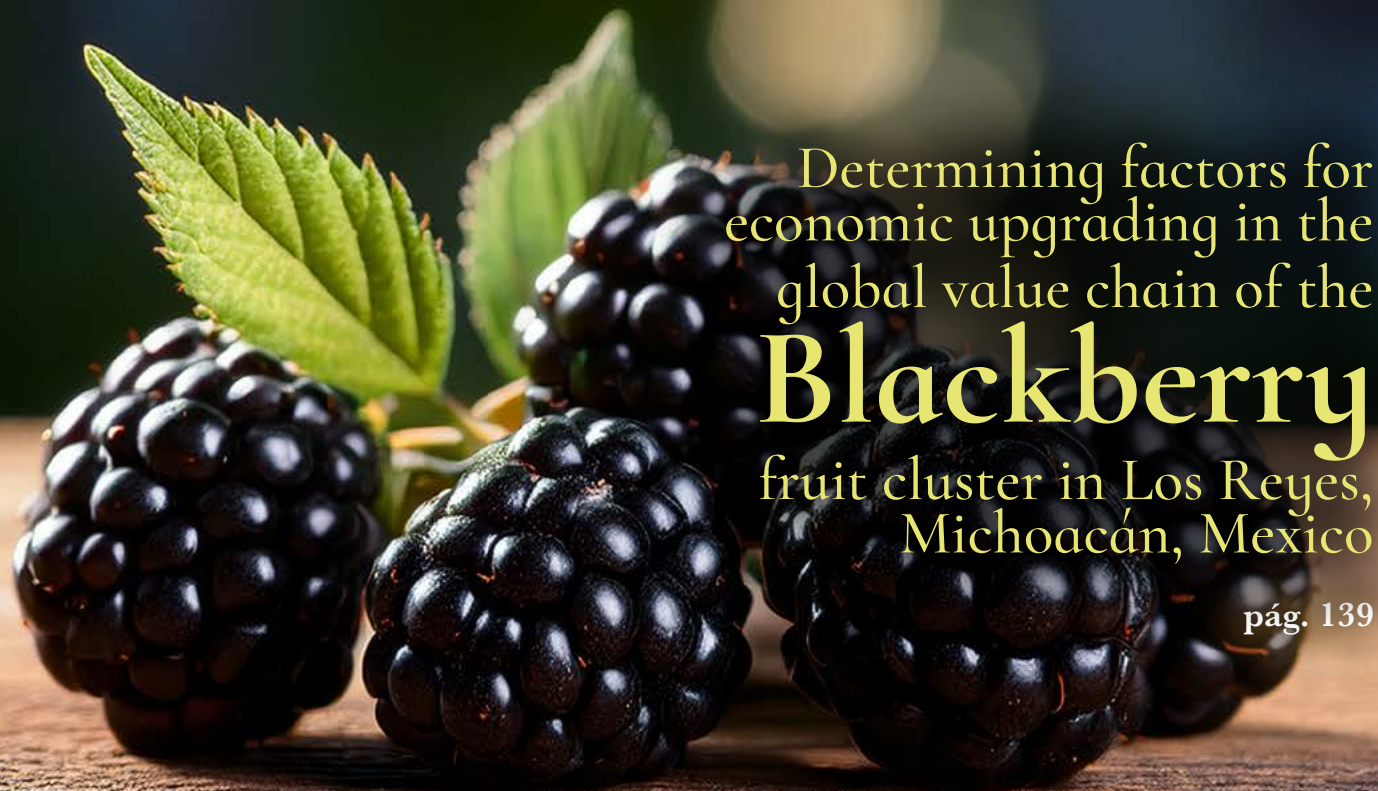


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 0000-0002-3399-3622


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Contacto principal
Jorge Cadena Iñiguez
Guerrero 9, esquina avenida Hidalgo,
C.P. 56220, San Luis Huexotla, Texcoco,
Estado de México.
✉ agroproductividadeditor@gmail.com

Contacto de soporte
Soporte
5959284703
✉ agroproductividadesoporte@gmail.com

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Resultados y Discusión: Puede presentarse en una sola sección. En caso de presentarse de forma separada, la discusión debe enfocarse a comentar los resultados (sin repetirlos), en términos de sus características mismas, su congruencia con la hipótesis planteada y sus semejanzas o diferencias con resultados de investigaciones similares previamente realizadas.

Conclusiones: Son la generalización de los resultados obtenidos; deben ser puntuales, claras y concisas, y no deben llevar discusión, haciendo hincapié en los aspectos nuevos e importantes de los resultados obtenidos y que establezcan los parámetros finales de lo observado en el estudio.

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

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Potential use of *in vitro* plants in the decoration of events and spaces

Cadena-Zamudio, Jorge David¹; Ramírez-Mosqueda, Marco A.^{1*}; Bautista-Aguilar, José Roberto²; Aguirre-Noyola, José Luis¹; Armenta-Medina, Alma¹

¹ Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Centro Nacional de Recursos Genéticos, Blvd. de la Biodiversidad #400 Rancho las Cruces Tepatitlán de Morelos, C.P. 47600, Jalisco, México.

² Facultad de Ciencias Agrícolas, Universidad Veracruzana, Circuito Gonzalo Aguirre Beltrán, Zona Universitaria, 91000 Xalapa, Veracruz, México.

* Correspondence: marcomoqueda02@hotmail.com

ABSTRACT

Objective: To evaluate the ornamental potential, technical viability, and acceptance of *in vitro* plant arrangements as a compact, low-maintenance alternative for decorating spaces and events among young consumers.

Design/methodology/approach: Arrangements were created using *Stevia rebaudiana* and three orchid species in glass jars containing colored and gelled MS medium. A total of 100 university students (ages 18-30) were surveyed using Likert-scale items, demographic questions, and three open-ended questions. Data were analyzed using one-way ANOVA (gender), Tukey's test, average rating categorization (>3.5), principal component analysis, price histograms, and linguistic processing of open responses to generate word clouds.

Findings: Perceptions were uniform across genders ($F \leq 2.78$; $p > 0.10$), with only personal purchase intention showing a significant difference ($F = 4.40$; $p = 0.041$). Average scores exceeded 3.5 in aesthetics, innovation, and practicality. Willingness to pay was concentrated in the 100-200 MXN range (one plant) and 150-250 MXN (two or more). The keywords "aesthetic," "innovative," "natural," and "unique" dominated positive responses, while concerns focused on durability, heat, and cost.

Limitations/implications: The study used non-probabilistic sampling limited to young individuals from Jalisco; more diverse populations are needed.

Originality/value: *In vitro* arrangements are perceived as novel, aesthetic, and low-maintenance ornamental products, with minimal gender influence on their evaluation. Adjusting plant density and improving thermal stability may support differentiated pricing strategies and expand commercial adoption in national and international markets.

Keywords: *in vitro* plant culture; ornamental arrangements; consumer perception; willingness to pay; tissue culture.

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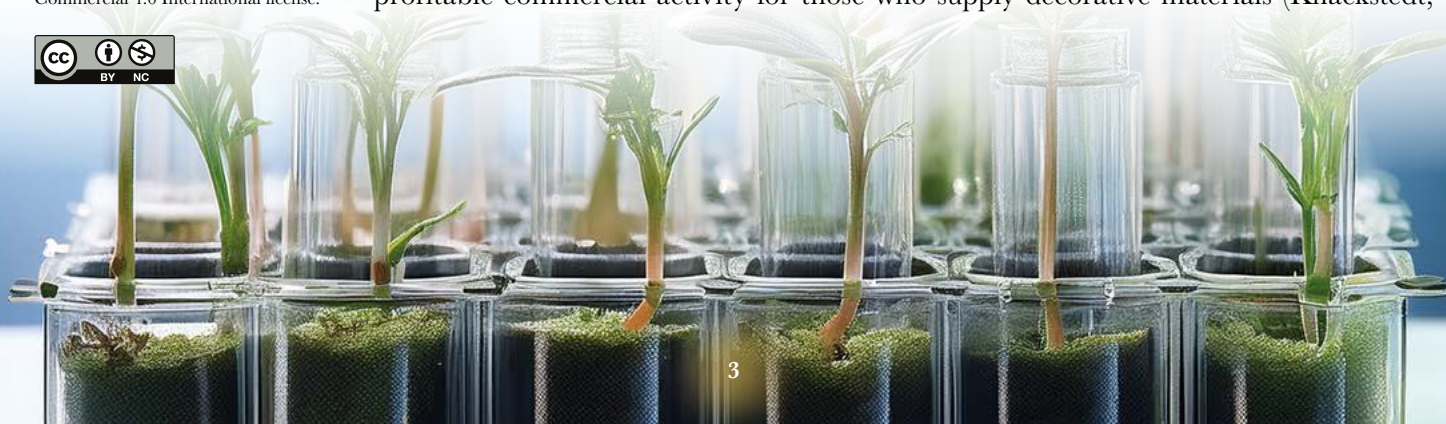
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INTRODUCTION

The ornamentation of physical spaces within homes, offices, social halls, etc., is a recurring practice that, in addition to fulfilling an aesthetic function, has become a profitable commercial activity for those who supply decorative materials (Knackstedt,



2012; Patel and Kharabe, 2023). The use of floral arrangements is one of the space ornamentation practices applied for many years; however, nowadays artificial flowers are used due to their long-lasting nature and minimal care requirements (Paiva *et al.*, 2020). The use of inanimate objects with vibrant colors and peculiar shapes has flooded the market with high demand. Nevertheless, a large portion of the population still prefers living nature, such as plants, due to their visual appeal and positive environmental effects (Dravigne *et al.*, 2008). On the other hand, science has developed tools to cultivate plants and microorganisms under laboratory conditions, almost always inside glass containers (jars, flasks, Petri dishes, etc.) (Phillips and Garda, 2019). For this, various culture media compositions and incubation conditions have been investigated to allow living organisms to be maintained and developed *in vitro* (Sudheer *et al.*, 2022). Specifically, plant tissue culture has enabled the aseptic maintenance and development of plant tissue using artificial culture media and controlled incubation conditions (Mehbub *et al.*, 2022). In this regard, *in vitro* plants (inside a glass jar) grow and develop like normal plants. There are reports in which certain species have even flowered inside the culture jar, particularly some orchids (Kaur, 2022). The growth, development, and *in vitro* flowering characteristics can be harnessed for the creation of ornamental products, enhancing the feature of having a live plant inside a jar with an artificial nutrient medium. Unlike potted plants, *in vitro* arrangements offer a more compact, durable option with lower care requirements, making them ideal for indoor spaces and temporary decorations. Therefore, the aim of this study was to create and analyze the potential of *in vitro* plants for decorating spaces or events, as well as to assess their perception and level of acceptance among a group of young adults.

MATERIALS AND METHODS

Plant material

Stevia rebaudiana Bert. plants of the Morita II variety and three different orchid species (*Laelia anceps* Lindl., *Guarianthe skinneri* (Bateman) Dressler & W.E. Higgins, and *Cattleya dolosa* [Rchb.f.]) that were undergoing micropropagation were used in the preparation of *in vitro* arrangements.

Preparation of *in vitro* arrangements

Culture medium preparation

MS medium (Murashige and Skoog, 1962) supplemented with 30 g L⁻¹ of sucrose was used. McCormick[®] artificial food colorings were added. The selection of each coloring was based on personal preference; however, light-colored dyes are recommended to better visualize root development. The concentration of dyes ranged from 200 mg L⁻¹ to 500 mg L⁻¹, depending on the desired intensity. The pH of the culture medium was adjusted to 5.8±0.1, and 3 g L⁻¹ of Phytigel[®] was added as a gelling agent. Before use, aesthetically pleasing glass jars of various shapes and suitable sizes for proper plant growth were selected. The colored medium was dispensed into the jars. The quantity varied depending on the jar volume, but at least one-fourth of the container was filled with medium. The culture jars were sealed with aluminum caps and self-adhesive food-grade film. Jars with glass lids were

sealed with self-adhesive film. The culture media were sterilized in an autoclave at 1.5 kg cm⁻² and 121 °C for 15 min.

Planting of plant material

Under a laminar flow hood and using sterile surgical instruments (forceps, scalpel, etc.), the plant material undergoing micropropagation (*in vitro* multiplication) was individualized (complete plants with roots). Each plant was transferred into a previously prepared culture medium (only one plant per culture jar was used). The jars were sealed with self-adhesive film only (aluminum lids were removed to improve aesthetics), while those with glass lids were sealed with the lids and self-adhesive film. The jars were decorated with various materials (ribbons, beads, etc.). Cultures were maintained at room temperature (28±2 °C) under natural light (as found in a typical room or interior space).

Ornamental perception analysis of *in vitro* arrangements

A structured survey was administered to a non-probabilistic sample of 100 young adults (aged 18 to 30), all of whom were university students from various regions of the state of Jalisco. The objective was to understand their perception of the ornamental value of *in vitro* arrangements. Participant selection was conducted without discrimination based on race, ethnicity, social status, sexual orientation, or other personal characteristics, ensuring a balanced distribution between men and women. The instrument consisted of 30 items: 21 closed-ended Likert-type questions (numerically coded on a scale from 1 to 6, where 1 indicates the lowest level of agreement or acceptance and 6 the highest), two questions with categorical price intervals analyzed separately, six sociodemographic questions, and three open-ended questions. The Likert scales addressed dimensions such as aesthetic perception, functionality, purchase intent, and perceived disadvantages of the arrangements. Meanwhile, the open-ended questions explored the most attractive features, perceived concerns, and suggestions for improvement related to this form of plant decoration.

Statistical analysis

Analysis of variance (ANOVA) and Tukey Post Hoc test

A one-way analysis of variance (ANOVA) was conducted using RStudio software (version 4.4.2) to assess whether there were statistically significant differences between gender groups (independent variable) across each of the Likert-scale questions (dependent variable). The statistical model used was as follows:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Where: Y_{ij} represents the Likert score of participant j in group i ; μ is the overall mean of the responses; α_i is the effect associated with the group (in this case, gender); ε_{ij} is the random error, assumed to follow a normal distribution with mean zero and equal variance.

This analysis was performed individually for each Likert-scale question, considering the assumptions of normality and homogeneity of variances. In cases where the ANOVA result was significant or near the threshold ($p < 0.05$), a Tukey HSD post hoc test was applied to identify specific differences between groups.

Principal component analysis (PCA)

To explore latent patterns in the Likert-type responses and reduce data dimensionality, a Principal Component Analysis was applied to the numerically coded variables (values from 1 to 6) corresponding to the closed-ended survey questions. Prior to the analysis, it was verified that all included variables were numeric. No missing data were imputed, as the selected response set contained no missing values. The PCA was conducted without additional scaling since all variables shared the same ordinal metric. The analysis was performed using RStudio (4.4.2). The PCA function from the FactoMineR package was used, and the visualization was generated with factoextra, including 95% confidence ellipses.

Perceptual trend analysis using likert scale averages

To assess the general perceptual trend toward *in vitro* plant arrangements, average response scores were calculated for each Likert-type question. This coding was based on predefined dictionaries for each type of scale: frequency, agreement, attractiveness, preference, purchase intention, among others. Once the average scores per question were obtained, a new categorical variable named Trend was created, assigning a label of “Favorable perception (mean > 3.5)” or “Unfavorable perception (mean ≤ 3.5)” based on each item’s average value. The threshold of 3.5 was considered the midpoint on the 6-point scale, with a mean above this value indicating a generally positive tendency toward the evaluated attribute. RStudio (4.4.2) and ggplot2 were used to visualize these results.

Differentiated assessment of willingness to pay for *in vitro* plant arrangements

To explore the perceived economic value of *in vitro* arrangements among participants, the questionnaire included two specific items aimed at estimating their willingness to pay:

- (1) How much would you be willing to pay for a standard unit (250 mL jar, single plant)?
and
- (2) How much would you be willing to pay for a standard unit (250 mL jar, with two or more plants)?

Both questions provided predefined price categories in intervals and were analyzed independently. Relative frequencies (%) by category were calculated for each gender, enabling the identification of differentiated behavioral patterns. The use of proportions instead of absolute frequencies was justified by the need to make equitable comparisons between groups of different sample sizes, ensuring a proportional representation of preferences within each subgroup. This approach allowed for the identification of potential gender-

related trends in economic willingness toward these biotechnological products. Analyses and visualizations were conducted using RStudio (v4.4.2) with the ggplot2 package.

Semantic content analysis of open-ended responses using natural language processing

To identify deep, unstructured perceptions of *in vitro* plant arrangements, content analysis was conducted using three open-ended questions from the questionnaire. Responses were processed using natural language processing techniques in RStudio (v4.4.2). For each item, a manual Spanish-to-English translation dictionary was created, focusing on the most frequent keywords and their specific semantic context. A preprocessing routine was then applied, which included dictionary-based translation, conversion to lowercase, removal of punctuation, numeric digits, and English stopwords. From this cleaned text, a *TermDocumentMatrix* was generated to calculate the relative frequencies of each keyword. Word clouds were created using the wordcloud package, graphically visualizing the most recurring concepts in each question. This approach enabled the identification of the most valued aspects, participants' main concerns, and the most common suggestions for improving the *in vitro* arrangements.

RESULTS AND DISCUSSION

***In vitro* arrangement development**

In vitro arrangements were successfully created using the described methodology. Figure 1A shows *Stevia rebaudiana* (var. Morita II) plants in the *in vitro* multiplication phase, which were individually separated under a laminar flow hood for the arrangement process. Figure 1B presents a *Stevia* plant subcultured in a medium dyed blue with food-grade artificial colorants. It is important to note that the intensity or tone of the medium depends on the amount of dye used. In Figure 1C, a decorative element was added as a final step to enhance the aesthetic appeal and ornamental value of the *in vitro* arrangement. These *in vitro*-cultivated plants can survive, grow, and develop for several months without needing subculturing. In some cases, these plants can be removed from the jar (*in vitro* conditions) and transplanted into pots (*ex vitro* conditions) (Figure 1D). However, successful acclimatization to external environmental conditions depends on proper plant growth, strong root system development, and gradual adaptation to conditions outside the jar.

The correct selection of color and tone of the culture medium is a key factor in the aesthetic appreciation of *in vitro* plant arrangements. In Figure 2A, different orchid species are shown growing in media of varying colors, which can enhance the appeal to potential buyers based on their preference for a specific color or orchid species. The choice of containers also greatly influences the aesthetic value of these ornamental products. In Figure 2B, culture vessels of various shapes and sizes are displayed, offering a wide range of options for consumers. Additionally, planting different orchid species increases the ornamental interest of these arrangements.

The analysis of ornamental appreciation using a one-way ANOVA applied to 19 Likert-type items completed by one hundred university students revealed a notably uniform perception between men and women. In 18 of the statements, including evaluations of

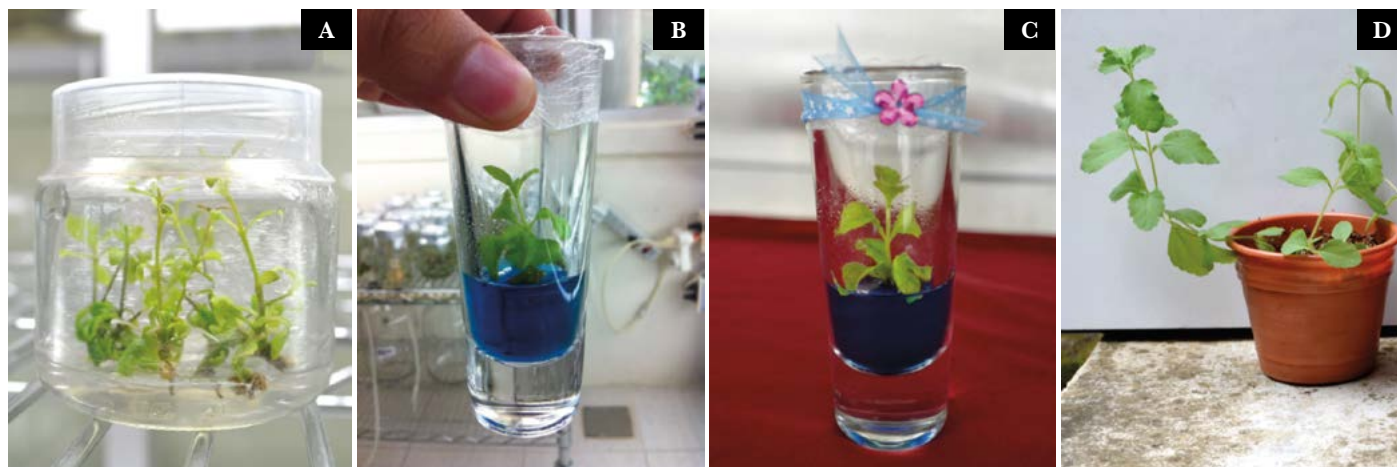


Figure 1. *In Vitro* Arrangement Process. A) *Stevia rebaudiana* plants during *in vitro* propagation. B) Arrangement assembly steps: plant separation, selection of aesthetically pleasing containers, preparation of colored culture medium, and *in vitro* planting. C) Final decoration of the arrangement with external adornments (e.g., ribbons, ornaments). D) Example of a plant successfully transplanted from *in vitro* (inside the jar) to *ex vitro* (potted) conditions.

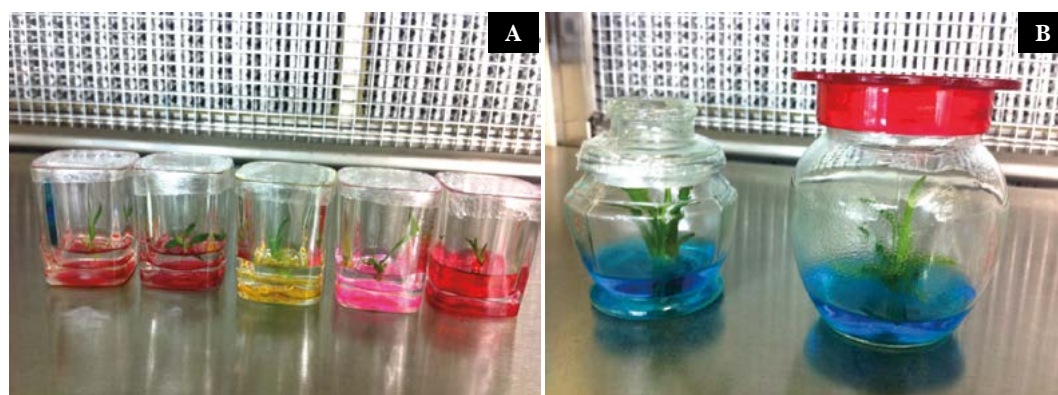


Figure 2. *In vitro* plant arrangements. A) Orchids of different species grown *in vitro*, and B) *In vitro* arrangement made with agave plants.

aesthetic appeal, perceived innovation, practical convenience, and overall willingness to recommend, F-values did not exceed 2.78 and all associated p-values were comfortably above the 0.10 threshold, indicating a clear absence of gender effects. Only the item concerning whether respondents would purchase an *in vitro* plant arrangement for personal decoration deviated from this pattern, as the factor “sex” explained a modest but statistically significant portion of the variance ($F=4.40$; $p=0.041$). Other questions, such as the frequency of using fresh flowers for decoration ($F=3.58$; $p=0.064$) and the price participants would be willing to pay for a 250 mL jar containing two or more plants ($F=3.50$; $p=0.067$), approached statistical significance, suggesting the possible presence of latent differences that a larger sample might confirm. Altogether, these findings describe a consumer landscape in which gender rarely shapes attitudes toward *in vitro* arrangements, although it subtly influences the specific act of purchasing and, to a lesser extent, the habitual use of flowers and price sensitivity. The only significant effect observed indicates

that marketing strategies could benefit from messages tailored to the gender showing greater purchase intent, while the product design itself can remain largely gender-neutral. Trends nearing statistical significance support the collection of additional data to determine whether these differences reflect genuine behavioral divergences or are artifacts of limited statistical power.

Principal component analysis (PCA)

Regarding the principal component analysis (PCA) applied to the Likert-type items, this approach enabled a visual exploration of general trends and latent differences in the perception of men and women toward *in vitro* plant arrangements (Figure 3). The analysis produced a bidimensional projection of individuals along the first two principal components (10.7% and 34.8%), which together accounted for the highest proportion of explained variance in the dataset. Each point represents a participant, coded by gender

Table 1. One-way ANOVA by gender for 21 Likert items on perception and acceptance of *in vitro* plant arrangements.

Questions	Df_Sex	Sum_Sq_Sex	Mean_Sq_Sex	F_value	Pr_F
Would you buy an <i>in vitro</i> arrangement as personal decoration?	1	8.00064935	8.00064935	4.39706823	0.04078867
How often do you use natural flowers for decoration?	1	6.23636364	6.23636364	3.5848945	0.06377191
How much would you be willing to pay for a standard unit 250 ml jar with two or more plants?	1	6.10974026	6.10974026	3.50315066	0.06677542
Would you give an <i>in vitro</i> arrangement as a gift for events birthdays weddings quinceaneras etc?	1	4.26038961	4.26038961	2.78348956	0.10113855
I consider this type of decoration innovative	1	2.4961039	2.4961039	2.50149796	0.11968804
How much would you be willing to pay for a standard unit 250 ml jar with a single plant?	1	2.03636364	2.03636364	1.34237901	0.25180883
The presence of visible roots adds visual interest?	1	3.36623377	3.36623377	1.31626552	0.25641613
How often would you buy this type of arrangement?	1	2.33766234	2.33766234	1.22219945	0.27392273
The color of the culture medium increases the arrangement's appeal	1	1.03896104	1.03896104	1.06010601	0.30786698
I consider it easy to transport the arrangement to an event	1	1.31493507	1.31493507	0.88081775	0.35223532
A single plant per jar conveys minimalism and sophistication	1	0.62402597	0.62402597	0.70788453	0.40392722
It would be easy for me to place the arrangement in my home or office	1	1.04331445	1.04331445	0.69413837	0.40864526
The shape and size of the jar influence its appeal	1	0.47337662	0.47337662	0.41513468	0.52215365
I would prefer this type of arrangement vs potted arrangements	1	0.5461039	0.5461039	0.35110397	0.55600865
How often do you use artificial flowers plastic fabric?	1	0.47337662	0.47337662	0.35022979	0.5564992
I prefer an <i>in vitro</i> arrangement over a bouquet of artificial flowers	1	0.62402597	0.62402597	0.34053635	0.56199507
Combining different species for example orchids on display is more striking	1	0.37402597	0.37402597	0.30324221	0.58417075
I am concerned about the durability of the culture medium in high temperatures	1	0.34350649	0.34350649	0.22177136	0.6396261
Low maintenance no watering is advantageous for me	1	0.0025974	0.0025974	0.00159068	0.96833596

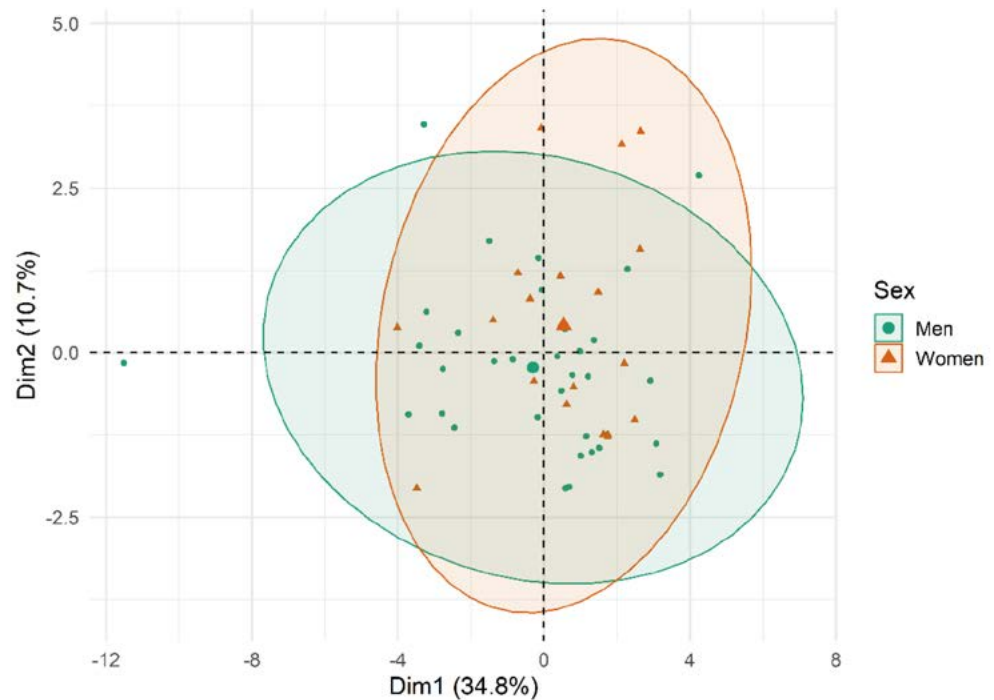


Figure 3. Principal Component Analysis applied to the responses from Likert-type items, differentiated by gender. Each point represents a participant projected in the space defined by the first two principal components. The overlap between groups indicates a high degree of similarity in perceptions between men and women regarding *in vitro* arrangements, aligning with the ANOVA results.

(male or female), and the resulting distribution reveals a significant overlap between the two groups. This convergence indicates a strong homogeneity in responses, consistent with the previous ANOVA results, which showed a minimal influence of gender as a discriminating factor across most of the evaluated variables. Nonetheless, subtle directional trends emerge, suggesting marginal variability in certain perceptual dimensions. For instance, within the quadrants where items associated with purchase intention or the product's visual appeal are clustered, a slight predominance of one gender over the other becomes visible, albeit without forming a clear structural separation. This observation further supports the conclusion that, although minor individual differences do exist, gender does not constitute a primary axis of variation in the overall perception of *in vitro* arrangements.

Analysis of perceptual trends using averages on Likert scales

Complementing the findings from the ANOVA and Principal Component Analysis (PCA), the distribution of responses for the 19 Likert-type items was analyzed through a trend plot (Figure 4). This visualization tool enabled an integrated view of the average ratings assigned by participants to each statement, as well as the overall direction of their opinions. The results revealed a generally positive trend toward *in vitro* arrangements, with most items scoring above the midpoint of the scale (> 3.5). Statements related to aesthetics, perceived innovation, and the intention to give these products as gifts stood out as the most favorably rated, reinforcing the broad acceptance of the proposed concept. In contrast,

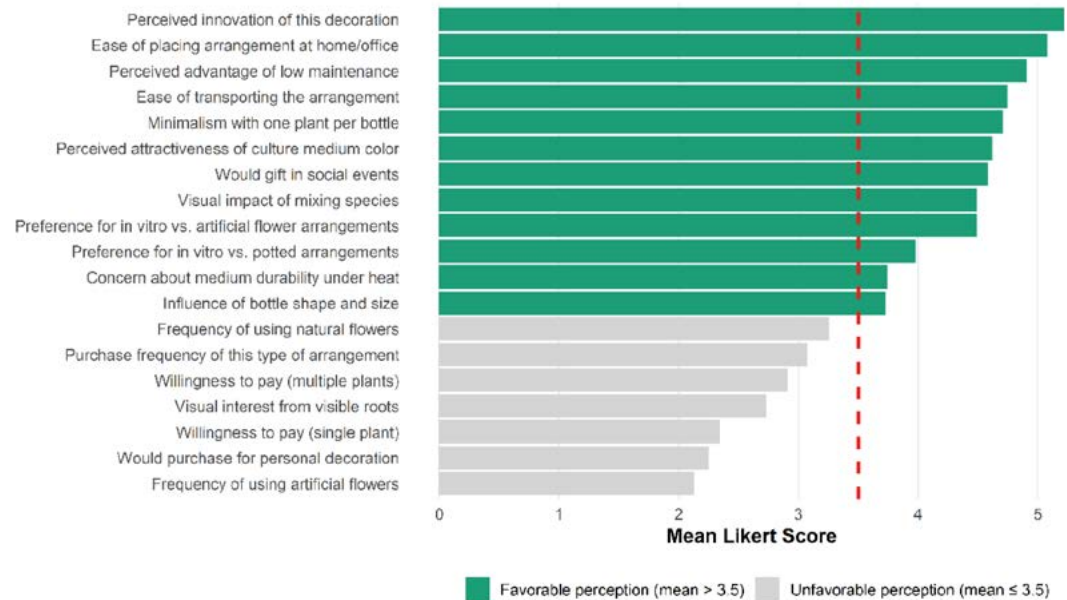


Figure 4. Average trend of responses to the 19 Likert-type items evaluating the perception of *in vitro* arrangements. The scores reflect a predominantly positive attitude toward the aesthetic, functional, and innovative aspects of the product. Values above the midpoint of the scale suggest widespread acceptance, while slight declines in certain items highlight areas that could be improved.

concerns regarding the durability of the culture medium and perceived pricing showed slight declines, indicating potential areas for improvement to enhance commercial appeal. This response pattern, consistent across male and female participants, supports the notion that gender does not substantially influence the overall evaluation. Furthermore, it suggests a favorable and relatively homogeneous perception of the product as an essential factor for its potential integration into the ornamental and personalized gift markets.

Differentiated assessment of willingness to pay for *in vitro* plant arrangements

Building upon the broadly positive and homogeneous perception of *in vitro* plant arrangements where aesthetic appeal, innovation, and ease of placement were highly valued and no significant gender differences emerged this analysis sought to better understand consumer market positioning by exploring willingness to pay under two distinct scenarios: jars containing a single plant and jars with two or more plants (Figure 5A and B). For the single-plant presentation (Figure 5A), responses clustered mostly within the 100-200 MXN range, suggesting a moderate but favorable valuation of the product. This indicates that while consumers recognize the arrangement's attractiveness, they expect its price to remain accessible. In contrast, when examining willingness to pay for jars with two or more plants (Figure 5B), there was a noticeable shift toward higher price categories, with the 150-250 MXN range being the most commonly selected. This change reflects consumers' inclination to assign value proportionally based on plant quantity and ornamental complexity. Comparing both formats highlights the potential of a differentiated pricing strategy: consumers are willing to pay more for arrangements with higher vegetative density, which presents an opportunity to segment offerings and cater to different market

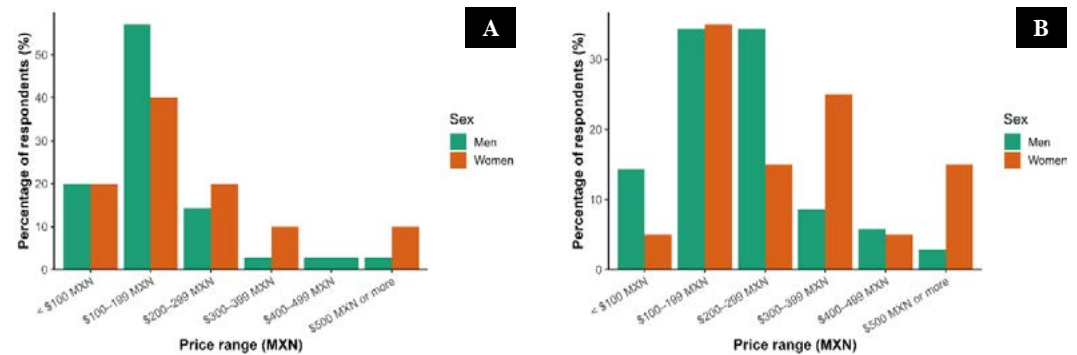


Figure 5. Distribution of responses regarding willingness to pay for a standard 250 mL *in vitro* plant arrangement, under two scenarios: (A) with a single plant and (B) with two or more plants. In both cases, responses clustered around intermediate price ranges, although a shift toward higher price categories was observed when multiple plants were offered per jar. This difference suggests a value perception proportional to the number of plants and provides key insights for establishing pricing differentiation and scaling strategies.

niches without compromising overall affordability. Altogether, these findings deepen our understanding of the aesthetic, functional, and economic factors that shape the acceptance of *in vitro* arrangements as a viable option in the ornamental and personalized gift markets.

Semantic content analysis of open-ended responses using natural language processing

Complementing the quantitative analyses, three open-ended questions were examined to capture a richer, more contextual perception from participants. Their responses were processed via text analysis, standardized, and translated into English, enabling the creation of word clouds that highlight the most frequently mentioned concepts.

In the first question, the semantic analysis revealed that the most recurrent terms were “aesthetic,” “innovative,” “natural,” “unique,” and “minimalist,” thus confirming and expanding the insights from the Likert items. The dominance of these terms indicates that participants value the arrangements not only for their functionality or practicality but also for their ability to evoke visual sophistication and novelty. The word cloud also highlights a strong emotional and symbolic component, where notions such as originality and “alive” carry significance (Figure 6). This result suggests that the appeal of *in vitro* arrangements transcends mere decoration, positioning them as a form of personal expression and connection with nature, even within artificial or urban environments.

In contrast to the positively valued attributes, the second open-ended question explored the main disadvantages or concerns perceived by participants regarding *in vitro* arrangements. The generated word cloud reveals that the most frequent terms were “durability,” “heat,” “fragility,” “evaporation,” and “cost,” highlighting critical dimensions that could hinder their widespread acceptance (Figure 7). Among the most common concerns were the potential degradation of the culture medium under high temperatures, as well as the fragility of the container and its handling in social or transportation contexts. These responses align with survey items that received slightly lower scores, suggesting that

while not predominant, these concerns are consistent and warrant attention in future stages of design and commercialization.

Finally, the third open-ended question invited participants to suggest improvements or additional features they considered important for *in vitro* arrangements. The generated word cloud highlighted “customization,” “color,” “lighting,” “labeling,” and “variety” as predominant terms, providing a clear view of consumer expectations regarding personalization, aesthetics, and product functionality (Figure 8). The suggestions reflect an interest in expanding design possibilities, including more attractive colors in the culture medium, integrated lighting, and labels explaining plant care or symbolism. Additionally, several participants mentioned a desire for a greater variety of species or creative combinations, suggesting a broader potential market if the botanical offering is diversified and adapted to different tastes or events. This set of spontaneous ideas confirms that, beyond being well received, the product sparks interest in evolving toward more versatile and personalized formats, which can be strategically leveraged to strengthen brand identity and increase perceived value.

Plant tissue culture (PTC) techniques have enabled the maintenance and development of plant tissues under controlled *in vitro* conditions, significantly contributing to the clonal propagation of commercially valuable plant species (Phillips & Garda, 2019; Zuzarte *et al.*, 2024). Such is the case of *in vitro* cultivation of *Stevia rebaudiana*, used as an alternative to its low germination and conventional rooting rates (Pande & Gupta, 2013), or orchids, whose micropropagation has facilitated the asymbiotic germination of seeds dependent on mycorrhizal fungi (Rasmussen, 2015; Yam *et al.*, 2018). In this context, the present study proposes an emerging and underexplored application of PTC: the design of *in vitro* ornamental arrangements, whose acceptance and feasibility were evaluated through quantitative and qualitative analyses.

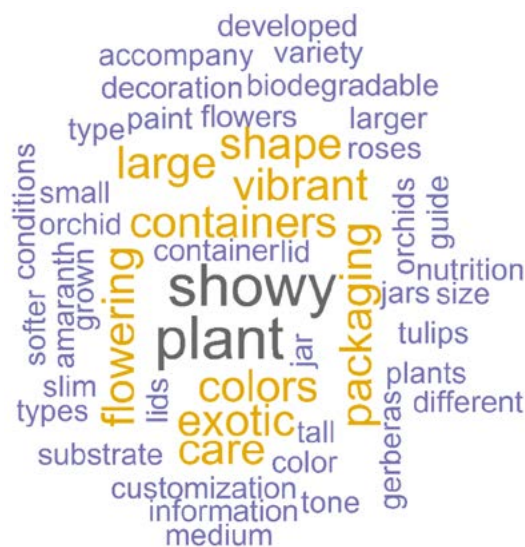


Figure 8. Word cloud generated from responses to the question: “Suggest improvements or additional features you would consider important for *in vitro* arrangements.” The most prominent keywords were customization, color, lighting, labeling, and variety, indicating participants’ desire for more personalized, informative, and visually appealing presentations.

The results obtained from the ANOVA analysis revealed notable homogeneity in responses between men and women, with no significant differences in most items. This uniformity was visually confirmed through principal component analysis, where both groups showed overlapping response patterns. These findings suggest that the product's evaluation is not influenced by gender, broadening its market potential. The analysis of average ratings showed a positive trend toward *in vitro* arrangements, particularly regarding their aesthetics, innovative nature, and practicality as gifts. However, certain items related to the durability of the culture medium and heat exposure revealed slight reservations, reflecting the technical limitations of PTC when used for ornamental purposes. In this regard, it is acknowledged that *in vitro* plants require stable environmental conditions, with temperature being a critical factor; temperatures above 34 ± 2 °C can denature the culture medium (Medina-Esquivel *et al.*, 2008). Nevertheless, placing them in protected indoor environments, such as offices, allows for proper conservation. Another challenge noted is transportation, due to the semi-solid state of the medium, although this can be mitigated by adjusting the concentration of gelling agents, such as Phytigel® (2-3 g L⁻¹) or Agar® (7-9 g L⁻¹), without compromising nutrient diffusion (Khalid *et al.*, 2024; Beruto *et al.*, 1999). Finally, although transplanting to *ex vitro* conditions is technically feasible, it involves a complex acclimatization process (Chandra *et al.*, 2010), which limits its immediate viability as a post-sale strategy. Regarding perceived economic value, willingness-to-pay graphs showed a preference for mid-range prices, with a higher willingness to pay when the arrangement included more than one plant. This finding allows for pricing strategies to be based on design complexity, maximizing profitability potential. The qualitative results added depth to the analysis. Spontaneous responses highlighted aesthetics, originality, and naturalness as the most valued aspects, while the main concerns focused on durability, heat, and cost. Constructively, participants suggested improvements such as lighting, species variety, and informative labeling, demonstrating not only interest but also clear expectations for product evolution. Overall, this study demonstrates that *in vitro* arrangements, beyond their aesthetic value, represent a viable innovation within the field of plant tissue culture. Their acceptance among young consumers, combined with the potential for scaled production in biofactories (Bello-Bello *et al.*, 2025), suggests that this ornamental application of PTC could become a promising line of development within applied plant biotechnology.

CONCLUSIONS

The results of this study confirm the high potential of *in vitro* arrangements as innovative ornamental products derived from plant tissue culture. Through a mixed-methods approach, integrating statistical analyses and natural language processing, it was demonstrated that this type of product is widely accepted by young consumers, who value it for its aesthetics, originality, naturalness, and ease of use.

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Vanadium differently affects sugarcane bud emergence and early growth

Carbajal-Vázquez, Víctor H.¹; Gómez-Merino, Fernando C.^{1*}; Trejo-Téllez, Libia I.¹; Hidalgo-Contreras, Juan V.²

¹ Colegio de Postgraduados Campus Montecillo. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. C. P. 56264.

² Colegio de Postgraduados Campus Córdoba. Carretera Córdoba-Veracruz km 348, Manuel León, Amatlán de los Reyes, Veracruz, Mexico. C. P. 94953.

* Correspondence: fernandg@colpos.mx

ABSTRACT

Objective: To evaluate the effect of vanadium on bud emergence and initial growth variables in sugarcane cv. CP 72-2086

Design/methodology/approach: A trial was conducted with sugarcane buds cv. CP 72-2086 in hydroponics under greenhouse conditions. Increasing doses of V (0, 15, and 30 μM) were applied, starting from NH_4VO_3 . Bud emergence and initial growth variables were evaluated. A GLM analysis of variance (ANOVA) was performed, comparing means by Tukey ($p \leq 0.05$).

Results: Vanadium demonstrated to have beneficial effects on the germination and initial growth of sugarcane seedlings.

Limitations on study/implications: The evaluations were done with a single source of V and a single variety of sugarcane.

Findings/conclusions: Vanadium showed a tendency to biostimulate bud emergence and initial growth of sugarcane.

Keywords: Poaceae, *Saccharum* spp., beneficial elements, inorganic biostimulation, CP 72-2086.

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INTRODUCTION

In Mexico, the average green sugarcane production volume was 53.6 million Mg for the 2022/23 harvest, and 49.1 million Mg for the 2023/24 harvest, showing a 4.5 million Mg decrease compared to the previous year (SIAP, 2024; SADER, 2024a; Zafranet, 2024; CONADESUCA, 2024). Regarding sugar production, the country produced an average of 5.3 million Mg in the 2022/23 cycle. Meanwhile, in the 2023/24 cycle, 4.7 million Mg were produced, with a deficit of 0.6 million Mg between the two cycles (SIAP, 2024; Zafranet, 2024; CONADESUCA, 2024).

It should be noted that sugarcane cultivation is carried out in 15 states in the country. Among the most productive states are Veracruz, Jalisco, and San Luis Potosí, which in 2023 reached production of 21.2, 7.2, and 5.2 million Mg of green cane, respectively (SIAP, 2024). This activity employs approximately 180,000 families in more than 267 municipalities, generating nearly 500,000 direct jobs and 2.4 million indirect jobs (SADER, 2024b). Within



the agroindustrial sector, sugarcane is the second most economically important product system, only after corn. In 2023, sugarcane production was approximately 53.6 million Mg, placing Mexico as the eighth-largest producer of this crop globally. Also in 2023, Mexico exported (mainly to the United States, Canada, Puerto Rico, Germany, Cuba, and Spain) 997,000 Mg of sugarcane, equivalent to 794 million dollars (SADER, 2024b).

In Mexico, sugarcane production is mainly based on four cultivars: CP 72-2086, Mex 69-290, Mex 79-431, and ITV 92-1424, of which, CP 72-2086 (parents: CP 62-374×CP 63-588) occupies about 36% of the cultivated area in the country (CONADESUCA, 2016). Some of the general botanical characteristics of the cultivar are: semi-erect growth habit of the stems, yellow-green color, internodes with slight zigzag, a round bud without pubescence, and long leaves of medium width. Table 1 shows the active mills in the 2022/2023 harvest and the percentage of use of the four aforementioned cultivars, according to the cultivation area per mill.

Table 1. Percentage of planted area occupied by cultivars CP 72-2086, Mex 69-290, Mex 79-431, and ITV 92-1424, by sugar mill in Mexico.

STATE		Sown Surface (%)			
		CP 72-2086	Mex 69-290	Mex 79-431	ITV 92-1424
CAMPECHE	LA JOYA	34.20	18.40	5.90	1.80
CHIAPAS	PUJILTIC	31.00	14.20	-	-
CHIAPAS	HUIXTLA	55.53	34.07	0.28	-
COLIMA	QUESERÍA	15.65	30.76	0.39	26.31
JALISCO	BELLAVISTA	33.64	7.29	8.69	27.89
JALISCO	JOSÉ MARÍA MORELOS		22.04	21.49	19.19
JALISCO	MELCHOR OCAMPO	13.22	-	-	23.23
JALISCO	SAN FRANCISCO AMECA	21.06	29.14	2.22	18.96
JALISCO	TALA	28.40	9.60	7.20	3.20
JALISCO	TAMAZULA	20.82	2.22	-	23.22
MICHOACÁN	LÁZARO CÁRDENAS	0.17	9.98	19.03	26.80
MICHOACÁN	PEDERNALES	1.00		2.00	3.00
MICHOACÁN	SANTA CLARA	1.50	2.00	15.00	66.00
MORELOS	CASASANO	48.52	-	-	14.99
MORELOS	EMILIANO ZAPATA	46.15	-	-	38.84
NAYARIT	EL MOLINO	2.98	7.46	2.16	14.13
NAYARIT	PUGA	4.61	18.26	-	-
OAXACA	ADOLFO LÓPEZ MATEOS	41.68	12.86	1.58	0.28
OAXACA	EL REFUGIO	4.94	33.29	0.82	32.17
OAXACA	LA MARGARITA	32.91	37.75	4.95	2.89
PUEBLA	ATENCINGO	15.36	15.27	20.96	9.93
PUEBLA	CALÍPAM		0.07	2.74	0.56
QUINTANA ROO	SAN RAFAEL PUCTÉ	50.73	46.45	-	0.81
SAN LUIS POTOSÍ	ALIANZA POPULAR	15.00	-	1.00	18.00
SAN LUIS POTOSÍ	PLAN DE AYALA	36.03	-	1.00	2.09
SAN LUIS POTOSÍ	SAN MIGUEL EL NARANJO	61.37	-	3.85	0.52

Table 1. Continues....

STATE		Sown Surface (%)			
		CP 72-2086	Mex 69-290	Mex 79-431	ITV 92-1424
SAN LUIS POTOSÍ	PLAN DE SAN LUIS	34.84	0.01	0.40	12.90
SINALOA	EL DORADO	95.60	-	-	-
TABASCO	SANTA ROSALÍA	42.96	41.00	10.84	-
TABASCO	BENITO JUÁREZ	8.74	21.58	11.07	-
TAMAULIPAS	EL MANTE	94.05	-	-	-
VERACRUZ	SAN JOSÉ	7.64	44.60	6.73	16.38
VERACRUZ	EL POTRERO	20.37	50.52	9.09	5.23
VERACRUZ	PROVIDENCIA	3.00	55.00	3.00	9.00
VERACRUZ	SAN MIGUELITO	4.58	68.29	15.50	1.52
VERACRUZ	CUAUTOTOLAPAM	23.51	50.05	0.79	14.93
VERACRUZ	SAN PEDRO	36.00	8.00	21.00	-
VERACRUZ	SAN CRISTOBAL	22.00	24.00	23.00	2.00
VERACRUZ	EL MODELO	67.50	43.50	51.30	20.00
VERACRUZ	CENTRAL MOTZORONGO	4.30	37.00	1.10	7.60
VERACRUZ	EL PROGRESO	28.65	38.89	-	13.87
VERACRUZ	CONSTANCIA	18.24	28.77	0.33	39.20
VERACRUZ	DEL CARMEN	4.82	64.56	8.27	-
VERACRUZ	EL HIGO	97.26	-	-	-
VERACRUZ	LA GLORIA	17.19	55.63	5.20	3.93
VERACRUZ	MAHUIXTLÁN	9.00	22.00	-	40.00
VERACRUZ	PÁNUCO	83.30	6.80	4.30	4.30
VERACRUZ	SAN NICOLÁS	13.00	71.00	3.00	1.00
VERACRUZ	TRES VALLES	34.00	24.00	6.00	2.00

Prepared by the authors using data from MAM (2024) corresponding to the 2022/2023 harvest cycle.

Despite its agricultural, economic, and social importance, sugarcane is a crop that employs little modern technology, which has led to pollution, loss of biodiversity, and low yields both in the field and in the factory (Herrera-Reyes *et al.*, 2023). These problems are being exacerbated by the impact of global climate change, which is expected to cause reductions in sugarcane productivity of nearly 20% of the current average in the state of Veracruz (Brígido-Morales *et al.*, 2023). Therefore, the implementation of sustainable measures to address these climate challenges is urgent. One of these alternatives that could contribute to the sustainability of agriculture is biostimulation (Di Sario *et al.*, 2025).

Inorganic biostimulation with beneficial elements, applied via leaf or root application in low doses, can improve physiological, biochemical, and molecular parameters in plants, allowing greater tolerance to abiotic stress factors and increasing productivity (du Jardin, 2015). Among the beneficial elements, vanadium stands out (Gómez-Merino *et al.*, 2021).

Located in group five of the periodic table and with an atomic weight of 23, V is a soft and ductile metal. It occupies the 22nd place in abundance in the Earth's crust, representing an average of 0.02% in soils and a concentration of 35 nM in seawater (Rehder,

2012). Its biologically active oxidation states are 3^+ , 4^+ , and 5^+ . Some of the species that hyperaccumulate V are beans (*Phaseolus vulgaris* L.) and maize (*Zea mays* L.), which can absorb and translocate V through transpiration to the aerial parts (Ameh *et al.*, 2019). Although overaccumulation of V in plant tissue has been documented to inhibit plant metabolism, there is evidence that low doses of this metal can have potentially beneficial effects (Chen *et al.*, 2020).

In seeds of chili peppers (*Capsicum annuum* L.) cv. Jalapeño Emperador and Poblano Capulín, doses of 25 and 50 μM V, respectively, benefited germination variables (Buendía-Valverde *et al.*, 2018). In seedlings of peppers (*Capsicum annuum* L.) cv. Misterio F1, root supplementation with 5 μM V improved initial growth, biochemical, and nutritional variables (García-Jiménez *et al.*, 2018). In maize seedlings cv. Xincaitian 1, treated with different doses of V, it was observed that V promotes the synthesis of thiol groups and reduces Hg toxicity by inhibiting its uptake (Hou *et al.*, 2019). In sugarcane (eight-month-old plants), doses of 10 and 20 μM V inhibit flowering and pith formation in cv. CP 72-2086; increases stem height and diameter in cv. Mex 79-341; and increases height in cv. Mex 69-290 (Sentís-Herrera *et al.*, 2018). The objective of this study was to evaluate the effect of vanadium on bud emergence and initial growth variables in cv. CP 72-2086.

MATERIALS AND METHODS

Characteristics of the plant material and location of the experiment

The plant material used in this study was obtained from the seed banks of the Colegio de Postgraduados, Campus Córdoba, located at Carretera Federal Córdoba-Veracruz km 348.5, Manuel León congregation, Amatlán de los Reyes, Veracruz, Mexico ($18^{\circ} 86' \text{ N}$, $96^{\circ} 85' \text{ W}$, at an altitude of 650 m). Sugarcane buds were obtained from 11-month-old plants. The cultivar CP 72-2086 (early maturing) was used. The experiment was carried out in a tunnel-type greenhouse (6.5x4.3x1.9 m and 2.8 m at the zenith) with plastic cover, anti-aphid mesh on the sides, and a white tarp on the soil surface. This facility belongs to the Plant Nutrition area of the Soil Science program at the Colegio de Postgraduados, Campus Montecillo ($19^{\circ} 46' 13'' \text{ N}$ and $98^{\circ} 90' 90'' \text{ W}$, at an altitude of 2,220 m).

Imbibition, Planting, and Treatment Application

The buds were separated from the sugarcane stems by making 8-10 cm cuts and imbibed for 18 h, depending on the treatment. The buds were treated with 0, 15, and 30 μM V, using ammonium metavanadate (NH_4VO_3 , Merk, Darmstadt, Germany) as a source. Buds were planted for study in 0.8 L black pots (13x14x18.5 cm) previously filled with a substrate mixture of white peat (Super Terra ST1 Hawita; Vechta, Germany) and tezontle volcanic rock (1:1/v:v). After sowing, the pots were watered with tap water, 200 mL per day, every two days. Five treatments with V directly applied to the substrate (50 mL per container) were made at 10-d intervals. The duration of this stage was 60 d.

Bud emergence and initial growth variables

Bud emergence data were collected daily for 30 days. The following variables were calculated from the germination data: 1) percentage of bud emergence (BE), 2) speed

coefficient of bud emergence (SCBE), and 3) speed of bud emergence (SBE), according to the following formulas (González-Zertuche and Orozco-Segovia, 1996):

$$BE = \frac{\sum n_i}{\sum (n_i t_i)} \times 100 \quad (1)$$

$$SCBE = \frac{\sum n_i}{\sum (n_i t_i)} \times 100 \quad (2)$$

$$SBE = \sum \frac{n_i}{t_i} \quad (3)$$

Where: n_i =number of buds emerged on day i , t_i =number of days after sowing.

After harvest (60 das), plant height (PH) was measured from the base of the substrate to the tallest leaf using a tape measure (Wiseup, ART-080102; Yiwu, China). Stem diameter (SD) was measured 5 cm from the base of the substrate using a digital caliper (Truper-14388; Shanghai, China). The number of leaves (NL) was measured by manual counting, and leaf area (LA) was determined using a leaf area integrator (LI-COR, model LI-3100C Area Meter; Lincoln, NE, USA). Fresh biomass weight (FB) was determined using an analytical balance (Adventurer Ohaus Pro AV213C; Parsippany, NJ, USA).

SPAD Units

At 60 days of age, SPAD (Soil Plant Analysis Development) units were measured using a portable SPAD-502[®] meter (Minolta, Tokyo, Japan).

Statistical Analysis

A GLM analysis of variance was performed using a randomized block treatment design and means comparison tests were performed using Tukey ($p \leq 0.05$).

RESULTS AND DISCUSSION

The results obtained are presented below, describing the variables evaluated in cultivar CP 72-2086.

Table 2 presents the results obtained with the variables measured in bud emergence in cultivar CP 72-2086. Regarding the percentage of BE variable, both doses of V allowed buds to reach 100% emergence, compared to the control treatment, which only reached 90%; the 15 μM V dose improved SCBE by 177% compared to the control treatment; no significant differences were found between treatments in SBE, although both doses tended to improve the variable.

Table 3 shows the effect of V on initial growth variables in cultivar CP 72-2086. No significant differences were observed in the variables evaluated. However, both doses (15

Table 2. Effect of vanadium (V) on bud emergence variables of sugarcane (*Saccharum* spp.) cv. CP 72-2086.

V (μM)	Bud Emergence (%)	Speed Coefficient Bud Emergence	Speed Bud Emergence (day^{-1})
0	90 b	2.78 ± 0.47 b	1.88 ± 0.22 a
15	100 a	4.92 ± 0.43 a	3.15 ± 0.24 a
30	100 a	1.85 ± 0.40 b	2.13 ± 0.57 a

Means \pm SD with different letters in each column indicate statistically significant differences between treatments (Tukey, $p \leq 0.05$).

Table 3. Effect of vanadium (V) on initial growth variables of sugarcane (*Saccharum* spp.) seedlings cv. CP 72-2086.

V (μM)	Stem Diameter (mm)	Plant Height (cm)	Number of Leaves
0	4.52 ± 0.34 a	34.9 ± 1.58 a	3.52 ± 0.11 a
15	4.85 ± 0.07 a	35.2 ± 0.33 a	3.88 ± 0.09 a
30	4.76 ± 0.08 a	34.5 ± 0.19 a	3.38 ± 0.17 a

Means \pm SD with different letters in each column indicate statistically significant differences between treatments (Tukey, $p \leq 0.05$).

and 30 μM V) show a tendency to increase SD; whereas, the 15 μM V dose tends to improve PH and NL, in both cases compared to the control treatment.

No significant differences were observed in the SPAD units and LA variables (Figure 1). In SPAD units, doses of 15 and 30 μM V tended to increase the variable by 8 and 12%, respectively. However, in LA, the 30 μM V dose reduced the variable by 20% compared to the control treatment, although this was not significant. Regarding fresh biomass (FB), the highest dose of V applied (30 μM V) reduced the variable by 18% compared to the control.

A significant portion of bioavailable V concentrations in soils, both for agricultural use and irrigation water, is a consequence of activities such as the burning of fossil fuels, wastewater, and the use of fertilizers containing V compounds. Soil V concentrations can range from less than 1 mg kg^{-1} to 9,200 mg kg^{-1} (Shaheen *et al.*, 2022), and such concentrations can have positive, null, or negative effects on plant biology. In this study, a higher percentage of bud emergence was observed in cv. CP 72-2086 with both V doses tested (15 and 30 μM V), which were statistically different from the control treatment, in which only 90% bud emergence was observed; that is, V enhanced total bud emergence (Table 2).

The SCBE and SBE variables examine both the speed and quantity of buds emerging in a given time; high values indicate greater short-term emergence (Soblarzo-Bernal *et al.*, 2021). In our study, both the SCBE and SBE values were higher at the 15 μM V dose compared to the control (Table 2), although only the SCBE was statistically different. The above may indicate possible interventions of V in the biosynthetic pathways of phytohormones (auxins, abscisic acid, gibberellins, and strigolactones) that influence cell elongation and/or division, in addition to developmental processes (Bajguz and Piotrowska-Niczyporuk, 2023).

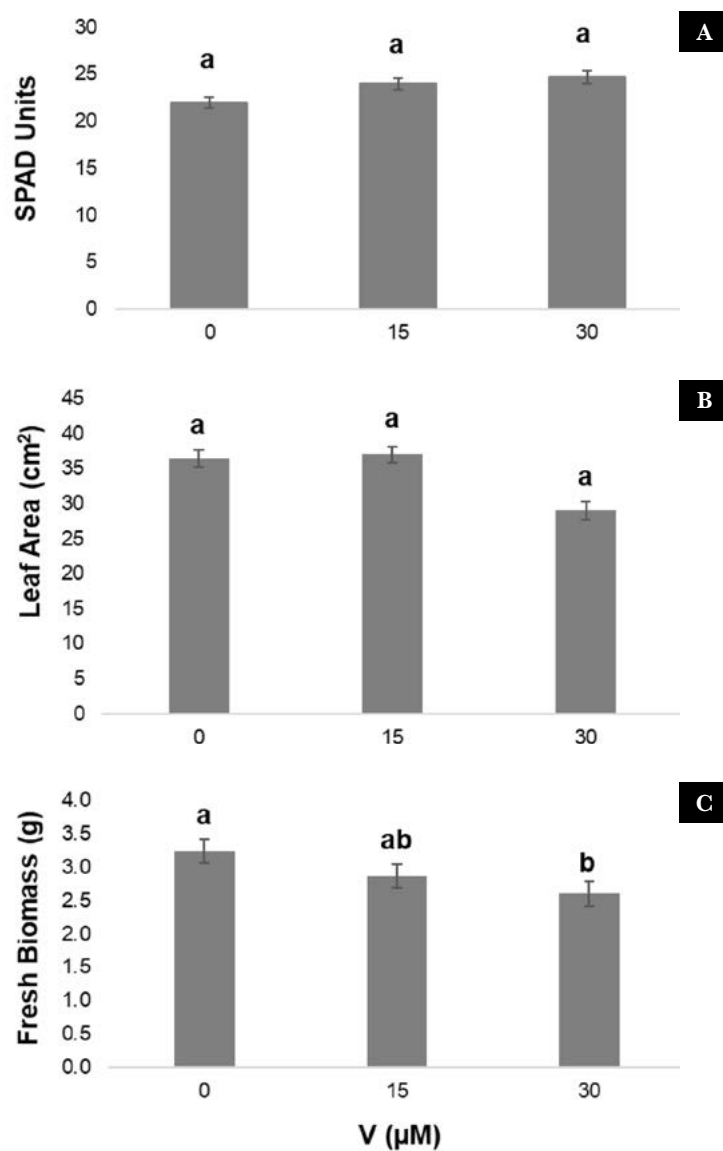


Figure 1. Effect of vanadium (V) on 60-day-old sugarcane (*Saccharum* spp.) seedlings cv. CP 72-2086. A) SPAD units; B) leaf area and C) fresh biomass. Means \pm SD with different letters indicate statistically significant differences (Tukey, $p \leq 0.05$).

V may be essential in the photosynthetic processes of different species, allowing increases in growth and development variables (Nalewajko *et al.*, 1995). The variables stem diameter, plant height, and number of leaves did not show significant effects compared to the control. However, slight tendencies for V to increase stem diameter and plant height were observed with doses of 15 μM (Table 3). Similar effects were observed in the same cultivar (CP 72-2086) when treated with 20 μM (Sentías-Herrera *et al.*, 2018). Regarding SPAD Units, the trend is upward, confirming that V can differentially influence the production of photosynthetic products among species.

V may influence tissue elasticity, allowing greater cell expansion and acting as a growth promoter, mainly because it is metabolized using the iron transport and storage proteins

transferrin and ferritin (Gresser and Tracey, 1990; García-Jiménez *et al.*, 2018). V has also been documented to regulate plant growth, acting mainly as a cofactor that enhances or inhibits the enzymatic activity of proteins, such as kinases and phosphatases (Harland and Harden-Williams, 1994). In our study, a tendency toward increased growth variables was observed with the low dose (15 μM V), but LA and FB were also reduced with the high dose (30 μM V), as shown in Figure 1.

CONCLUSIONS

In the CP 72-2086 variety, applying vanadium in irrigation water and as leaf sprays improved bud emergence and initial seedling growth, resulting in greater seedling vigor. These results may have important applications in sugarcane production systems and should be extended to a larger number of varieties, both commercial and those undergoing selection for validation in the laboratory, greenhouse, and field.

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Network value of agriculture and livestock production cooperatives in Mexico City: case study of the Agroab cooperative

Camacho-Bercherlt, Mirna¹; Rojas-Herrera, Juan J.¹; Santillán-Fernández, Alberto^{2*}

¹ Universidad Autónoma Chapingo. Departamento de Sociología Rural. Doctorado Interinstitucional en Economía Social Solidaria. Texcoco, Estado de México, México. C.P. 56230.

² Investigador por México SECIHTI y Colegio de Postgraduados campus Campeche. Sihochac, Champotón, Campeche, México. C. P. 24450.

* Correspondence: santillan.alberto@colpos.mx

ABSTRACT

Objective: To identify the marketing factors that have made it possible for the Agroab cooperative to remain in the preference of its consumers, by describing its value network.

Design/methodology/approach: To determine the success factors of the Agroab cooperative, the stakeholders and their interactions were documented using the value network methodology, identifying its suppliers, consumers, complementors and competitors.

Results: The Agroab cooperative has been in the market for 20 uninterrupted years, a period in which it has promoted the improvement of the living conditions of its members, creating value for its network of customers, complementors and suppliers. Likewise, it presents characteristics typical of fair trade, although it still needs to form marketing networks that are alternatives to conventional trade.

Limitations on study/implications: For future research, it is suggested to analyze the operation of the Agroab cooperative considering the different solidarity alternatives to the conventional market, describing its social company model and the cooperation networks it establishes in the production, transformation and marketing processes of products.

Findings/conclusions: Agroab is characterized by offering quality products at fair prices to its consumers through direct deals that eliminate intermediaries and contribute to the development of agribusiness.

Keywords: production cooperatives, marketing, Mexico City, social economy.

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INTRODUCTION

The current hegemonic capitalist system has provoked negative consequences for most of humanity (Houtart, 2014). Proof of this is the strong presence of adverse phenomena such as environmental deterioration, unemployment, labor exploitation, migration, poverty, economic crises and inequality. Consequently, it turns out to be an unsustainable economic model to preserve life on our planet (Ovejero-Bernal, 2008). To counteract these negative effects, an alternative is the so-called Social and Solidary Economy (SSE), which sustains a project for the construction of a just and human society, performing an increasing role in modern economies and societies by offering economic, social, political, environmental and cultural benefits for partners and communities, with the aim of contributing to the integral development of all, as well as contributing to local development (Toledo-Manzur, 2013).

One of the organizations that are part of SSE are agriculture and livestock production cooperatives, which conduct activities for the benefit of their partners and the people who make up their locality, alleviating marginalization and poverty through the generation of sources of employment, and promoting the trade of basic products with quality and

fair prices (Camacho-Bercherlt *et al.*, 2023). Hence, the importance of researching the marketing processes that these associations follow to achieve their economic and social objectives, given that they take on a double character in their operational functioning: as a company and as an association. However, within the understanding that the company is the means and not the ultimate or exclusive aim. Therefore, they are companies of people and not capital, based on the social property of the means of production and the company itself, which is of joint property (Rojas-Herrera, 2019).

Cooperatives have a dual objective: they must function as companies that compete in the market and at the same time guarantee equity, social inclusion and diversity in productive activities, integrating cooperative principles: voluntary and open adherence, democratic control of members, economic participation of members, autonomy and independence, education, training and information, cooperation between cooperatives, interest for the community (Magallón and Calderón, 2016).

However, the immense majority of agriculture and livestock production cooperatives are immersed in the conventional market, competing with private capitalist companies, so they must implement strategies that allow breaking the entry barriers for their products, placing them in the market, winning the preference of consumers, and therefore, ensuring a part of the conventional market to obtain the necessary benefits to reinvest and satisfy the needs of their members (Magallón and Calderón, 2016).

Among the studies that describe the participation of agriculture and livestock production cooperatives in the market, the ones that stand out internationally are those developed by Rojas-Herrera (2013) and Pérez-Sanz *et al.* (2019); in Mexico, Garduño de Jesús *et al.* (2021); and specifically in Mexico City (CDMX), Reygadas *et al.* (2015), Díaz and Rivera (2019), Luvían-Reyes *et al.* (2019), and Luvían-Reyes and Rosas-Baños (2021). All these studies agree that the marketing strategies of cooperatives: 1) Conduct their practices with ethics of service in compliance with their cooperative principles; 2) Adapt to local conditions, and based on these, they carry out strategic alliances that directly benefit producers and inhabitants of the community; 3) Contribute to local development by revitalizing the economy through the generation of self-employment and employment for people from the population; and 4) Attempt to conduct agroecological production to offer the market healthy products with organic quality that differentiates them from conventional products of the capitalist market.

Because the studies about agriculture and livestock production cooperatives are scarce, and even more so the studies that analyze marketing processes, this study had the objective of identifying the marketing factors that have made it possible for the Agroab cooperative to remain in the preference of its consumers, through the description of its value chain.

The Agroab cooperative was selected as study case because of its trajectory of more than 20 years in the market for fruit-based pulps, with a social and economic impact in its territory, promoting the economic progress of the stakeholders with which it relates through the generation of respectable jobs and supporting people who are devoted to agriculture; therefore, it represents an example highlighting agriculture and livestock cooperativism in Ciudad de México (CDMX).

MATERIALS AND METHODS

Study area

The Agroab cooperative is located in CDMX, between coordinates 19° 11' 28" latitude North and 99° 57' 07" longitude West. CDMX is found in the Valley of Mexico and divided administratively into 16 areas, among which the boroughs of Tlalpan, Milpa Alta, Tláhuac and Xochimilco are devoted to crop production. In these zones, there is production of corn, fruit trees, vegetables and animals for family consumption and local sales, but there is also production, at larger scale, of nopal, amaranth, vegetables, herbs, and ornamental plants destined for urban and regional markets (SIAP, 2024). Agroab is located in the town of San Pablo Oxtotepec in the borough of Milpa Alta, with a low degree of marginalization. However, the percentage of poverty in this borough in 2020 was 54.7%, that is, the highest in the entire CDMX, according to data from the National Council for Evaluation of Social Development Policy (*Consejo Nacional de Evaluación de la Política de Desarrollo Social*, CONEVAL, 2020).

History and consolidation of Agroab

Through semi-structured interviews and surveys applied to the legal representative (1) and members (5) of the Agroab cooperative, aspects about their production processes, marketing and growth expectations were documented. Due to the qualitative nature of the research, no statistical analysis was developed; the study was limited to describing the operations of Agroab, prioritizing aspects of cooperativism (mutual help, shared responsibility, democracy, equality, and solidarity).

Agroab value network

To describe the value network of the Agroab cooperative, the methodology proposed by Nalebuff and Brandenburger (1997) was followed, which allows the incorporation of the joint participation of various stakeholders (customers, suppliers, complementors, and competitors), with economic and non-economic connections that contribute to the value creation for their members and territory. Thus, the *customers* are classified as individuals or companies that purchase the product offered by the focus organization of the value network or producers; the suppliers as those stakeholders and institutions that provide what is necessary for the production of the good or service; the *complementors* as organizations and institutions that allow the client to value the product obtained; and *competitors* as those stakeholders with whom the producer disputes the market for the sale of their product or service (Muñoz and Santoyo, 2020).

The connections and empathy that Agroab has developed with the stakeholders that it considers as customers, suppliers, complementors, and competitors were gathered through semi-structured interviews and surveys applied to their members (6). The location of each of the stakeholders was developed through the spatial analysis software ArcMap (ESRI, 2015), with the aim of documenting the geographical influence of each stakeholder on the Agroab cooperative.

RESULTS AND DISCUSSION

History and consolidation of Agroab

The Agroab cooperative emerged in the year 2003, because of the need of creating a productive initiative for self-employment. For its constitution, it received support from the Ministry of Labor and Employment Promotion. The founding members had previous experience, and it was basically founded as a family cooperative, applying the principle of democratic control of the members (giving every partner voice and vote in decision making and in planning and organization processes of the cooperative). Their legal representative has undergraduate studies in Food Chemistry, which has allowed them to professionalize some of their processes; according to Fernández *et al.* (2010), professionalization is a defining component for a cooperative to be able to remain competitive in the market.

Agroab is devoted to the transformation of various fruits to obtain products such as pulps, concentrates for alcoholic beverages, and juice concentrates. In addition, they produce precooked organic potato chips, and they grow corn that they use as base ingredient in their various elaboration processes of other products. Agroab sells their raw materials wholesale (40 t), for the elaboration of new products, which is why their productive process is in function of the technical specifications requested by their customers. The price of products from Agroab are the result of several considerations such as production costs and surplus, frequency and amount purchased by the customer, always attempting to fix a fair price for the customer and, at the same time, to generate fair payment for the members. They are always based on the principle of economic participation of the members, for whom this represents 100% of their income because they are devoted exclusively to the activities of the cooperative.

Agroab members consider that they have remained in the market because they have consolidated an organizational structure where each member has specific functions; they apply manuals and internal regulations for work; they have plans for quality control and maintenance; and they comply with all the certifications, norms and requirements for its functioning. However, the most important thing is that they have been able to solve the internal conflicts between members, reconciling interests of each member for the collective wellbeing. In addition, every year they set growth goals of no more than 10% and they diversify the products offered.

Regarding the principles of cooperativism, Agroab complies with: the creation of opportunities for producers in economic disadvantage; fair commercial practices; payment of fair prices; saying no to child and forced labor; commitment with non-discrimination; gender equality and economic empowerment of women; guaranteeing good labor conditions; and developing capacities. However, there is a key aspect in the philosophy of cooperativism that Agroab is not addressing, and it is the fact of inserting itself into fair trade which would allow to gradually distance itself from the capitalist conventional market, and to prioritize caring for life and the environment (Diaz and Rivera, 2019).

Agroab value network

Agroab partners recognize as customers, suppliers, complementors, and competitors the stakeholders presented in Figure 1. In the case of customers and suppliers, they have



Figure 1. Value network of the Agroab cooperative obtained through direct interviews to partners and by reviewing their records.

developed a system for personal attention, which has allowed them to reinforce the bonds of commitment and loyalty. According to Muñoz and Santoyo (2020), when bonds of trust are strengthened in a value network, they tend to improve the competitiveness of the network by generating value for their members and territory.

The spatial location of the stakeholders involved in the Agroab value network allowed understanding the agreements of collaboration that they have with their customers and suppliers in a national context, while for the case of their complementors and competitors, they are in the same region as the cooperative (Figure 2). The diversity of customers and suppliers outside their region of influence minimizes the risk of remaining without sales or supplies in the face of environmental and social unforeseen circumstances, which strengthens the operations of the cooperative (Luvián-Reyes and Rosas-Baños, 2021).

In addition, the territorial proximity of Agroab with the stakeholders that strengthen it (complementors) and weaken it (competitors), increases the value of its products, according with Ireta-Paredes *et al.* (2018), since the estimation that users make of these products to satisfy their needs and wishes tends to be positive in face of the possibility of purchasing alternative products from the same region.

Agroab has managed to create an undisputed market space that frees them from the constant practice of competition, and in turn allows the territorial development, by generating employment and increasing the income and wellbeing of people in its region of influence, cataloged as a zone with high marginalization (CONEVAL, 2020), thus fulfilling the basic principle of the cooperatives: interest for the community (Díaz and Rivera, 2019).

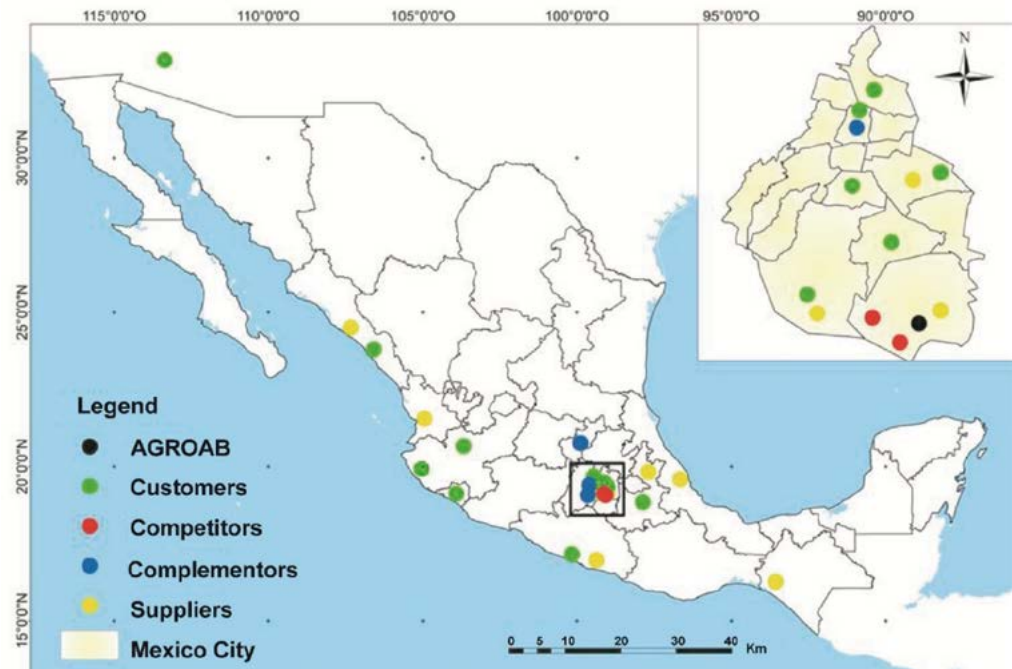


Figure 2. Spatial location of the stakeholders that make up the value network of the Agroab cooperative.

Customers

In the case of the sale of organic potato chips, Agroab's client is Campo Vivo Productos Orgánicos from Tlalpan (CDMX), an organization that is part of fair trade and ruled under principles of cooperativism. For the sale of products derived from fruit pulp, the following were identified as customers: Grupo Frutichela (Tlalpan, CDMX), company specialized in the elaboration of flavoring for micheladas, as well as sauces for chicken wings, shrimp and hamburgers; El Dorado (Iztapalapa, CDMX), which supplies hotels and restaurants from the Pacific coast in Mexico (Acapulco, Manzanillo, Puerto Vallarta, Mazatlán); Engloba Food Solutions (Zapopan, Jalisco), which sells fruit concentrates; Sirloin Stockade, which has a restaurant chain in CDMX, Estado de México and Puebla; and Jarabes Tucán (Acolman, Estado de México), which sells syrups and fruit concentrates. In addition, Agroab has managed to consolidate sales abroad (Los Ángeles, California, USA), with one or two shipments in the warm season of fruit products such as nance, guava and passion fruit, to produce an Italian-type beverage.

However, to consolidate this client portfolio, the Agroab members visited the companies that they estimated could purchase their products, to understand their needs and develop the products with the technical specifications of each customer with whom they signed exclusivity contracts and a confidentiality letter, to back both parties. According to Sánchez-Álvarez (2018), when a cooperative can develop specialized products for their customers, it is difficult for customers to replace it, because the quality of their products can be compromised and therefore their sales volumes.

Likewise, it is important to highlight that Agroab has been able to put into practice the principle of cooperation between cooperatives, forming local marketing networks that help

all the participants, as has been the case of the relationship sustained with the cooperatives Nopal Mexica and Pizca Campesina. Thus, from some ingredients that Agroab purchases wholesale, it usually sells Nopal Mexica the amount it needs, favoring it with a lower cost in the acquisition of inputs; and when Pizca Campesina has tomato surplus that it cannot place in the market, it takes it to Agroab to make purée and thus it can avoid losing part of its production.

Also, for 10 years they were suppliers of the CDMX government for community dining rooms, where they were able to sell a ton of their products daily, so the institutional sale was very important for the development of this cooperative, action that reinforces the proposal by Sánchez-Álvarez (2018), in the sense that public policies should be directed towards promotion, for the government to be the buyer of products from the SSE organizations, an action that can undoubtedly contribute in their advancement and sustainability.

Suppliers

The main supplier for Agroab is a Public Limited Company (PLC) that owns farms in many states of the country and storehouses in the central supply market of Iztapalapa (CDMX). This Limited Company purchases seasonal fruit from Chiapas, Veracruz, Guerrero, Sinaloa, Nayarit and Puebla, and the surplus or amounts that it cannot sell fresh due to size or color are sold to Agroab for their transformation. In this relationship, both parties benefit: the cooperative gets products at a low price, which helps it maintain fair prices for its customers, and for the PLC it represents extra income by selling the byproducts, exceeding fruit that they cannot sell fresh.

Another supplier is the Pizca Campesina cooperative, from which they buy organic potato in the borough of Tlalpan (CDMX); it bases its activities in the fulfillment of the cooperative principles and in the practice of organic agriculture. Some sporadic suppliers of Agroab are in the borough of Milpa Alta and they tend to be producers of seasonal fruits such as nance, guava and passion fruit.

The Agroab cooperative maintains a relationship of commitment and loyalty with its suppliers, purchasing everything that the producers offer to have a stable and safe relationship with them. Although the concept of listening to the customer has become common, the same thing does not happen when it comes to the suppliers; however, the cooperative does listen to them and works coordinately with them, since it considers that relationships with the suppliers are as important as relationships with the customers.

Complementors

The fundamental role of a complementor is to get customers to value products more. In this sense, the Agroab members recognize as main complementor the Ministry of Labor and Employment Promotion (*Secretaría de Trabajo y Fomento del Empleo, STyFE*), since it has benefitted the cooperative three times through its Social Program for Social Economy in Ciudad de México. In the year 2003, it was backed through the Subprogram for the Creation of Cooperative Societies for its constitution and the delivery of a training course, paying attendees the minimum wage; in 2006, it received support from the Subprogram for the Strengthening of Cooperative Societies to improve its facilities; and in 2012, it

participated in the Program for Cooperative Promotion, Strengthening and Integration (*Programa de Promoción, Fortalecimiento e Integración Cooperativa*, PPFIC), with the objective of receiving economic support to purchase equipment and machinery.

However, despite having received backing from the government, Agroab has maintained its autonomy, and its decisions continue to be made collectively and democratically, without external interference from political or government agencies, which shows its degree of adherence to the cooperative principle of autonomy and independence. In this sense, government participation is fundamental for cooperatives to be able to remain in capitalist markets, fostering specialized market niches, where quite often training is more important than facilitating infrastructure (Díaz and Rivera, 2019).

Agroab also considers as complementors three of its commercial partners, two of them located in Atizapán (Estado de México) and the other in Querétaro, because they sell Agroab's products and tend to be involved in reviewing formulas, costs, prices, presentation and image of the products. In addition, these partners help Agroab to get raw materials at a lower price.

Competitors

Since its beginnings, Agroab has competed with companies that produce fruit-based syrups, so its strategy was to seek new customers and offer products based on their needs. The only competitors that they recognize are: Tucán Concentrados y Jarabes, and Pulpas de Fruta San Pedro Atocpan, located in Milpa Alta (CDMX); these sell fruit concentrates in small proportions to Walmart, Bodega Aurrera, Superama, City Market, Fresko and Mercado Libre.

Agroab recognizes the fact that having consolidated customers with specific products greatly reduced its competition. This strategy has been described by Kim *et al.* (2016) as blue oceans, which are associated with unknown spaces in the market, uncontaminated by the competition (the demand is created and not disputed), where there is an abundance of opportunities for growth.

CONCLUSIONS

Analysis of the value network of Agroab allowed detecting the factors that have helped it to remain in the market for fruit-based pulps for two decades: it personally attends to all the stakeholders of its value network, and it fosters coo-petition with its competitors, that is, the practice of cooperating and competing, without destroying or crushing. Therefore, it has managed to create an undisputed market space that frees them from the constant practice of competition. In addition, personal treatment of suppliers and customers has also allowed it to address the specific needs of those stakeholders, achieving a safe commercial relationship with them, which is strengthened by contract signing, to promote mutual respect and cooperation.

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Organic biostimulants for vegetative growth of sugarcane (*Saccharum* spp.) in the nursery

Gabriel-Hernández, Magdiel¹; Osorio-Espinoza, Humberto^{1*}; Marroquín-Agreda, Francisco J.¹; Au-Cárdenas, Cesar. A.¹; Yereña-Yamallel, José I.²; Reyna-González, Ángel M.²; Méndez-Martínez, Kevin¹

¹ Universidad Autónoma de Chiapas. Huehuetán Chiapas, C. P. 30660, México.

² Universidad Autónoma de Nuevo León. Monterrey Nuevo León, C. P. 67700. México.

* Correspondence: humberto.osorio@unach.mx

ABSTRACT

Objective: To evaluate the effect of organic root enhancers (mycorrhizae, humic acid soil conditioner, and seaweed extracts) on the vegetative growth of sugarcane (*Saccharum officinarum* L.) seedlings under nursery conditions.

Design/methodology/approach: A completely randomized block design was used with four treatments: mycorrhizae, Bio-Organik[®] (based on humic acids), AlgaBest[®] (seaweed extract), and a control (Control); and five replicates. Destructive sampling was carried out at 15, 30, 45, 60, and 75 days after sowing (DAS), to measure plant height, root length, and root biomass.

Results: Mycorrhizae significantly increased plant height at 30 DAS (58.8 cm *vs.* 49.2 cm control). The seaweed extract enhanced early root biomass (0.19 g at 15 DAS), and late-stage root length (44.8 cm at 75 DAS). Both treatments outperformed the control (Tukey, * $p \leq 0.05$).

Limitations on study/implications: There was variable efficacy across growth stages; long-term field validation is needed.

Findings/conclusions: Organic biostimulants improve sugarcane seedling biomass, with mycorrhizae and seaweed extracts showing specific benefits for each stage. These offer sustainable alternatives for nursery propagation.

Keywords: *Saccharum officinarum*, arbuscular mycorrhizae, seaweed extracts, root biomass, sustainable agriculture.

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INTRODUCTION

The sugarcane agroindustry stands out for its significant impact on Mexico's economy. In 2023, this agroindustry reached a production of 55,589,515.48 tons, positioning Mexico as the eighth largest producer worldwide. This agroindustry generated 500,000 direct jobs and 2.4 million indirect jobs in 267 municipalities across 15 states (CONADESUCA, 2023). Sugarcane is a semi-perennial crop that is conventionally propagated through cuttings that have more than three viable buds to emerge (Rehman *et al.*, 2021). The use of chemical



products to stimulate rooting in sugarcane has been a common practice in agriculture, due to their effectiveness in accelerating root development. The root system is essential for the plant's initial development. In sugarcane, it is of vital importance for the regrowth and vigor of the shoot cycles (Pissolato *et al.*, 2021). The search for sustainable and efficient agricultural practices has led to the implementation of various biological technologies that promote plant development and improve crop productivity. Roots require water, nutrients, and oxygen for optimal development, and an imbalance of any of these resources in the soil will affect their development (Van Antwerpen *et al.*, 2022). Sustainable agricultural practices should boost the growth and prevalence of beneficial microbes. Several studies show that regenerative agriculture manifests soil health by improving microbial diversity and richness. There is a wide variety of regenerative agricultural practices such as mulching, cover crops, interspersed and mixed crops, no-till farming, among others, that would boost productivity (Singh *et al.*, 2023). Biostimulants are substances that promote plant growth, nutrition, and metabolism through different modes of action, although decidedly different from those related to fertilizers; they are supplied to plants at very low doses to induce beneficial effects (Nardi *et al.*, 2016; Yakhin *et al.*, 2017; Rouphael and Colla, 2018). The use of biological rooting agents such as mycorrhizae, humic and fulvic acids, and seaweed is emerging as a promising alternative that could significantly contribute to improving sugarcane production. Therefore, the objective of this research was to evaluate the effect of organic rooting agents on vegetative growth in sugarcane mini-cuttings, as a sustainable alternative under nursery conditions.

MATERIALS AND METHODS

Description of the study area

The study was carried out between April and August 2024, at the Experimental Field of the School of Agricultural Sciences of the Autonomous University of Chiapas, located at 15° 0' 30.68" N, 92° 24' 3.84" W, with an altitude of 33 meters above sea level (Figure 1).

The area has a semi-warm sub-humid climate and annual rainfall of 1100 and 4500 mm (INEGI, 2024). Geologically, the soil is predominantly composed of igneous rocks (>85%) and, to a lesser extent, sedimentary rocks (López-Pérez *et al.*, 2022). Mini-cuttings of sugarcane (*Saccharum officinarum* L.), variety CP 72-2086, were used, each with a viable

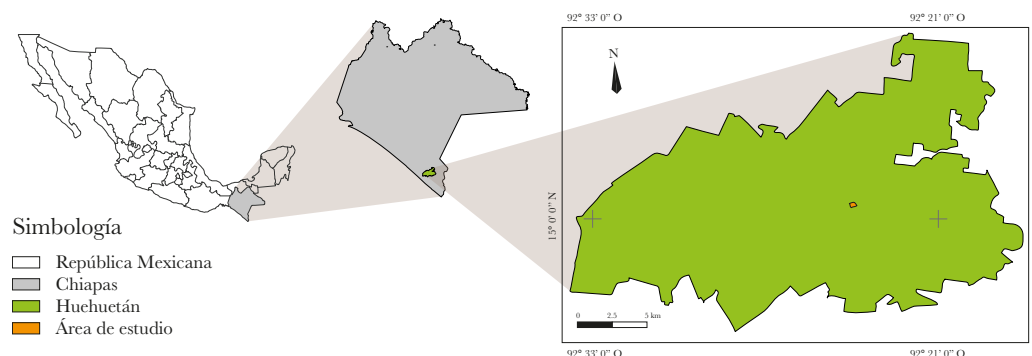


Figure 1. Geographic location of the study area.

bud to ensure sprouting. Sowing was conducted in polyethylene bags (15×23 cm) with perforations at the bottom to facilitate drainage.

Treatments

Four treatments were evaluated: T1. Mycorrhizae (*Rhizophagus intraradices*), containing 40 spores g⁻¹, applied at a dose of 5 g/plant; T2. Bio-Organik[®] (formulated with 25% phosphorus, 5% total nitrogen, 18.96% organic matter plus humic acid-based conditioners, and 51.04% diluents), at a dose of 1.5 L/ha; T3. AlgaBest[®] (containing 20% humic acids, 20% fulvic acids, 10% amino acids, 10% seaweed extract, 2% alfalfa plant extract, 1% carbohydrates and 37% diluents), at a dose of 2.0 L/ha; T4. Control (only water was applied). The treatments were distributed in a randomized complete block design with four treatments and five replicates. Destructive sampling was conducted at 15, 30, 45, 60, and 75 days after planting (DAS) and application.

Study variables

Three response variables were evaluated: Seedling height, measured with a graduated flexometer (accuracy ±0.1 cm), from the base of the stem (ground level) to the young vegetative apex; root length, determined with a flexometer (cm), from the root emergence point to the apex of the main root; and, root biomass. The roots were separated from the aerial part, washed with running water to remove the adhered substrate and dried in a forced-air oven at 75 °C for 72 h until constant weight. The research results were analyzed with the INFOSTAT version 2020e software, and the differences between treatment means with Tukey's test (P≤0.05).

RESULTS AND DISCUSSION

Height

Seedling height showed significant variations on one of the five sampling dates evaluated (Table 1). At 15 days after sowing (DAS), no significant differences were observed; however, numerical ones were observed between the biostimulants evaluated, with mycorrhizae and humic acids showing the highest values. However, at 30 DAS, the treatment with mycorrhizae showed a significantly greater effect compared to seaweed and the control, although similar to humic acids. At later dates, all biostimulants showed statistically similar behavior. These results coincide with those reported by Juntahum *et al.* (2022), who

Table 1. Means comparison of the height variable at 15, 30, 45, 60 and 75 DAS.

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS
Mycorrhizae	36.00 a	58.80 a*	67.00 a	64.20 a	78.40 a
Bio-Organik [®]	35.60 a	54.40 ab	71.40 a	70.20 a	78.60 a
AlgaBest [®]	32.40 a	50.40 bc	67.00 a	72.60 a	83.60 a
Control	31.80 a	49.20 c	65.40 a	63.80 a	77.60 a
% of CV	11.74	5.16	6.24	7.11	4.41

* Values with the same letter are equal according to Tukey's test at P≤0.05.

demonstrated that Arbuscular Mycorrhizal Fungi (AMF) positively influence the height of sugarcane seedlings grown in a nursery.

The use of individual sugarcane buds inoculated with mycorrhizae showed shoot regrowth during the early stages of development, with a significant effect observed up to four weeks after planting (DAS). These results suggest that mycorrhizal symbiosis can stimulate early seedling growth, probably due to greater efficiency in nutrient and water uptake during critical establishment phases (Musa *et al.*, 2020). Previous studies indicate that low inoculation doses (50 to 100 spores/plant) favor efficient symbiotic interaction. Therefore, early application of mycorrhizae at optimal concentrations could improve seedling conditioning before transplanting, optimizing their initial development (Moreno, 2022).

Root length

Root length showed significant differences ($p < 0.05$) at all evaluation dates (Table 2). At 15 DAS, the seaweed treatment showed significantly higher values than the other treatments. However, at 30 DAS in this trend, the mycorrhizal treatment showed greater root development, statistically outperforming the other biostimulants evaluated.

By the third sampling (45 DAS), mycorrhizae remained significantly different from the control and seaweed, but no statistically significant differences were found with respect to the humic acid treatment. By the fourth evaluation (60 DAS), seaweed again showed significant differences compared to humic acids and the control, although it showed similar results to the mycorrhizae treatment. By the last sampling (75 DAS), the observed trend continued. Both seaweed and mycorrhizae proved to be statistically superior to the other treatments evaluated.

Microbial biostimulants are a key agroecological tool for mitigating abiotic stress in crops. Their mechanism of action operates at multiple physiological levels: (1) hormonal regulation through modulation of indole-3-acetic acid (IAA), cytokinins, gibberellins, and abscisic acid; (2) production of ACC-deaminase, which reduces the levels of ethylene under stress conditions; (3) improved availability of essential nutrients; (4) induction of antioxidant enzymes that counteract oxidative stress (Bahera *et al.*, 2021; Del Buono, 2021).

Their impact on root architecture is particularly relevant. Studies with *Glomus intraradices*, *Exophiala* sp., and *Paecilomyces formosus* have shown that microbial inoculation under conditions of water stress significantly increases soil exploration (40 to 60% greater root length and hyphal development), consequently improving root hydraulic conductivity

Table 2. Means comparison of the root length variable at 15, 30, 45, 60 and 75 DAS.

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS
Micorrhizae	11.20 c	37.00 a*	51.80 a*	39.20 ab	43.60 a
Bio-Organik®	14.20 b	29.20 b	47.80 ab	38.60 b	34.60 b
AlgaBest®	17.20 a*	25.40 b	42.80 b	45.60 a*	44.80 a
Control	14.24 b	28.40 b	42.20 b	37.00 b	34.70 b
% of CV	9.67	7.28	6.72	8.74	7.47

*Values with the same letter are equal according to Tukey's test at $P \leq 0.05$.

(Aroca *et al.*, 2007; Khan *et al.*, 2015). These findings are consistent with our results, where mycorrhizal treatments showed greater root development, especially during critical establishment phases (30-75 DAS).

Recent studies show that seaweed extracts stimulate root development by modulating redox homeostasis, particularly through the regulation of antioxidant enzyme systems (Van Tol de Castro *et al.*, 2024). In the sugarcane crop, Arioli *et al.* (2024) demonstrated that these biostimulants significantly alter the composition of the rhizosphere microbiome, suggesting an indirect growth promotion mechanism through modifications in root-associated microbial communities.

The available studies have key limitations: (1) lack of specific characterization of plant-microbiome-biostimulant interactions, and (2) limited information on the temporal persistence of these effects. Our results partially coincide with these findings, where the AlgaBest[®] treatment (seaweed-based) showed variable efficacy depending on the phenological stage, being particularly effective in the initial (15 DAS) and final (60-75 DAS) studies of development.

Root biomass

The root biomass showed statistically significant differences between treatments (Table 3) at various sampling times. At 15 DAS, the seaweed treatment recorded the highest biomass (0.19 g), significantly exceeding the control and mycorrhizal treatments ($p < 0.05$), but similar to humic acids. At 30 DAS, no statistical differences were detected between treatments, which indicates homogeneous behavior at this early stage of development.

With sampling at 40 DAS, the mycorrhizal treatment presented the highest value of biomass (0.98 g), which is significantly higher than the treatments with humic acids, seaweed extract, and the control. At 60 DAS, mycorrhizae, seaweed, and humic acids outperformed the control. However, no statistical differences were found between mycorrhizae and humic acids, suggesting a favorable trend for biostimulants at this stage.

Finally, at 75 DAS, the treatment with seaweed extract reached the highest value (1.36 g), showing a statistically significant difference with respect to humic acids and the control, although not differing from the mycorrhizae. Recent studies affirm that seaweeds have a dual functionality as biostimulants and as soil improvers, promoting plant growth under stress conditions (Nephali *et al.*, 2020; Banakar *et al.*, 2022).

Seaweed extracts are rich in carbohydrates, enzymes and proteins, and can be used to reduce abiotic stress, increase nutrient utilization, and stimulate root growth, quality,

Table 3. Means comparison of the root biomass variable at 15, 30, 45, 60 and 75 DAS.

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS
Mycorrhizae	0.08 b	0.87 a	0.98 a*	0.89 a*	1.33 ab
Bio-Organik [®]	0.13 ab	0.81 a	0.82 b	0.75 ab	1.12 bc
AlgaBest [®]	0.19 a*	0.74 a	0.85 b	0.75 ab	1.36 a*
Control	0.10 b	0.73 a	0.67 c	0.62 b	1.03 c
% of CV	28.2	10.51	8.32	13.34	9.66

* Values with the same letter are equal according to Tukey's test at $P \leq 0.05$.

weight, and microbial activity in the root zone of different plants (Sible *et al.*, 2021; Lau *et al.*, 2022). Despite these beneficial results, microbial biostimulants may exhibit variable effects in different agricultural products. Therefore, further research is needed to explore specific microbes with specific functions (Khalil *et al.*, 2022).

Overall, biostimulant treatments promoted greater root biomass development compared to the control, with seaweed and mycorrhizae treatments being particularly notable, which show a sustained positive effect throughout the evaluation cycle.

CONCLUSIONS

Mycorrhizae (*Rhizophagus intraradices*) promoted greater growth in height (30 DAS) and root biomass (45 DAS), highlighting their role in early nutrient absorption.

The seaweed extract (AlgaBest®) showed a biphasic effect, stimulated initial root biomass (15 DAS) and root length in advanced stages (75 DAS), associated with its content of humic and fulvic acids and bioactive compounds.

Both biostimulants outperformed the control, confirming their potential as sustainable alternatives for nursery seedling production.

It is recommended to evaluate these treatments under field conditions to validate their persistence and profitability in complete production cycles.

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Socioeconomic profile and game theory in potential consumers of functional cheeses with bean flour

López-Santiago, Marco A.¹; Sánchez-Toledano, Blanca I.²; García-Vázquez, Rafael^{3*}; Valdivia-Alcalá, Ramón³

¹ Universidad Autónoma Chapingo-Unidad Regional, Universitaria de Zonas Áridas. Carretera Gómez Palacio-Chihuahua Km. 40, Bermejillo, Durango, México. C. P. 35230.

² Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Zacatecas. Calera de Víctor Rosales, Zacatecas, México. C. P. 98500.

³ Universidad Autónoma Chapingo-División de Ciencias, Económico Administrativas. Carretera México- Texcoco Km. 38.5, Texcoco, Estado de México, México. C. P. 56230.

* Correspondence: garciarafael154@gmail.com

ABSTRACT

Objective: To analyze the influence of consumer preferences and socioeconomic profile on the adoption of functional bean flour cheese.

Design/methodology/approach: The study was conducted in Zacatecas, Mexico, where a questionnaire was applied to consumers stratified by age. Significant variables were detected using a Kruskal-Wallis test, and three socioeconomic profiles were identified using a post hoc test. In addition, correlations were assessed through Pearson's correlation coefficient. Game theory was then used to evaluate strategic choices in different scenarios: willingness to purchase a functional food, health awareness, and motives for adoption.

Results: Relationships were identified between socioeconomic variables and consumer preferences, including income, gender, age, educational level, cheese price, consumption expenditure, label, and consumption frequency. Overall, game theory revealed distinct purchasing patterns: "Strong Consumers", "Conscious Consumers", and "Cautious Consumers".

Limitations on study/Implications: The research is limited to a specific geographic region, so caution is required when results are generalized. Key implications include market segmentation, functional food design, differentiated strategies, and consumer education.

Findings/conclusions: The importance of considering socioeconomic variables when analyzing consumption patterns and purchasing choices for functional foods is emphasized. The methodology used can serve as a basis for future research in diverse settings or to focus on different food products.

Keywords: Functional foods, Bean flour, Game theory, Agronomic products.

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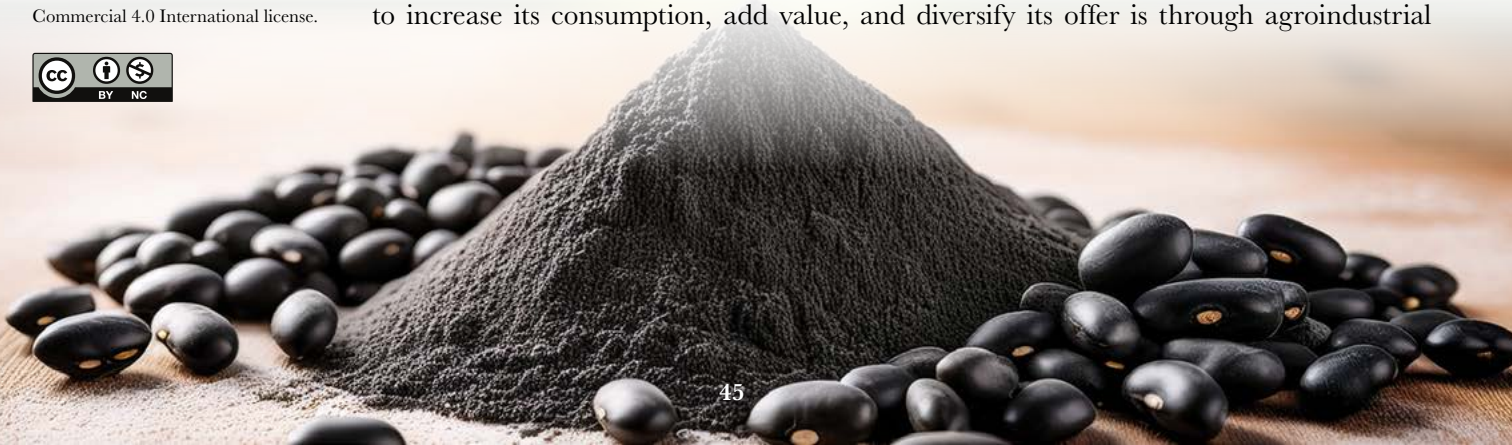
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INTRODUCTION

Black bean (*Phaseolus vulgaris*) is an important crop in Mexico; however, its value chain has weakened (Ramírez-Jaspeado *et al.*, 2020; García-Vázquez *et al.*, 2024). One way to increase its consumption, add value, and diversify its offer is through agroindustrial



transformation (Sánchez-Toledano *et al.*, 2021; Figueroa-González *et al.*, 2023). In this context, the development of functional foods has been investigated (Granato *et al.*, 2020), and the increasing interest of consumers for healthy products has generated a growing demand (Rojas-Rivas *et al.*, 2018; Baker *et al.*, 2022).

Among the key characteristics exhibited by functional foods, there are essential nutrients, increased wellbeing, and reduced disease risk (Granato *et al.*, 2020). Some studies have shown that agricultural products such as beans meet these criteria (Rojas-Rivas *et al.*, 2020; Sánchez-Toledano *et al.*, 2021). However, the intrinsic motivations and desires of individuals play an important role in food choice (Topolska *et al.*, 2021). Consequently, understanding consumer acceptance becomes crucial to identify potential market prospects (Palmieri *et al.*, 2022).

Previous research has highlighted the importance of the socioeconomic profile when it comes to the adoption of functional foods (Topolska *et al.*, 2021; Baker *et al.*, 2022; Mohammad, 2022). This serves as the driving force behind the present study, which has the objective of researching the influence of socioeconomic factors.

The use of game theory as an analytical framework is the innovative aspect of this work. This approach allows predictions and recommendations to be made in product promotion (Zhao *et al.*, 2018). It allows analyzing different factors that influence consumer behavior (Mousapour *et al.*, 2022), and it is a valuable tool to expand knowledge on different topics when evaluating strategic options (Choi *et al.*, 2020).

The study proposes an integrated view of consumer interaction with functional foods by bringing together key concepts from agriculture, nutrition, and game theory. Therefore, the main objective was to analyze the influence of consumer preferences and socioeconomic profile on the adoption of functional cheese made from bean flour. The hypothesis is that socioeconomic variables such as income, age, and spending patterns significantly influence the decision to adopt functional foods, specifically functional cheese made from bean flour.

MATERIALS AND METHODS

Characterization of the study sample

The study was conducted in the city of Zacatecas, Mexico, located at an altitude of 2,169 meters above sea level on the geographic coordinates 22° 56' 57" latitude North and 102° 42' 08" longitude West (Instituto Nacional de Estadística y Geografía [INEGI], 2023). A structured questionnaire was used to investigate consumer habits, socioeconomic characteristics, and strategic decisions regarding the willingness to purchase new functional products, consumer health awareness, and the reasons for adopting new functional foods in their diet.

The survey was applied to a sample of 100 consumers, stratified by age and with a confidence level of 90%. The sample size was validated using convenience sampling and finite population sampling. This strategy has been validated in similar studies (Sánchez-Toledano *et al.*, 2021). Subsequently, a discriminant survey was used to select participants, whose key question was whether they consumed cheese in their households. Respondents were presented with the functional cheese enriched with bean flour microcapsules, and they were given the opportunity to taste it before answering the questionnaire.

Methodological process

Significant variables related to consumption habits were identified using the Kruskal-Wallis test. This was done at 95% confidence level and significance level of $p \leq 0.05$. Three socioeconomic profiles were identified and characterized using this procedure and a post hoc test. To validate them, Pearson's correlation coefficient was applied, which revealed relationships between the variables and their bilateral significance.

Consecutively, game theory was used to create payoff matrices and to evaluate the strategic choices of these three profiles using Nash equilibrium.

The consumers were divided into three profiles: A, B, and C. Each profile was assigned a decreasing value (60, 40, and 20) depending on its importance within the sample. The assignment of values was adjusted considering the frequency of income distribution of each profile.

The responses obtained and the associated assumptions for the different scenarios were evaluated with the same values and parameters (60, 40, 20). These values, when combined with the frequency of response, reflected the relative importance of the options in each scenario and socioeconomic profile.

The establishment of matrices was based on the following assumptions:

Scenario 1. Willingness to purchase a new functional food.

- Strong consumers (60 points). They demonstrate a strong level of commitment, and they purchase even without trying the food. They are often the first to purchase within their social circle.
- Conscious consumers (40 points). They show a balanced approach, are open to trying new foods, but tend to be more cautious when it comes to early adoption.
- Cautious consumers (20 points). Cautious, they tend to delay their choice until the majority has already adopted them.

Scenario 2. Consumer health awareness.

- High awareness (60 points). Consumers who are highly committed to their health, consistently engage in self-reflection, and prioritize their well-being.
- Balanced awareness (40 points). This group shows neutral awareness. They are alert to changes and have a general awareness of their well-being.
- Moderate awareness (20 points). They exhibit less care, with daily awareness, but without deep reflection.

Scenario 3. Reasons for adopting a new functional food.

- Ecological and solidarity reasons (60 points). They seek to ensure that the functional food has "ecological" and "solidarity" principles.
- Natural and wellness reasons (40 points). Foods are intended to be "natural", "plant-based", "medicinal", and offer "energy and well-being".
- Practical reasons and lifestyle (20 points). Their adoption depends on "nomadism", "saving time" and "easy management".

The procedures were carried out in Excel spreadsheets and in the SPSS statistical package.

RESULTS AND DISCUSSION

According to the grouping category, “Economic income”, Table 1 shows the fundamental variables in consumption habits.

Then, a post hoc test was performed to determine the minimum significant difference, and three distinct groups were formed (Table 2).

Group A (<\$5,000) shows a predominantly female population, young, and with a tendency towards lower educational levels compared to the other groups. The price of one cheese is shown to be a defining factor in its consumption. Likewise, moderate weekly consumption and low spending were observed, although they do pay attention to the product label, reflecting a willingness to invest moderately in this food.

In general, young consumers tend to analyze the information before purchasing a product. They also tend to evaluate several alternatives, with price being one of the aspects that they prioritize most (Santoso and Prastiwi, 2023).

In the case of Group B (\$5,001 to 15,000), it is predominantly made up by men. Most are young adults with a university education. A negative relationship is observed with the price of cheese, suggesting that they don't pay much attention to this aspect. Despite this,

Table 1. Kruskal-Wallis test statistics and key variables in consumption habits.

Significant variables	Kruskal-Wallis H	Degrees of freedom	Asymptotic significance
Gender	6.01	2	0.05
Age	18.48	2	0.00
Level of education	19.78	2	0.00
Price of a cheese	6.73	2	0.03
Consumption spending	17.28	2	0.00
Label	6.48	2	0.04
Weekly consumption	12.78	2	0.00

Table 2. Consumption habits in relation to economic income through *post hoc*.

Income	Gender ^a	Age ^b	Level of education ^c	Price of one cheese ^d	Consumption ^e	Label ^f	Weekly consumption ^g
Group A. <\$5,000	0.55	-3.65	-1.94	0.57	-2.02	0.55	-1.34
Group B. \$5,001 a 15,000	-0.08	-2.61	-0.13	-0.01	-0.53	-1.00	-1.23
Group C. >\$15,001	-0.47	6.92	2.07	-0.56	2.56	0.65	2.58

^a Categorical variable. 1=Male; 2=Female.

^b Categorical variable. 1=15 a 19; 2=20 a 24; 3=25 a 29; 4=30 a 34; 5=35 a 39; 6=40 a 44; 7=45 a 49; 8=50 a 54; 9=55 a 59; 10=>60.

^c Categorical variable. 1=Primary school; 2=Secondary school; 3=High School; 4=University; 5=Graduate studies.

^d Dichotomous variable. 1. Yes, and 0. No.

^e Categorical variable. 1=Less than 50; 2=51 to 100; 3=101 to 150; 4=151 to 200; 5=201 to 250; 6=251 to 300; 7=301 to 350; 8=more than 351.

^f Categorical variable. 1=Always; 2=Sometimes; 3=No, never; 4=Not aware.

^g Categorical variable. 1=200 gr; 2=300 gr; 3=400 gr; 4=1 kg; 6=Más de 1 kg.

their consumption spending is only slightly higher than that of Group A, and their weekly consumption is similar.

Group C (>\$15,001) presents notable differences, since although it predominantly made up by men, it includes older individuals with postgraduate education. They pay less attention to the price of one cheese but do pay more attention to the product label. In contrast to the first two groups, their consumption spending is higher, as is their weekly consumption.

Some studies such as Topolska *et al.* (2021) and Szakos *et al.* (2022) have shown that older adults tend to be the most committed to consuming this type of food. Furthermore, the relevance of the information on the label is notable for these consumers (Baker *et al.*, 2022).

Under these assumptions, the study indicates that there is a correlation between income and age, educational level, price of one cheese (negative correlation), consumption spending, and weekly consumption (Table 3). It is revealed that as age increases, income also increases. Similarly, there is a correlation with the variable “Educational level”, so it can be inferred that higher educational levels are linked to higher spending on cheese consumption, as well as an increase in weekly consumption frequency.

In contrast, the price shows a negative correlation, indicating that when income is high, people are more willing to purchase it regardless of this factor. The information is relevant in supporting and substantiating the data presented in the groups, while highlighting the influence of the variables on consumption habits. Accordingly, the existing literature shows that the acceptance of functional foods is conditioned by different socioeconomic variables such as income, gender, age, educational level, and price, among others (Mohammad, 2022; Palmieri *et al.*, 2022; García-Vázquez *et al.*, 2024).

Table 4 shows the payoff matrix obtained for scenario 1. Analyzing the Nash equilibrium, there is indication that Profile A has a willingness to purchase a new functional food like a conscious consumer (40/40); this profile tends to be more reluctant to adopt a functional food early. Profile B, on the other hand, tends to have the characteristics of a cautious consumer type (60/60). Meanwhile, strong consumers are found in Profile C (20/20), which may suggest that they are willing to pay more for this experience.

Table 3. Pearson’s correlation between key variables of consumption habits.

Income ¹	Gender ²	Age ³	Educational level ⁴	Price of one cheese ⁵	Consumption spending ⁶	Label ⁷	Weekly consumption ⁸
1	-0.242	0.392**	0.455**	-0.261**	0.372**	0.008	0.329**
-0.242*	1	0.192	-0.175	-0.182	0.007	-0.162	0.00
0.392**	0.192	1	-0.34	-0.308**	0.452**	-0.151	0.379**
0.455**	-0.175	-0.034	1	0.046	0.194	0.081	0.197*
-0.261**	-0.182	-0.308**	0.46	1	-0.329**	0.227*	-0.170
0.372**	0.007	0.452**	0.194	0.329**	1	-0.003	0.368**
0.008	-0.162	-0.151	0.081	0.227*	-0.003	1	0.188
0.329**	0.000	0.379**	0.197*	-0.170	0.368**	0.188	1

Asterisks () and double asterisks (**) indicate statistical significance levels; (*p≤0.05, **p≤0.01).

Table 4. Socioeconomic profiles and willingness to purchase a new functional food.

	Strong consumers (20)	Conscious consumers (40)	Cautious consumers (60)
Profile A (40)	40/20	40/40	40/60
Profile B (60)	60/20	60/40	60/60
Profile C (20)	20/20	20/40	20/60

In an action plan, it is suggested to focus on attracting cautious consumers and to consider heterogeneity in marketing (Guinea *et al.*, 2020; Tian *et al.*, 2022). In turn, knowledge of individual preferences becomes relevant (Alongi and Anese, 2021).

Table 5 reflects how different socioeconomic profiles interact with levels of health awareness.

Values are intertwined in the decision-making process of consumers regarding health and food. Along these lines, Profile A is oriented toward balanced awareness, Profile B is characterized by moderate awareness, and Profile C is distinguished by high awareness; this distinctive feature suggests that these consumers continually reflect on their health status, which leads them to seek out functional foods that allow obtaining benefits.

In this framework, functional foods have gained significant attention (Obayomi *et al.*, 2024; Vignesh *et al.*, 2024). Specifically, it has been shown that bean flour has nutritional quality and health benefits (Figueroa-González *et al.*, 2023; García-Vázquez *et al.*, 2024).

The profiles and reasons for adopting a new functional food show differences (Table 6). When making decisions between food options, Profile A seeks ecological and solidarity attributes. Profile B consumers prioritize reasons related to naturalness and well-being. Meanwhile, Profile C shows interest in practical and lifestyle-related reasons; specifically, consumers belonging to this profile value pragmatic aspects in their choice of foods for adoption.

The study by Vorage *et al.* (2020) points out that the reasons for adoption reveal differences according to socioeconomic variables and motivations. Elements such as hedonism, well-being, and natural incentives become relevant.

Table 5. Socioeconomic profiles and consumer health awareness.

	High awareness (20)	Balanced awareness (40)	Moderate awareness (60)
Profile A (40)	40/20	40/40	40/60
Profile B (60)	60/20	60/40	60/60
Profile C (20)	20/20	20/40	20/60

Table 6. Socioeconomic profiles and reasons for the adoption of a new functional food.

	Ecological and solidarity reasons (40)	Natural and well-being reasons (60)	Practical and lifestyle reasons (20)
Profile A (40)	40/40	40/60	40/20
Profile B (60)	60/40	60/60	60/20
Profile C (20)	20/40	20/60	20/20

In these scenarios, game theory proved to be an efficient and applicable method for modeling consumer behavior (Choi *et al.*, 2020). Differences in preferences were observed based on socioeconomic profile.

New trends in the market are opening new possibilities for functional foods (Palmieri *et al.*, 2022; Sgroi *et al.*, 2024; Süfer, 2025). Therefore, identifying the reasons for consumption and the willingness to purchase agricultural products becomes necessary.

CONCLUSIONS

The research offers valuable insights into how consumer profiles and preferences influence the introduction of new agricultural products in the functional food industry. It also emphasizes the importance of considering socioeconomic factors when analyzing consumption patterns and purchasing choices for these types of foods. The methodology employed can serve as a basis for future studies in diverse settings or focusing on different food products. Furthermore, the findings can be compared with similar studies to identify commonalities and disparities that may have broader implications.

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Estimation of lactation persistency in a population of Rambouillet ewes utilizing five mathematical methods

Castillo-Hernández, Gabriela¹; Hernández-Mendo, Omar¹; Suárez-Espinosa, Javier²; Alcántara-Carbajal, José L.¹; Mendoza-Pedroza, Sergio I.¹; Torres-Hernández, Glafiro^{1*}

¹ Colegio de Postgraduados-Campus Montecillo. Programa de Ganadería. Montecillo, Edo. de México. C.P. 56264. México.

² Colegio de Postgraduados-Campus Montecillo. Programa de Estadística. Montecillo, Edo. de México. C.P. 56264. México.

* Correspondence: glatohe@colpos.mx

ABSTRACT

Objective: The aim of the study was to estimate the lactation persistency (LP) of Rambouillet ewes using five mathematical methods.

Design/methodology/approach: Production records of 45 Rambouillet ewes were used, with an average live weight of 65 ± 7.1 kg, from one to three lambings, with one and two lambs, which were weaned at six and eight weeks of age. To estimate the LP, the following five mathematical methods were used: P_1 (regression of the rate of decline in milk production after reaching peak production, in mL d^{-1}), $P_2 [-(\mathbf{b} + 1)\ln \mathbf{c}]$, where \mathbf{b} and \mathbf{c} are parameters of the Wood model ($Y_t = \mathbf{a}t^{\mathbf{b}} e^{-\mathbf{c}t} + \varepsilon_i$, without units), P_3 (the ratio between milk production in the second third of lactation and the first third, in %), P_4 (the ratio between milk production in the last third of lactation, and that of the first third, in %), and P_5 (milk production between the lactation peak and the end of lactation, in %).

Results: Results of the five methods to estimate the LP were: $P_1 = -6.3 \pm 0.4$ mL, $P_2 = 4.8 \pm 0.1$, $P_3 = 108.9 \pm 3.7\%$, $P_4 = 72.0 \pm 3.2\%$, and $P_5 = 11.1 \pm 12.7\%$.

Limitations on study/implications: All the methods generated estimators whose magnitude was not satisfactory compared to other studies in the literature, because the comparisons were made mainly with studies on dairy ewes and cows. In the P_5 method, a standard error greater than that of the estimator itself was obtained.

Findings/conclusions: The most reliable methods to estimate the LP in this population of Rambouillet ewes were P_1 , P_2 , and P_4 .

Keywords: Milk production, lactation curve, production systems.

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INTRODUCTION

Sheep milk production has lower participation worldwide compared to cow's milk; however, in countries such as Spain, sheep milk production systems represent a model

of great economic importance and even constitute an alternative kind of livestock production for disadvantaged rural areas (García-Díaz *et al.*, 2012). In Mexico, information about the production and composition of milk from non-dairy ewes is very limited, as is the case of Rambouillet sheep (Ochoa-Cordero *et al.*, 2002) and Katahdin sheep (Chay-Canul *et al.*, 2020). However, milk production systems with dairy sheep breeds are relatively recent, due to the introduction of germplasm from dairy breeds, such as East Friesian, Awassi, Lacaune, and Assaf; therefore, information on production rates and lactation characteristics is limited (Ángeles-Hernández *et al.*, 2018; Velarde-Guillén *et al.*, 2022).

A lactation curve (LC) describes the amount of milk produced during the lactation period. A critical phase of the LC is lactation persistency (LP), which refers to the ability of a cow, ewe, or goat to maintain milk production after reaching its peak production (Swalve and Gengler, 1999; Cobuci *et al.*, 2003). LP is of great importance in dairy cattle, since it benefits both in the reduction of feed costs (Dekkers *et al.*, 1998), and in some reproductive aspects (Dobson *et al.*, 2007). Therefore, in dairy cattle it has been preferred to prolong the LP, instead of increasing production at peak lactation (Schutz *et al.*, 1990), which is also applicable to ewes and goats. To estimate the LP, different mathematical methods have been used that are based on different criteria such as: multiple linear regression (Louda and Doney, 1976; Kominakis *et al.*, 2002), using the Wood method $[S = -(\mathbf{b} + 1) \ln \mathbf{c}]$, where parameters \mathbf{b} and \mathbf{c} must be estimated from the Wood model ($Y_t = \mathbf{a} t^{\mathbf{b}} e^{-\mathbf{c}t} + \varepsilon_i$, Nezamidoust *et al.*, 2013), due to the relationship between milk production in advanced stages of lactation in relation to initial stages, or else, using information on genetic values (Torshizi *et al.*, 2019), and others.

In Mexican Rambouillet sheep, a wool-producing breed, Ochoa-Alfaro *et al.* (2009) measured milk components and found contents similar to those of some dairy breeds, particularly in the total solids content (mean: $16.8 \pm 12.6\%$). This suggests a possible shift in the production system, focusing it on cheese production, since current wool production is not profitable for Rambouillet sheep breeders due to competition from synthetic fibers in the international market. This has encouraged continued research in this breed on aspects such as LC (currently under study) and, especially, LP, with the purpose of using this information in genetic improvement programs by selecting ewes with outstanding performance in this characteristic. The objective of the present study was to use the same database of this population (Ochoa-Alfaro *et al.*, 2009) to estimate the LP of Rambouillet ewes by comparing five methods reported in scientific literature. Most studies on this topic have been conducted with dairy cows, so information on ewes and goats is very limited. The study's hypothesis was that there are differences in the results from the five methods used to estimate lactation persistency in Rambouillet ewes.

MATERIALS AND METHODS

Animals and management

The study was conducted using milk production records of 45 Rambouillet ewes with a live weight of 65 ± 7.1 kg, from one to three lambings, with one and two offspring

weaned at six and eight weeks, belonging to the sheep flock of the School of Agronomy, Autonomous University of San Luis Potosí, at 1835 m above sea level (22° 09' 10" N and 100° 58' 41" W). The climate is cold and dry, with an average temperature of 17.8 °C and annual rainfall of 271 mm (García, 2004). The ewes were confined and fed daily with 3.5 kg fresh alfalfa and 1.7 kg of a commercial feed that had 14% crude protein and 2.96 Mcal kg⁻¹ of digestible energy. The ewes received similar amounts of the diet in the morning and afternoon (08:00 h and 13:00 h). Ewes were hand-milked twice daily (08:00 h and 13:00 h), starting on the first week post-lambing, without using any stimulus to allow milk let-down. During the lactation period, ewes were milked every seven days, with the lambs being separated from their dams at 16:00 h the day before milking. Additional management details are given in Ochoa-Alfaro *et al.* (2009).

Statistical Analysis

To estimate the LP of sheep, the following five mathematical methods were used:

Method 1 - (Kominakis *et al.*, 2002):

$$P_1 = \hat{\beta}_k = \frac{\sum_{i=1}^n X_{ik} Y_{ik} - n \bar{X}_k \bar{Y}_k}{\sum_{i=1}^n X_{ik}^2 - n (\bar{X}_k)^2}$$

Where: $\hat{\beta}_k$: slope of the regression line of the k -th ewe, expressing the rate of decline in milk production after the peak performance of the k -th ewe ($k=1, \dots, 45$). Y_{ik} : milk production of the k -th ewe on the i -th milk production day of the test day ($i=1, \dots, 84$). \bar{Y}_k : average milk production on the test day in the k -th ewe. \bar{X}_k : average days of milk production in the k -th ewe. n_k : days of milk production of the k -th sheep.

Method 2 - (Wood, 1970):

$$P_2 = S = -(\mathbf{b} + 1) \ln \mathbf{c}$$

Where: \mathbf{b} and \mathbf{c} : parameters to be estimated from the lactation curve. \ln : natural logarithm.

To obtain parameters \mathbf{b} and \mathbf{c} from this second method, it was necessary to start from the Wood model (1967), whose mathematical function is: $Y_t = \mathbf{a} t^{\mathbf{b}} e^{-\mathbf{c}t} + \varepsilon_t$, where Y_t : milk production obtained on day t ; \mathbf{a} : scale factor, or milk production at the beginning of lactation; \mathbf{b} : slope of the curve in the ascending phase; e : natural logarithm; \mathbf{c} : slope of the curve in the descending phase; ε_t : random error. By means of a logarithmic transformation, this model became: $\ln(y_t) = \ln \mathbf{a} + \mathbf{b} \ln t - \mathbf{c}t$, and when solving by linear regression, the final values of parameters \mathbf{a} and \mathbf{c} were 901.2 mL and -0.01299 , respectively, values that were used in the estimation of Method 2.

Methods 3 and 4 - Johansson and Hansson (1940):

$P_3 = R_{2:1}$ rate between milk production in the second third of lactation after lambing and the first third (%)

$P_4 = R_{3:1}$ rate between milk production in the last third of lactation after lambing and the first third (%)

Method 5 - (Keskin and Dag, 2006):

$$P_5 = \frac{\sum_{i=1}^k (pi + 1) / pi}{k} \times 100$$

Where: pi : production of the i -th milk record starting at the time of maximum milk production (peak lactation). k : record number between the time of peak lactation until the end of lactation.

RESULTS AND DISCUSSION

Table 1 presents the results of the persistency estimators, according to the five methods used in the study.

The literature on lactation persistency in non-dairy ewes is very limited. Therefore, information from dairy cows and ewes will be presented for illustrative purposes only. Readers are advised to interpret the results with great caution, especially those from dairy ewes, since, on the one hand, there are differences in the zootechnical function of the ewes (dairy *vs.* non-dairy), in addition to the differences in feeding and general management of these populations.

The average P_1 , expressed as a regression coefficient, indicates that after milk production reached the peak of lactation, Rambouillet ewes lost on average, 6.3 mL d^{-1} , a value slightly higher than the average obtained by Kominakis *et al.* (2002) in Boutsico sheep from Greece (-3.3 mL d^{-1}), which are dairy ewes. This indicates that, using this method, the persistency of Rambouillet ewes is lower than that of Boutsico ewes.

Table 1. Averages and standard errors (SE) of the lactation persistency estimators in Rambouillet ewes, according to the methods used*.

Persistency measures	Average	Standard error
P_1 (mL d^{-1})	-6.3	± 0.4
P_2	4.8	± 0.1
P_3 (%)	108.9	± 3.7
P_4 (%)	72.0%	± 3.2
P_5 (%)	11.1%	± 12.7

*: described in the Materials and Methods section.

The estimated average of P_2 was slightly lower than that obtained by Franci *et al.* (1999) in Massese dairy ewes from Italy, who used the same method in 3 types of lactations, named: Type 1 (short lactation in the fall with a duration of 136 days), Type 2 (short lactation in the spring with a duration of 93.7 days), and Type 3 (long lactation with a duration of 204.8 days), having obtained averages of 5.32, 4.93, and 5.35, for Type 1, Type 2, and Type 3 lactations, respectively, noting that the average estimated in this study is very close to that obtained by these authors (Franci *et al.*, 1999) in Type 2 lactation. In Suffolk and Columbia ewes, meat and wool producers, mated with North Country Cheviot, Dorset, Finnsheep and Romney Marsh sires, Torres-Hernández and Hohenboken (1980) obtained an average of 5.1 in P_2 , a slightly higher value than that of the present study. On the other hand, Nezamidoust *et al.* (2013) found an average of 6.9 in P_2 in Makui ewes from Iran, which are used in that country for milk, meat and wool production. From the results of these studies (Torres-Hernández and Hohenboken, 1980; Franci *et al.*, 1999; Nezamidoust *et al.*, 2013), it is observed that, in all cases, the persistency averages were greater than the average persistency obtained in the Rambouillet ewes of this study, although the average of this study was further from the average of the studies that used dairy ewes.

Methods 3 and 4, which are persistency measures that use proportions (Johansson and Hansson, 1940), have been most widely used in dairy cows and, unlike the other methods, are measures that indicate the rate of decline in milk production (in %) relative to the production level at the beginning of lactation. The average P_3 in this study was too large compared to the average obtained by Madsen (1975) in Red Danish dairy cows from Denmark using the same method, which was 87.62%. Using the same method, Pesántez *et al.* (2014) found an average P_3 of 69.4% in Anglo Nubian \times Criolla goats from Ecuador. Also in goats, other averages of P_3 obtained have been: 59.2% in Bornova goats from Turkey (Takma *et al.*, 2009), 64.2% in Dhofari goats from Egypt (El-Wakil and Fooda, 2013), and 60.2% in Beetal goats from Pakistan (Waheed and Kan, 2013). In the same population of Red Danish dairy cows, Madsen (1975) obtained an average of 75.9% of P_4 , a value higher than the average obtained in this study. On the other hand, in Dairy-mead dairy sheep from New Zealand, Marshall *et al.* (2023) used a method similar to the P_4 method developed by Madsen (1975) and interpreted as $P_{3;1}$, in which these authors (Marshall *et al.*, 2023) related the amount of milk produced in the last third of lactation with the amount of milk produced in the first third of lactation, expressed in %. Thus, the more persistent the curve, the closer its value will be to 100%; the average they found was 57.8%. In tropical dairy goats in Colombia, Henao *et al.* (2017) used the method where $P = (\text{average milk production during lactation} / \text{peak production}) \times 100$, proposed by Montaldo *et al.* (1997), and obtained a persistency rate of 64.3%. The results for P_3 and P_4 from previous studies also show higher persistency rates than those obtained in the Rambouillet sheep in the present study.

Regarding Method 5, Keskin and Dag (2006), calculated the value of P_5 in native Akkaraman sheep from Turkey, using the models Wood ($Y_t = \mathbf{a}t^{\mathbf{b}} e^{-\mathbf{c}t}$), quadratic ($Y_t = \mathbf{a} + \mathbf{b}t + \mathbf{c}t^2$), and cubic ($Y_t = \mathbf{a} + \mathbf{b}t + \mathbf{c}t^2 + \mathbf{d}t^3$), and having obtained averages of P_5 of 86.8, 79.7, and 81.6%, for the Wood, quadratic, and cubic models, respectively. This

also means greater persistency rates than those of the Rambouillet sheep in this study. It is not advisable to use this P_5 method because its standard error was greater than the value of the estimator, which could be attributed to the sample size used in the present study. In Mexico, Velarde-Guillén *et al.* (2022) estimated the LP in crosses of sheep, mainly East Friesian and Awassi, using the method where $P = (Y_{150} - Y_{42}) / (150 - 42) \times 100$, where: Y_{150} and Y_{42} represent milk production on days 150 and 42 of lactation, respectively. These authors (Velarde-Guillén *et al.*, 2022) explain that this definition of persistency indicates the slope of the regression line from day 42 to 150 of lactation, expressed in $g\ d^{-1}$ and that, furthermore, the more negative its value, the lower the LP will be. Thus, the average obtained by the authors of this study was $-3.5\ g\ d^{-1}$.

After reviewing the previous studies in the literature carried out on ewes, goats and cows, it can be stated that the differences between the present study and the studies reviewed here can be attributed mainly to the fact that most of the reviewed studies to measure the LP have used animals specialized in milk production, and it must be taken into account that the zootechnical function of Rambouillet sheep in Mexico is for wool production. However, it is important to note that their average is comparable to the LP averages obtained by Madsen (1975) in Red Danish dairy cows using the P_4 method, Torres-Hernández and Hohenboken (1980) in crosses of meat and wool sheep using the P_2 method, and Kominakis *et al.* (2002) in Boutsiko dairy ewes using the P_1 method.

CONCLUSIONS

Sheep milk production systems in Mexico are recent, due to the introduction to the country of dairy sheep breeds such as East Friesian, Awassi, Lacaune, and Assaf. A lactation curve represents milk production during the lactation period, while lactation persistency describes the ability of a cow, ewe, or goat to maintain constant milk production after reaching peak milk production. A high persistency of the lactation curve is highly desirable due to the benefits in feed costs and some reproductive aspects. In the present study, lactation persistency was estimated using five methods (P_1 , P_2 , P_3 , P_4 , and P_5) reported in scientific literature. Based on the magnitude of the estimators obtained, the best methods to estimate lactation persistency in Rambouillet sheep were P_1 , P_2 , and P_4 .

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Hyper-intensive culture of white shrimp (*Penaeus vannamei*) usin photoheterotrophic systems

Rodríguez-Olague, Delia¹; Martínez-Cárdenas, Leonardo^{2*}; Castillo-Vargasmachuca, Sergio-Gustavo¹; Claro-De los Santos, Raúl²; Arámbul-Muñoz, Eulalio³; Hernandez Crisantema⁴

¹ Universidad Autónoma de Nayarit, Programa de Doctorado en Ciencias Biológico-Agropecuarias, Carretera Tepic-Compostela km 9, Xalisco, Nayarit, México. C.P. 63780.

² Universidad Autónoma de Nayarit, Secretaría de Investigación y Posgrado, Ciudad de la Cultura Amado Nervo s/n. Tepic, Nayarit. México. C.P. 63190.

³ Universidad Autónoma de Baja California Instituto de Investigaciones Oceanológicas, km 107 carretera Tijuana, Ensenada, Baja California, México. C.P. 22860.

⁴ Centro de Investigación en Alimentación y Desarrollo, A.C. Unidad Mazatlán Mazatlán, Sinaloa, México

* Correspondence: leonardo_martinez@uan.edu.mx

ABSTRACT

Objective: The objective of this review is to analyze the published information regarding hyper-intensive cultures of white shrimp (*Penaeus vannamei*) in photoheterotrophic systems.

Design/methodology/approach: The research focused on the collection of information in the databases of Elsevier, Scielo, Wiley online library, Pubmed, Springer, Scopus and Science direct. The information was limited to the white shrimp *Penaeus vannamei*.

Results: There are several types of studies in shrimp farms, highlighting those focused on experiments in laboratories or at a pilot level to study water quality, biological variables, yield, production and survival. The crops are classified as extensive which is characterized by having a low density of shrimp per unit area, the semi-intensive where there is a higher density than the extensive and hyper-intensive system where fertilizers, artificial feed and aeration are used to maintain adequate water quality conditions. It was found that water quality, solid waste, the type of microalgae and environmental factors affect the development, performance and survival of white shrimp (*P. vannamei*).

Study limitations/implications: white shrimp (*P. vannamei*) culture is widely studied; however, there is little information about the use of hyper-intensive photoheterotrophic cultures in this species.

Findings/conclusions: Hyperintensive photoheterotrophic cultures have a great potential for development for improved shrimp production, since they are floc-based cultures and are similar to biofloc cultures; However, there is little research done on photoheterotrophic cultures, which can give better response results in terms of development, production and water quality.

Keywords: aeration, aquaculture, growth performance, photo heterotrophic.

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INTRODUCTION

White shrimp (*Penaeus vannamei*) is mainly produced in Ecuador, India, and Vietnam. Mexico ranks fourth in global shrimp production, with 238,000 tons produced in 2022, of which 82.7% came from the states of Sinaloa and Sonora (CONAPESCA, 2022). Shrimp



farming is a crucial industry due to its sustained growth, compared to other agro-industrial food production activities such as fishing, livestock, and agriculture (Khanjani *et al.*, 2024). This industry faces challenges related to environmental impact, frequent epizootics, and dependence on animal-derived inputs, particularly fishmeal for feed production (Moreno-Figueroa *et al.*, 2021). To address these issues, the aquaculture industry seeks intensification, as there is growing competition for space and resources like water and feed with other industries like livestock and agriculture (Vázquez-Vera & Chávez-Carreño, 2022). Additionally, there is a need to reduce production costs to increase profit margins and competitiveness (Lei *et al.*, 2024).

New strategies have been implemented, such as intensive cultivation systems, which manage water consumption efficiently (Ende *et al.*, 2024). White shrimp (*P. vannamei*) can be successfully produced in systems with limited or no water exchange (Lei *et al.*, 2024). Intensive shrimp farming has shown advantages over conventional aquaculture practices, characterized by high yields, small dimensions, and reduced environmental impact (Khanjani *et al.*, 2023; Long *et al.*, 2023).

Hyper-intensive shrimp farming systems include biofloc, photoheterotrophic, and recirculation systems, among others. This review aims to analyze scientific information on photoheterotrophic systems for white shrimp (*P. vannamei*) cultivation.

MATERIALS AND METHODS

Information was gathered from databases like Elsevier, Scielo, Wiley Online Library, Pubmed, Springer, Scopus, and Science Direct, focusing on white shrimp (*Penaeus vannamei*).

RESULTS AND DISCUSSION

Shrimp farming systems

Most studies on shrimp farming have focused on experiments or pilot-scale research to investigate water quality, biological variables, yield, and survival (Nugraha *et al.*, 2023; Da Silveira *et al.*, 2020; Khanjani *et al.*, 2023; Long *et al.*, 2023; Li *et al.*, 2024). The Food and Agriculture Organization (FAO, 2020) classifies farming systems into three categories: i) extensive: characterized by low shrimp density, ii) semi-intensive: higher density than extensive systems, with increased water exchange rates and supplemental feeding, and iii) hyper-intensive: utilizing fertilizers, artificial feed, and aeration to maintain optimal water quality.

Hyper-intensive shrimp farming methods

Biofloc technology system

Biofloc systems are relatively low-cost, sustainable, and environmentally friendly compared to conventional systems (Samsuri *et al.*, 2024). This system promotes the formation of flocs containing bacteria, fungi, protozoa, rotifers, and microalgae by adding carbon sources to regulate the carbon-to-nitrogen ratio in water (Khanjani *et al.*, 2024). Biofloc is rich in nutrients such as crude protein, lipids, and extracellular enzymes beneficial to aquatic animals (Long *et al.*, 2023; Martínez-Porchas *et al.*, 2020). Biofloc technology recycles waste into natural feed, reducing dependence on external feed in shrimp farming,

increasing shrimp growth, feed efficiency, water quality by controlling the C:N ratio, and improving biosecurity by reducing pathogen risks and water exchange requirements (Iber *et al.*, 2025).

Recirculation system (RAS)

RAS utilize industrialized means to artificially control water conditions in cultivation tanks, creating optimal conditions for shrimp growth and survival (McMillan *et al.*, 2003; Nugraha *et al.*, 2023). RAS occupy limited land areas, produce high yields, and have high profitability (Du *et al.*, 2021), are environmentally sustainable, and control most of the aquaculture production process (Ende *et al.*, 2024). In RAS, *P. vannamei* is grown with less water and a lower environmental impact, operating year-round under controlled conditions, offering benefits such as improved nutrient recycling, waste management, disease control, profitable land use, affordable electricity and lower biological contamination (Halim *et al.*, 2025).

Photo-heterotrophic system

The photoheterotrophic system (FH) technology utilizes a co-culture of bacteria a heterotrophic microorganisms that grow as flocs under controlled conditions (Khanjani *et al.*, 2023). These organisms stabilize water quality by converting unconsumed food and waste into non-toxic compounds (Khanjani *et al.*, 2023). These conditions promote the development of suspended organic matter flocs in the water, enhancing nitrogen recycling (produced by shrimp in single-cell protein synthesis) through nitrogen cycle oxidation-reduction relationships and the addition of carbon-rich substrates (Khanjani & Sharifinia, 2022). This floc is ingested through shrimp grazing (Ayazo-Genes *et al.*, 2019). Particulate organic matter and microorganisms in the microbial trophic chain serve as a food source in floc-based cultures due to interactions between water, organic matter, substrate and diverse microorganisms (Ayazo-Genes *et al.*, 2019).

White shrimp (*P. vannamei*) culture using a photoheterotrophic system

Super-intensive systems produce aquatic organisms sustainable and bio-securely (Da Silveira *et al.*, 2020). A super-intensive system can provide welfare for at least 150 shrimp per m², producing over three crops annually with a yield of 4.5Kg/m³ in greenhouse conditions (Li *et al.*, 2024).

Martinez-Porchas *et al.* (2020) reported that autotrophic and heterotrophic microorganisms create a micro-biofloc, a soft and compact aggregated material composed of: algae, bacillus bacteria supplied through commercial probiotics, detritus, organic particles, and protozoa that control water quality by converting unconsumed food into non-toxic compounds.

Arambul-Muñoz *et al.* (2019) determined the optimal cultivation density of *P. vannamei* in a circular photoheterotrophic intensive system with grow-out tanks in line with minimal water replacement. The authors reported that water quality during the 98-day grow-out period was within the suitable range for *P. vannamei*. Additionally, they indicated that growth rate and survival decreased as density exceeded 300 organisms per m³. Moreno-

Figuroa *et al.* (2017) determined the performance of a hypersaline photoheterotrophic system for intensive culture of *P. vannamei* shrimp with minimal water replacement in lined ponds and reported shrimp growth to a marketable size at fifteen weeks, suggesting that two shrimp harvests per year could be produced. They reported a 95% confidence in production of 12.1 to 14.7 tons/ha at harvest, with a mean weight of 13.1 g and a survival rate of 84.2%.

Water quality

Water quality influences biomass production in intensive photoheterotrophic cultures of white shrimp (*P. vannamei*) (Moreno-Figuroa *et al.*, 2021). Nugraha *et al.* (2023) reported improved water quality through reduced ammonia levels using a biofilter to control ammonia produced as byproduct of animal protein catabolism.

On the other hand, Vinasyam *et al.* (2024) reported that adding microbial groups (bacteria, archaea, algae, protists, zooplankton and fungi) and particles to biofloc improves water quality by decreasing ammonia concentration (NH₄-N). Huang *et al.* (2022) found that adding diatom microalgae to shrimp cultures positively impacts water quality enhancing shrimp development, yield, survival and weight gain. Arambul-Muñoz *et al.* (2019) evaluated the water quality of an intensive photoheterotrophic white shrimp (*Penaeus vannamei*) system in circular-lined grow-out tanks with minimal water replenishment and different stocking densities, where the parameters were dissolved oxygen concentration (mg L⁻¹), temperature (°C), pH and salinity, in addition to total ammonium nitrogen (TAN) (mg L⁻¹), nitrite-N (mg L⁻¹), nitrate-N (mg L⁻¹) and orthophosphate-P concentrations (mg L⁻¹). These authors observed a significant increase in the concentrations of TAN, nitric nitrogen and total phosphorus in the treatments, the level of dissolved oxygen in all treatments was above 5 mg L⁻¹, they found a higher concentration of nitrates and phosphorus, which could be associated with greater nitrification and heterotrophic activity, inorganic nitrogen and total phosphorus were reduced because the photoheterotrophic system promotes the development of microbial communities.

Solid waste

Feeding in intensive or super-intensive shrimp farms releases a considerable volume of waste that rapidly contaminates the pond water and directly affects the shrimp (Nguyen *et al.*, 2024). Solid waste often occurs in the form of feces, feed scraps, and dead organisms that accumulate in the system, causing eutrophication, oxygen depletion, and precipitation (Saputra *et al.*, 2017). McMillan *et al.* (2023) reported that sediments and suspended particles contain organic matter that, when degraded by bacteria, consume oxygen, reducing its bioavailability for cultivation systems, solid residue (RS) removal must equal waste production (Lei *et al.*, 2024), as accumulated residues can deteriorate water quality (Arambul-Muñoz *et al.*, 2019).

Samsuri *et al.* (2024) reported that harmful effects of solid residues include gill damage, reduced growth rates, mortality, disease, obstruction of biological filters and ammonium production through mineralization. Wastewater with a stocking density of 750 to 1250 shrimp/m² has been reported to contain an average total suspended solids (TSS) of 798

to 924 mg/L, dissolved organic matter of 81,227 to 88,641 mg/L, total nitrogen (TN) of 9.8389 to 14.4260 mg/L, and total phosphate (TP) of 7.8770 to 11.8720 mg/L (Hidayat *et al.*, 2019).

Other factors that Influence the crop

Temperature is an abiotic factor that influences the growth and development of organisms such as shrimp (Moreno-Figueroa *et al.*, 2021). Increased temperature reduces the oxygen content of water while simultaneously increasing the oxygen demand of organisms two- to threefold with a 10 °C temperature increase (Kir *et al.*, 2023). Furthermore, variations in ambient salinity levels can also modify the organism's osmotic and ionic balance, thus causing an increase or decrease in metabolic energy demand (Ponce-Palafox *et al.*, 2019). Additionally, increased salinity results in higher concentrations of flagellated organisms, while decreased salinity increases the concentrations of ciliated organisms (Fraga, 2009). Ponce-Palafox *et al.* (2019) reported that controlling salinity at 25.5 g/L yields optimal specific growth rate, productivity, and survival of Pacific white shrimp (*Penaeus vannamei*). For his part, Carranza (2020) determined the oxygen consumption of shrimp at different salinities (4, 12, 22 and 32 practical salt units UPS) and temperatures (27 and 31 °C) and reported that organisms subjected to 32 UPS had a higher oxygen demand, while organisms that were at lower salinities had a lower oxygen demand and the respiration rate was reduced, as was the osmoregulatory process and energy consumption.

On the other hand, climatic conditions have been reported to negatively impact shrimp cultivation. Prolonged intense rainfall (Le *et al.*, 2024) causes up to 50% mortality in cultivated populations within 3-4 days after intense rainfall. Additionally, food consumption decreases by 10% for each degree Celsius drop in water temperature, as rainfall cools cultivation water by 3-5 °C (Buike, 2018).

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CONCLUSIONS

Hyper-intensive photoheterotrophic cultivation systems hold great promise for development. Given their similarity to biofloc systems, due to their floc-based nature, they warrant further investigation, potentially yielding enhanced productivity and water quality.

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Evaluation of the growth and development of oil palm (*Elaeis guineensis* Jacq.) under two nursery production systems

Martínez-Valdés, Martin G.¹; Esparza-Morales, Alexander G.¹; Sánchez-Gutiérrez, Facundo¹; Velázquez-Martínez, José R.²; Hernández-Gallegos, Minerva A.³; Pozo-Santiago, César O.^{1*}

¹ Universidad Autónoma de Chiapas, Facultad Maya de Estudios Agropecuarios, Carretera Catazajá-Palenque Km 04, Catazajá, Chiapas, México. C.P. 29980.

² Universidad Juárez Autónoma de Tabasco.División Académica de Ciencias Agropecuarias, Km. 25. Carretera Villahermosa-Teapa. Km 25, C.P. 86298. Centro, Tabasco, México.

³ Universidad Juárez Autónoma de Tabasco.División Académica Multidisciplinaria de Jalpa de Méndez, Carretera estatal libre Villahermosa-Comalcalco, 27 S/N, ranchería Rivera Alta, C.P. 86200. Jalpa de Méndez, Tabasco, México.

* Correspondence: cesar.pozo@unach.mx

ABSTRACT

Objective: To evaluate the growth and development of two varieties of oil palm (*Elaeis guineensis* Jacq.) under nursery conditions, planted in bags and tubes.

Design/methodology/approach: The research was carried out in a nursery located in Macuspana Tabasco, Mexico, under a randomized block experimental design, with four treatments and four repetitions each. Two commercial varieties of *E. guineensis* were studied: Deli×Compacta and Compacta×Ghana, in two tube and bag containers. The variables recorded were height, number of leaves, and stem diameter every 15 days for 180 days. Data were analyzed using ANOVA and Tukey's mean tests.

Results: Significant differences were found between the treatments in plant height; T3 was the one that recorded the greatest increase with 32.33 ± 0.51 cm. In relation to the variables number of leaves and stems, the tube treatments obtained the highest averages with both germplasms compared to the bag treatments.

Limitations of the study/implications: The probable variability of germplasm impacts the development of the plant with respect to the containers.

Findings/conclusions: The tube treatments achieved greater plant height, number of leaves, and greater stem diameter. It is considered that the tube treatments allow the development of robust plants.

Keywords: Containers, height, increase, number of leaves, plant quality.

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INTRODUCTION

Oil palm cultivation is considered profitable due to its high yield, which is approximately 4.3 million tons of palm oil per million hectares; this represents a source of employment in producing countries and economic development for the country (Mohamad-Zaki *et al.*, 2025). Producers face challenges in seedling germination and production due to low efficiency of the techniques used to obtain and develop them, which impacts the

production rate and demand for the fruit (Beveridge *et al.*, 2022). Therefore, it is important to inquire about conditions that favor seedling development, such as containers and type of substrate for the plant; these practices have had positive effects on plantations such as coffee, eucalyptus, acai palm, among others (Paim *et al.*, 2023).

One of the practices that has allowed the production of higher quality seedlings with uniform growth vigor has been nursery growth (Gúzmań-Camposeco *et al.*, 2021). This can be carried out in two ways; the first is the simple or single-stage system, which involves directly planting the germinated seed in polyethylene bags and allowing them to develop for 10 to 12 months, followed by transplanting to the field (Lédo *et al.*, 2019). The other is the double or two-stage nursery system, which begins with a pre-nursery stage where the germinated seeds are planted in a polyethylene bag, for a period of three to four months; a pre-selection of vigorous and uniform seedlings is made, and then comes the nursery stage, where pre-selected seedlings are transplanted into larger bags for a period of seven to nine months so that at the end of this period, they are transplanted to the field (Adileksana *et al.*, 2020). There are some specifications that have become important given the improvements that have been observed in some other crops, such as the size and characteristics of the bags for growing, since this ensures the protection and development of the seedling (Furcal-Beriguete *et al.*, 2023). Establishing ideal agronomic conditions allows the existence of production materials that meet the demands of the environment, physical and chemical environment of the soil, which impacts crop yield and longevity, among others (Kouame *et al.*, 2021).

To verify that the nursery conditions implemented favor the development of the seedlings, it is important to measure biometric variables, such as root size, height, number of folioles, number of leaves, and stem diameter (Furcal-Beriguete *et al.*, 2023; Gúzmań-Camposeco *et al.*, 2021).

Therefore, the objective of this study was to evaluate the growth of two varieties of oil palm (*Elaeis guineensis* Jacq.) using tube containers and bags in the nursery.

Hypothesis: The use of different production systems has an effect on oil palm seedlings, which will be reflected in their growth and development.

MATERIALS AND METHODS

This study was carried out in the experimental plot “Predio la Sombra”, located at 13.5 km on the Zapatero-Jonuta federal highway, latitude North 17.841086 W, 92.198414 N, with average temperature of 27 °C (National Institute of Statistics and Geography, 2022).

Experimental design

The nursery was established and monitored for 180 days, according to a standardized technological package (Gremial de Palmicultores de Guatemala, 2017). A randomized complete block experimental design was used, with four treatments that include two varieties (Deli×Compacta and Compacta×Ghana), planted in two types of containers (bag and tube); each treatment had four repetitions and each of the repetitions had 100 experimental units (plants) (Table 1).

Table 1. Randomized complete block experimental design to determine nursery development of *E. guineensis* Jacq.

Treatments	Description	Repetitions	N° of plants	Plants by treatment
T1	D×Ct*	4	100	400
T2	D×Cb**	4	100	400
T3	C×Gt***	4	100	400
T4	C×Gb****	4	100	400
Totals		16	400	1600

*Deli×Compact-Tube; **Deli×Compact-Bags; ***Compact×Ghana-Tube; **** Compacta×Ghana-Bags.

The polyethylene bags used in the present study were black, 40 to 45 cm high by 28 cm wide, caliber 0.015 and 0.020 cm, with perforations in the lower third and the bottom; the tubes were made of grayish black polypropylene, 13 cm high and 15 cm wide, with internal grooves along the tube and open at the bottom (Mashani-Ahmad *et al.*, 2022). The experiment was set up in a planting frame of 1×1×1 meters, with 80 cm streets. Later, the nursery was established in which the plants were placed in 40×53 cm bags, made of 500 caliber plastic, at a distance between bags of 100 cm.

Registry and analysis of variables

The variables height, number of leaves, and diameter of the stem were measured every 15 days with a total of 12 measurements over 180 days (six months) (Gremial de Palmicultores de Guatemala, 2017). The ANOVA statistical analysis was performed, followed by Tukey's means comparison as a post-hoc test, with a confidence level of 95%. Previously, the normality and homoscedasticity of the data were verified (Beltrán-Rodríguez *et al.*, 2022), all of this with the InfoStat-Statistical Software version 2017 (InfoStat, 2017).

RESULTS AND DISCUSSION

Increase in height in the nursery

The two treatments that used tubes (T1 and T3) showed greater height compared to those produced in bags ($p < 0.0001$) (Table 2). T3 (C×Gt) recorded a greater increase in average height, of 32.33 ± 0.51 cm after 180 days ($p < 0.0001$), compared to T1, T2 and T4 which had average height of 25.66, 11.25 and 8.8 cm, respectively.

Table 2. Mean values \pm SE of the height, number of leaves, and stem diameter variables of two oil palm varieties at 180 days.

Variable	Treatments				F16	P
	1	2	3	4		
Height	25,7±0,51 c	11,3±0,51 b	32,3±0,51 d	8,8±0,51 a	487.3	0.0001
Leaf	5,9±0,1 b	1,5±0,1 a	5,8±0,1 b	1,8±0,1 a	615.2	0.0001
Diameter	1,3±0,04 c	0,5±0,04 b	1,3±0,04 c	0,41±0,04 a	188.2	0.0001

T1: D×Ct, T2: D×Cb, T3: C×Gt, T4: C×Gb. Means with a common letter between rows are not significant ($p > 0.05$), Alpha=0.05, \pm SE: standard error.

When analyzing the accumulated growth in height over a period of six months (180 days), T2 and T4 in bags started with greater heights in the nursery compared to those in tubes (T1, T3); four months later, those in tubes equaled those produced in bags. At 180 days, tubes recorded greater growth in height compared to the bag treatments, with T3 showing the greatest growth with 68.37 ± 0.61 cm in height in the nursery, followed by T1 with 65.71 ± 0.61 , and to a lesser extent, T4 and T2 followed with 58.73 ± 0.61 and 54.97 ± 0.61 , respectively (Table 3). The greatest growth effect on plants is significantly observed 90 days after sowing. It is likely that at 45 days, they use the seed reserves and not the contribution provided in the soil directly, even when the elements for their nutrition and development that accompany their growth are applied in a standard way (Purnama & Afrillah, 2022).

With height, although not an indicator of survival, vigor is considered to be determined by the size of the bulb (Madrigal-Valverde & Garbanzo-León, 2018), which provides nutrient transfer capacity (Greenshields *et al.*, 2022). On the other hand, Cobas-López, Sotolongo-Sospedra and Almora-Ramos, (2020) mention that the tube technique helps to avoid plant dehydration; the substrates are kept in good condition and can be adapted to the cultivation field during transplanting (Mashani -Ahmad *et al.*, 2022).

Number of leaves

Treatments T1 and T3 in tubes showed a greater number of leaves, with average of 5.9 ± 0.1 and 5.8 ± 0.1 , respectively, during the 180 days ($p < 0.0001$), both treatments being statistically different from treatments T2 and T4 in bags ($p > 0.05$), which presented values of 1.5 ± 0.1 and 1.8 ± 0.1 leaves, respectively (Table 2).

Regarding the accumulated number of leaves over a period of six months (180 days), T1 and T3 in tubes recorded a higher average number of leaves at transplanting, with 12.95 ± 0.11 and 12.38 ± 0.10 , respectively. The T2 and T4 treatments in bags presented a lower number of leaves at transplanting, with average values of 10.98 ± 0.10 and 11.08 ± 0.10 leaves (Table 3).

Regarding the number of leaves, the difference between treatments was observed to be significant independently. The varieties grown in tubes presented higher values, when defined from day 90 onwards. Paim *et al.* (2023) reported that the growth of swamp palm (*Mauritia flexuosa* Lf.) seedlings showed a higher leaf height-diameter ratio than those grown in 20×30 cm polyethylene bags, which could be due to better transpiration of the plant, nutrient proportion, and good photo assimilation.

Ibarra-Ruales and Reyes-Cuesta (2015) mention that with the use of tubes, plants present less stress, and this promotes growth in the palm's aerial organs in the nursery (Radin *et al.*, 2018).

Stem diameter

The stem diameter of the treatments evaluated showed significant differences ($p < 0.0001$). T1 and T3 in tubes showed greater significant differences with respect to T2 and T4 in bags, presenting average values of 1.3 ± 0.04 , 1.3 ± 0.04 and 0.5 ± 0.04 , 0.41 ± 0.04 , respectively (Table 2).

Table 3. Increase (over a six-month period) in height, number of leaves, and stem diameter in *E. guineensis* Jacq. for 180 days.

Variables	T1	T2	T3	T4
Height (cm)	65,71 ± 0,61	54,97 ± 0,61	68,37 ± 0,61	58,73 ± 0,61
N° of leaves	12,95 ± 0,11	10,98 ± 0,10	12,38 ± 0,10	11,08 ± 0,10
Diameter (cm)	2,21 ± 0,03	2,19 ± 0,03	2,11 ± 0,03	1,95 ± 0,02

For stem diameter, comparisons of the data accumulated during the six months (180 days) between the tube and the bag treatments were decisive; there was greater growth in diameter in treatments T1 and T3 compared to T2 and T4; the treatments in bags (T2 and T4) finished their nursery cycle with an average stem thickness of 20.7 millimeters, while those in tubes (T1 and T3) had an average of 21.6 millimeters (Table 3).

Castro-Garibay *et al.* (2018) report higher values of variables in seedlings, such as dry weight in root aerial and length, better quality plants, greater biomass obtained in the aerial/root ratio, height and diameter, due to their probable capacity for adaptation and survival, although the effect of the genotype must be considered (Ibarra-Rurales and Reyes-Cuesta, 2015).

A container must promote the efficient use of nutrient resources, for example, phosphorus and potassium found in the substrate, which will directly correlate with the growth of the plant (Hapsos *et al.*, 2020; Basyar *et al.*, 2023), something that this study also noted since there was better growth and development with the tube treatments.

Generating plants using this method requires careful agronomic management, especially in the container itself with variables such as stem height and thickness, although a cone-shaped pot is recommended to reduce growth time, transportation costs, transplanting, among others. In palm, growth in tubes is considered to increase the number of plants per unit area, and labor and input efficiency in the activity (Chaves-Vargas, Peraza-Padilla and Sancho-Barrantes, 2020).

CONCLUSIONS

Oil palm seedlings (*Elaeis guineensis* Jacq.) grown in tube-type containers showed greater growth in height, number of leaves, and stem diameter compared to those produced in bags, with no significant differences between the varieties. This indicates that the use of tubes favors optimal plant development in the nursery.

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Characterization of the *milpa* system in four rural communities

Epifanio-Rayón, José M.¹; Rojas-Hernández, Saúl²; Romero-Rosales, Teolincacihuatl³; Olivares-Pérez, Jaime²; Toledo-Aguilar, Rocío⁴; Olea-Reséndiz, María de J.⁵

¹ Universidad Autónoma de Guerrero. Maestría en Ciencias Agropecuarias y Gestión Local, Unidad Tuxpan. Carretera Iguala-Tuxpan Km 2.5, C. P. 40101, Iguala de la Independencia, Guerrero, México.

² Universidad Autónoma de Guerrero. Facultad de Medicina Veterinaria y Zootecnia No. 1. Carretera Altamirano- Iguala km 2.5, Col. Querenditas, Cd. Altamirano, Guerrero. México. C. P. 40660.

³ Universidad Autónoma de Guerrero. Facultad de Ciencias Agropecuarias y Ambientales. Unidad Tuxpan, Carretera Iguala-Tuxpan, km 2.5. CP. 40101, Iguala de la Independencia, Guerrero, México.

⁴ Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias - Campo Experimental Iguala. Carretera Iguala-Tuxpan, km 2.5. CP. 40101, Iguala de la Independencia, Guerrero, México.

⁵ Universidad Autónoma de Guerrero. Preparatoria Popular Tuncingo 135 - Domicilio conocido S/N. Localidad, Tuncingo. Acapulco, Guerrero, México.

* Correspondence: saulrh@hotmail.com

ABSTRACT

Objective: To analyze the social and productive characteristics and the adoption of agroecological practices in family production units (FPUs) of the *milpa* system in four localities of the municipality of Tecoaapa, Guerrero.

Design/Methodology/Approach: Local agricultural producers were interviewed for a descriptive analysis. Social, technological, seed conservation, and marketing aspects of the FPUs were characterized. The research likewise included a bibliographic review.

Results: Most of the producers were men between 40 and 80 years old, with low educational levels. A high rate of youth migration (30%) was recorded. FPUs are mainly owned by families (85.7%). As a consequence of the rugged terrain, these units make a limited use of agricultural machinery. Native corn grains and bean and squash seeds are sown in the FPUs; however, some corn varieties have been lost. The income resulting from commercialization is low. Producers mainly use hand tools. As a result of their health issues and production benefits, 46.7% of producers are willing to adopt agroecological practices.

Study Limitations/Implications: Youth migration and low education levels limit continuity and adoption of technologies. The terrain hinders the use of machinery, consequently impacting productivity.

Findings/Conclusions: Despite the challenges, an interest to strengthen traditional systems through agroecological practices has arisen. These efforts could contribute to seed and grain conservation and local agricultural sustainability.

Keywords: agroecology, rural migration, native seeds, *milpa* system, family production units.

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INTRODUCTION

Milpa is an association between corn, maize, and squash. In addition, polyculture can be associated with a wide variety of domesticated and wild plants. This system provides an average of 40-70% of the food consumed by the rural population which, paradoxically, records an increasingly severe food deficit (Statista, 2021).

The *milpa* is the union of the lore, technology, and agricultural practices required to obtain the food from the land and through human labor, in order to meet the basic needs of peasant families. In addition, *milpa* plays a major role in the diet and is the basis of food sovereignty in many regions of Mexico (Vizcarra-Bordi, 2019).

The crops of the *milpa* are perfectly intertwined, because plants get sunlight at different heights, bean can fix more nitrogen than other crops, and squash leaves reduce weeds, retaining moisture in the soils (Gouttefanjat, 2020; Harguindeguy, 2021).

The Costa Chica of Guerrero is known by its marginal areas inhabited by indigenous people (Hernández-Soc, 2019). This region has a family-based economy; producers work in rustic production systems, obtain low yields, and frequently use agrochemicals (Hernández-Flores, 2007). Production usually takes place in a *milpa* system, where plants such as corn, squash, bean, and jamaica interact (INEGI, 2019).

The characterization of traditional agricultural systems in rural communities is fundamental to understand the production dynamics that prevail in highly marginalized areas. The municipality of Tecoanapa, Guerrero, has strong cultural roots. It heavily depends on traditional agricultural methods and germplasms. Additionally, this region has been the subject of very few scientific and social research.

Consequently, the objective of this research was to characterize the *milpa* system in four rural communities of the municipality of Tecoanapa. The aim was to identify the main associated crops, agricultural practices, and the role of local lore in this traditional system. The purpose of this characterization was to provide useful information that addresses regional agricultural challenges, related to climate change, soil degradation, and field abandonment.

The resulting information can help to identify key traditional practices that can be used to design locally-adapted and sustainable production strategies. In addition, they can be used to develop rural extension programs and public policies aimed to improve the productivity and sustainability of agroecosystems.

MATERIALS AND METHODS

Study area

The study was conducted in four rural communities of the municipality of Tecoanapa, Guerrero: 1) El Pericón (16° 58' 45" N and 99° 19' 18" W, at 640 m.a.s.l.), 2) Los Saucitos (16° 59' 22" N and 99° 15' 47" W, at 630 m.a.s.l.), 3) Huamuchapa (16° 56' 23" N and 99° 31' 59" W, at 720 m.a.s.l.), and 4) Villa Hermosa (16° 58' 00" N and 99° 58' 00" W, at 760 m.a.s.l.) (INEGI, 2020).

The following three criteria were used in the selection of the localities: i) their location within the municipality; ii) their marginalization level; and iii) the persistent use of *milpa* systems as a main agricultural practice. The first criterion allowed the research team to cover different agroecological subregions. The second criterion was based on the data obtained from Consejo Nacional de Evaluación de la Política de Desarrollo Social (CONEVAL, 2020), according to which these localities were classified as areas with a high level of social marginalization. The final criterion included the use of native plant materials and lore. These facts were verified in the preliminary interviews conducted with

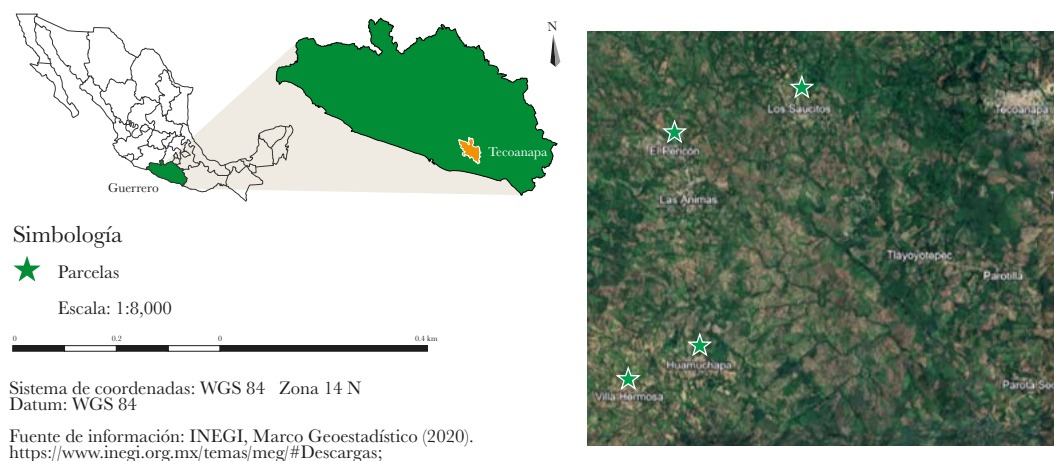


Figure 1. Location of the four localities under study, municipality of Tecoaapa, Guerrero, Mexico.

local producers and rural development technicians. These communities are characterized by subsistence economies, mainly based on rainfed agriculture, a limited productive infrastructure, and a restricted access to technical services. Most of the population belongs to indigenous or farmer groups with strong cultural roots, favoring the preservation of traditional agricultural practices. In addition, agro-climatic conditions slightly change from one locality to another, allowing a comparison of the characteristics of the *milpa* systems, under different ecological contexts but in the same municipality. The basic information of the communities was complemented with data from INEGI (2020), CONEVAL (2020), and direct field observation, in order to guarantee data reliability and scientific precision in the study design.

Sample size and selection

Sixty producers were interviewed. Producers with *milpa* systems (N=240) were chosen from the total population of each community (stratified sampling). The study included 250 (n=14), 300 (n=15), 280 (n=16), and 240 (n=15) agricultural producers from El Pericón, Los Saucitos, Huamuchapa, and Villa Hermosa, respectively. The inclusion criterion of the surveys was to select producers that still work under *milpa* systems. The management practices of the production units were characterized in semi-structured P2P interviews with key informants (*milpa* system producers) using participatory techniques.

Measured variables

The variables measured in this study included: i) social characteristics (household composition, age, education, and land ownership); ii) characteristics of the FPU's (area, crop type, and labor force); iii) agricultural practices (type of tillage, rotation, crop association, and use of fertilizers and inputs); iv) agricultural technology (machinery, equipment, irrigation system, and sowing and harvest tools); v) seed conservation (selection, storage, and exchange); vi) commercialization type (market type and self-consumption); and vii) level of adoption of agroecological practices.

These variables were chosen based on studies about farmer agroecosystems and traditional production systems (such as the milpa system), as well as in similar researches about traditional agriculture systems in Mexico (Enríquez and de Oca, 2022; Leguizamón *et al.*, 2023; Mena-Jiménez *et al.*, 2024). These variables help to understand the milpa system both from a technical and a cultural point of view, as a practice associated with socioeconomic, environmental, and symbolic features. In addition, the variables identify factors that play a role in the sustainability, resilience, and transformation of the milpa system, under a context of a high marginalization in a rural area.

Statistical analysis of the data

The database was codified and input in Microsoft Excel 2016. A descriptive statistic was developed of each interviewed group per locality. The purpose was to obtain a diagnosis of the FPU. In addition, the SPSS v. 29.0 software was used for the analysis.

RESULTS AND DISCUSSION

Social characterization

Thirty percent of young people (18-25 years old) migrate and, consequently, most of the producers are old men, as well as women and children. These results are similar to the data from INEGI (2020) for the 2015-2020 period: 29.4% women and 70.5% men migrated from Guerrero. These figures account for 4.6% of the total migrant population. Migration is mainly caused by the lack of opportunities and income in the agricultural sector for the young population. International and domestic migration, the role of women in agriculture, and the generational change are the main causes of abandonment of the fields. Consequently, reappraising traditional agricultural systems and their contribution to food security is fundamental (Ramírez, 2021).

Characteristics of the family production units (FPUs)

The results show that 85.7% of the agricultural production units are family-based and that only 13.3% hire external workers (Table 1). Huamuchapa is the community with the highest hiring rate (18.8%). The income in the four localities is generated by the head of the family, followed by his sons, daughters, brothers, and sisters. External workers are hired only for heavy tasks. The workers are mostly men, between 15 and 78 years old. These results are similar to those reported by Conaculta (2020), which highlighted that women participation in the agricultural production has survived from generation to generation. In addition, they play a major communitary role: women are in charge of the selection of the best corncocks and the preparation of food.

The interviewees were 38- to 85-years old. The head of the families had a low education level: they learned to write and read, but they only attended one or two years of primary

Table 1. FPUs in four communities of Tecoanapa, Guerrero, Mexico.

	Saucitos (15)	Pericón (14)	Huamuchapa (16)	V. Hermosa (15)
Familial (%)	86.6	85.7	81.2	86.6
Workers (%)	13.4	14.3	18.8	13.4

school. Eighty-three-point-three percent of the producers of the four localities did not attend school, while 46.7% attended basic education and 15% attended secondary school. The highest rates of uneducated producers were recorded in Huamuchapa (43.8%) and El Pericón (42.9%) (Figure 2). Old uneducated producers are reluctant to adopt new technologies and to delegate responsibilities to young people. In addition, 70.5% of the local population has migrated to the cities in search of employment. INEGI (2020) points out that basic education lays the foundation for a higher or academic education. Education is a great opportunity for Mexicans (and consequently for Mexico) to reach their full potential.

Agriculture

One of the main activities of these localities was rainfed corn production integrated to a *milpa* system (Figure 3). Crops are established in plots with <math><60^\circ</math> slopes and producers use native grains and seeds. Most corn grains are used for self-consumption. Twenty-five

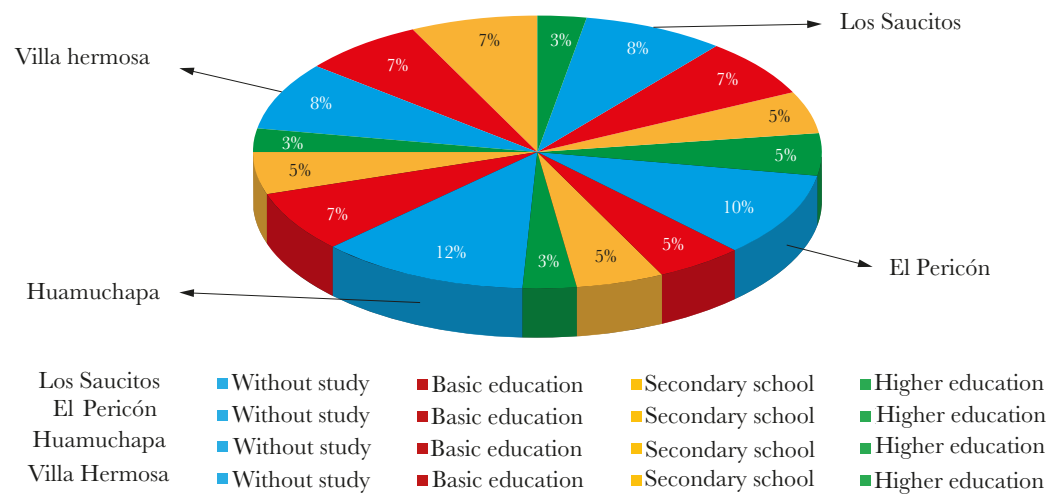


Figure 2. Education level of producers of FPU in Tecoanapa, Guerrero.



Figure 3. Plot of the *milpa* system located in Los Saucitos, Tecoanapa, Guerrero.

percent of the grains are occasionally sold in local stores or used to feed animals. Beans are also included in rainfed milpa systems. One-hundred percent of the producers use mainly native black bean seeds. The most commercialized product is squash seeds. Not enough income is produced by this production system to support a family.

Magdaleno (2016) mentioned that most of the genetic diversity of corn is native to Mexico and is grown as native varieties in the fields of farmers, who have grown these crops for generations. Farmers prefer using native grains to prepare tortillas for their daily meals and family and communitary feasts (Castillo, 2016).

Agricultural technology

Overall, the FPU's do not employ agricultural machinery; rather they use manual tools for agricultural labors (Figure 4). Very few farmers use rental tractors, because 80% of the plots of these communities is made up of very steep slopes. In addition, these plots are difficult to access. Consequently, most farmers work their plots in the traditional way. One-hundred percent of the interviewees apply fertilizers and herbicides to their crops. Regarding the sowing density per hectare, farmers require 15, 10, and 6 kg/ha for corn grains, bean seeds, and squash seeds, respectively.

Varoto *et al.* (2019) pointed out that producers must grow their crops in these rugged areas, with steep slopes and hills. This situation limits water retention and delays agricultural processes.

Meanwhile, Moreno *et al.* (2020) mentioned that traditional agriculture provides sustainable food and seeks to implement good agricultural practices, using local technology, employing family labor, and occasionally hiring local workers.

Seed conservation for sowing

In Los Saucitos, grains are mainly conserved putting the corncobs into boxes and storing them in a dark room. The corncobs are evenly arranged on top of one another; subsequently, sulphur (S) is applied every five layers to preserve them and protect them from *Sitophilus zeamais* (Motschulsky), also known as maize weevil. This procedure allows

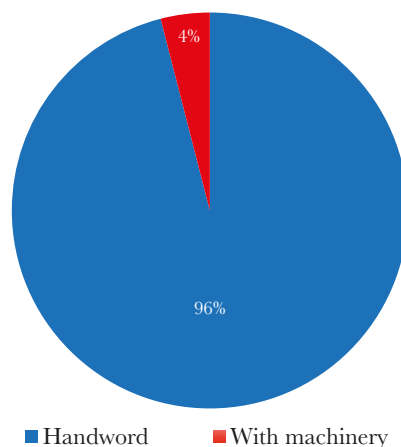


Figure 4. Work types in the four communities of Tecoanapa.

producers to keep grains in good condition (whole corncobs, without holes). Ten percent of producers uses this method. In addition, 8% of producers prefer plastic containers, because they are easier to use, although they require more space. Furthermore, some producers put the corncobs in the hearth of the kitchens, following the believe that log smoke protects them from pests (7%) (Figure 5).

Montenegro De Wit (2019) pointed out that native seeds are inseparable from the localities that have supported them throughout work organization and lore management. Finally, Torres (2016) mentioned that conserving native corn grains and squash and bean seeds is essential to strengthen the food security of local populations.

Commercialization

Figure 6 shows that the commercialization of the agricultural produce of the milpa system generate incomes between MXN\$8,000 and MXN\$9,000 for the four localities. Ten percent of producers obtained more than MXN\$10,000 in profits. The produce was commercialized in local markets; consequently, producers are part of the local

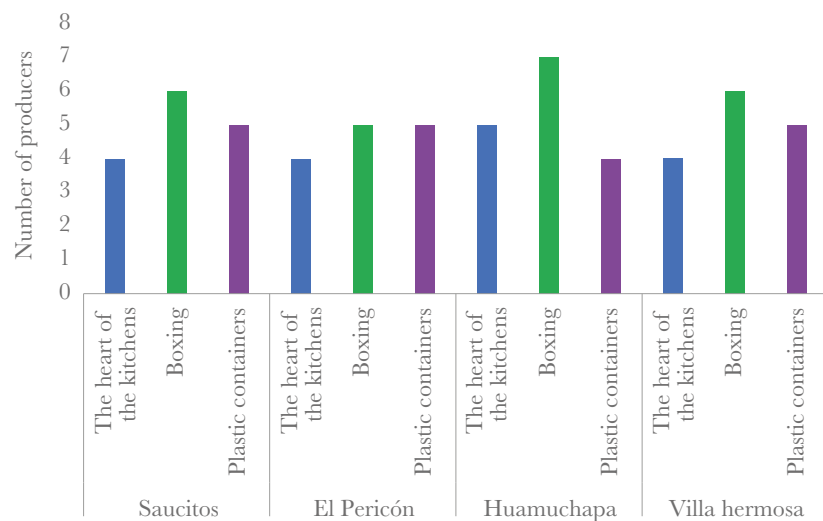


Figure 5. Seed and grain conservation for sowing in the four localities of Tecoanapa.

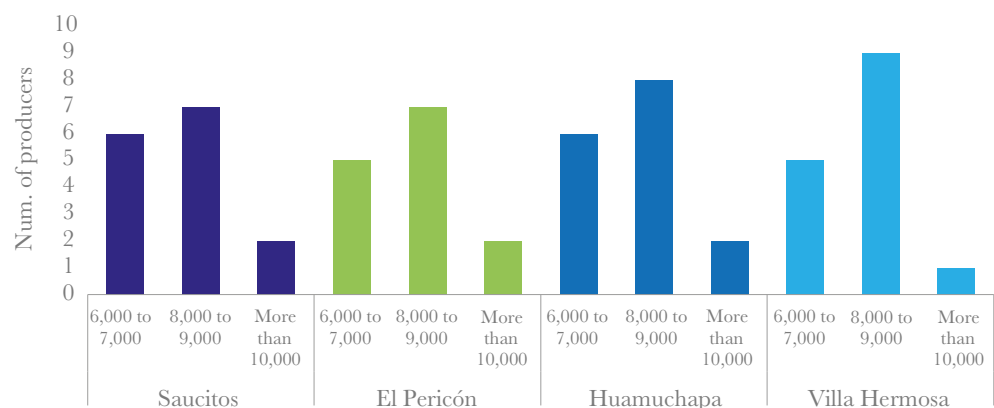


Figure 6. Income of the producers of the communities of Tecoanapa, Guerrero, Mexico.

trade economy. Meanwhile, Otero (2015) highlighted the importance of producers and consumers. On the one hand, producers are a fundamental element and are related to the way in which products are commercialized. On the other hand, consumers are interested in a given product. Finally, Muñoz-Máximo *et al.* (2017) determined that production areas receive income from local, regional, and nearby markets. They pointed out that, up to a certain point, this measure can help producers to refrain from abandoning their *milpas*.

Adoption of agroecological technology

Forty-six-point-seven percent of producers said that using agroecological products largely helps to reach a healthy life. Meanwhile, 33.3% of the producers are willing to reduce the use of agrochemicals in their plots, in order to obtain a healthier produce. Eleven-point-seven percent of the producers are willing to learn more about the use of agroecological products and, finally, 8.3% adopted agroecological products to conserve and improve their grains and seeds (Figure 7). García *et al.* (2021) pointed out that agroecology is interested in lore as an element that can provide a transition of the production systems. Lore is born from environmental experiences and the direct relationship of farmers with the ecosystem in which they live.

Regarding the adoption of agroecology, producers were questioned about their understanding of agroecological practices. Their answers included the application of manure, new farming methods, and knowledge or innovation regarding new equipment and machinery. Most of the answers from producers of Los Saucitos (53.3%) and El Pericón (57.1%) were focused on the conceptualization of agroecological practices such as farming methods, while the producers of Huamachapa (36.5%) believe that agroecological practices only include applying organic manure. One-hundred percent of the producers in Los Saucitos, El Pericón, and Villa Hermosa are willing to adopt agroecological practices, because they benefit agriculture; meanwhile, only 25% of the producers in Huamuchapa are willing to adopt agroecology, because they think that its effects are important for agriculture.

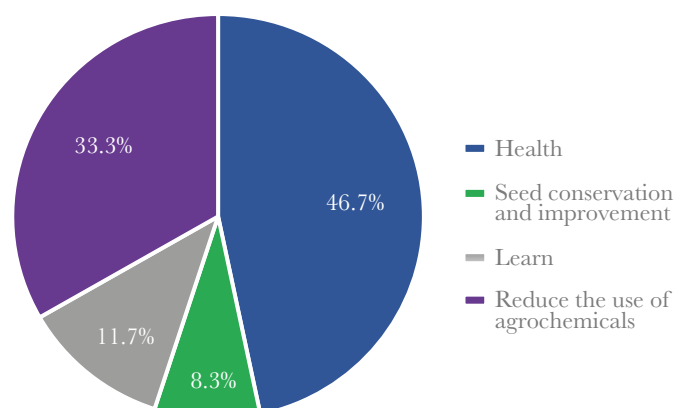


Figure 7. Reasons for the adoption of agroecological practices in the plots of the four communities of the municipality of Tecoaapa.

Questioned about their reasons for adopting these practices, the producers answered: improvement of food and health, reduction of costs, and their desire to learn about them. In Los Saucitos, 53.3% of the producers mentioned good health, while in El Pericón, 57.1% of the producers answered that they were interested in the improvement of food and its association with a higher production. In Huamuchapa, 50% of the producers mentioned health as a priority and pointed out that the wide use of agrochemicals has caused health issues. The producers of Villa Hermosa also mentioned health as a main reason to adopt agroecology (Table 2).

Table 2. Adoption of agroecology in the communities of Tecoanapa, Mexico.

Variables	Saucitos		El Pericón		Huamuchapa		V. Hermosa	
	F*	%	F	%	F	%	F	%
Adopting agroecology	8	53.3	8	57.1	6	37.5	7	46.7
Practice of agroecology	15	100	14	100	4	25.0	15	100
Agricultural importance	15	100	4	28.6	6	37.5	3	20.0
Importance of agroecology	6	40.0	8	57.1	8	50.0	10	66.7

*F: Frequency.

García *et al.* (2021) pointed out that agroecology seeks to be a transition element of the production systems. These systems are born from environmental experiences, a direct relationship with the ecosystems, daily processes, and responsible productive practices. Meanwhile, Pástor (2017) discussed the association of traditional agriculture with rainy seasons (rainfed agriculture) and crop rotation, based on an agroecological reasoning that seeks to balance and preserve ecosystems. Finally, Linck (2018) mentioned that a transition to sustainable agricultural systems must involve the reproduction of local ecological lore, based on a different logic, focused on giving and receiving benefits from ecosystems.

CONCLUSIONS

Most of the producers of the FPU in the municipality of Tecoanapa are men between 40 and 80 years old. They have little access to education opportunities and approximately 10% of them are illiterate. Migration and field abandonment by the new generations pose a threat to the continuity of traditional agricultural practices and the conservation of seeds and grains. A loss of native corn grains was recorded: none of the interviewees still sow native *Conejo* corn grains and only 40% worked with *Olotillo* corn grains. Despite these issues, producers are interested and willing to adopt agroecological practices and to take part in activities aimed to strengthen their production systems.

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Effect of *Moringa oleifera* Lam, leaves in the *in vitro* germination of *Guarianthe aurantiaca* (Bateman) Dressler & W.E. Higgins

Mendoza-García, María G.¹; Ortiz-Fernández, Alejandro²

¹ Tecnológico Nacional de México, Campus Instituto Tecnológico Superior de Coalcomán, Av. Tecnológico No. 371, Emiliano Zapata, Coalcomán de Vázquez Pallares, Michoacán., México, C. P. 60840.

² Tecnológico Nacional de México, Campus Instituto Tecnológico Superior de Calkiní, Av. Ah Canul S/N por Carretera Federal, Calkiní, Campeche, México, C. P. 24900.

* Correspondence: guadalupe@itscoalcoman.edu.mx

ABSTRACT

Objective: To evaluate an alternative culture medium based on moringa (*Moringa oleifera*) leaves and enriched with growth regulators that could improve seed germination efficiency in a controlled environment. This alternative method was compared with conventional methods; in addition, the benefits of using nutrient-dense natural components for the species *G. aurantiaca* were analyzed.

Design/Methodology/Approach: Dehydrated moringa leaves were used to prepare the *in vitro* culture media. Their effect on the germination of *G. aurantiaca* was evaluated using a completely randomized experimental design. Meanwhile, an analysis of variance and Tukey's test were used to compare means. The variables included different moringa leaf (7, 10, and 15 g/L⁻¹) and growth regulator concentrations. The dependent variable was germination percentage.

Study Limitations/Implications: The availability of *G. aurantiaca* seeds was a limiting factor.

Results: After 47 days, the MS medium with GA₃ (0.1 mg/L⁻¹) achieved the highest germination rate (91%). Ninety-seven days after sowing, the moringa leaf-based medium, with a 10 g/L⁻¹ concentration and enriched with GA₃ (0.1 mg/L⁻¹), achieved an 89% germination rate.

Findings/Conclusions: The culture medium enriched with moringa leaves is an efficient alternative for the *in vitro* germination of *G. aurantiaca*. These results established for the first time that the moringa-based culture medium is an effective alternative for the germination and conservation of this species.

Keywords: *Guarianthe aurantiaca*, micropropagation, organic extracts.

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INTRODUCTION

Orchids belong to one of the most diverse monocotyledon plant families: Orchidaceae. This family includes approximately 700-800 genera and a total of 35,000 species (Piña, 2020). Orchids are mainly distributed in warm and humid regions, but they cannot be found in deserts and cold areas. Mexico is a megadiverse country that stands out by its high percentage of endemic orchids (López and Rangel, 2018). There are about 1,260 orchid species and they are distributed in 170 genera. Mexico has 444 endemic species, which account for 40% of its orchid diversity (Tejeda Sartorius *et al.*, 2017; López and Rangel, 2018). The Mexican states with the highest orchid diversity are



Oaxaca, Chiapas, Michoacán, Guerrero, Puebla, Morelos, Jalisco, San Luis Potosí, and Veracruz (Morales, 2019).

Guarianthe aurantiaca is an epiphytic orchid that grows in warm weather (20-30 °C) and at 300-1,800 m.a.s.l. (Zaragoza, 2013). Although this species is not included in the NOM-059-SEMARNAT-2010, a decrease in its population has been recorded (Mondragón, 2009). As a result of the small size of its seeds and its dependence on mycorrhiza, it has a low natural germination rate (2-3%); consequently, the *in vitro* cultivation of plant tissue is a key tool for its conservation (Pérez-Martínez, 2016; Valdés-Infante *et al.*, 2012).

In recent years, protocols for the germination and growth of orchid seeds have been developed using organic extracts such as water and coconut milk (Gutiérrez-Zavala *et al.*, 2021), banana homogenate (Utami and Hariyanto, 2019), iron chelate (Bertolini *et al.*, 2014), pineapple juice and purée (Camilo, 2017), potato homogenate or potato dextrose agar (De Stefano *et al.*, 2022), and media with almond milk and tomato (Calevo *et al.*, 2020; Ng and Saleh, 2010). All these organic extracts are growth regulators that favor *in vitro* germination (De Stefano *et al.*, 2022).

Moringa (*Moringa oleifera*) is native to India and it has adapted to tropical climates. It is known for its high content of minerals (calcium, iron, potassium, and zinc) and essential vitamins (beta-carotene, folic acid, A, B12, and C) (Flores, 2021; Glover-Amengor *et al.*, 2016; Ndhlala and Tshabalala, 2023). Moringa has bioactive compounds, such as glycosides, isothiocyanates, glucosinolates, and flavonoids (apigenin) which provide it with antioxidant and anticancer properties (Patil *et al.*, 2022; Perveen *et al.*, 2023; Sreeja *et al.*, 2021). In addition, under controlled conditions, moringa leaf extract improves germination and reduces microbial contamination (Ndhlala and Tshabalala, 2023; Patil *et al.*, 2022). It also has phytohormones (such as auxin and cytokinin) that favor cell and root growth (Perveen *et al.*, 2023; Sreeja *et al.*, 2021). Meanwhile, its antioxidant and antimicrobial properties minimize oxidative stress (Glover-Amengor *et al.*, 2016; Flores, 2021). Therefore, the objective of this study was to use moringa as an *in vitro* culture medium to improve the germination and development of *G. aurantiaca*.

MATERIALS AND METHODS

Experiment location

This study was carried out in the Ciencias Químico-Biológicas lab of the Instituto Tecnológico Superior de Coalcomán (ITSC), located at 18° 47' 27" N and 103° 10' 31" W, Ciudad de Coalcomán de Vázquez Pallares, Michoacán, Mexico.

Plant material collection

The orchid seed capsules of *G. aurantiaca* were collected at 1,000 m.a.s.l, in the surroundings of the municipal seat of Coalcomán de Vázquez Pallares (18° 78' 33" N and 103° 16' 67" W). The capsules reached maturity at approximately five months. This information was established based on the monitoring of this species and the recording of its flowering and pollination stages, as well as the phenological characteristics of its fruits.

The pruning tool used to collect the leaves was previously disinfected with alcohol (70%). The leaves were washed with distilled water. Afterwards, the leaves were dehydrated at 60 °C for 48 hours. Finally, they were crushed in a mortar with a pestle.

Culture media

The *in vitro* culture media were MS (100%; Murashige and Skoog, 1962) and a moringa-based medium (7 g/L⁻¹, 10 g/L⁻¹, and 15 g/L⁻¹). All the media received 30 g L⁻¹ of sucrose, 2 g L⁻¹ of activated carbon, and growth regulators such as Bencylaminopurine (BAP; 0, 0.05 and 0.1 mg/L⁻¹) and gibberellic acid (GA₃; 0, 0.1 and 1.0 mg/L⁻¹). The pH was adjusted to 5.75 ± 1 with a 1N concentration of NaOH and NaCl. Figatel (2.5 g L⁻¹) was used as gelling agent. Subsequently, the media were steam sterilized, at 121 °C for 15 minutes in an autoclave.

Disinfection of orchid seed capsules

The capsules were washed under running water and immersed in a solution of 70% ethanol for 15 minutes. Subsequently, they were placed in a 20% NaOCl solution for 30 minutes. Afterwards, three washes with sterile distilled water were performed to remove the solutions. Finally, the capsules were flamed up. An inoculating needle was used to inoculate the seeds, spreading them in Petri dishes with culture media. The experimental units were kept at 28 ± 2 °C, during a photoperiod of 16 h (light) and 8 h (darkness). Changes in the experimental units were monitored during the incubation period. In addition, the physiological changes of the seeds (germination and initial development of the seedlings) were recorded.

Evaluated variables

The dependent variable was quantitatively determined. The seeds per cm² were those in which the embryo emerged from the coat seed, causing the testa rupture, the protocorm formation, and the appearance of the first leaf primordia. The independent variables were 100% MS and the 7, 10, and 15 g/L⁻¹ moringa media, enriched with BAP (0, 0.05, and 0.1 mg/L⁻¹) and three GA₃ (0, 0.1, and 1.0 mg/L⁻¹).

Experimental design and statistical analysis

The experimental design consisted of a randomized complete block design. Thirty-six treatments with a 4 × 3 × 4 three-way factorial design were conducted, with four repetitions each (36 × 4). The study factors included three BAP concentrations (0, 0.05, and 0.1 mg/L⁻¹) and three GA₃ concentrations (0, 0.1, and 1.0 mg/L⁻¹), resulting in a total of 9 treatments with the 100% MS and each of the moringa concentrations (7, 10, and 15 g/L⁻¹). The data were set forth as the mean ± the standard error of the mean (SEM). The dependent variable and the germination percentage were subjected to a one-way ANOVA, based on a general linear model (GLM). The Kolmogorov-Smirnov and the Levene tests were priorly used to verify the assumptions of normality and the homogeneity of variance, respectively. Welch's ANOVA was applied in heteroscedasticity cases to correct unequal variances and to guarantee the validity of the analysis. Using the Minitab 2021

and SPSS 2022 statistical software, treatments with significant differences ($P < 0.05$) were subjected to a mean comparison test (Tukey's test with 95% reliability), in which $*P < 0.05$ and $**P < 0.001$ were statistically significant.

RESULTS AND DISCUSSION

In stage 0, *G. aurantiaca* seeds had not germinated yet, because they had been recently placed in the *in vitro* culture medium (Figure 1A). The seeds were still adapting to the new environment, but no germination signs were visible yet. In stage 1 (4 days after sowing), the volume of the seeds started to increase, due to water and nutrient absorption, a process known as imbibition (Figure 1B). In stage 2 (day 18), the embryo had swollen and the color of the seeds had clearly started to turn green—a sign of photosynthetic activity and embryo development (Figure 1C). This change is particularly noticeable in culture media supplemented with a 0.1 mg/L^{-1} concentration of gibberellic acid (GA_3). As the germination process unfolded, most of the seeds in stage 3 had developed an intense green color and the embryo was significantly thicker and longer. In this stage, known as protocorm development, seeds occupy more space and the testa begins to break, a clear sign that germination is underway, leading to the formation of the first leaf primordia (Figure 1D). This process ended approximately 47 days after the sowing and was followed by the development of the second leaf primordia (Figure 1E). Finally, the first seedlings started to develop (Figure 1F).

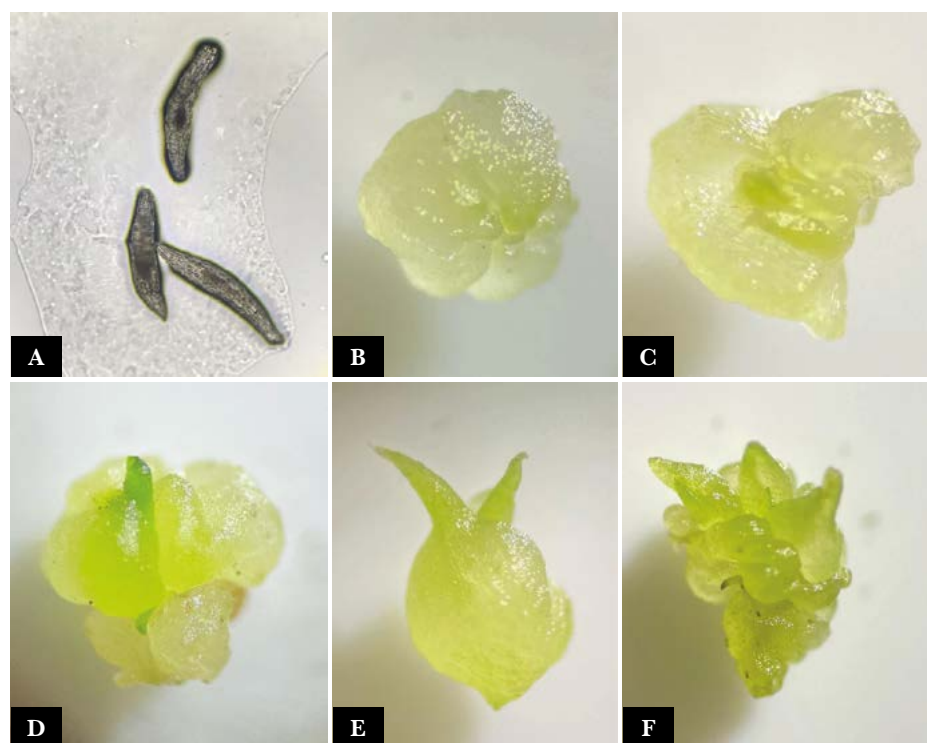


Figure 1. Germination of *G. aurantiaca*. A) Seeds with a viable embryo. B) Imbibition: water and nutrient absorption. C) First signs of green and protocorm development. D) Emergence of the second leaf primordia. E) Germinated seeds. Michoacán, Mexico (2024).

The data analyzed showed the dispersion and core tendency values of the germination variable (%). The ANOVA clearly shows that the germination percentage variable recorded significant differences ($p < 0.05$) between treatments, with the MS (100%) treatment at 47 days and the moringa treatment (7, 10, and 15 g/L⁻¹) at 98 days (Table 1).

Tukey's multiple comparison test (95% reliability) confirmed that treatment 4 (0.1 mg/L⁻¹ of MS+GA₃) is statistically different from the other treatments: it causes a better germination (91%) than the control (42.67%) in *G. aurantiaca* (Figure 2). A 7 g/L⁻¹ concentration of moringa showed significant differences ($p < 0.05$) with regard to the control (Table 1). Figure 2 shows significant responses (**) by T2 (0.05 mg/L⁻¹ of BAP) and T4 (0.1 mg/L⁻¹ of GA₃). T4 recorded an 80% germination, while control only achieved a 43% germination (Figure 2). Meanwhile, T5 (0.05 mg/L⁻¹ of BAP) was also highly effective, with a 70% germination (Figure 2). Table 1 shows that similar results were obtained with the 10 mg/L⁻¹ moringa concentration, recording highly significant differences (**), with a 95% reliability for the response variable ($p < 0.05$) of T4 (0.1 mg/L⁻¹ of GA₃). For its part, control recorded a 90% germination (Table 2), indicating a highly effective germination boost (Figure 2). T5 (0.05 mg/L⁻¹ of BAP) was also effective, recording a 75% germination.

The interaction between factors resulted in significant statistical differences for the 15 g/L⁻¹ moringa-based culture medium (Table 1). These results are significant (**) for the control group, T2 (0.05 BAP), T3 (0.1 BAP), T4 (0.1 GA₃), T5 (0.05 BAP and 0.1 GA₃) and 9 (0.1 BAP and 1.0 GA₃). T4 (0.1 GA₃) obtained an 86% germination, while T5 (0.05 mg/L⁻¹ of BAP) recorded a 78% effectivity. Meanwhile, T3 (0.1 mg/L⁻¹ of BAP) achieved a 50% germination and the combination of 0.05 mg/L⁻¹ of BAP and 0.1 mg/L⁻¹ of GA₃ resulted in 45% germination. These results suggest that 0.1 mg/L⁻¹ of GA₃ and 0.05 mg/L⁻¹ of BAP are the most favorable concentrations for the *in vitro* germination de *G. aurantiaca*, while higher GA₃ concentrations do not significantly improve the process.

All the treatments achieved the *in vitro* germination in a MS medium. Aung *et al.* (2022) reported similar results with the use of MS in orchids, because mature seeds of the *G. aurantiaca* species have reserves of nutrients and endogenous growth regulators that help germination (Santiago-Jerónimo *et al.*, 2024). Meanwhile, De Stefano *et al.* (2022) achieved a 90% germination at 98 days with moringa leaves, confirming their potential substitution with the micro and macroelements of MS salts. Therefore, they corroborated that the use of organic extracts improves the *in vitro* germination process, as a result of its content nutrients which are essential for the development of physiological processes (Ndhala and Tshabalala, 2023). These elements include calcium (Ca), iron (Fe), potassium (K), and zinc (Zn), as well as vitamins, amino acids, sugars, and phenolic compounds

Table 1. Descriptive statistics results as a response to the germination percentage variable.

Concentration	Time Days	F	Sig.	Dev. Stand.	R ² square	R ² (adjusted)	R ² (pred)
MS al 100%	47	21.743	0.000	31.510	86.56%	82.58%	76.11%
Moringa 7 g/L ⁻¹	98	57.839	0.000	19.570	94.49%	92.85%	90.20%
Moringa 10 g/L ⁻¹	98	82.183	0.000	16.567	96.06%	94.89%	92.99%
Moringa 15 g/L ⁻¹	98	132.354	0.000	13.340	97.51%	96.78%	90.20%

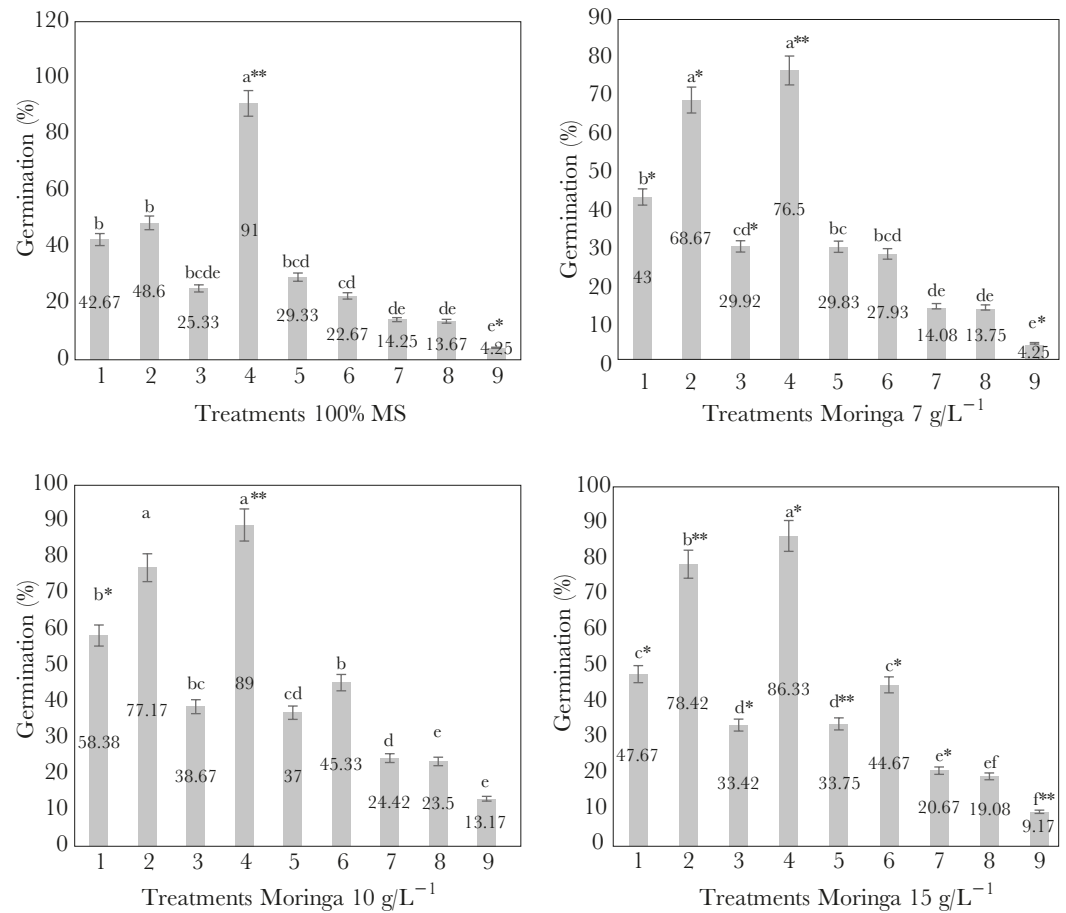


Figure 2. Effect of the MS culture medium and moringa leaves concentration (7, 10, and 14 g/L⁻¹) with various combinations of the BAP (0, 0.05, and 0.1 mg/L⁻¹) and GA₃ (0, 0.1, and 1.0 mg/L⁻¹) growth regulators, as a response to the *in vitro* germination behavior of *G. aurantiaca*. Means that do not share a letter have significant differences (P<0.05* and P<0.001**). Michoacán, Mexico (2024).

(Nunthanawanich *et al.*, 2016). These results match the findings of Álvarez (2021) who applied banana, pineapple, and coconut milk to *Vanilla planifolia* Jacks for 90 days. Choza *et al.* (2016) reported similar results with the combination of coconut milk, apple purée, and banana purée, which effectively helped the *in vitro* germination of the *Stanhopea tigrina* Bateman and *Cattleya* sp. orchids. Adding a mixture of tomato and banana pulp to the medium achieves a 70% germination in 30 days for *Catasetum integerrimum* and *Brassabola* sp. (Victoriano *et al.*, 2016). In a similar study, Salazar (2012) germinated *Cattleya mendelii* orchids in a coconut milk-enriched MS culture medium.

GA₃ (1.0 mg/L) recorded the highest percentage with MS (91%) and a 10 g/L⁻¹ concentration of moringa leaves (89%). Recent research also supports these findings, concluding that moderate concentrations of GA₃ are particularly effective with several plant species (Salazar-Mercado, 2012).

Meanwhile, the effectiveness of 0.05 mg/L⁻¹ of BAP with MS (48%) and moringa (78%) could be attributed to its capacity to stimulate cell proliferation and tissue differentiation in plants. As a cytokinin, BAP promotes cell division and shoot development, which

are essential for germination and the initial growth of seedlings (Ariza *et al.*, 2018). In this study, these synergic effects explain the high germination percentages recorded by moderate BAP concentrations.

However, treatments that combined BAP and GA₃ particularly with the highest GA₃ concentration (1.0 mg/L⁻¹) recorded lower germination percentages, perhaps as a consequence of antagonistic effects between high concentrations of these growth regulators. Prior studies have proved that excessive regulator concentrations can cause phytotoxicity, inhibiting seed development and resulting in lower germination (Davies, 2010). The presence of phytotoxicity in these treatments could have interfered with the normal physiological processes of the seeds, consequently reducing germination.

Likewise, the combination of 0.1 mg/L⁻¹ of BAP and 1.0 mg/L⁻¹ of GA₃ were less effective and only recorded a 9% germination. These unexpected results could indicate that these specific concentrations created an unfavorable environment for the seeds: perhaps seed metabolism was inhibited or the enzymes required for germination could not be activated.

CONCLUSIONS

A culture enriched with moringa and the BAP and GA₃ growth regulators was an efficient medium for the *in vitro* germination of *G. aurantiaca*. The application of 0.1 mg/L⁻¹ of GA₃ and 0.05 mg/L⁻¹ of BAP in both media was the most effective way to boost species germination, achieving 90 and 80% germination percentages, respectively.

These findings contribute to the understanding of the physiological elements involved in the germination of *G. aurantiaca*, proving that the application of specific concentrations of BAP y GA₃ can optimize the *in vitro* germination process.

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Effect of pig farming wastewater on the physiological quality of habanero pepper (*Capsicum chinense* Jaq.) seeds

Hernández-Pinto, Carlos D.¹; Vega De-Lille, Marisela I.^{1*}; Giacomán-Vallejos, Germán¹; Hernández-Núñez, Emanuel²

¹ Universidad Autónoma de Yucatán. Facultad de Ingeniería, Av. Industrias No Contaminantes por Periférico Norte S/N. Mérida, Yucatán. México, C.P. 97302.

² Instituto Tecnológico Superior de Calkiní. Departamento de Posgrado e Investigación, Av. Ah Canul S/N por carretera Federal, Calkiní, Campeche, México, C. P. 24930.

* Correspondence: marisela.vega@correo.uady.mx

ABSTRACT

Objective: to evaluate the effect of pig farming wastewater on the viability and vigor of habanero pepper seeds. **Design/Methodology/Approach:** treatments (T) applied were T1: 20% wastewater+80% tap water; T2: 40% wastewater+60% tap water; T3: 60% wastewater+40% tap water; T4: 80% wastewater+20% tap water; and T5: 100% wastewater; T6: was the control (100% tap water). The experiment was established in a completely randomized design with five replicates. Physiological variables and vigor in the seeds were evaluated through the calculation of seed germination rate and seedling emergence rate, as these express the germination capacity and vigor of seeds. Also, morphological variables in the seedlings were measured.

Results: in germination and emergence, seeds in T1 treatment and the control T6 were superior to T5. A similar trend was also observed in the emergence rate, T1 treatment and the control T6 reached the highest values. T1 and control T6 recorded the greatest plant height compared to the other treatments. Similar results were obtained for root length among all treatments, except T5, which showed seedlings with the lowest growth and vigor.

Limitations/Implications of the study: only five fixed percentages of pig farm wastewater were used in this study; So, it remains unknown about any effect that other concentrations of wastewater may have on the evaluated attributes of seed and plants of *C. chinense*.

Findings/Conclusions: pig farming wastewater at 100% decreased seed physiological attributes. In contrast, treatments with wastewater dilutions increased seed viability, vigor, and seedling growth.

Keywords: germination, emergence, growth, wastewater.

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INTRODUCTION

In recent years, population growth has led to a greater demand for food. Under this scenario, agricultural and livestock production systems have increased their productivity based on increasingly larger planting areas, a greater number of reproductive females, and larger numbers of animals in production, among other strategies (Rodrigues de Souza *et al.*, 2023). On the other hand, the increase in animal load in confinements increased the amount of waste generated in production (Rodrigues de Souza *et al.*, 2023). So, these



strategies had a negative impact on the environment due to the amount of manure and effluents generated by animals, which generally end deposited in agricultural soils and underground water bodies (Shashvatt *et al.*, 2017).

Waste from pig farming typically contains excrement, urine, water, food scraps, dust, among others. These generally contain nutrients and chemical elements such as nitrogen, phosphorus, potassium, calcium, magnesium, and some others, such as iron, copper, and zinc, which can be used in crop production and to improve the physical composition of soils (Guardini *et al.*, 2012; Silva *et al.*, 2015; Loss *et al.*, 2017; Mergen Junior *et al.*, 2019). Considering the above, some alternatives have been searched to try mitigating and reducing the impact of waste on the environment and on human health. One among those alternatives is to reuse wastewater for agriculture irrigation, which can benefit both the environment and the producer (Matos & Matos, 2017).

Due to the mineral content present in wastewater, it can be used as a fertilizer for soils and crops (Bastos, 2016; Debortolli *et al.*, 2018). Among the benefits of reusing wastewater in the soil is that it can reduce production costs by decreasing the use of commercial fertilizers for crops. Crops, in turn, can function as a natural bio-filter, which would improve the treatment of waste. Likewise, by using wastewater as a fertilization method in crop production, results similar to conventional management, or even an increase in production can be obtained. As, in contrast to the conventional form of production, wastewater use would represent a reduction in environmental pollution and an improvement of those agronomic characteristics that are required in a crop (Barbosa *et al.*, 2014).

On the other hand, inappropriate management of pig farm wastewater can have the opposite of expected effects, since it is usually used without considering the crop to be produced and its requirements. In many cases, an excess of use can originate poisoning and a reduction in crop yield, also, it can cause greater environmental pollution (Matos, 2014; Rodrigues de Souza *et al.*, 2023). In this context, it is imperative to generate information on the interaction of wastewater with plants and to evaluate possible forms of production by reusing pig production waste. Therefore, the objective of the research was to evaluate the effect of pig farming wastewater on the viability and vigor of habanero pepper seeds.

MATERIALS AND METHODS

Experimental site and plant material

The experiment was conducted in the laboratory of the Faculty of Environmental Engineering under the Autonomous University of Yucatán. Seeds of a commercial variety of habanero pepper (*Capsicum chinense*) were used.

Experimental design and treatments

This research was developed with a completely randomized experimental design. The treated pig wastewater used in the experiment was obtained from a pig farm that has a wastewater treatment plant. This facility consists of a sump, an anaerobic bio-digester, and oxidation lagoons, composed of a part with aeration and another with ozonation where the treated wastewater for agricultural use is obtained. Seeds and seedlings were produced and irrigated with treated wastewater and with wastewater dilutions based on the following

treatments T1: 20% wastewater + 80% tap water; T2: 40% wastewater + 60% tap water; T3: 60% wastewater + 40% tap water; T4: 80% wastewater + 20% tap water; and T5: 100% wastewater; T6: was the control (100% tap water). Seedlings produced in the control were supplemented with 190 mg L^{-1} of NPK (Poly Feed, 19N-19P-19K, Mexico City, Mexico). Each treatment consisted of five replicates.

Variables evaluated

Physicochemical parameters (pH, electrical conductivity, chemical oxygen demand, total nitrogen and total phosphorus) of the wastewater and the dilutions corresponding to each treatment were analyzed, as well as the tap water used in the control treatment, in accordance with the Mexican Official Standard NOM-001-SEMARNAT-2021.

Physiological variables of the seeds were determined as effects in relation to seed imbibition with the dilutions of wastewater. Germination and germination rate (GR) of the seeds were evaluated according to Hernández-Pinto *et al.* (2020); the latter was counted for 14 days and a seed was considered germinated when the radicle was observed. The seeds were placed in Petri dishes of 90 mm diameter, in each dish 25 seeds were placed on paper towels, moistened with the dilutions (4 mL each) based on the established treatments. Four Petri dishes were established per treatment, each one represented a replicate; at a temperature of $22 \pm 1 \text{ }^\circ\text{C}$, 60% of relative humidity, and placed in total darkness.

The seedlings emergence test was done in polystyrene trays with germinating substrate (Sunshine mix 3), placing one seed per cavity. Emergence was counted daily for seven days after sowing (das). The percentage (%E) and emergence rate (ER) were calculated from the data obtained (Hernández-Pinto *et al.*, 2020). Once the seedlings emerged (at 4 das), trays were moved to a greenhouse for growth. Irrigation with the wastewater dilutions and inorganic fertilization began at 12 das. The experiment was arranged in a completely randomized design with five replicates.

Morphological variables of the seedlings were also measured; height, which was measured with a tape measure from the base of the root ball to the apical bud; stem diameter was obtained with a digital Vernier caliper 1 cm apart from the root ball; and root length was measured using the ImageJ software. These variables were evaluated at 40 days. Fifty seedlings were evaluated as the experimental unit.

Statistical analysis

Data obtained as percentage were transformed with the arcsine square root. An analysis of variance ($p \leq 0.05$) was performed for all variables; where significant statistical difference was found among treatments, a multiple comparison of means was applied (Tukey, $p \leq 0.05$). The analyses were performed using Statistica[®] v.7 (Statsoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Chemical analysis of the water showed significant statistical differences among treatments. Treatments with a higher proportion of wastewater (T5 and T4) had a higher

pH and a much higher electrical conductivity (EC), than the other treatments. In contrast, treatment T1 and control T6 had significantly lower EC and pH. The trend was similar for most of the parameters evaluated and for chemical oxygen demand (COD) (Table 1). Dilutions of the wastewater reduced the chemical parameters. The lower EC of the dilutions in treatment T1 and the control T6 allowed for better assimilation of the mineral content present in the wastewater, which enhanced seedling growth.

In this regard, Sambo *et al.* (2019) indicated that EC and pH affect and influence the absorption of nutrients required by plants and consequently their growth and development. Nitrogen content was significantly higher in those treatments with a higher amount of wastewater (T4, 491 mg L⁻¹ and T5, 544 mg L⁻¹), this concentration decreased in relation to the percentage of wastewater present in the treatments. On the other hand, a higher concentration of phosphorus was observed in those treatments T1 (10.32 mg L⁻¹) and T2 (9.62 mg L⁻¹) with a lower percentage of wastewater, compared to the rest of the treatments. The concentration of these elements positively influenced the viability and vigor of seeds.

Treatments with higher nitrogen concentrations had lower results in the variables evaluated, which could be due to interference with the functions of phosphorus in the seeds and seedlings evaluated in those treatments. In addition to increasing the EC, which hindered the interaction and action of minerals on the physiological attributes of the seeds. In this regard, Bilal *et al.* (2020) mentioned that an EC greater than 3000 $\mu\text{S cm}^{-1}$ reduces the absorption of water and nutrients necessary for plant growth and development. In contrast, treatments T1 and T2, and the control T6 with lower mineral concentrations recorded better results in the evaluated variables.

Regarding COD, treatments T1, T2 and the control T6 presented a lower degree of contamination in comparison to treatments T4 and T5, which were higher compared to the others. The dilutions of the wastewater made it possible to improve the quality of the irrigation water used for seeds and seedling production, as well as decreasing contamination. As indicated by Banach *et al.* (2009) who mentioned that COD quantifies the amount of total organic matter present in a liquid sample and it is used to determine the level of water contamination.

Table 1. Chemical parameters of wastewater and wastewater dilutions in treatments.

Treatments	pH	EC ($\mu\text{S cm}^{-1}$)	COD (mg L ⁻¹)	TKN (mg L ⁻¹)	TP (mg L ⁻¹)
T1	7.73±0.015 NS	2041±0.041 e	152±0.01 c	100.3±0.31 d	10.32±0.30 a
T2	8.6±0.020 NS	3207±0.079 d	215±0.02 b	203±0.39 c	9.62±0.46 a
T3	8.72±0.027 NS	4488±0.105 c	233±0.05 b	294±0.51 b	8.7±0.76 b
T4	8.87±0.041 NS	5680±0.109 b	440±0.13 a	491±0.75 a	5.84±0.80 d
T5	8.96±0.052 NS	6620±0.130 a	503±0.33 a	544±1.02 a	6.48±0.91 c
T6 (Control)	6.56±0.011 NS	971±0.034 f	≤20±0.01 d	6.72±0.27 e	6.17±0.14 c

T1: 20% wastewater + 80% tap water; T2: 40% wastewater + 60% tap water; T3: 60% wastewater + 40% tap water; T4: 80% wastewater + 20% tap water; and T5: 100% wastewater; T6: was the control (100% tap water); pH: hydrogen ion concentration in water; EC: Electrical conductivity; COD: Chemical oxygen demand; TKN: Total Kjeldahl nitrogen; TP: Total phosphorus. Data are means + standard error. Different letters in the same column indicate significant statistical difference (Tukey, $p \leq 0.05$). NS: non-significant.

Seed viability and vigor

Highly significant differences in seed germination were found starting on the fourth day. Control T6 (control) and treatment T1 had the highest germination (98% and 96%, respectively), compared to the other treatments; whereas treatments T2 and T3 were statistically similar. In contrast, treatments T4 and T5 had the lowest germination, 52% and 31%, respectively; T5 was at least 69% lower than T1 and the control T6 (Figure 1 A). A similar trend was observed for seedling emergence; treatment T1, 94% and the control T6, 98% were statistically superior to the rest. Most treatments were similar in seedling emergence, except treatment T5, which had the lowest emergence rate (72%) (Figure 1 B).

It was evident that pig farming wastewater influenced the germination capacity and vigor of the seeds. The dilutions of wastewater enhanced seed germination and seedling emergence in a shorter time (4 and 1 day), statistically similar to the control T6 (100% tap water). In contrast, treatments that had a higher percentage of wastewater in their composition decreased viability and vigor in the evaluation. The high viability of the seeds and the emergence in treatments with a low percentage of wastewater is likely due to a higher concentration of phosphorus in relation to total nitrogen, compared to the other dilutions. This could enhance the greater germination and emergence by promoting the decrease of abscisic acid and the activity of metabolic enzymes that initiated the germination process in less time.

It is known that some minerals such as N, Mg, K, Ca, P, among others, are related to the viability of seeds, and participate in the synthesis of proteins and concentration of abscisic acid (Lott *et al.*, 1995). Also, the minerals K, Ca, Mg, Mn, P and Fe are initiators of enzyme activators, promoting protein synthesis during seed development and germination (Xu *et al.*, 2002; Iwai *et al.*, 2012). This allows for greater availability of reserves to the embryo during the germination and emergence process, which in turn enhanced germination capacity and vigor in less time.

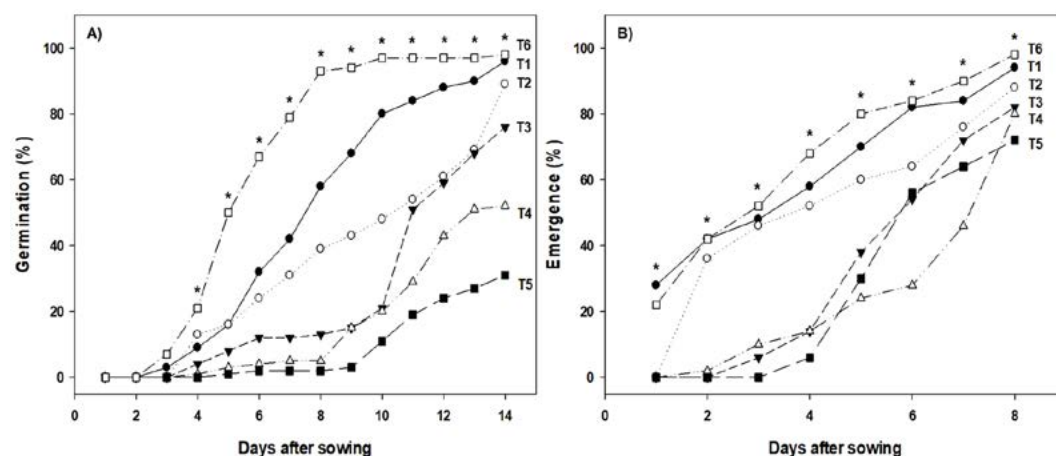


Figure 1. A: seed germination dynamics and B: seedling emergence of Habanero pepper (*Capsicum chinense*) produced with pig farm wastewater dilutions T1: 20% wastewater + 80% tap water; T2: 40% wastewater + 60% tap water; T3: 60% wastewater + 40% tap water; T4: 80% wastewater + 20% tap water; T5: 100% wastewater; T6: 100% tap water (control). Data are means. * indicates significant statistical difference (Tukey, $p \leq 0.05$); $n = 100$.

Highly significant differences were found in seed germination and plant emergence rates of habanero pepper seeds. In the GR, treatment T1 and control T6 recorded the highest values (18.86 and 10.19 seeds germinated per day), respectively 89% and 88% higher than T5, which obtained the lowest value (2.19 seeds germinated per day) (Table 2). Similarly, in ER, T1 and the control T6 (with 13.09 and 13.24 seedlings per day) statistically surpassed the rest of the treatments; closer behind was T2, which obtained 9.08 seedlings per day; whereas T3, T4, and T5 only were statistically similar to each other (4.26, 3.98, and 3.49 seedlings per day, respectively) (Table 2).

There was evidence that the dilutions of wastewater made it possible to enhance and homogenize seed germination and seedling emergence due to the presence of minerals in the wastewater. Xu & Kafkafi (2003) and Mori *et al.* (2012) mentioned that the presence and concentration of minerals in contact with the seeds, increase the activity of enzymes and metabolic reactions. Consequently, there is a greater availability of reserves and energy during the germination process, improving the viability and vigor of the seeds.

The aforementioned is in agreement with the homogenization of germination and emergence of seedlings in less time observed in this study, contrary to what was observed in treatments with a higher concentration of wastewater, which negatively influenced the percentages obtained (Table 2).

In growth variables, diluted wastewater significantly influenced by inducing plant growth. Control T6 (8.53 cm) and treatment T1 (7.78 cm) were superior to all others, T2 with 7.13 cm was closer. However, the high concentration of wastewater was not favorable for vegetative growth, as observed in T3, T4, and T5, which had the lowest values (respectively 6.59, 6.59, and 3.57, cm) (Table 3). In stem diameter, control T6 had the greatest thickness (2.07 mm), treatment T1 with 2.02 mm was statistically similar. The low concentration of wastewater that was present in that treatment increased stem thickness in seedlings by at least 74%. In contrast, a 100% concentration of wastewater decreased this variable (T5, 0.97 mm) by up to 66% (Table 3).

Table 2. Seed germination rate and seedling emergence rate of Habanero pepper (*Capsicum chinense*) watered with pig farming wastewater dilutions.

Treatments	GR (seeds per day)	ER (seedlings per day)
T1	18.86±0.025 a	13.09±0.32 a
T2	10.19±0.27 b	9.08±0.67 b
T3	6.38±0.35 c	4.26±0.71 c
T4	4.07±0.38 d, e	3.98±0.73 c
T5	2.19±0.80 e	3.49±1.07 c
T6 (Control)	20.63±0.22 a	13.24±0.27 a

T1: 20% wastewater + 80% tap water; T2: 40% wastewater + 60% tap water; T3: 60% wastewater + 40% tap water; T4: 80% wastewater + 20% tap water; T5: 100% wastewater; T6: 100% tap water (control). Data are means ± standard error. Different literals in the same column indicate significant statistical difference (Tukey, $p \leq 0.05$); $n=100$.

Table 3. Morphological responses of Habanero pepper (*Capsicum chinense*) seedlings produced with dilutions of pig farm wastewater.

Treatments	Plant height (cm)	Stem diameter (mm)	Root length (cm)
T1	7.78±0.08 a	2.02±0.02 ab	6.04±0.27 a
T2	7.13±0.08 b	1.90±0.02 bc	5.23±0.20 b
T3	6.59±0.06 c	1.84±0.02 cd	4.19±0.24 c
T4	6.59±0.10 c	1.73±0.02 d	3.30±0.16 d
T5	3.57±0.32 d	0.97±0.09 e	1.47±0.09 e
T6 (Control)	8.53±0.06 a	2.07±0.01 a	6.33±0.20 a

T1: 20% wastewater + 80% tap water; T2: 40% wastewater + 60% tap water; T3: 60% wastewater + 40% tap water; T4: 80% wastewater + 20% tap water; T5: 100% wastewater; T6: 100% tap water (control). Data are means ± standard error. Different letters in the same column indicate significant statistical difference (Tukey, $p \leq 0.05$).

In root length, the presence of minerals in the wastewater favored greater root growth as observed in treatments T1 with 6.04 cm and the control (T6, 6.33 cm) (Table 3). The wastewater allowed greater root growth which increased the height of the seedlings and improved stem development; as a consequence, seedlings have a greater probability of survival in the field after transplantation. In this regard, Villar-Montero *et al.* (2004) indicated that the presence of minerals such as N, P and K in interaction with the seedlings influences quality and tolerance because of a greater amount of reserves and elements available to survive the transplant. Mineral composition of the wastewater with the prepared dilutions promoted vegetative growth and development of seedlings because they had mineral elements available for assimilation.

Likewise, Gorbe and Calatayud (2010) and Torres-García *et al.* (2019) mentioned that N and P are essential during plant growth due to their role in the various photosynthetic activities and energy transfer in metabolic processes. Higher available energy is expressed in plant growth, as increases in height, stem diameter, root length, number of leaves, among others. Overall, an inversely proportional relationship was observed in regard to seed viability and the concentration of pig farm wastewater. That is, a high concentration of pig farm wastewater decreased the germination capacity of the seeds, whereas a low concentration of it (pig farm wastewater in dilution) increased seed germination capacity and seedling emergence (Figure 2).

CONCLUSIONS

Pig farm wastewater contains a higher amount of chemical elements. In contrast, wastewater dilutions decreased mineral content and increased seed attributes. Treated pig farm wastewater (100%) decreased seed viability and vigor. In contrast, treatments with wastewater dilutions increased and enhanced seed germination and seedling emergence. Wastewater dilutions influenced seedling morphology by increasing height, stem diameter, and root length. Therefore, pig farm wastewater diluted is a viable alternative that modifies and enhances seed physiological quality, and improves seedling production of Habanero pepper in a shorter time.



Figure 2. Impact of treated pig farm wastewater on the physiological quality of Habanero pepper (*Capsicum chinense*) seeds. T1: 20% wastewater + 80% tap water; T2: 40% wastewater + 60% tap water; T3: 60% wastewater + 40% tap water; T4: 80% wastewater + 20% tap water; T5: 100% wastewater; T6: 100% tap water (control).

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Comparative evaluation of multicriteria methods versus the traditional approach in the prioritization of public projects in Mexico

Pérez-Ramírez, Rodrigo¹; Martínez-Damián, M.A.^{1*}; Garza-Bueno, L.¹; Mora-Flores, S.¹; Pérez-López, A.¹

¹ Colegio de Postgraduados-Campus Montecillo, Programa de Socioeconomía, Estadística e Informática-Economía, Carr. México - Texcoco km 36.5, Montecillo, Texcoco, Estado de México, México, C.P. 56264.

* Correspondence: angel01@colpos.mx

ABSTRACT

Objective: To compare the performance of the basic method used by Mexican public entities with various advanced multicriteria decision-making (MCDM) methods, aiming to prove that the latter are better suited to evaluate the competitive allocation of public resources.

Design/Methodology/Approach: The performance of six MCDM methods and two variants of the Weighted Sum Method (WSM) were assessed as part of the prioritization of 100 simulated projects, replicating the 2017 model of the Programa de Apoyo para Productores en Zonas de Atención Prioritaria (FAPPA). The analysis, conducted using R, included a linearity validation of rankings to measure the variability in ranking.

Results: The model currently used in public programs (WSM without normalization) has low discriminative capacity, with 98% ties between proposals. In contrast, methods that incorporate normalization reduced this percentage to 41%, with variations depending on the algorithm used. In contrast, the model currently used in public programs (WSM without normalization) exhibits low discriminative capacity, with 98% ties between proposals. In contrast, methods incorporating normalization reduced this percentage to 41%, with variations depending on the algorithm used. Evaluation based on Distance from Average Solution (EDAS) and Complex Proportional Assessment (COPRAS) recorded the rankings with the highest concordance ($R^2=0.92$), whereas Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) and WSM recorded the lowest results ($R^2=0.23$). Clear differences in stability, discrimination, and complexity were recorded. Multi-Objective Optimization on the basis of Ratio Analysis (MOORA) stood out based on its balance between accuracy and simplicity.

Study Limitations/Implications: The study is limited to 100 FAPPA proposals, which may limit the generalisation of the results.

Findings/Conclusions: The comparison of multicriteria methods for public allocation emphasizes normalization, while the algorithmic structure influences proposal discrimination. Well-evaluated projects were consistently identified, reinforcing the validity of the model. Method selection should balance accuracy and feasibility to enhance transparency and equity.

Keywords: Public resource allocation, normalization, evaluation of large number of alternatives, multicriteria analysis.

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INTRODUCTION

Efficient resource allocation represents a structural challenge for grant-allocation agencies, especially when demand exceeds budget availability. This difficulty becomes more noticeable as the number of requests increases, demanding strict, transparent, and multi-criteria evaluation processes (Torres *et al.*, 2024).

In Mexico, public programs such as Desarrollo Forestal Sustentable para el Bienestar (CONAFOR), Apoyo a la Infraestructura Hidroagrícola (CONAGUA), and elements of the Programa de Fomento a la Agricultura, Ganadería, Pesca y Acuicultura (SADER) operate

under competitive grant schemes, in which resources are allocated to the top-evaluated projects according to the criteria established in the operating rules of each program.

The most used methods in these processes are checklists and the Weighted Sum Model (WSM), also known as Simple Additive Weighting (SAW). On the one hand, checklists help to identify the presence or absence of criteria through binary assessments or simple scales, without considering their relative weight (Concha *et al.*, 2020). The WSM, on the other hand, assigns a value to each alternative as the sum of weighted products, assuming that all criteria can be subjected to a linear aggregation.

While these methods are useful in simple contexts, they have limitations when they deal with criteria that have different natures, scales, or units—for example, public programs that take social, technical, economic, and environmental dimensions into consideration. In such cases, data normalization is necessary to guarantee that variables can be compared. The lack of data normalization—a common situation in this type of evaluation—can both distort the results by favoring criteria with broader ranges (Kolios *et al.*, 2016; Ciptayani and Dewi, 2018; Soria *et al.*, 2023) and diminish the accuracy of the evaluation (Altamirano and Lucero, 2023). Ochoa *et al.* (2019) and Pérez and Martínez (2019) agree that the basic methods currently used by the Mexican government fail to capture the complexity of the problems or adequately prioritize a multitude of proposals. In contrast, specialized literature has documented that the effectiveness of advanced Multicriteria Decision Methods (MCDM) enables the integration of multiple dimensions and the comparison of heterogeneous alternatives (Álvarez *et al.*, 2023). These methods include techniques such as the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) (Hwang and Yoon, 1981), Weighted Aggregated Sum Product Assessment (WASPAS) (Zavadskas *et al.*, 2012), Complex Proportional Assessment (COPRAS) (Zavadskas, Kaklauskas and Šarka, 1994), Evaluation based on Distance from Average Solution (EDAS) (Keshavarz Ghorabae *et al.*, 2015), and Multi-Objective Optimization on the basis of Ratio Analysis (MOORA) (Brauers and Zavadskas, 2006). These techniques have been widely validated in international contexts to address alternative selection problems (Ayan and Abacıoğlu, 2022).

In this context, this study aims to compare the performance of the basic method used by Mexican public entities with various advanced MCDMs, seeking to show that the latter achieve a better evaluation of problems involving the competitive allocation of public resources. The hypothesis is that traditional methods have significant limitations when ranking projects in contexts with multiple criteria and large volumes of proposals, while MCDMs overcome these shortcomings by providing more accurate results, tailored to the complexity of the problem.

To this end, a case study for the year 2017 was developed based on the evaluation model of the Programa de Apoyo para Productores en Zonas de Atención Prioritaria (FAPPA). On the said year, the federal government allocated MXN\$536.63 million to finance 3,682 productive projects out of a total of 19,471 proposals received, which implies an acceptance rate of 18.91% (SAGARPA, 2018). This scenario offers an ideal context to compare the performance of the traditional method with the advanced MCDM performances under real-life conditions (*i.e.*, high competitiveness and multiple criteria).

MATERIALS AND METHODS

A non-experimental, quantitative, and comparative design was used to evaluate the performance of the various prioritization methods applied to the competitive allocation of public resources. The evaluation framework used by the FAPPA during the fiscal year 2017 was replicated, as established in its operating rules. This model, based on the WSM without data normalization, was compared with five advanced MCDMs (TOPSIS, WASPAS, COPRAS, EDAS, and MOORA), in addition to WSM with data normalization.

To assess their performance in scenarios with many alternatives, 100 hypothetical project profiles—designed to simulate real-life conditions of competition for funding—were analyzed. The algorithms were implemented and processed in the R software (version 4.1.2). As part of the validation process, pairs of methods were subjected to a comparative analysis of linearity to assess how each alternative position varies in the different rankings generated. This analysis quantifies the direct relationship between the ranking assigned by each pair of methods, where a linearity value equal to 1 indicates that both methodologies place each alternative in the same position, consequently enabling an accurate identification of the variability in the ranking of each proposal between different methods.

Case Study Description

The first stage to select the best alternative is to define the criteria and weights that reflect their relative importance. Table 1 shows the criteria and weights established in the 2017 FAPPA operating rules. Meanwhile, Table 2 includes the evaluation of the 100 investment proposals to which the said rules were applied. Finally, Figure 1 provides a step-by-step description of the application of each of the MCDMs used in this study.

RESULTS AND DISCUSSION

Table 3 details the scores and rankings obtained after applying six MCDMs to a set of 100 investment proposals, while Figures 2 through 8 show the performance of each method in the assignment and ranking of the scores. The results reveal substantial differences in the discriminatory capacity of the different methods. In particular, the model adopted by the FAPPA program—based on the WSM method without data normalization—displays a critical limitation: 98% of the proposals receive identical scores (Figure 2), preventing an effective ranking and jeopardize transparency, and equity in the allocation of public resources.

In contrast, the MCDM methods applied with normalization processes recorded a considerable improvement in the discrimination of alternatives. On average, the proportion of ties was reduced to 41%—a significant improvement over the original scheme. Data normalization both improve comparability between criteria expressed on different scales and reduces the weight of variables with high variance, resulting in more equitable and accurate evaluations, which are consistent with specialized literature (Kolios *et al.*, 2016; Altamirano and Lucero, 2023).

However, the improvement achieved through normalization was not uniform across all methods. For example, in the case of WASPAS (Figure 6), rating percentage matches

Table 1. Criteria and weights established in the 2017 FAPPA operating rules.

Criterion and Weight	Indicator	Global Weight	Unit of Measurement	Response Levels	Score
Social (30%)	Inclusion	0.2	Number of members directly benefiting from the Investment Project	More than 35	100
				From 21 to 30	50
				From 11 to 20	25
				Fewer than 10	0
	Expected Employment	0.1	Number of direct jobs expected	More than 13	100
				From 9 to 12	75
				From 4 to 8	50
				From 1 to 3	25
			None or not specified	0	
Technical (35%)	Technical Assistance and Training	0.05	The Technical Assistance Programme includes specific training activities for members in productive terms	Yes	100
				No	0
	Innovation	0.1	The Project considers the use of new technologies to improve the production process	Yes	100
				No	50
	Expected Percentage Increase in Production Volume	32	Percentage	More than 10	100
				From 6 to 10	75
From 4 to 5				50	
From 1 to 3				25	
			Less than 1 or not specified	0	
Economic (25%)	Internal Rate of Return (IRR) with subsidy	0.05	Percentage	Greater than 30	0
				From 21 to 30	50
				From 10 to 20	100
				Less than 10 or not specified	0
	Marketing	0.2	Scheme	National/International	100
				State level	75
				Regional	50
				Local	25
Environmental (10%)	Sustainability	0.1	Number of actions considered to mitigate the environmental impact of the project	More than 2	100
				2	50
				1	25
				Not specified	0

Table 2. Evaluation of 100 investment projects from the FAPPA program (2018).

Project	C1	C2	C3	C4	C5	C6	C7	C8	Project	C1	C2	C3	C4	C5	C6	C7	C8
1	25	100	100	50	25	0	50	100	51	0	50	100	50	50	0	25	50
2	0	50	100	100	75	0	75	50	52	50	25	100	50	50	50	75	0
3	0	50	100	100	100	0	25	100	53	50	25	100	50	50	0	25	50
4	0	75	100	100	75	0	75	50	54	25	50	100	50	25	0	25	50
5	0	50	0	50	25	50	25	100	55	50	50	100	50	50	0	25	50
6	0	75	100	50	75	0	75	50	56	50	25	100	50	50	0	25	50
7	25	100	100	100	75	100	50	50	57	50	25	100	50	50	100	25	50
8	25	100	100	50	50	50	50	100	58	25	50	100	50	0	0	25	0
9	25	75	100	50	0	0	25	50	59	25	25	100	50	25	0	25	50
10	0	50	100	50	0	0	25	50	60	25	25	100	50	50	0	25	50
11	50	75	100	50	25	0	25	50	61	50	25	100	50	50	0	25	25
12	100	100	0	50	25	100	25	25	62	50	50	100	50	50	50	25	50
13	25	75	100	50	50	0	25	50	63	0	50	100	50	25	0	25	100
14	50	75	100	50	25	0	75	50	64	0	50	0	50	25	0	25	50
15	100	100	100	50	25	100	25	100	65	0	50	100	50	25	0	25	50
16	50	75	100	50	25	0	25	50	66	50	25	100	50	25	50	25	0
17	0	50	100	50	50	0	25	0	67	50	25	100	50	25	0	25	50
18	50	75	100	50	25	100	50	50	68	50	25	100	50	25	0	25	100
19	25	75	100	50	25	0	50	50	69	0	50	100	50	25	100	50	50
20	25	75	0	50	25	0	50	50	70	0	50	100	50	25	0	50	25
21	100	100	100	100	25	0	25	25	71	50	25	100	50	25	0	25	50
22	50	75	100	100	75	0	25	50	72	50	25	0	50	50	0	25	50
23	0	50	100	50	25	0	25	50	73	0	50	100	50	25	100	25	50
24	0	50	100	50	25	0	75	100	74	25	25	100	100	25	0	25	50
25	25	75	100	50	25	0	25	50	75	50	25	100	50	25	0	25	25
26	25	75	100	50	25	0	25	25	76	0	50	100	50	25	0	25	50
27	50	75	100	50	75	0	25	50	77	0	50	100	50	25	0	25	50
28	25	75	100	50	25	0	25	50	78	0	50	100	50	50	0	25	50
29	0	50	100	50	25	0	75	50	79	0	50	100	50	25	50	50	50
30	50	75	100	50	25	50	25	0	80	0	50	100	50	25	0	25	50
31	0	50	100	50	25	50	25	50	81	0	50	100	50	50	0	25	0
32	25	75	100	50	50	0	50	50	82	50	25	100	50	50	0	25	50
33	0	50	100	50	50	0	25	50	83	0	50	100	50	25	0	50	50
34	50	75	100	50	75	0	25	50	84	50	25	100	100	25	50	25	50
35	50	75	100	50	50	100	25	50	85	0	50	100	50	25	0	25	50
36	25	50	100	100	50	0	25	50	86	25	50	100	50	25	0	25	50
37	25	25	100	100	25	0	25	50	87	50	50	100	50	50	0	25	50
38	50	75	100	100	50	50	75	100	88	50	75	100	50	25	100	25	50
39	25	75	100	50	75	0	25	100	89	0	50	100	50	25	0	25	25
40	25	25	100	50	50	0	25	50	90	50	75	100	50	25	0	25	25
41	50	50	100	50	0	0	50	0	91	0	50	100	50	50	0	25	50

Table 2. Continues....

Project	C1	C2	C3	C4	C5	C6	C7	C8	Project	C1	C2	C3	C4	C5	C6	C7	C8
42	50	75	100	50	25	0	25	50	92	0	50	0	50	75	50	25	50
43	0	50	100	50	50	0	25	50	93	0	50	100	100	25	0	25	100
44	0	50	100	50	50	0	75	50	94	50	75	100	50	25	0	25	50
45	50		100	50	50	100	25	50	95	0	50	100	50	50	50	50	50
46	0	50	100	50	50	0	25	50	96	0	50	100	50	25	0	50	100
47	0	50	100	50	0	0	25	50	97	0	50	100	50	25	0	25	50
48	0	50	100	50	50	0	50	50	98	50	75	100	50	75	100	25	0
49	25	25	100	50	50	0	25	50	99	25	75	100	50	25	0	25	50
50	25	50	100	50	50	0	25	50	100	50	75	100	100	25	0	25	50

Where: C1=Inclusion, C2=Expected Employment, C3=Technical Assistance and Training, C4=Innovation, C5=Expected Percentage Increase in Production Volume, C6=Internal Rate of Return (IRR) with subsidy, C7=Marketing, C8=Sustainability.

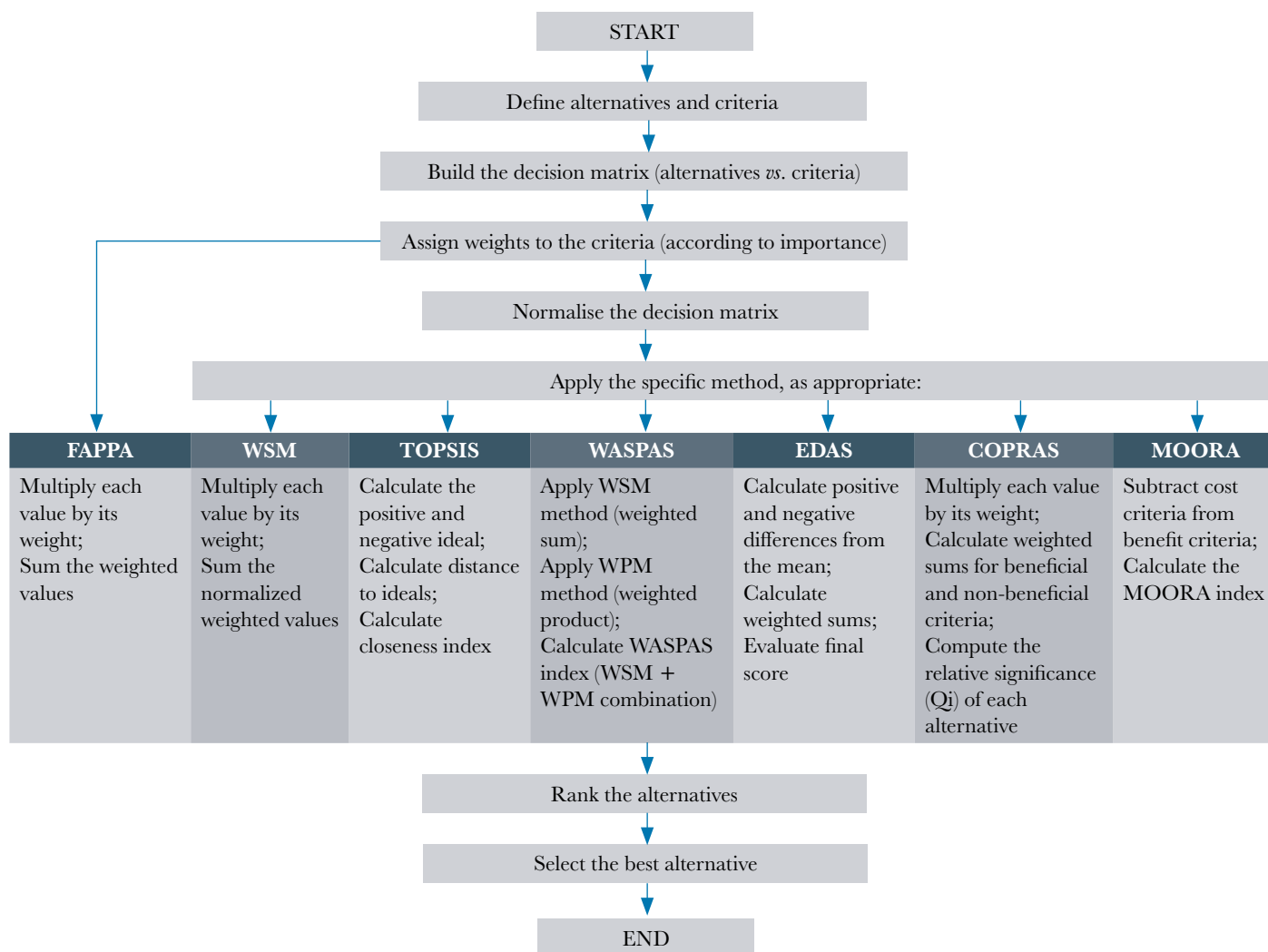


Figure 1. General process for multicriteria evaluation using multicriteria methods.

Table 3. Ranking of the top 20 alternatives according to the method applied.

FAPPA		SWM		TOPSIS		EDAS		MOORA		WASPAS		COPRAS	
Project	Ranking	Project	Ranking	Project	Ranking	Project	Ranking	Project	Ranking	Project	Ranking	Project	Ranking
38	1	38	1	15	1	38	1	38	1	38	1	15	1
7	2	7	2	38	2	15	2	15	2	7	2	38	2
15	2	4	3	12	3	7	3	7	3	15	3	7	3
4	4	2	4	21	4	12	4	21	4	8	4	12	4
8	4	15	5	22	5	18	5	12	5	18	5	21	5
21	4	22	6	98	6	35	6	22	6	35	6	98	6
22	4	8	7	27	7	8	7	4	7	62	7	18	7
2	8	3	8	34	7	98	8	8	8	57	8	35	8
3	8	6	9	52	9	21	9	52	9	88	9	52	9
6	10	21	10	7	10	22	10	98	10	84	10	8	10
12	10	14	11	14	11	52	11	2	11	4	11	22	11
14	10	39	12	35	12	57	12	14	12	2	12	57	12
18	10	27	13	57	13	14	13	18	13	21	13	14	13
27	10	34	13	39	14	88	14	35	14	22	13	88	14
34	10	52	15	3	15	62	15	27	15	6	15	45	15
35	10	18	16	4	16	27	16	34	15	14	15	4	16
39	10	1	17	62	17	34	16	6	17	3	17	27	17
98	10	98	18	18	18	45	18	3	18	52	18	34	17
1	19	35	19	45	19	39	19	39	19	12	19	62	19
52	19	32	20	6	20	4	20	57	20	27	19	2	20

Where: FAPPA: Programa de Apoyo para Productores en Zonas de Atención Prioritaria, SWM: Weighted Sum Method, TOPSIS: Technique for Order of Preference by Similarity to Ideal Solution, EDAS: Evaluation based on Distance from Average Solution, MOORA: Multi-Objective Optimization on the basis of Ratio Analysis, WASPAS: Weighted Aggregated Sum Product Assessment, COPRAS: Complex Proportional Assessment.

remained high (72%). This result indicates that, despite its usefulness, normalization on its own is not sufficient to ensure an effective differentiation between proposals. This situation suggests that the algorithmic structure of the method also significantly influences its discriminatory capacity, as pointed out by Zavadskas *et al.* (2016), who underscored that the sensitivity of MCDMs depends both on the data transformation and on the way in which the partial evaluations are weighted and aggregated.

Although TOPSIS, EDAS, MOORA, WSM (with normalization), and COPRAS reduced the number of proposals with identical ratings to 41%, it is still considerably high, considering the magnitude of the evaluated set. This persistence can be partially attributed to the structural constraints of the FAPPA scoring system, which classifies all criteria as benefits and uses a 5-level ordinal scale (0, 25, 50, 75, and 100 points). This design restricts the specificity of the analysis and favors duplicate values, especially among proposals with similar performance.

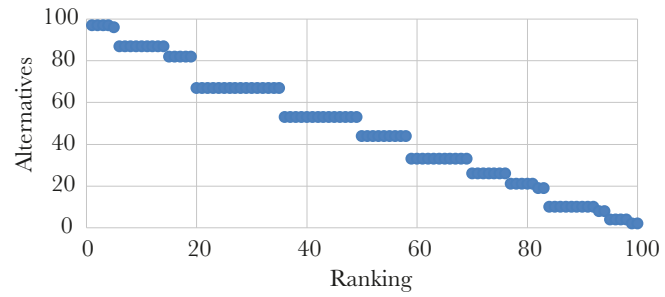


Figure 2. Distribution of the project ranking under the FAPPA model.

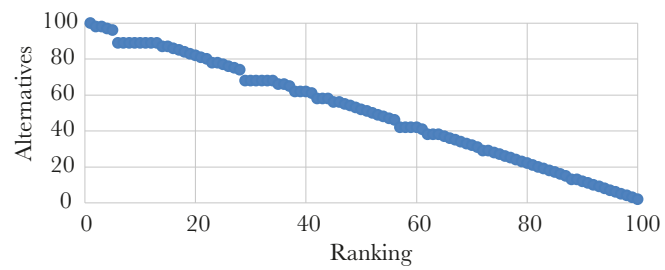


Figure 3. Distribution of the project ranking under the WSM model.

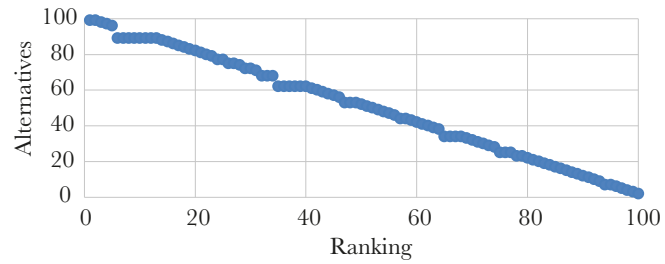


Figure 4. Distribution of the project ranking under the TOPSIS model.

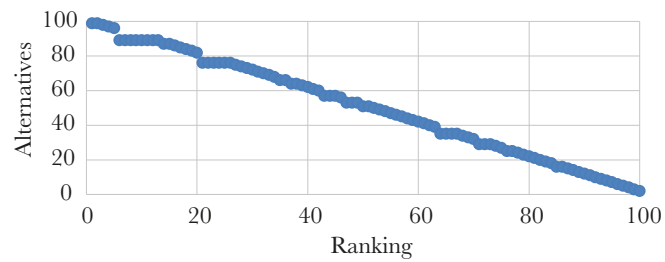


Figure 5. Distribution of the project ranking under the EDAS model.

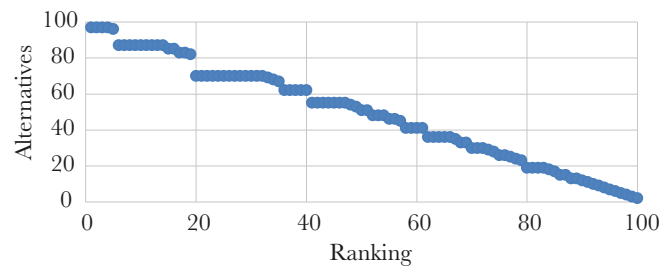


Figure 6. Distribution of the project ranking under the WASPAS model.

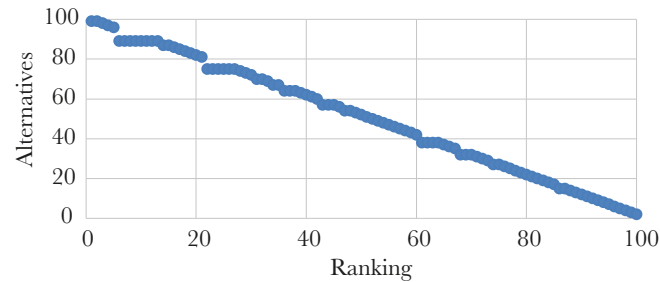


Figure 7. Distribution of the project ranking under the COPRAS model.

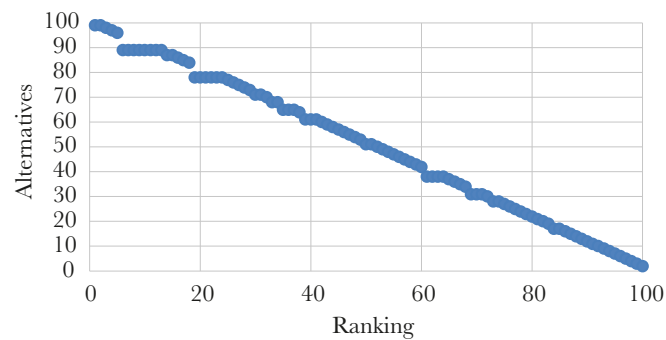


Figure 8. Distribution of the project ranking under the MOORA model.

Regarding the ranking generated by each method, the best alternatives placement was highly consistent. Projects 38, 15, and 7 received the top positions in most of the methods evaluated, indicating that their overall assessment was stable. However, slight discrepancies were identified in the third position: project 12 and project 4 held that position in the case of TOPSIS and WSM, respectively. These variations reflect underlying methodological differences: while TOPSIS is based on the relative distance to ideal and nonideal solutions, WSM uses a weighted linear aggregation, which responds differently to small changes in individual criteria.

Figure 9 compares the rankings assigned to each project per pairs of methods analyzed. The X and Y axis show the positions generated by the first and the second method, respectively. Each figure includes the R^2 coefficient of determination, which quantifies the linearity degree between both arrangements, providing a measure of relative consistency in the ranking of alternatives.

EDAS and COPRAS had the greatest correspondence; its coefficient of determination ($R^2=0.9245$) proved that there is a highly consistent ranking between them. This coincidence can be explained by the similarity in their structural approaches, both of which are centered on proportional evaluation relative to average or collective references and the transformation of normalized data that prioritize relative stability between alternatives.

For their part, TOPSIS and WSM (with normalization) recorded the lowest correspondence ($R^2=0.2301$). This low linearity reveals a substantial difference between the rankings generated by these methods, especially for the mid-performing alternatives.

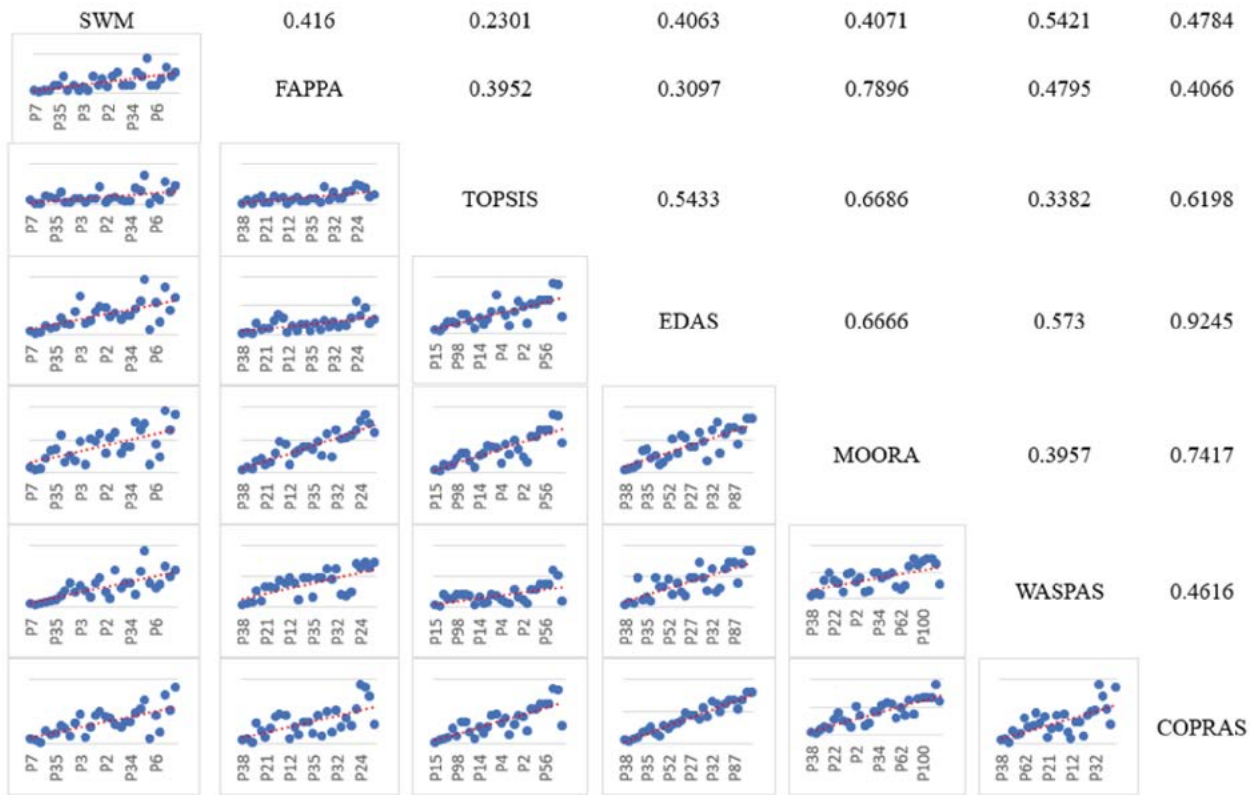


Figure 9. Analysis of the coefficient of determination (R^2) of the linear correlation between rankings generated by paired multicriteria methods.

These differences reflect how the aggregation logic —based on distances and direct weighting in the case of TOPSIS and SWM, respectively— can alter the relative position of these proposals, reinforcing the need to carefully select the evaluation method based on the application context.

The MOORA method showed intermediate variability and was consistent with several methods, including SWM, TOPSIS, EDAS, and COPRAS. Therefore, it is positioned as a strong methodological option for scenarios requiring a balance between operational simplicity and stability of results. As a whole, these findings underscore the value of linearity analysis as an internal validation tool, allowing for the assessment of the positional consistency of the alternatives, beyond the overall correlation offered by Spearman, Kendall, and other indicators (Martin, 2017).

Finally, from an operational implementation point of view, significant differences were identified in the computational complexity of the methods. The simple algorithmic structure and low technical demands of SWM, WASPAS, MOORA, and COPRAS makes them suitable for institutional contexts with limited resources. In contrast, given the need to calculate relative distances or deviations from ideal or average values, EDAS and TOPSIS require more intensive processing, which could represent a barrier in environments with limited analytical capabilities (Mayor, Botero, and González, 2016).

CONCLUSIONS

This study compared six multi-criteria methods with the traditional FAPPA program which is usually used to allocate public resources. The results show that MCDMs substantially increase distinction ability between proposals, reducing on average the percentage of projects with identical ranking from 98% to 41%. This improvement is largely attributed to the application of normalization processes, which contributes to the comparability between criteria measured on different scales. However, the improvement was not homogeneous: In the case of WASPAS, the matching results remained high (72%), which proved that the algorithmic structure of each method decisively influences its discriminatory capacity.

The linearity analysis showed high consistency between methods such as EDAS and COPRAS ($R^2=0.9245$) and low correspondence between TOPSIS and WSM ($R^2=0.2301$). These results confirm that the methodological choice can significantly alter the ranking of proposals. Furthermore, consistently well-evaluated projects (38, 15, and 7) were identified, providing cross-validation to the models.

In conclusion, the selection of the evaluation method should consider both the technical differentiation capacity and its operational feasibility in public allocation schemes. This work provides evidence that strengthens the transparency and equity in the distribution of public resources, through the adoption of MCDM methods with solid methodological foundations and adapted to the institutional context.

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Evaluation of two sources of choline on reproductive variables in primiparous ewes

Martínez-Cruz, Israel¹; Sánchez-Torres Esqueda, María T.^{1*}; Martínez-Aispuro, José A.¹; Cordero-Mora, José L.¹; Figueroa-Velasco, José L.¹; Nieto-Aquino, Rafael²; Ayala-Monter, Marco A.³; Cárdenas-León, Mario⁴

¹ Colegio de Postgraduados, Campus Montecillo, Programa de Ganadería. Texcoco Estado de México, México. C. P. 56264.

² Facultad de agronomía y Veterinaria. Universidad Autónoma de San Luis Potosí, México. Carretera San Luis-Matehuala Km 14.5 Ejido Palma de la Cruz, 78321

³ Universidad Autónoma de Guerrero-Facultad de Medicina Veterinaria y Zootecnia. C. P. 39086.

⁴ Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Laboratorio de Biología de la Reproducción, C. P. 14080.

* Correspondence: teresa@colpos.mx

ABSTRACT

Objective: to evaluate the reproductive response of primiparous ewes supplemented with herbal choline and synthetic choline during an estrus synchronization protocol.

Design/Methodology/Approach: ninety-two 15-month-old primiparous ewes were used, with initial body weight of 50 ± 1.25 kg. The ewes were distributed randomly in three (T) treatments: T1 (basic diet without choline), T2 (basic diet + 4 g of herbal choline), and T3 (basic diet + 4 g of synthetic choline).

Results: there were no differences among treatments for presentation and onset of estrus, the pregnancy rate, prolificacy, type of delivery (lambing), and insulin concentration ($p > 0.05$). But the concentration of progesterone did decrease ($p \leq 0.05$) before estrus with the addition of herbal choline to the diet.

Limitations/Implications of the study: the dietary addition of herbal or synthetic choline (4 g d^{-1}) did not improve reproductive variables, because the level of supplementation was low. Or else, the animals synthesized or consumed a sufficient amount of choline for their metabolic functions.

Findings/Conclusions: the inclusion of herbal or synthetic choline during synchronized estrus in primiparous ewes did not alter the reproductive variables, while progesterone concentrations were modified due to herbal choline supplementation.

Keywords: herbal, synthetic, progesterone, reproduction.

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INTRODUCTION

Choline is considered a conditionally essential vitamin in sheep, which is involved in the synthesis of proteins, phospholipids, acetylcholine, bone growth, membrane integrity, signaling functions, and development of the nervous system of the fetus. It is also an essential factor in the metabolism of fat in the liver and the regulation of methylation processes (NRC, 2007).



Because dietary choline is extensively degraded in the rumen and a limited amount passes through to the gut (Jayaprakash *et al.*, 2016), supplementation with choline protected from rumen degradation reduces deficiency, and improves productive behavior, health, and reproductive aspects in sheep (Li *et al.*, 2015; Tsiplakou *et al.*, 2016; Rodríguez-Guerrero *et al.*, 2018; Suárez-Suárez *et al.*, 2023).

Synthetic (CS) and herbal (CH) choline sources are available on the market; however, they are not regularly included in sheep feed. Choline chloride is the synthetic source commonly used in animal diets; however, under poor storage conditions, high hygroscopicity can accelerate oxidation of food vitamins and premixes (Tavcar-Kalcher and Vengust, 2007), low bioavailability (40-80%), and toxicity of secondary metabolic products (Jayaprakash *et al.*, 2016).

In addition, recent animal production restricts the use of synthetic compounds, which is why it is sought to incorporate alternative natural dietary supplements (from plants) into ruminant diets that are relatively non-toxic and environmentally friendly. Therefore, CS can be replaced by food additives from plants containing phosphatidylcholine, which show natural resistance to rumen degradation (Gutiérrez *et al.*, 2019). The objective of this research was to compare the effect of the addition of herbal or synthetic choline on the reproductive variables of primiparous ewes during an estrus synchronization protocol.

MATERIALS AND METHODS

The research was established at the Experimental Farm of the Colegio de Postgraduados, Texcoco, State of Mexico (19° 27' 18" N and 98°5 4' 26" W), at an altitude of 2220 m; the climate is temperate subhumid with rainfall in summer. The sheep were managed in accordance with the Official Mexican Standard NOM-062-ZOO-1999 for the use and care of animals intended for research.

Ninety-two 15-month-old primiparous sheep (Dorset×Kathadin), with an average initial live weight of 50 ± 1.25 kg and a body condition of 3 on a scale of 1 to 5 were used in the experiment. Zootechnical management before the start of the experiment consisted of deworming, vitaminizing (A, D and E), bacterin application (BOBACT[®] 8 ways), shearing and hoof-trimming.

The ewes were subjected to an estrus synchronization protocol (Figure 1) by applying synthetic hormones to submit them to a reproductive program during the months of October to December (reproductive season). The ewes were fed a base diet consisting of oat hay and dehydrated alfalfa on free-access, supplemented with 250 g of concentrate per animal per day; which was added with choline during the synchronization and mating period (36 days: 15 days before mating, and 21 days after mating).

The treatments (T) consisted of supplementing different types and dietary concentrations of choline incorporated into the concentrate. Base diet (forage+concentrate) without the addition of choline (T1, n=30); Base diet+4 g of herbal choline (T2; n=31); Base diet+4 g synthetic choline (T3; n=31). The source of herbal choline was the commercial product BioCholine[®] (Indian Herbs and Technofeed, Mexico) and the synthetic source was 50% choline chloride (Orffa Elovitals, Belgium). The composition of the concentrate (g kg^{-1}

DM) was as follows: corn (806.6), soybean paste (131.5), common salt (2), molasses (50), and mineral premix (10).

Regarding reproductive management (Figure 1), estrus presynchronization was performed by two applications of 125 μg of prostaglandin F 2α (cloprostenol[®]), on days -8 and 0 of the protocol. Six days after the second application of PGF 2α , an intravaginal sponge impregnated with 20 mg of progesterone (sponges with crotonone acetate) was inserted for 11 days.

The females received three mating events; the first at the beginning of the estrus, and two subsequent at intervals of 12 h. Return to estrus was detected in two periods (morning and afternoon) between days 14 and 17 after synchronized estrus and mating. The diagnosis of pregnancy was made 31 days after the last mating event (SONOVET 600[®] ultrasound with 7.5 Mhz transrectal linear transducer). The evaluation timeline is shown from the start of the estrus synchronization protocol (Figure 1).

Blood samples (5 mL) were collected from the jugular vein prior to placement of the sponge, and thereafter every 48 h, to determine the concentration of progesterone during the estrus synchronization period. Insulin concentrations (INS) were monitored in fasting conditions, on days 1 and 36 of the choline addition period. All samples were centrifuged for 20 min at 2500 $\times g$ to separate the blood serum, which was stored at $-20\text{ }^{\circ}\text{C}$ until hormonal analysis. To determine P $_4$ concentrations, a radioimmunoassay (RIA) was performed with a commercial kit PROGEST-CTRIA[®] (CIS-BIO INTERNATIONAL, France) with intra- and inter-assay coefficients of variation (CV) of 4.1 and 8.7, respectively, and sensitivity of 0.05 ng mL^{-1} . Plasma insulin analysis was also performed by radioimmunoassay (RIA) with a sensitivity of 4.09 ng mL^{-1} and intra- and inter-assay coefficients of variation of 3.2 and 4.6%.

The experimental design was completely randomized, each ewe was considered as an experimental unit. The presentation of estrus, percentage of gestation, prolificacy index and type of delivery (lambing) were analyzed with an X 2 test with the PROC FREQ procedure. The analysis of variance of estrus onset and insulin concentration was executed

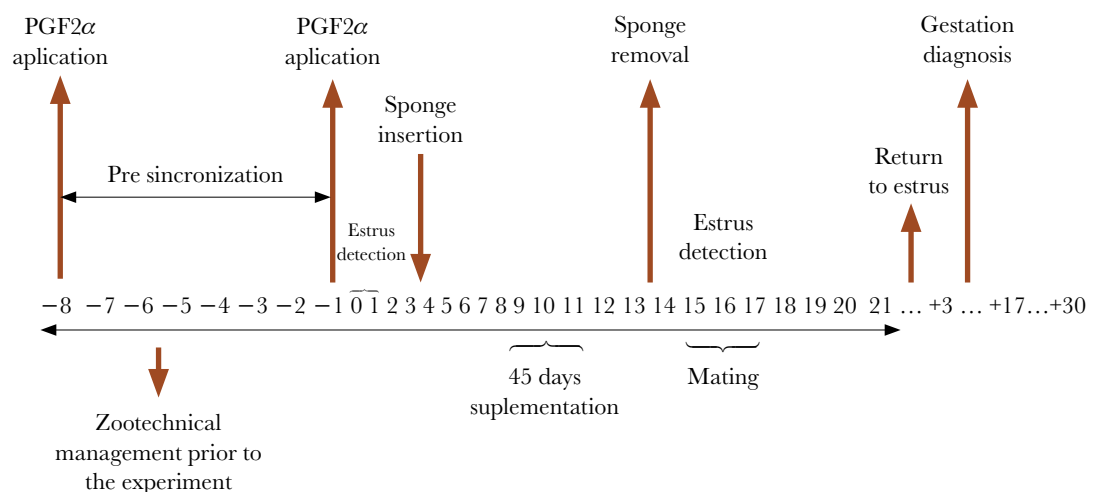


Figure 1. Experimental protocol in female sheep fed with herbal choline and synthetic choline.

with the PROC GLM procedure ($p \leq 0.05$). For the concentration of progesterone, the analysis of variance of repeated measurements over time was performed using the PROC MIXED procedure, which included treatment and day as fixed effects, as well as their interaction. For this procedure, the covariance structure was modeled with the effect of the ewes within the group. The comparison of means was performed with Tukey's test ($p \leq 0.05$). All variables were analyzed in SAS[®] 5.0 for Windows (SAS Institute, Inc., NC, USA).

RESULTS AND DISCUSSION

The presentation of estrus, estrus onset, and percentage of gestation (Table 1) were not influenced ($p > 0.05$) by the dietary addition of herbal or synthetic choline. Ardalan *et al.* (2009) noted that supplementing 60 g d^{-1} choline in cows in the first third of lactation period does not improve the percentage of pregnant cows. However, in Zaraibi goats, there is evidence that supplementation with CS ($10, 20$ and 40 g d^{-1}) increased the pregnancy rate (90-100%) compared to the control group (80%) (Habeeb *et al.*, 2018).

Choline supplementation to cows in the transition stage (before and after calving) positively affects the appearance of first post-delivery heat, open days, the number of services, and the pregnancy rate (Acharya *et al.*, 2019; Gutiérrez *et al.*, 2019; Mecionyte *et al.*, 2022). During the transition stage (dry period-early lactation), dairy cows often experience a negative energy balance, leading to mobilization of energy reserves, decreased dry matter intake, higher levels of non-esterified fatty acids (NEFAs), and consequent lower milk production. This predisposes early lactating cows to metabolic diseases, such as fatty liver syndrome and ketosis; in addition to reproductive problems.

Rumen-protected choline supplementation is a strategy to restrict the negative effects associated with negative energy balance in early lactating cows. What results in increased follicular development and fertility, as choline deficiency causes a decrease in the production of FSH and LH hormones. However, in research conducted where herbal or synthetic choline are evaluated, there seems to be no consistency in the benefits to the reproductive response (Humer *et al.*, 2019).

In the study conducted by Acosta *et al.* (2016) although CS supplementation to cows during the transition period did not affect the days to first ovulation; choline supplementation reduced the expression of mRNA factors (TNF, TLR4 and IL1- β) in follicular cells, which are associated with increased infertility. The difference in the response, this is the improvement of reproductive variables, between those dairy cows in the transition stage, and breeding sheep may be due to the fact that the sheep used our study presented a good body condition throughout the evaluation.

Dietary choline supplementation to dairy cows had shown lower incidence of metritis, endometritis, pyometra, placental retention (Furken & Hoedemaker, 2014), and reduced the presence of metritis-associated pathogens (Marques *et al.*, 2023).

Prolificacy. The addition of herbal and synthetic choline did not influence ($p > 0.05$) the prolificacy index and the type of lambing (single and double), which contrasts with what was found by Habeeb *et al.* (2018) who observed that adding CS (20 and 40 g d^{-1}) to the diet of Zaraibi goats increased the number of double and triple kiddings. Also, studies

in cows indicated that the group added with CS had a higher incidence of twin births compared to the control group (Guretzky *et al.*, 2006).

Insulin concentrations. The addition of choline (herbal and synthetic) during estrus synchronization and days after mating did not modify the concentration ($p>0.05$) of insulin in sheep blood serum (Table 1). Because choline supplementation to fattening lambs increased glucose concentration (Crosby *et al.*, 2017), it was inferred that there could potentially be an increase in insulin, however, this did not happen. In another study, dietary supplementation of CS (18.8% choline) with 50 or 100 g d⁻¹ per animal in multiparous Holstein cows in the transition stage resulted in a higher insulin concentration compared to the control treatment (Leiva *et al.*, 2015).

Progesterone (P₄; Figure 2). The P₄ levels of sheep that were supplemented with herbal choline decreased ($p\leq 0.05$) during the days that the exogenous source of P₄ (intravaginal sponge) was available compared to the control treatment. While ewes supplemented with synthetic choline only tended to reduce the level of P₄, without detecting differences ($p>0.05$) compared to the control treatment or the treatment with herbal choline; except on day 2 before the removal of the intravaginal sponge, where a clear decrease in the concentration of P₄ was observed compared to the control treatment ($p\leq 0.05$). Generally speaking, it can be seen that choline supplementation (herbal or synthetic) tended to reduce the level of P₄ during the time that one external source of P₄ (sponge) was present in the vagina of the ewe.

Results obtained in this research differ from those expected, as there is evidence that CS supplementation in goats increased the concentration of P₄ (Habeeb *et al.*, 2018). Also, in peripartum cows (before and after calving) the first peak of P₄ was increased during days 21-24 postpartum (Mecionyte *et al.*, 2022). Although, in the study conducted by Acosta *et al.* (2016), CS supplementation to cows during the transition period did not affect estradiol and progesterone concentrations.

Since cholesterol is a precursor for P₄ synthesis, and there is evidence that CH supplementation (4 g d⁻¹) to fattening lambs increased cholesterol concentrations (Crosby *et al.*, 2017; Rodríguez-Guerrero *et al.*, 2018; Martínez-Aispuro *et al.*, 2022), we hypothesized that a higher cholesterol concentration would influence P₄ concentration. In spite that,

Table 1. Reproductive response of ewes supplemented with different choline sources.

Reproductive variables	Treatments		
	T1 (n=30)	T2 (n=31)	T3 (n=31)
Estrus onset (%)	93.33 (28/30)	93.55 (29/31)	96.77 (30/31)
Beginning of estrus (h)	43.28±2.57	42.00±2.48	42.07±2.57
Gestation (%)	93.33(28/30)	93.55 (29/31)	87.10 (27/31)
Prolificacy index	1.22 (33/27)	1.24 (36/29)	1.30 (34/26)
Single birth %	73.33 (22/30)	70.97 (22/31)	61.29 (19/31)
Twin birth %	16.67 (5/30)	22.58 (7/31)	19.35 (6/31)
Insulin (ng mL ⁻¹)	0.64±0.08	0.55±0.08	0.58±0.08

T1 (control): base diet + 0 g per ewe; T2 (herbal choline): base diet + 4 g choline per ewe; T3 (synthetic choline chloride): base diet + 4 g choline per ewe.

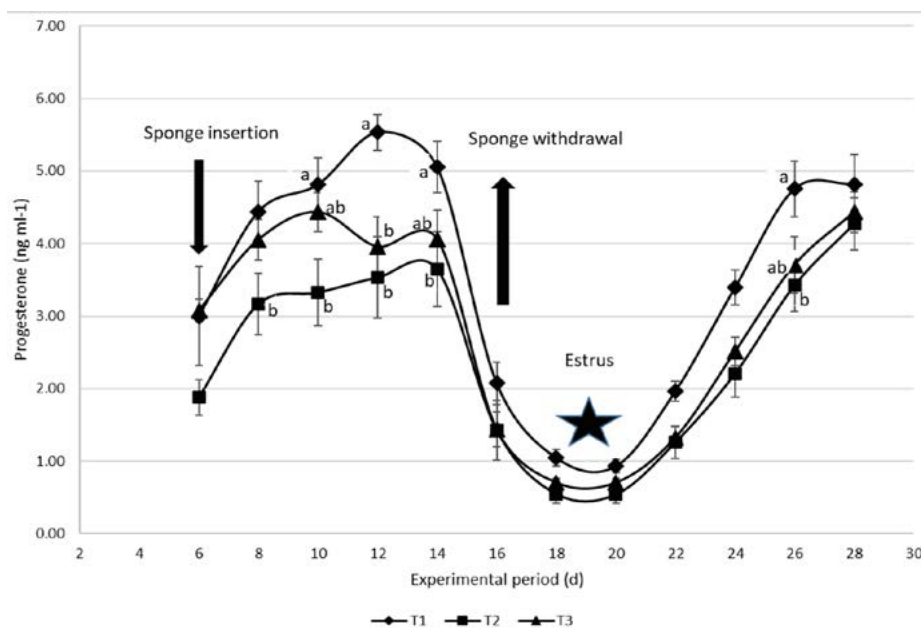


Figure 2. Concentration of progesterone (mean \pm standard error) in choline-supplemented ewes. T1 (choline-free base diet), T2 (base diet + 4 g of herbal choline) and (T3) base diet + 4 g of synthetic choline. a,b values with different literal are statistically different ($p \leq 0.05$).

regarding peripartum dairy cows, there are inconsistencies in the reports, such as choline supplementation can either increase (Soltan *et al.*, 2012) or decrease (Sun *et al.*, 2016) cholesterol concentration.

CONCLUSIONS

The inclusion of herbal or synthetic choline in the diet during synchronized estrus of primiparous sheep did not alter reproductive variables. Meanwhile, progesterone concentrations were modified due to herbal choline supplementation. It is possible that the beneficial effect of dietary choline addition on reproductive variables would be manifested in sheep that were in a negative energy balance.

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Remote monitoring of training and technology-transfer services in vanilla cultivation (*Vanilla planifolia* Jackson ex Andrews)

Mariscal-Aguayo, D. Valentina^{1*}; Estrella-Quintero, Heriberto¹; Salas Barboza, José Enrique²

¹ Universidad Autónoma Chapingo. Centro Regional Universitario Occidente, Rosario Castellanos 2332, Col. Residencial La Cruz. Guadalajara, Jalisco. México. C.P. 44950.

² Asesor independiente. Cenobio Moreno 146, Buenos Aires. Parácuaro, Michoacán. México. C.P. 60786.

* Correspondence: valmara@hotmail.com

ABSTRACT

Objective: to remotely monitor training and technology-transfer services (called “extensionism” in Mexico) using the Chapingo-AGROPEC Star[®] advisory and consultancy model to promote the development of vanilla small-scale agribusiness.

Design/Methodology/Approach: the study was implemented in Puebla and Veracruz, Mexico, with the participation of 81 producers and five advisors. The *ad hoc* designed digital platform AGROPEC Star[®] was used, which consists of assembled software tools for monitoring agribusiness management and advisory process. Advisors (agricultural extensionists) received training in both productive management, and the use of the software. Agricultural extensionists, in turn, provided technical assistance and training to the producers.

Results: data were collected from 40.68 hectares, with yields ranging from 50 to 300 kg ha⁻¹. The activities in technology-transfer included ground layering (51.85%), compost application (43.21%), pollination (38.27%), and weed control (37.04%).

Limitations/Implications of the study: in Mexico, vanilla is traditionally cultivated without technology, on small plots, where all the crop cultivation activities are done by the producers. Although productivity is low, it has potential.

Findings/Conclusions: digital monitoring with the advisory management platform enabled to verify those activities that agricultural extensionists and producers performed. Also, it allowed us the generation of indicators, and the identification of opportunities to improve vanilla crop productivity.

Keywords: advisory technicians, producers, agribusiness, training, technology-transfer services, agricultural extensionists.

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INTRODUCTION

Vanilla is a tropical terrestrial orchid with a climbing habit, which grows in the form of a vine. To produce its fruit, each flower must be individually pollinated. Due to the decline in the bee population that used to perform this task, producers are now responsible for manual pollination to obtain each fruit (a pod), so achieving production. Vanilla is a species that fetches a very high market value, with a current price of approximately 12 000 MXN pesos per kg of processed vanilla (Menchaca, 2018).



It is an aromatic product originating in Mexico and one of those with the highest demand in the soft drink and food industries. Between 2003 and 2016, vanilla production almost doubled, reaching a total of 512 tons (megagrams, Mg) in 2016. This was due to a 71.07% increase in the national average yield during that period. Of the 1059 hectares (ha) planted in 2016, 38.55% was managed mechanically, and 57.82% had technology applied to plant health. Of the 54.23% of the land planted with this crop, the cuttings were planted by producers with technical assistance; and 99% of production was established under rainfed conditions. Potential production for 2024 was estimated at 703 Mg, with an estimated export volume of 151 Mg (SAGARPA, 2017).

The cultivation and production of vanilla in the country have been historically linked to the Totonac indigenous region. For this reason, understanding and analysis of the behavior of this orchid would be incomplete without first establishing a profile of what this indigenous region still is (ASERCA, 2002). To do this, sociocultural and economic characteristics should be noted above all. In this region producers maintain plantations with little or no profitability, and in many cases the explanation lies in the producer's confidence that the price of green vanilla will increase, as occurred in 2004, when the price was \$400 MXN per kg of green pod (Barrera-Rodríguez *et al.*, 2011).

Producers are distinctively characterized because they had small areas dedicated to vanilla cultivation, ranging from a quarter to a half hectare, with low yields of green vanilla. There are four different production systems. In acahual (traditional, on secondary succession wild populations, in disturbed areas), under the shade of pichoco (*Eritrina* sp.), orange tree (*Citrus sinensis* L. Osbeck), or shade cloth (at 50% light). Each of those shows a specific level of technical development, and use of traditional knowledge in crop management (Damirón, 2004).

In 2017, the national record of yield was 0.54 Mg per hectare (Mg ha^{-1}). Whereas it was 0.25 Mg ha^{-1} in the municipality of San José Acateno (Puebla), and 0.6 Mg ha^{-1} in Papantla (Veracruz) (SIAP, 2017). This variability is explained by the cyclical nature of production, which depends on crop renewal and management practices, as well as on physical and climate factors (precipitation, temperature, and humidity in that region). In addition to traditional production systems and some technical aspects such as the type of support used, whether or not there was premature fruit drop, poor nutrition, presence of pests and diseases. Finally, yield is affected by high production costs due to manual pollination (Elorza *et al.*, 2007; Coro, 2009).

A recent approach to production analysis is developed through information and communications technology, which facilitates the exchange of information, the monitoring and comprehensive evaluation of various processes, and the management of training, human resources, and materials, among other important aspects. Together, this represents a valuable tool for the process known as Agricultural extension, that is, the formal and operational procedure for extending knowledge and transferring technology in order to be understandable to farmers and producers (Hernández and Santillán, 2021; Mariscal *et al.*, 2023).

Due to the importance of vanilla cultivation for the Mexican economy and the problems faced by producers in the Totonac region in vanilla management, the objective of the study

was to monitor online the advisory and training process for technicians and producers, through the Chapingo AGROPEC Star[®] advisory and consulting platform, designed *ad hoc* to advise and manage vanilla small-scale agribusinesses.

MATERIALS AND METHODS

Monitoring and analysis were conducted with 81 vanilla producers from the municipalities of San José Acateno (Puebla), Hueytamalco (Puebla), and Papantla (Veracruz) from November 2015 to July 2016. Five agricultural extensionists participated, they were selected based on their professional training, experience in vanilla cultivation, and knowledge of the lands in the study area. These technology advisors received training in the use of the digital platform and in the cultivation techniques for vanilla production.

Online monitoring was implemented through the Chapingo-AGROPEC Star[®] advisory and consulting platform (Mariscal and Estrella, 2008; Estrella and Mariscal, 2020) which consisted of assembled tools of information technology and communication within a specialized digital platform, an original interface developed to provide this systematic services (Agropec Star[®], 2000; Figure 1). The interface records the technical and cost management implemented during the vanilla production process; as well as, the training for agricultural extensionists and producers to provide subsequent monitoring. It also includes geolocation data, in Google Maps format, for the small-scale agribusiness participating.

The agricultural extensionists provided advice and training to producers to improve their capacities (capacity building) to implement technological innovations that would allow improving the productive development of a network in the value chain (Red



Figure 1. The Chapingo-AGROPEC Star[®] digital platform with a specialized interface for online monitoring of the agricultural extension effort in value chains. Select the specific network for vanilla cultivation (*Vanilla planifolia* Jackson ex Andrews) in the “Red Vainilla” option at the Main menu.

Vainilla). Scheduled activities were monitored in the field and online, as well as the diagnosis and performance of the small-scale agribusinesses, according to the production process in question. In this regard, emphasis is placed on the maximum usefulness of online monitoring, as it proved to be targeted, dynamic and cost-effective.

RESULTS AND DISCUSSION

The systematic and updated information records of this project can be consulted at <https://www.agropecstar.com/proyectos/38#> Query type, as a registered user, choose from the main menu, the option for Vanilla network (“Red Vainilla”).

The inclusion of 81 small-scale agribusinesses was achieved, reaching a total of 40.68 ha dedicated to vanilla cultivation (Table 1). The average production area per producer was 0.50 ha, which coincides with the results of Damirón (2004) and Barrera *et al.* (2009), who indicated plot sizes of 0.25 to 0.5 ha for producers in the Totonacapan region, which includes several municipalities in the Mexican states of Puebla and Veracruz.

The geolocation coordinate pairs (UTM) that delimit the edges at the Cardinal directions of the area of influence of the evaluated small-scale agribusiness are (to the North) 20.29617 N, -97.26039 W; (to the South) 20.00720 N, -97.26213 W; (to the East) 20.09670 N, -97.17213 W; and (to the West) 20.16488 N, -97.42673 W. The specific coordinates of each agribusiness can be viewed on the cartography of the digital-monitoring system interface.

Ninety percent of producers are over 60 years old, with a maximum education level of three years in elementary school. Vanilla production is a secondary source of income; they do so solely out of tradition or because they inherited the land with the crop. Yields range from 50 to 300 kg of green pods per hectare. Fifty percent of producers are natives, from the Totonac Indigenous people.

Farmers do manually the flower fertilization for the orchid reproduction, from mid-February to the end of April. Furthermore, in many cases, replanting is not implemented, which is why the plant density per hectare is low. Sanitary control is scarce and sometimes nonexistent. Weed removal is done manually by the producer himself. Pest and disease control is not implemented. Leaf litter and decomposing wood are used as natural fertilizer, and commercial inorganic fertilizers are not applied.

Table 1. Small-scale agribusinesses and surface area (hectares, ha) evaluated by agricultural extensionists in the remote monitoring of Vanilla cultivation.

Extensionist's name	Agribusiness (No.)	Area (ha)
Alfredo Rodríguez Moreno	20	13.93
Eduardo Orlando Sánchez Días	20	10.04
Benjamín Torres Espejel	1	0.25
José Luis Pérez Hernández	20	7.81
Liliana Lucas Brito	20	8.65
Total	81	40.68

Training

Producers received training as workshops in five production disciplines. Marketing; Business Organizational Development; Soil Fertility; Pest and Disease Management; and Crop Management. These workshops were held at 27 events in different towns to facilitate the travel of participants. The workshops were attended not only by the 81 farmers participating in the study, but also by other producers interested in vanilla cultivation. As a result, a total of 313 attendees were gathered (Table 2). This represented almost four-folded (3.86 times) the expected attendance; it indicates the need to implement more training workshops, involving all producers in the region who are dedicated to (or interested in) vanilla production.

These workshops were conducted in response to the problems identified in the diagnostic assessment. Emphasis was placed on capacity building among producers and the need to adopt the proposed technology to improve production processes (Figure 2). Current production systems are characterized by a low level of technological development. Overall, this is due to the use of traditional crop management, where vanilla cultivars are established in wild plant populations of secondary succession called “acahuales.” In these populations, planting densities are estimated to be low, as it is the area covered. Host plants (phorophytes) vary and are selected based on the tree species already present on the site, in particular farmers use non-introduced trees (*i.e.*, native species).

In general, there is no pest and disease control program, and when it is implemented, it is not done at the right time. Because of this, the incidence of diseased vanilla plants is high, so that in many cases only 10% of the plants produce flowers. Production does not occur until the fourth year and not in the third as it should. Furthermore, productivity is low and the plants are not very vigorous; most of the time, a plant produces only once and tends to die, which coincides with what was reported by ASERCA (2002), Barrera *et al.* (2009), and Rocha *et al.* (2018).

Evidence of technology transfer

As a result of agriculture extension services, training, and capacity building provided to producers, coupled with participants’ interest in improving the vanilla production process, several technologies were indeed transferred, including ground layering (42 producers), compost application (35 producers), pollination (31 farms), and weed control (30 producers).

Table 2. Training workshops provided to participating farmers and other producers interested in vanilla cultivation.

Workshop	Events (No.)	Attendees (No.)
Crop management	6	64
Pest and disease management	7	81
Soil fertility	5	71
Commercialization	4	42
Organizational development of enterprises	5	55
TOTAL	27	313



Figure 2. Workshops provided to producers. A: vanilla marketing strategies; B: soil fertility, preparation of Bocashi organic fertilizer; C: pest and disease control, preparation of bioinsecticides and biofungicides; D: manual pollination; E: general crop management; F: pest and disease monitoring; and G: compost application.

Other activities adopted to a lesser extent were production estimation, flowering record, pruning, and irrigation, which also had an impact on the existing traditional production system (Table 3).

Laborers and activities that were implemented, as well as the transferred innovations based on this study, favored the following aspects in vanilla cultivation.

Reactivation of the vanilla production process. Vanilla production continued despite the adverse conditions present over time. Producers never abandoned the system; rather, knowledge extension and technology transfer efforts encouraged producers to

Table 3. Technologies transferred and implemented by the vanilla producers evaluated in this study.

Tasks	Producers recording the task	
	(No.)	(%)
Layering vines	42	51.85
Applying compost manually	35	43.21
Manual pollination	31	38.27
Manual weed control	30	37.04
Guiding vines	28	34.57
Making planting basins	25	30.86
Manual pesticide application	20	24.59
Manual harvesting	17	20.99
Pest monitoring	16	19.75
Manual herbicide application	14	17.28
Plant losses	12	14.81
Estimating harvest	11	13.58
Measuring flowering	6	7.41
Irrigation	6	7.41
Pruning	5	6.17
Pruning support tree	4	4.94
Manual shade regulation	3	3.70
Planting	2	2.47
Sanitizing support tree	1	1.23
De-capping	1	1.23
Manual fertilizer application	1	1.23
Replacing support tree	1	1.23
Plant sanitation	1	1.23
Removing diseased fruits	1	1.23

reactivate production on each of the host trees they rely on to establish a vanilla plant. Producers' interest persists, even though vanilla cultivation is not their primary source of income.

Acquisition and establishment of cuttings. An average of 25 vanilla plants was established per producer using their own resources, without the support of any government program or agency, thereby increasing planting density.

Preparation and use of organic fertilizer. This practice was implemented and Bocashi fertilizer was produced. It was applied to a portion of each producer's plantation. For Bocashi preparation available resources were used and materials from the same plot or location, such as banana stems, composted sawdust, decomposing tree branches and trunks, and other materials.

Preparation of bioinsecticides and biofungicides. Bordeaux mixture and sulfocalcium mixture were prepared as alternative products to prevent major vanilla pests and diseases.

Irrigation. Some producers already had irrigation systems for other crops; therefore, they were suggested to irrigate their vanilla crop to improve its yield. Irrigation was successfully applied by 7.41% of producers, primarily in the municipality of Papantla, Veracruz.

Organizational principles. The foundations were established for collective work to begin. In the case of producers in Puebla, for the possibility of joining the legally constituted group “Vanilla Producers of San José Acateno SPR de RL,” which would allow them to share the benefits of a consolidated group.

There are other innovations that could not be transferred or implemented because the study period was short. Among those are the establishment of “mother orchards” to generate high genetic quality germplasm, plantation renewal, increased planting densities, application of commercial fertilizers, use of modern production systems (such as shade cloth). Also, construction of facilities to process the product, intensive production systems using orange trees as host plants, and ongoing technical advice. Some authors such as Hernández (2014), Vargas and Gámez (2014), Rocha *et al.* (2018) and Santillán *et al.* (2018) conducted evaluations of these technologies in vanilla cultivation and reported encouraging results focused on improving the production process, increasing the number of plants per hectare, comprehensive crop management, pest and disease control, plantation renewal, and producers’ organization.

Chapingo-AGROPEC Star[®] digital agricultural innovation platform was transferred as technology. The adoption of the digital innovation platform by the agricultural extensionists enabled remote, updated and online monitoring of the agricultural extension and technology assimilation processes.

It was found that most producers who restart this activity do so with little or no profitability, since they hope for an improvement in the price of green vanilla, which is the most common way this product is sold (Jaramillo *et al.*, 2012; FND, 2017). Thus, increasing yields per hectare, planted area, and improving structure in land-use can positively change the competitiveness of vanilla as an agricultural product.

The effects of these variables are not definitive explanations for the sector’s growth, but they do constitute a link in the search for the causes and variables that determine the competitiveness of this production system. Since it is in the planted area where soil preparation and planting efforts are noticed, which in turn impact yields. As well as do innovations in the implementation of production techniques, and the effects of better management practices (Cruz *et al.*, 2013; Santillán *et al.*, 2018).

Production costs

Due to the physiological cycle of vanilla cultivation, production and harvest stages occur only once a year; harvest is done in December. Unfortunately, the period covered by this research did not coincide with the harvest season, then, it was not possible to account for any changes in productivity factors and profitability for the production cycle evaluated. However, with 49.38% of the producers studied, we got records of the production costs summed up to July 31, 2016. It was observed that 90% of them had a cost per hectare between \$1060 and \$1900 MXN, and only 5% recorded expenses exceeding \$2320 MXN (Table 4).

Table 4. Ranking of expenditures observed in producers who recorded this indicator.

Stratum (\$, MXN)	Agribusinesses	
	No.	(%)
Less than 1,060	15	37.50
Between 1,060 and 1,480	16	40.00
Between 1,480 and 1,900	5	12.50
Between 1,900 and 2,320	2	5.00
More than 2,320	2	5.00
Total	40	100.00

These costs, which are not for the complete production cycle, are very low compared to those reported by other authors. As reference, Vargas and Gámez (2014) reported production costs of vanilla up to \$25 000 MXN in the traditional system (“acahual”). Barrera-Rodríguez *et al.* (2011) mentioned a cost of \$23 MXN kg⁻¹ of vanilla produced in a system with orange trees as host plants, which means a cost per hectare of \$23 000 MXN with an average yield of 912 (kg ha⁻¹) which is obtained under this production system.

Santillán *et al.* (2018) indicated that vanilla crop yields are extremely variable, because they depend on the age of the plant, planting density, cultivation method (traditional or technical), source of moisture (rainfed or irrigation), soil characteristics, climate of the planting site, and crop management by the producer. However, according to the review by Rodríguez *et al.* (2023), the most efficient producers are those who begin to take into account the income derived from the crop and, those are the ones that produce vanilla with citrus as hosts, and under shade cloth.

Consequently, a remote monitoring platform contributes to technology transfer activities and capacity building in production systems. In the case of this study, it was pertinent to improve vanilla cultivation in at least some of the summarized aspects. Moreover, we consider vitally important to plan, incentivize, and rescue vanilla cultivation in Mexico through the design and implementation of public agrifood policies. Vanilla is a product native to our country that could become profitable enough for improving producers' household incomes. Otherwise, Mexican vanilla production could decline rapidly in the medium term (Luis *et al.*, 2020).

CONCLUSIONS

Through the Chapingo-AGROPEC Star[®] interface, online monitoring of the agricultural extension process was achieved for small-scale agribusiness producing vanilla. It was verified that vanilla is grown traditionally on small plots, and all the crop management is done by the producers and their family. In spite of the technological practices transferred and the use of innovation in production monitoring and records to improve crop management, farm lands productivity is still low.

Because there is potential to increase production, it is recommended that producers be provided with advice and training to improve technical crop management and increase

production efficiency. The recommendation is to continue recording information of the production process through the specialized digital interface, as there are areas of opportunity that, with proper attention, can improve the productivity and profitability of Mexican vanilla cultivation.

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Determining factors for economic upgrading in the global value chain of the blackberry fruit cluster in Los Reyes, Michoacán, Mexico

Morales-Arellano, Yunuén^{1*}; Ortega-Gómez, Priscila^{1*}

¹ Universidad Michoacana de San Nicolás de Hidalgo. Dirección postal: C. de Santiago Tapia 403, Centro, 58000 Morelia, Michoacán.

* Correspondence: yunuen35@hotmail.com, priscila.ortega@umich.mx

ABSTRACT

Objective: to measure the impact of variables such as innovation, public policy, dependency and inequalities among actors and at the level of organization so that the producer of blackberry fruit (*Rubus* sp.) can achieve upgrading in the global value chain (GVC).

Design/Methodology/Approach: a partial least squares technique was used for measurement, and SmartPLS[®] software for modeling and graphing relationships among variables.

Results: dependency, inequalities, innovation, and public policies largely explain the upgrading potential of producers of blackberry fruit in the global value chain. To the extent that these social actors can reduce dependency level and inequality; while innovation and public policies to support their production activities are developed.

Limitations/Implications of the study: there is no official record of blackberry fruit producers. The internal level of organization did not present a positive association with the economic upgrading of producers in the chain. However, it was positive in relation to external factors; it is important then to strengthen the individual positioning of each producer towards increasing productive capacity, as well as improving skills on marketing and sales of the product.

Findings/Conclusions: reduction in inequality and level of dependency that producers experience compared to the rest of the value chain, as well as innovation are the variables that positively impact economic upgrading of blackberry fruit producers in the municipality.

Keywords: blackberry fruit, stakeholders, social actors, economic upgrading, GVCs.

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INTRODUCTION

The terminology associated with the concept of global value chain includes various approaches. The one based on the theory of comparative advantages uses the term global value chain (GVC) which refers to the international insertion of stakeholders. This global-scale insertion occurs from the specialization of certain links in the chain whose processes make it possible to obtain a final good (Gereffi & Fernandez-Stark, 2016).

Updated research serves as a basis for evaluating the relationships, functions, behavior, performance, and outcomes of stakeholders interactions within these chains. Two of the central pillars of the study of global value chains are governance and economic upgrading.



These elements interact and directly influence the capacity of stakeholders in a chain to act, as well as to benefit from those markets where large profits can be achieved (Opoku-Mensah *et al.*, 2024).

Governance refers to the relationships of authority and power that determine the flow and allocation of financial, human and material resources allocated within a value chain (Lee *et al.*, 2017). According to Reyes *et al.* (2023), governance is described as the leaders management within an entire value chain. On the other hand, upgrading refers to the scaling up in economic terms of those stakeholders dedicated to a certain productive activity within a global dynamic. Thus, this term is used to highlight the paths of advancement of stakeholders for improvement. This means progress for economic purposes in their positioning in the global chain (Ponte *et al.*, 2023).

The concept of specific trajectories is developed in some published studies, referring to the potential for economic upgrading of an organization, through four types of trajectories that can be adopted towards upgrading. These trajectories are process improvement, which occurs when value is added to existing tasks; product improvement, occurring when the value of goods increases; functional improvements, when companies increase the overall qualification content of activities; and chain improvement, which is generated by a horizontal shift to new GVCs that produce greater added value per production unit (Bassetti & Troncoso, 2020).

There are various analyses of agricultural GVCs in Mexico for products such as avocado, berries, and coffee, which highlight low-tech global governance, which primarily falls on marketing companies (González-Ramírez *et al.*, 2020; Reyes *et al.*, 2023). Our research contributes with the case-study of the value chain of blackberry fruit locally produced in a municipality; considering the theoretical elements described above, then analyzing how the local producers of blackberry fruit are positioned as stakeholders in the value chain, within the economics environment in the municipality of Los Reyes, Michoacán, México. The objective of this study was to measure the impact of variables such as innovation, public policy, dependency and inequalities among actors and at the level of organization so that the producer of blackberry fruit (*Rubus* sp.) can achieve upgrading in the global value chain (GVC).

MATERIALS AND METHODS

As a background for the analysis, some factors were selected that can facilitate upgrading for the producer in the global dynamics. Such as, innovation, inequalities or imbalances, and dependency at the level of organization. Local governance dynamics is a key to analyze these factors.

Fernandez-Stark & Gereffi (2019) defined five types of governance which are identified within global value chains, we analyzed those types in adapting them to describe blackberry fruit value chain (Table 1).

Then, based on Lema *et al.* (2019) we selected innovation as a variable to economic upgrading. Those authors described four innovation trajectories in organizations, in terms of the type of capacity required in a company to innovate, and consequently entering GVCs. Inequalities or imbalances and the level of dependency among stakeholders and

Table 1. Types of governance identified in the global value chain of blackberry fruit (Los Reyes, Michoacán, México).

Market	Involves relatively simple transactions, specifications for products are transmitted to suppliers and manufactured.
Modular	Suppliers manufacture products to customer specifications, using generic technology that limits specific asset investments.
Relational	Complex interactions between buyers and sellers, creating high asset specificity
Captive	Small suppliers depends on larger buyers, characterized by a high degree of monitoring and control by lead firms.
Hierarchical	Vertical integration dominated by managerial control of affiliates and subsidiaries.

Fuente: Fernandez-Stark & Gereffi (2019), modified for this study.

social actors, according to Kano (2018), who observed these factors make vulnerable those positions (or links) with fewer resources and capacities in the GVCs. Also, public policies that can act as catalysts for the growth and expansion of a production cluster within the global value chains (Mudambi & Santangelo, 2016; De Marchi *et al.*, 2018).

Finally, the level of organization between companies, which increases the probability that local companies can have an advantage by owning an established brand in the market (Mudambi & Santangelo, 2016; Pietrobelli *et al.*, 2021). To measure the importance of each of the factors selected for analysis of the economic upgrading of the producers of blackberry fruit in the GVCs, the partial least squares (PLS) technique was used, which is a method to estimate the parameters of a system of equations that includes observed or manifested variables, and unobserved or latent variables (Evermann & Ronkko, 2023).

Creation of the structural model

Hair *et al.* (2017) indicated the stages for the creation of a structural equation model. A: model description, which consists of determining the relationships of the model with the latent variables (LV) and their indicators. B: validity and reliability of the model, which require to verify the main measurements at this stage through the analysis of internal validity; individual reliability; construct reliability; convergent validity and discriminant validity. Lastly, C: structural model assessment, where hypotheses are contrasted by examining path coefficients and running a Bootstrapping analysis.

Data collection for analysis was done through a survey applied to producers of blackberry fruit in the municipality of Los Reyes (Michoacan) Mexico. These social actors constitute the core of this economic activity, generating fruit production that is marketed and distributed to international markets. A 35-item Likert-type questionnaire was designed to measure innovation, public policies, imbalances and level of dependency among stakeholders, and the level of organization to evaluate economic upgrading of fruit producers in the GVCs. Each questionnaire was administered directly or digitally from May to August 2024, obtaining a total of 52 responses.

Then, we determined the operationalization of study variables, as well as defined the correspondent measurement indicators (Table 2).

Table 2. Operationalization of variables in the case-study of the value chain of blackberry fruit (Los Reyes, Michoacan, Mexico).

Conditions	Indicators	Item	PLS indicator
Innovation	Importance	1,2	IN1- IN2
	Development of new products	3	IN3
	Comercialization	4	IN4
	Development of new processes	5	IN5
	Diffusion of innovations	6	IN6
	Cooperation with research centers	7	IN7
Public policies	Activity growth	8	PP1
	Producer and government cooperation	9	PP2
	Network	10	PP3
	Usefulness	11	PP4
	Integration level	12	PP5
	Practice regulations	13	PP6
Actor dependence	Price improvement	14	PP7
	Extensive network buyers	15	AD1
	Price negotiation capacity	16	AD2
	Integration level	17	AD3
	Earning profits	18	AD4
	The need to sell in advance	19	AD5
Organization level	Debt level	20	AD6
	Trading companies control	21	AD7
	Frequency of business meetings	22	OL1
	Cooperation between producers degree	23	OL2
	Agreements compliance	24	OL3
	Diffusion of personal knowledge	25	OL4
Upgrade in GVC	Bargaining power of purchase conditions	26	OL5
	Group formation	27	OL6
	Government cooperation	28	OL7
	Conditions	29	UG1
	Up-grading needs	30	UG2
	Up-grading preferences	31	UG3
	Advantage in sale terms	32	UG4
	Economic and commercial evolution	33	UG5
	Sales and marketing ability	34	UG6
	Skills enhacement	35	UG7

Determining the sample for the producers survey

Historically, the municipality of Los Reyes is one of the main in terms of agriculture production areas in the state of Michoacan (Mexico). Due to a high concentration of stakeholders for the analysis, we selected this area; with the limitation that there is no

official record that determines the total number of producers in the municipality. In 2022, the Mexican Secretariat of Agriculture and Rural Development (SADER) called for producers to register for the “Strategic Project for the Rescue and Comprehensive Care of Blackberry Fruit Cultivation,” which aimed to address plantations health problems caused by several agents, through the provision of financial resources. To that official program, 150 producers were registered, which lands cover the municipalities of Los Reyes, Peribán, Tocuambo, and Tacámbaro (NotiGuía, 2022). Based on this record, which constitutes one of the few available lists of producers in the region, we determined the sample for this study that we consider as representative, since it was integrated by a third of the producers.

Graphing the structural model

Variable modeling was done using the software SmartPLS[®] version 4, which allowed for the creation of the structural model and the measurement of indicators related to the selection variables. To run the model, as the endogenous (LV) latent variable (this is, the dependent variable) we considered the economic upgrading of producers in the global value chain, which is determined by the causal factors evaluated, according to the theory on GVCs.

RESULTS AND DISCUSSION

In 2023, total blackberry fruit production in Mexico, according to data from the national Agri-Food and Fisheries Information Service (SIAP, 2024), recorded a total of 236 989 tons (Megagrams, Mg) produced nationwide. Of which 90.29% were obtained from the state of Michoacán, followed by the state of Jalisco, with 0.6% of national production. These data shows the importance of the state of Michoacán in blackberry fruit production. In regard to Los Reyes municipality, blackberry fruit production (2018 to 2023) had the trend summarized in Table 3.

The representativeness and importance of the municipality studied is high, as it constitutes one of the geographical areas with the highest production in the state from 2018 to 2022. In 2023, a decrease in the percentage of production and representativeness of Los Reyes is noted, as the municipalities of Peribán and Tocuambo (also in the state of Michoacán) significantly increased their blackberry fruit production.

Table 3. Blackberry fruit production in the municipality of Los Reyes (Michoacan), compared to the total produced in the state of Michoacán (Mexico).

Agricultural year	Total production in Los Reyes (ton)	Total production in Michoacan (ton)	Share of total production (%)
2018	119,944.00	246,801.38	48.60
2019	137,246.40	285,601.55	48.06
2020	94,374.00	201,335.53	46.87
2021	86,448.00	197,486.75	43.77
2022	86,448.00	205,066.16	42.16
2023	77,430.00	213,991.35	36.18

Source: Mexico’s Agri-Food and Fisheries Information Service (SIAP, 2024).

As a result of the series of regression equations, the path coefficients (Hair *et al.*, 2017) were obtained. The graphic representation of the model created, with the interrelations of variables is shown (Figure 1).

The proposed model integrates path coefficients and the relationship between each of the indicators and the study variable. This estimate shows that innovation, level of dependency and inequality, as well as public policies, explained 47.8% of the variance in economic upgrading of producers in GVCs.

If we agree to consider that this study is a pioneer approach, since there are no previous studies that support the factors required for this productive activity, as determinants for producers upgrading in GVCs, the percentage we obtained is satisfactory. Thus, the variables we selected for analysis would largely determine the chances for stakeholders towards reaching better positions (upgrading) in the global value chains.

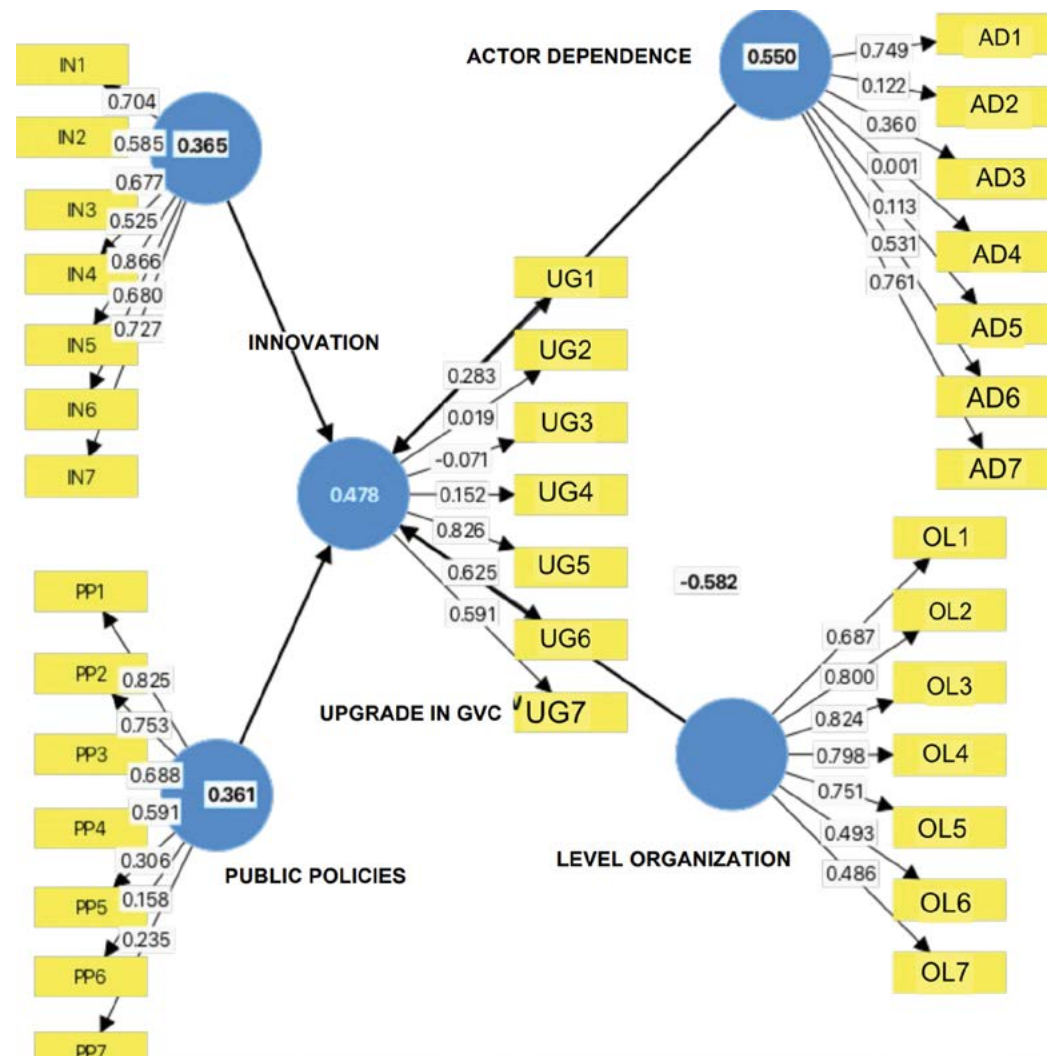


Figure 1. Model of interrelations of the variables for economic upgrading of producers of blackberry fruit (Los Reyes, Michoacán, Mexico) in the global value chains.

The strongest relationship between upgrading and the analysis variables was found in level of dependency and inequality; then, in innovation, and afterwards in public policies. The level of organization was not positively related to the level of upgrading, as it was indicated by the path coefficients (Table 4).

It was evident that innovation explained 36.5% of the variance of the economic upgrading of producers in global value chains. This emphasizes the importance of innovation in production. According to Rikap (2018), the growing gaps between the innovation capacity of companies within a productive branch force non-innovators companies to prefer being dominated. On the other hand, in opinion of Sako & Zylbergberg (2019), economic upgrading only occurs when companies increase added value, and appropriate it within a value chain.

Public policies explained 36.1% of the variance in the producer's ability to upgrade their economic position in the GVC. According to De Marchi & Alford (2022), the facilitating and regulatory role of the Government is required, one that allows strategies to improve participation in value chains and an increase in the capture of value from production activities. In agreement with Filippo *et al.* (2024), we highlight the need for public policies that promote productivity, sustainability and resilience, in order to improve integration and adaptation to new global conditions.

Even more crucial was the factor related to level of dependency and inequality, which explained 55% of the economic upgrading of producers in the GVCs. If we agree on considering that in the municipality studied, the marketing companies make it possible to export the product to international markets; also considering that those companies are primarily formed with foreign capital, the need to reduce producer's level of dependency can be understood. In order to achieve such a reduction, those aspects related to the most important sources of inequality need to be addressed; such as income distribution, added value, risks, and the environmental and social costs caused by production activities (Ponte & Bair, 2023).

The behavior of the impact of the selected indicators, on each of the study variables, is expressed through the external coefficients of the items (Table 5).

Based on the coefficients shown in the table above, the emphasis is important on the interpretation for each of the causal factors, and to the resultant variable.

Innovation: all indicators were positively related to the study variable, confirming the importance of each of the elements considered for the innovation trajectories described by Lema *et al.* (2019).

Table 4. Results of the path coefficients in the model that explain economic upgrading of blackberry fruit producers (Los Reyes, Michoacán, Mexico).

	Upgrade
Innovation (IN)	0.365
Public policies (PP)	0.361
Actor dependence (AD)	0.55
Organization level (OL)	-0.582

Table 5. Measurement of external coefficients that influence on the indicators for economic upgrading of the blackberry fruit producer (Los Reyes, Michoacán, México).

	IN	PP	AD	OL	UG
IN1	0.704				
IN2	0.585				
IN3	0.677				
IN4	0.525				
IN5	0.866				
IN6	0.68				
IN7	0.727				
PP1		0.825			
PP2		0.753			
PP3		0.688			
PP4		0.591			
PP5		0.306			
PP6		0.158			
PP7		0.235			
AD1			0.749		
AD2			0.122		
AD3			0.36		
AD4			0.001		
AD5			0.113		
AD6			0.531		
AD7			0.761		
OL1				0.687	
OL2				0.8	
OL3				0.824	
OL4				0.798	
OL5				0.751	
OL6				0.493	
OL7				0.486	
UG1					0.283
UG2					0.19
UG3					-0.071
UG4					0.152
UG5					0.826
UG6					0.625
UG7					0.591

IN: innovation; PP: public policies; DD: level of dependency and inequality; LO: level of organization; E: economic upgrading.

Public policies (PP): current public policy does not clearly address the regulation of commercial practices (PP6) used by the exporting companies. Although some institutional reforms have been implemented to improve foreign trade, policies to address transformation, reconversion, or access to production and commercial incentives are limited. These conditions increase dependence on certain export markets (Baena-Rojas *et al.*, 2023).

Imbalances and level of dependency (DD): selling price to the producer (DD4) does not allow them to individually sustain autonomous processes of production or sales. Producers are generally tied to pre-harvest sales contracts (DD5), signed at imbalanced and unfavorable conditions.

Level of organization (LO): all external coefficients on the level of organization were positively related to the economic upgrading of the producer.

Economic upgrading in the value chain: there are no overall conditions (E2 and E3) for producers upgrading in global value chains, mainly due to the lack of skills on marketing and sales of the product.

Results on the structural model assessment

The results of reliability and internal consistency, both evaluated through Cronbach's alpha coefficient (CA) and the composite reliability of the construct (CR) as measures of internal consistency, presented in each case and for at least two factors, the expected level of indices (0.7, modest reliability in the early stages of the research; 0.8 for basic research). The convergent validity criterion considered the average variance extracted (AVE); this measure is only applicable to VLs composite of reflective indicators. When 0.5 is the lowest limit, AVE is acceptable; this means that more than 50% of the variance of the construct was explained by selected indicators. In this way, indicators fitting is significant and they are highly correlated (Hair & Alamer, 2022).

Table 6. Coefficients for the proposed model in the reliability and internal consistency tests.

	Cronbach's Alpha	Composite Reliability	Average Variance Extracted (AVE)
Actor dependence	0.526	0.491	0.326
Upgrade IN GVC	0.474	0.548	0.619
Innovation	0.817	0.849	0.573
Organization level	0.845	0.893	0.596
Public policies	0.842	0.448	0.521

In addition, results are presented of the measure of discriminant validity, this is, the Heterotrait-Monotrait ratio (HTMT). In this test, the limit proposed by Roemer *et al.* (2021) is 0.85, to be considered as a valid and reliable measurement (Table 7).

Table 7. Test results of the Heterotrait-Monotrait (HTMT) ratio, the measure of discriminant validity of the proposed model.

	ACTOR DEPENDENCE	UPGRADE IN GVC	INNOVATION	LEVEL ORGANIZATION	PUBLIC POLICIES
Actor dependence					
Upgrade in GVC	0.844				
Innovation	0.686	0.658			
Level organization	0.806	0.56	0.761		
Public policies	0.819	0.653	0.687	0.843	

CONCLUSIONS

The reduction in inequality and level of dependency that producers experience compared to the rest of the value chain, as well as innovation are the variables that positively impact economic upgrading of blackberry fruit producers in the municipality under study. Innovation oriented to the internal processes of production and agriculture management is the most needed, as well as that related to the capacity to generate value-added products, such as new varieties, or improved production cycles.

The public policy variable is relevant to effectively reduce inequality and producers dependence to other actors in the global value chain, as well as to promote innovation. This study is the first approach towards identifying the potential options and mechanisms to the economic upgrading of blackberry fruit producers. In order to improve their position in production and sales, within the international dynamics of global value chains.

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Effect of the price of yellow maize (*Zea mays* L.) imports on animal protein consumption in Mexico

Luquez-Gaitan, Carlos E.¹; Hernández-Mendoza, Natividad²; Zavala-Pineda, María J.²; Leos-Rodríguez, Juan A.^{1*}

¹ Universidad Autónoma Chapingo. Texcoco, Estado de México, México. C. P. 56230.

² Universidad Politécnica de Texcoco. Texcoco, Estado de México, México. C. P. 56250.

* Correspondence: jleos45@gmail.com

ABSTRACT

Objective: to determine the effect of the price of yellow maize imports on animal protein consumption, through time series analysis, to provide empirical evidence of the effect of trade policies on the animal protein consumption by the population in Mexico.

Design/Methodology/Approach: the Food Trade Dependency Index (FTDI) was calculated to measure the degree of dependence on imports of this grain. Data from the National Survey of Household Income and Expenditure (ENIGH in Mexico) were used to account for household expenditure on consumption of beef, chicken meat and pork, eggs, and milk, in three basic baskets of goods. Regressions were performed with the Autoregressive Vector (ARV) method and Granger causality tests.

Results: results indicated that variations in the price of yellow maize imports have a direct impact on the price of animal protein. This, in turn, can affect Mexico's population access to essential sources of animal protein, especially in low-income households.

Limitations/Implications of the study: this research did not consider other factors that can also influence the price of animal protein; such as, production costs, domestic demand and seasonal variations in protein consumption. This fact can limit the comprehensive understanding of the causal relationship found with the study variables.

Findings/Conclusions: this research highlighted the vulnerability of the Mexican agrifood industry to international trade dynamics. It also underlines the importance of designing strategies to enhance competitiveness in the domestic production of yellow maize.

Keywords: competitiveness, apparent national consumption, food trade dependency.

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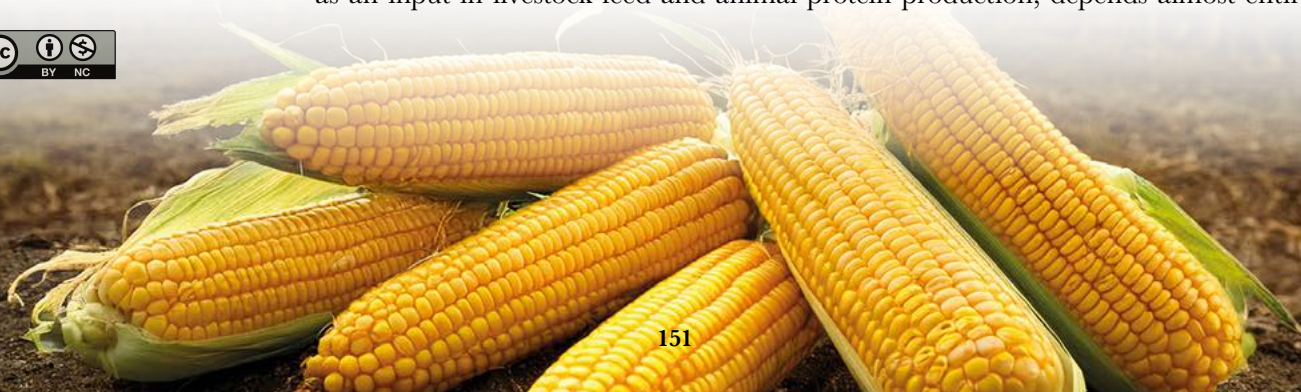
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INTRODUCTION

Maize is a fundamental component in Mexico's agrifood system, not only because of its cultural and social importance, but also because of its strategic role in the diet of the population and in the livestock industry. In 2022, Mexico produced around 27 million tons (Megagrams, Mg) of maize, of which 90% corresponded to white maize intended mainly for human consumption (FAOSTAT, 2024). However, demand for yellow maize, used as an input in livestock feed and animal protein production, depends almost entirely on



imports (SIAP, 2024). This dependence generates economic vulnerability to fluctuations in international prices of yellow maize, which can have a significant impact on the production costs of basic food products. In this regard, Zahniser *et al.* (2019) mentioned that Mexican imports of yellow maize are used for industrial processing, mainly for diets in the production of beef, pork, chicken meat, eggs, and milk.

The basic basket of goods for Mexicans includes food products derived from animals that are fed with yellow maize. So, it is interesting to study the relationship between maize imports and the prices of products in the basic basket of goods, as they would be directly influencing inflation levels in the country. In the current national context, since the decree on February 13, 2023 (DOF, 2023) that prohibits imports of genetically modified maize for direct human consumption, this supply is limited in Mexico's national territory, which aggravated vulnerability in terms of food security. Over the years it is observed that the national consumption, covered only by domestic production, is continuously decreasing (Figura 1).

According to García-Salazar *et al.* (2023), the Food Trade Dependency Index can be expressed as the proportion of imports in relation to ANC. The value of this indicator went from 15% in 1994 to 36% in 2022, which is mainly due to the low maize prices that were caused by the trade preferences established in the North American Free Trade Agreement (NAFTA), then ratified in the current United States-Mexico-Canada Agreement (USMCA). In the period from 1994 to 2022, Mexico's national production, consumption and imports grew 46%, 104% and 492% respectively, which is consistent with the calculations of the degree of food dependency in the same period.

In this research, the effect of yellow maize imports on the consumption of animal protein *per capita* was determined, through time series analysis, to provide empirical evidence of the effect of trade policies on the animal protein consumption by the population of Mexico. The hypothesis states that changes in imports of yellow maize

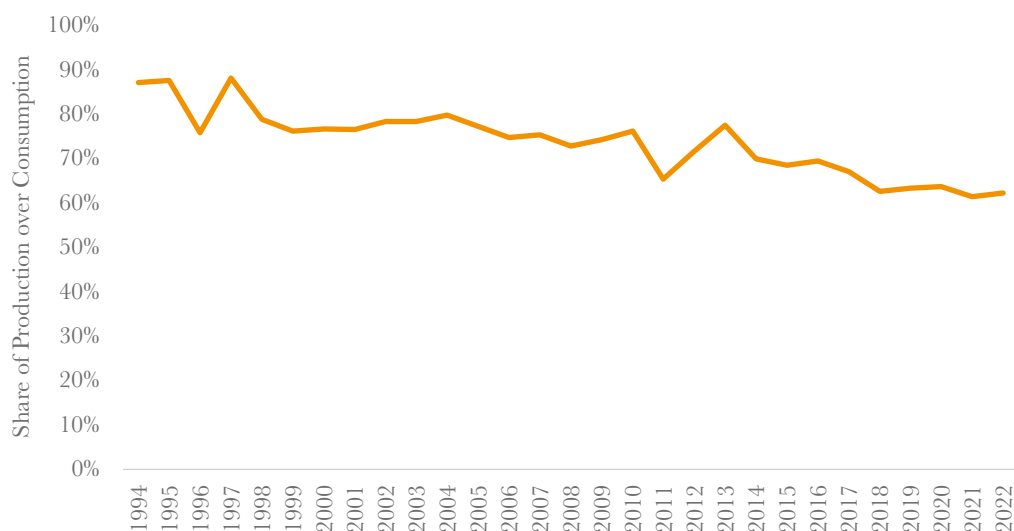


Figure 1. Share of production in the Apparent National Consumption (ANC), from 1994 to 2022. Source: prepared by the authors using data from FAOSTAT (2024).

are related to changes in the price of animal protein, and therefore to the national consumption of this protein *ceteris paribus*.

MATERIALS AND METHODS

Information data sources

Data collection was done through official sources. In the case of trade flows, two sources were used: i) Database of the United States Department of Agriculture (USDA, 2024) which allowed obtaining information on trade between the United States and Mexico, and ii) Database of the United Nations Food and Agriculture Organization (FAOSTAT, 2024), from which the total trade flows of each country were obtained, and also, homogenized information on production.

The data for the characterization of Mexican production (yield, production volume, and irrigation use) were obtained from the agricultural end-of-cycle database of Mexico's Agrifood and Fisheries Information Service (SIAP, 2024). The average annual expenditure that households make on food goods was determined. Data for this estimation were obtained from results of the Mexico's National Survey of Household Income and Expenditure (ENIGH, 2022), by the Mexico's National Institute of Statistics and Geography (INEGI, 2024).

For the terms of this research, we accounted for the average household expenditure in three different basic baskets of goods (Table 1). In addition, the average expenditures per household on chicken meat, beef, pork, eggs, and milk, were considered individually. Data processing was performed in the econometric software STATA[®] 17.

In 2005, due to a demographic reconciliation caused by the II Count of Population and Housing 2005 at the request of Mexico's Secretariat of Social Development under the Federal Government, INEGI deployed the extraordinary survey of the ENIGH 2005 (INEGI, 2005). Since the information reported in 2004 was incomplete, the data presented in that year were discarded, and the information of 2005 was used twice, as if it were from 2004.

Table 1. Characteristics of the basic baskets of goods that were used.

	Basic basket (DOF) ¹	Basic basket (SEGALMEX) ²	Food basket (CONEVAL) ³
Purpose	Established for measuring inflation through the Consumer price index.	Established for assuring the access of the poor to basic foods.	Established by The National Council for the Evaluation of Social Development Policy (CONEVAL) in order to measure food poverty. It calculate the cost of satisfying the minimum caloric requirements of an individual.
Composition	Contain fifty three products including food, cleaning items, personal hygiene items.	Contain forty products including corn, beans, rice, eggs, milk and non food items.	Take into account only food items considering nutrition recommendations.

Source: elaborated by the authors based on the Official Gazette of the Mexican Federation (DOF, 2020), the Mexico's Secretariat of Agriculture and Rural Development (SADER, 2019), and CONEVAL (2022). From this point on, these will be referred as 'Canasta DOF'¹, 'Canasta 40 (SEGALMEX)² and 'Canasta Alimentaria'³.

Food trade dependency index

The Food Trade Dependency Index (FTDI) is an indicator that measures the degree to which a country depends on imports to meet its domestic food needs (García-Salazar *et al.*, 2023). The FTDI is particularly useful in contexts such as Mexico, where imports of basic foodstuffs, such as maize, have increased significantly, affecting food self-sufficiency and access to affordable food of the most vulnerable population (FAO, 2024).

The calculation of the FTDI was done based on the methodology used by the United Nations Food and Agriculture Organization (FAO, 2010) to calculate the trade dependency index on cereal imports (%). Other authors (García-Salazar *et al.*, 2023; Hernández *et al.*, 2023) have used this index to address issues of food security and international trade. According to FAO & Banco Mundial (2021), this methodology can be complemented with statistical data-collection tools and multidimensional assessment models to identify vulnerabilities associated with international trade and their impact on national food systems.

The calculation of the food trade dependency index responds to the following expression:

$$FTDI = \frac{M}{(P + M - X)}$$

where, M : volume of imports, P : domestic production, and X : volume of exports, of yellow maize.

Within the context of this research, the FTDI reflects the relationship between the volume of yellow maize imports and its apparent national consumption. So, the higher the quotient, it is interpreted as a greater food trade dependency on imports.

Data interpolation

The ENIGH data are collected every two years; so, 16 observations were obtained in the study period 1994-2022. According to Harrell (2015), to guarantee reliable results and avoid problems such as multicollinearity during the estimation of the econometric model, it is desirable to have between 10 and 20 observations for each estimated parameter. In addition, some complex techniques, such as structural equation models (Bentler and Chou, 1987) or autoregressive vector models (ARV) require larger sample sizes (Hamilton, 1994).

Therefore, in order to strengthen the time series to achieve statistical consistency in the estimators, we used interpolation of the data (Arévalo-Ovalle *et al.*, 2021) for the average consumption expenditure on chicken meat, beef, pork, eggs, milk, and those in the three basic baskets of goods previously described. In this way, the time series was composed of 29 annual observations for each variable. The linear interpolation method was used because the data showed an almost linear behavior among the available observations.

Autoregressive vector models (ARV) and causality in Granger's sense

According to Hamilton (1994), ARV models are especially useful when i) we seek to analyze the intertemporal and bidirectional relationship among several endogenous

variables; ii) there is not clearly defined economic or structural theories; or iii) the aim is to analyze how one variable responds to a “shocking event” in another variable, and how this effect propagates over time.

Once the ARV model has been estimated, it can be used to identify and understand the mechanisms by which the effects of the variables included in the model are transmitted (Rossi & Wang, 2019). This analysis can be performed by means of a causality test, and be complemented by the analysis of impulse-response functions. All of which allows examining how a shock in one variable affects the others over time (Guerrero, 1987).

The Granger causality test, proposed by Clive W. J. Granger in 1969 is used in econometrics and time series analysis to evaluate whether past information from one time series helps to improve predictions of another time series, beyond what could be predicted using only past information from the second variable.

The available data on the average import prices of yellow maize, the information collected from the ENIGH, and the objective of this research are consistent with the characteristics described above that make plausible the estimation of an ARV model, and the application of the Granger causality analysis.

The determination of the order of the ARV model was delimited through the Akaike Information Criterion-AIC (Akaike, 1974), which penalizes complex models to avoid overfitting. This means that although a more complex model may better fit the data, the AIC introduces a penalty for using more parameters, this feature is useful in contexts where sample sizes are limited (Hurvich & Tsai, 1989).

RESULTS AND DISCUSSION

The FTDI was calculated to determine the level of dependence that Mexico has on imports of yellow maize. Figure 2 shows the evolution of the index during the study period (1994-2022).

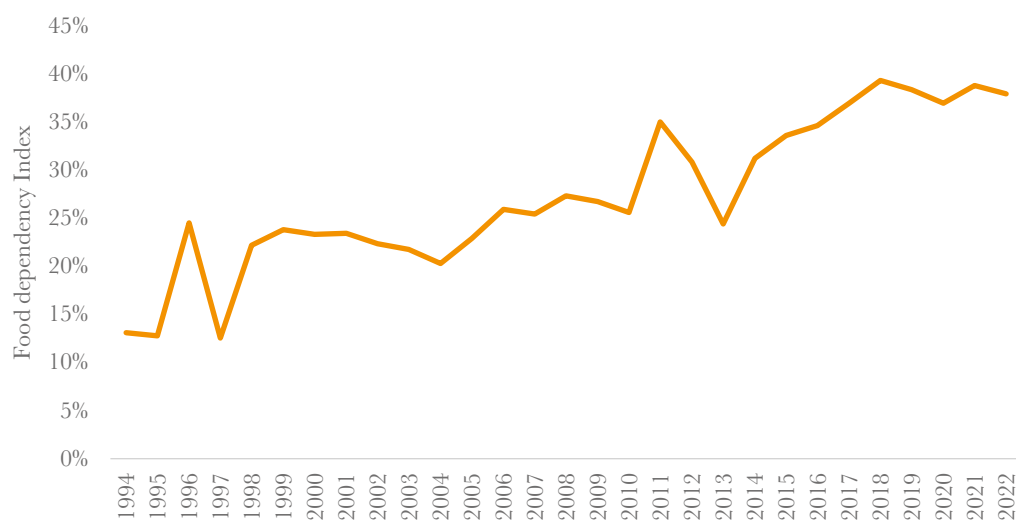


Figure 2. Evolution of the Food Trade Dependency Index (FTDI) of yellow maize in Mexico in relation to the United States, 1994-2022. Source: elaborated by the authors with data from FAOSTAT (2024) and USDA (2024).

According to FAO (2010), in situations in which the FTDI value is greater than 25%, the analyzed country is interpreted as being at critical levels of food dependence. Only in the years 1994 to 2005 did the FTDI not exceed that value.

During the period 2000-2010, it is observed that although there was a growth in dependence on imports, this value only exceeded the maximum limit established by FAO (2010) by two percentage points. In that space of time, NAFTA (recently, USMCA) fostered a more robust trade flow between Mexico and the U.S., allowing the latter to be consolidated as Mexico's main supplier of yellow maize.

From 2011 onwards, there has been a significant increase in the FTDI, with values close to 40%, due to the almost total absence of national production of yellow maize. According to Cruz Herrera *et al.* (2021), recent policies that seek to promote food self-sufficiency in Mexico have had a limited impact on reducing this dependence.

Based on that analysis, the specification of an ARV model was proposed in which the average price of yellow maize imports was estimated as an explanatory variable, and the average consumption expenditure on proteins from animals fed with imported yellow maize were estimated as explained variables. Those are chicken meat, beef, pork, eggs, and milk; in addition, the average expenditure in the three basic baskets of goods mentioned was included. The average expenditures per household in those three selected basic baskets of goods were analyzed (Table 2).

Table 2. Average expenditure per household (MXN \$) in three selected basic baskets of goods (1994-2022).

Year	Basic basket (DOF)	Δ %	Basic basket (SEGALMEX)	Δ %I	Food basket (CONEVAL)	Δ %
1994	2403		255		236	
1996	1991	-17.2	377	48.2	314	33.0
1998	3105	56.0	511	35.6	421	34.0
2000	3091	-0.4	649	27.0	524	24.3
2002	2512	-18.7	960	47.9	713	36.1
2005	2632	4.8	1269	32.2	802	12.5
2006	2311	-12.2	765	-39.7	571	-28.9
2008	2295	-0.7	832	8.7	956	67.6
2010	912	-60.2	778	-6.4	669	-30.1
2012	3608	295.4	1314	68.8	848	26.9
2014	3535	-2.0	1095	-16.6	811	-4.3
2016	3835	8.5	1232	12.5	943	16.2
2018	4990	30.1	1320	7.1	1476	56.5
2020	6457	29.4	1759	33.3	1363	-7.6
2022	6685	3.5	2172	23.5	1701	24.7
Average	3357	22.6	1019	20.1	823	18.6
Standard Deviation	1604	0.83	515	0.28	420	0.29
Standard Error	414	0.22	133	0.08	109	0.08

MXN \$: Mexican pesos. Source: generated by the authors with data from the ENIGH (2024).

A substantial difference can be observed in the averages and standard deviations, especially in the data of the basic basket ‘Canasta DOF’, which can be explained by the inclusion of goods that are not essential for certain segments of the population. The percentage variations and volatility of the data indicate that price and consumption dynamics have been particularly sensitive to macroeconomic events such as economic crises or inflation. Since abrupt falls in expenditure in the three baskets from 2009 to 2010 are observed, reflecting the decrease in the median of real labor income as part of the effects from the 2008-2009 economic crisis in Mexico. Likewise, according to data from the Bank of Mexico (2024), the increase in the costs of the three baskets, especially in recent years, reflects a direct impact of inflation on the prices of basic goods.

The years 1996, 2002 and 2008 showed that households, despite had reduced their expenditures on the basic basket ‘Canasta DOF’, which refers to the broadest basket of goods studied; they in fact increased their spending on ‘Canasta 40 (SEGALMEX)’ and ‘Canasta Alimentaria’. Therefore, the demand for basic foodstuff has an inelastic behavior compared to basic non-food goods.

The average household expenditure on livestock products as a proportion of expenditure in the ‘Canasta Alimentaria’ basic basket was analyzed to understand the relative importance of each product over time, regardless of the growth or decrease in total household expenditure. Likewise, the total of the joint participation of these goods for each year analyzed is presented (Table 3).

Fluctuations in percentage share reflect the influence of economic factors, such as income, prices, and changes in the accessibility of these products (Martínez *et al.*, 2019).

Table 3. Percentage share of average household expenditure on livestock products within the ‘Canasta Alimentaria’ (the basic basket including only foodstuff).

Year	Chicken*	Beef*	Pork*	Eggs	Milk	Total
1994	1.5	4.3	3.2	0.7	1.6	11.3
1996	2.0	5.3	3.5	1.0	2.4	14.2
1998	2.1	5.3	3.4	1.2	2.0	14.0
2000	2.1	4.6	3.4	1.0	1.8	12.9
2002	2.8	4.4	3.1	1.1	1.9	13.3
2005	3.8	5.0	3.2	5.6	2.8	20.5
2006	5.4	7.4	4.2	2.1	3.0	22.1
2008	3.4	4.9	2.9	1.5	2.0	14.7
2010	5.5	7.4	4.5	2.1	3.0	22.6
2012	4.1	24.8	5.9	6.7	2.7	44.1
2014	9.6	9.2	6.5	2.9	3.3	31.5
2016	5.4	9.9	4.6	3.5	4.9	28.2
2018	4.6	12.7	4.0	2.8	2.5	26.5
2020	8.1	12.6	6.4	3.1	2.8	33.0
2022	5.7	8.8	5.2	3.4	3.7	26.9
Average	4.3	8.4	4.2	2.5	2.6	22.1

Source: elaborated by the authors with data from the ENIGH 2022. *Data included expenditures on raw meat for household consumption, these do not include prepared or processed meals.

Beef continues to be the product with the greatest weight in expenditure, although its variability highlights the need to observe its behavior compared to other more accessible products such as chicken meat or eggs.

The Akaike Information Criterion (AIC) was used to determine the optimal number of lags in the estimation of the ARV model. In general, the lag with the lowest AIC value for each variable was selected. The results indicated that, of the eight dependent (explained) variables analyzed, two showed an optimal lag in the second period; three, in the third; and the remaining three, in the fourth period. This selection sought to ensure the best specification of the model. In all cases, the exogenous variable used was the average price of yellow maize imports.

Values of the coefficients for each regression in the ARV model are shown (Table 4). In the causality test in Granger's sense, the calculated values of the χ^2 (Chi square) statistic are presented. Statistical significance is noted in parentheses.

The statistical significance of the regressions shows that the lag coefficient in the ARV models for the variables 'Canasta DOF', 'Canasta 40 (SEGALMEX)', and 'Canasta Alimentaria', expenditure on chicken meat, expenditure on beef, expenditure on eggs, and expenditure on milk was statistically significant with ($p \leq 0.05$). Therefore, the average price of imports of yellow maize had a relevant impact on these dependent variables.

In all regressions, the expected sign of the coefficients was positive as expected, because the increase in the price of yellow maize was transferred to higher prices in livestock products. However, the total expenditure on these products was not significantly reduced because the demand for these products responds more quickly to changes in other factors; such as, taste and preferences, changes in substitute products, and consumer incomes (Purcell, 2000).

Regarding causality in Granger's sense, there was significant evidence ($p \leq 0.01$) that, lags in the price of yellow maize cause changes in all dependent variables. The values of the χ^2 (Chi square) statistic were high; statistical significance of each estimate allowed us to ensure valid results. In this way, it was possible to demonstrate causality in Granger's sense of the price of yellow maize imports on the expenditure for those goods selected in this

Table 4. Results of ARV model regressions and causality tests in Granger's sense.

Variable	Lag 0	Lag 1	Lag 2	Lag 3	Lag 4
Basic basket (DOF)	16.61	15.3	15.08	15.002*	15.03
Basic basket (SEGALMEX)	14.09	12.84	12.52	12.55	12.39*
Food basket (CONEVAL)	12.94	12.05	11.94	11.61	11.59*
Chicken spending	8.33	0.54	7.46	7.05*	7.1
Beef spending	10.34	9.77	9.34*	9.4	9.37
Pork spending	7.81	6.12	5.85*	5.92	5.99
Egg spending	8.06	7.59	7.23	7.21	7.04*
Milk spending	6.58	6.02	5.64	5.5*	5.58

Source: elaborated by the authors with data collected from the ENIGH (2022) and USDA (2024) processed with the STATA[®] 17 software.

research. Our hypothesis is accepted that changes in imports of yellow maize are related to changes in the price of animal protein, and therefore to its consumption. Thus, trade dependence makes the stability of the prices of the basic basket vulnerable. Despite what was found by Orozco-Cirilo *et al.* (2023), in the case of the price of maize (per bushel) and the prices of Mexican pork, who reported a non-significant relationship after performing multiple regressions.

Yellow maize is one of the main components in the feed of the animals used for protein production (Castillon, 2021), those that were chosen in the set of goods analyzed in our research. In livestock production systems, feed represents around 60 to 70% of the costs (Núñez, 2017); therefore, causality in the consumption of basic goods of livestock origin, on the part of the price of the input, is congruent. Beckman *et al.* (2024) showed that pursuing food security for maize in Mexico could increase maize prices by 24.8%, also that Mexican households would have to spend between 6.7% and 13.9% more on food.

The growing percentage of SEGALMEX strategic products, in the basic basket of food products, indicates that more and more consumption in Mexico is destined to the most essential good, which is food. This can be interpreted as a loss in the purchasing power of households. It was proved that yellow maize imports cause, in Granger's sense, variations in the consumption of selected livestock products. Such a transmission of variations is consistent with another study (González & Martínez, 2015), which determined that the price of maize was causal in the tortilla price, because unit transmission elasticity was found between the two price series.

CONCLUSIONS

Causality was demonstrated in Granger's sense. Thus, the prices of yellow maize imports explain the variations in the expenditure on livestock products. Trade policies and fluctuations in the international prices of yellow maize may have a direct impact on the economic access of households to livestock products. Also, trade variations can also affect producers, particularly those with lower purchasing power. The increase in spending on animal protein from livestock farming in Mexico is closely linked to the dependence on imports of yellow maize as a key input in livestock farming.

This signifies structural vulnerability of the Mexican agrifood sector to fluctuations in international trade, which can compromise the sustainable access to livestock-origin food in the medium and long terms. Moreover, if there were significant increases in import prices. Thus, we emphasize the importance of generating strategies to reduce the levels of dependence on imports of yellow maize, in order to guarantee food security, economic feasibility, and, indirectly, social stability in Mexico.

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Ginger (*Zingiber officinale* Roscoe), a natural additive with antimethanogenic properties in ruminants

Gutiérrez-Fidencio, Miguel^{1,4}; Ramírez-Mella Mónica^{2,3,5}; Crosby Galván, María M.¹; Ramírez-Bribiesca, Jacinto E.¹; López-Rosas, Itzel^{2,3,5}; Hernández-Rodríguez, Martha¹; Sánchez-Villarreal, Alfredo^{2*}

¹ Colegio de Postgraduados Campus Montecillo. Montecillo, Texcoco. Estado de México. México. C. P. 56230.

² Colegio de Postgraduados Campus Campeche. Champotón. Campeche. México. México. C. P. 24450.

³ Secretaría de Ciencia, Humanidades, Tecnología e Innovación, México. Benito Juárez, Ciudad de México. México. C. P. 03940.

⁴ Tecnológico Nacional de México, Instituto Tecnológico de Ciudad Valles. Ciudad Valles, San Luis Potosí. México. C. P. 79010.

⁵ Tecnológico Nacional de México, Instituto Tecnológico de Chiná. Chiná, Campeche. México. C. P. 24520.

* Correspondence: asanchezv@colpos.mx

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ABSTRACT

Objective: To describe ginger (*Zingiber officinale* Roscoe) properties and its potential to reduce ruminal methane production.

Design/methodology/approach: A literature search and review of scientific articles published over the past 20 years was conducted, focusing on the use of ginger as an antimicrobial agent in animals of zootechnical interest, as well as its application as an antimethanogenic additive in ruminants.

Results: The metabolite profile of ginger is described, along with the effects of ethanolic, methanolic, and/or aqueous extracts, essential oils, and ginger meal in *in vitro* assays evaluating antimicrobial and antimethanogenic activity in cattle, sheep, and goats. Additionally, the scope and potential areas of opportunity for its field application are discussed.

Limitations on study/implications: No *in vivo* studies have been conducted on the use of ginger as an antimethanogenic additive in ruminants.

Findings/conclusions: Ginger rhizome meal has been shown to reduce methane production *in vitro*, suggesting its potential as an antimethanogenic additive. However, *in vivo* studies are needed to confirm this effect, identify the active compounds responsible, and clarify their mechanism of action in the rumen.

Keywords: Ruminal methanogenesis, ruminal microbiota, greenhouse gases, gingerols and shogaols.

INTRODUCTION

The livestock sector contributes to greenhouse gas (GHG) emissions, with cattle production being responsible for 62% of global livestock-related emissions, equivalent to 5 gigatonnes of CO₂-e. Additionally, enteric fermentation accounts for 44% of methane (CH₄) emissions (FAO, 2022). Methane production not only contributes to global warming



but also compromises livestock productivity, as between 4% and 15% of the total energy consumed by the animal is lost during CH₄ synthesis (Bodas *et al.*, 2008).

Many of the strategies aimed at reducing CH₄ production in ruminants focus on modifying ruminal characteristics through dietary interventions (Patra, 2012), as the ruminal microbiota is directly responsible for the biosynthesis of this gas (Patra *et al.*, 2017). However, none of these strategies have proven entirely effective, as their impact on CH₄ reduction is temporary, often costly, and may even be toxic to the ruminant (Moss *et al.*, 2000; Patra, 2012). Therefore, it is essential to search for new dietary alternatives with the potential to reduce ruminal methanogenesis, without generating harmful residues that negatively affect the animal or pose risks to consumers of milk and meat. One natural alternative could be the inclusion of ginger (*Zingiber officinale* Roscoe) in the diet of domestic ruminants, as it exhibits antimicrobial properties (Mao *et al.*, 2019) and acts as a modulator of microbial populations (Teng *et al.*, 2018). The antimicrobial effect of this plant has been tested in various animal species of zootechnical interest, including some ruminants (Kim *et al.*, 2012; Soroor & Moeini, 2015), with reports of reduced ruminal CH₄ production resulting from the use of both ginger rhizome meal (Patra *et al.*, 2010; Soroor & Moeini, 2015; Khejournsart *et al.*, 2021; Altınçekiç *et al.*, 2021; Gutiérrez-Fidencio *et al.*, 2023) and aerial part extracts (Kim *et al.*, 2012).

Although the antimicrobial and antimethanogenic effects of ginger in ruminants have been documented, its mechanism of action remains unknown, and thus, the effectiveness of its application under field conditions is still uncertain. Based on this context, the objective of this review is to contribute to the understanding of ruminal methanogenesis and its environmental impact, as well as to examine the effect of dietary ginger on CH₄ production in the rumen, its inclusion levels, the most suitable form of presentation in complete diets, its potential limitations, and its utilization as a feed additive.

MATERIALS AND METHODS

A systematic review was conducted on scientific articles addressing the chemical properties of ginger, its use as an antimicrobial agent in animals of zootechnical interest, and its use as an antimethanogenic additive in ruminants, published over the past 20 years. Articles were obtained from academic repositories and search engines such as Google Scholar, NCBI, and Scopus, using the following keywords individually or in combination, in English: “*Zingiber officinale*,” “ginger,” “ginger meal,” “ginger extract,” “ginger properties,” “gingerols,” “shogaols,” “ruminant,” “ruminal methanogenesis,” “rumen microbiota,” “greenhouse gases,” “methane.”

RESULTS AND DISCUSSION

Ruminal Methane

According to the UN (2023), the global population is expected to reach 10.4 billion by the year 2100, which will result in a greater demand for food inputs, including animal-based protein. Globally, cattle are among the main providers of milk and meat, both of which are important sources of high biological value protein (FAO, 2022). However, the cattle sector contributes more than 5 gigatonnes of CO₂-e emissions within the livestock

industry —6.5 and 8.5 times more than buffaloes and small ruminants, respectively (Figure 1). Of the total GHG emissions from livestock, 44% corresponds to enteric CH_4 produced by cattle (FAO, 2022).

Ruminal Methanogenesis

In the rumen, CH_4 is synthesized by methanogenic archaea, which reduce remnants of carbon dioxide (CO_2), hydrogen (H_2), formic acid, and methylamines through various metabolic pathways. These compounds are produced as a result of the fermentative activity of other microorganisms (Patra *et al.*, 2017). Methanogenic archaea are found in the rumen both free-living interacting as part of a microbial consortium composed of ruminal bacteria and fungi within biofilms that cover feed particles and as endosymbiotic and ectosymbiotic associates, mainly with ciliated protozoa (Figure 2; Valle *et al.*, 2015). It is estimated that between 9 and 37% of ruminal CH_4 is produced by these microorganisms (Newbold *et al.*, 2015; Eckard *et al.*, 2010; Huws *et al.*, 2018). Therefore, studying dietary strategies aimed at reducing methanogenesis necessarily involves considering the various groups within this complex microbial consortium. Reducing CH_4 production would not only decrease greenhouse gas emissions from the sector but also improve feed efficiency and productivity of production systems (Johnson and Johnson, 1995).

Some strategies to reduce ruminal CH_4 production have involved the use of ionophores, the addition of chemical compounds such as bromochloromethane and nitrocompounds, or energy and protein supplementation in low-quality diets. More natural alternatives include the use of forage legumes with high condensed tannin content and/or the addition of secondary metabolites such as saponins, tannins, and essential oils (Patra, 2012).

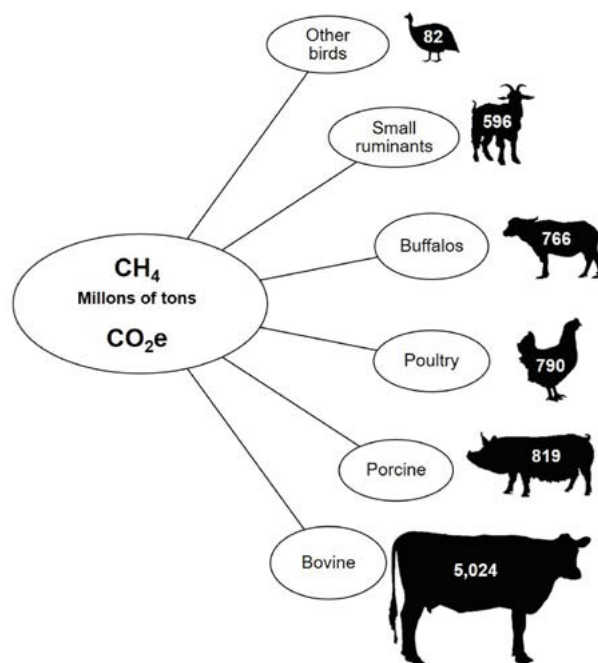


Figure 1. Methane production from the main species of zootechnical interest, presented in million tonnes of CO_2 -equivalents. Prepared based on data from the FAO (2022).

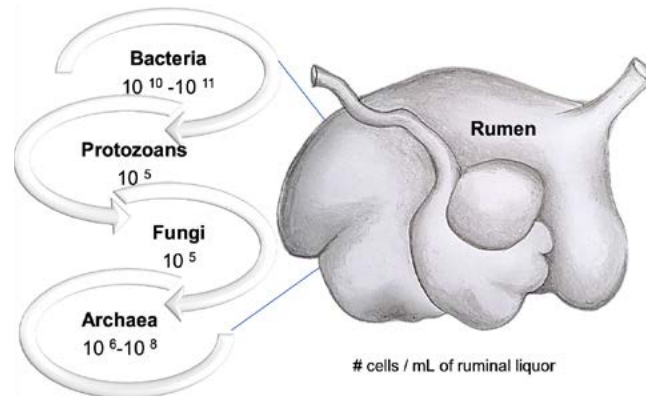


Figure 2. Estimated proportion of bacteria, protozoa, fungi, and archaea comprising the ruminal microbiota. Illustration by the authors.

However, some of these strategies do not guarantee the welfare and/or productivity of the ruminant or have only a partial or short-lived effect (Moss *et al.*, 2000), most likely due to the resilience of the microbial consortium. With the aim of utilizing plant resources with intrinsic natural properties that can modify the ruminal microbiota and consequently reduce CH_4 production, a review of the scientific literature on ginger was conducted, which could have potential as an additive or supplement.

Antimicrobial and Antimethanogenic Activity of Ginger in Ruminants

Ginger (*Zingiber officinale*) is a tropical and subtropical plant native to China and India, attributed with various biological activities, including antimicrobial properties (Figure 3A; Mao *et al.*, 2019). Its biological activity is due to the content of different secondary metabolites, such as terpenes, sesquiterpenes, monoterpenoids, and phenolic compounds, mainly 6-, 8-, and 10-gingerol and 6-, 8-, and 10-shogaol (Figure 3B). These latter compounds are the most abundant and provide ginger with its characteristic aroma and pungent flavor (Sanwal *et al.*, 2010; Mao *et al.*, 2019).

A Biological activities

1. Respiratory protectant
2. Antioxidant
3. Antidiabetic
4. Cardiovascular protectant
5. Anti-inflammatory
6. Anticarcinogenic
7. Antiemetic
8. Antimicrobial



B Phenolic compounds

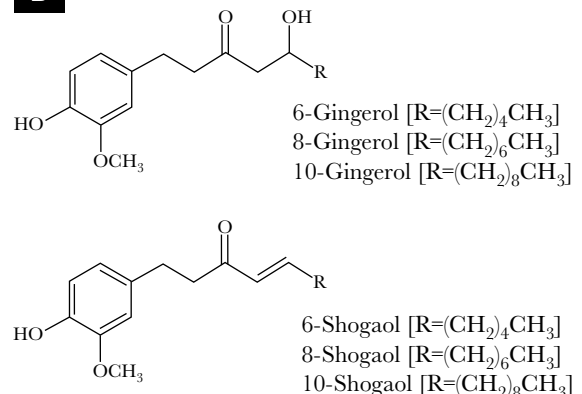


Figure 3. Biological activities (A) and main phenolic compounds of ginger (B). Illustration by the authors based on information from Mao *et al.* (2019).

Gingerols, the main metabolites of fresh ginger, are chemically known as phenolic β -hydroxyketones and are precursors of other bioactive compounds in ginger (Gopi *et al.*, 2016). Their importance lies in their antimicrobial potential and inhibition of biofilm formation (Kumar *et al.*, 2014; Kim *et al.*, 2015; Borges *et al.*, 2016). Shogaols are products of the dehydration of the β -hydroxyketone group of gingerols (Shukla *et al.*, 2019); therefore, they are more abundant in dried or dehydrated ginger. Shogaols are considered the metabolite with the greatest bioactive potential even though they are found in lower concentrations (Samwal *et al.*, 2010). Shogaols are thermodynamically more stable than gingerols at high temperatures (Hu *et al.*, 2011), an important characteristic when emphasizing on the extraction of a metabolite of interest. The amount and ratio of gingerol/shogaol will depend on the physical state of the rhizome, whether fresh (Jacotet-Navarro *et al.*, 2016; An *et al.*, 2020) or dried (Ok *et al.*, 2012; Guo *et al.*, 2017), post-harvest storage time (Sanwal *et al.*, 2010; Yeh *et al.*, 2014), among other factors.

The preparation of ginger extracts is affected by drying, maceration, and root extraction methods (Delasasso *et al.*, 2022), type and concentration of solvent used (Jacotet-Navarro *et al.*, 2016; Ko *et al.*, 2019; Shukla *et al.*, 2019; An *et al.*, 2020; Tanweer *et al.*, 2020), type of industrial processing, drying and extraction time and temperature, pH (Ok *et al.*, 2012), and the origin of the ginger crop (Yudthavorasit *et al.*, 2014; Ozola *et al.*, 2019).

The antimicrobial effect of ginger has been tested in various animal species. In mice, a modulatory effect of microRNAs contained in exosome-like nanomembranes from ginger on the intestinal microbial populations of the rodent was described (Teng *et al.*, 2018). In livestock species, antimicrobial effects have been evidenced in laying hens and broiler chickens, as well as a modulatory effect on intestinal microorganisms in quail (Dieumou *et al.*, 2009; Shanoon *et al.*, 2012; Salmanzadeh, 2015). In ruminants, *in vitro* studies demonstrated an antimicrobial effect on ciliated protozoa associated with methanogens (Kim *et al.*, 2012), as well as a reduction in total ruminal bacteria (Soroor and Moeini, 2015); however, studies using aqueous, ethanolic, and/or methanolic ginger extracts reported an increase in ruminal protozoa numbers in an *in vitro* assay with ruminal fluid from buffaloes (Patra *et al.*, 2010).

Regarding the effect of ginger on the reduction of ruminal CH₄ production, *in vitro* studies have shown that essential oils (Altınçekic *et al.*, 2021) and ginger powder (Soroor and Moeini, 2015; Kurniawati *et al.*, 2018; Khejornsart *et al.*, 2021; Gutiérrez-Fidencio *et al.*, 2023) can reduce the production of this gas by up to 27% and 46%, respectively, without significantly affecting other fermentation variables. These results were dose-dependent; however, no changes in ruminal CH₄ production were observed when ginger extracts were used (Patra *et al.*, 2010).

Optimal Dose of Ginger in the Diet of Ruminants

An optimal dose of ginger in the diet of ruminants for reducing ruminal methanogenesis has not been established, due to the variability of results among studies. This variability originates from the form in which ginger is incorporated and the processing it undergoes, which alters its chemical profile (see previous section). In this regard, ginger rhizome powder is the form that has shown the most promising results in reducing CH₄ in the rumen

(Soroor and Moeini, 2015; Khejornsart *et al.*, 2021; Altınçekiç *et al.*, 2021; Gutiérrez-Fidencio *et al.*, 2023), compared to other presentations such as extracts from the aerial part of the plant (Kim *et al.*, 2012), or ethanolic, methanolic, and/or aqueous extracts (Patra *et al.*, 2010), and essential oils (Kurniawati *et al.*, 2018) from the root. Gutiérrez-Fidencio *et al.* (2023) demonstrated through *in vitro* studies using ruminal fluid from cattle in southeastern Mexico that the inclusion of 5% to 40% ginger rhizome powder reduced CH₄ production by 45.9% and 52.1%, respectively, as lower concentrations had no effect. In fact, this study reported the highest CH₄ reduction compared to others conducted with ginger powder at inclusion rates of 7.5% (Khejornsart *et al.*, 2021) and between 15% and 30% (Soroor and Moeini, 2015). Although the effect on ruminal methanogenesis is promising and Gutiérrez-Fidencio *et al.* (2023) confirmed this effect using different ginger powders of various origins, further *in vivo* studies are needed before ginger powder can be recommended as a natural anti-methanogenic additive for ruminant livestock.

Efficient Utilization of the Qualities of Ginger Rhizome

To date, there are no studies identifying which metabolites or other components of ginger are responsible for its anti-methanogenic activity in ruminants. It is crucial to determine which molecules cause this effect in order to obtain consistent results with ginger—although this also applies to any foliage or plant-based additive. In the case of ginger, this will require chemical analysis of its phenolic compound profile using high-precision techniques such as HPLC. Additionally, it is important to consider that the composition of ginger's main metabolites changes depending on whether it is used fresh or dried. For example, in ginger juice, the most abundant metabolite is gingerol (Gopi *et al.*, 2016; Wohlmuth *et al.*, 2005), whereas dehydration transforms gingerol into shogaol (Ozola *et al.*, 2019; Teng *et al.*, 2019).

The gingerol and shogaol content in ginger (Ghasemzadeh *et al.*, 2018; Mao *et al.*, 2019; Dalsasso *et al.*, 2022) can also be affected by several factors, such as the genotype or variety (Wohlmuth *et al.*, 2005; Sanwal *et al.*, 2010; Pawar *et al.*, 2011; Salmon *et al.*, 2012), the cultivation origin (Yudthavorasit *et al.*, 2014; Ozola *et al.*, 2019), and agroclimatic conditions during cultivation, such as altitude, temperature, soil pH, and phosphorus availability (Sahoo *et al.*, 2023). Other factors include the plant's stage of maturity (Bailey-Shaw *et al.*, 2008), post-harvest storage time and conditions (Sanwal *et al.*, 2010; Yeh *et al.*, 2014), and finally the dehydration process, whether by heat or lyophilization (Ok and Jeong, 2012; An *et al.*, 2016). Identifying the agent responsible for the antimethanogenic effect would allow for the manipulation of certain cultivation conditions and/or extraction processes to optimize ginger's chemical profile.

Preference for Diets Containing Ginger

The presence of gingerols and shogaols gives ginger a sweet aroma that may be attractive to cattle (Ginane *et al.*, 2011), which is an advantage for its use as a feed additive in ruminants. However, these same metabolites also produce an astringent sensation that could affect intake (Wohlmuth *et al.*, 2005), since ruminants rely on their senses of taste and smell, in addition to sight and touch, to select their food (Harper *et al.*, 2016). Proposing

ginger rhizome powder as an additive for livestock feeding implies that it must be palatable to the animal; therefore, preference and feeding behavior tests will be necessary to assess the acceptance of ginger in the diet and thus determine its viability as an antimethanogenic additive for ruminants.

Perspectives on Understanding the Effect of Ginger on the Rumen Microbiota

The use of ginger in ruminant diets could have an effect on the rumen microbiota by impacting microbial populations directly involved in CH₄ synthesis or by altering the metabolism of other microorganisms, thereby reducing CH₄ precursor substrates. This could be studied using highly sensitive molecular techniques, such as digital droplet PCR (ddPCR), to analyze the proportions of microbial groups and/or subgroups (Ángeles-Mayorga *et al.*, 2022). On the other hand, for a more in-depth analysis, it is necessary to employ Next Generation Sequencing (NGS) tools, either through the generation of metaprofiles, which typically provide an overview of microorganisms from phylum to family (and sometimes genus) levels, with over 80% identification certainty using databases such as Greengenes (DeSantis *et al.*, 2006) or the Ribosomal Database Project (Cole *et al.*, 2014); or through untargeted sequencing approaches aimed at obtaining full metagenomes, which include the total DNA of the complex rumen fluid sample.

Therefore, analyzing the changes in microbial populations due to the effect of ginger and/or any of its components or metabolites would allow us to elucidate the cause of the anti-methanogenic effect. Ideally, the application of molecular and/or genomic tools would enable the evaluation of the effect of additives or dietary components in ruminants, not only ginger, which has been the focus of this review.

CONCLUSIONS

Ginger reduces ruminal methane production in cattle and sheep under *in vitro* conditions. It is more effective to provide ginger in the form of rhizome powder, with an appropriate inclusion rate of 5% in total mixed rations. However, the mechanism and the metabolite responsible for the anti-methanogenic effect of ginger are still unknown. This could be analyzed through HPLC chemical profiling, as well as molecular and genomic analyses of the rumen microbiota; the latter would help to understand the underlying basis of ginger's effect on ruminal methanogenesis. *In vivo* diet trials using ginger and preference tests will be necessary to propose the use of rhizome powder as an anti-methanogenic feed additive.

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Pectobacterium brasiliense, causal agent of rhizome rot in ginger (*Zingiber officinale* Roscoe)

Sosa-Pérez, Eligio¹; Luna-Martínez, Edith¹; Ortega-Acosta, Candelario¹; Rivera-Sosa, Martín¹; Aranda-Ocampo, Sergio^{1*}

¹ Colegio de Postgraduados. Posgrado de Fitosanidad-Fitopatología, Carretera México-Texcoco Km 36.5, Montecillo, Estado de México, México. C.P. 56264.

* Correspondence: arandasergio63@gmail.com

ABSTRACT

Objective: To isolate and identify the causal agent of ginger rhizome rot in Jalpan, Puebla and to evaluate its pathogenicity and *in vitro* sensitivity to bactericides.

Design/methodology/approach: Bacteria with similar morphology were isolated from ginger rhizomes with rot. Strain CPB04 was biochemically characterized and identified by 16S rRNA gene sequencing. *In vitro* sensitivity was evaluated with nine commercial bactericides and pathogenicity in ginger rhizomes and organs of 11 plant species by injection of a suspension containing 3×10^8 CFU mL⁻¹.

Results: The biochemical characterization of strain CPB04 showed similarity with *Pectobacterium*. Sequencing of the 16S rRNA gene of strain CPB04 identified it with 100% similarity to *Pectobacterium brasiliense* strain CP047495.1. *P. brasiliense* CPB04 was sensitive *in vitro* to copper formulations and resistant to kasugamycin. It caused organ rots in sweet potato (*Ipomoea batatas*), onion (*Allium cepa*), chili (*Capsicum annum*), jicama (*Pachyrhizus erosus*), tomato (*Solanum lycopersicum*), ginger (*Zingiber officinale*), potato (*Solanum tuberosum*) and radish (*Raphanus sativus*).

Implications of the study: *Pectobacterium brasiliense* is a highly virulent pathogen with potential to infect new hosts. Rot diseases are a global problem in agriculture. This is the first report in Mexico of *P. brasiliense* as a causal agent of rot in ginger.

Findings/conclusions: *Pectobacterium brasiliense* is the causal agent of ginger rhizome rot in Jalpan, Puebla. The tuberous root of jicama may be a new host of *Pectobacterium brasiliense*. *Pectobacterium brasiliense* is sensitive *in vitro* to copper oxychloride.

Keywords: *Pectobacterium brasiliense*, ginger, rot, 16S rRNA.

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INTRODUCTION

Ginger (*Zingiber officinale*) is a monocotyledonous plant belonging to the family Zingiberaceae. The underground rhizomes of ginger are consumed as fresh vegetables and spices, and have a long history due to their high economic, nutritional, and medicinal value (Chawla *et al.*, 2021). Globally, India is the leading ginger producer with 1,844,000 tons. Mexico ranks 21st in ginger production with 4,405 tons and 17th as an exporter with 1,673 tons (FAO, 2023).

In Mexico, ginger is mainly cultivated in the states of Oaxaca, Guerrero, and Puebla (SIAP, 2023). However, around 70% of the ginger consumed in Mexico is produced in the



northern Sierra of Puebla state, with an approximate production of 2,870 tons, highlighting the municipalities of Jalpan, Pantepec, and Xicotepéc as the main producers (SIAP, 2023). The most well-known and widely cultivated ginger in Mexico is common ginger (*Zingiber officinale*). Due to its high nutritional and medicinal value, the demand for production and consumption has increased in recent years (García *et al.*, 2023).

Globally, research on diseases affecting ginger cultivation is scarce compared to other agricultural crops. However, ginger is known to be highly susceptible to both bacterial and fungal diseases that affect the plant and its rhizome (Kyaw *et al.*, 2022; Meenu & Kaushal, 2017). Among these, rhizome rot caused by bacteria is a highly destructive disease that significantly impacts rhizome yield and postharvest quality in all countries where it has been reported (Huang *et al.*, 2020). Bacterial pathogens identified worldwide as causal agents of rhizome rot include *Enterobacter cloacae* in China and the United States (Liu *et al.*, 2021; Nishijima *et al.*, 2004; Zhao *et al.*, 2022); *Bacillus pumilus* and *Serratia marcescens* in China (Huang *et al.*, 2020; Peng *et al.*, 2013); *Erwinia chrysanthemi* in the United States (Stirling, 2002); and *Ralstonia solanacearum* in Thailand (Kyaw *et al.*, 2022).

In 2024, in the community of La Garza, municipality of Jalpan, Puebla, ginger producers were affected by rhizome rot both in the field and in storage, with an estimated incidence of 30%. This disease is currently considered the most important one, causing significant economic losses for local producers. Infected rhizomes initially exhibit external water-soaked lesions, followed by internal tissue rot. Based on the characteristics of these symptoms, this study assumes that the rhizome rot is caused by bacteria. To date, no research has been conducted on diseases affecting ginger cultivated in Mexico, particularly on rhizome rot. The objective of this study was to isolate and identify the causal agent of ginger rhizome rot in Jalpan, Puebla, Mexico, and to evaluate its pathogenicity and *in vitro* sensitivity to bactericides.

MATERIALS AND METHODS

Sample Collection

In 2024, four composite samples, each consisting of five ginger rhizomes showing symptoms of rot (Figure 1A), were collected in the community of La Garza, municipality of Jalpan, located in northern Puebla state (20° 23' 24" N and 98° 00' 54" W) (Figure 1B). The samples were analyzed at the Phytopathogenic Bacteria Laboratory of the Colegio de Postgraduados, Montecillo campus, in Texcoco, State of Mexico.

Bacterial Isolation

Ginger rhizomes were surface-disinfested with 70% ethanol for 1 minute, followed by three rinses with sterile distilled water. The rhizomes were then cut longitudinally, and 0.5 cm segments were taken from the internal tissue at the interface between healthy and diseased areas showing rot symptoms. The tissue segments were macerated in a mortar with 500 μ L of sterile distilled water, and 20 μ L of the suspension were plated onto Petri dishes containing Wilbrink's agar medium (Koike, 1965). The plates were incubated at 28 °C for 48 h. Individual bacterial colonies were purified from the resulting growth and

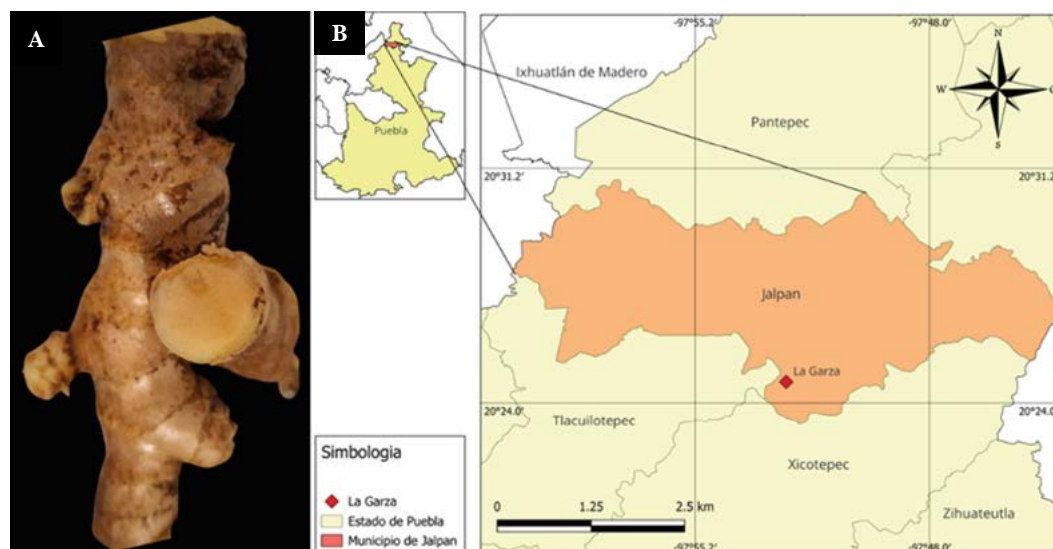


Figure 1. (A) Ginger rhizome with rot symptoms collected from the community of La Garza; (B) Municipality of Jalpan, Puebla.

preserved at $-80\text{ }^{\circ}\text{C}$ in nutrient broth with 20% glycerol. From these bacterial isolates, strain CPB024 was selected for further studies.

Physiological and Biochemical Characterization

The physiological and biochemical characterization of strain CPB024 was carried out following the protocols described by Borkar (2017) and Schaad *et al.* (2001). *In vitro* sensitivity to bactericides was evaluated using a modified agar diffusion method (Liu and Filiatrault, 2020): 100 μL of a bacterial suspension containing 10 CFU mL^{-1} of strain CPB024 were inoculated onto square Petri dishes (120 \times 120 mm) (Thermo Fisher Scientific, USA) containing nutrient agar medium (BD Bioxon, Mexico). The inoculum was evenly distributed on the surface of the culture medium using a Drigalski loop. Sterile filter paper discs (0.5 cm in diameter) previously soaked in bactericide solutions were then placed on the agar surface, with three replicates each. The plates were incubated for 72 h at 28 $^{\circ}\text{C}$. The sensitivity of strain CPB024 was determined by the formation of an inhibition zone around the filter paper disc containing the bactericide. Seven bactericide formulations were evaluated at the label-recommended dosage: Cobrezate[®] (50% copper oxychloride + 36% mancozeb); Intermicin 500[®] (0.235% oxytetracycline + 71.8% tribasic copper sulfate + 2.2% streptomycin); Kasumin[®] (2.3% kasugamycin); Oxicob[®] (85% copper oxychloride); Quatz[®] (quaternary ammonium compound); Sagol[®] (22% copper oleate); and Copper Sulfate.

Molecular and Phylogenetic Identification

Genomic DNA was extracted using the 2% CTAB method (Doyle and Doyle, 1990) from pure cultures grown on nutrient agar for 48 h at 28 $^{\circ}\text{C}$. Molecular identification was performed by amplification and partial sequencing of the 16S rRNA gene using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R

(5'-GGTTACCTTGTTACGACTT-3') (Heuer *et al.*, 1997). Polymerase chain reactions (PCR) were carried out under the conditions described by Mejía-Sánchez *et al.* (2019) using a Touch C1000 thermal cycler (Bio-Rad). PCR products were sequenced by Macrogen Inc. (South Korea). Consensus sequences were compared with those in the National Center for Biotechnology Information (NCBI; <https://www.ncbi.nlm.nih.gov/>) database using the BLASTn tool. A phylogenetic tree was constructed based on aligned sequences using the maximum likelihood method, applying the Tamura-Nei evolutionary model and 1,000 bootstrap replicates to assess branch robustness. Phylogenetic analysis was performed using MEGA X software.

Pathogenicity Tests

Pathogenicity tests were conducted using strain CPB024 isolated from ginger rhizomes exhibiting rot symptoms. Ginger rhizomes were surface-disinfested sequentially with soapy water, 70% ethanol, and three rinses with sterile distilled water. Subsequently, rhizomes were inoculated by injecting 50 μL of a bacterial suspension adjusted to 3×10^8 CFU mL^{-1} using the McFarland standard scale. In addition to ginger, the pathogenicity of strain CPB024 was evaluated following the same disinfection and inoculation protocol described above on organs of plant species belonging to 10 botanical families: Amaryllidaceae [onion bulb (*Allium cepa*)], Apiaceae [carrot tuberous root (*Daucus carota*)], Brassicaceae [radish tuberous root (*Raphanus sativus*)], Cactaceae [pitahaya stem (*Hylocereus undatus*)], Convolvulaceae [sweet potato tuber (*Ipomoea batatas*)], Fabaceae [jicama tuberous root (*Pachyrhizus erosus*)], Marantaceae [pencil plant rhizome (*Goepfertia ornata*)], Rosaceae [apple fruit (*Malus domestica*)], Solanaceae [chili pepper fruit (*Capsicum annum*) and tomato fruit (*Solanum lycopersicum*)], potato tuber (*Solanum tuberosum*), and Zingiberaceae [turmeric rhizome (*Curcuma longa*) and shell ginger (*Alpinia zerumbet*)].

The treatments were maintained in a humid chamber at room temperature (~ 24 °C). Each treatment was performed with three replicates. In the control, the same volume of sterile distilled water was injected instead of the bacterial inoculum.

RESULTS AND DISCUSSION

Bacterial Isolation

From the four ginger samples with rot symptoms collected from the study region, colonies with similar morphological characteristics (circular, convex, mucoid, and moist-appearing colonies) were most frequently isolated on Wilbrinks agar medium. Among these, strain CPB024 was selected for further studies.

Physiological and Biochemical Characterization

Strain CPB024 was Gram-negative, catalase positive, oxidase negative, exhibited oxidative and fermentative metabolism of glucose, reduced nitrates, produced H_2S ; it utilized arabinose, mannose, and sucrose as carbon sources and showed pectinolytic activity on potato slices (Table 1). This metabolic profile is characteristic of bacteria in the Enterobacteriaceae family, which includes the genus *Pectobacterium* (Borkar *et al.*, 2017; Czajkowski *et al.*, 2015; Schaad *et al.*, 2001), a pathogen with a broad host range

Table 1. Physiological and biochemical characterization, pathogenicity, and *in vitro* sensitivity to bactericides of *Pectobacterium brasiliense* CPB024 isolated from ginger rhizomes with rot symptoms.

Test	Strain		Pathogenicity CPB024			In vitro sensitivity CPB024
	CPB024 Ginger ¹	PbTet5 Cactacea ²	Family	Specie	Organ	
Gram stain	-	-	Amaryllidaceae	Onion (<i>Allium cepa</i>)	Bulb	+ Cobrezate (Copper oxychloride + Mancozeb)
Levana	-	ND	Apiaceae	Carrot (<i>Daucus carota</i>)	Tuberous root	+ Intermicin 500 (Oxytetracycline + Tribasic copper sulfate + Streptomycine)
Oxidase	-	-	Brassicaceae	Rábano (<i>Raphanus sativus</i>)	Tuberous root	+ Oxibac (Copper oxychloride)
Arginine	-	-	Cactaceae	Pitahaya (<i>Hylocereus undatus</i>)	Stem	- Kasumin (Kasugamycin)
Catalase	+	+	Convolvulaceae	Sweet potato (<i>Ipomoea batatas</i>)	Tuberous root	+ Quatz (Quaternary ammonium)
Nitrate reduction	+	ND	Fabaceae	Jicama (<i>Pachyrhizus erosus</i>)	Tuberous root	+ Sagol (Cupric oleate)
Starch hydrolysis	-	ND	Marantaceae	Calathea (<i>Goepertia ornata</i>)	Rhizome	- Cupric sulfate
Oxidative/fermentative metabolism	+/+	+/+	Rosaceae	Apple (<i>Malus domestica</i>)	Fruit	-
Phosphatase	-	-		Chili (<i>Capsicum annuum</i>)	Fruit	+
Gelatin hydrolysis	+	ND	Solanaceae	Tomato (<i>Solanum lycopersicum</i>)	Fruit	+
Gas production	-	ND		Potato (<i>Solanum tuberosum</i>)	Tuber	+
Indole	-	-		Turmeric (<i>Curcuma longa</i>)	Rhizome	-
Growth at 7% NaCl	+	+	Zingiberaceae	Ginger (<i>Zingiber officinale</i>)	Rhizome	+
Growth at 8% NaCl	+	ND		Shell ginger (<i>Alpinia zerumbet</i>)	Rhizome	-
Growth at 36 °C	+	+				
H ₂ S production	+	ND				
Esculin	+	ND				
-Methyl glucoside	-	+				
Arabinose	+	ND				
Cellobiose	+	ND				
D-glucose	+	ND				
Dulcitol	-	ND				
Inositol	+	ND				
Lactose	+	+				
Maltose	-	+				
Mannitol	+	ND				
Mannose	+	ND				
Melezitose	-	ND				
Melibiosa	+	ND				
Sorbitol	-	-				
Sucrose	+	ND				
Trehalose	+	+				
Xylose	+	ND				

¹ *Pectobacterium brasiliense* CPB024 isolated from ginger.

² *Pectobacterium brasiliense* Tet5 isolated from *Neobuxbaumia tetetzo*. Source: Mejía-Sánchez *et al.* (2019).
ND=Not determined.

affecting at least 35% of angiosperms (Toth *et al.*, 2021), and is distributed worldwide across diverse ecological niches (van der Wolf *et al.*, 2021). Additionally, strain CPB024 showed 87% similarity with the metabolic profile of strain Tet5 (accession No. MF403054) of *Pectobacterium carotovorum* subsp. *brasiliense* isolated from *Neobuxbaumia tetetzo* in Puebla, Mexico (Mejía-Sánchez *et al.*, 2019); which was reclassified as *Pectobacterium brasiliense* (Portier *et al.*, 2019) (Table 1).

Molecular and Phylogenetic Identification

The partial amplification of the 16S rRNA gene of strain CPB024, isolated from ginger rhizome, was deposited in GenBank under accession number PV017467. BLASTn analysis of this sequence showed 100% identity with various sequences belonging to *Pectobacterium brasiliense*, among which is sequence CP020350 (strain 1692), identified as the causal agent of cucumber fruit rot and considered highly virulent in a wide range of plant species in Shanxi Province, China (Meng *et al.*, 2017), and phylogenetically related to the strain isolated from ginger in this study (Figure 2).

Pathogenicity Tests

Inoculation of strain CPB024, isolated from ginger with rot symptoms (identified as *Pectobacterium brasiliense* in this study), caused rot in ginger rhizomes seven days after inoculation (dai) (Figure 3). The symptoms were similar to those observed in rhizomes from Jalpan, Puebla. No symptoms were observed in the control rhizomes injected with sterile distilled water. The bacteria re-isolated from the rotted rhizomes exhibited the same morphological characteristics as the inoculated strain, fulfilling Koch's postulates.

In ginger, rhizome rot has been associated with infections by the fungi *Fusarium* spp. and *Pythium* spp. (Meenu and Kaushal, 2017); however, *Pectobacterium brasiliense* is considered the most destructive and important pathogen causing ginger rhizome rot, leading to

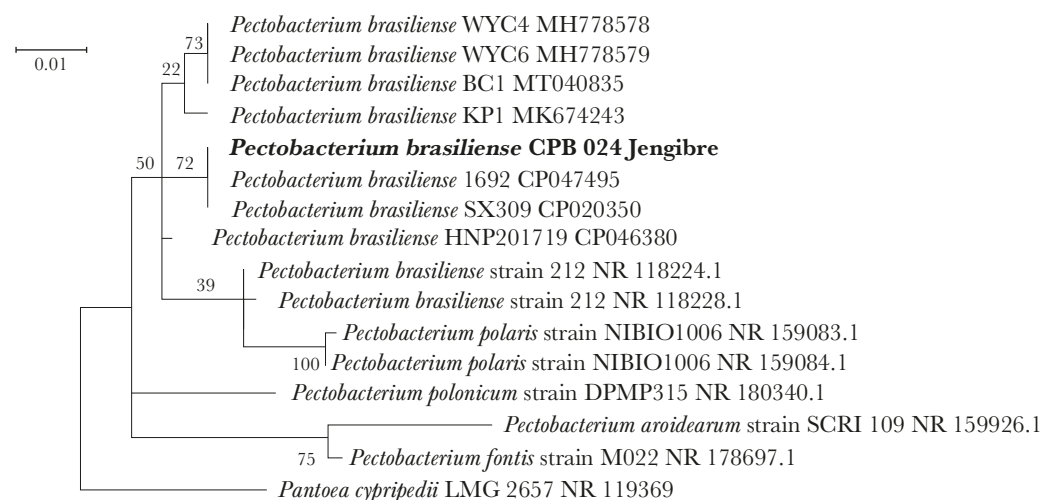


Figure 2. Phylogenetic tree based on the partial sequencing of the 16S rRNA gene of the genus *Pectobacterium* using the Maximum Likelihood method, constructed in MEGA X, with 1000 bootstrap replicates. The ginger sequence from this study is highlighted in bold (GenBank accession number PV017467). *Pantoea cyripedii* was used as the outgroup.

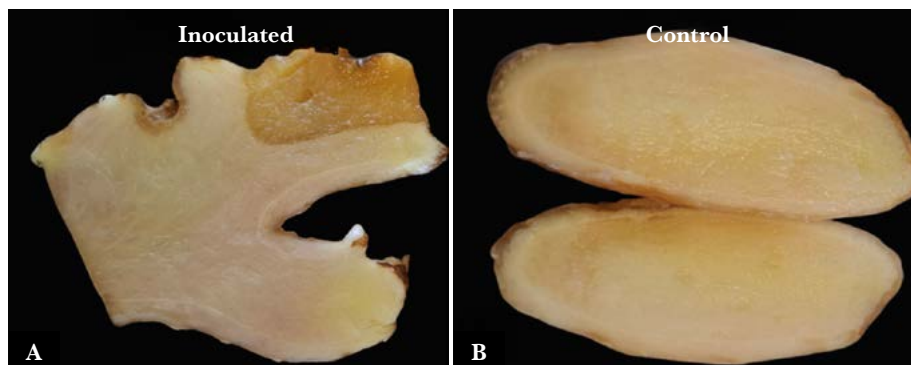


Figure 3. Rot symptoms on ginger rhizome at 7 days post-inoculation (dpi) with strain CPB024 of *Pectobacterium brasiliense*.

production losses ranging from 50 to 90% (Wang *et al.*, 2022, 2024). The identification of *P. brasiliense* as the causal agent of ginger rhizome rot in Jalpan, Puebla, is consistent with the first report of *P. brasiliense* causing ginger rhizome rot in China (Wang *et al.*, 2022). Until now, *P. brasiliense* had been reported in Mexico causing rot in the cactus *Neobuxbaumia tetetzo* in Zapotitlán de las Salinas, Puebla (Mejía-Sánchez *et al.*, 2019; Vargas-Peralta *et al.*, 2021).

Inoculation of *Pectobacterium brasiliense* CPB024 on plant organs from 10 different families caused rot symptoms at 2 days post-inoculation (dpi) in tomato, chili pepper, onion, sweet potato, jicama, radish, and potato; however, no symptoms were observed in apple, pitahaya, carrot, calathea, shell ginger, and turmeric (Table 1) (Figure 4).

Until 2021, *P. brasiliense* had been reported as a pathogen on 19 plant species across 10 families; since then, its host range has expanded to include additional species within the families Aizoaceae, Cucurbitaceae, and Zingiberaceae (Park *et al.*, 2023a; Song *et al.*, 2023; Wang *et al.*, 2022), making it an emerging global problem (Oulghazi *et al.*, 2021).

In this study, the rot caused by the inoculation of *P. brasiliense* CPB024 is consistent with results reported in other investigations involving *P. brasiliense* strains inoculated in tomato, chili pepper, and potato (Duarte *et al.*, 2004; Mejía-Sánchez *et al.*, 2019), as well as

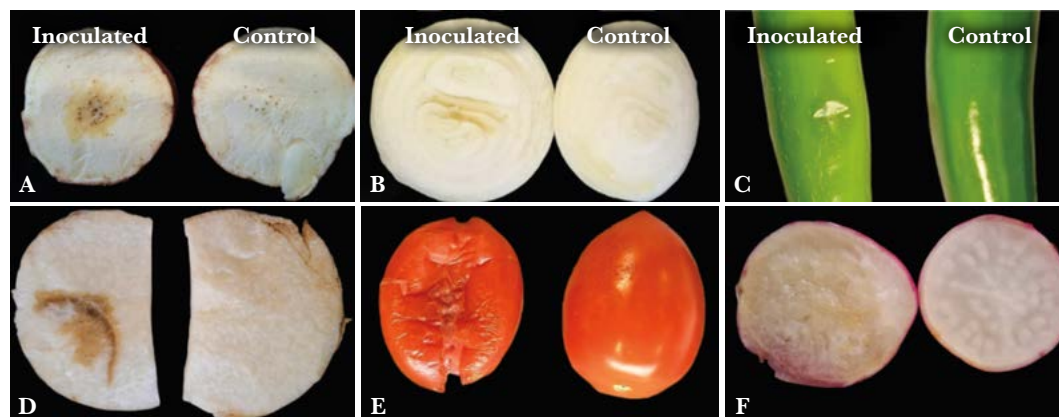


Figure 4. Rot symptoms in: A) Sweet potato, B) Onion, C) Serrano chili, D) Jicama, E) Tomato, and F) Radish at 48 days post-inoculation (dpi) with *P. brasiliense* CPB024.

in radish, onion, and sweet potato (Li-ping *et al.*, 2020; Mejía-Sánchez *et al.*, 2019; Park *et al.*, 2023b). However, to our knowledge, there are no reports of rot caused by *P. brasiliense* inoculation in the tuberous root of jicama.

The worldwide distribution of *Pectobacterium brasiliense* suggests that it adapts to both tropical and temperate climates as well as high humidity conditions (Wang *et al.*, 2022). Therefore, the prevailing environmental conditions in Puebla and other states where ginger is cultivated could favor the establishment and spread of this pathogen in Mexico.

In Jalpan, Puebla, the origin of the planted rhizomes as well as the source of *P. brasiliense* inoculum are unknown. Previous studies have documented that *P. brasiliense* has been isolated from water and the rhizosphere of weeds, and can be easily transmitted to other cultivation areas through irrigation systems, soil, and during the storage of planting material (Oulghazi *et al.*, 2021). In Mexico, Mejía-Sánchez *et al.* (2021) detected *P. brasiliense* in the intestines of larvae and adults of the insect *Lagocheirus lugubris* Dillon (Coleoptera: Cerambycidae) and in adults of *Glyphidops flavifrons* (Bigot) (Diptera: Neriidae), both closely associated with the rot disease of the columnar cactus Tetecho in Zapotitlán, Puebla; highlighting the possible role of insects as potential vectors of *P. brasiliense*. Other factors identified that favor infection in other *Pectobacterium* species include saturated or flooded soils, wounds caused to planting material during harvest, and infection by other soil pathogens such as fungi and nematodes (Stirling *et al.*, 2002; Charkowski, 2018).

Interestingly, in this study, the control ginger rhizomes injected with sterile distilled water showed rot symptoms similar to those inoculated (7 days after the start of the assay); from the tissue of the rotten rhizomes, colonies with the same morphological and metabolic characteristics of *P. brasiliense* were isolated. Studies on the endophytic microbiota in ginger rhizomes with rot symptoms and asymptomatic rhizomes revealed the presence of bacteria from the Enterobacteriaceae family, including *P. brasiliense*, in both types of rhizomes; however, a higher relative abundance of these populations was found in the rotten rhizomes compared to the asymptomatic ones (Huang *et al.*, 2022; Wang *et al.*, 2024). This suggests that *P. brasiliense* could be an endophyte in ginger rhizomes, which would be relevant in the *P. brasiliense*-ginger pathosystem due to the asexual propagation of this crop and the use of rhizomes for new plantations in other cultivation areas.

Currently, no effective control method against *P. brasiliense* has been documented, making it necessary to develop management strategies against this pathogen. Control recommendations based on copper sprays have been frequently suggested for managing diseases caused by *Pectobacterium* spp., along with other strategies such as the use of healthy planting material, sanitary practices, and biological control (Azaiez *et al.*, 2018; Charkowski, 2018). In this study, *P. brasiliense* CPB024 was sensitive *in vitro* to the bactericidal formulations Intermicin 500 (Oxytetracycline+tribasic copper sulfate+streptomycin), Cobrezate (Copper oxychloride+Mancozeb), Oxicob (Copper oxychloride), and Quatz (Quaternary ammonium) (Table 1). Quaternary ammonium salts are an emerging group of antibacterial agents used as disinfectants that inhibit bacterial biofilm formation on surfaces (Nadagouda *et al.*, 2022); however, they have been little studied in agriculture except as disinfectants in greenhouse structures. In contrast, the bactericidal activity of copper is widely documented globally in agriculture (Charkowski, 2018; Czajkowski *et al.*,

2015). Therefore, it is important to evaluate the biological effectiveness in the field of the copper-based formulations identified in this study for the control of *P. brasiliense* in ginger cultivation in Jalpan, Puebla. Likewise, it is necessary to deepen the understanding of the *P. brasiliense*-ginger pathosystem, particularly regarding the epidemiology, distribution, and genetic variability of this pathogen, as well as the susceptibility of the cultivated ginger varieties. This is crucial for the development of efficient control strategies against *P. brasiliense* in Puebla and other states in Mexico where ginger is grown.

CONCLUSIONS

Pectobacterium brasiliense is the causal agent of rhizome rot in ginger plants from Jalpan, Puebla. Plant organs of species belonging to the Solanaceae family are more susceptible to rot caused by *P. brasiliense*. Jicama, which belongs to the Fabaceae family, may be a new potential host of *P. brasiliense*. *P. brasiliense* is resistant to antibiotics such as kasugamycin and sensitive to copper-based compounds; among them, formulations containing copper oxochloride may be an effective tool for managing *P. brasiliense* in ginger crops in Mexico. This is the first report of *P. brasiliense* causing rhizome rot in ginger in Mexico.

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Comparison of models for estimating the planted area of *Agave* spp. for mezcal production in Mexico

Cruz-Ramírez, Angel Saul¹; Martínez-Gutiérrez, Gabino Alberto^{2*}; Morales-García, Isidro²; López-García, María del Rosario³; Castillo-Martínez, Carlos Roman¹

¹ Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias y Forestales, Centro Nacional de Investigación Disciplinaria en Conservación y Mejoramiento de Ecosistemas

² Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Oaxaca.

³ Secretaría del Trabajo y Previsión Social.

* Correspondence: gamartinezg@ipn.mx.

ABSTRACT

Objective: The aim of this study was to compare forecasting models for the planted area of agave mezcalero from 2023 to 2027, using data from the 1982-2022 period through autoregressive integrated moving average (ARIMA) models, in order to promote the efficient management of natural resources.

Design/methodology/approach: To validate the forecasting efficiency, an out-of-sample comparison was conducted between ARIMA models and simple forecasting methods such as the mean and naïve approaches.

Findings: The results identified a growth scenario for the planted area of agave mezcalero during the 2023-2027 period. The most accurate model, the autoregressive (4,0,0), projected that the maximum planted area would be reached in 2024, ranging from 22,723 to 60,280 hectares, followed by a gradual decline starting in 2025.

Research limitations/implications: The predictions are constrained by the availability and quality of the databases. The main limitation faced by the study was the lack of historical data.

Originality/value/conclusions: It is concluded that the ARIMA model can be an efficient tool for estimating the planted area of agave intended for mezcal production. The cultivation of agave mezcalero is currently in a context of overproduction. The information generated may be useful for planning and investment decisions by the industry, agave producers, and government authorities.

Keywords: ARIMA, *Agave angustifolia*, agricultural forecasting models, time series, non-timber forest products.

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INTRODUCTION

In Mexico, the planted area (PA) of espadín agave (*Agave angustifolia* Haw.) for mezcal production is experiencing unprecedented growth (Lira *et al.*, 2022a; Olvera-Vargas *et al.*, 2022). In 2023, the Agrifood and Fisheries Information Service (SIAP, by its acronym in Spanish) reported a PA of 24,506 hectares nationwide (SIACON-SADER, 2024). This represents a 69% increase compared to 2018, when 14,460 hectares were reported.

The Mexican Regulatory Council for Mezcal Quality (COMERCAM, by its acronym in Spanish) publishes a statistical report presenting data from its affiliated members. In 2023, it reported 64.79 million agave plants nationwide, representing a 152% increase compared to 2020 (25.66 million plants) (COMERCAM, 2024). Additionally, it provided data on the “registered area” with agave, which includes various types of forest vegetation and therefore differs from the planted area (PA) reported by SIAP.

Mezcal production represents the main value-added product derived from *Agave angustifolia* Haw. For this reason, it is identified in SIAP’s publications as agave mezcalero (AM) or Agave espadín, a name that refers to the elongated and pointed shape of its leaves. AM has a rich cultural history and, over the past fifteen years, has become an economic development opportunity for marginalized communities in Mexico (Fonseca & Chalita, 2021; Martínez *et al.*, 2014). This crop is essential for small-scale artisanal farming systems: according to the report published by COMERCAM (2024), 125,000 families earned income from mezcal production in 2023.

However, studies on the agave mezcalero value chain (VC) highlight issues related to competitiveness (Espejel *et al.*, 2019; Moctezuma-López *et al.*, 2023), organization (Martínez *et al.*, 2014; Sánchez-Gómez *et al.*, 2022), market dynamics (Lira *et al.*, 2022a; García-Vásquez *et al.*, 2018), deforestation, and natural resource pollution (Antonio *et al.*, 2017; Palma *et al.*, 2016). It is worth noting the significant presence of small-scale producers and the predominance of communal land tenure as key characteristics of this value chain (González *et al.*, 2023; Lira *et al.*, 2022b).

The *Agave* genus has generated significant economic benefits in the regions where it is cultivated (Moctezuma-López *et al.*, 2024; Landa-Vidal *et al.*, 2023). However, over the past 40 years, high uncertainty has arisen due to price fluctuations (Antonio & Terán, 2008; Palma *et al.*, 2016). Sharp changes in prices between periods have caused substantial financial losses for producers involved in this crop (Martínez *et al.*, 2014).

The increase in monoculture plantations of *Agave angustifolia* has led to a higher incidence of pests and diseases (Figueroa-Castro *et al.*, 2017). This is mainly caused by the improper use of agrochemicals and the lack of experience among new producers, whose numbers increase every year (Rodríguez *et al.*, 2020; COMERCAM, 2024). Additionally, negative socioeconomic and environmental impacts have been documented in the regions where this crop is developed, such as the decline in the price of agave piña (Palma *et al.*, 2016), soil erosion due to planting in rows along the slope direction (Lira *et al.*, 2022b), and deforestation to establish new cultivation areas (Antonio *et al.*, 2017; Antonio & Smit, 2012), among others. However, statistically based information on future changes in the growth or trend of the PA of *A. angustifolia* remains scarce.

Due to the economic and environmental importance of agave, it is essential to create conditions that ensure it remains profitable for small- and medium-scale agave mezcalero producers and the industrial sector involved in this value chain (Valencia-Sandoval *et al.*, 2020; Camacho-Vera *et al.*, 2021; Cervantes-Luna *et al.*, 2022). One possible solution to the overproduction of raw material could be agroforestry systems for establishing new plantations (Antonio *et al.*, 2017). These agroforestry systems reflect traditional practices inherent to peasant culture.

The establishment of new plantations, whether agroforestry or monoculture systems, must be planned taking into account the trends in demand and availability of AM (Martínez *et al.*, 2014). The planning aims to ensure that agroforestry systems, although a viable option, are implemented sustainably and efficiently (Antonio *et al.*, 2017). The same applies if the choice continues to favor monoculture production systems.

The years required to reach maturity in agave cultivation (6-8 years) and the species cultivated contribute to increased financial risk linked to fluctuations in the price of the “piña” as raw material (Antonio *et al.*, 2017; Valencia-Sandoval *et al.*, 2023). If trends in the expansion of the PA and thus the supply of raw material are unknown, the stakeholders involved may not be prepared to respond to market changes, potentially facing severe economic losses and continuing to impact the vegetation in the territories where agave mezcalero is cultivated. Therefore, the objective of this research was to compare autoregressive integrated moving average (ARIMA) models for estimating the growth of the planted area with agave mezcalero in Mexico, based on statistical data, to promote efficient management of the natural resources involved in this value chain.

MATERIALS AND METHODS

Characteristics of the agave mezcalero planted area database

The PA of AM reported by SIAP includes information on different agave species, both cultivated and wild (*Agave* spp), with Agave espadín (*A. angustifolia* Haw.) being the predominant species in plantations. The SIAP methodology for data collection on agave is based on a monitoring process that involves agricultural authorities, beneficiaries of agricultural programs, and key producers (SIAP, 2019). SIAP publicly provides only the historical annual databases for the PA (SIACON-SADER, 2024). In this study, the annual data for PA were used to perform a time series analysis.

Statistical Analysis

The study was conducted at the national level using a database covering the period from 1982 to 2022. The time series data correspond to the variable PA, measured in hectares (ha), and were obtained from the Agro-Food Information System for Consultation (SIACON-SADER). The dataset was divided into two time series: a training period spanning 1982 to 2014 and a validation period from 2015 to 2022.

Data were analyzed using R software version 4.1.2 (R Core Team, 2023) employing autoregressive integrated moving average (ARIMA) models, following the procedure described by Hyndman & Athanasopoulos (2021). This method was selected because it allows estimation with univariate data. This allows to optimize resources by not requiring the collection of information on other variables that might affect the variable of interest in the study.

Stationarity of the series was verified using the Augmented Dickey-Fuller (ADF) and Kwiatkowski-Phillips-Schmidt-Shin (KPSS) tests. The ARIMA methodology is based on autoregressive (AR) models (p), moving average (MA) models (q), and autoregressive moving average (ARMA) models (p, q) (Box *et al.*, 2016). If, in addition to these components,

the model includes an integration component (d), it is called an ARIMA (p, d, q) model (Shumway & Stoffer, 2019).

To understand the behavior of the data series, an exploratory analysis was conducted, including the creation of a line plot as well as autocorrelation function (ACF) and partial autocorrelation function (PACF) plots. The series was identified as non-stationary and stationarity was induced through logarithmic transformation (West, 2022) and double differencing (Shumway & Stoffer, 2017). Stationarity tests were performed on the differenced series using the ADF and KPSS tests. Once the series was identified as stationary, the optimal ARIMA model was selected using the Auto ARIMA algorithm in R (Hyndman & Khandakar, 2008).

The transformed model was compared with another model without logarithmic transformation but with the same level of differencing and autoregressive order identified by the Auto ARIMA algorithm procedure in R. Additionally, a manually selected model was included to compare its accuracy with the previous models. Following the manual procedure described by Hyndman & Athanasopoulos (2021), the autoregressive order (p) and moving average order (q) were identified using the ACF and PACF functions and plots. The Akaike Information Criterion (AIC) was used as the selection measure to choose between the manually identified models and those selected by the Auto ARIMA function algorithm, opting for the model with the lowest AIC value.

Additionally, as a reference point, a comparison was made with two simple forecasting methods: the mean and Naïve methods. The performance of the five models was evaluated with out-of-sample data through accuracy tests for an eight-year forecast period. Parameter estimation was carried out using the conditional least squares estimation method. This method is one of the most popular in statistical inference due to its accuracy and reliability in data analysis (Harring & Harring, 2022). To verify the suitability of the estimated model for the analyzed data, residuals were evaluated using a line plot, an ACF plot, a residual histogram, and the Ljung-Box test (Box *et al.*, 2016).

A forecast was made for the years 2023-2027 using the model with logarithmic transformation and two differencing steps, which showed the highest accuracy according to the statistical tests performed. According to Shumway & Stoffer (2017), transforming the data can cause issues when interpreting the results. For this reason, an adjusted ARIMA model was estimated based on the information obtained in the previous steps for the components of the ARIMA model. This last model did not apply transformation to the original data in order to establish a reference point for evaluating the back-transformation process in the transformed time series. Subsequently, upper confidence intervals were estimated for the predictions.

RESULTS AND DISCUSSION

Stationarity analysis

The training data series showed a positive trend during the period 1982-2014 (Figure 1). Stationarity tests were applied to this series. In the Augmented Dickey-Fuller (ADF) test, whose null hypothesis states the presence of a unit root (it is non-stationary), a p-value of

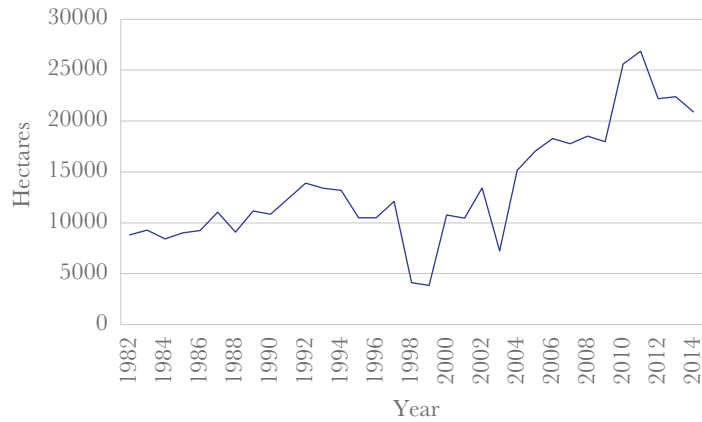


Figure 1. Training data series for the ARIMA model, period 1982-2014, of the planted area of agave mezcalero in Mexico.

0.8259 was obtained, which is greater than 0.05. Therefore, the null hypothesis was not rejected, indicating that the series is non-stationary.

In the Kwiatkowski-Phillips-Schmidt-Shin (KPSS) test, whose null hypothesis states that the series is stationary, the test statistic was 0.6178. This value is below the critical value at the 1% significance level (0.739), but above the critical values at the 2.5% (0.574), 5% (0.463), and 10% (0.347) levels. Consequently, the null hypothesis was not rejected, suggesting that the series can be considered stationary at the 1% significance level.

Since the results of the ADF test indicated that the series was non-stationary, natural logarithm transformations and differencing were applied to induce stationarity prior to fitting the ARIMA model. After the first differencing of the logarithmic series, the ADF test still indicated non-stationarity, while the KPSS test suggested the opposite.

A second-order differencing was therefore applied to the series. Following this procedure, both tests (ADF and KPSS) confirmed that the series was stationary (Figure 2). Subsequently, the automatic ARIMA model selection process was carried out using the Auto ARIMA function in R with the transformed data.

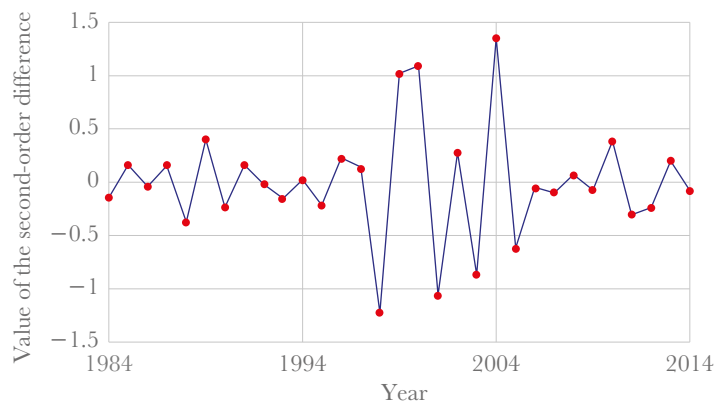


Figure 2. Second-order difference of the natural logarithm of the planted area of agave mezcalero in Mexico, period 1982-2014.

Model Selection

The ARIMA (4,0,0) model with zero mean (*i.e.*, no intercept included) presented the lowest AIC value. This model suggests significant autocorrelations up to four years back in the time series. To validate these results, the model's performance was evaluated using out-of-sample data and forecast accuracy tests.

To obtain forecasts in the original units of the time series, the inverse of the transformation previously applied to the data was performed. In order to assess the benefit of the logarithmic transformation on model accuracy, it was compared to an ARI (4,2,0) model. This model includes four lags and second-order differencing, but without applying a logarithmic transformation to the data.

According to Hyndman & Athanasopoulos (2021), ARIMA model components can be manually selected based on ACF and PACF plots. Following this approach, the models AR (2,0,0) (AIC=44.65), ARMA (2,0,2) (AIC=36.64), and ARMA (2,0,1) (AIC=34.35) were identified. The AIC values of these models were compared with the AR (4,0,0) model selected by the Auto ARIMA function, which had an AIC of 35.29. Although the AR (4,0,0) model showed a lower AIC than the first two models, the ARMA (2,0,1) model had a slightly lower AIC. Therefore, the ARMA (2,0,1) model was also included in the out-of-sample comparison.

Out-of-sample comparison

The out-of-sample evaluation of the ARIMA model's efficiency was conducted for the 2015-2022 period. The performance of the AR (4,0,0) model was compared against an ARI (4,2,0) model and an ARMA (2,0,1) model, as described in the previous section. The comparison with simple forecasting methods (SFMs) was used as a reference framework.

SFMs can outperform more complex models in certain contexts, such as financial indices or stock market data, according to Hyndman and Athanasopoulos (2021). The AR, ARMA, and ARI models were compared to the SFMs: Naïve (the last observed value) and mean (Figure 3). The estimation results indicated that the AR model better captured the behavior of the PA.

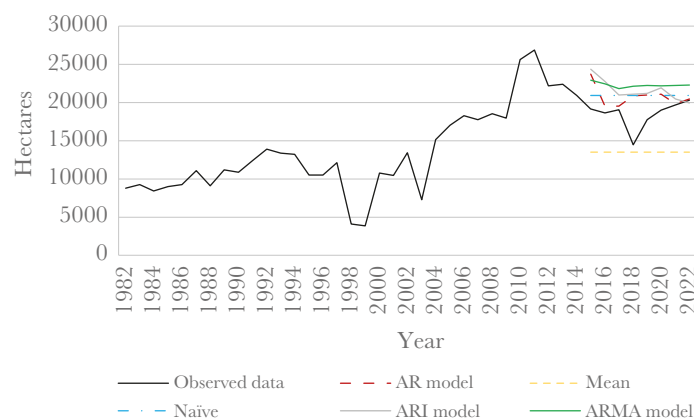


Figure 3. Comparison of forecasting methods for the planted area of agave mezcalero in Mexico, period 2015-2022.

Forecast accuracy was evaluated based on genuine forecasts (Box *et al.*, 2016; López-García *et al.*, 2022) using indicators such as: root mean square error (RMSE), mean absolute error (MAE), mean absolute percentage error (MAPE), mean absolute scaled error (MASE), autocorrelation of residuals at lag 1 (ACF1), and Theil's U statistic. For the mean error (ME) and the mean percentage error (MPE), the smallest deviation from zero was considered (R Core Team, 2023). The AR (4,0,0) model deviated the least from the actual observed data, obtaining six of the eight most efficient values in the accuracy tests (Table 1).

Forecast 2023-2027

The AR (4,0,0) model was applied to the transformed data for the period 1982-2022. The ADF test applied to the transformed series yielded a p-value of 0.01, allowing rejection of the null hypothesis and indicating stationarity. The KPSS test returned a statistic of 0.061, which is below the critical values at all significance levels, reinforcing this conclusion.

Using the Auto ARIMA function, the AR (4,0,0) model with zero mean presented the lowest AIC (33.38). Residual analysis revealed oscillations around zero without discernible patterns and constant variance. The residual ACF plot showed autocorrelations within confidence limits. The Ljung-Box test ($p=0.7228$) did not reject the null hypothesis of no autocorrelation. All these findings suggest an adequate model.

An ARI (4,2,0) model without logarithmic transformation was also fitted. It presented an AIC of 753.37. The autoregressive component of order 4 indicates dependence up to four lags, and the two differentiations performed reflect the need to induce stationarity.

Forecasts were made with both models for the period 2023-2027 (Figure 4). The AR (4,0,0) model projected a peak in 2024 of 22,723 ha, followed by a declining trend until 2027, reaching 20,965 ha. In contrast, the ARI (4,2,0) model estimated an increasing trend, with a peak in 2027 of 24,039 ha.

The document analysis highlighted the need to carefully examine the growth projections of AM plantations that the ARIMA model can identify. Upper confidence intervals for the point estimates were constructed using the ARIMA methodology. The results correspond to the two models with the lowest AIC values: the AR model with transformed data and the ARI model with untransformed data (Table 2).

Table 1. Accuracy test of the models.

Model	ME	RMSE	MAE	MPE	MAPE	MASE	ACF1	Theil's U
Mean	5012.24	5285.07	5012.24	26.37	26.37	2.32	0.15	2.19
Naïve	-2408.95	2934.69	2408.95	-14.11	14.11	1.12	0.15	1.31
ARI(4,2,0)	-3075.87	3737.79	3160.51	-17.71	18.13	1.46	0.17	1.48
AR(4,0,0)	-2245.29	3119.58	2245.29	-13.26	13.26	NA	0.01	1.23
ARMA(2,0,1)	-3765.24	4109.93	3765.24	-21.48	21.48	NA	0.06	1.79

ME: Mean Error; RMSE: Root Mean Squared Error; MAE: Mean Absolute Error; MPE: Mean Percentage Error; MAPE: Mean Absolute Percentage Error; MASE: Mean Absolute Scaled Error; ACF1: Autocorrelation of Errors at Lag 1; Theil's U statistic.

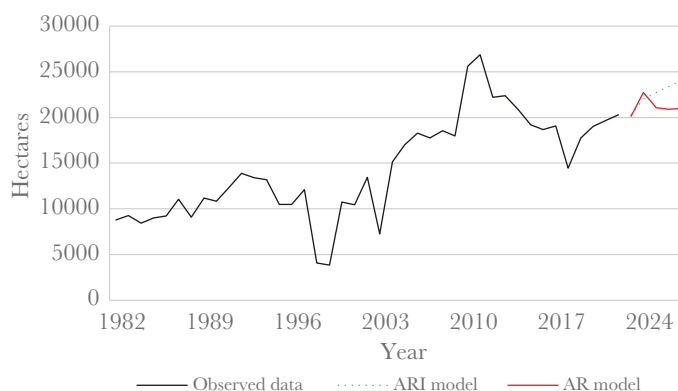


Figure 4. Comparison of forecasting methods for the planted area of agave mezcalero in Mexico, period 2023-2027.

Table 2. 95% Upper Confidence Intervals for the Forecast of agave mezcalero Planted Area (hectares) from 2023 to 2027.

Año	AR (4,0,0)	Upper confidence interval for AR model at 95%	ARI (4,2,0)	Upper confidence interval for ARI model at 95%
2023	20,128.27	38,341.02	20,275.81	26,874.14
2024	22,723.36	60,280.33	22,033.64	31,138.74
2025	21,064.68	49,735.97	22,729.64	34,339.05
2026	20,913.96	53,397.18	23,363.45	37,851.83
2027	20,965.33	54,305.34	24,039.87	42,192.84

AR: Autoregressive model forecast; ARI: Integrated autoregressive model forecast.

The projections indicate a significant growth trend in the area allocated for AM plantations, which could have important implications for the use of agricultural land and productive planning in the sector. The difference in projections between the two models may reflect different interpretations of the trends in the AM planted area data. The AR model captures a possible short-term significant increase in supply, while the ARI model indicates a steadily increasing trend.

The results of the ARIMA models for the PA of AM in Mexico were consistent with the observed data in 2023. Although the analysis was conducted with data up to 2022, the estimates aligned with the figures reported by COMERCAM (2024) and SIAP (SIACONSADER, 2024) for the year 2023. In the case of COMERCAM, the validation was performed using data on “registered plants.”

Martínez *et al.* (2014) reported an average density of 1,740 plants per hectare in the master plan for the maguey-mezcal production system. Meanwhile, OEIDRUS (2011) recorded different densities in the most recent agave plantation census, considering 2,000 plants per hectare for semi-intensive planting systems. According to COMERCAM (2024), in 2023, 64,792,341 agave plants were registered by its associates for the PA of AM in Mexico.

By dividing the number of plants registered by COMERCAM in 2023 by the average planting density reported by Martínez *et al.* (2014), a national planted area of 37,237

hectares is estimated for 2023. Using the planting density reported by OEIDRUS (2011), the estimated area would be 32,396 hectares. In contrast, SIAP (SIACON-SADER, 2024) reported a national PA of 24,506 hectares for the same year. These values fall within the confidence intervals projected by the AR model presented in Table 2 for 2023.

In this ARIMA model estimation, the diversity of agave species included in the AM cultivation must be taken into account. According to the OEIDRUS (2011) census conducted in 2008, 97.4% of the registered plantations corresponded to *Agave espadín*. Similarly, in 2023, COMERCAM (2024) reported that 86.23% of the mezcal produced came from this same species. Therefore, the increase in AM PA could mainly refer to *Agave espadín* and not necessarily reflect the situation of other wild species used in mezcal production. One factor that could be contributing to the expansion of the planted area with AM is the inclusion of this species in governmental programs for establishing new plantations. Escobar-Flores and Sandoval (2022) document that the “Sembrando Vida” program promoted the repopulation of agaves in the state of Durango. In this context, proper planning is required to ensure that the new plantations meet the objectives of these programs.

ARIMA models provided estimates consistent with the observed data, presenting confidence intervals that encompass possible future values. This demonstrates that ARIMA models are useful tools for managing uncertainty in forecasting agricultural variables. Castañeda *et al.* (2021) and Elsamie *et al.* (2021) provide additional evidence of their applicability in crops such as passion fruit and cotton, respectively. The PA of AM is part of the agricultural agave market. The effectiveness of ARIMA models in analyzing time series of agricultural products has been demonstrated in studies on cotton (Korivi *et al.*, 2023) and cabbage (Yang & Hu, 2013). The results obtained in this research reinforce the usefulness of these models to analyze time series with complex and non-stationary patterns, as occurs in the AM market.

Previous research highlights multiple applications of ARIMA models, ranging from estimating vanilla production (Luis-Rojas *et al.*, 2020) to estimating the planted area of staple crops such as maize (Tipi & Erdal, 2021) and wheat (Fawzy *et al.*, 2019). Tipi and Erdal (2021) emphasize the relevance of these models for estimating cultivated areas, a key aspect for agave and other crops. More recently, this methodology has been applied to crops such as apple (Ajit *et al.*, 2021) and papaya (Shafiya *et al.*, 2022).

The cited studies confirm the widespread use of ARIMA models in the analysis of agricultural data, where trend identification is fundamental for food security and economic stability, as in the case of the PA of AM. However, it must be considered that predictions are limited by the quality and availability of data. Unexpected events, such as natural disasters, changes in public policies, or economic crises, can significantly alter projections (Box *et al.*, 2016; Shumway & Stoffer, 2019).

CONCLUSIONS

The cultivation of AM is currently experiencing a context of overproduction. Statistical analysis results indicate that in 2024 a production peak could be reached, equivalent to nearly three times the maximum planted area historically recorded by SIAP. From 2025 onward, a decline in the planted area is projected.

Among the compared models, the autoregressive model (4,0,0) showed the best performance in predicting the planted area of AM in Mexico during the 2023-2027 period. This model presented the lowest value in the Akaike Information Criterion and achieved better results in the precision tests conducted, outperforming other evaluated models, including the mean model, the naïve model, the integrated autoregressive model (4,2,0), and the autoregressive moving average model (2,0,1).

The results obtained confirm the feasibility of using integrated autoregressive moving average models to estimate the planted area of AM. The analysis indicates potential growth in this area between 2023 and 2024, with a possible maximum value within the range of 22,273 to 60,280 hectares for the year 2024. Subsequently, a decreasing trend is projected, with an estimated range between 20,965 and 54,305 hectares for 2027.

The estimates generated by the integrated autoregressive models were consistent with the observed data and those reported for 2023 by COMERCAM and SIAP, especially regarding the upper limit of the confidence interval. The agreement between the model's projections and the official data reinforces the validity of the statistical analysis performed. This scenario of growth in the planted area can support decision-making in agricultural public policy, as well as the design of investment strategies by the business sector.

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Typology of coffee producers in Tlachichilco, Veracruz, Mexico

Méndez-Cortés, Vianeth¹; García-Salazar, José A.^{2*}

¹ Universidad Veracruzana, Campus Tuxpan, Facultad de Ciencias Biológicas y Agropecuarias, Carretera Tuxpan Tampico Kilómetro 7.5, C.P. 92870. Tuxpan de Rodríguez Cano, Veracruz, México.

² Colegio de Postgraduados, Programa de Economía Carretera México-Textcoco km 36.5, Montecillo, Textcoco, Estado de México, México. CP. 56230.

* Correspondence: jsalazar@colpos.mx

ABSTRACT

Objective: To characterize the coffee-growing units in the municipality of Tlachichilco, Veracruz, Mexico, in order to establish a typology of producers and identify key characteristics to inform public policy development.

Design/methodology/approach: Data were obtained through semi-structured surveys applied to n=54 coffee producers in Tlachichilco, Veracruz, selected using convenience sampling. A Principal Component Analysis (PCA) was conducted, and a Technological Index (TI) was estimated. For the cluster analysis, the Cluster procedure and Ward's method from the SAS statistical package were used.

Results: Three types of coffee producers were identified: subsistence (64.8%), intermediate (29.6%), and transitional (5.5%). The subsistence group showed the lowest yield, income, and technological index, while the transitional group had the highest yield and income, reaching 1,400 kg/ha and 42,000 pesos per year, respectively. The lowest technological index was found in the subsistence and transitional groups, while the intermediate group displayed characteristics between the first and third groups.

Limitations of the study/implications: The typology of producers was established specifically for Tlachichilco; therefore, similar studies are needed in other coffee-producing regions of Veracruz.

Findings/conclusions: Coffee producers face productive and economic limitations, resulting in a predominantly subsistence-oriented coffee sector. To increase production, it is necessary to adopt technologies that help control weeds, pests, and diseases, along with continuous training.

Keywords: Technological index, subsistence, transition, main components.

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INTRODUCTION

Coffee production (*Coffea arabica* L.) is important for the economies of developing countries, as its cultivation, processing, transportation, and commercialization generate millions of jobs worldwide (SADER, 2022). The topography, altitude, climate, and soils of Mexico enable the production of high-quality coffee and the development of varieties ranked among the best in the world, making coffee one of the main agricultural export products of the country.

In 2022, global coffee production reached 170 million 60-kg bags (USDA, 2024), with five countries accounting for 73% of total output: Brazil produced 36.8% of the global supply, Vietnam 17.5%, Indonesia 7%, Colombia 6.6%, and Ethiopia 4.9%. In the same year, Mexico ranked eleventh (SIAP, 2023).



In Mexico, two types of coffee plants are cultivated: *Coffea arabica*, which occupies the largest area with 95% of total cultivation, and *Coffea canephora* (commonly known as Robusta), which is gradually expanding its cultivated area and is primarily used for the production of instant coffee (Escamilla *et al.*, 2021). The Arabica variety grows at altitudes ranging from 500 to 2,000 meters above sea level, has a mild flavor, and contains 1.5% caffeine; in contrast, Robusta contains 2.5% caffeine and has a more bitter taste (SADER, 2015). In 2024, the main coffee-producing states in Mexico were Chiapas, Veracruz, and Puebla, with 393,000, 256,000, and 228,000 tons, respectively, accounting for 83% of the country's total production (SIAP, 2024).

In Veracruz, coffee production takes place in 82 municipalities where approximately 86,000 producers reside, including Indigenous peoples such as the Náhuatl, Totonac, and Popoluca. The main characteristics of coffee production in the state are as follows: it is carried out on small plots located in highland areas; production units are situated in Indigenous regions with varying degrees of poverty and marginalization; many families depend on this activity for their livelihood; and coffee production is a significant source of foreign exchange (Hermida, 2018).

There are three main coffee-producing regions in Veracruz: the northern region, which includes Huayacocotla and Papantla; the central region, which comprises Atzalan, Misantla, Coatepec, Huatusco, Córdoba, and Zongolica; and the southern region, where Tezonapa and Los Tuxtlas are located. Among the coffee-producing municipalities in the state, 23% have Indigenous populations and are classified as areas of high or very high marginalization; additionally, these municipalities report the lowest *per capita* income in the state. Of all the municipalities, fifteen are of industrial importance and are located in the metropolitan areas of Córdoba and Orizaba (Gobierno del Estado de Veracruz, 2019).

Tlachichilco is located in the northern region of the state of Veracruz and is a coffee-producing municipality. It covers an area of 226 km², comprises 64 communities, and has a population of 10,900 inhabitants, of whom 48.7% are men and 51.3% are women. The municipality is situated at an altitude of 790 meters above sea level, and most of the population lives in rural areas, where seasonal migration is common due to limited employment opportunities in agriculture.

Coffee production units in Veracruz are characterized by their heterogeneity, limited technical and financial capacity, and marketing challenges (Ávila-Foucat, 2017). Producer typology is one of the most commonly used tools for designing targeted programs in the agricultural sector, and its success depends on the effectiveness of appropriate stratification (Ruiz-Ramírez *et al.*, 2020). Heterogeneous producers within the same geographical region should not be treated as a homogeneous group; instead, differentiated agricultural policies should be developed, and typologies should be created to identify the strengths and limitations of each group of producers (Cuevas *et al.*, 2019). Therefore, the objective of this study was to develop a typology of coffee production units in the municipality of Tlachichilco, in northern Veracruz, as a basis for identifying differences among producers and analyzing the economic activity in order to design public policies that improve the quality of life of the local population.

MATERIALS AND METHODS

The study was conducted in the municipality of Tlachichilco, located in the northern region of the state of Veracruz. A structured questionnaire divided into seven sections was used to carry out the research. It included the identification of the producer, characterization of the family fruit production unit, marketing, other relevant data supporting coffee cultivation, availability of resources and infrastructure, and the technical-productive characterization. Data collection took place from January to March 2022 through a questionnaire administered to small-scale producers with coffee farms.

The statistical sample was calculated using data from a registry of $n=125$ coffee producers in the municipality. The sampling design was determined based on finite population sampling (Aguilar-Barojas, 2005), using the following formula:

$$n = \frac{(p)(q)(N)(z^2)}{E^2(N-1) + z^2(p)(q)} \quad 1)$$

Where N is the total population of the study area, consisting of 125 coffee producers; n is the sample size; p is the estimated percentage of positive variability: 50%; q equals $100-p$ (negative variability); E is the allowed estimation error or precision (10%); and z is the confidence level, with z from tables equal to 1.96. Substituting these values, the following sample size was obtained:

$$n = \frac{(0.50)(0.50)(125)(1.96^2)}{0.1^2(125-1) + 1.96^2(0.50)(0.50)} = 54 \text{ questionnaires} \quad 2)$$

A total of 54 questionnaires, including quantitative and qualitative variables, were administered. Data collection used a non-probabilistic convenience sampling method, since producers were included based on their availability to be interviewed until the required number of interviews was completed. Communities with the highest coffee production index were considered as the stratification criterion.

Data obtained from the questionnaire were recorded in Excel spreadsheets and then exported for further analysis. To reduce the dimensionality of the variables, a Principal Component Analysis (PCA) was performed, using variables selected by the program to identify interdependencies among variables (Tharwat, 2016).

To complement the information from the principal components, a Technological Index (TI) was developed. At the technological level, knowledge of activities and applied science through technological innovation has allowed developed countries to increase productivity and reduce costs (Mejía and Ramírez, 2015). Agricultural technology has features that promote efficient and sustainable farming methods, helping farmers to thrive in today's industry. Using information obtained from the producers, a Technological Index (TI) was calculated, defined by three components (Benítez-García *et al.*, 2015; Rosales-Martínez *et al.*, 2018).

The first component was agricultural practices and pest control, which was assigned a weighting of 0.50 and is composed of five activity subcomponents: a) Soil analysis (with a weighting of 0.20); b) Weed control (0.20); c) Pest and disease control (0.20); d) Pruning (0.20); and e) Fruit thinning (0.20). The second component, corresponding to crop and species management activities, was assigned a weighting of 0.25, and includes five subcomponents: a) Clearing (with a weighting of 0.20); b) Raised beds or ridges (0.20); c) Preparation of planting holes (0.20); d) Transplanting (0.20); and e) Planting pits on ridges (0.20). The third component corresponds to species and varieties, considering the number of varieties with or without resistance to pests and diseases, and was assigned a weighting of 0.25; it has three subcomponents corresponding to the following varieties: a) Costa Rica (0.25); b) Oro Azteca (0.50); and c) Typica or Criolla (0.25). The sum of the weightings in each principal component (0.50, 0.25, and 0.25) equals 1; and the sum of the subcomponents in each component also equals 1.

To describe the characteristics of the producers, a principal component analysis (PCA) was performed using the SAS statistical package. A correlation matrix between variables and a mean difference test were generated. The data analysis consisted of descriptive analysis followed by a mean difference test. The Kaiser criterion was used to determine the number of components, including only those with eigenvalues greater than 1 (Demey *et al.*, 1994). For the PCA, 11 variables measuring productive and socioeconomic aspects of the producers were considered.

The variables analyzed were as follows: age, years of schooling, total land area, percentage of self-consumption, membership in a producers' organization, species or varieties cultivated, area planted with coffee, yield in the last year, coffee price, income from coffee sales, and technological index. For the cluster analysis, the Cluster procedure and Ward's method from the SAS statistical package were used.

RESULTS AND DISCUSSION

Of the 54 producers interviewed in the study region, 85% were male and 15% female, with an average age of 62 years for both sexes. This variable ranged from a minimum of 32 to a maximum of 88 years (Table 1), which aligns with the maximum age reported by Dussán-Lubert (2006). The schooling level of coffee producers in the municipality of Tlachichilco averaged 4.1 years of study, corresponding to a basic education level up to the fourth grade of primary school, similar to that reported by Benítez-García *et al.* (2015) in a study conducted on coffee growers in Cuetzalan, Puebla.

The average land area available to the producers was 3.8 ha. This low average corresponds to subsistence agriculture, where most producers cultivate maize, beans, cinnamon, and coffee—the latter being harvested for sale. However, according to Benítez-García *et al.* (2015), the coffee-growing area is characterized by smallholder producers. In the municipality of Tlachichilco, producers have on average 1.4 ha planted with coffee. They consider coffee cultivation as a complementary economic activity to their income. Producers in this region diversify their activities in other sectors to obtain additional income and thereby meet the basic needs of their families.

Table 1. Statistics of the socioeconomic variables used in the study.

Variable	Description	Mean	Standard Deviation	Minimum	Maximum
X1	Age (years)	62.5	13.4	38	84
X2	Schooling (years)	4.1	3.9	0	15
X3	Total land area (ha)	3.8	1.5	1	8
X4	Percentage of self-consumption (kg)	15	9.7	0	50
X5	Years of experience in coffee production (years)	22.4	12.8	2	50
X6	Coffee varieties handled (1,2,3)	1.9	0.6	1	3
X7	Coffee-planted area (ha)	1.4	0.6	0.3	2
X8	Yield (kg/ha)	417.1	411.9	0	1500
X9	Coffee selling price (\$/kg)	18	11.7	5	35
X10	Coffee sales revenue (\$)	10,070	13,187	0	52,500
X11	Technological index	2	1	0.5	3.5

Source: prepared by the authors with field data.

The average length of time producers have been dedicated to coffee-growing activities in the region is 22 years. Most are experienced coffee growers, a result similar to that reported by Vázquez-López *et al.* (2022) for coffee producers in northern Chiapas, where 36% of surveyed producers have between 11 and 22 years of experience in coffee production.

For the interpretation of variables, Principal Component Analysis (PCA) was used. This method transforms multidimensional information into a few variables that explain a large part of the fluctuations of the original variables, as well as their interrelationships.

According to the criterion of including only those values greater than 1, three principal components were selected (Table 2), which explain 69.58% of the variation among coffee producers. As these values drop below one, the proportion of variance becomes less significant (Amat, 2017).

Table 2. Proportion of absolute and cumulative variance of the principal components.

Variable	Eigenvalue	Difference	Proportion	Cumulative
X1	4.4762	2.5022	0.4069	0.4069
X2	1.9740	0.7706	0.1795	0.5864
X3	1.2034	0.3523	0.1094	0.6958
X4	0.8511	0.1762	0.0774	0.7732
X5	0.6749	0.1140	0.0614	0.8345
X6	0.5609	0.1409	0.0510	0.8855
X7	0.4200	0.0145	0.0382	0.9237
X8	0.4055	0.1474	0.0369	0.9605
X9	0.2581	0.1131	0.0235	0.9840
X10	0.1450	0.1141	0.0132	0.9972
X11	0.0309		0.0028	1.0000

From the three main values of the correlation matrix, variables were constructed to determine the principal components. Principal Component 1 (PC1) explains 40.69% of the total variance and largely reflects the differences between producers and their production systems. Principal Component 2 (PC2) accounts for 17.95% of the variance, while Principal Component 3 (PC3) explains 10.9% of the variance.

Table 3 describes the characteristics by component of the coffee producers. The dominant variables for the first group (PC1) were identified as the age of the producers (X1), the experience in coffee production (X5), the area planted with coffee (X7), production yield (X8), the selling price of coffee (X9), and income from coffee sales (X10). According to SAGARPA (2014), in rural areas the aging of the population has been more pronounced, mainly due to the migration of young people in productive age. Ramírez *et al.* (2023) report that the coffee harvest runs from January to May, with the heaviest picking occurring from mid-February to mid-March; during these months, conditions of lower temperature, precipitation, and relative humidity prevail. Another important factor in this component is the technological index, which encompasses the cultural practices carried out by coffee producers. As Hernández-Ruíz *et al.* (2018) point out, the technological index is considered the basis for defining strategies that contribute to achieving better performance of the production system.

The second group, CP2, is represented by the variables (X2) and (X9), corresponding to the producer's level of education and the coffee selling price. De Brito *et al.* (2018) report that education is a determining factor for improving the quality of life of rural inhabitants. In the municipality of Tlachichilco, most producers have an education level equivalent to primary school. Galindo (2007) mentions that age and level of education determine the degree of acceptance and adoption of new technologies for the development of agricultural crops in Zacatecas; younger producers with higher levels of education are more willing to implement technological recommendations. The

Table 3. Correlation matrix for the three most relevant principal components.

Variable	CP1	CP2	CP3
X1	0.3017	-0.3735	0.1541
X2	-0.2096	0.4746	0.1754
X3	0.1480	-0.4143	-0.3177
X4	0.2493	0.1668	-0.4999
X5	0.3239	-0.3443	-0.0182
X6	0.0687	-0.2508	0.6241
X7	0.3877	0.0600	-0.0015
X8	0.3961	0.1686	0.2098
X9	0.3068	0.3530	0.1184
X10	0.3924	0.2283	0.2600
X11	0.3407	0.2134	-0.2815

CP1: Principal Component 1; CP2: Principal Component 2; CP3: Principal Component 3.

coffee selling price (variable X9) characterizes the producers in Group 2. In the region, the price of coffee is determined by intermediaries according to supply and demand conditions. A similar study conducted by Tomas-Torres *et al.* (2018) in a community in the state of Guerrero points out that intermediaries pay very low prices; during each harvest there is uncertainty due to price fluctuations, and there is no organization to carry out marketing. Additionally, the coffee producers do not add value to the beans to improve the product's price.

For component CP3, the dominant variables describing the group of producers are income obtained (X10) and coffee variety (X6). According to Tablas *et al.* (2021), the main problems affecting coffee production and marketing are phytosanitary issues, technical challenges, market difficulties, and poor organization. The results of the component characteristics highlight the lack of organization among the producers.

The data resulting from the principal component analysis were used for cluster integration. The Ward method was applied to minimize variation within groups and to obtain well-defined clusters. The clustering technique allowed the definition of three groups of producers: 1) Subsistence; 2) Intermediaries; and 3) In transition.

The results presented in Table 4 represent the existence of three types of producers classified as smallholders. Rojas-Herrera and Olgún-Pérez (2018) point out that this type of producer needs to organize collectively to strengthen their individual efforts and face competition. The findings regarding the number of typologies identified in this research are similar to those reported by Rosales-Martínez *et al.* (2018) for the Fortín District, Veracruz; by Leguizamo *et al.* (2023) for the municipality of Temascaltepec, State of Mexico; and by Merlín-Uribe *et al.* (2018) for the Sierra Madre of Chiapas. The latter study identified three typologies of coffee growers and analyzed the relevance of the role of women and youth in the productive and survival strategies in smallholder coffee farming. The results of the typologies found show a relationship between the cultivated area and the technological level of the producers. Given the resource constraints and the socioeconomic context of the producers, similarities can be identified among coffee growers at the national level.

Table 4. Main variables by type of producers in the municipality of Tlachichilco, Veracruz.

Variable	Group 1	Group 2	Group 3
	64.80%	29.60%	5.50%
	Subsistence	Intermediates	In transition
Age (years)	58.8	62.2	56
Schooling (years)	4.5	3.3	3
% of self-consumption	12	20.9	18.3
Coffee varieties	1.7	2	2
Coffee area (ha)	1.2	2	2
Yield (t ha ⁻¹)	160.7	793.7	1,400
Coffee sale price (\$ kg ⁻¹)	12.6	26.5	35
Sales revenue (\$)	1,908.2	20,625	49,000
Technological index	1.5	2.8	3.0

Group 1, corresponding to subsistence producers, comprises 64.8% of the coffee growers and the most important characteristics are as follows. The average age of this group is 58 years, similar to the 56 years reported by Benítez-García *et al.* (2015) for coffee producers in Cuetzalan, Puebla. The average schooling is 4.5 years of study. The main coffee variety they cultivate is Costa Rica 95, which is characterized by its resistance to rust and high productivity (Escamilla *et al.*, 2015). The average cultivated area is 1.2 ha, consistent with Ruiz-García *et al.* (2020), who report that 95.4% of all coffee producers fall into the category of smallholders with cultivated areas not exceeding 3 ha.

The coffee yield obtained in Group 1 is 360.7 kg per hectare, similar to that reported by Leguizamo-Sotelo *et al.* (2023) for the municipality of Sultepec, State of Mexico. The coffee variety influences the selling price; in this group, the Costa Rica variety stands out. These plants are short in stature, have bronze-colored shoots, high productivity, and good adaptability in areas ranging from 800 to 1,400 meters above sea level (Julca-Otiniano *et al.*, 2023).

The average price of green coffee of the Costa Rica variety is 12.60 pesos per kilogram and is sold in sacks of 25 and 50 kg. Despite the low yields obtained by this group, the producers consider coffee cultivation an important activity that generates income for their families throughout the year.

Group 2, considered the intermediate group, comprises 29.6% of the producers. The average age of this group is 62 years. The average schooling level is 3.3 years, indicating incomplete primary education and significant educational lag for this type of producer. Camero and Del Pino (2021) point out that low schooling is a limitation for the adoption of new technologies.

The average technological index in this group is 2.8, higher than that of Group 1. The average price per kilogram of coffee is 26.5 pesos for the Oro Azteca variety, with an annual income of 20,625 pesos, representing more of a subsistence option than wealth accumulation.

According to Rosales-Martínez *et al.* (2018), there is an urgent need to implement strategies for the reproduction of their coffee agroecosystems and to reduce vulnerability to situations such as the drop in international coffee prices, as well as environmental and plant health contingencies.

Group 3, called the transitional group, includes 5.5% of the producers. The average age is 56 years, similar to Group 1. This group has the highest average income from coffee sales, amounting to 42,000.00 pesos in 2022. The selling price for the Oro Azteca variety is 28.00 pesos per kg, similar to that reported by Benítez-García *et al.* (2015e) for coffee producers in Cuetzalan, Puebla, where prices ranged from 25.93 to 29.93 pesos per kg.

It is also similar to the findings of Orona-Castillo *et al.* (2024), who reported that the main problems for producers are the low prices received for their products, coffee marketing, and the long distances they must travel to manage their coffee plantations. The technological index for Group 3 was 3, higher than that observed in Groups 1 and 2.

In this study region, producers across all three groups lack technology for production; activities are carried out using only the basic necessities for cultivation. Therefore, it is necessary to provide specialized technical advice and continuous training for coffee

plantation renewal, as well as to promote the use of machinery and equipment technology to modernize production processes (Villareal, 2023).

CONCLUSIONS

Through principal component analysis, three types of coffee producers were identified in the municipality of Tlachichilco, Veracruz. The first group, called subsistence producers, comprises more than 60% of the producers; they cultivate the Costa Rica 95 variety on plots smaller than three hectares with low yields. The second group, called intermediate producers, represents nearly one-third of the producers and is characterized by having a Technological Index below the average. The third group, called transitional producers, accounts for 5.5% and includes producers with the highest income as well as the highest price for the Oro Azteca variety. For the three groups of coffee producers, age was a dominant factor; the majority are older producers who are encouraging new generations to work in the coffee plantations with the goal of increasing income. The typology of producers provides useful information for decision-making aimed at defining activities to enhance the development of the main producing areas.

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Effect of sex ratio on reproduction, fecundity, and fertility of Montezuma dwarf crayfish (*Cambarellus montezumae*, Saussure 1857) in closed aquaculture systems

García-Olvera, Javier¹; Gallego-Alarcón, Iván¹; González-Blanco, Gehovana¹; White-Olascoaga, Laura²; García-Mondragón, David^{1*}

¹ Universidad Autónoma del Estado de México. Instituto Interamericano de Tecnología y Ciencias del Agua. Dirección postal: Instituto Literario 100, Centro, Toluca, Estado de México, México, C.P. 50000.

² Universidad Autónoma del Estado de México. Facultad de Ciencias, Instituto Literario 100, Centro, Toluca, Estado de México, México, C.P. 50000.

* Correspondence: dgarciamo@uaemex.mx

ABSTRACT

Objective: to evaluate the effect of sex ratio on reproductive aspects of dwarf crayfish (*Cambarellus montezumae*) in aquaculture recirculating systems.

Design/Methodology/Approach: three treatments with female-to-male sex ratios (2:1, 3:1, and 5:1) were used. The organisms were collected from the wild, acclimated, and subsequently placed into a recirculating aquaculture system. Reproductive variables such as the number of fertilized eggs and the number of offspring released per female were measured.

Results: sex ratio had no effect ($p > 0.05$) on fecundity, fertility, or frequency of ovigerous (egg-bearing) females per treatment. Fertilization rate exceeded 50%. This suggests the existence of other independent factors (either on females or males) which contribute to reproductive success.

Limitations/Implications of the study: this study confirms the polygamous behavior of males, which is a characteristic of other crustacean. It is found, then, that reproduction can be optimized by modifying female to male ratios. In addition to considering factors such as water quality, female size, and environmental conditions.

Findings/Conclusions: sex ratio is not a key factor for improving Montezuma dwarf crayfish reproduction in controlled systems. We recommend prioritizing the optimization of environmental conditions, and selecting high-quality broodstock to increase reproductive efficiency.

Keywords: crayfish, Mexican acocil, sex ratio, reproduction.

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INTRODUCTION

The importance of aquaculture in global food security has increased in recent years, particularly in crustacean production systems. Some of the most important species in terms of production volume and commercial value are *Litopenaeus vannamei* [Boone, 1931] Pacific white shrimp; *Procambarus clarkii* [Girard, 1852] red swamp crayfish; *Eriocheir sinensis* [H. Milne-Edwards, 1853] Chinese mitten crab; *Penaeus monodon* [Fabricius, 1798] giant tiger prawn; *Macrobrachium nipponense* [De Haan, 1849] East Asian river prawn; and *Macrobrachium rosenbergii* [De Man, 1879] giant river prawn (FAO, 2018).

Due to the growing importance of their commercial value, some of these species are introduced to environments out of natural distribution (Norzagaray Campos *et al.*, 2012; Platas-Rosado and Vilaboa-Arroniz, 2014). This leads to problems associated with technological, ecological, and even cultural value, since studies and improvements in production processes are directed at those species with the highest economic value (Cerón-Ortíz *et al.*, 2019; FAO, 2024).

Furthermore, there is a risk of displacement of native species, destruction of their habitat and even the loss of traditional knowledge due to population decreasing of those species that were the basis of the diet in riverside communities (Moctezuma, 1996; Arredondo-Figueroa *et al.*, 2011).

The economic importance of endemic species is low worldwide (FAO, 2024). Mexico is a country with great potential for native aquaculture species. However, a lack of scientific studies promoting the generation of new technologies has limited the development of local species cultivation (Norzagaray Campos *et al.*, 2012; Platas-Rosado and Vilaboa-Arroniz, 2014).

Montezuma dwarf crayfish, known in Mexico as acocil (*Cambarellus montezumae* Saussure 1857), is widely distributed in Central Mexico in rivers and related freshwater ecosystems (Avilés-Quevedo and Vázquez-Hurtado, 2006). It is described with a great potential for aquaculture because of a great adaptation capacity to different conditions in terms of space, water quality, and feed (Arredondo-Figueroa *et al.*, 2011; Cerón-Ortiz *et al.*, 2015).

Although studies on this crayfish have focused mainly on aspects of geographical distribution (Álvarez and Rangel, 2007; Barba-Macías *et al.*, 2015) and taxonomy (Gutiérrez-Yurrita and Morales-Ortíz, 2002; Rojas *et al.*, 2002), some studies regarding cultivation have demonstrated the aquaculture potential of the species (Latournerié Cervera *et al.*, 2006; Cerón-Ortiz *et al.*, 2015; Gallardo-Pineda *et al.*, 2015; Cerón Ortiz *et al.*, 2019).

The development of new aquaculture production systems must consider multiple factors such as the species used, life cycle, environmental requirements, and reproductive maturity time, among others (Thieman and Palladino, 2010). To a large extent, reproductive success depends on environmental and ecological parameters. In particular, egg production in crustaceans depends on water temperature, feeding, sexual maturity, spawning frequency, fecundity, and fertility (Lizárraga-Cubedo *et al.*, 2008).

Another factor that determines the structure and reproductive capacity of a population is the sex ratio (Piferrer, 2009). Selecting the correct sex ratio optimizes the reproductive time and effort of both males and females to achieve the desired number, volume, and quality of spawning.

Some studies conducted on crayfish reported optimal sex ratios of 2:1 (two females per one male) (Arredondo-Figueroa, 2011); even 1:1 (Cerón-Ortiz *et al.*, 2019), which are attributed to antagonistic behavior, density, and intrapopulation competition, among others. Likewise, reproductive parameters such as fecundity and fertility are reported in association with morphometric characteristics of females (Álvarez and Rangel, 2007; Arredondo-Figueroa *et al.*, 2011). Even the size at first reproduction was observed as

relevant (Cerón-Ortiz *et al.*, 2019); in addition to other factors such as environmental quality or food availability (García-Padilla, 2014).

Most studies in crustaceans consider females as the determining factor of reproductive parameters such as fertility and egg fecundity. However, male sperm quality is also a variable that must be considered in the reproductive success of a population, which can be indirectly evidenced in the quality of fertilized embryos and the production of viable eggs (Pérez-Rodríguez, 2017). It is also important to consider whether male sperm reserves allow them to mate with more than one female in the same reproductive period and what is the maximum number of females that a single male can fertilize (Carmona-Osalde *et al.*, 2004; Gowland-Sainz, 2018).

Based on the above, to solve the question How does the sex ratio affect reproduction, fecundity, and fertility in crayfish farming? We posed the hypothesis that the male is the variable which determines reproductive success and spawning quality in crayfish farming. The objective of the study was to evaluate the effect of sex ratio on reproductive aspects of Montezuma dwarf crayfish (*Cambarellus montezumae*) in an aquaculture recirculating system.

MATERIALS AND METHODS

Collection and acclimatization

Wild Montezuma crayfish (*Cambarellus montezumae*) were obtained and divided by sex into two recirculating ponds at an average temperature of 18 ± 2 °C and a water exchange rate of 500 mL per minute. Acclimatization was implemented for three weeks; organisms were fed with shrimp feed “El Pedregal” brand (45% protein, 10% fat); feed portion was 6% of the biomass every two days. Oviparous females found after collection and acclimatization were discarded of the experiment and isolated from the experimental group.

Experimental design

The experiment was established in a recirculating aquaculture system at the facilities of the aquaculture bioengineering laboratory of the Inter-American Institute of Water Sciences and Technology (19° 23' 58" N, 99° 42' 52" W; altitude 2609 m).

After acclimatization of organisms, 30 females and 9 males (39 experimental organisms) were randomly selected; biometric measurements were performed. Total body weight (TW), which was measured with an analytical balance [Bel Engineering MG314Ai with 0.0001 g readability]; and total length (TL), measured from the tip of the acumen to the posterior margin of the telson (Álvarez and Rangel, 2007). They were subsequently arranged in 60 L experimental units in a 3×3 randomized block design. Treatments consisted of three sex ratios: T1: two females per one male (2:1); T2: three females per one male (3:1); T3: five females per one male (5:1). The organisms used in the experiment were randomly assigned to each block, ensuring that they had similar morphometric characteristics. Males had an average length of 46.9 mm and an average weight of 1.8 grams; Females had a length range from 28 to 45 mm, and weight from 0.7 to 2.3 grams (Table 1).

The experiment lasted 14 weeks (April-July 2024) at an average temperature 16.7 ± 1.6 °C, with a minimum of 12 °C and a maximum of 22 °C. The presence of

Table 1. Distribution of experimental organisms (*Cambarellus montezumae*) by treatment (sex ratio).

Treatment	TLf	TWf	TLm	TWm
T1	34.3 mm	1.7 g	47.7 mm	1.6 g
T2	34.4 mm	1.5 g	45.7 mm	2.2 g
T3	37.6 mm	2.0 g	47.3 mm	1.6 g

TWf: average female weight; TLf: average female length; TWm: average male weight; TLm: average male length.

ovigerous females was checked every third day; when an ovigerous female was identified, it was removed from the system and replaced with another with similar morphometric characteristics.

In order to reduce possible antagonistic behaviors between organisms, each experimental unit had two PVC shelters (standard tube, 1/2" in diameter × 1 1/2" long) for each experimental organism.

Additionally, the experimental organisms were provided with balanced shrimp feed crushed with a mortar to ensure conditions close to ad libitum feeding.

Fecundity (number of eggs per female)

Fecundity, the number of eggs released and retained by a female under the abdomen in a single spawning (Hernández and Palma, 2003), was evaluated by counting eggs attached to the pleopods; in addition, we recorded female total weight and length (Figure 1).

Fertility (number of offspring per female)

To estimate the number of live offspring released by an ovigerous female, the organisms were placed individually in 4 L containers (Figure 2), which contained a PVC



Figure 1. Ventral view of a fertilized female *Cambarellus montezumae* with eggs (black mass) that remain attached to the pleopods by a mucilaginous substance throughout incubation. Photo: Javier García-Olvera.



Figure 2. Montezuma crayfish larvae (*Cambarellus montezumae*) attached to the abdomen of an ovigerous female. Photo: Javier García-Olvera.

shelter with complete water replacement every third day until hatching and expulsion of their offspring.

At the time of expulsion of the crayfish fry (none attached to the female's abdomen), the female was removed from the 4 L container. Biometry was performed of total female weight and length, and the offspring inside the container were counted.

Statistical analyses

The data obtained were analyzed using the statistical software IBM SPSS[®] for Windows v. 24.0; A non-parametric Chi-square test was applied to the number of ovigerous females to find differences ($p \leq 0.05$) among treatments. Data on fecundity and fertility were verified as normal distributed (Shapiro-Wilk). After normality was proved, data were analyzed with a one-way analysis of variance test. In case of the opposite, this is, a non-normal distribution, a Kruskal-Wallis test for independent samples was applied.

RESULTS AND DISCUSSION

Frequency of ovigerous females

The number of females in the different treatments is shown in Table 2. The percentage of ovigerous females was 45%, 53%, and 50% of the females available for treatments T1, T2, and T3, respectively. Non-significant differences were found among treatments ($p \leq 0.05$).

Results show that, at least in the ratios evaluated, males had the same reproductive capacity (close to 50%) to fertilize available females, regardless of the sex ratio. A question arises then about why in any evaluated sex ratio, successful fertilization was only achieved in approximately 50% of females. There are factors inherent to the biology of the species that were not controlled in the experiment, such as mate selectivity, dominance, mating behavior or resistance, etc. (Barki, 2008). Those factors could be the cause for that 50% of non-ovigerous females, rather than the loss of energy experienced by males, due to the number of fertilized females.

Table 2. Frequency distribution of ovigerous (*Cambarellus montezumae*) females by treatment.

		Females		Total
		Non-ovigerous	Ovigerous	
Treatment	1	6	5 (45%)	11
	2	9	10 (53%)	19
	3	15	15 (50%)	30
Total		30	30	60

A polygamous behavior is reported in other decapods, such as the Red-clawed lobster (*Cherax quadricarinatus* [von Martens, 1868]), whose polygamous behavior is used to benefit culture, since sex ratio does not influence their reproduction. Authors of that study suggested to use female-biased ratios to increase the number of fertilized females per culture area (Yeh and Rouse, 1995). Thus, this polygamous behavior can be used in conjunction with a higher female-to-male ratio to optimize Montezuma dwarf crayfish reproduction.

On the other hand, the absence of competition among males may be a factor that facilitates copulation with a greater number of females. Arredondo-Figueroa *et al.* (2011) reported that in 1:2 ratios (female to male), males suffered higher mortality due to competition. Those authors suggested to consider this in setting reproduction densities, both for other studies and for batch culture. Since the presence of two or more males in the same pond could negatively influence reproduction rates, due to antagonistic competition behaviors for territoriality and sexual partners.

In spite of the polygamous role, reproductive efficiency, and lack of competition experienced by male crayfish, it is evident that the participation of the adult female is also crucial in the egg fertilization process. Females identify and determine the best environmental conditions for spawning (temperature, water quality, food availability, among others), and the influence of the culture environment on those conditions (Hernández and Palma, 2003). Some characteristics of the breeders, such as sexual maturity, morphometry, or age also have influence.

Rodríguez-Serna y Carmona-Osalde (2002) and Arredondo-Figueroa *et al.* (2011) reported that the metabolic expenditure of the culture is less when it is carried out in recirculating aquaculture systems, since organisms save more energy for reproduction. Therefore, when close to ideal environmental conditions exist within a controlled production system, the effect of the proportion of ovigerous females can be attributed more to behaviors inherent to the biology of the species and not to the effect of the reproductive capacity of males. This indicates that at least the reproduction frequency of females is not determined by the sexual proportion of males and therefore is a cultivation parameter that is not decisive in the efficiency of obtaining ovigerous females (García-Padilla, 2014).

Fertility (number of eggs per female)

The completely random distribution of females and males in the treatments allowed for representativeness of weights and sizes to evaluate their possible effect. Fertility results did not show significant differences among treatments ($p < 0.05$). The data suggest, at least in

the proportions evaluated, that regardless of the sex ratio used in reproduction, Montezuma dwarf crayfish fertility is not affected by the availability of females per male. Lizárraga-Cubedo *et al.* (2008) reported that the number of available partners was a limiting factor for male reproduction under natural conditions. However, under culture conditions male crayfish were capable of copulating with the largest number of available females without suffering any energy expenditure that significantly affects fertility.

Although in other crustaceans it has been reported that dominant males tend to limit their sperm release to increase the chances of fertilization of females (Barki, 2008), in the case of the proportions evaluated in Montezuma crayfish in our study, this phenomenon did not occur or it was insignificant. On the other hand, although energy expenditure is greater in females than in males (Lizárraga-Cubedo *et al.*, 2008), the number of eggs females produce is a factor related to the size of the abdomen, rather than to sexual ratio. This agrees with Álvarez and Rangel (2007) and Arredondo-Figueroa *et al.* (2011).

This situation seems to indicate that a large part of the effects on fecundity in the crayfish depend on the characteristics of the female. Such as female body size (García-Padilla, 2014) or on the environment, as quality of the medium, feed type and quality (Montemayor Leal *et al.*, 2010) or temperature (Gallardo-Pineda *et al.*, 2015). This effect of female characteristics on fecundity has been described in other species of cambarides, as *Procambarus clarkii*; *Cambarus longulus longulus* Girard 1852; *Orconectes kentuckiensis* Rhoades 1944; *Orconectes rusticus* Girard 1852; or *Cambarus shufeldtii* Faxon, 1884 (Barki, 2008).

Female weight, female length and correlations to fecundity

The average number of eggs ($\bar{x} = 62$) per ovigerous female in this study had limits within a minimum of 17 and a maximum of 115 (Figure 3). Thus, this average number of eggs obtained exceeds those found under similar conditions ($\bar{x} = 45$) in recirculating aquaculture systems (Arredondo-Figueroa *et al.*, 2011), those ($\bar{x} = 41$) in 40 L tanks (Cerón-Ortiz *et al.*, 2019); or those ($\bar{x} = 50.7$) under natural conditions (Álvarez and Rangel, 2007). This fact demonstrates that the reproductive conditions in recirculating aquaculture systems, especially those used in our study, were optimal for reproduction and allowed organisms to reach a reproductive capacity similar to, or even higher than, that in natural conditions.

The correlation between total body weight and total body length of females ($r^2 = 0.817$) was high in terms of body proportion. However, the body weight to fecundity ($r^2 = 0.568$) and total length to fecundity ($r^2 = 0.460$) correlations did not show evidence that female crayfish could produce a greater number of eggs as their weight or size increases (Figure 4). For most crustaceans (Pandian, 2016) and in other studies on Montezuma crayfish (Moctezuma, 1996; Álvarez and Rangel, 2007; Arredondo-Figueroa *et al.*, 2011), high correlations between fecundity and size are reported. This is due to the number of eggs is limited by the incubation space available in the female abdomen (Hernández and Palma, 2003; Álvarez and Rangel, 2007).

Regarding the size and weight at which ovigerous females were obtained, from a total body weight of 0.8 g and a total body length of 30 mm, fecundities higher than 45 eggs per clutch were obtained (Figure 3, Figure 4) which is the overall average found under

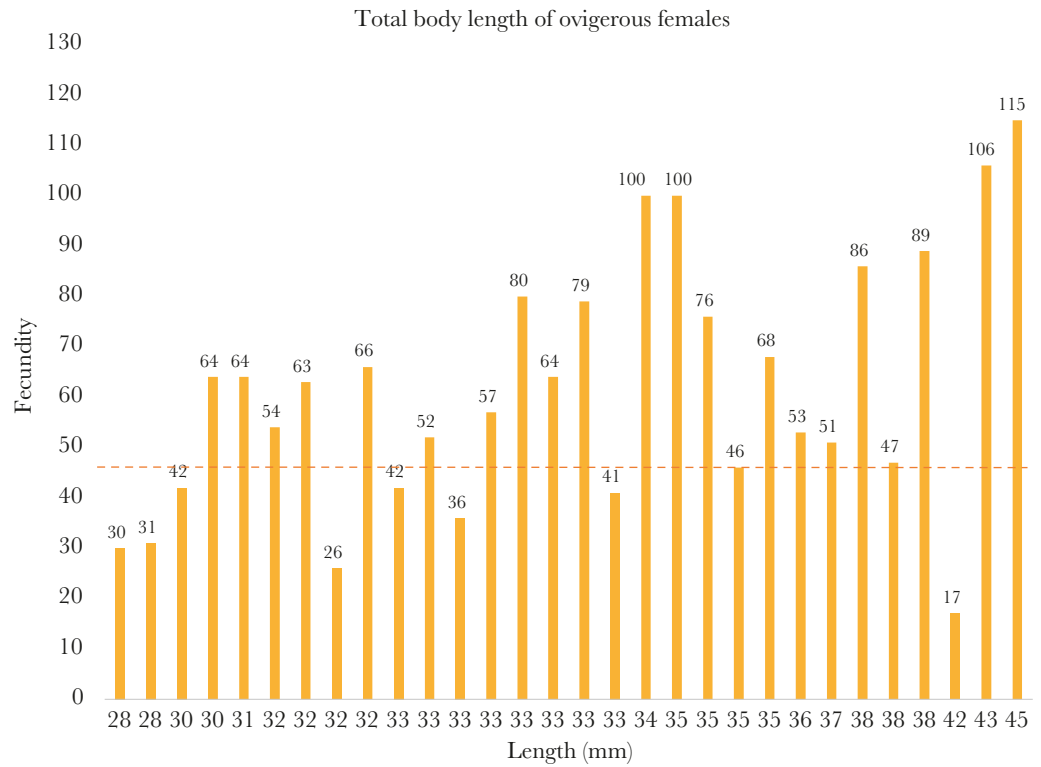


Figure 3. Average number of eggs produced individually according to size (total length) of females (*Cambarellus montezumae*).



Figure 4. Number of eggs produced individually according to body weight of females (*Cambarellus montezumae*).

similar conditions in other studies (Arredondo-Figueroa *et al.*, 2011). This data agrees with Álvarez and Rangel (2007) who referred obtaining ovigerous females from 24 mm and suggested a capture length of 30 mm for the size of the breeders. These biometric data can be a reference for the selection of females in Montezuma crayfish farming.

However, it is essential to emphasize that the size of the females is not the only variable of interest that should be used to estimate and obtain an optimal reproductive capacity in the breeders in Mexican acocil farming. There are other variables that can significantly affect fecundity such as age of the females, season of the year, physicochemical conditions of the water, size of the eggs, or feed availability, among others (García-Padilla, 2014; Pandian, 2016). These variables should be considered in subsequent studies to determine the best conditions for obtaining optimal fertility for this culture.

Fecundity results suggest that female-biased reproduction proportions can promote the production of a greater number of eggs per pond (Yeh and Rouse, 1995), due to the possibility of containing a greater number of females per square area in the culture systems and therefore a greater egg productivity.

Fertility (number of offspring per female)

A total of 444 hatched crayfish fry were obtained, with a $\bar{x} = 15$ average of offspring per ovigerous female (Figure 5), with a minimum of 0 and a maximum of 86. Fertility results showed non-significant differences among treatments ($p > 0.05$). Therefore, in the



Figure 5. Montezuma crayfish fry (*Cambarellus montezumae*) expelled in a single spawning by a single female. Photo: Javier García-Olvera.

sex ratios evaluated, one male can copulate with a greater number of females without affecting the quality of the spawning or the hatching of the offspring.

Crayfish fry hatched and were released from the female abdomen between 30 and 50 days after being fertilized (Figure 5), with a \bar{x} = 44-days average incubation period. The discrepancy found in relation to other studies, which used a similar methodology (Moctezuma, 1996; Cerón-Ortiz *et al.*, 2019), could indicate that the quality of the spawning is not significantly affected by sex ratio, but is determined by the incubation conditions of the eggs. This could affect the survival of those eggs initially carried by the female.

Gallardo-Pineda *et al.* (2015) reported in *Cambarellus patzcuarensis* [Villalobos 1943], that increasing temperatures may reduce the incubation period, which could explain the hatching ranges found. Furthermore, the fact that higher temperatures have effects on female survival and fertility opens the possibility of in vitro incubation of fertilized eggs to increase the hatching percentage and optimize the offspring obtained. This possibility is important for subsequent research.

CONCLUSIONS

Sex ratio between males to females had non-significant effects on reproductive indicators, such as fecundity and fertility. Males used had the same reproductive capacity. However, there may be biological factors inherent to the females that determine reproductive success, such as dominance, territoriality, size, weight, age, size at first reproduction, among others. Finally, in the number of fertilized and hatched eggs, no evidence was found of significant differences related to the number of females with which the male mated. In terms of productivity, this information can be used for reproductive strategies in conservation, and commercial production systems of Montezuma dwarf crayfish.

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Semiautomatic detection of coastal mangroves with Landsat Level-2

Escobar Flores, Jonathan G. 1 Sandoval, Sarahi 2*

¹ Instituto Politécnico Nacional, CIIDIR Unidad Durango, 34220, Mexico

² CONAHCYT – Instituto Politécnico Nacional, CIIDIR Unidad Durango, 34220, Mexico

* Correspondence: sarahisandoval@gmail.com

ABSTRACT

Objective: A model for rapid detection of coastal mangrove cover was devised. The idea is that it can be applied by users with basic knowledge of remote sensing and GIS.

Design/methodology/approach: The model is based on calculating the first three principal components (PC) from bands corresponding to the visible, near infrared, and shortwave infrared regions in Landsat Level-2 images. The model was tested for three RAMSAR sites located in different hydroclimatic conditions in Mexico: Laguna Guasima on the upper Gulf of California coast, Puerto Arista on the Pacific Ocean coast, and Laguna Madre on the Gulf of Mexico.

Results: It was found that the first PC in the three RAMSAR sites explains 80 to 90% of the variation and corresponds mainly to areas that include crop fields or urban infrastructure. The second PC, with cumulative variance of 8 to 14%, corresponds mainly to mangrove cover, and the PC with the lowest percentage of cumulative variance (<5.0%) is invariably open water.

Limitations on study/implications: The advantage of using Landsat Collection Level 2 is that there is an archive managed by the USGS of imagery from virtually all over the world that is over 50 years old. This model is not able to identify mangrove species.

Findings/conclusions: The advantages of this proposed model are: 1) it uses Collection 2 Level-2 images, which have radiometric and atmospheric corrections; 2) since it is carried out in ArcGIS Model Builder, it can be automated, making it intuitive and enabling the results to be exported to a Python script; and 3) the model can be replicated accurately with the QGIS model builder tool.

Keywords: Mangrove, Landsat, Remote sensing, Principal components, Mexico.

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INTRODUCTION

Mangrove swamps are among the main ecosystems that contribute to mitigation of climate change. They protect coasts from storms and soil erosion, and control flooding, and are considered to be “blue carbon” (store large amounts of carbon) due to their high rate of carbon sequestration compared to other ecosystems. Thus, they provide a diversity of ecosystem services that benefit human communities (Herrera-Silveira *et al.*, 2016; Taillardat *et al.*, 2018; Zeng *et al.*, 2023).

The remote sensing techniques available at this time seem promising for the study and monitoring of mangrove cover, but their use requires extensive knowledge in the area of remote sensing, which the relevant decision makers generally do not have. For example, Saoum and Sarkar (2024) analyzed the changes in mangrove forest dynamics over the last 20 years in Bangladesh, using Google Earth Engine (GEE) and the IDRISI program to detect forest changes with the CA-Markov model. Another example is the research of Jia *et al.* (2023), who also use GEE and algorithms such as OBIA and Random Forest to estimate the global distribution of mangrove cover. In order for users to be able to replicate this research, a background in programming, geographic information systems, remote sensing and classification algorithms would be required. Often, those who manage these lands do not have this set of skills.

Principal component analysis (PCA) is a simple technique that is easy to replicate in virtually any geographic information system. The main objective is to summarize the information contained in a group of variables into a new, smaller set without losing a significant part of the information (Chuvieco, 2019). Satellite image acquisitions in adjacent bands of the spectrum tend to detect redundant information due to the fact that different canopies on the ground present similar behavior when their electromagnetic spectrum lengths are close to each other. PCA is widely used for a variety of purposes in the study of mangrove swamps. For example, Tossi *et al.* (2022) propose a mangrove disturbance index for Iranian mangrove swamps that is generated by means of a PCA generated from WorldView-2 and Sentinel-2 multispectral images. These authors note that the advantage of using PCA over other object extraction methods are that 1) it removes correlated features, and 2) it eliminates the problem of multicollinearity (Brauner and Shacham, 2000).

This study proposes the use of images from any Landsat Collection Level-2 platform for extracting mangrove cover. The method has the advantage that the data collected are scientific products that provide images of the spectral reflectance of the Earth's surface as it would be measured at ground level in the absence of atmospheric scattering or absorption. USGS Landsat Collection 2 offers improved processing, geometric accuracy, and radiometric calibration compared to the previously available Collection 1 products.

MATERIALS AND METHODS

Three RAMSAR sites in Mexico were selected, to represent the different hydroclimatic conditions of the coasts of Mexico. The first area was Laguna Guasima, located in northern Sonora state (Figure 1a). This lagoon is dominated by mangrove swamp of the Gulf of California ecoregion, which is in the transition between tropical and subtropical climates. The Gulf is exposed to climate variability on a large scale (1200 km), including the El Niño Southern Oscillation (ENSO) and the Pacific Decadal Oscillation (Lluch-Cota *et al.*, 2013; Páez-Osuna *et al.*, 2016). The second area was Puerto Arista in the state of Chiapas (Figure 1b) a Ramsar site and also a natural protected area (sanctuary), located in the state of Chiapas. This Ramsar site has the tallest mangroves (35 m) in North America and is one of the few flooded mangrove forests in Mexico (Mendoza, 2000). The climate in this area is tropical humid (Aw) with a mean annual temperature of 28 °C and an annual precipitation of 2500 mm to 3000 mm. The vegetation around the mangrove forest is medium height

sub-evergreen forest and low sub-deciduous forest (Hernández-Hernández and Chávez, 2021). The third site is La Laguna Madre in the state of Tamaulipas (Figure 1c), another coastal mangrove swamp, on the Gulf of Mexico. This lagoon is of vital importance for the redhead (*Aythya americana*), as more than 75% of the global population of this duck spends the winter there each year (Onuf, 2007). The salinities of the lagoon are higher than those of the neighboring sea nearly all year round (Sánchez-Ramírez and Ocaña-Luna, 2015) and the main resource harvested is oysters, accounting for 79% of the extraction in Tamaulipas (Téllez *et al.*, 1999).

Landsat Level 2 data and processing

The images used in the study are from Landsat satellites and were downloaded from the United States Geological Survey (USGS) website www.earthexplorer.com. Specifically, the images used were from Landsat 9 OLI-2 (operational land imager) for Laguna Guasima, Landsat 5 TM (thematic mapper) for La Laguna Madre, and Landsat 8 OLI for Puerto Arista with date of acquisition in cloud-free months and before the rains

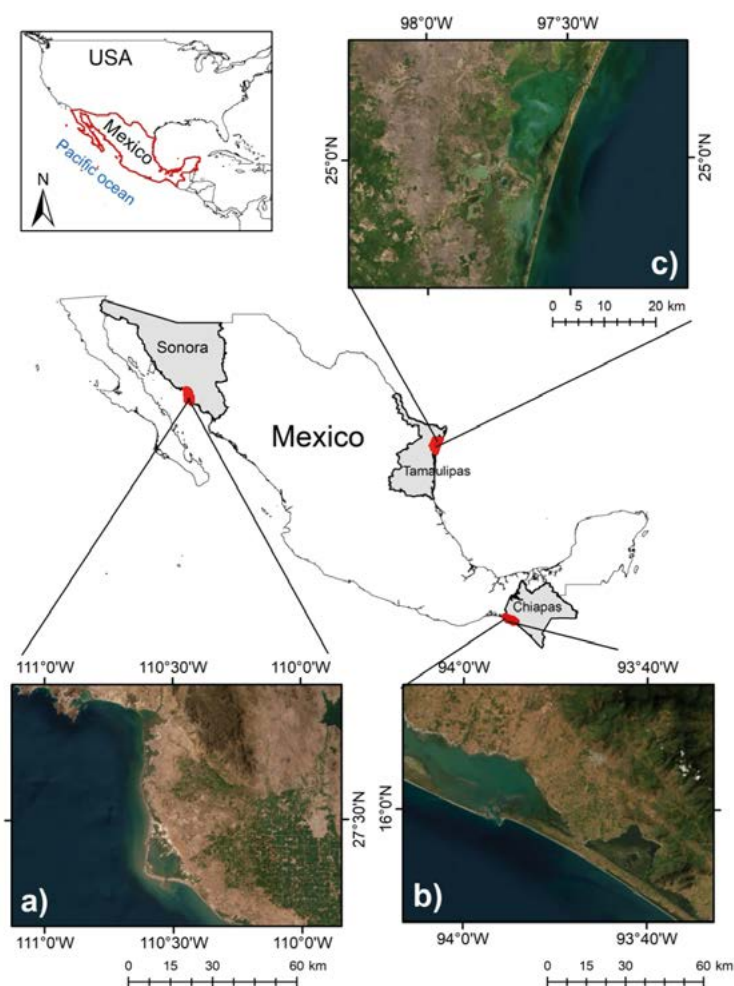


Figure 1. Study areas: Ramsar wetlands in coastal regions of Mexico. 1a. Laguna Guasima, Sonora, 1b. Puerto Arista, Chiapas, 1c. Laguna Madre, Tamaulipas in Mexico.

(Téllez *et al.*, 1999; Hernández-Hernández and Chávez, 2021) (Table 1). The purpose of using different Landsat products is to determine whether the different radiometric resolutions affect the PCA result, and if not, then users can conduct multitemporal analyses with these Landsat products.

The spectral bands of these images have temporal consistency within the same band and are therefore suitable for comparison purposes (Wulder *et al.*, 2016; Teixeira *et al.*, 2020).

The images are from Collection 2 Level-2, which have the advantage of being radiometrically calibrated and have high precision orthorectification using ground control points and a digital elevation model (DEM). In addition, the images had been atmospherically corrected by the Landsat Ecosystem Disturbance Adaptive Processing System (LEDAPS, version 3.4.0) for Landsat 5-7 and the Landsat Surface Reflectance Code (LaSRC, version 1.5.0) for Landsat 8 and 9 (USGS Landsat Science Products, <https://www.usgs.gov/landsat-missions/landsat-science-products>) (Vogelmann *et al.*, 2016).

To obtain the bands in TOA (top of the atmosphere) values for both TM and OLI sensors, users of Level-2 imagery need only multiply by the scaling factor and subtract the additive factor as shown in the following Equation (1).

$$TOA\ Reflectance = (band * 0.0000275) + (-0.2) \quad \text{Eq. 1}$$

Using PCA in ArcGIS

The model builder in ArcGIS was used to carry out an automatization process to extract both mangrove cover polygons. This tool has the advantage that it can be exported to a

Table 1. Bands from Landsat level 2 satellite data for each of the three study locations.

Area	Date of acquisition	Sensor	Radiometric resolution	Spectral bands used (micrometers)	Resolution (meters)
Laguna Guasima	April 7, 2024	Landsat 9- OLI 2	14 bits	Band 2 (0.45-0.51) Band 3 (0.53-0.59) Band 4 (0.64-0.67) Band 5 (0.85-0.88) Band 6 (1.57-1.65) Band 7 (2.11-2.29) Band 10 (10.6-11.19)	30
Puerto Arista	April 4, 2023	Landsat 8-OLI	12 bits	Band 2 (0.45-0.51) Band 3 (0.53-0.59) Band 4 (0.64-0.67) Band 5 (0.85-0.88) Band 6 (1.57-1.65) Band 7 (2.11-2.29) Band 10 (10.6-11.19)	30
Laguna Madre	May 10, 1993	Landsat 5-TM	8 bits	Band 1 (0.45-0.52) Band 2 (0.52-0.60) Band 3 (0.63-0.69) Band 4 (0.76-0.90) Band 5 (1.55-1.75) Band 6 (10.4-12.5) Band 7 (2.08-2.35)	30

Python script. The first step is to scale the atmospherically corrected bands to be used in the principal component analysis (PCA). These bands correspond to the visible, near infrared, and mid-wave infrared regions of the electromagnetic spectrum Atmospheric correction is already provided for each of the Landsat image bands from Collection Level-2, so it is only necessary to convert the values to TOA. The next step is to delimit the bands to the area of interest (Ramsar sites) so that all bands can be included in the PCA. The PCA process in ArcGIS basically consists of the following: 1) calculate the variance–covariance matrix of the spectral bands, 2) extract the eigenvalues that represent the magnitude of the vector of each successive component, and 3) produce a raster with the components that retain the information (Figure 2).

Extraction of mangrove

The PCA raster was reclassified following the Jenks natural breaks method (Jenks, 1997), whose main objective is to generate homogeneous groups that maximize the variance between class means and minimize the variance within classes (Chen *et al.* 2013). This procedure is carried out with the Reclassify function in ArcGIS (Figure 2). Three classes were defined, which correspond to the first three components, which contain the highest variance in a multispectral image (Chuvieco, 2019). The result of the reclassification was an integer raster containing integer values of 1 for pixels corresponding to PC1; values of 2 for PC2 pixels, a principal component which has been reported to have a close relationship with dense vegetation areas (Estornell *et al.*, 2012) and mangroves (Sunkur *et al.*, 2023); and values of 3 for PC3, which corresponds to pixels with low multispectral variability, which

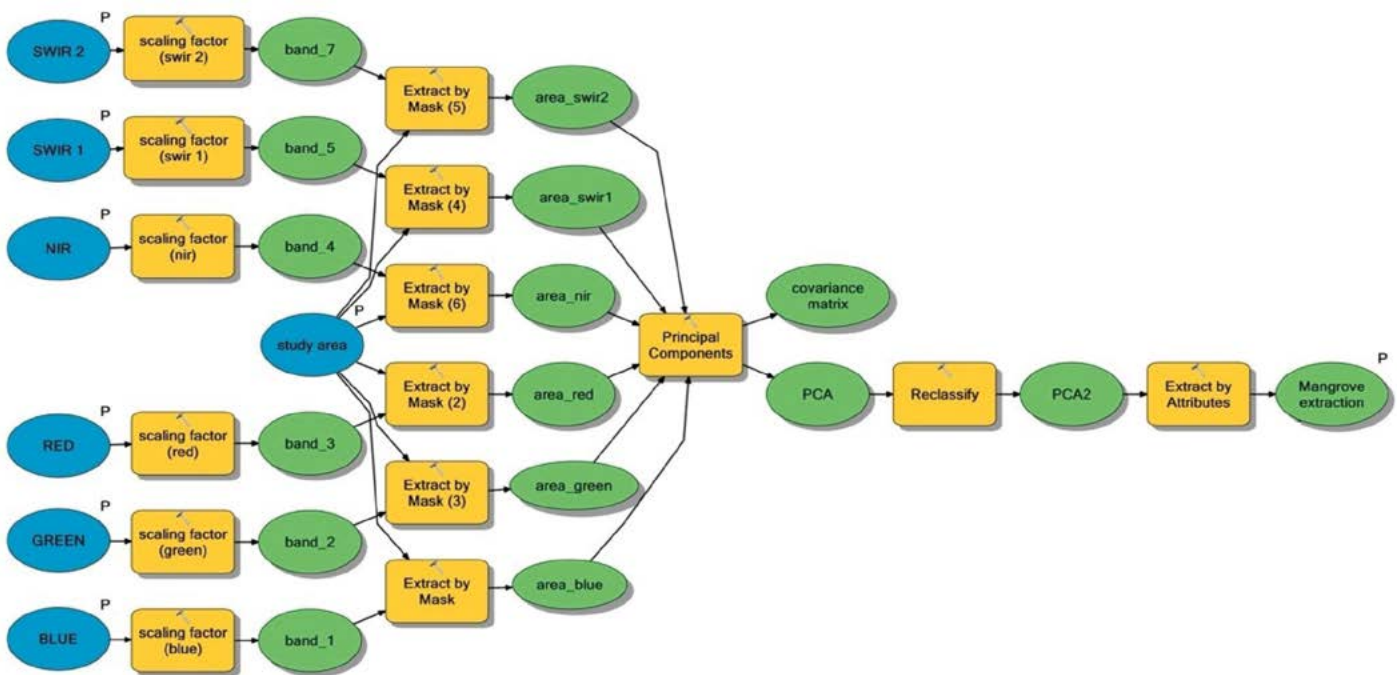


Figure 2. Automation model for extraction of mangrove cover and corresponding LST values. Blue ovals have a P superscript indicating that users only have to load the spectral bands of their site of interest. Yellow squares are geospatial processes and green ovals are the result of each geoprocess.

has been reported to be related to water bodies (Balázs *et al.*, 2018). Pixel classification by Jenks's method has proven to be useful for mangrove classification when using spectral bands from satellite images (Nuarsa *et al.*, 2010).

RESULTS AND DISCUSSION

In all Ramsar sites, mangrove cover was clearly delimited in PC 2 with cumulative variances of 3% to 14%. The mangrove swamp with the lowest percentage of variance was Laguna Guasima (Table 2, Figure 3). PC 3 for the three sites had cumulative variance of less than 5% and in the images always corresponded to bodies of surface water (Table 2, Figure 2), while PC 1 ranged from 80% to 92% of cumulative variance for the three sites and corresponded to areas of cropland, urban land, and vegetation (Table 2, Figure 3). For the eigenvectors of PC 2 it was found that in Laguna Madre the spectral band that made the greatest contribution was the NIR, while for Puerto Arista it was SWIR 1 and SWIR 2, and for Laguna Guasima it was the red and green bands (Table 2). In PC 1 all eigenvectors were positive and the highest values were found in the SWIR bands for all three areas (Table 3). In PC 3, in Laguna Madre and Puerto Arista the eigenvector with the highest value was in the red band while for Puerto Arista it was the NIR band (Table 3).

PCA is a multivariate statistical technique that is very useful in remote sensing because it reduces the dimensionality of the spectral bands (Harsanyi and Chang 1994). For example, in our study, transforming the multispectral images composed of three visible bands, one near infrared and two mid-wave infrared, resulted in a set of uncorrelated bands (orthogonal) that facilitated the detection of mangrove cover and water bodies. The detection of mangrove cover in PC 2 coincides with the detection of dense vegetation areas in other investigations such as in Valencia, Spain (Estornell *et al.* 2012). For the case of mangrove cover in Puerto Arista, the eigenvector value of the infrared band is indeed the highest (Table 3). PCA is considered one of the most efficient methods for mangrove cover detection; for example, Green *et al.* (1998) mapped mangrove cover in the Turks and Caicos Islands using various sensors; Landsat, SPOT, and Compact Airborne Spectrographic Imager. They tested different classification methods, from visual interpretation to supervised and unsupervised classification and concluded that PCA has up to 92% accuracy. Once again, the eigenvalues of NIR and red were the

Table 2. Cumulative variances obtained for the first 3 PCA in the study areas.

Ramsar site	Sensor and date	Ocean	Cumulative variance (%) and principal land cover
Laguna Guasima	Landsat 9 OLI 2 07/04/2024	Gulf of California	PCA 1=92.79 Ground PCA 2=3.94 Mangrove PCA 3=2.75 Water
Puerto Arista	Landsat 8 OLI 05/04/2023	Pacific Ocean	PCA 1=88.96 Ground PCA 2=8.87 Mangrove PCA 3=1.90 Water
Laguna Madre	Landsat 4 TM 03/10/1993	Gulf of México	PCA 1=80.30 Ground PCA 2=14.13 Mangrove PCA 3=5.00 Water

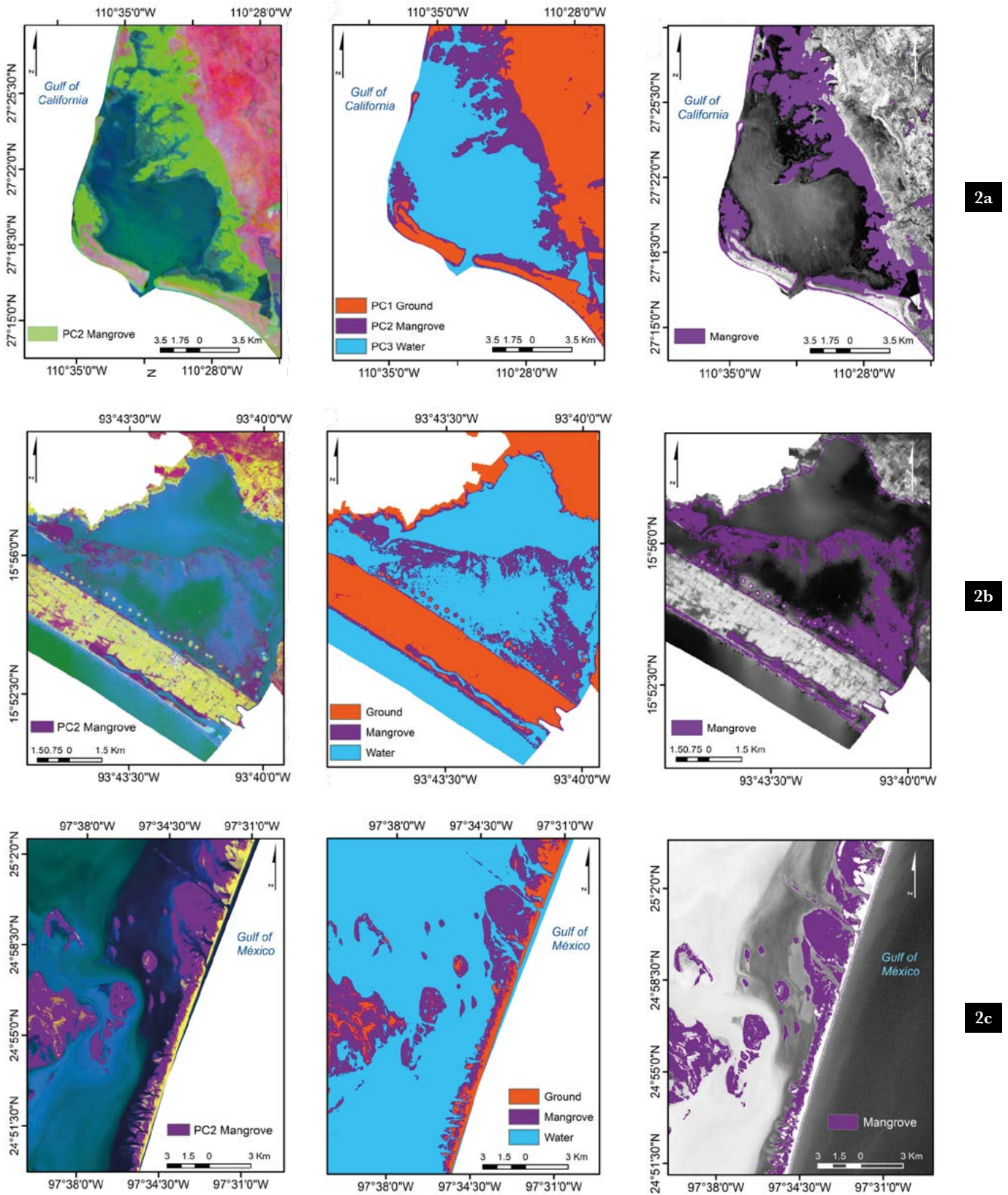


Figure 3. Studied mangrove swamps on Mexican coasts; 2a) Laguna Guasima, Sonora; 2b) Puerto Arista, Chiapas; 2c) Laguna Madre, Tamaulipas.

Table 3. Values of eigenvectors obtained for the first 3 PCs of the study areas.

Mangrove	Band	PCA1	PCA2	PCA3
Laguna Guasima	SWIR-1	0.543	-0.200	-0.528
	SWIR-2	0.576	-0.277	-0.166
	NIR	0.431	-0.259	0.829
	Red	0.292	0.552	0.027
	Green	0.292	0.552	0.027
	Blue	0.126	0.453	0.073
Puerto Arista	SWIR-1	0.324	0.459	-0.182
	SWIR-2	0.585	0.445	-0.366
	NIR	0.721	-0.664	0.152
	Red	0.140	0.259	0.594
	Green	0.085	0.173	0.481
	Blue	0.084	0.231	0.475
Laguna Madre	SWIR-1	0.521	-0.347	-0.150
	SWIR-2	0.680	-0.022	-0.459
	NIR	0.442	0.766	0.443
	Red	0.205	-0.391	0.451
	Green	0.129	-0.277	0.480
	Blue	0.107	-0.249	0.369

highest in PC 2. A similar pattern in cumulative variance was found to that reported by Sunkur *et al.*, (2023) in mangrove cover in Madagascar, but they use Sentinel-2 satellite images, finding cumulative variances for PC 2 between 5% and 8%, which is similar to the variances reported in this study (Table 2). PCA, besides being useful for mangrove cover detection (Kuenzer *et al.*, 2011) can also be used for change detection as reported by Prerna *et al.* (2015) in their investigation of mangrove swamp in the Gulf of Kachchh. Here, in addition to extracting mangrove cover with PCA, it was possible to identify dense and sparse mangroves, and to detect an increase in cover, from 30.69 km² in 1999 to 38.81 km² in 2010.

The advantage of using Landsat Collection Level 2 is that there is an archive managed by the USGS of imagery from virtually all over the world that is over 50 years old. Collection 2 images have better geolocation as well as radiometric calibration (Landsat 5, 8, 9) and contain global surface reflectance and surface temperature bands, which allows for multi-temporal analyses (Crawford *et al.*, 2023). Another advantage of Collection 2 is the atmospheric correction; traditional algorithms were based on dark objects and assumed that the TOA spectral reflectance of an object was equal to atmospheric reflectance (Chavez, 1996); however, these algorithms did not correct for atmospheric variations such as scattering and absorption captured by the image. In contrast, the surface reflectance obtained from Collection 2 Level-2 images is atmospherically corrected with the Landsat Ecosystem Data Adaptive Processing System (LEDAPS) algorithm (Masek *et al.*, 2006). This algorithm uses the solar spectrum vector (6SV) by band and retrieves the aerosol

optical thickness (AOT) independently using a dense and dark vegetation (DDV) approach (Crawford *et al.*, 2023).

CONCLUSIONES








This paper proposes a practical geoprocessing model for identifying and measuring mangrove cover using principal component analysis (PCA). The advantages of this method are: 1) it proposes the use of Collection 2 Level-2 images, which have radiometric and atmospheric corrections, 2) implementing the model in ArcGIS Model Builder enables an automated model that is intuitive, and allows the results to be exported to a Python script, and 3) the model can be replicated accurately with the QGIS model builder tool.

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Effect of partial or total substitution of fish meal by poultry by-product meal in diets of juvenile fish *Dormitator latifrons* (Richardson, 1844)

Aguirre-García, Zinnia E.^{1,2} ; Méndez-Soltero, Leticia² ; Barreto-Curiel, Fernando^{4*} ; Ponce-Palomera, Kevin O.² ; Vega-Villasante, Fernando² ; López, Lus M.⁴ ; Badillo-Zapata, Daniel^{2,3*} 

¹ Universidad de Guadalajara, Centro Universitario de la Costa, Programa de Doctorado en Ciencias en Biosistemática, Ecología y Manejo de Recursos Naturales y Agrícolas (BEMARENA). Av. Universidad 203, delegación Ixtapa, C. P. 48280, Puerto Vallarta, Jalisco, México.

² Laboratorio de Calidad de Agua y Acuicultura Experimental, Departamento de Ciencias Biológicas Centro Universitario de la Costa, Universidad de Guadalajara. Av. Universidad 203, delegación Ixtapa, C. P. 48280, Puerto Vallarta, Jalisco, México.

³ Investigadoras e Investigadores por México. Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI). Av. Insurgentes Sur 1582, Col. Crédito Constructor, Demarcación Territorial Benito Juárez. C. P. 03940, Ciudad de México.

⁴ Universidad Autónoma de Baja California (UABC), Facultad de Ciencias Marinas. Carretera Transpeninsular Ensenada - Tijuana No. 3917. Colonia Playitas C. P. 22860. Ensenada, Baja California, México.

* Correspondence: danielbad00@hotmail.com, fbarreto8@uabc.edu.mx)

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ABSTRACT

Objective: The present study evaluated the impact of replacing fishmeal (FM) with Poultry by-product meal (PBM) in balanced diets for *Dormitator latifrons*, testing four replacement treatments (0, 33, 67 and 100%).

Design/methodology/approach: The experiment was conducted using 180 specimens with an initial weight of 33.80 ± 0.08 g distributed in 600 L tanks fed at 2% of their total biomass, for 80 days.

Results: The results indicated that the inclusion of up to 33% of PBM did not significantly affect biological parameters compared to the control diet. However, the 100% PBM diet negatively affected blood parameters (such as glucose and protein) compromising the nutritional status of the fish. Although the proximate composition of muscle tissue was similar between treatments, the fatty acid profile showed deficiencies of Fatty acid composition (EPA) and docosahexaenoic acid (DHA, C22:6n3) in the 100PBM diet, essential for optimal growth and development.

Limitations of the study/implications: Poultry by-products represent a viable and sustainable alternative in the formulation of fish diets, with significant economic and environmental benefits. However, their use must be optimized through research that guarantees an adequate nutritional balance.

Findings/conclusions: In conclusion, the use of PBM up to 33% as a substitute for FM in the diet of *D. latifrons* is feasible, maintaining an adequate performance without compromising the flesh quality. Higher substitutions can impact negatively on both growth and physiological health of fish species, but also the nutritional quality of flesh, highlighting the need to adjust essential nutrient profiles in PBM-based diets. This approach combines sustainability and performance in the aquaculture of this species.

Keywords: Aquaculture; nutrition; by-products; animal welfare; native fish



INTRODUCTION

By-products of the food industry, defined as whole bodies or animal parts derived from the processing, manufacture or extraction of a primary product, mainly food intended for human consumption, represent a significant source of waste [1]. It is estimated that more than 1.3 billion ton of food waste, including by-products, are produced annually, equivalent to 13.8% of total world production [2]. This alarming figure has significant environmental, economic, and social implications [3]. In response to the growing demand for sustainable and environmentally friendly practices, increased emphasis has been placed on finding alternative applications for these waste materials. One promising strategy is the use of agro-industrial by-products as ingredients in diets formulated for aquaculture. Aquaculture has experienced accelerated growth in recent decades, consolidating its position as the most rapidly expanding food production system.

In Mexico, research on species with aquaculture potential has been predominantly directed towards exotic species such as tilapia (*Oreochromis niloticus*), however, there are native species with high culture potential that have outstanding nutritional characteristics and could be exploited in aquaculture. *D. latifrons*, known in different regions as Pacific fat sleeper, chopopo, chame, puyequé or popoyote, is a native species distributed along the Pacific coast, from California (USA) to Peru [4]. It inhabits freshwater environments such as riverbanks, marshes, coastal lagoons, and estuaries, and shows a preference for waters with temperatures between 21 °C and 30 °C. It has the ability to tolerate brackish water and low oxygen levels of up to 0.4 mg L⁻¹ [5].

Aquaculture faces the challenge of producing high nutritional quality feeds that reduce costs, minimize environmental impact and achieve an optimal feed conversion factor (biomass gained per kilogram of feed provided) [6]. A crucial aspect of this challenge is to design diets that promote growth, decrease dependence on fishmeal (FM) as the main protein source and increase sustainability [7]. Poultry by-product meal (PBM) is a by-product of poultry processing and its quality depends on factors such as raw material composition, heating process, water and fat extraction, as well as cooking time [8]. This meal contains 30% to 65% protein and it has been shown that its inclusion in diets for aquatic organisms can significantly reduce the use of fishmeal without compromising growth [9]. Therefore, the main objective of this study was to evaluate the partial and total replacement of fishmeal with poultry by-product meal in diets formulated for juvenile *D. latifrons*, measuring growth and animal welfare indices.

EXPERIMENTAL DESIGN AND METHODOLOGY

Preparation of diets

The study was carried out at the Laboratory of Water Quality and Experimental Aquaculture (LACUIC) of the Centro Universitario de la Costa, in Puerto Vallarta, Jalisco, Mexico. Four balanced isoproteic (30% total protein) and isolipidic (8% total lipids) diets were formulated following the methodology and nutritional requirements established by Badillo-Zapata *et al.* [10] in order to replace 0, 33, 67 and 100% of the fish meal with poultry by-product meal. The composition of ingredients is detailed in Table 1.

Table 1. Ingredients (g kg^{-1}) and proximal composition (g kg^{-1} dry weight), of the control diet (0PBM) and three experimental diets (33PBM, 67PBM and 100PBM) containing different levels of fishmeal substitution by poultry by-product meal. “Pet food grade” (PBM) used to feed *D. latifrons* juveniles, for 80 days.

	Experimental Treatments			
	0PBM	33PBM	67PBM	100PBM
Poultry by-product meal ^a	0	129	225	369
Fish meal ^a	368	235	130	0
Fish Oil	53	43	35	20
Corn flour	426	440	457	458
Corn starch	55	55	55	55
Gelatin	60	60	60	60
Vitamins and Minerals ^b	30	30	30	30
Vitamin C ^b	5	5	5	5
Sodium Benzoate	2	2	2	2
Choline Chloride	1	1	1	1
Alpha Tocopherol	0.1	0.1	0.1	0.1
Total	1000	1000	1000	1000
Proximal composition (g/kg)				
Protein	330.3	320.9	340.1	350.1
Lipids	81.8	78.7	82.3	79.9
Ash	103.5	93.5	83.9	77.8
Carbohydrates (ELN)	484.4	506.9	493.7	492.2

NFE (g/kg) = $100 - (\text{g/kg crude protein} + \text{g/kg total lipid} + \text{g/kg ash})$. NFE includes fiber.

^aPoultry by-product meal (65.5% CP; 12.0% LC) and fish meal (68% CP; 8.0% LC) were obtained from Proteínas Marinas y Agropecuarias S.A. de C.V. in Guadalajara Jalisco, Mexico. ^bDSM Nutritional Products Mexico SA de CV. Rovimix[®]; Vitamin and mineral mix (g/kg): *p*-aminobenzoic acid 1.45; biotin 0.02; myo-inositol 14.5; nicotinic acid 2.9; Capantothenate 1.0; pyridoxine-HCl 0.17; riboflavin 0.73; thiamine-HCl 0.22; menadione 0.17; α -tocopherol 1.45; cyanocobalamine 0.0003; calciferol 0.03; L-ascorbyl-2-phosphate-Mg 0.25; folic acid 0.05; choline chloride 29.65; retinol 0.015; NaCl 1.838; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 6.85; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 4.36; KH_2PO_4 11.99; $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot 2\text{H}_2\text{O}$ 6.79; Fe-citrate 1.48; Ca-lactate 16.35; $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ 0.009; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.17; CuCl_2 0.0005; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 0.04; KI 0.008; CoCl_2 0.05 and Stay-C (Vitamin C) donated by DSM, Nutritional Products Guadalajara, Mexico.

Fish breeding, feeding and biometrics

A total of 180 juveniles with an average weight of 33.80 ± 0.08 g and a length of 16.7 ± 0.6 cm were selected from the LACUIC laboratory stock and randomly distributed in 12 tanks of 600 L, with 15 organisms per tank. Each experimental unit was connected to a canister filter (EF-05 SERIES) with constant aeration and a 30% weekly freshwater replacement. Water temperature was maintained at 23.0 ± 1.0 °C using a heater (Thermal Pro 200W Lomas), and the dissolved oxygen level was maintained at 5.5 ± 1.0 mg L^{-1} , measured with a Hanna oximeter[®] (HI9146-04). Water pH was recorded at 8.1 ± 0.3 , determined using a pH meter (HI8314), and the photoperiod was maintained naturally at 12 hours of light and 12 h of darkness. Feed was supplied once a day (at 16:00 h), and the amount administered in each tank corresponded to 2% of the total biomass of each experimental unit. An initial biometry was performed using a digital balance (OHAUS[®] PR2201) with

an accuracy of ± 0.01 g. At the end of the experiment, after 80 days, final biometry was performed to determine growth, fish feed efficiency and other performance indices, which were calculated using the following formulas:

Specific growth rate:

$$SGR = \frac{(\ln \text{ final weight} - \ln \text{ initial weight})}{\text{time (days)}} \times 100$$

Weight increment:

$$\% \text{ Weight gain} = \left[\frac{(\text{final weight} - \text{initial weight})}{\text{initial weight}} \right] \times 100$$

Food Conversion Ratio (FCR):

$$FCR = \frac{\text{dry weight feed consumed (g)}}{\text{wet weight gain (g)}}$$

Blood parameters

15 fish were randomly selected from each experimental treatment (five specimens per pond) for been anesthetized. Anesthesia was performed according to Aréchiga-Palomera *et al.* [11]. Once the fish showed erratic swimming, blood samples were collected under aseptic conditions by caudal vein puncture, using a 1 mL insulin syringe with 30G \times 13 mm needle, following the protocol described by Stoskopf [12].

Hematological profile

For the hematological profile, basic parameters were determined: hemato-crit (HCT), respiratory burst (NBT), erythrocyte count (RBC), and white blood cell count (WBC). HCT was determined using the microhematocrit technique [13]. NBT was evaluated following the methodology of Ibrahim *et al.* [14]. For erythrocyte and leukocyte counts, a 20 μ L sample of EDTA-K2 blood was placed in 4 mL of Natt-Herrick's solution. In a Neubauer chamber with 1/400 mm² and 1/10 mm depth, it was filled with 5 μ L of the dilution. The analysis was performed by observation under a Quasar Qm20 2500x Binocular Professional Microscope. Erythrocytes were counted in the central grid, from which five squares of 0.0025 mm² each were selected, and leukocytes in four large squares, from each corner, with an area of 1 mm² each.

Fatty acid composition

Samples were taken from the four diets and from the muscle tissue of the fish from each experimental unit (n=5). Extraction of fatty acids and transmethylation was performed using the technique described by Parrish *et al.* [15]. To separate and quantify fatty acids,

an AGILENT gas chromatograph (GC 7820) was used, equipped with a Split/Splitless injector, a flame ionization detector (FID) and an AGILENT capillary column (122-2361 DB-23) 60 m × 0.25 mm with an internal diameter of 15 μ m. Calculations were performed using the GC Chemstation Data Analysis software. The initial injection temperature was 120 °C for 1 min, then raised to 190 °C at a rate of 25 °C/min, then increased to 230 °C at a rate of 6 °C/min and nitrogen (N₂) was used as carrier gas at 1.0mL/min. Fatty acids were identified by comparison with the retention times of the standard mixture of 37 components FAME, PUFA1 and PUFA3 (Supelco/Sigma-Aldrich®).

Proximal analysis

Poultry by-product meal, experimental diets and muscle tissue were subjected to proximate analysis: quantification of crude protein (method 960.52), crude lipids (method 920.97) and ash (method 942.05) following the standard methods of the Association of Official Analytical Chemists [16].

Statistical analysis

All data are presented as mean and standard deviation ($X \pm SD$). Normality (Shapiro Wilk) and homoscedasticity (Bartlett) tests were performed. When the statistical assumptions were met for the data of biological indices, blood parameters, fatty acids and proximate analyses, a one-way analysis of variance (ANOVA) with an $\alpha=0.05$, was applied among the four treatments. To identify differences between treatments, a Tukey's posterior test ($P<0.05$) was used. All analyses were performed with Minitab® 19.1.1 software.

RESULTS

Biological indexes

The results obtained show that there were no significant differences in the initial weight between the treatments (Table 2). However, the final weight showed a significant reduction ($p<0.05$) in the 100PBM group (64.8 ± 9.4 g) compared to the other treatments, while the 0PBM group obtained the highest final weight (88.9 ± 16.7 g). The specific growth rate (SGR) also decreased significantly in the 100PBM treatment ($1.3 \pm 0.1\%$) compared to 0PBM and 33PBM. Similarly, the weight gain (%) was lower in 100PBM ($49.3 \pm 74.5\%$), while 0PBM obtained the highest value ($139.2 \pm 23.1\%$). Regarding the feed conversion ratio (FCA), a tendency to decrease with increasing PBM in the diet was observed, being significantly lower in 100PBM (0.4 ± 0.2), suggesting lower feed efficiency. Finally, the survival rate was lower in the 100PBM treatment ($77.7 \pm 6.6\%$) compared to the other groups, which maintained values higher than 95%. These results suggest that a high inclusion of PBM in the diet negatively affects the growth and survival of the fish.

Blood parameters

The results show that hematocrit (HCT) was significantly reduced ($p<0.05$) in the 100PBM treatment ($26.5 \pm 1.1\%$) compared to the other treatments, while there were no significant differences between the 0PBM, 33PBM and 67PBM groups (Table 3). Regarding the erythrocyte (RBC) count, a progressive decrease was observed as the

Table 2. Biological indices in juvenile poplar (*D. latifrons*) after being fed for 80 days with diets containing different levels of fishmeal to poultry by-product meal (PBM) substitution.

Biological indexes	Experimental treatment			
	0PBM	33PBM	67PBM	100PBM
Initial weight (g)	33.9±0.1	33.7±0.1	33.8±0.0	33.8±0.0
Final weight (g)	88.9±16.7 ^a	72.2±15.2 ^b	78.2±19.4 ^{ab}	64.8±9.4 ^c
Specific growth rate (%) ¹	1.5±0.2 ^a	1.4±0.2 ^b	1.4±0.2 ^{ab}	1.3±0.1 ^c
Weight gain (%) ²	139.2±23.1 ^a	104.8±16.4 ^b	126.2±7.9 ^a	49.3±74.5 ^c
FCA ³	0.8±0.1	0.6±0.1	0.7±0.05	0.4±0.2
Survival (%)	97.7± 3.8	95.5± 7.7	97.7± 3.8	77.7± 6.6

Values are means ± standard deviation, different superscripts mean significant differences (P<0.05).

¹TEC=(ln final weight–ln initial weight)/time (days)×100. ²Weight gain (%)=[(final weight–initial weight)/initial weight]×100; ³FCA=feed dry weight consumed (g)/wet weight gain (g).

Table 3. Blood parameters of juvenile poplar (*D. latifrons*) after being fed for 80 days with diets containing different levels of fishmeal to poultry by-product meal (PBM) substitution.

Parameters blood	Experimental treatments			
	0PBM	33PBM	67PBM	100PBM
HCT (%) **	33.3±2.0a	32.3±1.5a	33.2±1.7a	26.5±1.1b
NBT (abs)*	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
RBC(×10 ⁶ /μL)	1.9±0.0a	2.0±0.1a	1.0±0.0b	0.8±0.0c
WBC(×10 ³ /μL)	14.3±1.2b	12.0±1.0b	13.5±1.0b	18.1±1.0a
Protein (g/dL)	6.5±1.0a	6.4±1.2a	5.5±1.0b	4.3±1.0c
Albumin (g/dL)	1.5±0.2b	1.6±0.1a	1.5±0.2b	1.1±0.2c
Globulin (g/dL)	5.0±1.0a	4.7±1.2a	3.9±1.0a	3.1±1.0b
A/G Ratio	0.3±0.0	0.3±0.1	0.4±0.1	0.3±0.1
Glucose (mg/dL)	110.2±4.3b	129.7±7.6a	76.3±6.8c	66.7±3.7d
Cholesterol (mg/dL)	175.7±3.0d	225.2±4.2b	246.7±2.7a	188.9±1.4c
Triacylglycerides (mg/dL ⁻¹)	661.6±3.2a	444.0±5.1d	509.2±4.3c	643.4±7.5b

Values are means ± standard deviation, different superscripts mean significant differences (P<0.05).

Hematological profile: hematocrit (HCT), respiratory burst (NBT), erythrocytes (RBC) and leukocytes (WBC). Biochemical parameters: protein, albumin, globulin, A/G ratio, glucose, cholesterol and triacylglycerides of blood and plasma under laboratory conditions for 80 days.

inclusion of PBM in the diet increased, being significantly lower in 67PBM and 100PBM compared to 0PBM and 33PBM. On the other hand, the leukocyte (WBC) count was significantly higher in the 100PBM group (18.1±1.0×10³/μL) compared to the other treatments. The levels of total proteins, albumin and globulin decreased significantly in the 100PBM treatment compared to the other groups. Regarding metabolic parameters, glucose showed a significant decrease in 67PBM and 100PBM compared to 0PBM and 33PBM groups. Likewise, cholesterol levels were higher in 33PBM and 67PBM treatments, while triglycerides had their highest value in 0PBM group and the lowest in 33PBM. These results indicate that a higher inclusion of PBM in the diet negatively affects several hematological and biochemical parameters in fish.

Biochemical composition of the tissue

The table presents the results of the proximate composition and fatty acids in proximal muscle under different experimental treatments (0PBM, 33PBM, 67PBM, 100PBM) (Table 4 y 5). Regarding saturated fatty acids (SFA), no significant differences were observed between treatments, with values ranging from 27.8 ± 0.6 to 32.1 ± 1.7 . Monounsaturated fatty acids (MUFA) showed a significant increase in the 100PBM treatment (34.3 ± 0.3) compared to the other treatments. Polyunsaturated fatty acids (PUFA) presented their highest concentration in the 67PBM treatment (35.2 ± 0.5), highlighting a high content of C18:2n6 (30.9 ± 2.5). Regarding n3 fatty acids, a progressive decrease was observed from 22.8 in 0PBM to 1.0 in 100PBM.

The proximate muscle composition showed consistent values of protein (840.5 ± 0.2 to 840.3 ± 0.2 g/kg), lipids (50.7 ± 0.2 to 50.9 ± 0.4 g/kg) and ash (60.1 ± 0.8 to 60.3 ± 0.7 g/kg) across all treatments, indicating that these did not significantly affect the basic muscle composition.

Table 4. Fatty acid (FA) composition of the control diet (0PBM) and three experimental diets (33PBM, 67PBM and 100PBM) containing different levels of fishmeal substitution for “Pet food grade” poultry by-product meal (PBM) used to feed *D. latifrons* juveniles, for 80 days.

Experimental treatments				
AG	0PBM	33PBM	67PBM	100PBM
C14:0	5.6	4.7	3.7	3
C15:0	0.8	0.7	0.6	Nd
C16:0	21.3	22.1	22.1	28.2
C18:0	5.2	5.8	6.1	7.9
C24:0	0.3	Nd	Nd	Nd
Σ SFA ¹	33.2	33.3	32.5	39.1
C16:1n-7	6.1	6.0	5.9	6.2
C18:1n-7	2.8	2.8	2.4	2.2
C18:1n-9	15.6	18.4	22	29.7
Σ MUFAS ²	24.5	27.2	30.3	38.1
C18:2n-6	8.9	11.4	13.6	10.2
C18:3n-3	1.9	1.7	1.5	0.5
C20:3n-3	1.8	1.9	1.8	0.8
C20:5n-3	6.4	5.2	3.8	Nd
C22:5n-3	1.0	0.8	0.6	0.5
C22:6n-3	9.1	6.9	4.8	0.5
Σ PUFAS ³	33.4	36.0	38.6	46.5
Σ n3	44.7	43.7	42.8	40.4
Σ n6	8.9	11.4	13.6	10.2
EPA/DHA	0.7	0.8	0.8	0

Fatty acid composition values are the mean (\pm SD) of two replicates. Nd not detected.

¹Total saturated fatty acids included C13:0, C14:0, C15:0, C16:0, C18:0 and C24:0.

²Total monounsaturated fatty acids included C16:1n-7, C18:1n-9 and C18:1n-7.

³Total n-3 and n-6 polyunsaturated fatty acids included C18:2n-6, C18:3n-3, C20:3n-3, C20:5n-3, C22:5n-3 and C22:6n-3.

Table 5. Fatty acid composition and proximate analysis of poplar (*D. latifrons*) muscle tissue after being fed for 80 days with diets containing different levels of fishmeal to poultry by-product meal (PBM) substitution.

Experimental treatments				
AG	0PBM	33PBM	67PBM	100PBM
C14:0	2.4±0.4a	1.7±0.2b	1.0±0.4c	0.8±0.0c
C15:0	0.6±0.4a	0.6±0.1a	Nd	0.3±0.0a
C16:0	18.1±0.8c	20.5±0.7b	19.4±1.0bc	24.6±0.9a
C18:0	8.6±0.3a	6.7±0.3b	6.8±0.7b	6.2±0.5b
C24:0	0.8±0.0a	0.9±0.0a	0.4±0.0b	Nd
∑SFA ¹	30.7±0.9	30.5±0.5	27.8±0.6	32.1±1.7
C14:1	0.7±1.7a	0.5±0.9a	1.8±2.8a	Nd
C15:1	0.5±0.0a	Nd	0.3±0.2b	Nd
C16:1	2.6±0.2a	2.9±2.3a	2.5±0.2a	4.8±0.4a
C18:1n9	15.5±0.7d	21.3±1.2b	18.1±1.0c	25.1±0.3a
C18:1n7	3.8±0.1a	3.8±0.2a	4.0±0.5a	4.3±0.2a
C20:1	0.3±0.1b	0.4±0.0b	0.8±0.4a	Nd
C24:1	0.8±0.1a	0.4±0.2b	Nd	Nd
∑MUFAS ²	23.2±1.1b	29.0±0.5b	25.6±0.4b	34.3±0.3a
C18:2n6	11.2±0.4c	14.7±0.8b	30.9±2.5a	16.1±0.5b
C20:3n3b	0.4±0.0b	0.2±0.2b	0.5±0.0b	1.0±0.3a
C20:4n6	0.3±0.0a	0.2±0.0b	Nd	Nd
C20:4n3	4.9±0.3a	3.7±0.3b	1.8±0.1c	Nd
C20:5n3	4.4±0.2a	2.1±0.1b	0.5±0.1c	Nd
C22:5n3	3.9±0.1a	3.5±0.2a	0.9±0.0b	Nd
C22:6n3	8.9±0.4a	5.7±0.3b	0.6±0.2c	Nd
∑PUFAS ³	20.5±1.4b	20.7±1.1b	35.2±0.5a	16.1±0.0c
∑n3	22.8	15.4	4.5	1.0
∑n6	11.5	15.0	30.9	16.1
EPA/DHA	0.5	0.4	0.9	0
Proximal muscle composition (g/kg dry basis)				
Protein	840.5±0.2	840.4±0.7	840.4±0.4	840.3±0.2
Lipids	50.7±0.2	50.9±0.4	50.8±0.6	50.8±0.3
Ash	60.1± 0.8	60.3±0.7	60.1±0.8	60.2±0.7
Carbohydrates (ELN)	48.7±1.1	48.4±1.1	48.7±0.6	48.6±1.0

Fatty acid composition values are the mean (±SD) of two replicates. Nd not detected.

¹Total saturated fatty acids included C13:0, C14:0, C15:0, C16:0, C18:0 and C24:0.

²Total monounsaturated fatty acids included C16:1n-7, C18:1n-9 and C18:1n-7.

³Total n-3/n-6 polyunsaturated fatty acids included C18:2n-6, C18:3n-3, C20:3n-3 and C20:5n-3, C22:5n-3 and C22:6n-3.

Values are means ± standard deviation of 100% of methylated fatty acids. Different superscripts mean significant differences (P<0.05).

DISCUSSION

The present study provides valuable information on the replacement of fishmeal with a significant amount of PBM in balanced diets for *D. latifrons* on biological indices, blood parameters and fatty acid composition of fish flesh. The result obtained in this experiment shows that the inclusion of PBM up to 33% had no adverse effects on biological indices (specific growth rate, weight gain, feed conversion factor and survival). A meta-analysis exploring the effects of replacing fishmeal with PBM found no significant differences in growth performance for most species studied [9]. This is likely due to the high crude protein content, favorable amino acid profile and lack of anti-nutritional factors usually associated with plant-based proteins [17]. However, like other animal-based proteins, variation in the quality of nutritional composition is common, largely due to alteration in raw material structure, quality and processing specifications, such as cooking process temperature, which is critical in determining the quality of the final product [18]. This variation results in deficiencies in certain essential amino acids, higher ash content and variability in digestibility. In this context, it has been shown that PBM can be used within diet formulations for aquatic organisms such as Nile tilapia (*Oreochromis niloticus*), catla (*Catla catla*), rohu (*Labeo rohita*), striped bass (*Morone saxatilis*), tench (*Tinca tinca*), trout (*Oncorhynchus mykiss*), grouper (*Epinephelus malabricus*), golden pompano (*Tachinotus ovatus*), juvenile gilthead sea bream (*Sparidentex hasta*) and juvenile totoaba (*Totoaba macdonaldi*) [7,19,20]. FM replacement percentages vary by species, however, replacement ranges from 15% to 67% have been observed that do not generate significant differences compared to organisms fed 100% FM [8,21-23] 150 or 500 g kg⁻¹ of fish meal protein was substituted by MBM (MBM15, MBM50. Particularly, it has been observed that proportions of 15, 25 and 35% PBM in juvenile gilthead sea bream (*Sparidentex hasta*) and 67% in totoaba (*Totoaba macdonaldi*), increase weight gain and improve feed conversion factor [24]. However, the importance of protein quality is highlighted, noting the lower availability of certain essential amino acids (AA) in PBM compared to fishmeal, which has the ideal AA profile for most aquaculture species [25]. In general, PBM protein is known to contain lower levels of methionine and lysine compared to FM, which is considered the limiting factor for growth in many species, such as Florida pompano (*Trachinotus carolinus*), African catfish (*Clarias gariepinus*), humpback grouper (*Chromileptes altivelis*), trout (*Oncorhynchus mykiss*), striped bass (*Morone saxatilis*), gibel carp (*Carassius auratus gibelio*) and sea bream (*Sparus aurata*) at higher dietary PBM inclusions [8,25-30] 150 or 500 g kg⁻¹ of fish meal protein was substituted by MBM (MBM15, MBM50.

Hematological profile and biochemical parameters, are fundamental tools for assessing fish welfare, health, immune system response, short- and long-term effects of “suboptimal” culture conditions, water quality, potential disease outbreak and nutritional status [31]. These parameters can be influenced by a wide variety of factors, including species type, season of the year, temperature, salinity, pH, presence of contaminants, nutrition, culture density, presence of diseases, farm conditions, and sampling method, among others [32].

Hematocrit (HCT) values are closely related to the activity and habitat of fish [33]. In this experiment, HCT ranged between 26.5% and 33.3%, these values, are similar to what was reported in the same species Todd [34] 39.1%. Ruiz-González *et al.* [35] 28%

and Santana-Piñeros *et al.* [36] 38%. El hematocrit remained stable in the 0PBM, 33PBM, and 67PBM treatments, but showed a significant reduction in 100PBM, suggesting a possible alteration in erythropoiesis or a deficiency of essential nutrients for red blood cell production [37]. This result is consistent with the decrease in RBC (red blood cell) count in the groups with higher inclusion of PBM, which could indicate an adverse effect on the oxygenation of the organism [38].

On the other hand, WBC values are similar to those of other demersal and sedentary species, such as the goldfish *Carassius auratus* ($10.1-14.7 \times 10^3$ cells μL^{-1}), the black goby *Gobius niger* ($8.0-10.8 \times 10^3$ cells μL^{-1}) and the African catfish *Clarias gariepinus* ($11-11.1 \times 10^3$ cells μL^{-1}) [33,39,40]. In *D. latifrons* our WBC values were very low, similar to the values of Santana-Piñeros *et al.* [36] ($8.9-10.3 \times 10^3$ cells μL^{-1}). WBC values decreased in 33PBM and 67PBM compared to 0PBM, which could reflect a lower immune activation. However, in 100PBM, a significant increase was observed, which could suggest an inflammatory response or a possible immune challenge associated with the high level of PBM in the diet. Despite these changes in leukocytes, neutrophil oxidative activity, measured by NBT, remained constant in all treatments, indicating that PBM did not significantly stimulate the production of reactive oxygen species in these cells [41].

Plasma protein levels also showed a decreasing trend as PBM in the diet increased. Both albumin and globulin decreased in treatments with higher inclusion of PBM, which could suggest a lower availability of proteins in the diet or an increase in their catabolism [42]. This could be related to a lower supply of essential amino acids in diets with high amounts of PBM or to a lower efficiency in nutrient absorption [43]. The A/G ratio remained stable, indicating that the reduction in plasma proteins proportionally affected both components.

Energy metabolism also showed important changes. The blood glucose levels differ considerably among fish species [44]. In the present study glucose levels were drastically reduced in 67PBM and 100PBM, which could indicate a lower availability of carbohydrates or an alteration in the regulation of energy metabolism [33]. The lipid profile presented variations, with an increase in cholesterol in 33PBM and 67PBM, followed by a decrease in 100PBM. This could be related to changes in lipid absorption or synthesis due to the composition of the diet.

The specific range of cholesterol and triacylglycerides levels in *D. latifrons* are not widely documented, however, it is known that fish triacylglyceride levels are usually relatively low, and variations may depend on factors such as diet, health status, time of year and environment [33]. Triglycerides, on the other hand, decreased with the partial inclusion of PBM, but in 100PBM they increased again, suggesting a possible alteration in lipid metabolism in the presence of high levels of PBM.

Taken together, these results indicate that moderate levels of PBM can be well tolerated without significantly affecting blood parameters, but a total inclusion of PBM in the diet can compromise red blood cell production, protein homeostasis and energy metabolism. It is recommended to evaluate micronutrient supplementation strategies to mitigate the adverse effects observed at high PBM inclusions and to conduct additional studies to better understand the physiological mechanisms involved.

The fatty acid (FA) composition of muscle tissue of most fish species is strongly influenced by the FA content of the diet [45]. Poultry by-product meal generally contains high levels of monounsaturated fatty acids (MUFAs) particularly oleic acid (OA, C18:1n9) and polyunsaturated fatty acids particularly linoleic acid (LA, C18:2n6), but contains low levels in essential fatty acids (EFAs) such as the long-chain polyunsaturated fatty acids LC-PUFAs of series n-3 (EPA and DHA) [46]. In the present study the inclusion of hydrolyzed feather meal (PBM) in the diet significantly altered the fatty acid profile of proximal muscle, increasing saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), while polyunsaturated fatty acids (PUFA), especially those of the n3 series, progressively decreased with increasing PBM [47]. The increase in palmitic acid (C16:0), present in vegetable oils such as palm and in animal fats, and stearic acid (C18:0), abundant in animal fats and butters, suggests a greater accumulation of SFA due to the lower availability of PUFA in PBM compared to other protein sources, such as fishmeal [7]. Likewise, the increase in oleic acid (C18:1n9), characteristic of vegetable oils such as olive and canola, indicates a possible metabolic adaptation to the lipid profile of the diet, although the reduction in myristoleic acid (C14:1), originating from dairy fats and fish oils, and pentadecenoic acid (C15:1), of bacterial origin and present in some dairy products, suggests a lower bioavailability of these fatty acids in PBM [48]. The drastic decrease in n3 PUFA, particularly eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3), both present in fish oils and marine microalgae, in 100PBM is worrying, since these compounds play essential roles in fish health, including the regulation of anti-inflammatory processes and the development of the nervous system [49]. The reduction of these fatty acids may affect the nutritional quality of the muscle and its value for human consumption, suggesting the need to supplement these diets with sources rich in n3 PUFA, such as marine oils or microalgae, to mitigate the negative effects of the total replacement of fishmeal by PBM [50].

CONCLUSIONES

Replacing fish meal (FM) with hydrolysed feather meal (PBM) in fish diets resulted in significant changes in several physiological, biochemical and muscle composition parameters, highlighting both its advantages and limitations. In haematological terms, the reduction in haematocrit and red blood cells at the highest levels of PBM could suggest a lower efficiency in blood oxygenation, while the increase in leukocytes at 100PBM indicates a possible immune or inflammatory response. Biochemical parameters revealed a negative impact on plasma protein and albumin levels, which could be related to a lower bioavailability of certain essential amino acids in PBM. Furthermore, the reduction of polyunsaturated fatty acids (PUFA), especially those of the n3 series, together with the increase of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), suggests a negative effect on the nutritional quality of muscle, affecting its value both for fish health and for the final consumer. However, the stability of the proximal composition of muscle in terms of protein and lipids suggests that PBM may be a viable protein source, provided that supplementation strategies are implemented to mitigate its nutritional deficiencies, particularly in essential fatty acids and limiting amino acids. In conclusion, although PBM

represents a promising alternative for the reduction of FM use in aquaculture, its inclusion should be carefully formulated to avoid adverse effects on the physiology, health and final quality of the product.

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Bibliometric analysis of scientific research: bioeconomy and climate change

Torres-Tadeo, César M.¹; Platas-Rosado, Diego E.¹; Tadeo-Castillo, Clotilde I.²; Gutiérrez-Salas, Yesenia V.¹; Pérez-Vázquez, Arturo^{1*}

¹ Colegio de Postgraduados. Tepetates, Manlio Fabio Altamirano, Veracruz, México. C. P. 91700.

² Universidad Veracruzana. Costa verde, Boca del Rio, Veracruz, México. C. P. 94294.

* Correspondence: parturo@colpos.mx

ABSTRACT

Objective: To offer a comprehensive bibliometric study of how bioeconomy research has been developed concerning climate change, finding the most researched issues, as well as the least researched issues and trends in scientific publications during the last ten years.

Design/methodology/approach: To conduct this study, the academic database Scopus was used. The selection criteria were the keywords “bioeconomy” and “climate change”. The selected study period was ten years (2014-2024), considering all types of documents such as articles from scientific journals, reviews, book chapters, conferences, among others. The obtained data was processed in VOSviewer software tool to construct bibliometric network map.

Results: The bibliometric data analyzed was a total of 791 scientific publications related to bioeconomy and climate change in the last ten years. Production has shown a significant increase, with notable trends since 2016-2023, being the year with major publications (165), reflecting a growing interest in the interaction of both fields. Subjects with major scientific production are environmental sciences, agriculture, biological sciences and energy, while the subjects with lesser scientific production are veterinary, psychology and nursing.

Limitations on study/implications: The scope’s study exclude another scientific database.

Findings/conclusions: Some of the identified emerging trends are: interdisciplinarity, reflecting an increase in research combining environmental, economic and social sciences to address complex problems related to bioeconomy and climate change. And the focus on public policies, which presents a recent interest in how policies can integrate the bioeconomy to face climate challenges.

Keywords: co-occurrences, VOSviewer, circular economy.

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INTRODUCTION

According to Lewandowski (2017), the bioeconomy concept evolution began in the 1960s decade on the proposal of Zeman, who utilized the concept to name an economic order that recognizes the biological bases of almost all economic activities, and then, It was defined for the first time by Juan Enriquez Cabot (1998) stating that, the application of discoveries in genomics will conduce to a restructuration in the roll of enterprises and industries, and it will change the world economy. Described the creation of a new economic sector: Biological Science. He didn’t use the bioeconomy concept, but this may be the first debate that would lead later to recent concepts. In the document, aspirational principles and criteria for a sustainable bioeconomy are stated. According to FAO (2021), bioeconomy is defined as the production, use, conservation and regeneration of biological resources, including related knowledge, science, technology and innovation, to provide sustainable solutions within and across all economic sectors and enable a transformation towards a sustainable economy.

MATERIALS AND METHODS

Scopus was used as the main source for bibliographic data collection. The search was made using the combination of terms such as “bioeconomy and climate change”, selecting publications from the period 2014 to 2024. The analysis was conducted using basic statistical tools in Microsoft Excel, together with the advanced capabilities of VOSviewer for graphical visualization. VOSviewer is a tool developed by the Center for Science and Technology Studies (CWTS). It was used to generate bibliometric maps and visualize the relationships between the analyzed documents. A bibliometric network map was generated from conceptual bibliographic data showing the relationships between different documents and the co-occurrence of keywords within the field of study. This map allows us to identify thematic groups and emerging trends in research on bioeconomy and climate change. This methodological approach not only helps to understand the current state of knowledge in this field but also guides future research by identifying gaps in the existing literature.

RESULTS AND DISCUSSION

Figure 1 shows the increase in scientific publications related to the keywords: bioeconomy and climate change. In 2015 only seven documents were identified where the relationship exists in the concepts. From 2020 onwards, more considerable production was achieved with 74 articles, finding 165 in 2023. In the second half of the year 2024, 137 documents were found. According to Pyka *et al.* (2021) bioeconomy modeling must consider variables such as climate change, biodiversity, circular biomass use, consumer behavior related to biomass and bioproduct use, and innovation and technological change. Wohlfahrt *et al.* (2019) propose an integrated assessment to tackle specific bioeconomy sustainability issues, among them, climate change.

Figure 2 shows the top ten authors in the topic. Blumberga, D., is the one with the most scientific publications, nine during the analysis period.

Figure 3 represents the group of the ten most publications by affiliation has 213 publications in the analyzed period (2014-2024). The main three affiliations show their

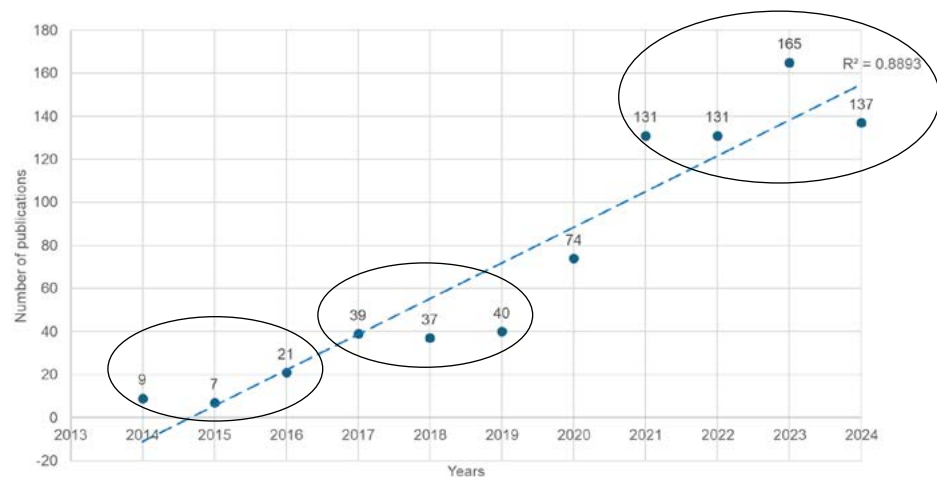


Figure 1. Temporal evolution of scientific production of bioeconomy and climate change.

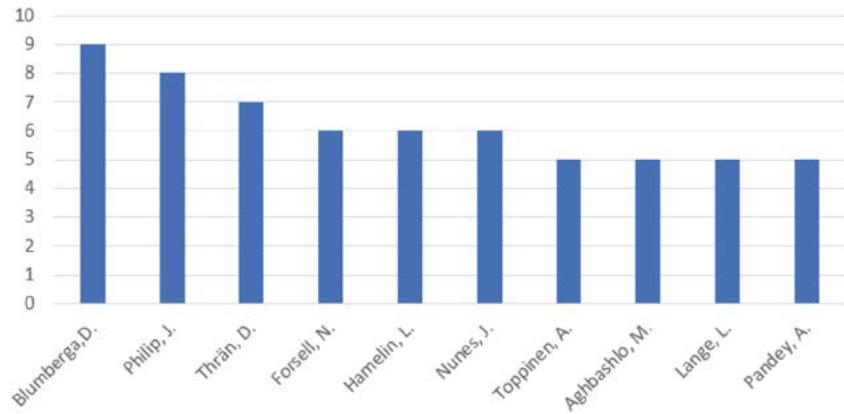


Figure 2. Publications by author on bioeconomy and climate change.

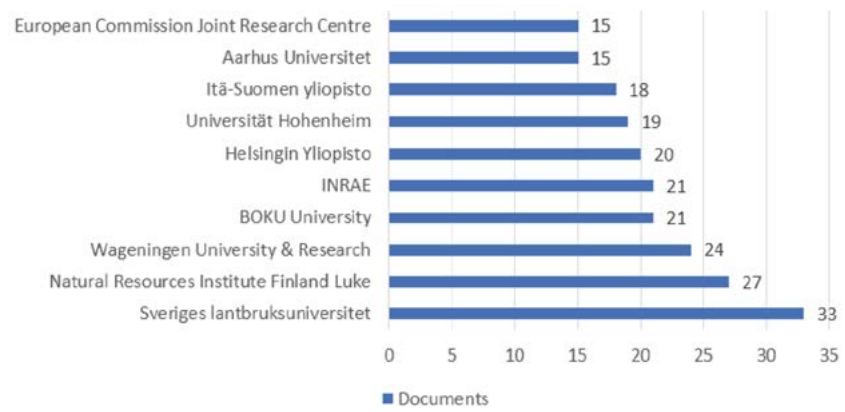


Figure 3. Publications by affiliation of bioeconomy and climate change.

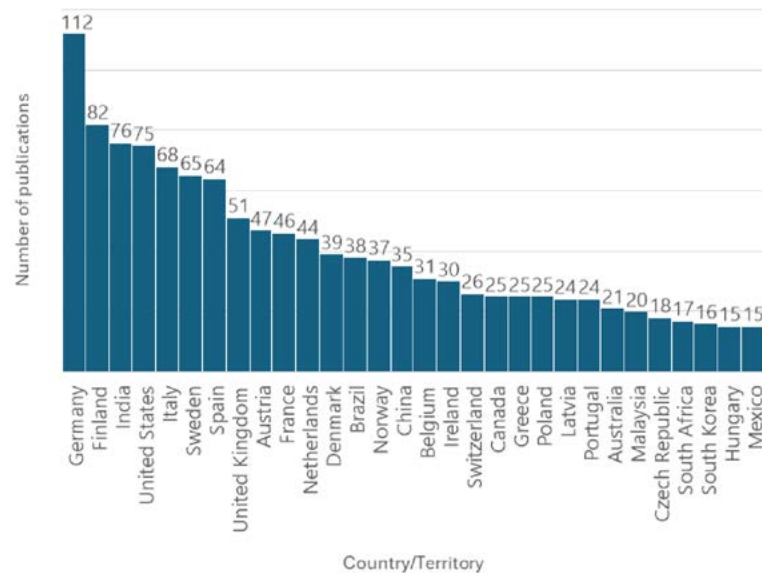


Figure 4. Publications by country on bioeconomy and climate change.

leadership in the scientific production of the studied knowledge field, with 39% of the publications in the mentioned group. Resulting: Sveriges Iantbruksuniversitet (33), Natural Resources Institute Finland Luck (27) and Wageningen University & Research (24).

Analysis criteria taken of sample universe selected was a minimum of five documents with a minimum of five countries. The countries where belong authors most cited are Germany 109 documents and 2356 cites, Finland 79 documents and 1989 cites, India 74 documents and 2353 cites, United States 72 documents and 1459 cites, Italy 65 documents and 1759 cites (Figure 5).

It is appreciated that most of the documental production on the field corresponds to articles (416, 52.6%), reviews (175, 21.1%) and book chapters (113, 14.3%). The percentage of each type of document is presented in Figure 6.

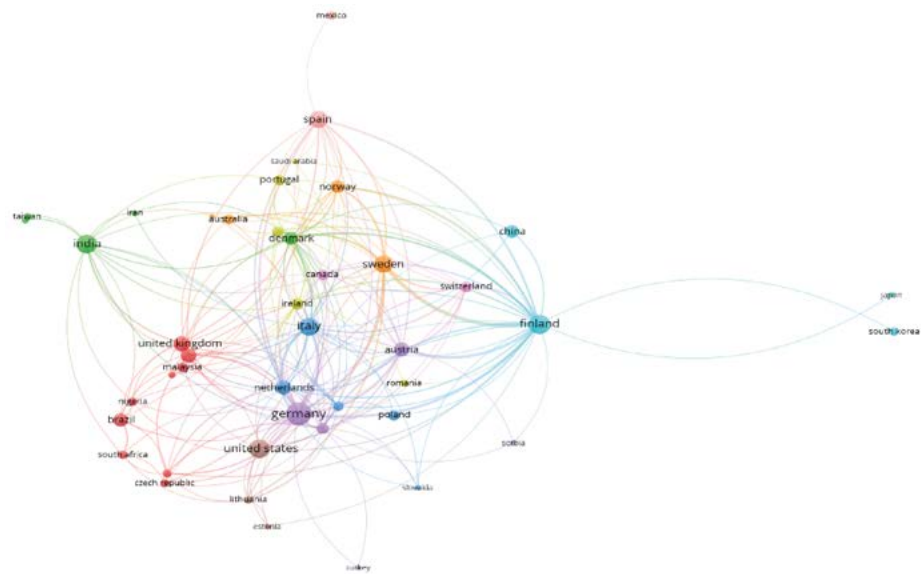


Figure 5. Citation level network map of the countries that produce scientific documents on bioeconomy and climate change.

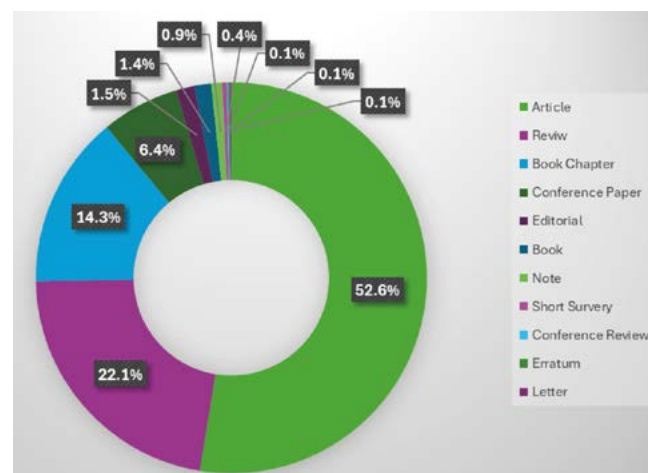


Figure 6. Percentage of publications by type of document.

In Figure 7. The citation level-network map of the documents on bioeconomy and climate change, the group of documents that cite each other, and the leader per group can be clearly observed. The most cited documents are written by Parisi 2015, Stegmann 2020, Antar 2021, Koul 2022, and Venkatamohan 2016.

Table 1 shows the title, author, source, year, and number of cited, of the two most cited documents in the research.

Analysis criteria taken of sample universe selected was minimum one document per author and three cites per author. Only 34 authors are connected.

The five most cited authors are: Parisi Claudia, with 4 documents and 568 cites; Amulya 4 documents and 552 cites; Junginger and Martin 4 documents and 526 cites; Viagani and Mauro 2 documents and 505 cites; Lendo and Marc 3 documents and 494 cites (Figure 8).

Figure 9 indicates the number of scientific publications by subject area, where it can be seen a dominant number of documents related to bioeconomy and climate change on environmental science (415), agriculture and biological science (242), energy (230), engineering (158), and social sciences (142). The percentage of publications by subject area is showed too.

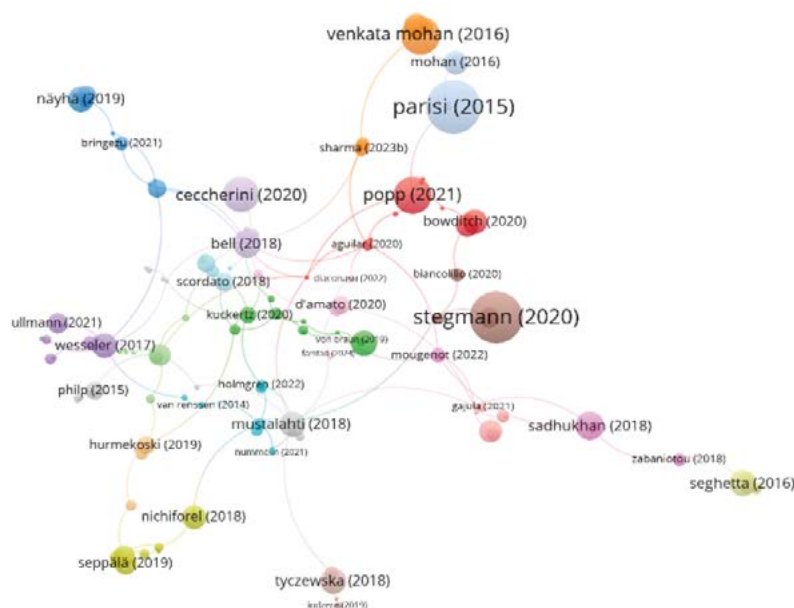


Figure 7. Citation level network map of the documents on bioeconomy and climate change.

Table 1. The most cited documents.

Author	Title	Source	Year	Citations
Claudia Parisi, Mauro Vigani, Emilio Rodríguez-Cerezo	Agricultural Nanotechnologies: What are the current possibilities?	Nanotoday	2015	490
Paul Stegmann, Marc Londo, Martin Junginger	The circular bioeconomy: Its elements and role in European bioeconomy clusters	Resources, Conservation & Recycling	2020	428

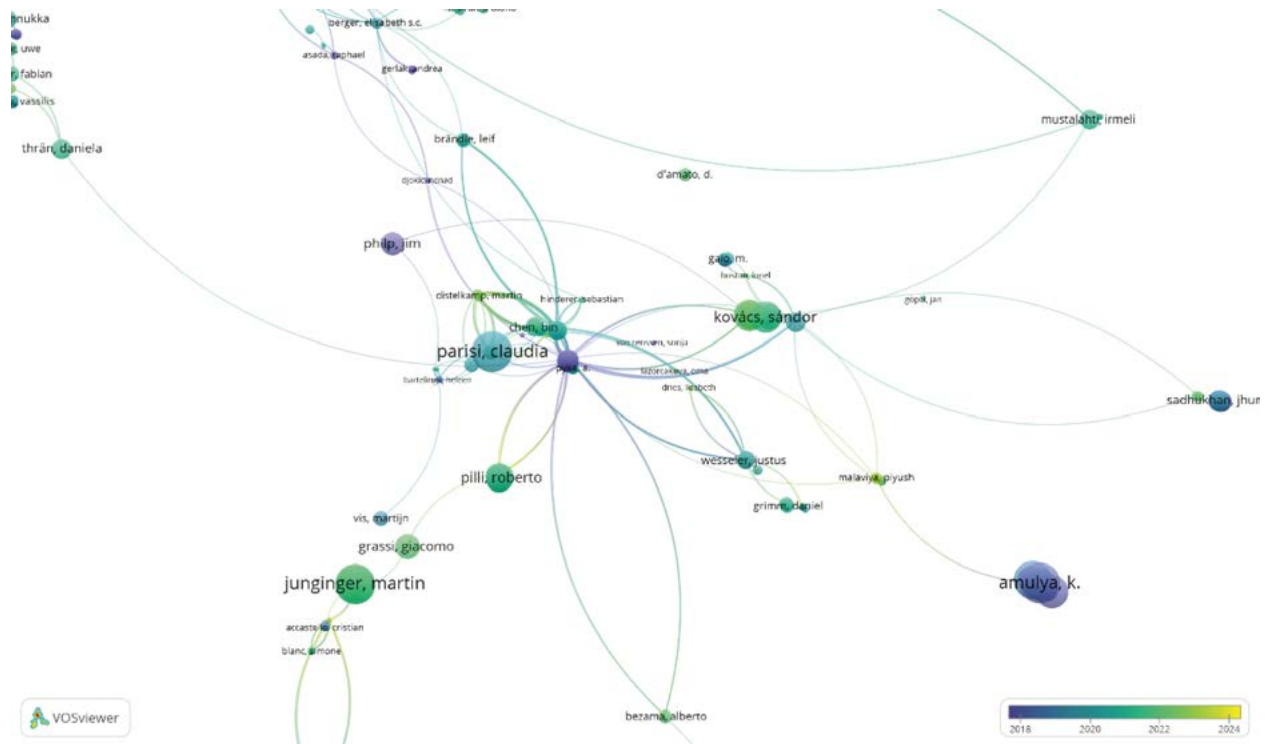


Figure 8. Citation level per authors network map of the documents on bioeconomy and climate change.

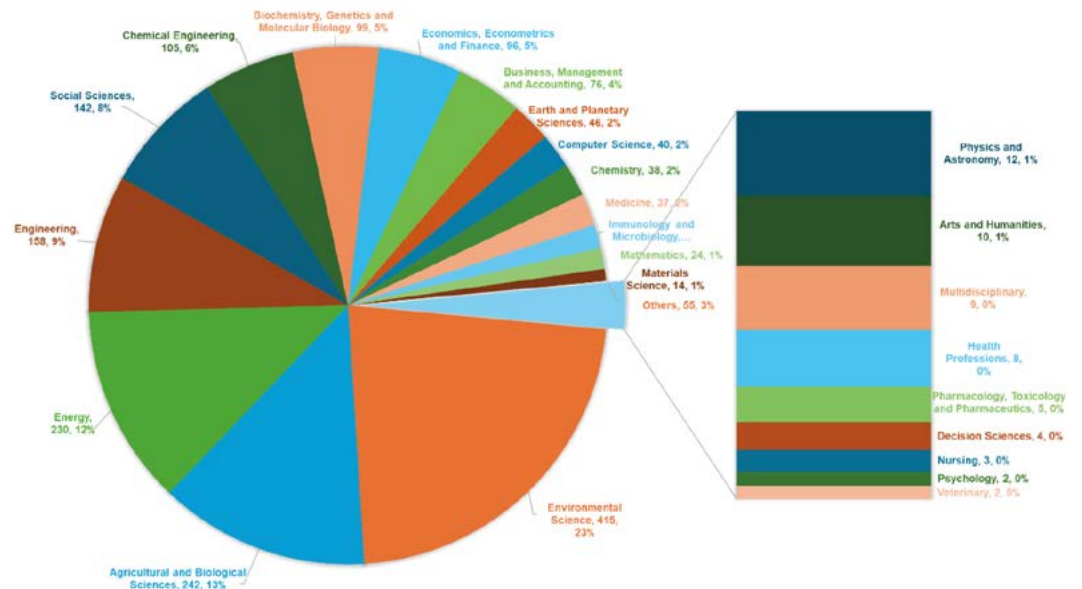


Figure 9. Percentage of publications by subject area.

The map in Figure 10 shows the density of the co-occurrence, in all keywords from 415 documents classified by Scopus in environment category to know, which are the most and lesser researched issues. According to the Vosviewer statistics, the most repeated keywords are: biomass with 111 co-occurrences, sustainable development 100, sustainability 77,

In Figure 13 we can see that bioeconomy and climate change indeed belong to the same cluster represented by the red color. Visualizing clearly in a graphic way how these terms are related to each other, it can also be seen that these two terms show co-occurrence more frequently with the following keywords: sustainable development, biomass, sustainability, forestry, biodiversity, climate change mitigation, greenhouse gases and economy. And the words showing less co-occurrence are methodology, conceptual framework, public policy, use, wood product, ecosystem service, runoff and bibliometrics.

Biancolillo *et al.* (2020) indicates that bioeconomy is considered frequently a key component for global challenges like climate change and ecosystem degradation. Perišić, *et al.* (2022) state that bioeconomy seeks to reduce the dependence on fossil fuels, prevent or reduce climate change. The bibliometric analysis of Ordoñez Olivo & Lakner (2023) is focused on the scientific production of different sectors of bioeconomy in countries of Latin America and the Caribbean. Liobikienė & Miceikienė (2023) Compares the European Green Deal strategies objectives with the pertinence of the development of the bioeconomy. According to Toplicean & Dăcu (2024), their review examines the most important elements of circular bioeconomy related to the agricultural sector. This bibliometric analysis shows that the concept of bioeconomy in scientific literature is recent in the various investigations related to the topic. Focusing on countries that develop and implement bioeconomy strategies at policies level, the information makes us reflect on the potential of leveraging the bioeconomy in agroecosystems, to reduce their vulnerability to climate change.

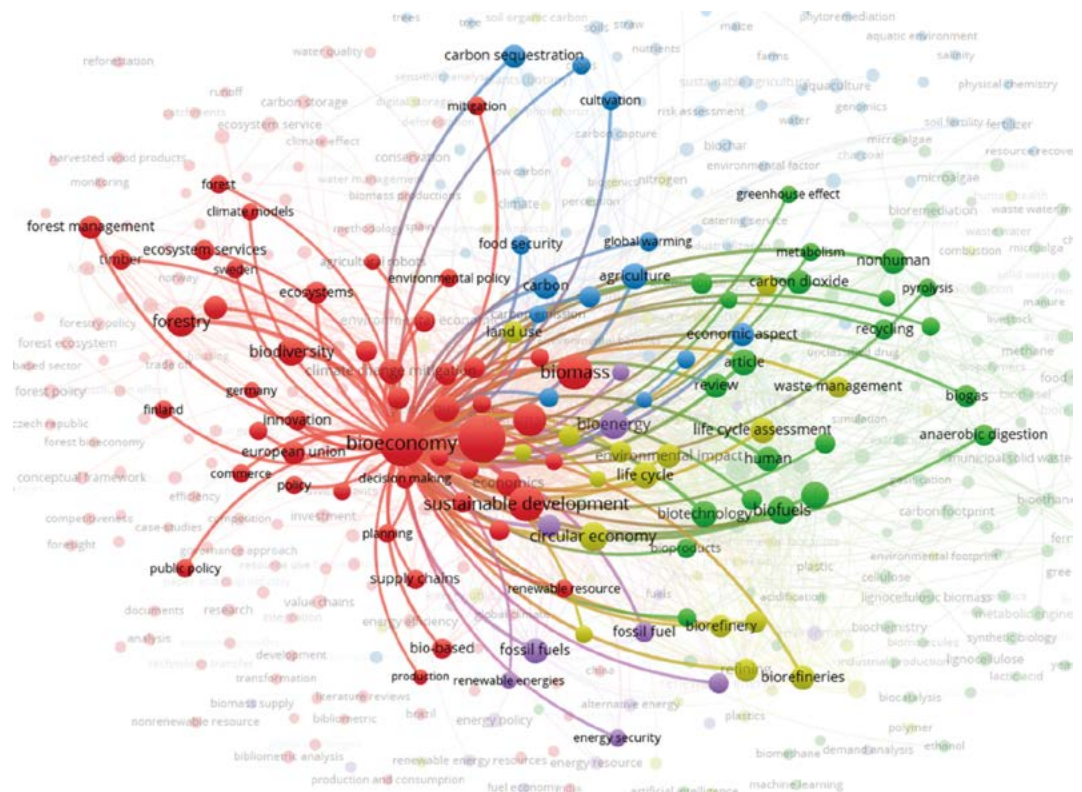


Figure 13. Bibliometric map focused on co-occurrence in the red bioeconomy node.

Bioeconomical agroecosystems are those that have gone through a process of knowledge transformation in their controllers over time, into nonlinear innovation guidelines between innovation agents and producers. Research on knowledge value networks is proposed to understand the causes of functionality, as well as the motivations for adopting innovations in agroecosystems, adopting diversification strategies, and creating new products and services to address the effects of climate change on rural families.

CONCLUSION

The issues bioeconomy and climate change needed depth analysis and to identify the challenges and opportunities that both fields of knowledge faces in social and economic terms, such as in environmental policies (analysis of policies that promote the bioeconomy as a tool to combat climate change). In contrast, the topics that have received less attention are socioeconomic impacts of the bioeconomy (studies on how bioeconomy practices impact local communities), biodiversity and bioeconomy (relationship between biodiversity conservation and bioeconomic practices), emerging technologies (technological innovations in bioeconomy that could influence climate change mitigation). Some of the emerging trends found are: interdisciplinarity reflected in an increase in research that combines environmental, economic, and social sciences to address complex problems related to bioeconomy and climate change. And the focus on public policy, which presents a recent interest in how policies can integrate the bioeconomy to address climate challenges.

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Relationship between private profitability and technical assistance in family-operated rabbit farms

Ortiz-Heredia, María Angelica¹; Domínguez-Olvera, Daniel Alonso^{2*}; Herrera-Haro, José Guadalupe¹; Rogers-Montoya, Nathaniel Alec²; Martínez-Castañeda, Francisco Ernesto³; Luis-Chincoya, Hector¹

¹ Colegio de Postgraduados. Programa de Recursos Genéticos y Productividad. México. C. P. 56264.

² Universidad Nacional Autónoma de México. Facultad de Medicina Veterinaria y Zootecnia. México. C. P. 05410.

³ Universidad Autónoma del Estado de México. Instituto de Ciencias Agropecuarias y Rurales. México. C. P. 50090

* Correspondence: iazdalonso@gmail.com

ABSTRACT

Objective: To evaluate private profitability (PP) in small-scale rabbit farms and establish the cause-effect ratio between investment in technical advisory services and PP.

Design/methodology/approach: An unrestricted random sampling was applied, and the Policy Analysis Matrix was used to calculate the private budget. Additionally, PP was determined at market prices, considering changes in the technical advisory services (TAS) variable. A simple linear regression was used to assess the relationship between TAS and PP, given the low heterogeneity among the production units in the sample.

Results: Based on the observed technical values, private profitability, the private cost ratio, value added to total income, break-even point (in units), feed proportion in the cost structure, and annual gross profit were 0.544, 0.453, 0.549, 0.503, 89%, and 0.494, respectively.

Limitations: The study examines a production method with little to no TAS, relying solely on projections to estimate the impact of investment in this area.

Conclusions: TAS enhance resource efficiency, significantly affecting technical indicators due to low overall production. While they have little effect on revenue, they reduce private profitability and net profit, two key measures of economic efficiency.

Keywords: Policy Analysis Matrix, private prices, linear regression, small-scale farms, competitiveness.

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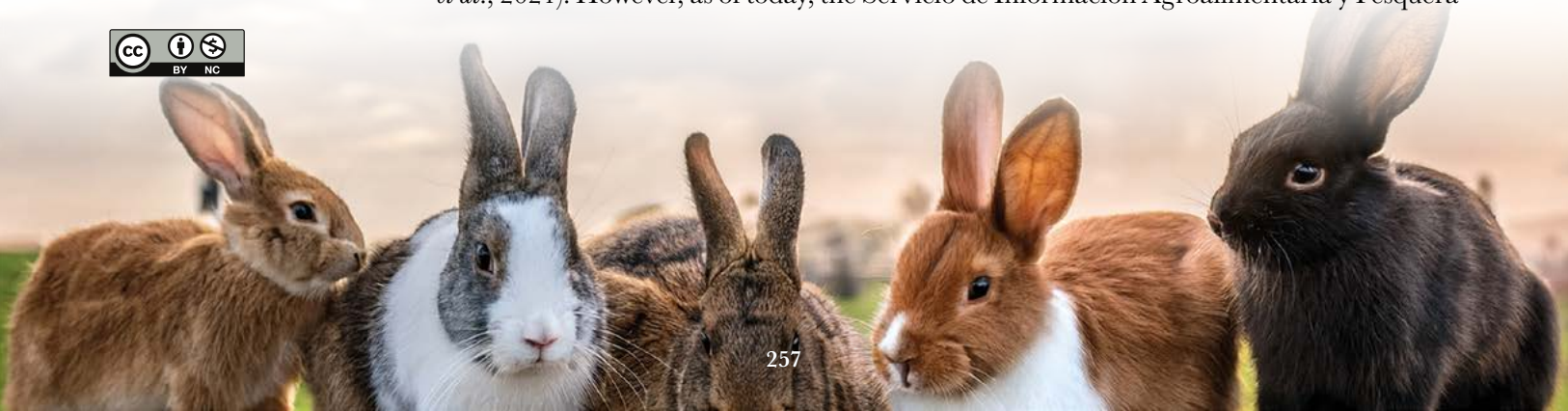
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INTRODUCTION

In Mexico, rabbit production operates on a small scale, as only 17% of this activity is carried out industrially (Gómez Soto, 2019). Rabbit breeding is predominantly conducted in backyard systems with family support, serving as a supplementary activity to primary occupations. From 2000 to 2018, the sector's average annual growth rate was 0.004% (Vélez *et al.*, 2021). However, as of today, the Servicio de Información Agroalimentaria y Pesquera



(2024) provides no official data on its potential, contribution to livestock production value, profitability, or competitiveness. According to FAOSTAT (2023), Mexico produced around 8,400 tons of rabbit meat, representing 1.42% of global production and ranking seventh among the leading producers. However, three countries account for 83% of total output. Rabbit farming is prevalent in Central Mexico, adaptable to land-based and landless systems, and contributes to food security and income generation (Gómez Soto, 2019). Meyer *et al.* (2021) emphasize the resilience of backyard production systems such as rabbit farming, in responding to future pandemics and the rise in meat demand, highlighting their valuable role in protein production and distribution. Its hardiness, adaptability, and high reproductive capacity support its production and facilitate adoption by rural and urban families (Gómez Soto, 2019). Vélez *et al.* (2021) report that 35% of producers operate on a small-scale, family-based model, 50% on a medium-scale with a similar structure, and the rest as commercial enterprises. In 2012, Mexico produced approximately 15,000 tons of rabbit meat (SPCDF, 2012), experiencing slow yet consistent growth, ranking 20th globally (Gómez Soto, 2019).

The State of Mexico and Mexico City are the country's leading producers (Gómez Soto, 2019). Based on the abovementioned figures, even a slight improvement in the sector's productivity at any level could significantly impact the total national production value. In 2007, Temascalapa ranked 50th among Mexico's rabbit meat-producing municipalities. Backyard rabbit farming demands minimal time and financial investment and relies on low or no technological inputs, constraining productivity and profitability. Compared to technified systems, which use advanced technology and focus on large-scale production, fewer animals are managed, and feed conversion efficiency is lower (Alonso *et al.*, 2006). Despite this, it plays a role in ensuring adequate nutrition for the population. However, production deficiencies may occur, and technical indicators vary widely. Rising total and unit production costs can threaten the viability of rabbit meat farming, given its susceptibility to economic fluctuations such as inflation, devaluation, and crises (Jiménez-Jiménez *et al.*, 2019).

The Policy Analysis Matrix (PAM), developed by Monke and Pearson (1989), has been widely applied in livestock production (Martínez *et al.*, 2015; Hernández-Martínez *et al.*, 2008; Magaña-Magaña *et al.*, 2002; Sosa-Montes *et al.*, 2000). This method is based on the accounting identity $Profit = Revenue - Cost$ ($P = R - C$), integrating economic principles related to production, processing, trade, and policy (Monke & Pearson, 1989). It aims to assess the impact of government policies on economic indicators and evaluate resource use efficiency (Hernández-Martínez *et al.*, 2008). The PAM method identifies market distortions and evaluates the degree of resource misallocation (Hernández-Martínez *et al.*, 2008), providing key indicators such as private profitability, private cost ratio, break-even point, value added to total revenue, and net profit, that facilitate a comprehensive evaluation and comparison of farms. Its estimation method enhances the accuracy of business performance analysis (Matus & Puente, 1992). Hence, this study aims to: 1) evaluate the private profitability of family-operated rabbit farms in Temascalapa, and 2) examine the impact of investment in technical assistance on private profitability.

MATERIALS AND METHODS

Ten surveys were conducted in Temascalapa, State of Mexico, between July 1 and July 15, 2023. Using Equation 1 and the variance from the Private Economic Indicators of Production Units (PEIPU) derived from a pilot sample, the sample size was determined for unrestricted random sampling in infinite populations (Sukhatme & Sukhatme, 1970). The PEIPUs were assessed using the Policy Analysis Matrix (PAM) by Monke and Pearson (1989). The analysis relied on accounting records to evaluate private revenues and costs (private budget), adjusting costs to market prices as of July 2023.

Private profitability (PP) is calculated as the difference between revenue (R) and the total costs of tradable inputs (CTI) and domestic factors (CDF), as defined in Equation 2.

The private cost ratio (PCR) compares the efficiency of different production systems. It is calculated as the ratio of domestic factor costs to value added at private prices, where value added equals total revenue minus input costs (Equation 3).

Value added to total revenue (VATR) is the difference between revenue and the cost of tradable inputs. It represents both the compensation for domestic production factors and the producer's profit, highlighting the production's impact on the sector (Equation 4).

The break-even point (BEP), measured in units, represents the production level needed to cover total fixed (TFC) and variable costs (TVC) without yielding a profit (Equation 5).

Net profit (NP) represents the share of revenue remaining after deducting total costs (TC, Equation 7) incurred by the activity (Equation 6).

$$n_i = \frac{t_{\alpha/2}^2 S_N^2}{d^2} \quad \text{Equation 1}$$

Where: n_i represents the sample size for variable i ; S_N^2 is the population variance estimator for variable i ; $t_{\alpha/2}^2$ is the t -table value at a 0.05 significance level; and d denotes estimation precision, defined as 8.0% of the mean ($0.08 * \bar{x}$).

$$PP = I - (CTI + CDF) \quad \text{Equation 2}$$

Where: PP represents Private Profitability, CTI denotes the cost of tradable inputs, and CDF refers to the cost of domestic factors.

$$PCR = \frac{CDF}{I - CTI} \quad \text{Equation 3}$$

Where: PCR represents the private cost ratio, CTI denotes the cost of tradable inputs, CDF refers to the cost of domestic factors, and I represents the revenue.

$$VATR = \frac{I - CTI}{I} \quad \text{Equation 4}$$

Where: $VATR$ represents the value added to total revenue, I is revenue, and CTI denotes tradable input costs.

$$BEP = \frac{TVC}{I - TFC} \quad \text{Equation 5}$$

Where: *BEP* represents the break-even point, *TVC* denotes total variable costs, *I* is revenue, and *TFC* refers to total fixed costs.

$$NU = \frac{I - TC}{I} \quad \text{Equation 6}$$

Where: *NU* represents net utility, *I* is revenue, and *TC* denotes total costs.

$$TC = TVC + TFC \quad \text{Equation 7}$$

Where: *TC* represents total costs, *TVC* denotes total variable costs, and *TFC* refers to total fixed costs.

Evaluation of Technical Advisory Services

A simple linear regression analysis was conducted to assess the effect of technical assistance services (TAS), measured as a regression coefficient. The only independent variable included in the model was TAS expenditure in different production units, while the response variables included key economic indicators (PP, PCR, VATR, NU, and BEP). Analyses were performed using the PROC MEAN procedure (to calculate the mean and standard error) and PROC REG (for simple linear regression) in SAS OnDemand for Academics (SAS Institute, Cary, North Carolina, USA, License u63361883).

RESULTS AND DISCUSSION

The largest sample size estimated for the most variable PEIPU was 9.8 units, based on the predefined precision. Given that the preliminary random sampling included 10 family-operated rabbit farms, the collected data can be considered representative of Temascalapa (Table 2).

In Temascalapa, family-operated rabbit farms have an average of 22 breeding females and utilize 32% of their production capacity. The average litter size is 10.12 kits, with a 20% mortality rate during lactation. According to Vélez *et al.* (2021), rabbit farm inventories range from 24 to 257 does. The most common breeds include California, New Zealand, Chinchilla, Mariposa, and their crossbreeds. Genetic management is basic, relying solely on phenotypic traits and desirable maternal characteristics for breeding replacement selection. Rabbits are slaughtered at an average age of 130 days and a weight of 2.4 kilograms. Their diet primarily consists of locally sourced forage and household food scraps, supplemented with 10% specialized rabbit feed.

Rabbit sales are distributed as follows: 55% live, 32% as carcass, and 13% as cooked meat. Abawiera *et al.* (2023) notes the importance of rabbit commercialization, showing a revenue difference of \$68.19 USD between rabbits sold live versus as carcasses. They report a net income of \$116.80 USD for live rabbits, compared to \$86.72 USD for carcass sales.

Adanguidi (2020) highlights that processors are the main beneficiaries in the rabbit meat production and commercialization chain. They supply 29% of the tradable intermediate products but capture 41% of the total added value. Despite covering 33% of the value chain's total costs, they receive 45% of the profits.

Table 1 presents the matrix's cost structure based on the original technical data. Commercial inputs account for 82%, which is high but reasonable, given the absence of miscellaneous expenses and the 12% share of internal factors. Since feed accounted for most of the marketable factors and had a low cost, this aligns with Mondin *et al.* (2021), who emphasize the decisive role of feed in rabbit production. In contrast, the Dirección General de Producciones y Mercados Agrarios from Spain (2020) reported that feed represents just under 50% of total production costs, as their system required greater investment in other cost components. Since no specialized literature with methodologically comparable results was found, a mirror comparison was conducted with other production systems in Mexico. For example, Hernández-Martínez *et al.* (2008) notes that in backyard systems, low investment leads to a higher proportion of marketable inputs; not because of their high value, but due to their larger share of the total cost structure. Martínez *et al.* (2015) determined that marketable inputs accounted for 86% of costs in a backyard pig farm, a slightly higher value than found in this study. This could be due to how labor and professional staff expenses are considered, as these costs are minimal in this system due to the low time investment and reliance on family labor, which represents 9% of total costs. Consequently, the share of internal factors increases to 12%, while the remaining costs decrease to 5%. Meanwhile, Domínguez *et al.* (2023) found a lower proportion of 64% for this category in a semi-technified system, suggesting a higher investment in infrastructure, specialized labor, health management, medication, and other factors.

Table 2 shows the average economic indicators for family farms in Temascalapa. The break-even point, measured in kilograms of meat sold, is achieved when 50% of total production is marketed. Private profitability in the observed matrix was 54%, a value deemed favorable compared to other production systems. In contrast, Domínguez *et al.* (2023) reported break-even points above 75% in a semi-technified piglet production system. Adanguidi (2020) found cost-benefit ratios between 59% and 83%, with economic returns varying from positive to negative in the rabbit value chain.

Private profitability in pig production was 12% (Martínez *et al.*, 2015), 18% (Hernández *et al.*, 2018), and 34% for semi-technified systems (Domínguez *et al.*, 2023). Posadas-

Table 1. Structure of the total average production cost at private prices for rabbit farms in Temascalapa.

Concept	Proportion n=10	SEM
Tradable Inputs	82.43	3.40
Internal Factors	12.04	4.89
Indirectly Tradable Inputs	5.55	1.61
Miscellaneous Expenses	0.00	0.00
Total Cost	100.00	NA

Domínguez *et al.* (2014) reported profitability between 7% and 26% in smallholder dairy systems, depending on barn size. In contrast, the evaluated system achieved 54%, an exceptionally high value due to low investment and the rabbits' adaptability to different conditions.

These differences are due to the fact that private profits at the normal level are considered when their value approaches zero, while a higher value indicates above-normal profitability. Losada-Espinoza *et al.* (2014) reported profitability exceeding 104% in three piglet production systems, emphasizing that profitability is a valuable indicator of individual productive and economic efficiency but is not easily comparable across farms. This is because "profitability results are residual and may originate from systems with varying input levels to produce goods that also differ significantly in price" (Hernández-Martínez *et al.*, 2008). Unlike private profitability, the private cost ratio enables comparisons between production systems by indicating the system's ability to cover internal costs while remaining competitive. A positive value signifies both profitability and competitiveness. In this study, the private cost ratio was 45% (Table 2), confirming the system's financial viability. Golban & Golban (2020) highlight that in markets with low product consumption; competitiveness can be improved by shifting from domestic rabbit farming to well-organized, enterprise-level systems that benefit from economies of scale. In this transition, government support through financing programs is essential. A low VATR indicates the contribution of rabbit meat production to the regional economy. In this study, the VAIT was 54%. Adanguidi (2020) reported a value-added ratio between 0.76 and 1.65 across different rabbit production systems.

Hernández-Martínez *et al.* (2008) reported a 23% value for family pig farms. This economic index measures the activity's contribution to direct employment, primarily driven by marketable inputs, with a smaller share from internal factors such as labor. This aligns with the sector's generally low impact on employment.

Evaluation of Technical Advisory Services

The analysis found that spending on technical advisory services does not affect private economic indicators, except for profitability. This is due to the system's inefficiency, which demands minimal investment.

Table 2. Private price budget summary for rabbit farms in Temascalapa, with estimated average values based on a regression model incorporating technical assistance services (TAS) as an independent variable.

Indicator	Mean n=10	SEM	n Estimated
Break-even point (in units)	50.31	1.79	7.60
Private Profitability*	54.44*	2.20	9.80
Private Cost Ratio	45.27	1.72	8.66
Value Added in Total Income	54.97	1.47	4.29
Net Profit	49.43	1.95	9.34

* Significant effect of technical advisory services ($p < 0.05$).

Greater investment in technical advisory services improves efficiency, but it also raises costs, which can eventually reduce private profitability.

CONCLUSIONS

The rabbit farms in Temascalapa, despite their low production levels, are profitable and competitive. Technical advisory services have a limited negative impact on private profitability, since they improve production efficiency.

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