse; FCUC=Percentage of sugarcane filter cake used for composting; CREG=Compliance with Regulations; ns=Not significant.

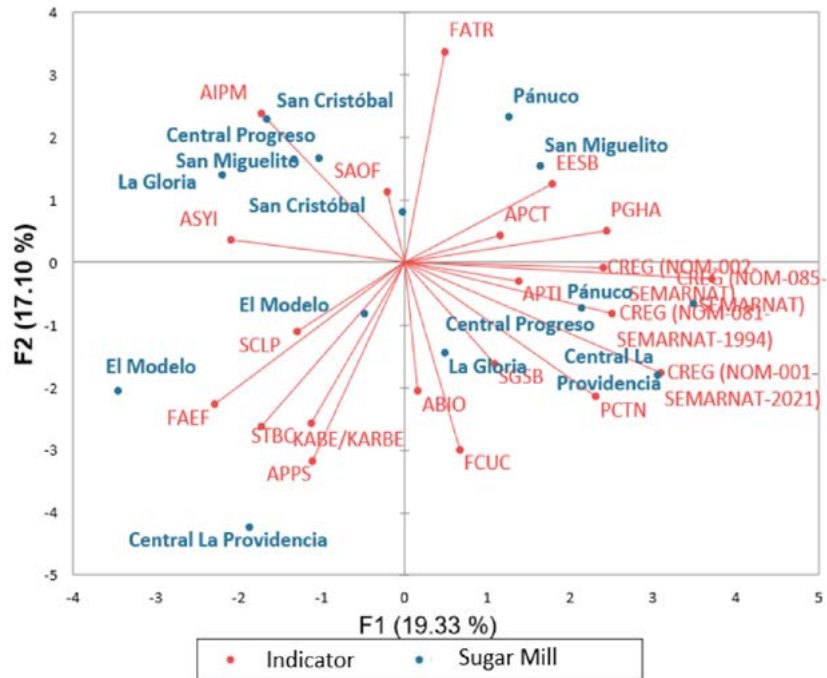


Figure 1. Representation of indicators per sugar mill.

APCT=Percentage of the area planted with conservation tillage; APPS=Percentage of the area planted with the pineapple system; APTI=Percentage of the area planted with technical irrigation; FATR=Percentage of the area fertilized based on technical recommendation; SAOF=Percentage of area with organic fertilizers applied; ABIO=Percentage of area with biofertilizers applied; AIPM=Percentage of area served with integrated pest management; STBC=Percentage of area served with biological control; SCLP=Percentage of area served with the Campo Limpio Program; PGHA=Percentage of area harvested green; ASYI=Average sugarcane yield (t/ha); PCTN=Production cost per ton (\$/t); EFA=Factory efficiency (%); KABE/KARBE= KABE / KARBE ratio; SGSB=Percentage of steam generated with sugarcane bagasse; EESB=Percentage of electrical energy cogenerated from sugarcane bagasse; FCUC=Percentage of sugarcane filter cake used for composting; CREG=Compliance with Regulations.

yield. This situation may indicate that sustainable management practices favor crop yield. El Modelo and La Providencia recorded the highest SCLP, FAEF, KABE/KARBE, STBC, and APPS indicators. These indicators demonstrate that these sugar mills have implemented strategies to promote sustainable practices, improving their field yield and factory efficiency. Meanwhile, La Providencia, Pánuco, Central Progreso, and La Gloria recorded a greater compliance with the NOM-085, 081, 001 and 002 Mexican official standards (SEMARNAT). In addition, they obtained the highest FATR, EESB, APCT, PGHA, APTI, SGSB, ABIO, and PCTN indicators.

Conservation tillage at the sugar mills is a major practice for crop sustainability. Only 29% of the sugar mills in the sample implemented this technique. Compared with the 2013/2014 harvest, Central Progreso recorded a decrease in the implementation of conservation tillage during the 2022/2023 harvest. In contrast, Pánuco increased the implementation of this practice from 0% to 30%. Less than 1% of sugar mill producers use the double furrow system; instead, they plant sugarcane as a main monocrop. The technified

irrigation area significantly increased at several sugar mills: Central La Providencia (11%), Central Progreso (18%), and La Gloria (1%). Meanwhile, Pánuco decreased by 12%. The percentage of the area fertilized based on technical recommendations decreased by 8% in the 2022/2023 harvest, mainly because of a 73% reduction in Pánuco. The use of organic fertilizers remained unchanged, and they were only used in 3% of the sugarcane crop area. Less than 1% of the producers apply biofertilizers, providing an opportunity to improve sustainable agricultural practices. Forty-three percent of the sugar mills use integrated pest management. However, since the 2013/2014 harvest, the area managed with these practices has decreased by an average of 30%. The indicators for the area under the Programa Campo Limpio and the area harvested green were better in the 2022/2023 harvest than in the 2013/2014 harvest, recording increases of 14% and 5%, respectively. Compared with the 2013/2014 harvest, the average sugarcane yield (t/ha^{-1}) decreased by 10 t/ha^{-1} in the 2022/2023 harvest. Pánuco, Central Progreso, and San Miguelito recorded the largest decrease in yield per hectare, with 35, 19, and 14 ha less than in previous period, respectively. Compared with the 2013/2014 harvest, the production cost per ton (MXN\$/t) for the 2022/2023 harvest increased by 136%. Central La Providencia recorded a significant increase in sustainable practices compared with the 2013/2014 harvest, regarding technified irrigation area (12%), area fertilized based on technical recommendations (70%), integrated pest management (22%), Programa Campo Limpio (60%), and harvested green area (9%). However, decreases in conservation tillage (1%), the double furrow system (4%), and the application of biofertilizers (2%) were registered. The Central Progreso sugar mill increased the technified irrigation area by 18% and the use of biofertilizers by 2% compared with the 2013/2014 harvest. Nevertheless, it recorded decreases in conservation tillage (28%), chemical fertilization (30%), use of organic fertilizers (11%), Programa Campo Limpio (15%), and average sugarcane yield (19%). El Modelo did not report improvements in any of the sustainability indicators between the 2013/2014 harvest and the 2022/2023 harvest, which provides an opportunity to improve the sustainability of its sugarcane fields. La Gloria registered increases in technified irrigation (1%), biological control (24%), and application of organic fertilizers (2%). However, this sugar mill decreased its integrated pest management (82%), Programa Campo Limpio (6%), and average yield (2%). Compared with the 2013/2014 harvest, Pánuco improved its integrated pest management by 31%, conservation tillage by 30%, and biological control by 27%. In addition, this sugar mill reduced chemical fertilization by 72% and yield by 35%. Neither San Cristóbal nor El Modelo reported any sustainability indicator improvements. Both sugar mills should make significant improvements in their sustainable practices. San Miguelito improved its conservation tillage (41%), application of organic fertilizers (6%), and Programa Campo Limpio (13%). However, its overall average yield decreased by 14 t/ha^{-1} . Factory indicators for the 2022/2023 harvest did not record much variability regarding the 2013/2014 harvest. A 13% increase in electricity cogenerated from sugarcane bagasse and a 36% increase in the sugarcane filter cake used for composting were recorded. The standard compliance indicator of sugar mills is essential to assess their commitment to environmental regulations. This indicator considers compliance with four standards:

- **NOM-002-SEMARNAT 1996** establishes the maximum permissible limits of pollutants in wastewater discharged into urban or municipal sewage systems.
- **NOM-085-SEMARNAT-2011** regulates the maximum permissible emission levels of indirect heating combustion equipment and their measurement.
- **NOM-001-SEMARNAT-2021** defines the permissible limits of pollutants in wastewater discharges into water bodies owned by the nation.
- **NOM-081-SEMARNAT-1994** establishes the maximum permissible limits of noise emission from fixed sources and their measurement method.

In the 2013/2014 harvest, only Pánuco complied with two of these standards: NOM-002-SEMARNAT-1996 and NOM-085-SEMARNAT-2011. None of the other sugar mills included in the sample complied with the aforementioned standards.

However, a significant improvement regarding the compliance with regulations was recorded in the 2022/2023 harvest.

- **La Providencia Central** complied with the four abovementioned standards.
- **La Gloria and Pánuco** complied with three standards: NOM-085-SEMARNAT-2011, NOM-001-SEMARNAT-2021, and NOM-081-SEMARNAT-1994.
- **Central Progreso** complied with three standards: NOM-002-SEMARNAT 1996, NOM-085-SEMARNAT-2011 and NOM-001-SEMARNAT-2021.
- **San Miguelito** complied with two standards: NOM-085-SEMARNAT-2011 and NOM-081-SEMARNAT-1994.
- **El Modelo** and **San Cristóbal** only complied with the NOM-081-SEMARNAT-1994 standard.

This analysis registered progress regarding the compliance of sugar mills with environmental regulations. Nevertheless, some sugar mills can still improve their sustainability and regulatory compliance.

CONCLUSIONS

The analysis provided a detailed overview of the behavior of sustainability indicators, identifying progress, trends, and improvement opportunities in the implementation of sustainable practices in these sugar mills. This overview provides a deeper understanding about the current sustainability status and challenges faced by the sugarcane agroindustry of Veracruz. The results from the sustainability indicator analysis of the sugarcane agroindustry of Veracruz showed significant progress and opportunity areas. Sugar conglomerates such as La Margarita, Zucarmex, and Grupo Azucarero del Trópico stand out in integrated pest management, application of organic fertilizers, and sugarcane yield. However, the overall adoption of sustainable practices —such as conservation tillage and the use of organic fertilizers and biofertilizers— is very low. A notable improvement was observed in the compliance with environmental regulations from the 2013/2014 harvest to the 2022/2023 harvest: Central La Providencia complied with all four main standards.

Nevertheless, the average yield decrease and the production cost increase highlight the need for the constant improvement of agricultural practices and resource management. Sugar mills such as El Modelo and San Cristóbal can significantly improve their sustainability and regulatory compliance. The analysis of sustainability indicators of sugar mills in Veracruz, in ten harvests (from the 2013/2014 harvest to the 2022/2023 harvest), shows both progress and opportunity areas for the implementation of sustainable practices. Although a progress on certain sustainability indicators has been reported, there are still significant challenges and improvement potential in key practices, such as the use of biofertilizers, integrated pest management, and chemical fertilization. The performance variability among sugar mills highlights the requirement for a more homogeneous approach and a greater adoption of sustainable practices, in order to strengthen sustainability in the sugarcane sector of Veracruz.

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From Garden to Table: *Viola* spp., Ornamental and Edible Flowers

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ABSTRACT

Objective: To provide a comprehensive review of the ornamental, cultural, nutraceutical, and food-related importance of the genus *Viola*, with an emphasis on the edible species *Viola cornuta* and *Viola × wittrockiana*.

Design/methodology/approach: An extensive review of recent scientific literature was conducted, focusing on the diversity, morphology, traditional uses, nutraceutical value, and production of two *Viola* species, highlighting their use as ornamental and edible flowers.

Results: The species *Viola cornuta* and *Viola × wittrockiana* display outstanding ornamental and nutritional attributes. Both are rich in relevant bioactive compounds such as flavonoids, anthocyanins, and carotenoids, which contribute to their nutraceutical and functional properties in gastronomy. Their demand as edible flowers has grown significantly, driven by consumer trends favoring differentiated, healthy, and value-added food products. This review describes their taxonomic diversity, morphological characteristics, phytochemical composition, nutraceutical potential, and the main challenges and prospects associated with their production and use in floriculture and the food industry.

Limitations of the study/implications: There remains a need to generate specific agronomic and food safety information for these species, particularly under Mexican production conditions, to ensure the quality and safety of flowers intended for human consumption.

Findings/conclusions: Edible *Viola* species represent a high value-added resource with great potential to diversify Mexican floriculture and the functional food market, provided that sustainable production systems are implemented and research on agronomic and food safety aspects is strengthened.

Keywords: Edible flowers, floriculture, phytochemicals, nutraceuticals.

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IMPORTANCE OF ORNAMENTAL AND EDIBLE PLANTS

Ornamental plants are defined as those cultivated for their aesthetic qualities, such as the beauty of their form and colors, their foliage, flowers, fruits, and, when present, their fragrance. They are widely used in gardening, landscaping, and interior design [1]. These plants provide multiple benefits to humans, including positive effects on emotional and physiological health, social dynamics, and economic development, through sensory, biochemical, and productive mechanisms [2]. Beyond their aesthetic value, ornamental plants play a significant role in environmental



improvement through various mechanisms: providing pollen for pollinating organisms, thermal regulation, oxygen production, modulation of rainfall patterns, mitigation of desertification, carbon capture, reduction of urban glare, control of water and soil erosion, buffering of climate impacts through windbreak barriers, and reduction of atmospheric, water, and noise pollution [3].

The use of ornamental plants is associated with a wide range of emotional and mental health benefits, including reduced anxiety and stress levels, improvement of attention deficit symptoms, positive visual responses to fractal patterns, decreased depressive symptoms, enhanced memory retention, increased happiness and life satisfaction, mitigation of post-traumatic stress disorder, stimulation of creativity, improved productivity and concentration, alleviation of dementia-related effects, and improved self-esteem [4]. Additionally, plants provide cognitive benefits that also influence quality of life. These include strengthening of place attachment and community belonging, reduction in crime rates, increased disaster resilience, access to locally produced food, promotion of socialization among children, improved academic performance, and therapeutic effects in community settings [5]. Ornamental flowers, in particular, act as cultural catalysts by promoting social cohesion, intergenerational learning, and the expression of human values, while reinforcing cultural identity, social health, and the connection with nature [6].

In terms of physiological well-being and its impact on quality of life, ornamental plants have demonstrated positive effects in multiple areas: improved sleep quality, increased birth weight, reduced diabetes incidence, alleviation of eye discomfort, strengthening of the immune system, regulation of circadian rhythm, improved rehabilitation processes, decreased cardiovascular and respiratory diseases, lower mortality rates, enhanced digestion, reduced allergies, increased physical activity, and cognitive development [4].

On the other hand, edible flowers (EFs) are specific floral structures that contain bioactive compounds with potential health benefits when consumed [7,8]. Since ancient times, these flowers have been used as food ingredients, flavoring agents, and decorative elements in culinary preparations, and they are currently experiencing a resurgence in contemporary gastronomy [9].

The growing interest in EFs is largely attributed to changing food consumption patterns, which are increasingly focused on selecting healthier nutritional options. In addition to their aesthetic and organoleptic contribution to various culinary preparations, EFs are now recognized for their nutritional value due to their content of bioactive compounds with multiple health benefits [10].

Floriphagy, defined as the practice of consuming flowers as food, represents an ancient cultural tradition in various regions that continues to grow in popularity today [11]. This practice has long been part of the food culture of Indigenous peoples in the Americas and other parts of the world, who have appreciated and consumed flowers since ancient times. Representative examples of this tradition include the consumption of *Cucurbita maxima* flowers in Mexico, *Calendula officinalis* in Europe, and *Jasminum officinale* in Asia [12].

The EFs from different species provide a wide variety of bioactive compounds that determine their organoleptic characteristics—such as flavor and aroma—and their potential nutritional benefits. These bioactive metabolites, including antioxidants,

vitamins, minerals, and various phytochemicals, confer not only sensory appeal but also nutritional value. Their incorporation into the diet can enhance the overall nutritional profile of food [13].

From a nutritional perspective, flowers can be divided into pollen, nectar, petals, and other parts. Pollen is a source of proteins and carbohydrates, saturated and unsaturated fatty acids, carotenoids, and flavonoids. Nectar contains a balanced mixture of sugars (fructose, glucose, and sucrose), along with free amino acids, proteins, inorganic ions, lipids, organic acids, phenolic compounds, alkaloids, and terpenoids, among others. Petals and other flower parts are rich in vitamins, minerals, and antioxidants [14].

THE GENUS *Viola*: DIVERSITY AND CHARACTERISTICS

The genus *Viola*

The family Violaceae is cosmopolitan and comprises 26 genera and approximately 1,100 species of trees, shrubs, lianas, and herbs [15]. The genus *Viola* is the largest within this family, with 664 species, divided into two subgenera, 31 sections, and 20 subsections [16]. It is characterized by plants that are generally herbaceous and distributed in temperate regions, and it is defined by several apomorphies, with few exceptions, such as non-articulated peduncles (meaning they lack an abscission zone at the level of the bracteoles), solitary flowers, calyx appendages, a clearly spurred lower petal (rarely sacciform or gibbous), and a blade shorter than the lateral and upper petals [16,17].

Importance of the genus

Certain species of the genus *Viola* are cultivated as ornamental plants for their floral display, such as the pansy hybrids *V. × williamsii* and *V. × wittrockiana*, as well as some cultivars of *V. sororia*, *V. palmata*, and *V. prionantha* [16]. Moreover, many more wild species have been introduced as novelties for specialized gardeners. The flowers of *V. odorata*, a commonly cultivated species, along with some wild species, are used for condiments, jellies, syrups, and sweets. The young leaves of several *Viola* species are occasionally consumed as an aromatic herb rich in vitamin C, and the flower syrup has been used for medicinal purposes [17].

The genus *Viola* groups its cultivated varieties, or cultivars, into four main subgroups: B1: pansies; B2: violas; B3: violetes; and B4: cornuta hybrids. Within this classification, modern pansies are distinguished by presenting a well-defined central blotch or “eye” on the flower, a characteristic that differentiates them from the other subgroups. In ornamental horticulture, the term “pansy” is commonly used to refer to annual flowering hybrids with large, multicolored flowers, mainly intended for bed decoration. In contrast, the term “viola” is used to describe smaller varieties, whether annual or perennial, with a more delicate growth habit [18].

General morphological description

The genus *Viola* consists of annual or perennial herbs, rarely suffrutescent, with leaves and stipules of variable morphology. Its flowers, typically zygomorphic and hermaphroditic, appear individually on non-articulated peduncles, generally with

two bracts. The five sepals are slightly differentiated from one another and bear basal appendages of diverse shapes. The petals are unequal, with the lower petal equipped with a spur. The five stamens possess anthers with elongated connectives in the form of membranous appendages, with the two lower stamens being nectariferous. The tricarpellary ovary produces capsules with thickened valves that open by explosive dehiscence. The seeds, usually ovoid, present a conspicuous elaiosome, a straight embryo, and plano-convex cotyledons [19].

HISTORY AND CULTURAL SIGNIFICANCE OF VIOLAS

The earliest records of the use of violas (*Viola* spp.) in Europe date back to Ancient Greece, where they were sold at the Agora of Athens, praised in the poetry of the time, used for medicinal purposes, and played an important role in myths such as that of Persephone. They were also used in garlands, mentioned in the *Odyssey*, and maintained their relevance throughout the Middle Ages and the Renaissance, when they became symbolically associated with the Virgin Mary and humility [16,20].

In the Americas, viola nurseries were established on both coasts, in New England along the Atlantic and in California along the Pacific. However, the locality most closely linked to the history of these flowers in the United States was Rhinebeck, New York, where varieties such as *Viola* ‘Marie Louise’ and *Viola* ‘Lady Hume Campbell’ were widely cultivated [20].

Today, pansy flowers are used as garnishes in salads and desserts; the bluish or purplish petals have been used to prepare syrups and jellies, and the tender leaves of several *Viola* species are cooked as a leafy vegetable [16].

During the first half of the 20th century, nurseries and catalogs across Europe and the Americas documented numerous iconic cultivars of *Viola cornuta* L., such as ‘Admiration’, ‘Alba’, ‘Papilio’, ‘Gelber Triumph’, and ‘Lord Nelson’, which were widely distributed and contributed to consolidating the popularity of this *Viola* species in gardens and green spaces. Today, many of these historical cultivars are still available through specialized markets, demonstrating the enduring ornamental value and potential of *Viola cornuta* for contemporary horticulture [21].

The history of the pansy (*Viola* × *wittrockiana*) is closely linked to that of the European violet (*Viola* spp.). The name pansy comes from the French word pensée, which means “thought” or “remembrance.” During the 19th century, systematic hybridization efforts led by horticulturists such as Lord Gambier and his gardener William Thompson in England resulted in the development of larger flowers with more striking color patterns, giving rise to the modern *Viola* × *wittrockiana*. This achievement stimulated the expansion of pansy cultivation throughout Europe and North America, where they quickly became very popular ornamental flowers, noted for their diversity of colors and their ease of propagation from seed [22].

EDIBLE FLOWERS OF THE GENUS *Viola*

The demand for EFs is growing worldwide, not only for their flavor, color, and aroma, but also for their health benefits. They are a rich source of phytochemicals with potential

health-promoting properties [23]. The use of EFs has been documented for thousands of years; however, they still represent only a small fraction of the market and are currently considered a culinary curiosity [24]. Different *Viola* species are known for their traditional use as medicinal agents with analgesic, antitussive, febrifuge, hypnotic, pain-relieving, and anti-inflammatory properties; moreover, they are considered edible flowers in certain cultures [25]. The species *Viola cornuta*, *V. tricolor*, and *V. × wittrockiana* are currently the three most consumed edible *Viola* species [24].

The growing interest in EFs is driving growers and technicians to seek cultivation methods that ensure high yields and quality production without losing sight of sustainability [26].

***Viola cornuta*: Horned violet**

This species produces one of the most popular EFs cultivated as bedding and potted plants [27]. Violas are short-lived annual or perennial plants that grow 10 to 15 cm tall and produce flowers approximately 2.5 cm in diameter (Figure 1). From 2015 to 2020, sales of *Viola* in the United States increased from approximately 95 to 100 million dollars, and in the same period, the number of *Viola* growers rose from 1,714 to 1,869. These flowers are among the most widely used for aesthetic food decoration. They are commonly used to decorate cakes and desserts and have also gained popularity in the kitchen for their vibrant colors and nutraceutical value [23].

***Viola × wittrockiana*: Pansy**

The pansy, *Viola × wittrockiana*, is another species of this genus with edible flowers. In addition, it is an important and attractive ornamental plant with a wide variety of flower colors (Figure 2) [28]. This species is considered an unconventional food plant (UFP) because it is not currently used in food, even on a seasonal basis, but it has high nutritional potential and is also a source of bioactive compounds such as flavonoids and anthocyanins [29]. It is an herbaceous plant characterized by its remarkable tolerance to low temperatures and its ability to flower early, traits that make it a key species in commercial floriculture. Its versatility has made it a suitable option for residential gardens, urban green areas and public spaces [30].



Figure 1. Floral color diversity of *Viola cornuta* plants.



Figure 2. Diversity of flower colors in the species *Viola* × *wittrockiana*.

Initially, this species was selected as an interspecific hybrid resulting from crosses between species of the *Viola* section *Melanium* Ging. (B1), specifically *V. tricolor*, *V. lutea*, and *V. altaica* [31]. Due to its hybrid origin, the modern variety offers a wide range of flowers in terms of shapes, colors, and sizes. The main types of garden pansies include rayless violas, with single-color flowers lacking dark-colored veins, and luxury pansies characterized by large blotches on the three lower petals [30]. Although pansies are perennial plants in cold climates, in regions of the southeastern United States they are cultivated as cool-season annuals and rarely survive the high summer temperatures [32].

PHYTOCHEMICAL COMPOSITION AND NUTRACEUTICAL VALUE OF *Viola* spp. FLOWERS

To date, approximately 370 compounds have been isolated from flowers of various species within the genus *Viola*, including flavonoids, coumarins, alkaloids, lignans, sesquiterpenes, three types of terpenoids, phytosterols, fatty acids, phenolic acids, cyclotides, and other compounds. Cyclotides and flavonoids are the two most abundant groups of compounds isolated from *Viola* species, with cyclotides also considered characteristic components of these plants [33].

The flowers of *Viola cornuta* represent a significant source of bioactive compounds, particularly polyphenols and flavonoids. In addition, non-anthocyanin phenolic compounds have been identified, with quercetin-3-O-rutinoside ($7.5 \pm 0.1 \text{ mg g}^{-1}$ of extract), chrysin-6,8-di-C-glucoside ($6.52 \pm 0.04 \text{ mg g}^{-1}$ of extract), and quercetin-3-O-(6-O-rhamnosylglucoside)-7-O-rhamnoside ($1.27 \pm 0.01 \text{ mg g}^{-1}$ of extract) representing the main constituents [25]. Other flavonoids, such as luteolin, apigenin, kaempferol, and isorhamnetin, are also present in smaller amounts. This composition supports the potential of *V. cornuta* as a natural source of antioxidants, with a possible contribution of vitamin C and carotenoids as reported by other studies [34]. Additionally, this species is rich in myrcene and α -farnesene [11].

The species *Viola* × *wittrockiana* produces an edible flower exhibiting exceptional nutritional composition and nutraceutical potential. Throughout its floral development, this plant exhibits appreciable levels of proteins, carbohydrates, and beneficial fatty acids, with a high proportion of linoleic acid, accompanied by relevant amounts of palmitic and

linolenic acids. Its phytochemical profile reveals the presence of bioactive compounds such as carotenoids, anthocyanins, tannins, and flavonoids, all of which are associated with notable antioxidant capacity [35].

The petals are rich in polyphenols, especially flavonoids such as rutin, violanthin, vicenin-2, and orientin, as well as various anthocyanosides derived from delphinidin, cyanidin, petunidin, and malvidin. These compounds contribute to a remarkable antioxidant capacity, as demonstrated by ABTS, DPPH, ORAC, and FRAP assays, which confirm a positive correlation between phenolic content and free radical scavenging activity [36].

On a dry weight basis, the flowers contain high amounts of carbohydrates ($80.27 \text{ g } 100 \text{ g}^{-1}$) and proteins ($10.14 \text{ g } 100 \text{ g}^{-1}$), highlighting their nutritional contribution compared to other edible flowers. Their energy value is approximately $376.67 \text{ kcal } 100 \text{ g}^{-1}$. The lipid profile is dominated by saturated fatty acids, mainly palmitic acid, followed by arachidic and myristic acids; among unsaturated fatty acids, the presence of linoleic acid, an essential omega-6 fatty acid, is significant. The petals exhibit considerable levels of phenolic compounds ($73 \text{ to } 1033.5 \text{ mg gallic acid equivalents } 100 \text{ g}^{-1}$), anthocyanins ($0.54 \text{ to } 543.49 \text{ mg } 100 \text{ g}^{-1}$), and flavonoids ($69.38 \text{ to } 824.84 \text{ mg } 100 \text{ g}^{-1}$), with variations depending on petal color [29]. In addition, they contain carotenoids, mainly lutein, zeaxanthin, β -carotene, violaxanthin, and antheraxanthin, which contribute to their bioactive potential [37].

These characteristics suggest that *V. × wittrockiana* could be used as a functional ingredient to enhance the nutritional value of various foods, representing a promising option within the edible flower market due to its potential health benefits [35].

PROSPECTS AND CHALLENGES IN THE PRODUCTION OF *Viola* spp.

Potential of Mexican floriculture

Floriculture in Mexico represents a strategic component within the agricultural sector, resulting in high profitability and employment generation capacity, surpassing even several conventional crops. This activity has shown remarkable growth in recent decades, driven mainly by geographic, edaphoclimatic, and biodiversity advantages that position the country as a territory with high potential to become a leader in the production and export of traditional ornamental species [38].

At the territorial level, flower production is concentrated in states such as Jalisco, State of Mexico, Morelos, Puebla, and Michoacán, regions that offer optimal agroecological conditions and a constantly modernizing production infrastructure [39]. A representative example is Mexico City, where over 100 varieties of ornamental plants are cultivated, mainly under intensive potted and greenhouse production systems. Among the most relevant crops of the autumn-winter season is the Dutch tulip, which in 2019 occupied an area of 2.2 hectares, generating approximately 326,700 plants, with a market value reaching 9,088,794 Mexican pesos [40].

However, despite the implementation of public policies and support programs to promote this subsector, the dynamics of foreign trade have faced structural challenges. Imports of floricultural products have increased at a faster rate than exports, generating a

persistent trade balance deficit [38]. This imbalance demonstrates that, although Mexico possesses considerable productive and biological potential, it is essential to strengthen competitiveness, biosecurity, innovation, and integration with international markets in order to fully capitalize on the opportunities in this sector.

Consumption trends of *Viola cornuta* and *Viola* × *wittrockiana*

Species of the genus *Viola*, particularly *Viola cornuta* and *Viola* × *wittrockiana*, occupy a prominent place within the edible flowers segment due to their long-standing tradition of use and their growing popularity today. These ornamental flowers are highly valued for their morphological and organoleptic characteristics: they display a wide range of vibrant colors, single-color or multicolor presentations, diverse floral shapes with varying diameters, and a velvety texture that complements their sweet and refreshing flavor. These attributes make them a versatile resource for gourmet cuisine and functional foods [11].

The interest in incorporating *Viola* spp. and other edible flowers into the human diet has intensified in response to the increasing prevalence of inadequate eating habits, which are associated with the development of various metabolic and chronic degenerative diseases. This situation has boosted the search for novel foods that improve the nutritional profile of the diet and provide bioactive compounds with potential health benefits [41].

Although the use of flowers in gastronomy dates back to ancient times, there is currently a sustained trend toward their use as functional ingredients, driven by growing consumer awareness of the link between food and well-being. This trend is further reinforced by lifestyles that prioritize the consumption of fresh, local, and healthy foods, as well as by increasing scientific evidence documenting the nutritional, antioxidant, and nutraceutical properties of edible flowers. All these factors have positioned *Viola cornuta* and *Viola* × *wittrockiana* as species with high added value for the food industry, responding to the demand for differentiated and functional products [42].

To fully capitalize on this emerging trend, it is essential that *V. cornuta* and *V.* × *wittrockiana* production systems incorporate sustainable agronomic practices that ensure the phytosanitary quality and food safety of flowers intended for human consumption. The adoption of greenhouse cultivation technologies, integrated pest management, and biofortification strategies can contribute to maximizing the content of bioactive compounds and optimizing shelf life. Likewise, the certification of good agricultural practices and the generation of added value through product traceability and differentiation will enable access to gourmet and functional food market niches, meeting the demands of increasingly informed and discerning consumers. This synergy between agronomic innovation, biological quality, and commercial strategies will strengthen the competitiveness of the national floriculture sector and consolidate various *Viola* species as benchmarks in the high-value edible flower market.

Production and sustainability challenges in *Viola cornuta* and *Viola* × *wittrockiana*

The species *Viola cornuta* and *V.* × *wittrockiana* have been established as key ornamental crops thanks to physiological attributes such as their high tolerance to low temperatures and their early flowering capacity, making them strategic in seasonal floriculture. Traditionally,

breeding programs in *V. × wittrockiana* have focused on maximizing vegetative vigor, promoting rapid establishment, and ensuring continuous and abundant flowering. However, specific morphological traits are now also prioritized, such as plant compactness, the development of larger, round-shaped flowers, and the presence of straight petals held on short peduncles, all of which directly influence ornamental quality and commercial acceptance.

One of the main challenges during the nursery stage is controlling excessive seedling elongation, a phenomenon that compromises the uniformity and final plant quality [30]. In addition, floriculture faces limitations associated with the postharvest phase: crops are often exposed to high thermal fluctuations and inadequate irrigation practices along the retail chain, increasing the incidence of water stress. It is estimated that such conditions can cause up to 15% yield loss during commercialization by directly affecting the aesthetic quality of the flowers. Consequently, studies aimed at strengthening *V. cornuta* tolerance to water deficit stress [43] and *V. × wittrockiana* frost resistance under different irrigation regimes [44] have been constantly increasing in recent years.

From a reproductive perspective, since the propagation of these species is primarily carried out through sexual reproduction, another technical challenge lies in optimizing seed quality and viability, an aspect that is currently being actively researched in *V. cornuta* to ensure efficient and uniform production [45].

In terms of food sustainability, the growing consumer interest in innovative and healthy products has driven the development of an emerging industry dedicated to the production and commercialization of edible flowers. In addition to their sensory appeal (unique colors, textures, and flavors), various *Viola* species can provide bioactive compounds with nutraceutical properties, expanding their potential as functional food.

However, this market faces significant challenges related to food safety, since not all ornamental flowers are suitable for human consumption, as some may contain toxic secondary metabolites or antinutritional compounds [37].

Furthermore, given that edible flowers are often consumed fresh or minimally processed, there is a considerable microbiological risk. Therefore, it is essential to guarantee proper agricultural practices, strict phytosanitary controls, and hygienic postharvest handling to reduce microbial load. The implementation of effective preservation, packaging, and storage methods not only helps minimize contamination but also preserves the nutritional quality and stability of the bioactive compounds present in the flowers [10].

Summing up, the comprehensive challenge for producers, researchers, and marketers is to adopt sustainable production systems that integrate genetic improvement, efficient agronomic management, food safety, and value-added strategies, thereby ensuring the competitiveness of *Viola cornuta* and *V. × wittrockiana* as high-value ornamental and edible crops.

CONCLUSIONS

The genus *Viola*, and specifically the species *Viola cornuta* and *V. × wittrockiana*, represents a valuable resource with great potential for diversification within both the floriculture and functional food industries. In addition to their undeniable ornamental value, these species

produce edible flowers rich in bioactive compounds, including polyphenols, flavonoids, anthocyanins, and carotenoids, which contribute to their antioxidant capacity and potential health benefits.

The increasing demand for edible flowers, driven by consumer preferences for innovative, healthy, and visually appealing products, highlights the need to develop sustainable production systems that ensure product quality, safety, and added value. To achieve this, it is essential to integrate agronomic innovation, genetic improvement, efficient postharvest management, and rigid food safety protocols.

Although *Viola cornuta* and *V. × wittrockiana* are already consolidated as important ornamental crops, their emerging role as functional food ingredients presents significant opportunities for the Mexican floriculture and food sectors. However, the challenges related to seed viability, postharvest preservation, food safety, and market differentiation must be addressed to fully exploit their potential.

In conclusion, promoting sustainable production, scientific research and innovation focused on these species will be key to strengthening their competitiveness as high-value edible and ornamental flowers, positioning them as a strategic alternative for responding to market trends and consumer demands for differentiated and health-promoting products.

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Organic and conventional production of tomato fruit and seed

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ABSTRACT

Objective: To evaluate the effect of two organic and one conventional management practices on the production and quality of fruit and seed from two native and one commercial tomato varieties.

Design/Methodology/Approach: The evaluated treatments included three substrate mixtures: vermicompost + tezontle (3 mm) at a 70:30 v/v ratio (S1), vermicompost + tezontle (5 mm) at a 50:50 v/v ratio (S2), and 100% tezontle (5 mm) (S3). Treatments S1 and S2 were completely organic (fertilized with bat guano and hydrolyzed fish), while S3 was fertilized with the Steiner solution. The following tomato varieties were used: Moneymaker (V1), Campeche 40 (V2), and Riñón (V3). The experiment was conducted in a greenhouse for 120 days.

Results: indicated that the highest fruit yield was obtained with the S3 × V1 combination (2.7 kg plant⁻¹). However, the S2 substrate produced fruits of superior quality, with a firmness of 7.74 N, a titratable acidity of 0.83%, and total soluble solids of 6.87 °Brix, similar to S1. Vitamin C content did not significantly differ between substrates. V3 recorded higher values in titratable acidity (0.94%), vitamin C content (4.1 mg/100 g), and total soluble solids (6.91 °Brix) than the other varieties. Regarding seed quality, S2 and S3 yielded superior physical and physiological characteristics, including 92 and 95% germination percentages, respectively. Likewise, they had an emergence speed of 2.9 and 3.3 radicles/d⁻¹ in aged seeds, respectively.

Findings/Conclusions: These findings suggest that the S2 mixture could be recommended for the commercial organic production of tomatoes.

Keywords: substrate, organic agriculture, vermicompost, quality.

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INTRODUCTION

Tomato is a highly important crop both in Mexico and the whole world, due to its significant economic contribution and its value as a source of vitamins, minerals, and antioxidants. Its nutritional value includes essential minerals such as Ca, P, K, and Na, as well as vitamins A, B1, and B2. These components and their bioactive substances contribute positively to human health (Andrade-Sifuentes *et al.*, 2020). Mexico holds the



11th place worldwide in tomato production, accounting for 1.7% of global production, behind countries such as China, India, and the United States of America. However, it contributes 19% of the total export volume, making it the main tomato exporter worldwide (FAOSTAT, 2020). From 2003 to 2017, Mexico produced an average of 2.5 million tons of tomatoes, recording an average annual value of nearly MXN\$15 billion pesos (SIAP, 2020).

In Mexico, the price of organic tomatoes can be up to 5.8 times higher than conventional ones. Combined with increased yields from crops grown in greenhouses (Murillo-Amador, 2010), this situation results in a significant increase in economic benefits for producers. However, conventional and organic farming methods have considerably different impacts on the environment and human health. As a consequence of the use of pesticides, conventional agriculture contributes to greenhouse gas emissions, water pollution and poses a risk to human health. In contrast, organic agriculture makes a significantly lower contribution to the carbon footprint, mitigates soil erosion, and promotes the restoration of natural ecosystems, formerly polluted by toxic pesticide residues (FAO, 2004). In recent years, Mexico—where 2 million ha are used for chemical-free agriculture—has emerged as one of the world leaders in organic food production. According to the Mexican Society of Organic Production (SOMEXPRO: Sociedad Mexicana de Producción Orgánica), approximately 700,000 ha are used for organic production in Mexico, with an additional 100,000 hectares in the process of converting to organic farming. The leading states in organic food production (Chiapas, Oaxaca, Michoacán, Chihuahua, and Guerrero) account for 82.8% of the total land area dedicated to organic agriculture in Mexico (Borbón *et al.*, 2021).

According to the latest report from the Foreign Agricultural Service of the United States Department of Agriculture, organic product trade between Mexico and the United States is increasing and reached record levels in 2023. Organic tomatoes are one of the top five crops exported to the United States and Canada. For Mexico, selling its organic products to these countries is clearly a priority, given their proximity and efficient transportation channels, despite having numerous free trade agreements with other countries (USDA, 2023).

Throughout its life cycle, tomato production depends on the synergistic interaction of essential factors such as genotype, soil, climate, and water. Yield varies depending on the growing site, the variety used, and the management practices adopted. Furthermore, the use of non-adapted varieties, suboptimal soil and water quality, or an inappropriate planting date can limit both the quantity and quality of the harvest (Murillo-Amador *et al.*, 2010).

Few current studies discuss the effect of organic production on tomato fruit and seed quality. Furthermore, scientific information about this production system in Mexico is scarce, despite its economic relevance and the benefits of consuming natural foods. For a production system to be classified and certified as organic, it must originate from seed produced under organic conditions. Accordingly, ensuring the availability of high-quality organic seed provides the justification for this investigation. Consequently, the aim of this study was to compare the yield, fruit quality, and seed quality of three tomato varieties grown in conventional and organic production systems.

MATERIALS AND METHODS

Experimental Site and Plant Material

The crop was grown in a 500 m² and 3.5 m high greenhouse, in a tunnel with side ventilation and a milky-white thermal polyethylene cover that provided 25% shade. The greenhouse is located at the Experimental Agricultural Field of the Universidad Autónoma Chapingo (UACH: Autonomous University Chapingo) in the State of Mexico, at 2,240 m.a.s.l., with the following coordinates: 19° 20' 55.15" N and 98° 50' 69.32" W. Three indeterminate tomato cultivars were used: 1) **MoneyMaker (MM)**, a round-shaped fruit cultivar widely used as a reference in genetic and physiological studies (Ruíz *et al.*, 2021); 2) the native cultivar **C-40**, with kidney-shaped fruit, originally from Campeche and provided by Mr. Iván Ramírez Ramírez (engineer) from the Plant Breeding Program at the Colegio de Postgraduados (Delgado-Vargas *et al.*, 2022); and 3) the native cultivar **Riñón**, with kidney-shaped fruits, traditionally grown using organic practices at the "Las Cañadas" Agroecological Center in Huatusco, Veracruz. Sowing was carried out on February 23, 2023, in 200-cell polystyrene trays using peat moss (sphagnum, Premier Tech Horticulture[®]) as the substrate. Fertigation with 50% Steiner solution began 15 days after sowing.

Substrates

Transplanting took place at 35 days after sowing (das) into 40 × 40 cm black polyethylene bags (pots), each filled with 15 L of substrate and one plant per pot. To select the appropriate substrate mixture for the organic treatments, a minimum total porosity test was performed. Substrate mixtures were chosen based on their suitability within the optimal range for total pore space and for water and air retention for root development. The substrates used in this experiment met the criteria established by Bilderback *et al.* (2005), which include 50% to 85% total pore space, 10% to 30% air capacity, and 25% to 35% water retention. For this purpose, five substrate mixtures with different ratios of vermicompost (Madre Tierra[®] Company) and tezontle were analyzed (results not shown). The 40:60 mixture (tezontle:vermicompost) with 3 mm tezontle particles (Substrate 1, S1), and the 50:50 mixture with 5 mm tezontle particles (Substrate 2, S2), were selected and compared with Substrate 3 (S3 or control), which consisted only of tezontle with particles smaller than 5 mm and was managed using a conventional (non-organic) hydroponic system.

Crop Management

The pots were arranged in double rows at a density of 3.8 plants m⁻². Three days after transplanting (dat), 1 mL L⁻¹ of amino acids was applied. *Fusarium oxysporum* was detected at 12 dat, and as result, 3 mL L⁻¹ of Bayer[®] Cupravit was applied three times, at 15 days intervals starting from the day of detection. Powdery mildew (*Oidiopsis taurica*) was also detected at 75 dat, requiring the application of 7 mL L⁻¹ of *Bacillus subtilis* QST 713 (SERENADE, Bayer[®]) every 15 days until the end of the crop cycle.

The main pest observed was the whitefly (*Bemisia tabaci*). In the organic treatments, 5 g L⁻¹ of Cactucentro[®] diatomaceous earth was applied at 17 dat to control the infestation.

Additionally, a dose of 20 mL L⁻¹ of Cactucentro[®] potassium soap combined with neem was applied once per week until the end of the crop cycle. In the conventional treatment (S3), the commercial insecticide Confidor[®] 350 SC was applied at 17 dat. Subsequently, the same potassium soap and neem mixture used in the organic treatments was also applied. Blue and yellow polypropylene chromatic traps were interspersed throughout the experimental area. The plants were pruned to a single main stem, removing senescent leaves every 7 d and leaving only 5 fruits per bunch. The apex was pinched after the fifth bunch appeared, leaving two leaves at the top to protect the fruits from excessive solar radiation. Nutrition in the organic treatments included three components: vermicompost incorporated into the substrate; diluted bat guano (Vita[®]) applied at 10 mL L⁻¹ per plant weekly from transplant until 60 dat; and the organic fertilizer Vitalex (Química Sagal[®]) and the pH regulator DAP-PLUS (Química Sagal[®]), both applied as foliar spray at 5 mL L⁻¹ and 0.1 mL L⁻¹, respectively, twice per week starting at 72 dat. Plants in the organic treatments were irrigated with 0.5 L of well water per plant during the first 30 dat. From transplant to harvest, an average of 1 L per plant was applied. In the conventional treatment (S3), the nutrient solution (Sánchez and Escalante, 1989) was applied via fertigation using the same irrigation schedule.

Experimental Design and Treatments

A randomized complete block design (RCBD) with a factorial arrangement was used in the greenhouse, considering three levels of the variety factor (MM, C-40, and Riñón) and three levels of substrate factor (S1: 40:60 T/V, S2: 50:50 T/V, and S3: unmixed T), resulting in nine treatments. Each treatment was replicated three times, with six plants per experimental unit. For the evaluation of fruit and seed quality, a single batch was formed from the harvested fruits collected for this purpose. Therefore, variables related to quality were analyzed using a completely randomized design with a factorial arrangement consisting of the same nine treatments, defined by the combination of the three substrate levels (S1, S2, and S3), each with its corresponding management, and the three tomato varieties evaluated.

Variables Evaluated

Throughout the growing season (120 dat), air temperature (°C) and relative humidity (%) inside the greenhouse were recorded every 30 min using a Hobo Onset[®] MX2301A sensor. Climatic variables were used to explain the results. Fruit yield per plant (FY) was determined by summing the weights of five fruits harvested at the ripening stage or when they were fully red. These fruits were randomly selected from the first to the fourth bunch. In addition, the following information was obtained from five other fruits from the fourth bunch: firmness (FF) at two opposite sites in the equatorial region using a Greenwich[®] FDV-30 texturometer; titratable acidity (TA) in 10 mL of juice; vitamin C (VITC) in 5 g of fruit, using the Tillman titration method; and total soluble solids (TSS) with an Atago[®] PAL-1 digital refractometer. To measure these last three variables, the AOAC (2005) methodology was followed. Fruit color was measured in a chamber illuminated with diffused light from

LED lamps. Digital images (610 × 813 pixels, JPG format) were captured from four sides of five fruits using a Canon[®] EOS Rebel T5 camera. The images were processed using PlantCV version 4.2.1 in Python 3.9, through the Jupyter Notebook web-based interface version 4.0.11. The CIE-LAB color model was used to segment the fruit and remove the background from each image. Average values and modes for each channel of the RGB, CIE-LAB, and HSV color models were then calculated. Seeds were extracted from 20 fruits from the fourth bunch, harvested at ripeness stage 6. The fermentation method was used to extract the seeds by hand. The fruit pulp and juice were left to ferment for 24 h. The seeds were then washed with running water, using a colander to remove mucilage residues. The clean seeds were left to dry on paper towels at room temperature (25 °C) for 7 d. Subsequently, a single batch was formed, enabling the analysis of variance for these variables to be performed using a completely randomized block design (CRBD). The seeds were then placed in paper envelopes and stored at room temperature for two months prior to use.

To assess physical seed quality, the weight of one thousand-seed weight (TSW) was measured following the standard procedure of ISTA (2021). Four replicates were used, each consisting of eight samples of 100 seeds. The seeds were weighed using a PA2202 balance (Ohaus[®]) with a precision of 0.001 g, and the average weight per replicate was multiplied by 10 to obtain the TSW. Hectoliter weight (HW) was estimated by measuring the volume displaced by 1 g of seeds in a 10 mL test tube. Seed length (SL) and width (SW) were measured using four replicates of 25 seeds each, through image processing with ImageJ[®] software version 1.54f. For this purpose, seeds were captured in a scanner HP[®] ScanJet 4890 at 600 dpi. To assess physiological seed quality, a germination test was conducted. Four replicates of 25 seeds each were sown in plastic boxes (11 × 11 × 3.5 cm) on filter paper moistened with distilled water. The boxes were placed in a germination chamber at 25 °C under constant light. After 14 d, the germination (G) percentage was determined following the ISTA (2021) criteria. Additionally, radicle emergence rate (RER) was evaluated through daily counts. At the end of the test, the radicle emergence rate index (radicles d⁻¹) was calculated, based on the resulting data, using Maguire's germination rate index (1962):

$$RER = \sum (ni / ti)$$

Where: *ni* = number of seedlings germinated in the time interval. *ti* = the time in days from sowing to the day of counting.

In order to assess seed vigor, an accelerated aging test was performed (Delgado-Vargas *et al.*, 2022). Four replicates of 25 seeds were placed in plastic boxes, which were then subjected to 45 °C and 100% relative humidity for 72 h in a Thelco 3480 conventional drying oven (Thermo Electron Corporation[®]). After this treatment, the seeds were sown, using a germination test similar to the one previously described, in which germination percentage (GAAA) and radicle emergence rate (RERA) were evaluated.

Data Analysis

Data were analyzed using R Studio version 3.6.4 for both DEBA and DECA designs. An analysis of variance (ANOVA) was conducted, followed by Tukey's HSD test at a significance level of $\alpha=0.05$. Percentage data were transformed using the arcsine square root transformation: $\arcsin\left(\sqrt{(X/100)}\right)$. If the color-related response variables did not meet the assumptions of normality, a Kruskal-Wallis test was applied, followed by Conover's post hoc pairwise multiple comparison test.

RESULTS AND DISCUSSION

Environmental Conditions

The recorded temperatures exceeded the optimal range for the crop, which is between 23 and 26 °C (Table 1). Growth and production of this species —classified by Delgado-Vargas *et al.* (2022) as sensitive to high temperatures— are reduced when exposed to temperatures above this range.

A low seed production was observed in the harvested fruits (a variable not measured), a situation likely attributable to adverse temperature conditions during anthesis of the first four bunches (from April 20 to July 5, 2023). During this period, the average temperature was 30.1 ± 2.9 °C and the average maximum temperature was 39 ± 3.5 °C —values that exceed the optimal range for this crop. Temperatures above 35 °C have a negative impact on pollination: they induce an elongation of the stigma that hinders contact between the pollen in the stamens and the stigma (Jarma-Orozco *et al.*, 2012). Although the average maximum relative humidity recorded in the morning ($60 \pm 20\%$) was adequate for pollination (Jarma-Orozco *et al.*, 2012), high temperatures may have contributed to the low seed set.

Fruit Yield and Quality

The ANOVA results revealed that the substrate factor (S) had a significant effect ($P \leq 0.05$) on fruit yield (FY). The variety factor (V) and the interaction between substrate and variety had a highly significant effect ($P \leq 0.01$). The highest FY was recorded with the S3 substrate, which outperformed the organic treatments; however, no significant differences were found between the organic treatments (Table 2). The complete and balanced nutrient solution provided improved nutrition. Similar results have been reported in tomato by De Grazia *et al.* (2007), Rodríguez *et al.* (2008) and Cruz *et al.* (2009).

Regarding the effect of the variety factor on FY, V1 recorded a higher yield than V2 and V3. The best treatment was the S3V1 interaction, which corresponded to the level with the

Table 1. Average and standard deviation (SD) of maximum, mean, and minimum air temperature (AT) and relative humidity (RH) recorded in the greenhouse during the growing cycle.

| Variable | Maximum | SD | Mean | SD | Minimum | SD |
|----------|---------|------|------|------|---------|------|
| AT (°C) | 37.7 | 2.7 | 30.1 | 2.6 | 19.6 | 1.9 |
| RH (%) | 66.1 | 11.4 | 39.0 | 11.4 | 27.1 | 10.1 |

AT: air temperature. RH: relative humidity.

Table 2. Comparison of means for individual factors and their interaction on fruit-related variables in three tomato varieties grown under two organic production treatments and one conventional treatment.

| SV | FY (kg plant ⁻¹) | FF (N) | TA (%) | VITC (mg 100 g ⁻¹) | TSS (° Brix) |
|------|---------------------------------|-----------|-----------|-----------------------------------|-----------------|
| S1 | 0.80 b | 7.62 a | 0.79 ab | 3.0 a | 6.50 a |
| S2 | 0.81 b | 7.74 a | 0.83 a | 2.8 a | 6.87 a |
| S3 | 1.46 a | 5.09 b | 0.69 b | 2.7 a | 5.75 b |
| LSD | 0.65 | 1.50 | 0.007 | - | 0.72 |
| V1 | 1.58 a | 6.90 ab | 0.59 c | 2.0 c | 5.85 b |
| V2 | 1.14 b | 8.16 a | 0.79 b | 2.4 b | 6.37 ab |
| V3 | 0.35 c | 5.39 b | 0.94 a | 4.1 a | 6.91 a |
| LSD | 0.42 | 1.55 | 0.004 | 0.29 | 0.73 |
| S1V1 | 1.25 c | 7.05 bcd | 0.65 d | 2.43 d | 6.25 b |
| S1V2 | 0.76 d | 9.37 a | 0.84 bc | 2.77 c | 6.60 ab |
| S1V3 | 0.25 e | 6.45 cd | 0.90 b | 3.79 b | 6.66 ab |
| S2V1 | 1.31 c | 8.07 abc | 0.65 d | 1.96 ef | 6.80 ab |
| S2V2 | 0.87 d | 8.85 ab | 0.81 c | 2.27 de | 6.39 b |
| S2V3 | 0.40 e | 6.30 cd | 1.04 a | 4.42 a | 7.43 a |
| S3V1 | 2.17 a | 5.57 d | 0.46 e | 1.75 f | 4.50 c |
| S3V2 | 1.79 b | 6.27cd | 0.72 d | 2.29 de | 6.12 b |
| S3V3 | 0.68 c | 3.42 e | 0.88 bc | 4.33 a | 6.63 ab |
| LSD | 0.92 | 2.12 | 0.004 | 0.34 | 0.98 |

SV: source of variation; FY: fruit yield; FF: fruit firmness; TA: titratable acidity; VITC: vitamin C; TSS: total soluble solids; LSD: least significant difference. Means with the same letter in the same column are statistically equal (Tukey $\alpha=0.05$).

highest performance for each individual factor (Table 2). Of the three varieties studied, only V1 (Moneymaker) is the result of a plant breeding program in a scientific research center and has become a reference in agronomic and physiological research (Delgado-Vargas *et al.*, 2022). This may explain why the yield of Moneymaker yield is higher than that of the other two varieties, which were selected for different traits. On the one hand, genotype V2 (C-40), native to the state of Campeche, is resistant to high temperatures (Delgado-Vargas *et al.*, 2022). On the other hand, V3 (Riñón) was chosen due to its traditional use in organic production systems.

Regarding fruit quality, the substrate factor had a highly significant effect ($P \leq 0.01$) on fruit firmness (FF) and a significant effect ($P \leq 0.05$) on total soluble solids (TSS) and titratable acidity (TA). Nevertheless, it had no effect on vitamin C (VITC). The variety factor had a highly significant effect ($P \leq 0.01$) on FF, TA, and VITC, as well as a significant effect ($P \leq 0.05$) on TSS. The interaction between factors (substrate \times variety) had highly significant effect on all the variables studied.

S1 and S2 recorded higher FF and TSS values than substrate S3. S1 and S2 also ranked higher in titratable acidity (TA), although only S1 surpassed S3 (Table 2).

On the one hand, Zahedi *et al.* (2010) indicated that the fulvic and humic acids found in the vermicompost used in treatments S1 and S2 likely contributed significant amounts of Ca—an essential element for the regulation of the physical properties of the fruit. Ca increases fruit size and promotes uniformity, elasticity, hydration, and skin firmness. It also improves postharvest fruit performance and promotes sugar formation, resulting in an increase of the °Brix index. Furthermore, Batu (2004) proved that Ca supplementation helps to minimize pectin decomposition and maintains the firmness of the pectic matrix, which supports the strength of the fruit walls.

On the other hand, the firmness values for all treatments were higher than those reported by Batu (2004) for 100% marketable fruits produced without Ca deficiency. Therefore, the type of Ca input in S1 and S2 enabled a sufficient assimilation. However, this was not the case for the hydroponic treatment, where Ca absorption may have been affected by the greenhouse environmental conditions and the lower moisture retention of the substrate composed solely of tezontle.

Variety 3 (Riñon) stood out in TA, VITC, and TSS; it also had the lowest FF (Table 2). Native varieties (including V3) are usually considered to have better flavor than improved varieties. For example, Juárez-López *et al.* (2009) reported a TA value of 1.01% for a native tomato variety, a result similar to that found in this study for V3 grown in S2 (Table 3). Furthermore, the TA values observed in V2 and V3 (both native varieties) exceeded the 0.4-0.6% range reported by Nuez (1995) for commercial tomatoes. However, these values were comparable to those observed for V1 in the present study.

Regarding the effect of substrate on fruit color, no significant differences were found in luminosity (CIE-L), red intensity (positive CIE-a*), yellow intensity (positive CIE-b*), hue, or chroma. However, the variety factor and its interaction with the substrate caused significant differences in the fruit color variables (Table 3).

Table 3. Post hoc pairwise multiple comparison (Conover's test) of the medians for the CIE-Lab color space channels, hue, and chroma of tomato fruits, as affected by the interaction between substrate and variety.

| VS | CIE-L | CIE-a* | CIE-b* | Matiz |
|------|-------|--------|--------|---------|
| S1V1 | 28 b | 40 a | 30 a | 5.5 a |
| S1V2 | 28 b | 37 b | 19 b | 351.5 a |
| S1V3 | 23 d | 32 c | 16 c | 353.5 a |
| S2V1 | 27 b | 38 b | 30 a | 7 a |
| S2V2 | 26 c | 38 b | 19 b | 356 a |
| S2V3 | 24 d | 32 c | 15 c | 354 a |
| S3V1 | 28 b | 37 b | 31 a | 7 a |
| S3V2 | 31 a | 40 a | 19 b | 355 a |
| S3V3 | 23 d | 32 c | 16 c | 356 a |
| LSD | 4 | 3 | 12 | 355 |

VS: variation source. CIE-L: luminosity; CIE-a*: a positive value indicates a reddish tint; CIE-b*: a positive value indicates a yellowish tint. LSD: least significant difference. Medians with the same letter in the same column are statistically equal ($\alpha=0.05$).

The Moneymaker (V1) variety had high luminosity and a red color with strong yellow hues. Its coloration was an intense, highly saturated red, contributing to a vibrant color. The native Campeche 40 variety (C40) also showed high luminosity, but displayed a red color with lighter yellow hues. Its lower color saturation, compared to Moneymaker, resulted in lighter red fruits. Variety 3 (Riñón) was characterized by lower luminosity and saturation, producing fruits with a light red color and pale-yellow hues, which gave them an overall orange appearance.

The combination of a tezontle-based substrate with particles smaller than 5 mm and hydroponic management, along with the C-40 variety, produced fruits with the highest luminosity. In contrast, the Riñón variety, cultivated under organic management, consistently exhibited the lowest luminosity and the lowest red intensity, regardless of the substrate. The most intense red coloration was observed in the Moneymaker variety grown in the 40:60 tezontle/organic vermiculite mix (S1V1), as well as in the C-40 variety under hydroponic conditions (S3V2). The Moneymaker variety also recorded the highest yellow values and the greatest chroma, regardless of the substrate.

Regarding fruit yield and quality, González-Fuentes *et al.* (2021) consider that organic nutrition does not necessarily result in higher yields compared to conventional nutrition. However, it does lead to higher-quality fruits, with growth and morphological characteristics that are similar to or better than those obtained with inorganic nutrition. These findings are consistent with the results of the present experiment.

Seed Quality

In terms of the physical seed quality variables, the substrate factor showed a highly significant effect ($P \leq 0.01$) on seed length (SL), seed width (SW), and thousand-seed weight (TSW). No substrate effect was observed on volumetric weight (VW). The variety factor only had a highly significant effect on SL. Meanwhile, the interaction between substrate and variety had a highly significant effect on all four variables evaluated. The means test showed that S2- and S3-levels of the substrate factor produced higher SL, SW, and TSW values than S1, although there were no statistical differences between S1 and S3 in the case of SL (Table 4). The TSW, SL, and SW values for the Moneymaker (V1) and C-40 (V2) varieties were similar to those reported by Delgado-Vargas *et al.* (2022), who studied the effect of high temperature on the seed quality of both varieties.

In the interaction between factors (substrate \times variety), the S2V2 treatment produced longer and wider seeds, being similar to S1V1 in the first variable and to S3V3 in the second. The highest TSW and VW values resulted from treatments S3V2 and S3V1 (similar to S3V3). Because some treatments resulted in bigger (longer or wider) seeds with the same weight, their accumulated reserves may be lower (Taylor, 2020). Furthermore, thickness—a third dimensional variable related to seed size which could have influenced TSW and VW values—was not measured.

Regarding seed physiological quality, the substrate factor (S) had a significant effect ($P \leq 0.05$) on germination (G), germination after accelerated aging (GAAA), and radicle emergence rate (RER) of artificially aged seeds. However, it had no effect on the emergence rate of unaged seeds (RUS). The variety factor (V) generated highly significant differences

Table 4. Comparison of means using Tukey's test for each independent factor and their interaction, based on seed quality variables from three tomato varieties grown under two organic and one conventional production treatments.

| SV | SL (mm) | SW (mm) | TSW (g) | VW (kg hL ⁻¹) | G (%) | RER (Rad d ⁻¹) | GAAA (%) | RERA (Rad d ⁻¹) |
|------|------------|------------|------------|------------------------------|----------|-------------------------------|-------------|--------------------------------|
| S1 | 3.58 b | 2.44 b | 2.63 b | 20.16 a | 80 b | 3.77 a | 52 b | 2.05 b |
| S2 | 3.76 a | 2.61 a | 2.95 a | 21.37 a | 92 a | 4.32 a | 76 a | 2.90 a |
| S3 | 3.68 ab | 2.58 a | 3.00 a | 21.86 a | 95 a | 5.33 a | 83 a | 3.25 a |
| LSD | 0.13 | 0.72 | 0.05 | 1.58 | 10 | 1.3 | 0.21 | 0.93 |
| V1 | 3.74 a | 2.54 a | 2.81 a | 21.54 a | 91 a | 3.68 b | 72 b | 2.45 b |
| V2 | 3.73 a | 2.53 a | 2.90 a | 21.23 a | 98 a | 7.05 a | 90 a | 4.06 a |
| V3 | 3.54 b | 2.58 a | 2.86 a | 20.62 a | 67 b | 2.60 c | 50 c | 1.69 c |
| LSD | 0.12 | 0.03 | 0.06 | 0.62 | 23 | 3.8 | 0.18 | 0.56 |
| S1V1 | 3.83 ab | 2.52 c | 2.6 e | 20.55 d | 80 b | 3.02 d | 48 c | 1.75 d |
| S1V2 | 3.53 e | 2.35 d | 2.7 d | 20.40 d | 97 a | 6.20 b | 92 a | 3.95 a |
| S1V3 | 3.37 f | 2.45 c | 2.6 e | 19.52 e | 56 d | 2.10 e | 17 d | 0.50 e |
| S2V1 | 3.79 b | 2.59 b | 2.8 c | 20.86 d | 95 a | 3.45 d | 82 ab | 2.75 bc |
| S2V2 | 3.90 a | 2.66 a | 2.8 c | 21.60 c | 100 a | 6.77 b | 88 a | 3.80 a |
| S2V3 | 3.58 de | 2.59 b | 2.9 b | 20.72 d | 67 c | 2.75 de | 59 c | 2.15 cd |
| S3V1 | 3.60 de | 2.46 c | 3.0 a | 22.27 ab | 99 a | 4.57 c | 85 ab | 2.87 b |
| S3V2 | 3.75 bc | 2.59 b | 3.0 a | 22.52 a | 99 a | 8.17 a | 91 a | 4.45 a |
| S3V3 | 3.68 cd | 2.71 a | 2.9 b | 21.71 bc | 79 b | 2.95 de | 74 b | 2.45 bcd |
| LSD | 0.10 | 0.07 | 0.04 | 0.56 | 16 | 5.4 | 0.15 | 0.44 |

SV: source of variation; SL: seed length; SW: seed width; TSW: thousand-seed weight; VW: volumetric weight; G: germination; RER: = radicle (Rad) emergence rate; GAAA: germination after accelerated aging; RERA: radicle (Rad) emergence rate after accelerated aging; LSD: least significant difference. Means with the same letter in the same column are statistically equal (α , 0.5).

($P \leq 0.01$) in RER, GAAA and RERA, and significant differences in G ($P \leq 0.05$). Finally, the $S \times V$ interaction caused highly significant differences in all variables. A seed of high physiological quality is considered to have better germination (higher percentage and speed) and vigor (greater resistance to storage under adverse conditions, such as accelerated aging) (Delgado-Vargas *et al.*, 2022). Furthermore, accelerated aging is used to test vigor —*i.e.*, the ability of seeds to germinate quickly and establish successfully, especially under adverse conditions (ISTA, 2021).

Similarly, for most of the seed physical variables, substrate levels S2 and S3 resulted in better values for G, GAAA, and RERA values than S1 (Table 4). Among the varieties, V2 showed superior values in G, GAAA, and RERA compared to the other two varieties. In the case of G, both V1 and V2 were better than V3. Regarding the interaction effect, the most notable result is that treatment S1V2 is in the top group for G, GAAA, and RERA. This is attributed to the strong influence of variety (V2), which compensated for the lower performance of substrate S1, the treatment with the lowest values for these variables (Table 4). The literature reviewed did not report data on tomato seed yield or quality under organic production systems, highlighting the relevance of the present findings. Overall, substrate S2 produced seeds with physical and physiological quality comparable to those from S3, which was managed conventionally and it is considered

optimal for tomato cultivation. According to Alcántar-González and Trejo-Téllez (2013), high nitrogen concentrations (>120 ppm) promote vigorous growth, increased flower production, and higher seed yield. This may help explain the observed results. Since S2 and S3 had seeds with similar physical and physiological quality characteristics, S2 can be recommended for tomato seed production under organic management systems.

CONCLUSIONS

Treatments with organic substrates S1 and S2 resulted in higher fruit quality than treatment S3 (conventional production), as indicated by greater firmness and higher total soluble solids. Substrate S2, a 50:50 (v/v) mixture of vermicompost and tezontle, also produced the highest titratable acidity. Although neither organic nor conventional substrates significantly affected fruit color, the C-40 variety (V2) grown under the conventional treatment (S3) exhibited the highest luminosity. In contrast, the Riñon variety, used in organic production, consistently showed the lowest luminosity and red intensity, regardless of substrate. The most intense red coloration was observed in fruits from the Moneymaker variety (V1) combined with organic substrate S1 and from the C-40 variety (V2) under conventional treatment S3. The highest fruit yield was recorded under the conventionally managed treatment S3. Both S3 and the organic managed S2 treatments produced the highest seed quality, based on seed length, seed width, thousand-seed weight, germination, germination after accelerated aging, and radicle emergence rate after accelerated aging. Since treatment S2 stood out in both fruit and seed quality traits, this mixture could be recommended for use in commercial organic tomato production.

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Analysis of meteorological drought in the Rodrigo Gómez Dam basin

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ABSTRACT

Objective: To identify the return periods of meteorological drought in the Rodrigo Gómez “La Boca” Dam basin, located in the state of Nuevo León.

Design/Methodology/Approach: Based on the Streak, Foley, and moving-average methods, a probabilistic approach was applied to annual rainfall data (1973-2015) from five climatological stations located within the Rodrigo Gómez Dam basin to forecast drought recurrence. Years in which at least two methods identified drought conditions were considered drought years for the purpose of calculating return periods.

Results: A meteorological drought is highly probable with a return period of 3 to 5 years.

Study Limitations/Implications: For the drought analysis, climatological stations with at least 30 years of rainfall records were required. The study area was reduced during the data collection, as two stations within the Rodrigo Gómez Dam area were excluded given their insufficient precipitation data.

Findings/Conclusions: This research confirmed the existence and persistence of drought-related problems at the dam. The results indicate a high probability of a meteorological drought with a return period of 3 to 5 years. This recurrence reinforces the need for a Drought Action and Prevention Plan in the state of Nuevo León, aimed at ensuring the sustainable management of water resources and minimizing impacts on the population.

Keywords: water security, water management, meteorological phenomenon, water stress, precipitation forecast.

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INTRODUCTION

Drought is a natural phenomenon characterized by the lack of water (mainly rainfall), in a given period and area, preventing humans from meeting their basic needs. This phenomenon impacts society, the agricultural sector, and the economy. Lobato-Sánchez (2016) defines drought as one of the phenomena with the greatest impact on the main sectors involved in water management, because the long-term precipitation deficit is reflected in hydrological and socioeconomic areas. Drought classification is complicated by its dependency on the approach with which they are analyzed and the magnitude of their impact. Given the lack of a universal definition, drought has not received enough attention and its analysis and research have been scattered. Wilhite and Glantz (1985) suggested differentiating between conceptual and operational definitions of drought. In general terms, conceptual definitions are developed to identify the boundaries of the concept, while operational definitions seek to characterize the drought with specific onset, severity, and duration data. The authors proposed classifying droughts from the point

of view of four scientific concepts: meteorological drought, agricultural drought, socio-economic drought, and hydrologic drought.

In Mexico, droughts are classified according to their intensity and impact. Based on their intensity, droughts are divided into abnormally dry, moderate, severe, extreme, and exceptional. Meanwhile, based on their impact, droughts are classified as agricultural, meteorological, and socio-economic droughts.

This study analyzes meteorological droughts, which are characterized by the lack of rainfall. Its main indicator is the climatic data of the study region. This type of drought occurs when the mean annual precipitation is lower than historical records, resulting in a lower storage volume of surface water in hydraulic structures and a decrease in groundwater recharge.

Various authors have provided their own definitions of this type of drought. For example, Palmer (1965) defines it as a meteorological anomaly characterized by a lengthy and abnormal lack of moisture in a given location. Its severity is determined by its duration and magnitude. Marcos (2001) bases his definition of drought on climate data, considering that precipitation deviates from the mean over a given period. This type of drought varies depending on the study region, as it is directly related to the characteristics of the regional climate. All over the world, droughts are a result of climate change and poor water resource management. In 1984, Africa faced one of the worst droughts in history, endangering the lives of 35 million people and causing one million deaths. In response to this phenomenon, drought was first recognized as a global issue in the first report of the World Commission on Environment and Development (1987).

The United Nations Educational, Scientific and Cultural Organization (2020) declared it a serious problem. It poses a risk to the health of the population, because the concentration and proliferation of pathogens are higher during droughts. Given the increased visibility of environmental issues, countries began to act accordingly.

Mexico became a pioneer in water management in 1989 with the creation of the National Water Commission (CONAGUA: Comisión Nacional del Agua), a public agency responsible for managing the country's water resources. In addition, various local operating agencies, such as basin councils, were established. In 1994, Mexico was the first country to sign the United Nations Convention to Combat Desertification. In the same year, Mexico developed its national action plan, which included a comprehensive analysis of the desertification and drought issues in the country (Ruiz and Febles, 2004).

Despite the efforts of the Mexican government to manage climate risks, the country has not been immune to climate crises. In 2011 and 2012, a severe drought affected more than 80% of the country, causing losses exceeding MXN\$16 billion in the agricultural sector, as well as water shortages in rural and vulnerable communities (Ortega-Gaucin, 2018). More than half of its territory is classified as arid or semi-arid, making Mexico susceptible to droughts. Its territory has a great climatic diversity, ranging from very warm areas in the south to deserts in the north. These natural conditions make Mexico more vulnerable to droughts.

There are three reasons why Nuevo León is particularly vulnerable to these types of natural phenomena: its location, its prevailing weather conditions, and the natural

availability of water. This state lies within the northern belt of the world's great deserts and it is part of an arid zone with a natural water deficit. The diverse climate of Nuevo León is the result of diverse orography within the state; however, the prevailing weather conditions are dry and semi-dry. The combination of all these natural conditions, coupled with the growing impact of climate change, makes Nuevo León a region of interest for the research and implementation of drought mitigation and prevention strategies. The objective of this research was to determine the return period of droughts for each season, with the goal of preventing and mitigating the short- and long-term effects of this phenomenon.

MATERIALS AND METHODS

The Rodrigo Gómez Dam, more commonly known as “La Boca,” is a hydraulic structure located in the municipality of Santiago, state of Nuevo León. It was built in 1961 by CONAGUA to supply drinking water to the city of Monterrey. It has a storage capacity of 40 hm³ and an area of approximately 455 ha. Its tributaries are the following streams: La Chueca, Cavazos, Cristalinas, Dolores, Escamilla, Puerco, and San Antonio (CONAGUA, 2015).

Beyond its hydraulic function, the dam is used for various recreational activities, such as sportfishing, water sports, and ecotourism.

This analysis required climate data from weather stations within the Rodrigo Gómez Dam basin. Since not all-weather stations located within the basin have uninterrupted precipitation records, three off-site stations were included for a more accurate characterization. “La Boca” and “El Cerrito” stations are located within the dam basin, while the “El Pajonal,” “Laguna de Sánchez,” and “Allende” stations are located outside of it.

The relevant information of each weather station (i.e., identification code, name, coordinates, and altitude) is summarized in Table 1.

This analysis used historical monthly precipitation data in millimeters (mm) from the aforementioned weather stations from 1973 to 2015 (Table 2). Based on these data, the mean annual precipitation for each station was calculated (Table 3), considering years when recorded precipitation was lower than the calculated annual mean as drought years.

Statistical models are increasingly used to identify the occurrence and duration of droughts, as a result of their significant usefulness in planning and managing water resources. Three drought identification methods were used to forecast meteorological droughts in the “La Boca” Dam basin.

Table 1. Specifications of the weather stations.

| ID Code | Name | Latitude | Longitude | Altitude (m) |
|---------|------------|-------------|---------------|--------------|
| 19069 | La Boca | 25° 25' 46" | −100° 7' 44" | 460 |
| 19015 | El Cerrito | 25° 30' 36" | −100° 11' 36" | 510 |
| 19018 | El Pajonal | 25° 29' 23" | −100° 23' 20" | 2576 |
| 19003 | Allende | 25° 17' 01" | −100° 01' 13" | 454 |

m = meters.

Source: Table developed by the authors, based on information from the SMN (National Meteorological Service of Mexico) (2023).

Table 2. Precipitation (mm) of the weather stations (1973-2015).

| Year | La Boca | El Cerrito | El Pajonal | Laguna de Sánchez | Allende |
|------|----------|------------|------------|-------------------|----------|
| 1973 | 1,839.30 | 1,561.30 | 543.00 | 903.00 | 1,937.00 |
| 1974 | 719.80 | 766.50 | 162.00 | 601.50 | 1,030.00 |
| 1975 | 978.90 | 945.50 | 318.00 | 582.00 | 1,503.10 |
| 1976 | 1,368.40 | 1,207.50 | 817.30 | 878.00 | 1,395.50 |
| 1977 | 851.90 | 609.00 | 592.20 | 693.00 | 850.00 |
| 1978 | 1,547.40 | 1,307.00 | 722.40 | 1,172.00 | 1,570.50 |
| 1979 | 984.70 | 791.90 | 587.90 | 224.50 | 945.00 |
| 1980 | 867.40 | 743.60 | 802.10 | 616.00 | 706.50 |
| 1981 | 1,116.70 | 974.40 | 888.90 | 516.50 | 1,410.80 |
| 1982 | 771.10 | 205.40 | 570.30 | 527.10 | 756.10 |
| 1983 | 1,157.90 | 901.20 | 111.00 | 276.00 | 1,224.20 |
| 1984 | 530.80 | 502.80 | 70.00 | 452.10 | 518.50 |
| 1985 | 749.70 | 826.20 | 653.00 | 482.00 | 893.50 |
| 1986 | 593.10 | 1,351.80 | 599.60 | 496.00 | 1,088.00 |
| 1987 | 869.50 | 936.70 | 478.00 | 719.00 | 1,039.80 |
| 1988 | 1,036.60 | 1,115.00 | 815.00 | 965.70 | 1,275.50 |
| 1989 | 613.80 | 833.20 | 459.50 | 474.50 | 835.00 |
| 1990 | 991.30 | 957.00 | 619.00 | 633.60 | 958.50 |
| 1991 | 854.20 | 897.00 | 513.00 | 484.00 | 1,036.50 |
| 1992 | 776.90 | 772.60 | 448.00 | 907.70 | 1,155.50 |
| 1993 | 1,154.90 | 1,258.00 | 497.00 | 525.50 | 1,176.00 |
| 1994 | 926.40 | 878.00 | 394.00 | 400.10 | 793.50 |
| 1995 | 1,132.60 | 853.30 | 285.00 | 268.00 | 105.50 |
| 1996 | 839.70 | 772.00 | 307.90 | 618.50 | 176.60 |
| 1997 | 966.90 | 724.30 | 641.40 | 648.80 | 826.30 |
| 1998 | 927.90 | 1,008.90 | 692.50 | 390.50 | 690.50 |
| 1999 | 789.00 | 261.50 | 360.50 | 722.00 | 467.50 |
| 2000 | 1,236.90 | 939.50 | 148.00 | 324.00 | 797.90 |
| 2001 | 1,233.40 | 939.30 | 475.00 | 795.80 | 1,128.30 |
| 2002 | 1,148.50 | 937.00 | 718.00 | 995.40 | 945.40 |
| 2003 | 1,565.70 | 129.50 | 707.20 | 803.00 | 1,302.70 |
| 2004 | 1,210.50 | 488.20 | 551.50 | 828.70 | 1,254.00 |
| 2005 | 1,396.70 | 1,010.40 | 592.60 | 1,458.90 | 1,224.50 |
| 2006 | 775.60 | 762.10 | 447.90 | 596.50 | 774.20 |
| 2007 | 1,039.50 | 682.60 | 497.40 | 851.30 | 760.90 |
| 2008 | 1,313.70 | 1,230.30 | 723.00 | 779.40 | 1,496.50 |
| 2009 | 666.70 | 624.30 | 551.80 | 793.20 | 817.00 |
| 2010 | 2,084.10 | 1,737.05 | 1,075.60 | 1,915.50 | 1,079.80 |
| 2011 | 637.70 | 542.10 | 281.00 | 416.40 | 605.70 |
| 2012 | 799.20 | 818.34 | 405.00 | 575.70 | 796.60 |
| 2013 | 1,393.90 | 1,366.50 | 963.00 | 1,625.40 | 1,313.00 |
| 2014 | 1,346.90 | 1,271.40 | 508.00 | 1,153.90 | 1,290.00 |
| 2015 | 991.50 | 987.10 | 588.20 | 707.90 | 1,029.30 |

mm = millimeters.

Source: Table developed by the authors, based on information from the SMN (2023).

Table 3. Mean annual precipitation.

| Station | Mean Annual Precipitation (mm) |
|-------------------|--------------------------------|
| La Boca | 1041.8 |
| El Cerrito | 539.1 |
| El Pajonal | 893.7 |
| Laguna de Sánchez | 716.2 |
| Allende | 999.6 |

mm= milimeters.

Source: Table developed by the authors, based on information from the SMN (2023).

1. **Runs or sequences method.** This method originated with the work of Yevjevich (1967), who first developed this model based on geometric distribution. Over time, he continued refining the method to incorporate techniques of series analysis to predict the occurrence and duration of droughts. The principle of this method is the analysis of periods that are below the mean (known as dry periods).

The equations used in this method are as follows:

$$Sequences = hpi - hp_{media} \quad (1)$$

$$Severity = duration \times magnitude \quad (2)$$

2. **Foley method (Residual Mass Curve).** This method examines the temporal behavior of dry and wet periods, as well as the monthly precipitation excesses and deficiencies in relation to the historical mean. According to this method, dry periods accumulate negative differences and consequently have a downward slope (Flores and Campos Aranda, 2015).

The following equation was used for the Foley method:

$$Foley = (hpi - hp_{media}) + (hpi - hp_{media2}) \quad (3)$$

3. **Moving-average method.** This technique smooths out irregular, random variations in a series of chronological precipitation events, with the goal of prioritizing the recording of wet and dry cycles. The threshold for this method is the mean of the accumulated rainfall. One of the special features of this analysis is that the variables are evaluated in groups of five. Wet periods are identified by comparing the moving-average line against the mean rainfall line. In rainy periods, the first line is above the second, while dry periods are below the mean (Campos-Aranda, 1988).

The main equation used in this method is:

$$\left[(hp_1) + (hp_2) + (hp_3) + (hp_4) + (hp_5) \right] / 5 \quad (4)$$

Once the drought periods have been identified at each weather station, the severity of the drought will be calculated. The results will indicate the yearly recurrence of this natural phenomenon. Further details about this calculation can be found in Campos Aranda (1988).

The return period calculation forecasts when an event of equal or greater magnitude will occur again. Mélice and Reason (2007) define the return period as the number of years when an extreme weather event is expected to be replicated or exceeded. The return period will be calculated with the following formula:

$$T_R = (\sum Recurrence) / (Periods - 1) \quad (5)$$

RESULTS AND DISCUSSION

After the three previously defined methods were used to identify droughts and return periods, the following results were obtained. According to the runs method (Table 4), 12 dry periods were identified at “La Boca,” making it the station where the greatest drought was recorded.

The results of the Foley method (Table 5) are more conservative than those of the runs method. Although both methods identify “La Boca” as the station with the greatest number of droughts, the Foley method only identifies seven dry periods.

The third method (moving-average) was the most limited of all, although it showed the highest recurrence rates (Table 6). This method identified two stations (“El Pajonal” and “Allende”) with three dry periods each.

More reliable results can be obtained through the classification as drought of those years in which at least two of the three applied methods identify the phenomenon at the same time (1982, 1983, 1984, 1985, 1986, 1991, 1992, 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2006, 2007, 2011 and 2012). To increase the accuracy of the values of the return and recurrence periods shown in the previous tables, the return period for each station and method analyzed will be calculated (Table 7), using the weighted mean of the results obtained by the three methods.

The AX.EXE software—a program developed by the Mexican National Center for Disaster Prevention (CENAPRED: Centro Nacional de Prevención de Desastres)—was used to adjust the various probability distribution functions to the annual precipitation data. The expected annual precipitation for the return periods of interest was calculated based on these adjustments (Table 8). The results of this analysis provide projections of the maximum annual precipitation that could occur in the future in the Rodrigo Gómez Dam basin.

Research conducted in the Rodrigo Gómez Dam basin highlights the high vulnerability to drought events faced by the region. A return period of 3 to 5 years is predicted in the study area, highlighting the urgency of implementing preventive measures.

Although Mexico played an important role in the development of integrated water management, it is currently an area with deficiencies and which is rarely updated. Mexico is naturally vulnerable to these types of climate events; however, other factors increase this risk, such as water overexploitation, ecosystem destruction, uncontrolled population growth,

Table 4. Results of the runs method.

| Station | Period | Year | Magnitude (mm) | Duration (years) | Severity | Recurrence |
|-------------------|--------|-----------|----------------|------------------|----------|------------|
| La Boca | 1 | 1974 | 322 | 1 | 322 | / |
| | 2 | 1977 | 189.9 | 1 | 189.9 | 3 |
| | 3 | 1980 | 174.4 | 1 | 174.4 | 2 |
| | 4 | 1982 | 270.7 | 1 | 270.7 | 1 |
| | 5 | 1984-1987 | 356 | 4 | 1424.1 | 1 |
| | 6 | 1989 | 428 | 1 | 428 | 1 |
| | 7 | 1991-1992 | 226.25 | 2 | 452.5 | 1 |
| | 8 | 1996 | 202.1 | 1 | 202.1 | 3 |
| | 9 | 1999 | 252.8 | 1 | 252.8 | 2 |
| | 10 | 2006 | 266.2 | 1 | 266.2 | 6 |
| | 11 | 2009 | 375.1 | 1 | 375.1 | 2 |
| | 12 | 2011-2012 | 323.4 | 2 | 646.7 | 1 |
| El Pajonal | 1 | 197-1975 | 299.1 | 2 | 598.2 | / |
| | 2 | 1983-1984 | 448.6 | 2 | 897.2 | 7 |
| | 3 | 1989 | 79.6 | 1 | 79.6 | 4 |
| | 4 | 1992 | 91.1 | 1 | 91.1 | 2 |
| | 5 | 1994-1996 | 210.1 | 3 | 630.4 | 1 |
| | 6 | 1999-2000 | 284.85 | 2 | 569.7 | 2 |
| | 7 | 2006 | 91.2 | 1 | 91.2 | 5 |
| | 8 | 2011-2012 | 196.1 | 2 | 392.2 | 4 |
| El Cerrito | 1 | 1974 | 127.2 | 1 | 127.2 | / |
| | 2 | 1977 | 284.7 | 1 | 284.7 | 2 |
| | 3 | 1980 | 150.1 | 1 | 150.1 | 2 |
| | 4 | 1982 | 688.3 | 1 | 688.3 | 1 |
| | 5 | 1984 | 390.9 | 1 | 390.9 | 1 |
| | 6 | 1992 | 121.1 | 1 | 121.1 | 7 |
| | 7 | 1996-1997 | 145.55 | 2 | 291.1 | 3 |
| | 8 | 1999 | 632.2 | 1 | 632.2 | 1 |
| | 9 | 2003-2004 | 584.83 | 2 | 1169.66 | 3 |
| | 10 | 2006-2007 | 171.37 | 2 | 342.75 | 1 |
| | 11 | 2009 | 269.4 | 1 | 269.4 | 1 |
| | 12 | 2011 | 351.6 | 1 | 351.6 | 1 |
| Laguna de Sánchez | 1 | 1974-1975 | 124.45 | 2 | 248.9 | / |
| | 2 | 1979-1986 | 267.43 | 8 | 2139.4 | 3 |
| | 3 | 1989 | 241.7 | 1 | 241.7 | 2 |
| | 4 | 1991 | 232.2 | 1 | 232.2 | 1 |
| | 5 | 1993-1996 | 263.2 | 4 | 1052.7 | 1 |
| | 6 | 1998 | 325.7 | 1 | 325.7 | 1 |
| | 7 | 2000 | 392.2 | 1 | 392.2 | 1 |
| | 8 | 2006 | 119.7 | 1 | 119.7 | 5 |
| | 9 | 2011-2012 | 220.15 | 2 | 440.3 | 4 |

Table 4. Continues...

| Station | Period | Year | Magnitude (mm) | Duration (years) | Severity | Recurrence |
|---------|--------|-----------|----------------|------------------|----------|------------|
| Allende | 1 | 1977 | 149.6 | 1 | 149.6 | / |
| | 2 | 1980 | 293.1 | 1 | 293.1 | 2 |
| | 3 | 1982 | 243.5 | 1 | 243.5 | 1 |
| | 4 | 1984 | 481.1 | 1 | 481.1 | 1 |
| | 5 | 1989 | 164.6 | 1 | 164.6 | 4 |
| | 6 | 1994-2000 | 448.5 | 6 | 2690.9 | 4 |
| | 7 | 2006-2007 | 232 | 2 | 464.1 | 5 |
| | 8 | 2009 | 182.6 | 1 | 182.6 | 1 |
| | 9 | 2011-2012 | 298.5 | 2 | 596.9 | 1 |

mm = millimeters

Note: The symbol “/” in Recurrence indicates no recurrence data available.

Table 5. Results of the Foley method.

| Station | Period | Year | Magnitude (mm) | Duration (years) | Severity | Recurrence |
|-------------------|--------|-----------|----------------|------------------|----------|------------|
| La Boca | 1 | 1973-1975 | 137.5 | 3 | 412.6 | / |
| | 2 | 1978-1980 | 91.4 | 3 | 274.1 | 2 |
| | 3 | 1983-1988 | 218.9 | 7 | 1532.1 | 2 |
| | 4 | 1989-1993 | 163.6 | 5 | 817.9 | 1 |
| | 5 | 1996-1999 | 160.9 | 4 | 643.7 | 2 |
| | 6 | 2005-2007 | 28.8 | 3 | 86.4 | 5 |
| | 7 | 2010-2012 | 131.9 | 3 | 395.6 | 2 |
| El Cerrito | 1 | 1978-1980 | 916.7 | 3 | 2750.1 | / |
| | 2 | 1983-1985 | 222.23 | 3 | 666.7 | 2 |
| | 3 | 1993-1997 | 576.54 | 5 | 2882.7 | 7 |
| | 4 | 2002-2004 | 678.22 | 3 | 2034.66 | 1 |
| | 5 | 2005-2007 | 1244.7 | 3 | 3734.02 | 1 |
| El Pajonal | 1 | 1973-1975 | 323.8 | 3 | 971.4 | / |
| | 2 | 1982-1984 | 360.73 | 2 | 721.47 | 6 |
| | 3 | 1990-1996 | 226.69 | 7 | 1586.8 | 5 |
| | 4 | 1998-2001 | 774.13 | 4 | 3096.5 | 1 |
| | 5 | 2005-2007 | 724.21 | 3 | 2172.63 | 3 |
| | 6 | 2010-2012 | 266.09 | 3 | 798.28 | 3 |
| Laguna de Sánchez | 1 | 1973-1975 | 65.6 | 3 | 196.8 | / |
| | 2 | 1978-1986 | 581.1 | 9 | 5229.7 | 2 |
| | 3 | 1988-1991 | 1635.4 | 4 | 6541.7 | 1 |
| | 4 | 1992-1998 | 2472.8 | 7 | 17309.7 | 1 |
| | 5 | 2010-2012 | 1143 | 3 | 3429 | 12 |

Table 5. Continues...

| Station | Period | Year | Magnitude (mm) | Duration (years) | Severity | Recurrence |
|---------|--------|-----------|----------------|------------------|----------|------------|
| Allende | 1 | 1983-1985 | 120.9 | 3 | 362.6 | / |
| | 2 | 1988-1990 | 23.4 | 3 | 70.2 | 2 |
| | 3 | 1992-2000 | 288.9 | 9 | 2599.7 | 1 |
| | 4 | 2005-2007 | 79.7 | 3 | 239.2 | 4 |
| | 5 | 2010-2012 | 368.5 | 3 | 1105.4 | 2 |

mm = millimeters

Note: The symbol “/” in Recurrence indicates no recurrence data available.

Table 6. Results of the moving-average method.

| Station | Period | Year | Magnitude (mm) | Duration (years) | Severity | Recurrence |
|-------------------|--------|-----------|----------------|------------------|----------|------------|
| La Boca | 1 | 1983-2001 | 201.4 | 19 | 3826.1 | / |
| | 2 | 2009 | 375.1 | 1 | 375.1 | 7 |
| El Cerrito | 1 | 1981-1986 | 752.98 | 6 | 4517.9 | / |
| | 2 | 1998-2009 | 742.51 | 12 | 8910.09 | 11 |
| El Pajonal | 1 | 1977-1978 | 118.2 | 2 | 236.4 | / |
| | 2 | 1984-1988 | 196.1 | 5 | 980.5 | 5 |
| | 3 | 1993-2004 | 160.1 | 12 | 1921.4 | 4 |
| Laguna de Sánchez | 1 | 1981-2002 | 215.9 | 3 | 647.8 | / |
| Allende | 1 | 1984-1988 | 939.15 | 5 | 4695.76 | / |
| | 2 | 1995-2003 | 692.26 | 9 | 6230.3 | 6 |
| | 3 | 2010-2013 | 954.8 | 4 | 3819.2 | 6 |

mm = millimeters

Note: The symbol “/” in Recurrence indicates no recurrence data available.

Table 7. Return periods (years).

| Station | Runs Drought Periods | Runs Return Period | Foley Drought Periods | Foley Return Period | Moving Averages Drought Periods | Moving Averages Return Period | Weighted Average | Rounded |
|-------------------|----------------------|--------------------|-----------------------|---------------------|---------------------------------|-------------------------------|------------------|---------|
| La Boca | 12 | 2.09 | 7 | 2.33 | 2 | 5.69 | 3.37 | 3 |
| El Cerrito | 12 | 2.09 | 5 | 2.75 | 2 | 9.14 | 4.66 | 5 |
| El Pajonal | 8 | 3.57 | 6 | 3.6 | 3 | 7.05 | 4.74 | 5 |
| Laguna de Sánchez | 9 | 2.25 | 5 | 4 | 1 | 2.57 | 2.94 | 3 |
| Allende | 9 | 2.38 | 5 | 2.25 | 3 | 5.36 | 3.33 | 3 |

Source: Table developed by the authors, based on information from the SMN (2023).

Table 8. Projection of precipitation (mm) for the calculated return periods.

| Station | Return Period | Mean Annual Precipitation (mm) | Expected Annual Precipitation AX.EXE (mm) |
|-------------------|---------------|--------------------------------|---|
| La Boca | 3 | 1,041.8 | 190.9 |
| El Cerrito | 5 | 539.1 | 1,171.0 |
| El Pajonal | 5 | 893.7 | 724.4 |
| Laguna de Sánchez | 3 | 716.2 | 650.1 |
| Allende | 3 | 999.6 | 1,099.7 |

mm = millimeters.

Source: Table developed by the authors, based on information from CENAPRED (2023).

industrialization, a deficient regulatory framework, and institutional opacity, to name a few. Unfortunately, Mexico lacks a public policy on drought prevention and mitigation. Its implementation would be key to develop a more efficient water and environmental management, as well as to reduce the vulnerability of Mexico to the growing effects of climate change. Arreguín-Cortés *et al.* (2016) suggest that, in order to ensure continuity, public policy needs to establish a new paradigm to address drought, shifting from a reactive to a preventive approach that transcends six-year administrations.

The lack of water literacy in Mexico makes people mistakenly believe that these types of problems are solely the responsibility of the authorities or that solutions are beyond the reach of common citizens. Droughts directly affect living beings, who depend on water for survival, and they also have a negative impact on the economy and health.

Esparza (2014) emphasizes that the large hydraulic structures built in the 20th century created a false sense of water abundance in Mexico. This perception has now been replaced by water waste and pollution, as well as by the concentration of the population in areas where there is not enough water to meet the basic needs. Drought prevention and planning not only reduces the negative impact on the environment and the economy, but also protects the well-being of communities. Barcia *et al.* (2014) emphasize that economic, social, and environmental consequences are more severe for populations that are not prepared to cope with them.

Droughts have economic, environmental, and social impact. The economic impact includes the loss of agricultural, livestock, forestry, and fishery production. The environmental impact directly affects ecosystems, altering their cycles, increasing erosion, and deteriorating water and air quality. Finally, the social impact can lead to food shortages, health problems, and conflicts between users and institutions responsible for water management; in addition this type of impact reduces the quality of life and increases poverty (Velasco *et al.*, 2005).

From an environmental point of view, the most damaging effects of droughts can be perceived in ecosystems, natural resources, flora, and fauna. These problems must be addressed: once an ecosystem has deteriorated, it is unlikely to survive the consequences of a drought (Vázquez *et al.*, 2007).

The drought in the Rodrigo Gómez basin not only impacts the population that depends on this source of drinking water, but also has a significant impact on the agricultural and

recreational activities connected with the dam. Furthermore, droughts harm aquatic habitats and the species that live in this body of water.

The results of this study provide a solid basis for informed decision-making regarding water management and drought prevention in Nuevo León. Droughts have diverse consequences that impact key sectors—including agriculture, one of the pillars of the regional economy—, jeopardizing both food security and the income of rural families. Furthermore, water scarcity impacts the supply of drinking water in the metropolitan area of Monterrey and has a negative impact on local flora and fauna.

CONCLUSIONS

Drought is a complex phenomenon that requires a multidisciplinary solution. Such a solution must implement sustainable measures that promote a balance between society and the environment. For this purpose, the institutions responsible for managing these extreme events must be supported. The results of this research can provide the authorities of the state of Nuevo León with a key tool to strengthen action plans and establish the necessary measures that ensure the well-being of the population impacted by these types of climatic events. Furthermore, these findings provide a basis for the implementation of public policies that not only address drought prevention and mitigation, but also foster community resilience, improve natural resource management, and promote more sustainable practices. Incorporating these results into governmental strategies will enable a more effective response to future climate challenges, guaranteeing the safety, health, and quality of life of the inhabitants of Nuevo León.

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Feasibility study of pitahaya (*Hylocereus undatus* (Haworth) D.R. Hunt) production in the Mixteca Poblana region

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ABSTRACT

Objective: To conduct a pitahaya production feasibility analysis in Tepexi de Rodríguez, Mixteca Poblana region, Puebla.

Design/Methodology/Approach: Twenty local producers were subjected to a panel technique. The research took place from May to June, 2023. The results were used to determine main economic indicators such as internal rate of return (IRR), net present value (NPV), and benefit-cost ratio (BCR).

Results: The plantation was established less than 6 years ago. Pitahaya cultivation is profitable. The NPV was >0 (MXN\$302,361.58), while the IRR recorded 28.29% —a percentage higher than the discount rate. Meanwhile, the BCR reached 1.35: for every MXN\$ invested, a profit of MNX\$0.35 was obtained.

Study Limitations/Implications: The lack of customers that guarantee the purchase of the fruit before or during the harvest is a limiting factor.

Findings/Conclusions: Finding commercialization channels and transformation processes for specific markets is very important. In addition, increasing the demand and improving the commercial positioning of pitahaya is fundamental. Pitahaya can be grown in different areas and its consumption benefits make it a feasible and profitable crop.

Keywords: Production costs, income, financial indicators.

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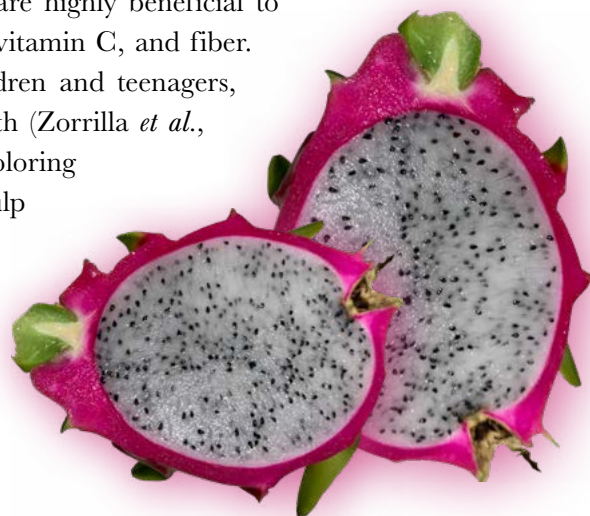
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INTRODUCTION

Pitahaya (*Hylocereus undatus* (Haworth) D.R. Hunt) has abundant nutritional and medicinal properties that are highly beneficial to humans, including phosphorous, calcium, vitamin C, and fiber. Its consumption is recommended for children and teenagers, because it strengthens their bones and teeth (Zorrilla *et al.*, 2004). In addition, pitahaya is a natural coloring agent: the intense colors of its skin and pulp are a result of its betacyanin content (Tze *et al.*, 2012). Pitahaya fruits are highly demanded in both domestic and international markets (García and Quirós, 2010). The adaptability of



pitahaya plants to harsh weather conditions makes them an option to replace traditional crops in marginal areas. Currently, this plant grows in the wild in 20 states of Mexico. However, it is grown as a commercial crop in some regions of the country. Pitahaya is an exotic fruit with a sweet taste and high-water content (Ortega *et al.*, 2018).

From 2010 to 2021, pitahaya was produced in the Mexican states of Yucatán, Tabasco, Sinaloa, Quintana Roo, Puebla, Nayarit, Jalisco, Guerrero, Guanajuato, and Aguascalientes. However, Quintana Roo and Yucatán produced approximately 92.4% of the total domestic production (SADER-SIAP, 2023).

The state of Puebla accounted for 6.12% of the domestic production during the same period, reaching a volume of 595.6 t and an average profit of 4.68 t/ha. In 2022, pitahaya was produced in the irrigation districts of Izúcar de Matamoros, Tecamachalco, and Tehuacán, accounting for 4.6, 45.7, and 49.7% of the state production, respectively (SADER-SIAP, 2023). This plant grows under semiarid conditions and, therefore, the extreme weather of the Baja Mixteca Poblana is ideal for its production.

Other studies have proved that growing pitahaya in Mexico is feasible. Michel *et al.* (2022) pointed out that its direct sale in Jalisco has a 2.66 BCR —*i.e.*, pitahaya is profitable and its profitability could be even greater without middlemen. Meanwhile, Ayala *et al.* (2023) indicated that pitahaya is a promising crop for producers, because it is profitable and does not require a high investment once the plant starts to grow, as long as the producer finds a buyer. Finally, Aguilar *et al.* (2017) also mentioned that pitahaya can be successfully grown and that it can reach a very favorable price abroad. In addition, it becomes profitable between the third and fourth year of production.

Therefore, the objective of this research was to conduct a pitahaya production economic feasibility study in the Mixteca Poblana region, particularly in the municipality of Tepexi de Rodríguez, Puebla. In addition, the characteristics of the producers and the production units were established.

Pitahaya is a profitable fruit in the area, has market potential, and can improve the wellbeing of the producers.

MATERIALS AND METHODS

Location

Tepexi de Rodríguez is one of the municipalities of the Mixteca Poblana. It is located in southern Puebla, at 18° 20' 24" and 18° 37' 42" N and at 97° 46' 00" and 98° 03' 18" W (INEGI, 2014). The altitude of the region ranges from 1,200 to 1,700 m.a.s.l. (Mindek, 2003). The area is divided into two regions with distinct climates: subhumid with summer rains (65.05%) and subhumid temperate with summer rains (34.95%). As a whole, the region has warm summer and temperate winters (Figure 1) (INEGI, 2010).

The soils of the Mixteca are basic, with a 6.8-8.7 pH (Altieri *et al.*, 2006). Leptosols cover approximately 65.42% of the region (INEGI, 2010). These soils are shallow and stony, with rocks in all or very close to the surface. These soils can be found in all types of weather (dry, temperate, humid) and are particularly common in mountain areas and shallow limestone plains. Their calcium content can immobilize minerals. In addition, their scarce depth and

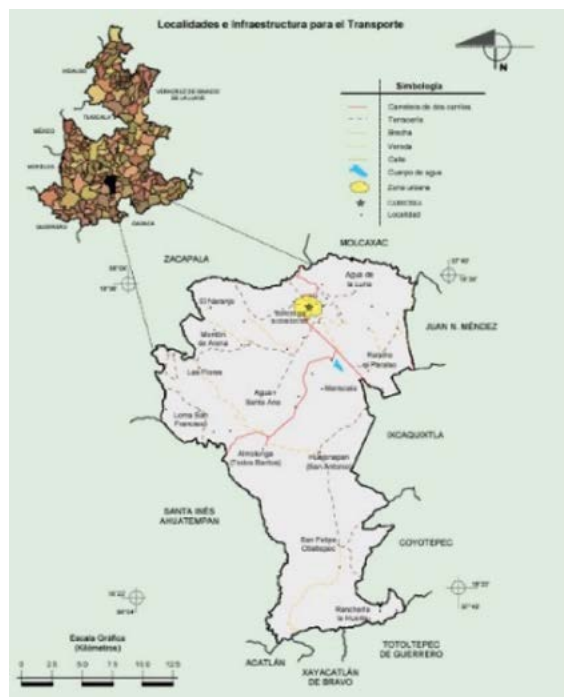


Figure 1. Study area of pitahaya (*Hylocereus undatus* (Haworth) D.R. Hunt) in Tepexi de Rodríguez, Puebla, Mexico. Source: INEGI, 2010.

high stone content limit agricultural practices and they require appropriate techniques to grow crops (IUSS Grupo de Trabajo WRB, 2007).

Agriculture is the main economic activity of the municipality and employs half of the active population of the area. The second most important economic activity is exploitation and exportation of marble, extracted from different local mines (Gobierno del Estado de Puebla, 2014).

Surveys

A direct survey was designed and applied based on Rojas (2022), who pointed out that this type of survey is appropriate when there is not enough data about the aspects to be researched or when the information cannot be obtained using other techniques. The survey included subjects related to the producer characteristics, production systems, economic feasibility, and commercialization of pitahaya in the region.

Producer panels

Data was obtained applying the survey to producer panels. The panel data methodology [Red Mexicana de Investigación en Política Agroalimentaria (Agroprospecta), 2009, 2010; Ireta *et al.*, 2015] takes into account groups of producers with similar technological level and growing area. The panel technique gathers a group of previously selected producers through a non-probabilistic and convenient sampling (Pimienta, 2000; Franco, 2018). The selection criteria were the willingness of producers with a similar technological level to participate in the survey.

Two panels were formed. Each panel was made up of ten pitahaya producers who received a direct invitation. Therefore, the survey was applied to 20 selected (expert selection) producers from a non-probabilistic sampling.

The panels were carried out from May to June, 2023.

Economic feasibility estimation

The indicators used to evaluate economic feasibility were: internal rate of return (IRR), net present value (NPV), and benefit-cost ratio (BCR). The results of these indicators were determined at the end of the cycle, once the initial investment and the total production (costs and income) were established (Mancilla *et al.*, 2020).

NPV was calculated with the following equation:

$$NPV = \sum_{t=1}^T \frac{B_t - C_t}{(1-i)^t} \quad (1)$$

The mathematical formal expression of IRR is an equation of the discount rate that equals NPV to 0 (Mancilla *et al.*, 2020).

$$IRR = \sum_{t=1}^T \frac{B_t - C_t}{(1-i)^t} = 0 \quad (2)$$

where: NPV = net present value (\$); IRR = internal rate of return (%); B_t = annual benefit (\$); C_t = annual costs (\$); i = interest rate (%); T = number of analysis periods or lifespan of the project; t = each period of the project (year 1, 2... T); $(1+i)^{-1}$ = discount factor.

Mancilla *et al.* (2009) established the following operation rule: if $BCR > 1$, the project is affordable; if $BCR < 1$, the project is unaffordable; and if $BCR = 1$, there is an indecision. According to Mancilla *et al.* (2009) the BCR can be calculated with the following equation:

$$\frac{B}{C} = \left[\frac{\sum_{t=1}^T \frac{B_j}{(1+i)^n}}{\sum_{t=1}^T \frac{C_j}{(1+i)^j}} \right] \quad (3)$$

where: B_j = annual benefit (\$); C_j = annual costs (\$); i = interest rate (%); n = number of analysis periods or lifespan of the project; j = each period of the project (year 1, 2... T).

RESULTS AND DISCUSSION

Characteristics of the producers

The producers are divided into 75% men and 25% women. They are heads of family who are in average 48-years old and have an age range of 39 to 52 years. Unlike the results

recorded by Ayala *et al.* (2023), who mention that 78% of the members of the community of San Luis Atolotitlan, Caltepec, Puebla, are 50+ years old (which involves an ageing problem in the rural sector), 60% of the producers in Tepexi de Rodríguez are younger than 50 years. Additionally, they have a basic education level: 78% had concluded primary school and 22% had attended junior high school. They are interested in learning, innovating, and adopting new technologies. According to INEGI (2020), the average education level in the state of Puebla is 9.2 years among the 15+ year old population (*e.g.*, third year of junior high school). This figure is lower than the national average (9.7 school years among the 15+ year population). According to SAGARPA-FAO (2014), analyzing the education level of agricultural producers is particularly important, given its implication in their capacity to implement productive technologies and to manage production units.

Characteristics of production units

In average, production surfaces reach 1 ha. Producers mentioned that pitahaya is a commercial product and that their plantations are less than 6-years old. The local climate is ideal for pitahaya production, given that this xerophyte can adapt to dry and arid environments and diverse environmental conditions (Montesinos *et al.*, 2015). This versatility makes it an alternative in zones where other crops are restricted (Ricalde and Andrade, 2009).

The following activities are carried out during the first year of the crop: sowing, installation of the support system, installation of the irrigation system, application of organic fertilizer, and weeding. The plantation is established using plant cuttings with the help of support, mostly consisting of concrete structures or natural supports. In average, 3,300 plant cuttings and 1,550 supports are required per hectare. A concrete support costs approximately MXN\$200, while a plant cutting costs MXN\$35.00, resulting in an investment of MXN\$521,843.10. The second-year maintenance tasks include the application of manure in May, as well as the application of varying quantities of fertilizers and pesticides in May, July, and August, depending on the conditions of the crop. The first harvest takes place during the second year. Although the production is rainfed, the initial plantation and production stages require additional water. Consequently, the May and June droughts have had a negative impact on production. The irregularity and lack of rain, as well as the high luminosity and temperatures damage and burn the plants during the winter-spring periods, reducing productivity and increasing production costs.

The fruit is harvested in three or four cuts, from June to October, recording an average production of 6 t/ha. This yield is possible as long as the plantation has been adequately cared for and has been irrigated at least twice.

Economic feasibility

The selling price is established according to quality. The base price per kg was MXN\$45.00.

Prices vary according to offer and demand: the price is high at the start of the season and then it substantially decreases as the product offer increases or other fruits appear in the market (Flores, 2003). Annual income is projected with a useful life of 5 years

(Table 1). No profits are obtained during the first year, because the plants do not bear fruits and only sowing activities are carried out during that period (year zero). Plants bear fruits approximately one year after transplanting. Producers are not aware of any technology that can diminish the time before the harvest.

Table 2 shows the results of the analysis of costs and income, cash flow, and benefits and expenditures. The said results take into account investment, working capital, and surrender value.

Table 3 shows the up-to-date estimated costs and benefits, taking into consideration an updated 10% factor, investment in year zero, and income from the production of pitahaya from year 1.

Table 1. Total income (MXN \$).

| Concept | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 |
|--------------|--------------|--------------|--------------|--------------|--------------|
| Price | 45,000.00 | 47,250.00 | 49,612.50 | 52,093.13 | 54,697.78 |
| Tons | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| Total income | \$270,000.00 | \$283,500.00 | \$297,675.00 | \$312,558.75 | \$328,186.69 |

Source: Table developed by the authors.

Table 2. Financial analysis, costs, benefits, and net flow.

| Year of operation | Total Income | Project Investments | | | | Valor de Rescate | | Net Cash Flow |
|-------------------|--------------|---------------------|------------------|---------------------|-----------------|------------------|-----------------------------|---------------|
| | | Total Expenses | Fixed investment | Deferred investment | Working capital | Residual value | Recovery of working capital | |
| 0 | | 31,104 | 487,283.10 | | 34,560.00 | | | 552,947.10 |
| 1 | 270,000.00 | 72,681.30 | | | | | | 197,318.70 |
| 2 | 283,500.00 | 76,315.37 | | | | | | 207,184.64 |
| 3 | 297,675.00 | 80,131.13 | | | | | | 217,543.87 |
| 4 | 312,558.75 | 84,137.69 | | | | | | 228,421.06 |
| 5 | 328,186.69 | 88,344.57 | | | | 23,932.77 | 34,560 | 298,334.89 |

Source: Table developed by the authors.

Table 3. Up-to-date financial analysis, costs, benefits, and net flow.

| Year of operation | Total costs (\$) | Total benefits (\$) | Discount factor 10.0% | Adjusted costs (\$) | Adjusted benefits (\$) | Discounted net cash flow (\$) |
|-------------------|------------------|---------------------|-----------------------|---------------------|------------------------|-------------------------------|
| 0 | 552,947 | 0 | 1.000 | 552,947.10 | 0.00 | -552,947.10 |
| 1 | 72,681 | 270,000 | 0.909 | 66,073.91 | 245,454.55 | 179,380.64 |
| 2 | 76,315 | 283,500 | 0.826 | 63,070.55 | 234,297.52 | 171,226.97 |
| 3 | 80,131 | 297,675 | 0.751 | 60,203.71 | 223,647.63 | 163,443.93 |
| 4 | 84,138 | 312,559 | 0.683 | 57,467.17 | 213,481.83 | 156,014.66 |
| 5 | 88,345 | 386,679 | 0.621 | 54,855.03 | 240,097.52 | 185,242.49 |
| Total | 954,557 | 1,550,413 | | 854,617.47 | 1,156,979.05 | 302,361.58 |

Source: Table developed by the authors.

Updating cash flow is necessary to determine a net value, taking into account the long-term capital costs, therefore correctly determining financial indicators. Without updated incomes and expenditures, it would not be possible to accurately determine the long-term capital cost (Mancilla *et al.*, 2009).

The NPV was higher than zero, while the IRR was higher than the discount rate. A 1.35 BCR indicates that a MXN\$0.35 profit was obtained per each Mexican peso invested (Table 4).

Although pitahaya is a profitable crop (Table 4), commercialization has been problematic throughout the region, because producers must seek buyers or wait for their arrival.

According to Ayala *et al.* (2023), middlemen usually arrive at local pitahaya plantations; however, if the middleman does not visit the producer, the harvest will be lost, due to the lack of defined commercialization channels. Producers sell their pitahaya to middlemen, who determine the buying and selling price of the fruit; nevertheless, producers could obtain greater profits if they sold directly to the final consumers. According to Michel *et al.* (2022), the direct commercialization of pitahaya would be a feasible, effective, sustainable, and more profitable alternative for producers. Producers would then establish the selling prices throughout the fruit season. Likewise, adding value would be another alternative for pitahaya producers.

Pitahaya is not widely known in the domestic market; therefore, promoting non-traditional products with development potential is a core task for the sustainable development of the agricultural sector. Additionally, fresh fruit does not have added value. However, given its nutritional value, pitahaya could have various market opportunities. Its properties help the successful treatment of several diseases (Lezama *et al.*, 2007). Appropriate market strategies could increase the demand for this fruit.

The prospects for domestic production lay mainly in the growth of internal consumption; therefore, advertising strategies are required to stress the exceptional characteristics of pitahaya, including its high nutritional content, health benefits, and versatile use in the kitchen. Such strategies could include using diverse communications channels (*e.g.*, social networks, TV, radio, and printed media) to reach the widest audience possible and organizing workshops, tastings, and cooking events to allow people to experience different ways in which they can consume pitahaya.

These activities could widen the consumer base, increase the commercial value of the fruit, and identify exportation opportunities to countries with high consumption of exotic fruits and gourmet products. Producers could also participate in international trade shows and establish alliances with distributing companies. According to Ayala *et al.* (2023), in

Table 4. Financial indicators.

| Financial indicator | Value |
|-------------------------------|--------------|
| Net Present Value (NPV) | \$302,361.58 |
| Internal Rate of Return (IRR) | 28.29% |
| Benefit-Cost Ratio (B/C) | 1.35 |

Source: Table developed by the authors.

order to position pitahaya in competitive markets and boost demand, a rural co-op system that allows producers to harvest and transform the fruit should be considered. Positioning pitahaya in the domestic market requires campaigns that make the population aware of its benefits and encourage its consumption. Pitahaya is a promising crop for small producers, as long as they establish appropriate agricultural management and demand grows.

CONCLUSIONS

Pitahaya thrives with minimum maintenance work and its production is profitable. Nevertheless, identifying commercialization channels for each harvest or a market niche specifically interested in pulp is essential. Meanwhile, the demand for this fruit should be increased beyond the local level. Its production on non-demanding zones is easy and feasible. It is not only a profitable fruit, but its consumption provides several benefits.

The profitability of pitahaya enables the improvement of its production, as well as the post-harvest management and development of its market positioning.

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