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Production of *Agave* spp. and Mezcal

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> Ogata N. (2003b).

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Analysis of Methodologies for Gender Studies

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ABSTRACT

Objective: To identify and analyze methodologies used in gender studies.

Design/methodology/approach: To analyze research methodologies applied to gender studies in agroecosystems, a theoretical review of the topic was conducted. A methodological approach was employed that included qualitative and quantitative analyses, as well as intersectionality as a key tool for understanding inequalities in access to resources, labor distribution, and decision-making processes. Various research methodologies for gender studies were identified, including discourse analysis, ethnography, and systematization of experiences.

Results: The inclusion of gender indicators in agroecosystem assessments is essential to highlight disparities and propose strategies to overcome them. There is a need for inclusive public policies that strengthen women's participation in agriculture. The intersectional approach examines how gender, ethnicity, social class, and age interact to shape inequalities. The use of digital technologies, such as Geographic Information Systems (GIS), allows for the mapping of disparities and supports the implementation of inclusive governance models that promote women's participation in decision-making processes.

Limitations of the study/implications: The implementation of inclusive policies may be constrained by institutional or sociocultural barriers in rural contexts.

Findings/conclusions: The analysis confirms that qualitative methodologies, such as ethnography and the systematization of experiences, allow us to recover the voices and practices of rural women, highlighting inequalities in the distribution of labor and access to resources. Quantitative and mixed methods provide tools for measuring structural gaps and understanding the relationships between gender, ethnicity, social class, and age in production processes.

The incorporation of digital technologies such as Geographic Information Systems (GIS), the inclusion of gender indicators, and the adoption of participatory governance models have been identified as significant innovations in this field. However, this study demonstrates that their application remains limited and requires further empirical exploration in diverse rural contexts.

Keywords: Gender studies, Agroecosystems, Qualitative and quantitative analysis, Gender indicators.

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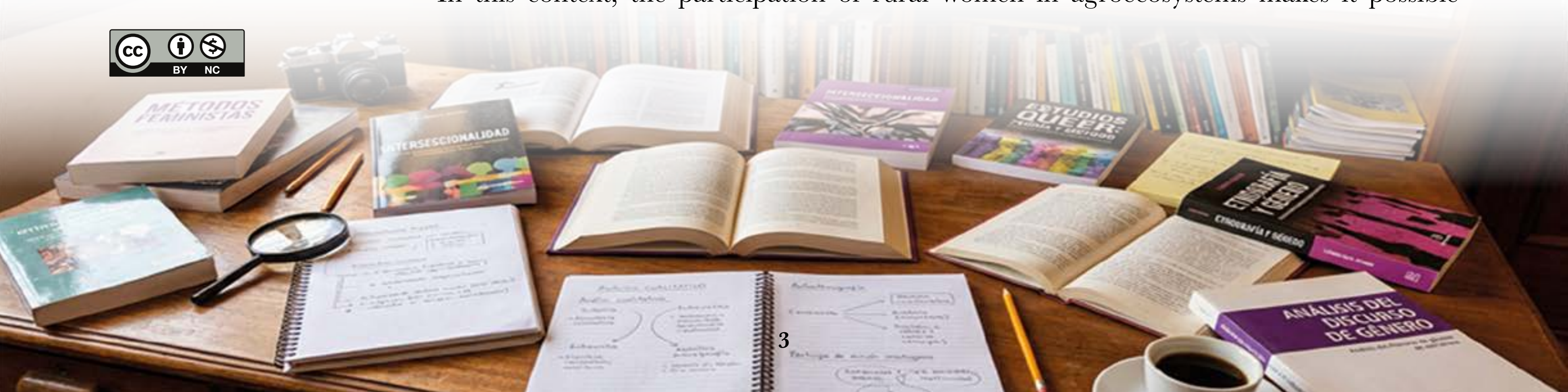
INTRODUCTION

Gender studies are essential for understanding and addressing gender inequalities, relationships, and interactions within our society. To approach this concept, it is necessary to conduct a theoretical systematization of gender studies —understood as a sociocultural phenomenon— with the aim of contributing to the improvement of social organization. In this context, the participation of rural women in agroecosystems makes it possible



to identify their role in processes of emancipation and empowerment. Women actively participate in generating income and employment, acting as providers, producers, and marketers of food and related products within these systems.

Considering the above, it becomes necessary to address a central problem: although there are various theoretical and practical approaches within gender studies, there is still an insufficient understanding of which methodologies are most effective for analyzing gender inequalities in agroecosystems. Therefore, the following research question is proposed: Which methodologies are most suitable for analyzing gender inequalities in agroecosystems?

The objective of this article was to discuss the different research methods used for gender analysis.

To better understand this analysis, it is necessary to review the main trends and conceptual developments in gender studies. Various authors, such as Lamas (1986), Amorós (1994), Ochoa (2002), and Valcárcel (2008), have analyzed the current of feminist thought known as feminism or gender equality, which remains central to contemporary gender debates. Specifically, they propose reflecting on the structural conditions that result in the systematic exclusion of women from traditionally spheres considered as "male domains."

Gender studies have developed diverse methodologies and approaches to characterize policies and women's participation in social processes. According to Lagarde (1996), the gender perspective provides the ability to "focus on, analyze, and understand the specific characteristics that define women and men, as well as their similarities and differences." From this perspective, "the life possibilities of both are analyzed —the meaning of their lives, their expectations and opportunities, as well as the institutional and everyday conflicts they must face, and the multiple ways in which they do so."

Gender as a concept arises from the distinction made in relation to sex, originating from research on several cases of children who were assigned to a sex that did not correspond to their genetic, anatomical, and/or hormonal characteristics. This distinction was first explored within feminist theory through Stoller's book Sex and Gender (1968), which demonstrated that gender refers to domains encompassing human behavior, emotions, thoughts, and fantasies —areas that do not necessarily have a biological basis.

Similarly, within the history of Western feminism, the currents that embrace enlightenment principles of human beings as free and autonomous are known as feminisms of equality or egalitarian feminisms. These schools of thought rest on a logical assertion: if the rights of man are truly universal, they must also include women. Any individual, whether identified as female or male, is coherent and rational, capable of deciding and acting according to the common sense inherent to all human beings.

In this regard, the gender movement enables an understanding of the complexities and challenges of feminism in the global context, while also providing tools for the processes of social change initiated by modernity. These contributions foster the construction of a fairer and more egalitarian world for all people, regardless of gender (Valcárcel, 2008; Ortega, 2013).

In this regard, several authors, such as Gayle Rubin, define a sex-gender system as a set of arrangements through which a society transforms biological sexuality into human products (Rubin, 1975). Therefore, Cobo (2005) explains, "it represents the transition from biological sexuality to human sexuality, from sex to gender."

According to the INSTRAW or International Research and Training Institute for the Advancement of Women (2017), "Gender refers to the range of socially constructed roles, relationships, personality traits, attitudes, behaviors, values, relative power, and influence that society assigns differently to men and women. While biological sex is determined by genetic and anatomical characteristics, gender is an acquired and learned identity that widely varies both within and across cultures".

According to Cirillo (2005), gender is a principle of order that reveals the existence and effects of power relations, difference, and unequal encounters. Throughout his life, every man experiences, to some extent, situations in which he exercises a certain degree of power —however minimal or even illusory it may be. Despite being democratic, rational, and sincerely convinced of the equal dignity of men and women, traces of an unconscious childhood fantasy often persist —one that sustains the belief in possessing something women do not, or in having a kind of natural right to power.

Gender is relational, as it does not refer exclusively to women or men but rather to the relationships between them (INSTRAW, 2017). The current value of these studies lies in their contribution to the social reflection on human relationships.

The analysis of the concept of gender defines it as the "set of characteristics and behaviors, as well as the roles, functions, and evaluations assigned dichotomously to each sex through socialization processes, sustained and reinforced by patriarchal ideologies and institutions." This concept is adapted to the context of each society and is redefined according to various factors, such as social class, ethnicity, age, and nationality, among others.

Currently, research on gender contributes to an epistemological intersection, "constituting a meeting point between social and political demands and contributions to the field of scientific and academic knowledge" (Jiménez, 2007). Therefore, it is necessary to systematize gender as a multidisciplinary field of knowledge within a diversity of pathways and alternatives yet to be explored. This article was developed with the aim of examining and analyzing gender assessment methodologies.

MATERIALS AND METHODS

Various methodologies applied in gender studies are described, with a particular emphasis on their application in agroecosystems. The information analyzed was obtained through a theoretical and methodological review of the approaches used in gender research, drawing from reference databases such as Web of Science® and Google Scholar®, using the keywords "Gender Methodologies" and "Gender and Agroecosystems." Documents were reviewed, including articles reporting research conducted in Latin American and European countries. The selected methods address the need to examine gender inequalities in agroecosystems from different levels of analysis. Qualitative methods allow for the understanding of human experiences, perceptions, and narratives (Ciencia Latina, 2023). While, quantitative methods enable the objective measurement of inequality indicators, while an intersectional approach provides a framework to understand the interaction between gender, ethnicity, age, and social class. The choice of methods offers a more comprehensive perspective on gender-related issues in agroecosystems, in contrast to traditional approaches that sometimes overlook social dimensions. A systematic document analysis method or systematic literature review was employed to search databases and select studies. The information collected was organized according to the methodological approaches used in gender studies.

RESULTS AND DISCUSSION

The incorporation of a gender perspective in society has generated significant debate in recent times. It is understood both as an epistemological starting point and as a critical/reflexive lens that provides valuable insights in the production of any scientific knowledge, humanistic understanding, or aesthetic proposal. The gender perspective challenges language, categories, methods, and assumptions that, by definition, render women and other feminized individuals invisible within social imaginaries, thereby erasing voices, actions, spaces, and worldviews of a substantial portion of humanity. Through a gender perspective, categorical tools are constructed that allow for the representation of human realities that would otherwise go unnoticed (Serret, 2008). Gender can be expressed in terms of identity, expression, sexual orientation, and sex assigned at birth, but not in a univocal or necessary manner.

According to Butler (2002), identity is understood as a relationship among sex, gender, sexual practice, and desire, viewed as the effect of a regulatory practice that can be defined as compulsory heterosexuality. This author further explains that expression can be a performative act that either reinforces or subverts gender norms, with its coherence produced in daily life and shaped by regulatory practices.

Table 1. Methodological Process for Gender Studies.

Stages	Description	
Bibliography search	Identification of the reviewed literature on gender methodologies in agroecosystems	Consultation of reference databases: Web of Science® and Google Scholar®, using the keywords "Gender Methodologies", "Gender and Agroecosystems "
Selection of documents	Definition of inclusion/exclusion criteria	Selection of articles with references to research in Latin American and European contexts
Classification of methodologies	Organization of the information collected according to methodological approaches	Qualitative: They seek to understand phenomena through the exploration of experiences (Discourse analysis, ethnography and systematization of experiences) Quantitative: They provide statistical indicators (wage gap, access to resources and political participation) Intersections/mixed: Social variables (gender, ethnicity, age, social class) are included
Analysis of Methodologies	Evaluation of the relevance and applicability of the methodologies	Identification of strengths, limitations and possibilities for innovation in gender studies of agroecosystems

The approaches to gender studies —from feminist, equity, and inclusion perspectives— are addressed by various researchers and theorists who draw on diverse social and political elements. Ortega (2013) asserts that the universality of rights necessarily includes both women and men. Complementarily, Valcárcel (2008) provides insights into the current complexities and challenges of feminism in the global context, as well as tools for the social change process initiated by modernity, contributing to the construction of a more just and equitable world for all individuals, regardless of gender.

Gender inequity is understood not only as an inequality between men and women but also as a complex system of power relations that is reproduced through social structures and hierarchies of masculinities. According to Connell (1995), these inequalities result from a gender structure that legitimizes male hegemony —a dominant cultural model that privileges certain ways of being a man (strong, heterosexual, competitive) while subordinating other masculinities and femininities. From another perspective, Butler (2007) argues that identities are neither natural nor universal but are social constructs that can be challenged through performative acts.

In the analysis and development of gender studies methods, researchers Osborne and Molina Petit (2008) highlight "feminist theory as a new perspective of study, such as a category for analyzing the relationships between the sexes, the differences in socio-sexual roles and characteristics of men and women, and, ultimately, as a critique of the 'natural' foundations of these differences."

Various authors have conducted research on gender methodology studies. Butler (1990) introduces the notion of gender performativity, implying that studies should analyze not only biological differences but also the social processes that construct gender identity. Similarly, Crenshaw (1991) defines terms such as gender, equity, intersectionality, and inequality, as well as their relationship with other factors like race and social class. DeVault (1999) proposes that qualitative methodologies, such as in-depth interviews and ethnographic studies, can better capture gendered experiences than exclusively quantitative methods. Walby (2005) emphasizes that studies should apply appropriate gender indicators to measure inequalities in access to resources, education, employment, and political power. Likewise, Sen (2000) argues that development should be assessed in terms of freedoms and opportunities, implying that a good gender study should offer recommendations to improve equity.

The critical contribution of this research is grounded in the participation and contributions of feminist thought to the development of gender theories, where the feminist perspective is validated as a category that describes and makes visible the situations of discrimination faced by women. Furthermore, it calls the government to integrate this perspective into all policies, programs, and legislative frameworks. The incorporation of a gender perspective is proposed as a key strategy to ensure that the interests and experiences of both women and men are considered equitably in the design, implementation, monitoring, and assessment of policies and programs across all sectors. Its main objective is to achieve equal benefits for all individuals and to prevent the perpetuation of inequality between the feminine and masculine, thereby promoting full equality between women and men.

According to research on gender studies, these are approached through various theoretical discussions that frame the methodology within two types of methodological orientations:

- It analyzes the method based on the theoretical discussion context, reflecting a marked difference determined by the epistemological framework in which it addresses gender research procedures and techniques as a field of activity.
- It understands the role of feminism in research and acknowledges the existence of specifically feminist research methods, thereby rejecting research from a traditional epistemology.

In relation to the above, in gender studies, as in social research, the choice of an appropriate method depends on various factors, with the object of study —that is, the problem to be analyzed— and the theoretical framework being the most decisive. These elements guide the methodological approach and ensure coherence between the research objectives and the tools used to address them.

In gender studies, various methods are employed, some of which have been used in research on equality-focused feminism. These methods have served as a reference for current studies and for addressing gender issues across different areas of society (Valcárcel, 2008).

The bibliographic review identifies seven methods, techniques, and instruments used in gender studies, including the non-statistical descriptive method, experimental method, ethnographic method, narrative method, projective method, discourse analysis, historical-documentary method, and systematization of experiences.

The assessment of gender requires a suitable methodology (Table 1) that allows for the analysis of its theoretical approach, research methodology, and its impact on understanding gender inequalities and interactions.

Definition of the Evaluation Objective

The objective of the gender analysis is to determine its quality, relevance, and contribution to the understanding of gender inequalities. To achieve this, various aspects of the study are analyzed, including its theoretical framework, methodology, data analysis, and impact on society or public policy formulation.Definition of the Conceptual Framework The evaluation involves reviewing the theoretical framework (Figure 1) used in the study. According to Scott (1986), gender should be understood as an analytical category that structures social and power relations.

An appropriate analysis must identify whether the study incorporates these perspectives and whether it avoids essentialist approaches that reinforce gender stereotypes.

Methodological Analysis of the Study

The choice of methodology in gender studies is key to ensuring an adequate analysis. According to Harding (1987), feminist methodologies should consider the experiences of the study subjects and avoid androcentric biases. When evaluating the study's methodology, it

Table 2. Methodology for Conducting Gender Studies.

Steps	Methodology
Definition of objectives and conceptual framework	• Set up the key concepts: gender, equality, equity, roles, stereotypes, intersectionality, etc. and delimit the focus of the study (academic, social, political, institutional). • Determine the specific objectives of the analysis.
Methodological analysis of the study	• Methodology used: qualitative, quantitative or mixed. • Data collection: interview, survey, documentary analysis, observation. • Sample and representativeness: gender diversity, vulnerable groups, intersectionality. • Gender indicators: wage gap, access to education, gender violence, political participation, among others.
Data evaluation, analysis and presentation	• Determine whether the study specifically incorporates the gender perspective. • Analyze the differences among men, women, and other gender identities. • Avoid the use of sexist language and stereotypes. • Include diverse voices and different experiences.
Impact and applicability of the analysis	• Investigate the findings if they can influence public policies or institutional practices. • Intervention strategies or corrective measures are proposed. • Investigate whether the study has limitations in terms of bias or lack of data.
Discussion	• Identification of strengths and weaknesses of the study. • Suggestions for innovating the methodology in future research. • Possible applications and areas for improvement in gender analysis.

Source: Author's own work.

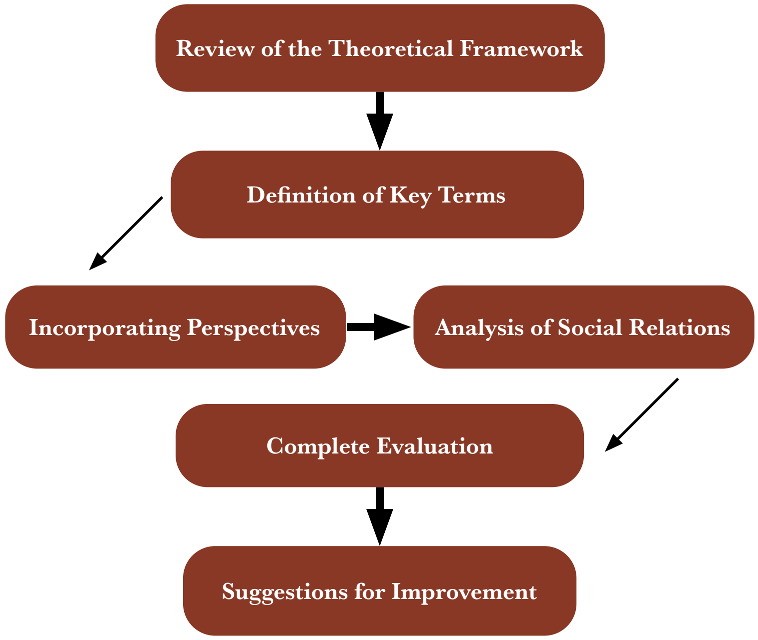


Figure 1. Definition of the Theoretical Framework. Source: Author's own work.

is important to review whether the data collection instruments are designed with a gender perspective. Additionally, it should be verified whether the sample is diverse and includes intersectional variables that allow for a more in-depth analysis of structural inequalities (Lugones, 2008).

Evaluation of Data Analysis and Presentation

Data analysis in gender studies must go beyond a simple comparison between men and women. Authors such as Connell (1995) emphasize the importance of examining hegemonic masculinities and their impact on gender relations.

It is important that the analyzed study disaggregates data by sex and other categories such as ethnicity, age, and socioeconomic status. Additionally, it should be evaluated whether the study avoids using sexist or stereotypical language in the interpretation of results (Hooks, 1984).

Evaluation of Impact and Applicability

A comprehensive assessment of a gender study should also consider its impact on society and public policy formulation. A key criterion is determining whether the study provides evidence to support decision-making in areas such as education, health, or employment. Additionally, it is relevant to analyze whether the study mentions its limitations and suggests future research directions to further explore the issue.

The applicability of gender studies within agroecosystems allows for the identification of inequalities and opportunities in the participation of men and women in the agricultural sector. Therefore, it is important to:

- **Analyze access to resources**: Assess equity in access to land, water, seeds, and technology.
- **Identify inequalities in decision-making**: Examine the participation of women and men in agricultural management and organization.
- **Evaluate the impact of public policies**: Determine how agricultural regulations affect women and men differently.
- **Improve sustainability and equity**: Design production strategies that promote fairer and more sustainable participation.

Gender analyses should synthesize the findings regarding the conceptual and methodological rigor of the study. A gender study must integrate updated theoretical approaches, apply a relevant and appropriate methodology, and provide a data analysis that reflects the complexity of disparities. Additionally, it should have a clear impact on knowledge generation and social transformation.

Methodologies for gender analysis, such as those proposed by Butler (1990), Crenshaw (1991), and Connell (1995), allow for the evaluation of gender studies and their contribution to the field of social studies.

Studies in agroecosystems are important for understanding the distribution of labor, access to resources, and participation in decision-making within the agricultural sector. Methodological and technological innovations can improve gender equity in rural contexts. According to the research conducted, in the review of studies applied to agroecosystems, it was identified that there is a limited presence of research on this topic.

Intersectional Approach in Agroecosystems

Gender cannot be analyzed in isolation, but in relation to other factors such as ethnicity, social class, generation, and age (Crenshaw, 1991). In agroecosystems, intersectionality allows for the identification of inequalities in access to land, technology, and financing. Innovations in this area are reflected in the use of mixed methodologies to capture differences in opportunities and barriers faced by rural women, as well as in the application of ethnographic studies (DeVault, 1999) to document the experiences of Indigenous and peasant women in agroecological production. In this regard, there are studies that help contextualize this category. The analysis of seasonal agricultural work from an intersectional perspective demonstrates how gender, social class, and migration intersect to produce the exclusion of women, revealing dynamics of inequality that are often overlooked (Güell, 2022).

Digital Technologies and Gender in Agroecosystems

Digital tools can enhance the collection and analysis of gender-related data in the rural sector. This includes the implementation of innovations such as geographic information systems (GIS) to map access to land and crop distribution from a gender perspective (FAO, 2019), as well as the use of mobile platforms for training in sustainable agricultural techniques targeted at women. Based on studies conducted in a rural laboratory, the analysis of impact and social appropriation of knowledge in science, technology, and innovation for food security among rural women reflects a reduction in gender gaps (Trivino *et al.*, 2023).

Gender Indicators of Agrecological Sustainability

The evaluation of the sustainability of agroecosystems must include gender-specific indicators (Walby, 2005). Innovations are aimed at developing indicators that measure women's participation in agroecological practices such as agroforestry and seed conservation, as well as assessing the traditional knowledge of indigenous women in enhancing the resilience of agroecosystems. According to the above, in Mexico, case studies such as Vida, a coffee-growing peasant organization in Veracruz, show that women's participation not only improves access to resources and technical assistance but also strengthens community networks and food security (Severiano *et al.*, 2024).

Inclusive Governance and Decision-Making Models

Historically, women have had lower participation in governance, limiting their access to opportunities such as land ownership, technical assistance, and decision-making processes (Flores, 2015; IICA & ETGJ, 2019). In this regard, innovations are focused on the creation of community councils with gender parity for territorial planning and management (Sen, 2000). In line with the above, some studies address the analysis of fair and equitable distribution for women engaged in agriculture, including generating household income, attending to family needs, and contributing to food security (Marín & Ivanova, 2024).

Care Economy and Agroecosystems

Women's work in agriculture is often invisible, as it includes unpaid care activities (Federici, 2012). Innovations in this area involve integrating the care economy into agroecological studies, accounting for women's unpaid labor in the sustainability of agroecosystems, as well as developing policies that facilitate rural childcare, access to healthcare, and financing for women farmers. In relation to the above, studies in rural communities in Mexico indicate that the lack of recognition particularly affects racialized women, who perform domestic and care work under conditions of exploitation and vulnerability (Espinal *et al.*, 2020).

The use of qualitative methodologies for gender studies in agroecosystems demonstrates that ethnography and the systematization of experiences provide concrete evidence of the livelihoods of rural women, enabling governments to design public policies that are better contextualized to the socio-cultural diversity of rural communities. In this sense, gender research in agroecosystems in Mexico has a practical impact on public policy, showing that social cohesion in all communities promotes the integration of their members with equity and equal opportunities (Reyes & Caro, 2022).

Regarding quantitative methodologies in these contexts, key indicators such as wage gaps, access to land, and political participation are used. Therefore, the FAO (2019) recommends incorporating these indicators into the formulation of policies with a gender perspective to ensure access to credit and productive resources that benefit both women and men.

Meanwhile, methodologies oriented toward intersectionality make use of key social variables such as gender, ethnicity, age, and social class. It is also important to understand that intersectionality should not be treated as a secondary approach or something subject to variability; rather, it must be coherent and consistent at every stage of public policy design (Venegas & Riquelme, 2024).

CONCLUSIONS

The present research contributes to the methodological debate in gender studies within agroecosystems by systematizing qualitative, quantitative, and mixed approaches and evaluating their scope. The analysis confirms that qualitative methodologies, such as ethnography and the systematization of experiences, allow for the recovery of rural women's voices and practices, highlighting inequalities in the distribution of labor and access to resources. Likewise, quantitative and mixed methods provide tools to measure structural gaps and understand the relationships between gender, ethnicity, social class, and age in productive processes. These findings constitute a specific contribution of this work, highlighting the need to integrate different methodological levels in gender analysis applied to agroecosystems. The incorporation of digital technologies such as Geographic Information Systems (GIS), the inclusion of gender indicators, and participatory governance models have been identified as significant innovations in this field. However, this study shows that their application remains limited and requires further empirical investigation across diverse rural contexts and gender approches.

Therefore, based on this study, three lines of action are proposed for future research. First, to advance toward mixed methodologies that combine statistical evidence with situated narratives, in order to achieve a more comprehensive understanding of inequalities. In public policy, to incorporate gender indicators in the evaluation of agroecological programs and to design mechanisms for equitable participation in the governance of natural resources. At the community level, to strengthen initiatives that recognize and value the care work carried out by rural women, integrating it as an essential component of agroecosystem sustainability.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Enhancing tomato (*Solanum lycopersicum* (L.) Mill. agroproductivity: A scientific exploration of native *Rhizobium* biofertilizers

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ABSTRACT

Objective: To evaluate the effect of applying native bacterial strains as biofertilizers on the growth and yield of tomato (*Solanum lycopersicum*) cultivated under conventional agricultural practices through a field experiment.

Design/methodology/approach: Soil fertility was assessed by analyzing physicochemical parameters, including pH, electrical conductivity, cation exchange capacity (CEC), total carbon, nitrogen, phosphorus, and the C/N ratio, using standardized laboratory methods. Additionally, the ability of native *Rhizobium* strains to act as Plant Growth-Promoting Bacteria (PGPB) was evaluated through nitrogen fixation, phosphate solubilization, auxin synthesis, siderophore production, and ACC deaminase activity tests. The biofertilization trial involved three treatments with different native *Rhizobium* strains, a chemical fertilizer control (Triple 17), and a non-inoculated control. Growth, chlorophyll content, and yield parameters were statistically analyzed using one-way ANOVA and Tukey's test ($p<0.05$).

Results: The soil exhibited slight clay characteristics, variable pH, and cation exchange capacity, with low nitrogen (N) and phosphorus (P) content. Native *Rhizobium* strains demonstrated significant potential as PGPB, showing nitrogen fixation, phosphate solubilization, indole-3-acetic acid (IAA) synthesis, and siderophore production. Biofertilization with these strains significantly improved ($p<0.05$) tomato plant growth, chlorophyll content, and fruit quality compared to controls.

Limitations on study/implications: Unusual climatic variations, limited irrigation access, and subpar phytotechnical management affect tomato crop yields due to significant genetic variability. Evaluating biofertilization in various production cycles is crucial.

Findings/conclusions: Native *Rhizobium* biofertilizers enhance tomato growth and quality, addressing agroproductivity challenges.

Keywords: Agroproductivity, biofertilizers, *Rhizobium*, tomato cultivation.

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INTRODUCTION

The exponential growth of the human population has intensified the demand for food, leading to a widespread use of agrochemicals in agriculture (Gupta *et al.*, 2015). To

address this challenge, sustainable agricultural practices integrate biological sciences for safe and environmentally friendly approaches. Biofertilizers have gained importance for efficiently promoting plant growth, enhancing soil fertility, and reducing environmental contamination, thereby contributing to innovative and sustainable agriculture (Singh *et al.*, 2021).

Biofertilizers, particularly plant growth-promoting bacteria (PGPB), offer a promising approach to improving agricultural resilience and improve food production. PGPB, like *Rhizobium* species, establish symbiotic relationships, stimulate plant growth, improve nutrient absorption, and strengthen disease resistance. Their ability to fix atmospheric nitrogen provides an eco-friendly alternative to synthetic nitrogen fertilizers, mitigating environmental impact (Soumare *et al.*, 2020). Notably, *Rhizobium* species are versatile, colonizing the rhizosphere of non-leguminous plants, making them a practical and safe biofertilizer option for various crops, especially those consumed raw (Flores-Félix *et al.*, 2019; Gen-Jiménez *et al.*, 2023).

In southern Mexico, agriculture plays a fundamental role as a primary source of regional and national food security. This research focuses on the application of PGPB in the cultivation of tomatoes (*Solanum lycopersicum*) in Chiapas, Mexico. Tomatoes are of paramount importance as a versatile and widely consumed vegetable, and their cultivation holds significant economic value. Some recent studies have explored the application of *Rhizobium* bacteria as biofertilizers for tomato crops, demonstrating their potential to improve yield and quality (Gen-Jiménez *et al.*, 2023). Therefore, the objective of this study was to evaluate the impact of applying native bacterial strains as biofertilizers on the growth and yield of tomatoes (*S. lycopersicum*) cultivated using conventional agricultural practices.

MATERIALS AND METHODS

Experimental site

The biofertilization trials were conducted in a commercial tomato cultivation (*Solanum lycopersicum*) located in the “El Diamante” locality in the municipality of Ocozocoautla, Chiapas (16° 59’ 23” N and 93° 44’ 32” W) at an average altitude of 640 meters above sea level (Figure 1).

Soil characterization

Soil samples were collected from five randomly selected locations within the experimental tomato plot, both before and after biofertilization. Soil texture was determined using granulometric analysis (Bouyoucos, 1962), while pH and electrical conductivity (EC) were measured with a digital pH meter. Cation exchange capacity (CEC) was evaluated according to the specifications outlined in the Official Mexican Standard NOM-021. Total carbon and total nitrogen were quantified using a FLASH 2000® auto-analyzer, and total phosphorus was assessed through the $HNO_3/HCLO_4$ solubilization method (Rincón-Molina *et al.*, 2020). Additionally, the C/N ratio was calculated. An additional soil sample from a non-biofertilized control was also collected.

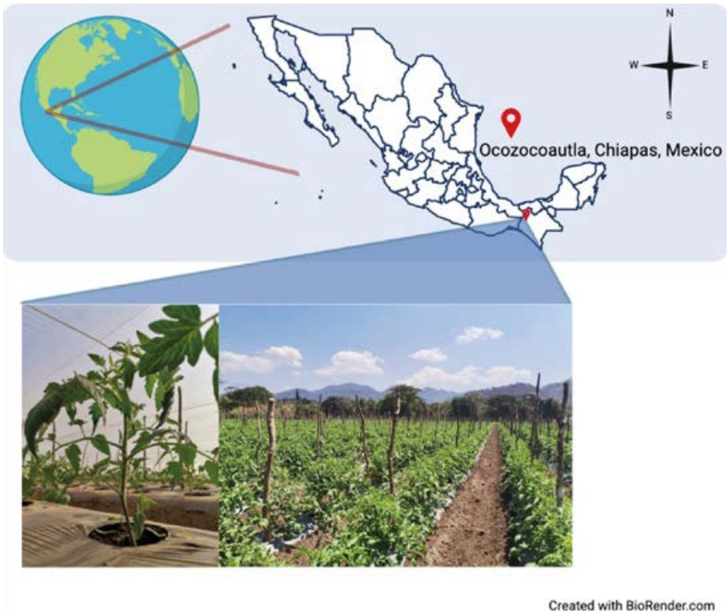

Figure 1. Location of the experimental plot of tomato cultivation.

Bacterial strains

The native nitrogen-fixing strains *Rhizobium calliandrae* LBP2-1, *Rhizobium mayense* NSJP1-1^{T}, and *Rhizobium jaguaris* SJP1-2 were used in this experiment (Rincón-Rosales et al., 2013).

Evaluation of the ability of bacterial strains as PGPB

Nitrogen fixation

The acetylene reduction assay (ARA) involved culturing bacteria in a nitrogen-free medium. After a 72-h incubation at 28 °C, cultures were exposed to 1% acetylene. Acetylene, generated by dissolving calcium carbide in tap water, was injected at a final concentration of 1% (v/v), replacing an equivalent air volume. ARA was assessed using a Varian 3300 gas chromatograph with a flame ionization detector (Navarro-Noya *et al.*, 2012).

Phosphate solubilization

Isolates, cultured individually in yeast-malt extract broth (YM medium) overnight, were adjusted to OD_{600} nm of 1.0. They were then inoculated into NBRIP medium with insoluble tricalcium phosphate ($Ca_3(PO_4)_2$) at pH 7.0, phosphate-solubilizing bacteria were identified by clear zones around colonies after 5 days at 30 °C. The phosphate solubilization index (PSI) was calculated following Liu *et al.* (2015).

Auxin synthesis

Indole-3-acetic acid (IAA) production was measured using the colorimetric Salkowski reagent method (Brick *et al.*, 1991). Bacterial isolates were cultured in YMB medium with L-tryptophan, incubated for 7 days at 28 °C. After centrifugation, supernatant was mixed with Salkowski reagent, incubated in the dark at 28 °C for 30 min, and absorbance was measured at 530 nm (O'Hara *et al.*, 1989). Auxin concentration was determined using a standard IAA curve.

Siderophores production

Bacterial isolates were cultured on chrome azurol-S (CAS) agar medium. Colonies exhibiting an orange halo after a 3-day incubation period at 28 °C were identified as positive for siderophore production. The diameter of the orange halo was measured, and the result was quantified as Siderophore Induced Droplet Formation (SID), following the methodology established by Alexander and Zuberer (1991).

ACC deaminase

The deaminase ACC activity was determined according to Glick (1995). A bacterial inoculum of 5 μl of 10^9 cel/mL (OD_{600}nm=0.2) of each of the isolates was inoculated in culture medium containing: 0.25 g K_2HPO_4; 0.05 g $MgSO_4.7H_2O$; 0.025 g $FeSO_4.7H_2O$; 0.25 g $CaCO_3$; 0.05 g NaCl; 0.0012 g $NaMoO_4.2H_2O$; 2.5 g glucose; 3.75 g agar; 240 ml distilled water, and 0.03% of ACC as the sole source of nitrogen. The Petri dishes were incubated at 30 °C for 4 d. Colonies were subcultured in fresh medium containing ACC and incubated under the same conditions to confirm growth.

Biofertilization trials in tomato crop (*Solanum lycopersicum*)

The biofertilization trial on "bola" variety tomatoes involved three treatments with native *Rhizobium* strains (T1=*R. calliandrae*, T2=*R. jaguaris*, T3=*R. mayense*), Triple 17 as positive control, and non-inoculated plants as negative control. Inoculation, initially via spray at 1×10^6 CFU/mL during transplant and a second application 25 days later, was performed on 25 plants per treatment in mulched furrows. Chemical treatments followed standard agronomic practices. After 120 days, various parameters were assessed, including plant height, total and root weight, chlorophyll content, and nitrogen and phosphorus levels, along with fruit number and weight.

Statistical analysis of experimental data

The data collected from the biofertilization trials consisted of 75 samples in total, with 25 samples per treatment, including triplicates. Given the sample size and replication structure, it was assumed that the data followed a normal distribution based on the central limit theorem, which states that with a sufficiently large sample size ($n\geq50$), the distribution of the sample mean approximates normality.

The data were subjected to a one-way analysis of variance (ANOVA) at a significance level of alpha (α)=0.05 using the statistical software Statgraphics Centurion XV.2 for all studied variables. When the ANOVA indicated significant differences among treatments, Tukey's honest significant difference (HSD) test was used for multiple pairwise comparisons ($p<0.05$) to identify specific differences between treatment means.

RESULTS AND DISCUSSION

Biofertilized soils displayed higher pH, Cation Exchange Capacity (CEC), Total Carbon (C), Total Nitrogen (N), and Total Phosphorus (P) but lower Electrical Conductivity (EC), indicating reduced salinity (Table 1). These physicochemical improvements are crucial, as increased CEC enhances the soil's ability to retain essential nutrients, directly benefiting plant growth (Dey et al., 2023). The increase in pH and reduction in EC observed after biofertilization align with findings reported by Gogoi *et al.*, 2004 and Helmy *et al.*, 2013, where biofertilizers mitigated soil acidity and salinity, improving overall soil health. CEC in biofertilized soils measured 13.7cmol/kg, and though C and P levels were adequate, total N content was relatively low. Both biofertilized and non-biofertilized soils exhibited a low C:N ratio, indicating slow mineralization and nitrogen release. The stability of the C:N ratio suggests that native *Rhizobium* strains may enhance nitrogen retention rather than promote rapid mineralization, which can be beneficial for sustaining long-term soil fertility (Chen *et al.*, 2024). These findings highlight biofertilization's positive impact on enhancing soil fertility and physicochemical properties, potentially improving tomato crop yields and overall agricultural sustainability.

It is important to emphasize that the observed reduction in electrical conductivity not only reflects decreased salinity but also implies a lower risk of ion toxicity to plants, particularly sodium and chloride ions, which are detrimental under arid or semiarid conditions (Seifi *et al.*, 2017; Geilfus, 2018). Moreover, the improved availability of total phosphorus suggests enhanced microbial activity, as *Rhizobium* strains are known to release organic acids that mobilize insoluble phosphate reserves in the rhizosphere (Etesami, 2023; Fadeh *et al.*, 2023). This dynamic is critical in tropical soils, where phosphorus is often immobilized in forms unavailable to plants. Native *Rhizobium* strains used as biofertilizers exhibited diverse Plant Growth-Promoting Bacteria (PGPB) qualities (Table 2). Notably, *R. jaguaris* demonstrated high acetylene reduction capacity (ARA), indicating strong nitrogen fixation. *R. calliandrae* showed the highest phosphate solubilization index (PSI=1.41), indicating proficiency in solubilizing both dicalcium and tricalcium phosphate (Figure 2). Such phosphorus solubilization is crucial for crop productivity, as phosphorus availability is often limited in agricultural soils (Ringeval *et al.*, 2017). Additionally, all three strains synthesized auxins, particularly *R. mayense* with notable indole-3-acetic acid (IAA) production at 34.8 mg/L. This auxin production is linked to improved root architecture, which enhances water and nutrient uptake, as demonstrated by Batista *et al.*, 2021.

Table 1. Physicochemical characteristics of soil in tomato crops enhanced by native *Rhizobium* Biofertilization.

Soil samples	pH	EC[a] (dSm^{-1})	CEC[b] Cmol kg^{-1}	Total C (%)	Total N (%)	Total P (%)	C:N ratio
Soil before biofertilizer	5.8±(0.04)[c]	0.87±(0.06)	10.5±(0.4)	1.17±(0.01)	0.65±(0.06)	1.46±(0.04)	1.8 ±(0.3)
Soil after biofertilizer	6.4±(0.07)	0.48±(0.04)	13.7±(0.5)	1.42±(0.03)	0.83±(0.04)	2.25±(0.06)	1.7±(0.5)
Chemical Fertilizer	5.4±(0.02)	2.69±(0.03)	18.0±(0.2)	2.28±(0.02)	0.95±(0.03)	3.22±(0.04)	2.4±(0.4)

[a] EC: Electrolytic conductivity; [b] CEC: Cation exchange capacity; [c]Mean of three replicates. In parentheses standard deviation.

Table 2. Multifunctional PGPB qualities of native *Rhizobium* strains.

Strain	ARA[a] (nmol C_2H_4 per culture h^{-1}	P-solubilization (PSI)[b]	IAA production (mg/L)	Siderophore production (SID)[c]	ACC-deaminase
Rhizobium calliandrae	425±(1.61)[d]	1.41±(0.62)	25.4±(0.98)	1.18±(0.75)	(+)
Rhizobium jaguaris	645±(1.41)	1.52±(0.48)	28.6±(1.12)	1.68±(0.95)	(+)
Rhizobium mayense	418±(1.35)	1.48±(0.58)	34.8±(1.34)	1.28±(0.67)	(+)

[a]ARA, Acetylene Reduction Assay); [b]PSI, Phosphate Solubilization Index. [c]SID, Siderophore Induced Droplet Formation. [d]Mean values of three replicates. The values in parenthesis are standard deviations. (+)=positive.

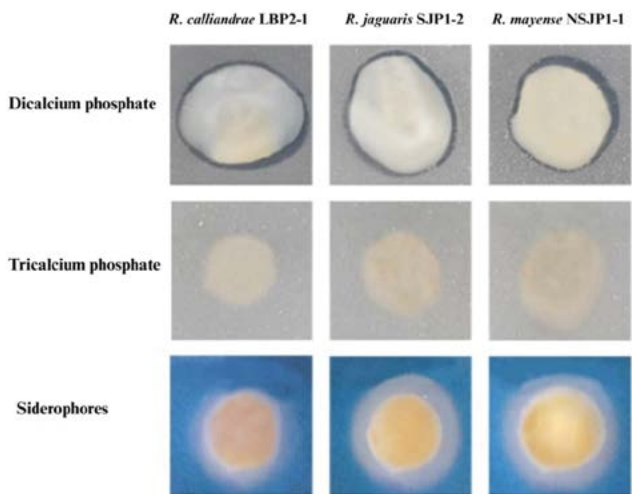

Figure 2. Halos of phosphate solubilization and production of siderophores by native *Rhizobium*.

These multifactorial traits are consistent with the concept of "multifunctional PGPB", where the synergistic expression of plant hormone production, nutrient solubilization, and stress alleviation (*e.g.*, through ACC deaminase activity) positions *Rhizobium* as more than just a nitrogen fixer. This redefines their potential for non-legume crops, aligning with Glick (2012) and Dheeman *et al.*, 2022, who emphasized the versatility of PGPB in a broad range of plant hosts. Furthermore, all three strains exhibited siderophore production, with *R. jaguaris* showing the highest percentage of siderophore production (SID=1.68) among the *Rhizobium* species analyzed. These findings align with previous studies demonstrating the capacity of rhizobacteria, including *Rhizobium* spp., to solubilize phosphorus through the secretion of organic acids (Taktek *et al.*, 2015).

Native *Rhizobium* strains' siderophore production is essential for plant iron uptake, critical in N_2-fixing systems and enzyme synthesis (Datta and Chakrabartty, 2014). Siderophores are low-molecular-weight iron-chelating compounds that bacteria release to scavenge ferric iron (Fe^{3+}) from the soil, especially under conditions of limited bioavailable iron, a common scenario in alkaline or calcareous soils. By forming stable Fe^{3+} -siderophore complexes, these molecules facilitate iron uptake not only for the bacteria but also for the host plant through rhizosphere exchange mechanisms (Ahmed *et al.*, 2014). In this context, the siderophore-mediated increase in iron availability can enhance photosynthetic activity, promote enzymatic function, and improve overall plant vigor.

This study emphasizes their diverse role as biofertilizers, enhancing soil fertility and plant growth. Inoculation significantly influenced various parameters in field-grown tomato plants, with biofertilized plants showing increased height and total weight, particularly with *R. calliandrae* and *R. mayense* application. The impact results from native *Rhizobium* strains' inherent abilities in N_2 fixation, phosphate solubilization, and auxin synthesis, vital for plant growth. Additionally, these strains play a crucial role in nutrient uptake, especially for essential elements like nitrogen and phosphorus (Gen-Jimenez *et al.*, 2023).

Rhizobium bacteria crucially influence biochemical and metabolic processes, enhancing crop quality as precursors in various pathways. Biofertilization, especially with native *Rhizobium* strains, significantly affects mineral concentration, carotenoids, and lycopene levels in tomatoes (Flores-Felix *et al.*, 2021). These findings underscore native *Rhizobium* strains' potential to positively impact tomato plants, improving crop yield and quality. Moreover, by modulating phytohormone signaling pathways, particularly auxin and ethylene, these bacteria can influence not only vegetative development but also

Table 3. Effect of biofertilization with native *Rhizobium* bacteria on morphometric and biochemical parameters in tomato cultivation.

Treatments	Plant height (cm)	Plant weight (g)	Chlorophyll Content (%)	Total N (%)	Total P (%)	Fruit (g)	Fruit/ plant
R. calliandrae	43.5 a*	264.3 a	39.5 ab	4.06 b	0.75 a	217.1 a	18.2a
R. jaguaris	42.9 a	152.3 c	42.0 a	3.12 c	0.60 a	206.8 ab	15.9b
R. mayense	41.5 a	242.6 a	41.4 a	2.78 c	0.62 ab	196.6 ab	17.9a
Chemical fertilizer	41.5 a	176.8 bc	44.7 a	6.41 a	0.48 b	187.6 ab	15.5b
Negative control	40.9 a	181.0 b	33.1 b	4.28 b	0.57 b	177.7 b	17.2ab
p-value	0.1677	0.0000	0.0002	0.0000	0.0003	0.0432	0.0002
¶HSD Tukey ($p<0.05$)	3.363	25.384	6.681	0.380	0.152	38.038	1.831

*Mean values were obtained from n=25 replicas; ¶HSD (Honestly Significant Difference). Means followed by the same letter are non-significant ($p<0.05$).

Figure 3. Effect of biofertilization with native *Rhizobium* bacteria on tomato growth and production.

reproductive processes such as flowering and fruit set. This interaction may explain the observed improvements in fruit yield and quality. The integration of *Rhizobium*-based biofertilization into tomato production systems thus presents a promising strategy not only for enhancing productivity, but also for improving the nutritional value and marketability of the crop in a sustainable manner. These results contribute to a growing body of evidence supporting the use of native, crop-adapted microbial consortia to optimize agroecosystem functioning while reducing dependence on synthetic inputs.

CONCLUSIONS

The application of native *Rhizobium* strains, such as *Rhizobium calliandrae*, *R. mayense*, and *R. jaguaris*, as biofertilizers significantly improved soil quality and positively influenced tomato plant growth. These strains demonstrated growth-promoting traits, including nitrogen fixation and phosphate solubilization, which were associated with increases in plant height and total biomass. The results of this study highlight the promising potential of native *Rhizobium* strains as sustainable biotechnologies for enhancing crop performance while contributing to improved soil health. Future research, including metagenomic analysis and next-generation sequencing, could further elucidate the significance of employing rhizobial biofertilizers in agricultural systems.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Fatty Acid Content in the Adipose Tissue of Lambs from Hair and Wool Meat Breeds

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ABSTRACT

Objective: To evaluate the fatty acid content of the intermuscular adipose tissue (IAT) and perirenal adipose tissue (PAT) of the lambs from hair breeds and their crosses with wool breeds.

Design/Methodology/Approach: Forty-two male lambs were used: 10 Blackbelly (BB), 6 Pelibuey (PB), 6 Katahdin (KT)×BB (KTBB), 10 Charollais (CH)×BB or PB (CHBP), and 10 Suffolk (SF)×BB or PB (SFBP). A linear fixed effects model was used and the following orthogonal contrasts were performed: C1) BB+PB *vs.* KTBB; C2) BB+PB *vs.* CHBP+SFBP; C3) BB *vs.* PB; and C4) CHBP *vs.* SFBP.

Results: The BB and PB lambs recorded the highest saturated fatty acids (SFA), while the CHBP and SFBP had the highest content of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in the IAT and PAT ($P<0.05$). Higher proportions of myristic and palmitic acid were found in the IAT and PAT of hair lambs ($P<0.05$). The wool breeds (CHBP+SFBP) had more palmitoleic and oleic acid in the IAT and PAT and linoleic acid in the IAT ($P<0.05$).

Study Limitations /Implications: Lamb meat is believed to be greasy and a higher content of healthy fats is sought for the benefit of consumers.

Findings/Conclusions: Hair lambs had more saturated fatty acids. Meanwhile, the higher content of monounsaturated and polyunsaturated fatty acids in wool lambs is important for the quality and nutritional value of their meat.

Keywords: Hair and wool lambs, adipose tissue, fatty acids.

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INTRODUCTION

Meat quality is usually associated with the age of the animal: a higher content of adipose tissue is deposited in the bodies of animals slaughtered at an older age (Della Malva *et al.*, 2016). People believe that sheep meat is greasy and can only be consumed as barbacoa (Rubio *et al.*, 2004; Partida *et al.*, 2017). The fat found in the carcass and the various ways in which meat is prepared (including barbacoa) mainly comes from intermuscular adipose tissue (IAT). The saturated fatty acid content in meats is important for human health

(Castro, 2002; Davis *et al.*, 2022) , since most meat consumers seek nutritious products with desirable organoleptic properties, considering, among other aspects, a greater content of monosaturated and polyunsaturated fatty acids, given their positive effect on human health (OMS/FAO, 2003).

Many more studies are required about the fatty acid content of the adipose tissue of lambs from hair and wool breeds, as well as their crosses. Additionally, Zhao *et al.* (2019) and Watkins *et al.* (2021) have highlighted that intermuscular fat is the key factor for the succulence and exceptional flavor of meat. The aim of this study is to determine the fatty acid content of the intermuscular and perirenal adipose tissue of lambs from pure hair breeds and their crosses with wool breeds specialized in meat production.

The hypothesis was that the difference in the fatty acid content of the intermuscular and perirenal adipose tissue between pure hair breeds and their crosses with wool breeds specialized in meat production could influence meat quality.

MATERIALS AND METHODS

The study was carried out in the Faculty of Animal Husbandry and Ecology of the Universidad Autónoma de Chihuahua (28° 38' N and 106° 04' W, at 1,435 m.a.s.l.). The annual average temperature is 17.0 °C and the annual average rainfall reaches 500 mm^3 (INEGI, 2016).

Biological material

The experiment included a total of 42 uncastrated male lambs: 10 Blackbelly (BB), 6 Pelibuey (PB), 6 Katahdin (KT)×BB (KTBB), 10 Charollais (CH)×BB ó PB (CHBP), and 10 Suffolk (SF)×BB ó PB (SFBP). They had a mean live weight ± standard deviation (SD) of 20.47±2.52 kg. The animals were housed in individual pens, where they were provided with a commercial diet (Table 1), consisting of 13.0% crude protein (CP) and 2.43 megacalories of metabolizable energy per kilogram of dry matter (Mcal ME/kg DM).

Before the start of the test, the lambs were dewormed with an Invermectine intake. They were given a 14-day period to adapt to the diet and the pens. They were weighted after a 16-hour fast every 14 days, from the start to the end of the 98-day experiment. At the end of the experiment, all the animals were slaughtered, after a 16-hour fast, according to the Official Mexican Standard for the Slaughter and Cutting of Animals (NOM-033-SAG/ZOO-2014). The head was cut at the occipito-atloid articulation, while the skin, the hooves, parts of the thoracic cavity (organs and glands), and the content of the abdominal and pelvic cavities (gastrointestinal content) were removed from the carcass. The empty carcass was refrigerated at 4 °C for 24 hours. Subsequently, the carcasses where cut in half along the spinal column. The left half was dissected and divided into muscles, bones, and fat. Fat samples were taken from the leg muscles were taken from each animal (intramuscular, IAT), as well from the kidneys (perirenal, PAT). Each sample weighted ≈30 g. The samples were vacuum-packed and frozen at −20 °C until they were subjected to a chemical analysis. Prior to the analysis, they were defrosted for 24 h at 4 °C. The fatty acid (FA) contented was measured using a gas chromatography, following method 996.06 of the AOAC (2012).

Table 1. Ingredients and chemical composition of the diet.

Ingredients	DM (%)
Rolled corn	57.60
Alfalfa hay	20.80
Cottonseed meal	11.80
Corn gluten meal	5.00
Cane molasses	3.60
Common salt (NaCl)	0.40
Mineral premix (Microfos MNA)	0.40
Calcium carbonate	0.40
Total	100.00
Chemical composition	
Dry matter (%)	86.70
Crude protein (%)	13.05
Calcium (%)	0.52
Phosphorus (%)	0.31
ME (Mcal/kg DM)[a]	2.43

Estimated based on (NRC, 1985).

Data analysis

The results were analyzed with a generalized linear model (GLM) with fixed effects, which included the effect of the paternal genetic group. Means were compared through orthogonal contrasts, using SAS procedures (SAS Inst. Inc., 2003): C1) BB+PB *vs*. KTBB; C2) BB+PB *vs*. CHBP+SFBP; C3) BB *vs*. PB; and C4) CHBP *vs*. SFBP.

RESULTS AND DISCUSSION

Table 2 shows the effect of the genetic group on the fatty acid content of the IAT, while Table 3 shows its effect on PAT. A significative effect ($P<0.05$) was recorded regarding the content of saturated FA (SFA) had on the IAT and PAT, when lambs from hair and wool (C2) breeds are contrasted, as well as when PB+BB *vs*. KTBB (C1) was compared with BB *vs*. PB (C3). BB and PB lambs recorded the highest SFA in IAT and PAT ($P<0.05$), which was key for their group; however, CHBP and SFBP specimens had a higher MUFA concentration ($P<0.05$) in IAT and PAT than BB and PB (C2) specimens. No significative differences ($P>0.05$) were recorded in SFA y MUFA percentages of the IAT and PAT between wool sheep groups (C4). Tavares *et al.* (2018) reported similar SFA and MUFA contents in the perirenal fat of Santa Inés lambs, while Karaca *et al.* (2016) reported similar results in the subcutaneous and tail adipose tissue of wool lambs. The IAT of wool crosses had a higher concentration ($P<0.05$) of polyunsaturated FA (PUFA) than the IAT of hair races (C2); no differences were found in the PAT of the other animal groups evaluated ($P>0.05$). Maleki *et al.* (2015) reported similar results in the subcutaneous fat of wool lambs. For their part, Karaca *et al.* (2016) reported a higher PUFA proportion in the subcutaneous and tail fat of wool lambs, possibly as a consequence of the various types of fatty deposits used in both studies.

Table 2. Effect of the genetic group on the fatty acid content of the intermuscular fat of lambs.

Fatty acids profile (% of total)	Genetic group					Orthogonal contrasts			
	BB	PB	KTBB	CHBP	SFBP	C1	C2	C3	C4
Saturated fatty acids	45.0±1.15	51.4±1.37	43.8±1.62	44.5±1.15	44.5±1.15	*	*	*	NS
Monounsaturated fatty acids	51.7±1.08	44.6±1.29	52.0±1.52	51.2±1.08	51.4±1.08	*	*	*	NS
Polyunsaturated fatty acids	3.3±0.27	4.0±0.32	4.2±0.38	4.3±0.27	4.1±0.27	NS	*	NS	NS
Myristic acid (C14:00)	4.1±0.30	5.3±0.36	4.1±0.42	3.2±0.30	3.6±0.30	NS	*	*	NS
Palmitic acid (C16:00)	27.3±0.74	28.2±0.88	27.5±1.05	24.7±0.74	25.3±0.74	NS	*	NS	NS
Stearic acid (C18:00)	13.6±0.80	17.9±0.96	12.2±1.13	16.7±0.80	15.6±0.80	*	NS	*	NS
Palmitoleic acid (C16:1 n-9C)	1.1±0.16	1.7±0.19	2.2±0.22	1.9±0.16	1.8±0.16	*	*	*	NS
Oleic acid (C18:1 n-9C)	50.58±1.12	43.0±1.34	49.8±1.58	49.3±1.12	49.6±1.12	NS	*	*	NS
Linoleic acid (C18:2 n-6C)	3.06±0.25	3.8±0.30	3.9±0.36	3.9±0.25	4.0±0.25	NS	*	NS	NS
Eicosatrienoic acid (C20:3 n-9C)	0.19±0.05	0.2±0.06	0.3±0.07	0.3±0.05	0.2±0.05	NS	NS	NS	*

Note: *=P<0.05; NS=Not significant; C1=BB+PB *vs*. KTBB; C2=BB+PB *vs*. CHBP+SFBP; C3=BB *vs*. PB; C4=CHBP *vs*. SFBP.

Table 3. Effect of genetic group on fatty acid composition in perirenal fat of lambs.

Fatty acids profile (% of total)	Genetic group					Orthogonal contrasts			
	BB	PB	KTBB	CHBP	SFBP	C1	C2	C3	C4
Saturated fatty acids	55.7±0.81	58.2±0.97	52.1±1.15	53.7±0.81	52.7±0.81	*	*	*	NS
Monounsaturated fatty acids	39.8±0.73	37.7±0.88	43.4±1.04	41.9±0.73	42.6±0.73	*	*	NS	NS
Polyunsaturated fatty acids	4.5±0.24	4.0±0.29	4.5±0.35	4.3±0.24	4.7±0.24	NS	NS	NS	NS
Myristic acid (C14:00)	3.5±0.2	3.8±0.3	2.7±0.3	2.7±0.2	2.4±0.2	*	*	NS	NS
Palmitic acid (C16:00)	25.8±0.6	26.0±0.8	23.9±0.8	23.8±0.6	22.9±0.6	*	*	NS	NS
Stearic acid (C18:00)	13.6±0.80	17.9±0.96	12.2±1.13	16.7±0.80	15.6±0.80	*	NS	*	NS
Palmitoleic acid (C16:1 n-9C)	0.5±0.1	0.6±0.2	1.0±0.2	0.9±0.1	0.9±0.1	NS	*	NS	NS
Oleic acid (C18:1 n-9C)	39.2±0.7	37.1±0.9	42.4±1.0	41.0±0.7	41.9±0.7	*	*	NS	NS
Linoleic acid (C18:2 n-6C)	4.4±0.2	4.0±0.3	4.4±0.3	4.2±0.2	4.5±0.2	NS	NS	NS	NS
Eicosatrienoic acid (C20:3 n-9C)	0.1±0.03	0.1±0.04	0.2±0.04	0.2±0.03	0.2±0.03	NS	*	NS	NS

Note: *=P<0.05; NS=Not significant; C1=BB+PB *vs*. KTBB; C2=BB+PB *vs*. CHBP+SFBP; C3=BB *vs*. PB; C4=CHBP *vs*. SFBP.

The IAT and PAT of hair lambs had a higher proportion ($P<0.05$) of myristic (40%) and palmitic (11%) acid than the IAT and PAT of wool lambs (C2). The IAT of PB and BB lambs had a higher content of stearic acid (29%) the PAT of KTBB ($P<0.05$). Meanwhile, the PAT of the same breeds had a higher content of myristic (35%) and palmitic (8%) acid than the PAT of KTBB ($P<0.05$). These findings match the results of Tavares *et al.* (2018) and Yagoubi *et al.* (2020), who reported a greater amount of palmitic and stearic acid in the visceral and perirenal fat of hair sheep. The higher concentration of these fatty acids in hair sheep could be associated with the gene expression found in the various deposits, which depend on the breed of the specimens (Yue *et al.*, 2016; Cui *et al.*, 2022). In contrast, the IAT and PAT of lambs from wool breeds (CHBP+SFBP) had more ($P<0.05$) palmitoleic (35 to 63%) and oleic (6 a 9%) acid; likewise, their IAT had more linoleic acid (8%) than the IAT of hair sheep (BB+PB) (C2). The IAT ($P<0.05$) of BB lambs had the highest oleic acid (18%) concentration among hair breeds (C1). Karaca *et al.* (2016) reported a lower oleic acid and a similar linoleic acid content, in both the subcutaneous and tail fat of wool lambs provided with a concentrate-based feed. However, Meale *et al.* (2015) reported a higher concentration of linoleic acid in the subcutaneous and renal adipose tissue of wool lambs, perhaps because these authors used different plant oils in the diet of the animals. The PAT of wool lambs recorded double the eicosatreinoic acid content ($P<0.05$) than the PAT of PB+BB (C2). Comparing wool breeds (C4), CHBP had 50% more eicosatreinoic acid than SFBP ($P<0.05$). The highest concentration of acid in wool breeds is caused by their higher amount of oleic acid, which is desaturated to produce eicosatreinoic acid (Sakuradani *et al.*, 2002). No significative differences were reported in the oleic acid proportions for the following contrasts: C1 and C4 for IAT and C3 and C4 for PAT ($P>0.05$). Neither were they found in the linoleic acid content of C1, C3, and C4 for IAT, nor for any PAT group. Finally, no significative differences were found in the proportion of eicosatreinoic acid for the following contrasts: C1, C2, and C3 for IAT and C1, C3, and C4 for PAT ($P>0.05$). Song *et al.* (2017) report lower concentrations of eicosatreinoic acid in the kidney and subcutaneous fat of crossed Dorper lambs fed with concentrates.

These findings have major implications for the meat industry and its consumers. On the one hand, the higher concentration of saturated fatty acids in hair lambs could be associated with sensory properties that consumers find valuable, such as succulence and flavor. On the other hand, the higher proportion of monosaturated and polyunsaturated fatty acids in wool lambs can provide a healthier nutritional profile. These differences help to diversity meat products, satisfying both the taste preferences of the consumers and their nutritional requirements.

CONCLUSIONS

Hair lambs have higher levels of saturated fatty acids, while wool lambs have more monosaturated and polyunsaturated fatty acids. These differences influence the sensory and nutritional quality of meat, highlighting the importance of the characteristics of the breeds regarding consumer preferences and health requirements.

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Colegio de Postgraduados

Impact of producer profiles on the sustainability of protected agriculture: analysis of the COMESA value network

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ABSTRACT
Objective: to analyze how producer profiles influence the sustainability of production units under protected agriculture, using historical customer records and the dynamics of a Mexican agribusiness, COMESA's value network, as a case study.
Design/Methodology/Approach: producers were segmented into three main groups —subsidized producers (inductees), entrepreneurs, and intermediary service providers— based on their financial and operational characteristics. Transaction recurrence was analyzed as an indicator of financial sustainability. Some tools of social network analysis were also applied to map their integration into the value network.
Results: entrepreneurial producers showed greater operational continuity and a significant share for COMESA's revenues. This financial power is reflected in more sustainable production units. In contrast, subsidized producers showed low transaction recurrence and weak loyalty, which limits the long-term sustainability of those production units.
Limitations/Implications of the study: despite this analysis is based on the records of a single company, our findings offer a replicable framework that can guide support strategies in similar contexts.
Findings/Conclusions: strengthening connections with strategic stakeholders —such as financial institutions, marketing companies, and technical advisors— is essential to improve the sustainability of production units, especially among the most vulnerable producers. Considering producer profiles in development programs can improve long-term results.

Keywords: protected agriculture, value network, productive sustainability.

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INTRODUCTION

Protected agriculture is established as an essential strategy to address challenges such as climate change, water scarcity, and food security. In Mexico, the area devoted to this type of crops has experienced significant growth in the last decade, reflecting producers'

ability to adapt to increasingly demanding environmental conditions and market demands. However, the success of these units is not based solely on technology, but also on network dynamics and the specific characteristics of farmers, who face challenges in terms of financing, technical training, and access to competitive markets. Worldwide, the adoption of protected agriculture systems has allowed for optimized yields and improved quality of agricultural products. Solid evidence demonstrates their potential to overcome environmental limitations and effectively respond to market demands.

In Mexico, official data show an upward trend in the total area cultivated under this model, underscoring its growing importance in the agrifood sector. For these reasons, this study focuses on analyzing the dynamics that influence the sustainability of production units under protected agriculture, considering the role played by the interaction between producers and other key actors in the value network. Our approach sought to contribute elements for the formulation of public policies and the design of business strategies that promote technological adoption, as well as the effective integration of producers into more resilient value networks. Through the case study of COMESA, a Mexican enterprise dedicated to the integral development for agribusiness, we examined how different client profiles relate to their ability to remain and consolidate within this productive system.

The objective was to analyze how producer profiles influence the sustainability of production units under protected agriculture, using historical customer records and the dynamics of a Mexican agribusiness, COMESA's value network, as a case study.

MATERIALS AND METHODS

The study focused on COMESA, a Mexican family business specialized in greenhouse construction and the provision of technologies for protected agriculture. Since its founding in 2012, COMESA has operated in 20 states in Mexico, allowing it to analyze a variety of agroecological conditions and levels of technological development. This background offers now a representative perspective on the production dynamics in the protected agriculture sector.

The analysis covered the period 2012 to 2023, integrating both the early years of consolidation of the company and recent changes in public policies, especially the reduction in subsidies since 2020 (Hernández Suárez, 2021). This timeframe facilitated the assessment of the impact of those transformations on sustainability and on the dynamics of producers within the value network. Quantitative information was obtained from the internal records of COMESA, which included detailed data on commercial transactions operated during the study period. Key client information was collected, such as the type of project, payments in the contract, frequency of purchases, and characteristics of the business relationship. All of which allowed us to integrate a robust database on business dynamics.

Additionally, semi-structured interviews were conducted with key actors in the value network to validate and enhance, with complementary information, the trends observed in historical records. According to Kvale (2007) recommendations, a flexible thematic guide was used to delve deeper into emerging issues during qualitative data collection. Ten interviews were conducted; six with entrepreneurial producers; three with technical service providers, two of them specialized in high-tech production and one with representation in the Secretariat of Agricultural Development; finally, one with a supplier who also acts as a competitor.

To visually analyzing and mapping the strategic interactions of COMESA with its environment, social network analysis tools were used; specifically, UCINET (Borgatti *et al.*, 2002) and Gephi (Bastian *et al.*, 2009). UCINET allowed the analysis of structural properties of the network, such as modularity; while Gephi facilitated the graphical representation of complex interactions, helping to identify patterns and areas for improvement.

The methodology was structured in three main stages. First, a segmentation of COMESA's customers was featured (Alvarado Chávez *et al.*, 2024) based on market segmentation principles (Kotler & Keller, 2016), as well as on the typology of entrepreneurial families (Islas Moreno *et al.*, 2023). This classification facilitated the grouping of customers according to common operational, financial and relational characteristics, thus allowing the analysis of their behavior within the value network. Second, recurrence of transactions was analyzed using statistical techniques that provided indicators on the impact of each segment on the financial stability of the company (Fernández, 2017). Finally, the interactions between the main actors related to COMESA were evaluated, considering how those relationships contribute to the creation of value, and to the sustainability of agricultural operations (Muñoz Rodríguez & Santoyo Cortes, 2020).

Our methodological approach was based on the conceptual definition of systemic innovation manager (Klerkx *et al.*, 2009). This approach emphasizes the role of intermediary individuals or organizations that facilitate interaction and capacity building in complex systems; promoting collaboration and the co-creation of innovative solutions. From this perspective, COMESA was analyzed as a systemic innovation manager within the value network of protected agriculture. The combination of quantitative and qualitative methods provided a comprehensive view of the commercial and relational dynamics of the company. This approach allowed us to identify critical factors influencing the sustainability of production units, and to offer a replicable methodological framework for other companies in the protected agriculture sector.

RESULTS AND DISCUSSION

Analyses showed that the profiles of producers directly influence the sustainability of their production units and their behavior within the value network. Based on COMESA's historical records, three customer segments were identified, subsidized producers (inductees), entrepreneurial producers, and intermediary service providers (Alvarado Chávez *et al.*, 2024). Each group presents distinct operational characteristics and interaction patterns, which affect their ability to remain in the protected agriculture market.

Subsidized producers, mostly small rural farmers, accessed technologies through government subsidies. Their participation focused on small-scale projects geared toward self-consumption, with limited technical integration and weak market connections. Although they represented 76% of the total clients served by COMESA, their economic contribution was 15% during the 2012-2023 period due to their low recurrence and limited investment capacity. This situation reflects a strong dependence on external resources and weak institutional coordination (Scott, 2014).

Muñoz Rodríguez *et al.* (2020) argued that, although subsidy-based programs seek to incentivize production and improve living conditions in rural areas, they do not address the structural causes that limit sustainable development, such as lack of access to markets and adequate technology. On the other hand, Aguilar Gallegos *et al.* (2013) emphasized that improving the sustainability of the subsidized group would require developing technical capacities, a better selection of beneficiaries, and to promote their integration into competitive markets. Furthermore, providing ongoing technical support, especially for larger, collaborative projects, would be essential to ensure operational and financial success.

In contrast, entrepreneurial producers consolidated their position as the most economically relevant segment. Representing only 18% of total clients, they generated 72% of COMESA's revenue. This group is composed of continuing professionals with advanced technology, new entrants with an entrepreneurial vision, and returnees with capital accumulated in other sectors, according to the typology of Islas Moreno *et al.* (2023). The ability of these participants to combine their own resources, private financing, and partial subsidies has allowed them to operate larger-scale greenhouses and maintain a sustained market presence.

A third segment, identified as intermediary service providers, played a prominent role in COMESA's early years of operation. This group consisted of professionals and companies that acted as project managers, channeling resources to end-producers through the technical support provided by COMESA. Between 2012 and 2018, those participants facilitated the execution of numerous projects financed by public subsidies. However, starting in 2020, their participation decreased significantly, and they ceased to appear as recurring clients. The reasons for this departure are linked to changes in subsidy policies, more rigorous evaluation criteria, and technical feasibility issues in the managed projects. The disappearance of this segment reflects a transition from the intermediation model to direct relationships between producers and specialized suppliers.

In the evolution of these segments and their economic participation, we observed how the reduction in subsidies starting in 2020 modified COMESA's revenue structure (Figure 1). The change in public policies primarily affected subsidized producers, who reduced their presence and transactions. In contrast, entrepreneurial producers maintained or even increased their investment; these participants demonstrated greater resilience facing institutional changes.

These findings are consistent with those reported by Muñoz Rodríguez y Santoyo Cortes (2020), who highlight that institutional coordination and engagement with strategic stakeholders largely determine access to key resources and continued production. Similarly, similar studies in Latin America have documented that farmers with a greater capacity to build strong relationships with suppliers, institutions, and markets tend to maintain more stable operations (Klerkx *et al.*, 2009).

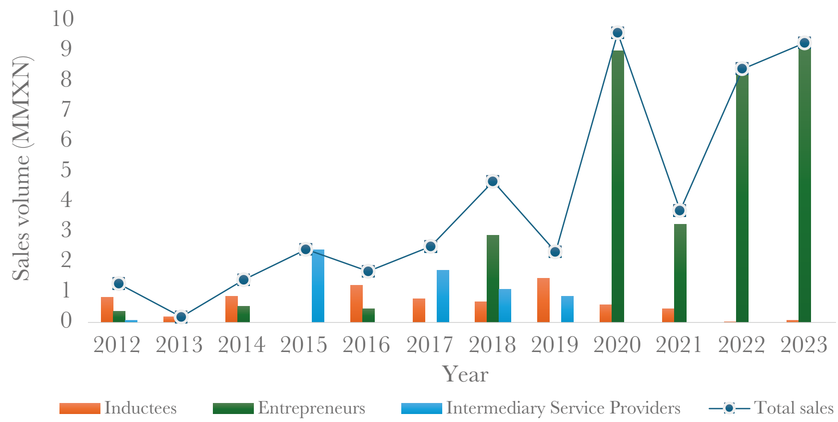

Figure 1. COMESA revenue records, by market segment during the period 2012-2023.

Transaction recurrence, recommendations and post-2020 adaptation

The recurrence of transactions reinforces the differences among the segmented profiles of producers. Entrepreneurial producers do subsequent transactions with greater frequency and economic volume, which indicates a more stable and long-term business relationship. This recurrence not only implies operational continuity, but also confidence and reinvestment capacity. These are key indicators of sustainability in the context of protected agriculture (Table 1).

Beyond purchasing power, transaction recurrence exhibits interaction dynamics based on mutual trust and reputation between producer and supplier. Producers who return to COMESA do so not only out of a need for expansion, but also because they perceive value in support, technical advice, and the quality of service received. This long-term relationship generates a multiplier effect within the network, as it allows for constant feedback, technological updates, and shared access to successful experiences.

COMESA data indicated that only 9% of its customers made recurring purchases during the 2012-2023 period; however, this group generated 56% of the company's total revenue. Sales recurrence is therefore emerging as a critical indicator of financial and operational sustainability. Recurring customers not only returned for new services but also strengthened their business relationship with the company, which expresses continuity in the operation of its production units.

Table 1. Distribution of recurring sales by segment in COMESA (2012-2023).

	Initial sale		Subsequent sales	
	Transactions	**Sales (MMXN)**	**Transactions**	**Sales (MMXN)**
Inductees	9	0.87	18	1.16
Entrepreneurs	7	12.82	11	10.02
Intermediary SP	6	0.49	9	1.18
Totales	22	14.18	38	12.36

Inductees, Entrepreneurs, Intermediary Service Providers.
Inductees (subsidized producers); SP: Service Providers; MMXN: Millions of Mexican pesos (2012-2023).

An analysis of transaction patterns showed that, starting in 2020, the proportion of customers making recurrent purchases increased significantly. This trend coincides with the market reconfiguration following the reduction of government subsidies. Such a decrease forced many producers to rely on their own resources and to seek more stable business relationships. In this context, entrepreneurial producers demonstrated greater adaptability, strengthening their ties with COMESA and other key participants in the value network.

In addition to recurring sales, the referral channel was also crucial; 97% of COMESA's sales during the period analyzed came from referrals from satisfied customers (Table 2). This dynamic underscores the importance of a positive customer experience as a driver of business growth. Recurrent producers not only generate direct income but also act as active promoters within the sphere of protected agriculture.

The combination of recurring and referrals represents a dual indicator of sustainability. On the one hand, it ensures constant economic flows; on the other, it expands the value network by attracting new customers with similar profiles. This dynamic reinforces the position of entrepreneurial producers as strategic players in the sector and confirms the relevance of their profile to the continuity of the production model.

From a public policy perspective, the results suggest that simply providing subsidies to small-scale producers is not enough. It is essential to support them with strategies that promote their progressive integration into value networks with higher density. Strengthening technical, financial, and organizational capacities should be an integral part of support programs, with differentiated schemes based on the profile of producers. Furthermore, supplier companies such as COMESA can play an active role in building relationships and transferring knowledge.

Value Network and the integration role of COMESA

The structural analysis of the value network allowed us to map the interactions between the main actors linked to protected agriculture in Mexico. Using UCINET (Borgatti *et al.*, 2002) and Gephi (Bastian *et al.*, 2009), different levels of collaboration and connectivity were identified among producers, marketers, suppliers, financial institutions, public entities and research institutions.

The general network of participants linked to greenhouse systems shows COMESA, with particular emphasis on its role as an articulation node (Figure 2). Its central location in the network reflects its ability to connect producers with other key participants, including

Table 2. Sales distribution and transaction recurrence in COMESA, as made by client-client referrals (2012-2023).

	Referral sales		Non-referral sales		Total sales	
	(MMXN)	(%)	(MMXN)	(%)	(MMXN)	(%)
Recurring customers	26.50	99.9	0.04	0.1	26.54	56
Non-recurring customers	19.51	93.2	1.43	6.8	20.94	44
Total	46.01	96.9	1.47	3.1	47.48	100

MMXN: millons of Mexican pesos.

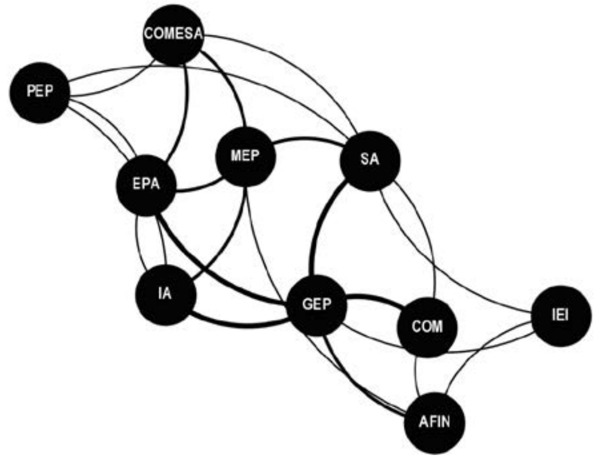

Figure 2. General linkage network in COMESA's value network. EPA: agricultural production specialists; COM: marketing companies; IA: agricultural inputs; SA: Secretariats of Agriculture; PEP: small production enterprises; MEP: medium production enterprises; GEP: large production enterprises; IEI: education and research institutions; COMESA: greenhouse builders; and AFIN: financing agencies.

financing agencies, educational institutions, and marketing companies. This position facilitates transfer of knowledge, technology adoption, and access to complementary services. All of which are critical elements for the sustainability of production units in protected agriculture.

The modularity analysis showed the existence of two large communities within the network (Figure 3). The first one, comprised of smaller-scale producers, shows limited connectivity, primarily with Government agencies. The second, comprised of medium- and large-scale producers, marketing companies, financial institutions, and research centers, presents a more cohesive and articulated structure. This segmentation suggests a structural barrier that prevents the integration of small producers into networks with greater technical and commercial capacities.

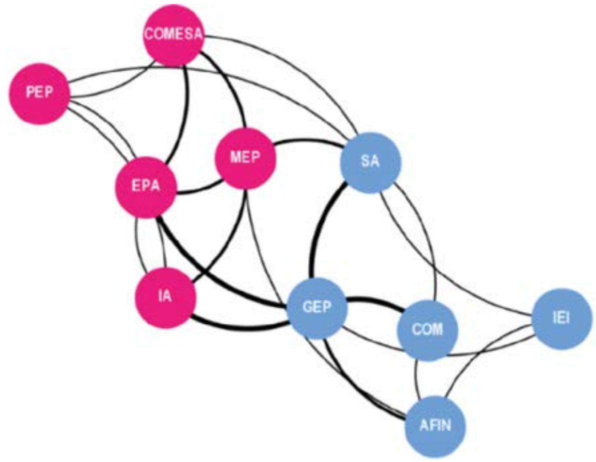

Figure 3. Modularity in COMESA's linkage network. EPA: agricultural production specialists; COM: marketing companies; IA: agricultural inputs; SA: Secretariats of Agriculture; PEP: small production enterprises; MEP: medium production enterprises; GEP: large production enterprises; IEI: education and research institutions; COMESA: greenhouse builders; and AFIN: financing agencies.

Within this articulated community, large production companies (GEP) are noticeable, operating on a significantly larger scale, with greenhouse surfaces exceeding 50 hectares. According to Pratt & Ortega (2019), these companies present a higher level of vertical integration, controlling everything from production to marketing and export of their products. They also use cutting-edge technologies, such as hydroponic crops and automated systems, and comply with strict international quality and safety standards to access export markets, especially in the United States.

The presence of GEPs establishes high standards of efficiency and competitiveness that, while raising the technological level of the sector, also generate significant gaps. Their disconnection from small and medium-sized producers restricts opportunities for knowledge transfer, adoption of advanced technology, and access to higher-value marketing channels. This structural fragmentation contributes to perpetuating inequalities within the network and reinforces the need for mechanisms that promote inter-segment linkages.

Entrepreneurial producers, located at the core of the densest network, maintain frequent ties with technical advisors, financial institutions, and strategic buyers. These connections strengthen their capacity to innovate, adapt, and remain competitive. In contrast, subsidized producers operate on the periphery of the network, with predominantly assistance-based ties, which limits their access to technology, training, and higher-value markets.

COMESA's role in this context transcends its commercial function. It acts as a systemic innovation manager, promoting connections between participants and facilitating capacity building among producers. This role is particularly valuable for strengthening the integration of marginalized segments within the protected agriculture system and promoting more balanced and sustainable collaboration schemes.

CONCLUSIONS

This study confirmed that the producer profile significantly influences the sustainability of production units under protected agriculture. Data showed that entrepreneurial producers have higher levels of permanence and transaction recurrence, which means investment capacity, growth vision, and articulation with strategic stakeholders in the sector. Sales recurrence identified patterns of operational continuity that demonstrate favorable conditions for the consolidation entrepreneurial producers; for example, their relationships within the value network. In contrast, subsidized producers exhibit more irregular participation, associated with financial limitations and weaker institutional ties.

The value network analysis highlighted COMESA as an integrating agent, due to facilitating links among producers, financing agencies, marketing companies, and research institutions. Articulation is key to strengthening technical and organizational capacities, especially in contexts where technical support is limited. Results offer valuable elements for the design of differentiated support, financing, and technological innovation strategies aimed at strengthening the permanence of producers in protected agriculture systems. Understanding the producer profile and their relational dynamics is a strategic component for public policies formulation and business decision-making in this sector.

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Bioconversion of poultry waste as growth enhancers and nutritional content in sugarcane

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ABSTRACT

Objective: Different doses of compost made from poultry residues and filter cake from sugarcane juice were evaluated, combined with mineral fertilizer, to determine the nutritional status and growth in sugar cane.

Design/methodology/approach: The agricultural yield of sugarcane and the concentration of minerals were studied through foliar analysis; the nitrogen content was determined through the micro-Kjeldahl method, while phosphorus, potassium, calcium, magnesium, iron, copper, zinc, manganese, and boron were quantified through inductively coupled plasma optical emission spectrophotometry and wet digestion spectrophotometry.

Results: The results show that the application of compost made from chicken manure presented the highest yield (120 t ha^{-1}), number of stems, and content of macronutrients, while nutrition with chemical fertilizers provided the highest content of micronutrients in sugarcane plants.

Findings/conclusions: This study has shown that the use of poultry waste compost favors the morphological development and nutritional content of the sugarcane crop.

Keywords: Compost, poultry sector, *Saccharum*.

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INTRODUCTION

The increase in anthropogenic activities has resulted in the generation of various residues of animal and plant origin that have an impact on the quality of the environment (Carnicer *et al.*, 2018). The poultry sector has increased the amount of organic solid waste associated with the formation of lixiviates that carry toxic substances and contaminate water sources and soil, causing eutrophication and degradation, and releasing greenhouse gases (Fernández-Nieto and Betancourt-González, 2019; Asses *et al.*, 2019).

Poultry production is the fastest growing sector in history (Molaey *et al.*, 2018). According to data from the United States Department of Agriculture (USDA), production in 2023 was 103.5 millions of t of chicken meat worldwide (FIRA, 2024). Furthermore, in Mexico

the poultry industry continues to be the most dynamic livestock activity, with a production of 3,9 millions of t of chicken meat and a growth of 2.7% compared to 2022 (3,781,735.284) (SIAP, 2024).

Poultry waste is a source of essential nutrients, nitrogen (N), phosphorus (P), potassium (K), for agricultural production (Thomas *et al.*, 2020; Wan *et al.*, 2020); however, if these are applied directly to the soil without previous treatment, they can bring with them pathogenic microorganisms, antibiotics, and compounds that interact with the endocrine system (steroidal hormones, phytoestrogens, pesticides and herbicides) which can be transferred to the crop, livestock, and the food chain with health risks. The direct application of manure to the ground can reach the water table (Pinos-Rodríguez *et al.*, 2012).

Considering the composting is a degradation process that can transform the organic substrates of agricultural residues into stabilized organic matter, which can inactivate the pathogenic microorganisms present and in thus, the nutrients will be more available to be absorbed by the crops (Fuquene and Yate, 2017; Freitag *et al.*, 2018). Bayrakdar *et al.* (2017) and Wang *et al.* (2017) mention that poultry waste generates environmental damage since they are considered sources of infection and are vectors of diseases that can spread through the soil, water, or air, which also causes a risk to nearby areas, as well as high emission of toxic gases to the atmosphere.

On the other hand, the cultivation of sugarcane is one of the most important resources in the world since it constitutes the main raw material to produce sugar. Consequently, worldwide in 2024, around a production of 183 million t of sugar cane and an agricultural yield of 72.6 t ha^{-1} (STATISTA, 2024). In Mexico, the sugar agribusiness is of great commercial importance and is the sixth largest sugar producer worldwide, having 50 active sugar mills distributed throughout the territory, which together represented a total of 5.3 4.7 million t of sugar and a cultivated area of 783 741 thousand hectares, of which 98% of the production is handled by small producers, obtaining a total yield of 63.4 t ha^{-1} (CONADESUCA, 2024).

However, the low productivity of the sugarcane crop is also due to the immoderate use of fertilizers, the extraction of nutrients by the stems, the burning of harvest residues, and the compaction from the use of heavy machinery (Araujo *et al.*, 2016). According to Rahmad *et al.* (2019), chemical fertilization with N, P and K is no longer able to maintain the optimal production of the sugarcane crop, since their intensive use reduces soil fertility and its capacity to provide essential nutrients is not sufficient (Shulka *et al.*, 2016). Nevertheless, to produce sugar cane, residues from the sugar industry have been applied; the so-called cachaza or filter cake, which is obtained as a result of the clarification process of the cane juices, is applied as organic fertilization for sugarcane production contributing to improve the soil and the environment (Velasco-Velasco, 2014). From this by-product of sugarcane, 30 to 50 kg per t of processed sugarcane is obtained (Salgado *et al.*, 2003).

However, with the application of organic fertilizer, greater benefits can be obtained in the development of plants compared to chemical fertilization in some growth and nutrition parameters, which also depends on the quality of the organic fertilizer (Bernui *et al.*, 2016; Maradiaga-Rodriguez *et al.*, 2019). The nutrition of the sugarcane crop is important because it requires thirteen essential elements for its production; six macronutrients where N, P, and K are called primary, calcium (Ca), magnesium Mg), and sulfur (S) secondary, and seven others, including boron (Bo), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), molybdenum (Mo), and chlorine (Cl), which correspond to microelements (Kingston, 2014). Therefore, it is of utmost importance to implement and develop sustainable nutritional programs, as well as to improve productivity and profitability for the producer, without neglecting the quality of the raw material and the improvement of soil conditions (SIAP, 2018). Thus, the objective of this work was to evaluate different doses of organic fertilizers obtained from poultry waste and filter cake on the development and yield of the sugarcane crop.

MATERIAL AND METHODS

Study area and location. The study was carried out in the supply area of Ingenio Central El Potrero, S. A. de C. V. The unit is located 20 km. from the City of Cordoba, Veracruz, Mexico. The agricultural field is located at 520 masl, 18° 53' 37.2" north (N) and 96° 47' 07.6" west (W). Its climate is warm subhumid with rains in summer (40%), with a minimum temperature of 16.8 °C and a maximum of 43.0 °C, its average annual rainfall ranges from 1400 to 1600 mm (SMN 2023).

Plant material. The CP 72-2086 sugarcane variety was used.

Composting material. The materials used to make the compost were: chicken manure with rice husk used as bedding for the birds from the "La Primavera 1" farm, located in Cuitlahuac, Veracruz, Mexico; incubator residues which contained empty shells, unhatched eggs, and dead chicks from the incubation plant located in Cordoba, Veracruz, Mexico, which belongs to Grupo Pecuario San Antonio SA de C. V; and finally filter cake from the storage unit of the Ingenio Central El Potrero sugar mill in the state of Veracruz, Mexico.

Composting. Composting was carried out for nine months following the methodology described by Román *et al.* (2013), at the Colegio de Postgraduados, Campus Cordoba, located at 650 masl, 18° 50' north (N) and 96° 51' west (W). From the selected organic residues, three mixtures were prepared; (T1) chicken manure, (T2) chicken manure and filter cake 1:1 (W/V), and (T3) incubator residues with filter cake 3:1 (W/V). One t piles were made, measuring 1 m high, 1 m wide and 2 m long; they were kept covered with transparent plastic, airing was done by hand turning every eight days. The temperature was measured every third day with a Traceable Model 4371 Digital Thermometer, Control Products, USA. The pH was verified by the method of Jackson (1979) every eight days. Water was added every eight days and humidity was measured through gravimetry taking 25 g samples; they were dried in a model 3488M Imperial V oven (Lib-line, IL, United States) at 70 °C for 72 hours.

Chemical analysis of composts. The N concentration was carried out by the micro-Kjeldahl method (Bremner, 1965); P, K, Ca, Mg, and Na were analyzed following the methodology described by Alcántar and Sandoval (1999), by means of inductively coupled plasma optical emission spectrophotometry, in a Model 725 ICP-OES equipment (Agilent; Mulgrave, Australia).

Evaluation morphological development. These variables were quantified following the methodology described by the IMPA (1983). At 9 months of age of the crop, 5 points were marked in a quincunx pattern, each 2 linear meters in every treatment and replicate; 10 stems were randomly selected for each point and the height and diameter of stems were recorded. For the number of stems, all the milling stems present at each point were counted.

Agricultural yield. At the time of harvest, rolls of 20 milling stems were formed from the four central rows and leaving two rows from the edge standing. Later, each roll per treatment was weighed and the yield was calculated.

Leaf mineral analysis. The concentration of N in leaf was performed by the micro-Kjeldahl method (Bremner, 1965) and the nutrients P, K, Ca, Mg, Fe, Cu, Zn, Mn, and B were quantified inductively coupled plasma optical emission spectrophotometry in an ICP-OES model 725 equipment (Agilent; Mulgrave, Australia), following the methodology described by Alcántar and Sandoval (1999).

Experimental design. Fourteen treatments were carried out with application of 4, 6, and 8 t ha^{-1} of compost, alone or in combination with mineral fertilizer, and an absolute control (Table 1). A completely randomized block design was used, with four replicates.

Table 1. Fertilization treatments, with composts, in template cycle of the CP 72-2086 variety of sugar cane cultivated for nine months.

Treatment	Dose
0	Absolute control
1	8 t ha^{-1} chicken manure compost
2	6 t ha^{-1} chicken manure compost
3	8 t ha^{-1} chicken manure compost and filter cake 1:1 (W/V)
4	6 t ha^{-1} chicken manure compost and filter cake 1:1 (W/V)
5	8 t ha^{-1} incubator residues compost and filter cake 3:1 (W/V)
6	6 t ha^{-1} incubator residues compost and filter cake 3:1 (W/V)
7	Mineral fertilizer by recommended dose (400 kg ha^{-1} of 20-10-10 and 20-00-30 during the first and second applications, each).
8	Mineral fertilizer by soil analysis (400 kg ha^{-1} of 92-120-2 and 46-60-2 during the first and second applications, each).
9	4 t ha^{-1} chicken manure compost+200 kg ha^{-1} mineral fertilizer 20-10-10 and 20-00-30 during the first and second application, each.
10	4 t ha^{-1} chicken manure compost and filter cake+200 kg ha^{-1} mineral fertilizer 20-10-10 and 20-00-30 during the first and second application, each.
11	4 t ha^{-1} incubator residues compost and filter cake+200 kg ha^{-1} mineral fertilizer 20-10-10 and 20-00-30 during the first and second application, each.
12	4 t ha^{-1} chicken manure compost+200 kg ha^{-1} mineral fertilizer 92-120-2 and 46-60-2 during the first and second application, each.
13	4 t ha^{-1} chicken manure compost and filter cake+200 kg ha^{-1} mineral fertilizer 92-120-2 and 46-60-2 during the first and second application, each.
14	4 t ha^{-1} incubator residues compost and filter cake+200 kg ha^{-1} mineral fertilizer 92-120-2 and 46-60-2 during the first and second application, each.

W/V (weight/volume), kg ha^{-1} (kilogram per hectare).

Statistical analysis. An analysis of variance (ANOVA) and Tukey means comparison tests (p≤0.05) were done with the SPSS V. 25 statistical software.

RESULTS AND DISCUSSION

Chemical composition of composts

Table 2 shows that there were statistically significant differences between the treatments from the effect of the organic residue and chemical fertilizer factors on the concentration of macronutrients. Composts made from chicken manure have the highest concentration value. Thus, the highest contents of N, K, Ca, and Na are found in the compost made from chicken manure, while the compost resulting from chicken manure and filter cake had the highest P and Mg contents.

The results of the present study showed that the chemical composition of the composts in treatments T1 (100% chicken manure) exhibited the highest content of N (4.30 g kg^{-1}), K (9.17 g kg^{-1}), and Na (3.93 g kg^{-1}), while T2 (poultry manure compost with the addition of 50% of sugar cane residues) reported the highest concentrations of P (12.49 g kg^{-1}) and Mg (10.81 g kg^{-1}). The higher N content in the chicken manure compost is likely due to the fact that chicken manure has a higher nitrogen content than other livestock manures (Zhu *et al.*, 2019). According to observations by Li *et al.* (2022), chicken manure compost has a higher nitrogen conversion because the characteristic microorganisms present in chicken manure are responsible for decomposing organic matter and fixing nitrogen during the composting stages. The higher P concentration observed in T2 may be due to the fact that filter cake has a higher P content than chicken manure (Suhartini *et al.*, 2020). Gonçalves *et al.* (2021) and Wongkoon *et al.* (2014) found that filter cake compost contains approximately 2.25 and 3.9 g kg^{-1} of P; therefore, the combination of filter cake with chicken manure likely increased the P concentration in T2.

Haroon *et al.* (2018) obtained high values of N, P, Ca, and Mg in chicken manure and cypress residues compost. This indicates that the chicken manure compost is rich in nutrients and their concentration increases as the degradation time increases; this is attributed to the effect of nutrient concentration due to losses from aerobic volatilization. Accordingly, stabilized poultry residues can be used in agriculture due to their high content of N, P, K, and beneficial microorganisms present (Hernández-Rodríguez *et al.*, 2013; Wan *et al.*, 2020).

Table 2. Effect of the organic residue factors on the concentration of macronutrients in compost.

Compost	N	P	K	Ca	Mg
	g kg^{-1}				
T1	4.30±0.08a	11.99±0.03b	9.17±0.10a	4.51±0.25a	9.24±0.34b
T2	2.22±0.12b	12.49±0.09a	8.58±0.20b	4.40±0.16a	10.81±0.15a
T3	3.80±0.17a	0.71±0.02c	2.94±0.02c	1.79±0.10b	4.35±0.17c

Means ± DE whit different letters in each column indicate significant statistical differences (P≤0.05). g kg^{-1} (gram per kilogram).

Morphological development

These variables showed a different behavior due to the interaction of climatic factors, mainly rainfall, presenting a dry period from January to May with an average of 20 mm, and subsequently from June to November the rains averaged 200 mm. The highest number of sugarcane stems (13.58±0.03) was obtained in treatment 1, made up of chicken manure (8 t ha^{-1}), while treatment 12, comprised of 4 t ha^{-1} of chicken manure compost + 200 kg ha^{-1} of mineral fertilizer (92-120-2 and 46-60-2), showed the greatest height and diameter of stems. Finally, the highest yield (120.79±0.23) was obtained with treatment 2, made up of 6 t ha^{-1} of chicken manure compost (Table 3).

In T1, the highest number of shoots is observed, probably because chicken manure compost contains a high N content, one of whose functions is structural (Cárdenas-Navarro *et al.*, 2004); an example of this is that Rengel *et al.* (2011) observed that during tillering, sugarcane plants increased N absorption, obtaining 24.9% higher stalk production. Another function of N is related to the crop yield of sugarcane in the field; field yield is observed in T2 (Sosa *et al.*, 2015). Treatment T2 is composed of composted chicken manure, which showed the highest N concentration, coinciding with Salgado-García *et al.* (2017), where sugarcane cultivars increased their yields as the N dose increased. The application of P to the sugarcane crop causes an increase in its growth (Patil *et al.*, 2020).

T3 consists of chicken manure and filter cake compost, resulting in a higher Mg content that influences the growth of sugarcane plants, because Mg is an element that forms part of molecules and has a structural function in plants; one of the main functions of Mg is as a constituent element of chlorophyll (Alcántar-González and Trejo-Téllez, 2007).

Table 3. Effect of organic residue factor on the morphological development of sugar cane cultivated for nine months and yield at harvest time.

Treatment	Stems (number)	Height (m)	Diameter (cm)	Yield (t ha^{-1})
0	10.22±0.02e	1.70±0.03h	2.31±0.03i	87.58±0.11i
1	13.58±0.03a	1.85±0.03ef	3.10±0.03c	93.86±0.13g
2	12.22±0.06b	1.90±0.005de	2.62±0.02gh	120.79±0.23a
3	12.08±0.08b	2.02±0.01a	3.04±0.02c	110.07±0.06c
4	11.42±0.02c	1.96±0.05bc	2.77±0.01f	103.03±0.20e
5	12.33±0.05b	1.92±0.03cd	2.91±0.01de	107.20±0.48d
6	11.51±0.01c	1.73±0.08h	2.95±0.05d	96.92±0.48f
7	10.84±0.08d	1.96±0.03bc	2.57±0.03h	107.03±0.29d
8	12.30±0.20b	1.97±0.05bc	2.61±0.01gh	89.97±0.19h
9	11.30±0.02c	1.83±0.05fg	2.85±0.03ef	103.25±0.12e
10	11.61±0.01c	1.95±0.05bc	2.77±0.05f	106.33±0.11d
11	12.33±0.08b	1.98±0.05b	2.66±0.08g	107.16±0.19d
12	11.66±0.03c	2.02±0.02a	3.42±0.01a	112.41±0.11b
13	10.46±0.03e	1.80±0.03g	2.55±0.03h	89.93±0.06h
14	11.46±0.03c	1.97±0.00bc	3.32±0.03b	107.07±0.48d

Means ± DE with different letters in each column indicate significant statistical differences ($P \leq 0.05$). m (meter), cm (centimeter), kg ha^{-1} (kilogram per hectare).

During the rapid growth development of the sugar cane crop, the period between 3 to 12 months of age (SADER, 2019), a positive effect was seen in the height variable in treatment T12 (chicken manure and fertilizer) with a height of (2.02±0.02 cm). This is probably due to the fact that chicken manure compost is rich in P, but it is highly soluble and leachable during application to the soil; however, if added with mineral fertilizers, the nutrient content increases and stabilizes in the soil, resulting in a balance of macro- and micronutrients and organic matter, which increases plant development (Mažeika *et al.*, 2021).

This coincides with the report by Nawaz *et al.* (2017), who mention that the application of compost and mineral fertilizer positively affects the growth of sugarcane. For their part, González *et al.* (2018) point out that stem height is an important growth parameter for sugarcane production since it depends on optimal edaphic conditions to estimate growth and development. Regarding stem diameter (3.32±0.03 cm) in treatment 14 (incubator residues and filter cake), it is considered a normal parameter, when compared with studies carried out by Unigarro *et al.* (2013), who report results with a diameter value between 2.75 and 2.97 m, which indicates adequate results for this variable. It should be noted that all treatments were superior to the control (without any treatment) presenting a diameter of 2.31±0.03. In this context, the number of stems is largely determined by the content of minerals present in the soil, being treatment 1 (chicken manure compost at 8 t ha^{-1}) the one thatpresented the highest number of stems (13.58±0.03). These results coincide with those mentioned by Rahmad *et al.* (2019), who say that when applying compost, nutrients necessary for growing sugarcane are added.

Macronutrients concentration in leaves

The results shown in Table 4 indicate a significant difference between treatments, with treatments 3 and 14 showing high N content, treatment 8 with P; however, treatment 1 had the highest concentrations of K, while treatment 7 had the highest concentrations of Ca, and Mg.

Regarding agricultural performance, the application of chicken manure compost showed the highest value, which is related to that reported by Liu *et al.* (2018), who mention that the application of compost increases productivity and improves soil conditions for the growth of sugarcane. Specifically, it coincides with what was stated by Nawaz *et al.* (2017) where they mention that a balanced fertilization based on compost or chemical fertilizer, not only guarantees the production of crops, but also provides greater benefits to producers, so compost application is the best option to counteract the effect of nutrient losses due to volatilization into the environment.

In any case, if the manure is applied to the soil, less than 10% of the N content is present as nitrate and ammonia (inorganic form), not in an organic form, which must be mineralized to be available for plants (Espinoza *et al.*, 2018). Another impact is on the quality of water, air, and soil as a result of the nutrient content (Chen *et al.*, 2018; Xiao *et al.*, 2019), affecting acidification, eutrophication, and ozone depletion, as mentioned by Skunca *et al.* (2015). Moreover, manure contains bacteria that can be spread to agricultural

Table 4. Effect of organic residue factor on macronutrients concentration in leaves of sugarcane cultivated over nine months.

Treatment	N	P	K	Ca	Mg
	g kg^{-1}				
0	1.71±0.019g	0.27±0.003ef	1.04±0.011h	0.25±0.002f	0.11±0.001f
1	2.21±0.024a	0.29±0.003ab	1.23±0.013a	0.32±0.003b	0.12±0.001cd
2	1.91±0.021d	0.27±0.003cd	1.12±0.012ef	0.32±0.003b	0.13±0.001c
3	2.25±0.025a	0.29±0.003b	1.17±0.013bc	0.27±0.003e	0.12±0.001de
4	1.96±0.021c	0.26±0.003fg	1.09±0.012g	0.26±0.003e	0.11±0.001fg
5	1.78±0.019ef	0.27±0.003def	1.22±0.013a	0.29±0.003d	0.11±0.001g
6	2.01±0.022b	0.27±0.003ef	1.18±0.013b	0.24±0.002g	0.11±0.001f
7	2.23±0.024a	0.28±0.003c	1.07±0.012g	0.33±0.003a	0.14±0.001a
8	2.00±0.022bc	0.30±0.003a	1.08±0.012g	0.25±0.002f	0.12±0.001de
9	1.63±0.018h	0.26±0.003fg	1.07±0.012g	0.24±0.002g	0.10±0.001e
10	1.49±0.016i	0.27±0.003cde	1.09±0.012fg	0.27±0.003e	0.10±0.001e
11	1.82±0.020e	0.27±0.003def	1.15±0.012de	0.29±0.003d	0.11±0.001g
12	1.77±0.019f	0.25±0.002h	0.99±0.011i	0.27±0.003e	0.13±0.001c
13	1.64±0.018h	0.25±0.002i	1.15±0.012cd	0.23±0.002g	0.12±0.001e
14	2.25±0.025a	0.26±0.002gh	1.01±0.011i	0.30±0.003c	0.13±0.001b

Means ± DE with different letters in each column indicate significant statistical differences ($P \leq 0.05$). g kg^{-1} (gram per kilogram).

soils with its application as an organic fertilizer (Li *et al.*, 2020; Fang *et al.*, 2018; Zhang *et al.*, 2020). Because of this, manure is considered an important source of pathogens (Neill *et al.*, 2018), and hence should not be applied to agricultural soils.

Micronutrients concentration in leaves

In the Regarding the content of micronutrients, in Table 5, the highest concentrations of Fe (88.5 mg kg^{-1}) and Mn (67.23 mg kg^{-1}) were observed in treatment 7, and Cu (5.0 mg kg^{-1}) in treatment 14. However, Zn (13.33 mg kg^{-1}) stood out in treatment 3. Finally, the highest concentration of B (8.41 mg kg^{-1}) was that in treatment 1. This showed significant differences between treatments.

Finally, a foliar analysis does not provide information for a given nutrient, but it does show the deficiencies to modify the fertilization program. Therefore, the values obtained in this study showed that mineral fertilization resulted in higher mineral content in foliar tissue. This could be due to the availability of nutrients and mineralization in the soil. Thus, these results are similar to those reported by Rangel *et al.* (2011), finding Fe, Mn, and B in greater quantity in leaf tissue and Cu and Zn deficiencies. For their part, Pérez *et al.* (2015) mention that the plant decreases nutrient absorption at 12 months of age due to the fact that it enters the maturation process, vegetative growth becoming slow. It is worth mentioning that minerals are elements with essential physiological functions for the metabolism of plants (Marín-Garza *et al.* 2018).

Table 5. Effect of organic residue factor on the concentration of micronutrients in leaves of sugarcane cultivated over nine months.

Treatment	Fe	Cu	Zn	Mn	B
	mg kg^{-1}				
0	71.9±0.80h	1.37±0.015e	11.4±0.12b	46.29±0.51f	5.01±0.056j
1	84.7±0.94bc	1.37±0.015e	11.6±0.13b	50.80±0.56c	8.41±0.094a
2	77.4±0.86fg	1.25±0.014g	11.47±0.12b	47.01±0.52ef	5.57±0.062g
3	86.3±0.96b	1.44±0.016d	13.33±0.14a	44.08±0.49g	5.51±0.061gh
4	69.7±0.77i	1.25±0.014g	10.97±0.12cde	46.85±0.52ef	7.32±0.081d
5	82.2±0.91d	1.50±0.016c	10.42±0.11f	47.48±0.53e	6.87±0.076e
6	79.5±0.88e	1.50±0.015c	10.91±0.12cde	46.29±0.51f	7.66±0.085c
7	88.5±0.99a	1.18±0.013h	10.79±0.12de	67.23±0.75a	7.94±0.088b
8	83.1±0.92cd	1.75±0.019b	10.91±0.12cde	52.85±0.59b	6.75±0.075e
9	68.2±0.76ij	1.44±0.016d	9.92±0.11g	41.00±0.45g	5.35±0.059hi
10	78.1±0.87ef	1.31±0.014f	11.04±0.12cd	44.87±0.50g	7.33±0.082d
11	75.5±0.84g	1.19±0.013h	10.73±0.12e	34.36±0.38i	7.68±0.085c
12	69.2±0.77i	1.31±0.014f	11.10±0.12c	48.74±0.54d	5.98±0.066f
13	66.4±0.74j	1.31±0.014f	10.91±0.12cde	34.44±0.38i	5.29±0.059i
14	83.2±0.93cd	5.00±0.055a	11.72±0.13b	47.56±0.53de	6.02±0.067f

Means ± DE with different letters in each column indicate significant statistical differences ($P \leq 0.05$). mg kg^{-1} (milligram per kilogram).

CONCLUSIONS

The application of compost made from poultry residues, such as chicken manure, had a positive effect on the morphological development of the sugarcane crop, while chemical fertilization based on the result of soil analysis stimulated the concentration of nutrients in plants. So, it is recommended to apply compost from poultry residues, alone or in combination with filter cake, to increase the agricultural production of crops. It was also shown that the final compost had a high level of maturity and was not phytotoxic for the crop.

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AGRO PRODUCTIVIDAD

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Canonical correlation analysis to identify the relationship of *in vivo* body measurements of rabbit (*Oryctolagus cuniculus* L.) with the carcass

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ABSTRACT

Objective: to determine the interrelations between morphological characteristics in live rabbits and carcasses, mainly loin and legs which are pieces of greater economic value in the carcass.

Design/ Methodology/ Approach: this study used the methodology of multivariate Canonical Correlation Analysis (CCA). Information was used from 139 rabbits of the New Zealand and California breeds, male and female, weaned at 35 days of age and carried in a fattening process up to 73 days. Morphological measurements were taken before and after slaughter to integrate a morphological traits database, which was divided into two subsets. First, Set X with the variables to be estimated (dependent) included those characteristics of the carcass before being chilled, *i.e.* non-chilled carcass weight (PCC), carcass loin weight (PLC), carcass leg weight (PPC), carcass loin width (ALC), carcass loin length (LLC), carcass leg length (LPC). The second, Set Y the predictor variables (independent) included the live measurements of body parts, live weight (PV), loin width (AL), loin length (LL), leg circumference (PP) and leg length (LP). No differences ($p>0.05$) were found between races or sexes, so database was analyzed in a general way.

Results: CCA results showed a strong association between the morphological characteristics measured in the live animal and those measured in the butchered carcass (CANAL, $rc=0.85$), which proved the CCA as relevant. Live weight (PV) and leg circumference (PP) were the factors most related to the important characteristics of the carcass (loin and legs).

Limitations/ Implications of the study: breeding stock selection is traditionally based on the visual evaluation of phenotypic characteristics, which is considered as subjective, thus limiting genetic progress. The proposal in this study was to consider an indirect selection, evaluating characteristics of the live animal that highly correlate with the characteristics of greater economic value of the carcass.

Findings/ Conclusions: indirect selection in rabbits can be an effective strategy to select future breeders, since animals above the average are obtained in the farm.

Keywords: canonical correlation analysis, rabbits, body measurements.

INTRODUCTION

Rabbit production in Mexico is concentrated in small farms located in rural and suburban areas around cities. These production units seek to obtain rabbit meat, a food of high nutritional value, low in fat and cholesterol (Villanueva-Díaz *et al.*, 2023), which can contribute to improve the diet of Mexican families. However, despite its benefits, the consumption of rabbit meat in Mexico is low, since it is limited to certain segments of the population; who eat it on special occasions, also as an exotic dish offered to national tourism on weekends.

There are around 10 000 rabbit production units in Mexico, with an annual *per capita* consumption of only 100 grams (SADER-SENASICA, 2019). This situation is partly due to the limited incorporation of rabbit meat into Mexican gastronomy, because it is perceived as an exotic product. Rabbit production in Mexico is semi-intensive, using breeds such as New Zealand, California and Chinchilla. Breeding stock selection is traditionally based on the visual evaluation of phenotypic characteristics, which can be subjective and limit genetic progress (Trocino *et al.*, 2019). Ideally, indirect selection should be considered, in order to evaluate characteristics of live animals that correlate with those of the carcass, such as yield of legs, thighs, and loin, which are pieces highly valued by consumers (Blasco *et al.*, 2018).

This practice implies the killing of the animal, which requires optimization for its initial application. Canonical correlation analysis (CCA) is a multivariate statistical technique that allows the identification and quantification of the relationships between two sets of variables. The CCA reduces the dimensionality of the data and makes it easier to interpret results. Previous research has shown that CCA is a powerful tool to identify relations between two homogeneous groups of variables in a dataset, based on pairs of linear combinations of these groups and the estimation of the variability of the data (Montes-Vergara *et al.*, 2020).

The procedure consists of dividing the set of variables into two subsets, the first consisting of p variables, denoted by the matrix X, of order (n, p); and the second by the q explanatory variables denoted as matrix Y, of order (n, q). The objective of the CCA is to analyze relations between multiple variables in order to find two linear functions. One of the X-variables, $V_1 = b_1X_1 + b_2X_2 + ... + b_LX_L$ and another of the Y-variables, $W_1 = a_1Y_1 + a_2Y_2 + ... + a_kY_k$ in such a way that the correlation between W and V is maximum. These linear combinations of functions (V, W) are called canonical variables, and the correlations between the corresponding pairs of canonical variables are called canonical correlations.

Studies that apply CCA in rabbit farming are scarce. This multivariate technique has proven to be highly effective because it surpasses conventional univariate approaches, allowing deeper and more precise analyses of the relationships between sets of variables (Atac & Altincekic, 2023). Therefore, the objective was to use the CCA technique to identify the interrelation between morphological characteristics of 73-day-old live rabbits and some carcass measurements, in order to determine which characteristics can be used as criteria for indirect selection of superior breeding stock.

MATERIALS AND METHODS

This research was implemented at the Experimental Rabbit Farm of Colegio de Postgraduados, Campus Montecillo, Texcoco, State of Mexico, in accordance with the regulations for the use and care of animals destined for research at Colegio de Postgraduados. The information was obtained from measurements made in 139 rabbits (78 of New Zealand breed, and 61 of California breed, 51% males and 49% females); 35 days-old (weaning) to 73 days-old (fattening), with an average initial weight of 754.5 g, and 2115 g at the end. Measurements were considered in live rabbits that included live weight (PV), loin width (AL), loin length (LL), leg circumference (PP), and leg length (LP). One day later, those animals were desensitized, slaughtered, and the parts cut from the dressed carcass were measured as, non-chilled carcass weight (PCC), carcass loin weight (PLC), carcass leg weight (PPC), carcass loin width (ALC), carcass loin length (LLC), carcass leg length (LPC).

Canonical Correlation Analysis (CCA)

The CCA was applied to know the relationship between two sets of variables. The first group of prediction or comparison variables, constituted by "p" (V=CANAL), denoted by the X-matrix of order (139 observations * 6 variables), which are non-chilled carcass weight (PCC), carcass loin weight (PLC), carcass leg weight (PPC), carcass loin width (ALC), carcass loin length (CLL), and carcass leg length (LPC).

The second set of "q" criteria variables (W=ANIMAL), denoted by the Y-matrix, of order (139 observations * 5 variables) which are, live weight (PV), loin width (AL), loin length (LL), leg circumference (PP) and leg length (LP), seeking to maximize the correlation between both canonical variables (V and W). Likewise, in linear combinations $V_i = a_1X_1 + a_2X_2 + ... + a_kX_k = X_{ai}$ and $W_1 = b_1Y_1 + b_2Y_2 + ... + b_LY_L = Y_{bi}$. The *ai* and *bi* are standardized canonical coefficients that can be used to determine which variables are redundant and to interpret the canonical variables. These coefficients indicate the relative importance of the set of variables measured in the carcass, and determine the value of the set of variables of the animal. On the other hand, coefficients can be unstable due to the presence of multicollinearity in the data (Mihalik *et al.*, 2022). For this reason, canonical weighing (canonical loadings) provided the importance of each canonical variable.

The correlations between Vq and Wp are called canonical correlations. The square root of canonical correlations (canonical roots or eigenvalues) represent the amount of variance in a canonical variable caused by another canonical variable. To know the canonical coefficients (b1, b2, b3, ... b6 & a1, a2, a3, ... a5), which maximize linear combinations the CCA was implemented, in which the following set of hypotheses was tested:

$$H_0 = p1 = p2 = p3 = p4 = p5 = 0$$

$$H_a = p1 = p2 = p3 = p4 = p5 \neq 0$$

In order to do this, the Wilks' Lambda statistic was used, which showed that under the null hypothesis (H0) the sets Vq and Wp were not linearly related. That is, in the case of this research, the set of variables measured in the carcass and the set measured in live animals were not linearly related.

$\Lambda m = \prod_{i=1}^{m} (1 - \lambda i)$ Wilk's Lambda equation;

where, Λm: canonical correlation coefficient; λi: quadratic canonical correlation; m: number of canonical correlations.

The significance of the canonical correlation coefficient can be tested using the Chi-square test shown below:

$$\chi^2 = -\left(N - 1\left(\frac{Kx + Ky + 1}{2}\right)\right) Ln\Lambda m$$

where, N: number of cases; Λm: canonical correlation coefficient; Kx: number of variables in set X; Ky: number of variables in the set Y.

The sets of V and W of canonical variables were estimated

$$CANAL = V = ZxBx \qquad ANIMAL = W = ZyBy$$

where Zx and Zy are standardized values and Bx, By are canonical coefficients.

The correlation matrix between variables was calculated using the weighted matrix and correlation coefficient.

$$Ax = RxxBx \qquad Ay = RyyBy$$

$$Pv_{xc} = \sum_{i=1}^{Kx} \frac{a^2 ixc}{kx} \qquad Pw_{yc} = \sum_{i=1}^{Ky} \frac{a^2 iyc}{ky}$$

where Pv: the proportion of the variance obtained from a set of variables through a canonical variable of the set, a^2: the square of the correlation and the number of variables in the set, and rd: $redundancy = \left[PV\left(r^2 c\right)\right]$.

Statistical analyses

Descriptive statistics were obtained for all the variables measured using PROC MEANS (SAS Institute Inc., 2013). An analysis of variance was performed using a fixed-effect model and two classification criteria with interaction to know the influence of race and sex on the criteria variables. Non-significant differences ($p>0.05$) were found between them, so a single dataset was considered.

The PROC CANCORR procedure was applied to know the relationship between the sets of variables V and W, in this study they represent the association between morphological characteristics of the carcasses and the live animals. Large canonical correlations do not always mean that there is a high correlation between the two sets because the CCA does not maximize the amount of variance due to one or more variables in one set for the other set of variables (Badii & Castillo, 2007). Therefore, it is suggested to calculate the redundancy measures for each canonical correlation in order to determine how much of the variance in one set of variables is attributed to the other set.

RESULTS AND DISCUSSION

The descriptive statistics of the morphological and carcass characteristics are presented in Table 1, to provide information for each morphological variable such as, average PV 2115.21g ± 229.21, an average PCC 1185 g ± 165.26 and a carcass yield 56.12% ± 5.73. Carcass yield values were similar to previous studies (Montes-Vergara *et al.*, 2020; Luis-Chincoya *et al.*, 2021). The economically important measurements of the non-chilled carcass PLC (258 g), PPC (349 g) and PCC were notable. These measures varied greatly (12% to 19%). Regarding the measurements in the live animal, those presented a minor variation, similar and homogeneous among them.

Pearson correlations between morphological characteristics of rabbits

Table 2 presents the correlations between the original variables. Regarding the correlations evaluated with variables in the live ANIMAL, those relevant were, PV that was correlated with loin width (AL, r=0.34) and loin length (LL, r=0.38); leg circumference (PP, r=0.31) and leg length (LP, r=0.46). While loin length (LL) was correlated with leg circumference (PP, r=0.48) and leg length (LP, r=0.60).

That is, to the extent that a rabbit has greater measurements in legs and loin, its live weight is greater. This coincides with what was reported by Montes-Vergara *et al.* (2020), where the weight of the non-chilled carcass increased as the measurements in the live animal were greater. In this way, body measurements in the live animal can be a way to predict the weight of the carcass, because both characteristics are closely related. In other zootechnical species (cattle), it is reported that live weight has a high correlation with height

Table 1. Descriptive statistics of the two sets of rabbit variables at the end of fattening.

Carcass	X Variables (Mean±SD)	CV (%)	Animal	Y Variables (Mean±SD)	CV (%)
PCC	1185.3±165.2	13.9	PV	2115.21±229.21	10.8
PLC	258.3±49.1	19	AL	60.14±6.55	10.9
PPC	349.8±41.7	11.9	LL	18.20±2.32	12.7
ALC	56.1±5.4	9.7	PP	13.78±1.87	13.6
LLC	15.3±1.4	9.5	LP	10.13±1.07	10.6
LPC	9.2±0.9	10.4			
PCC	1185.3±165.2	13.9	PV	2115.21±229.21	10.8

SD, standard deviation; CV, coefficient of variation, PCC, non-chilled carcass weight; PLC, carcass loin weight; PPC, carcass leg weight; ALC, carcass loin width; LLC, carcass loin length; LPC, carcass leg length; PV, live weight; AL, loin width; LL, loin length; PP, leg circumference; LP, leg length.

Table 2. Correlation matrix between morphological characteristics in rabbits, measured alive (ANIMAL) or in carcass cuts (CARCASS).

	PV	**AL**	**LL**	**PP**	**LP**	**PCC**	**PLC**	**PPC**	**ALC**	**LLC**	**LPC**
Animal											
PV	1.00	0.34	0.38	0.31	0.46	0.70	0.45	0.77	0.38	0.41	0.37
AL		1.00	0.11	0.36	−0.08	0.34	0.41	0.24	0.12	0.28	0.26
LL			1.00	0.48	0.60	0.34	−0.11	0.23	−0.09	0.28	0.25
PP				1.00	0.28	0.20	0.21	0.34	0.03	0.28	0.18
LP					1.00	0.32	−0.21	0.37	0.23	0.11	0.30
Carcass											
PCC						1.00	0.63	0.65	0.29	0.45	0.29
PLC							1.00	0.40	0.16	0.43	0.18
PPC								1.00	0.39	0.32	0.34
ALC									1.00	−0.02	0.04
LLC										1.00	0.43
LPC											1.00

PV, live weight; AL, loin width; LL, loin length; PP, leg circumference; LP, leg length; PCC, non-chilled carcass weight; PLC, carcass loin weight; PPC, carcass leg weight; ALC, carcass loin width; LLC, carcass loin length; LPC, carcass leg length.

at withers, and rump length, both measurements approximate well the size of the animal (Lakew *et al.*, 2017; Ozen *et al.*, 2021; Atac & Altincekic, 2023; Macena *et al.*, 2024).

Among the carcass variables (CANAL) correlations, the non-chilled carcass weight (PCC) was positive correlated with all the variables of cuts from the dressed carcass, mainly with the carcass loin weight ($r=0.63$), carcass leg weight ($r=0.65$) and carcass loin length ($r=0.45$). This means that the PCC is determined by the economically important characteristics of the carcass (PLC, PPC and LLC), without the need of considering other characteristics of little or null economic importance. This coincides with what was reported by Adamu *et al.* (2016), who mentioned that breed genetic component has an influence on non-chilled carcass weight.

Correlations between canonical variables

Although the characteristics measured in the live animal are important indicators of the measurements made in the carcass, it is difficult to interpret the simultaneous contribution of the variables included in each set to the relationship between them. To explain more clearly the interrelations between the carcass variables and those in the live animal, five canonical correlations were estimated to explain the correlations between the studied sets. Because the number of canonical correlations to be interpreted corresponds to the minimum number of characteristics within the periods evaluated in the live rabbit (ANIMAL) and in those of the carcass (CANAL) (Table 3).

The likelihood ratio test (Lr) showed that the first three canonical coefficients were significant (0.851, 0.562 and 0.414, $p<0.01$), *i.e.* the hypothesis of equality among canonical coefficients ($p<0.0001$) is rejected, which was corroborated by the tests of Wilks', Pillai,

Table 3. Pairs of canonical correlations between two sets of variables (Vi, Wi), eigenvalues, likelihood ratio, and probabilities.

Canonical	CC	ACC	CC^2	DF	Ev	Lr	Pr>F
1) W_1V_1	0.851	0.841	0.724	30	2.633	0.144	<0.0001
2) W_2V_2	0.562	0.525	0.315	20	0.461	0.525	<0.0001
3) W_3V_3	0.414	0.383	0.171	12	0.207	0.766	<0.0005
4) W_4V_4	0.266	0.248	0.070	6	0.071	0.924	0.1145
5) W_5V_5	0.069	0.028	0.005	2	0.005	0.995	0.7310

CC, canonical correlation; ACC, adjusted canonical correlation; CC2, canonical correlation squared; Df, degrees of freedom; Ev, eigenvalues; Lr, likelihood ratio; Pr>F, probability in relation to F.

Hotelling and Roy. This coincides with what was reported by Adamu *et al.* (2016). The first canonical correlation is the one that showed the highest correlation coefficient of the five analyzed, 0.851 (CC^2=0.72) and correlated the variable measured in the carcass (CANAL, V_1) with the canonical live variable (ANIMAL, W_1). The adjusted canonical correlation presented similar information. The other canonical correlations were lower; therefore, only the first canonical variable was considered, which is consistent with other studies (Lakew *et al.*, 2017; Kim *et al.*, 2018; Atac & Altincekic, 2023), that also considered the first canonical variable.

Based on the proposed hypotheses,

$$H_0 = p1 = p2 = p3 = p4 = p5 = 0$$

$$H_a = p1 = p2 = p3 = p4 = p5 \neq 0\,;$$

in which null hypotheses (H_0) is rejected.

In Table 4 we present the standardized canonical coefficients associated with the first pair of canonical variables. Those coefficients are of great importance because the original variables were not measured in the same units. Thus, standardized coefficients should be interpreted instead of the non-standardized coefficients. The correlations given by the matrices of canonical structure should also be examined. This table shows the effects of the carcass set (CANAL) on the variables included in it (Table 4). Therefore, the canonical variants (W_1 and V_1) represent the optimal linear combinations of the dependent and independent variables, which can be defined using the standardized canonical coefficients.

These estimated coefficients can be interpreted by the following equations, in which only the significant coefficients ($p<0.05$) were included.

$$V_1 = CANAL1 = 0.26PCC + 0.23\ PLC + 0.56PPC + 0.13ALC + 0.08LLC + 0.05\ LPC$$

$$W_1 = ANIMAL1 = 1.01\ PV + 0.03\ AL - 0.23\ LL + 0.24\ PP - 0.10LP$$

The first canonical variable, for the carcass (CANAL) variables (V1, V2, V3, V4, V5) shown in Table 4, is a standardized difference with more emphasis on PPC (rc=0.56)

Table 4. Interrelations between standardized canonical coefficients.

X−variables Set						
CARCASS	**PCC**	**PLC**	**PPC**	**ALC**	**LLC**	**LPC**
V1	0.26	0.23	0.56	0.13	0.08	0.05
V2	0.23	−1.1	0.32	0.11	0.17	0.30
V3	−0.50	0.32	0.02	0.81	−0.55	0.25
V4	0.03	0.21	0−.79	0.22	0.16	0.91
V5	−1.35	0.52	0.97	−0.39	0.04	−0.23
Y−variables Set						
ANIMAL	**PV**	**AL**	**LL**	**PP**	**LP**	
W1	1.01	0.03	−0.23	0.24	−0.10	
W2	−0.21	−0.09	0.32	−0.21	0.90	
W3	−0.02	0.18	−1.13	−0.24	0.85	
W4	−0.48	0.43	0.27	−0.48	0.33	
W5	−0.51	0.09	−0.51	1.06	0.39	

PCC, non-chilled carcass weight; PLC, carcass loin weight; PPC, carcass leg weight; ALC, carcass loin width; LLC, carcass loin length; LPC, carcass leg length; PV, live weight; AL, loin width; LL, loin length; PP, leg circumference; LP, leg length.

with PCC (rc=0.26) and PLC (rc=0.23). The coefficients for LLC and LPC were very small and close to zero. Whereas, for the first canonical variable, live variables (ANIMAL) showed a high correlation in live weight and leg circumference, with some negative signs for (−0.03) loin length and leg length (−0.10), but with higher weightings for live weight (PV, rc=1.01) and leg circumference (PP, rc=0.24). Higher coefficients are represented in Figure 1.

According to the equations, the correlations between PPC and PCC and the first canonical variable are positive, which means that as the values of PV and PP increase, also PCC, PLC and PPC increase in the carcass. Variables with the highest canonical weighting contribute to a greater extent to the multivariate relationship between morphological characteristics measured at the end of fattening in the live rabbit. Those will have an impact on the economically important cuts of the dressed carcass.

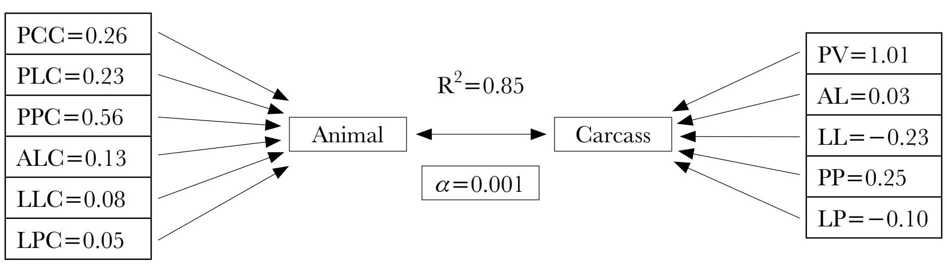

Figure 1. Maximum canonical correlation and standardized canonical coefficients: interrelation between characteristics of the animal and their effects on the carcass. PCC, non-chilled carcass weight; PLC, carcass loin weight; PPC, carcass leg weight; ALC, carcass loin width; LLC, carcass loin length; LPC, carcass leg length; PV, live weight; AL, loin width; LL, loin length; PP, leg circumference; LP, leg length.

In rabbit selection processes, if the PCC of a rabbit is to be predicted with the information of PV and PP, then rabbits with higher PV and PP will produce animals with a higher weight at slaughter age and consequently a higher weight of the cuts from the non-chilled carcass. This information is important to support indirect selection programs, when there are no productive records. The measurement of specific parts of the animal body can help to predict productive behavior, contributing with information for the selection of superior animals. Our results are similar to those reported by Mokoena y Tyasi (2021).

The weightings for carcass characteristics showed that the live weight (PV) of the animal has more influence than other characteristics in the construction of the canonical variable W1. The weightings for the carcass set (CANAL) are mainly influenced by carcass leg weight (PPC), non-chilled carcass weight (PCC) and carcass loin weight (PLC) to form the canonical variable V1 (Table 5).

The weightings of each set of canonical variables in the generation of interrelations (Table 6) for carcass variables (CANAL) are constituted by the non-chilled carcass weight (PCC), where legs weight (PPC) and loin weight (PLC) are outstanding in the canonical variable (V1), these constitute the pieces of highest economic value in the rabbit carcass. While the set of variables W1 is constituted by the live weight (PV) and the leg circumference (PP).

It was found that the canonical variables W1 and V1 presented the proportions of explained variance 66% and 51% respectively, of the total variation in the morphological characteristics measured in rabbits. Their respective values from the redundancy analysis were 31% and 21%.

Table 5. Canonical weightings of the original variables with their opposite canonical variables.

X-variables Set						
CARCASS	**PCC**	**PLC**	**PPC**	**ALC**	**LLC**	**LPC**
V1	0.85	0.66	0.90	0.42	0.49	0.40
V2	−0.07	−0.71	0.26	0.14	0.02	0.36
V3	−0.23	−0.05	0.04	0.75	−0.54	−0.03
V4	0.05	0.21	−0.24	−0.01	0.77	0.07
V5	−0.43	0.03	0.23	−0.31	0.27	−0.60
Y-variables Set						
ANIMAL	**PV**	**AL**	**LL**	**PP**	**LP**	
W1	0.94	0.51	0.23	0.27	−0.80	
W2	−0.23	0.79	0.08	0.29	0.04	
W3	0.18	−0.04	0.38	−0.14	0.94	
W4	−0.06	0.11	−1.09	−0.17	0.94	
W5	−0.15	−0.31	−0.60	1.13	0.16	

PCC, non-chilled carcass weight; PLC, carcass loin weight; PPC, carcass leg weight; ALC, carcass loin width; LLC, carcass loin length; LPC, carcass leg length; PV, live weight; AL, loin width; LL, loin length; PP, leg circumference; LP, leg length.

Table 6. Canonical correlations between carcass characteristics (Carcass) and canonical variables of live rabbits (Animal).

X-variables Set						
CARCASS	**PCC**	**PLC**	**PPC**	**ALC**	**LLC**	**LPC**
V1	0.73	0.56	0.76	0.39	0.42	0.35
V2	−0.04	−0.40	−0.16	0.08	0.01	0.20
V3	−0.10	−0.02	0.11	0.04	−0.23	−0.01
V4	0.01	0.06	0.01	0.30	0.11	0.20
V5	−0.04	0.01	0.05	−0.01	0.01	0.02
Y-variables Set						
ANIMAL	**PV**	**AL**	**LL**	**PP**	**LP**	
W1	0.82	0.37	0.18	0.39	0.25	
W2	0.13	−0.16	0.35	0.05	0.52	
W3	−0.02	−0.03	−0.29	−0.17	0.06	
W4	0.01	0.22	0.05	0.01	0.01	
W5	−0.01	0.01	0.01	0.06	0.01	

PCC, non-chilled carcass weight; PLC, carcass loin weight; PPC, carcass leg weight; ALC, carcass loin width; LLC, carcass loin length; LPC, carcass leg length; PV, live weight; AL, loin width; LL, loin length; PP, leg circumference; LP, leg length.

CONCLUSIONS

This study made it possible to establish the relation of morphological characteristics measured in live rabbits to those measured in dressed carcass cuts. Live weight of rabbits and leg circumference were the factors that most influenced the economically important characteristics of the carcass (loin and legs).

These results are of great importance for breeding stock selection programs, with the aim of improving carcass weight indirectly. Therefore, they can be criteria for rabbit producers when selecting outstanding breeders. The efficiency of indirect selection, based on characteristics in live rabbits, can be an effective strategy for early selection of breeding stock with better weights of carcass, loin and legs.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Development, quality, and yield of two habanero pepper hybrids (*Capsicum chinense* Jacq.) in three greenhouse cultivation systems

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ABSTRACT

Objective: To evaluate the plant development, fruit quality, and yield of two habanero pepper hybrids (*Capsicum chinense* Jacq.) in three cultivation systems under greenhouse conditions.

Design/methodology/approach: Plant height, stem diameter, chlorophyll index, fruit length, width and weight as well as the yield (fruits per plant, kg plant^{-1} and kg ha^{-1}) of the Chichen Itza and Megalodon hybrids grown on plastic mulch, coconut coir growth bags (CCGB) and soil under greenhouse conditions were evaluated. The data obtained were subjected to analysis of variance (ANOVA) under a completely randomized design with a factorial arrangement.

Results: The best vegetative development and highest chlorophyll index were observed in Megalodon hybrid grown on plastic mulch and CCGB. Chichen Itza hybrid produced the longest fruits, while Megalodon the widest ones; however, fruit weight was similar in both hybrids. Chichen Itza showed the highest number of fruits per plant, kg plant^{-1} and kg ha^{-1}.

Limitations on study/implications: The study was conducted during the winter-spring period; therefore, the results might vary in the summer-autumn period under the greenhouse conditions of the experimental site.

Findings/conclusions: The Chichen Itza hybrid showed the best productive performance. Similarly, the highest yield was recorded for both hybrids when directly sown in soil, with or without the use of plastic mulch.

Keywords: Plastic mulch, coconut coir, Chichen Itza, greenhouse, Megalodon.

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INTRODUCTION

Mexico is one of the main producers of several species of peppers (*Capsicum* spp.) worldwide, among which the habanero chili (*C. chinense* Jacq.) is widely cultivated in the Yucatan peninsula. This region is recognized as a center of genetic diversity of *C. chinense*, and its edaphoclimatic conditions have favored the existence of numerous landrace germplasm whose flavor, aroma, and spiciness distinguish them from habanero peppers grown in other parts of the world (Castillo-Aguilar *et al.*, 2019; Muñoz-Ramírez *et al.*, 2020). Paradoxically, these germplasms have small and heterogeneous fruits and low yields (Muñoz-Ramírez *et al.*, 2020), and although they are widely accepted in the local market (Flores-López & Sánchez-Osorio, 2020) they do not meet the quality standards of the national and international markets, making it necessary to develop and evaluate

new varieties and hybrids with better agronomic characteristics and higher yields (Tapia-Vargas *et al.*, 2016; Ramírez *et al.*, 2018).

On the other hand, to increase the yield of both native varieties and improved genotypes, various cultivation systems have been assessed. Thus, some authors have evaluated the direct sowing of this species into the soil (Castillo-Aguilar *et al.*, 2019), the use of plastic mulch (Torres-Bojórquez *et al.*, 2017) or growth bags (Muñoz-Ramírez *et al.*, 2020; Llamas *et al.*, 2024) under greenhouse conditions. Others have sown it under shade mesh using black polyethylene bags as containers (Ontiveros-Sajuan *et al.*, 2024). Likewise, some studies have evaluated the physicochemical characteristics of different types of soils as natural substrates for its cultivation (Borges-Gómez *et al.*, 2014) while others have focused on evaluating the use of alternative substrates such as coconut fiber (Muñoz-Ramírez *et al.*, 2020; Llamas *et al.*, 2024) mixtures of gravel and tezontle (López *et al.*, 2020), tezontle and coconut fiber (Meneses-Lazo *et al.*, 2020) and mixtures of vermicompost, gravel and river sand (Javier-López *et al.*, 2022) with variable results.

Under this context, the present study aimed to evaluate plant development, fruit quality and yield of two habanero pepper hybrids in three cultivation systems under greenhouse conditions.

MATERIALS AND METHODS

Experimental site

This study was carried out from February to September 2021 in a Gothic-type greenhouse at the Faculty of Biological and Agricultural Sciences of the University of Colima, located at the geographic coordinates 18° 57' 10" N and 103° 53' 46" W, at an altitude of 33 masl. The climate is warm semi-dry with summer rains, corresponding to the formula BS1 (h')w(w) with an average annual temperature and precipitation of 27.4 °C and 882.5 mm, respectively. The experimental soil was sandy loam (clay 17%, sand 77% and silt 6%) with an EC and pH of 2.5 mS cm^{-1} and 6.88, respectively.

Genetic material, plantlet production and trasplant

Seeds of two commercial habanero pepper (*C. chinense*) hybrids were used: Megalodon (Lark Seeds Company®, USA) and Chichen Itza (Bayer®, USA). They were sown at a depth of 0.5-1 cm in previously disinfected 200-cavity white polyethylene trays (Hortiblock®, Mexico) filled with Peat Moss substrate (BM8®, Canada). After sowing, the trays were covered with black bags for five days to induce germination. Once the seedlings emerged, they were uncovered and placed on tables to be watered and fertilized until transplanting, which was carried out after 49 days. The following fertilizers were applied for plant production: Triple 19 (Polyfeed® Haifa, 1 to 2.5 g L^{-1}), 12-42-12 (Polyfeed® Haifa, 1 to 2.5 g L^{-1}), Root factor (Agroscience®, 1.5 mL L-1), Startrak (Abonomex®, 1 to 2.5 g L^{-1}) and Maxirad (Coda®, 1.5 mL L^{-1}).

Greenhouse preparation, growing systems and topological arrangement

Sowing in soil with and without mulch. Manual weeding was performed, and the top 30 cm of soil was removed with a pick and hoe to form the seedbeds. Seedlings were transplanted at 30 cm from each other, with a row spacing of 1.5 m, resulting in a planting density of 21,978 plants ha^{-1}. Black and white plastic mulch was manually placed on the beds, and the edges were covered with soil. Fourteen growth bags (100×16×18 cm and 28.8 cm^3) were filled with fiber and coconut coir at a ratio of 70:30 and placed on partitions to insulate them from the soil. Three holes were made in each bag, spaced 30 cm from each other, and a seedling was placed inside. The distance between rows was 1.5 m resulting in a plant density like that used in direct sowing.

Nutrition and phytosanitary management

Nutrition was carried out through fertigation in four stages according to crop phenology. In stage one (adaptation), six fertigation treatments were applied in 21 days. The sources were: 272.7 g of phosphonitrate, 80.5 g of MKP, and 50.6 g of Nitro K Sulfur. During this stage, 200 mL of Root Factor and 150 g of Pow Humus were also applied to promote rooting. In stage two (development), 12 fertigation applications were made over 30 days, using the following sources: 220.2 g of phosphonitrate, 26 g of MKP, and 81 g of Nitro K Sulfur. In stage three (fruiting), 10 fertigation applications were made over 30 days with 170 g of phosphonitrate, 162.5 g of MKP, and 8.92 g of Nitro K Sulfur. In stage four (production), 28 fertigation applications were made over 60 days with 45 g of phosphonitrate, 28.2 g of MKP, and 108.2 g of Nitro K Sulfur. Throughout all stages, 50 g of micronutrients (Tradecorp A-Z®, Fe, Mn, Zn, B, and Mo) and 150 mL of calcium (Turgent Ca®, Agroscience) were applied weekly. The fertilizer quantities corresponded to a population of 400 plants.

For the phytosanitary management of the insects pest that were present (cryptic complex *Bemisia tabaci*, *Scirtothrips dorsalis*, *Liriomyza* spp., *Tetranichus urticae*, *Polyphagotarsonemus latus*) biorational products [Trilogy® (2.5 mL L^{-1}) and Ajick® (2.5 mL L^{-1})], biological [Bassi-Hit®, 2.5 g L^{-1}) and chemical [Imidacloprid (Imiland®, 1.5 mL L^{-1}), thiamethoxam + lambdacyhalothrin (Engeo®, 1.5 mL L^{-1}), Etoxazole (Tetrasan®, 1.0 mL L^{-1}) and Fenpyroximate (Avolant®, 1.5 mL L^{-1}) were used. For fungal diseases, biological fungicides (Magni-Root®, 2.5 g L^{-1}), biorational fungicides (Castell®, 2.5 mL L^{-1}) and chemical fungicides [Fosetyl-Al (Alleato®, 2.0 g L^{-1}), Azoxystronin + Metalaxyl (Uniform®, 1.5 mL L^{-1}) and Propamocarb + Fosetyl-Al (Previcur®, 1.5 g L^{-1})] were applied.

Treatments

Six treatments were evaluated, resulting from the combination of the two hybrids (Chichen Itza and Megalodon) and three cultivation systems (mulch, bag, and soil). Each plant was considered an experimental unit (replicate), and 12 plants were evaluated per treatment, for a total of 72 replicates.

Response variables

Agronomics. Beginning one week after transplanting, plant height, stem diameter, and chlorophyll index were assessed every two weeks. Plant height was measured using a telescopic aluminum rod from the ground to the apex. Stem diameter was measured with a digital vernier caliper, and chlorophyll index was measured with a CM1000 spectroradiometer (Field Scout®, USA).

Fruit quality. At harvest, 112 fruits were randomly selected per treatment. Their length and diameter were measured with a digital vernier caliper, and their weight was determined using an SF-400 digital scale.

Production and yield. The total number and weight of fruits harvested per plant were recorded during the 13 harvests made over a 99-day period. To calculate the yield in t ha^{-1}, the weight of all crops was added and multiplied by 21,978 plants ha^{-1}.

Statistical analysis

Data were analyzed using a completely randomized analysis of variance (ANOVA) with a factorial arrangement, and means were compared using the Tukey test ($p<0.05$). Statistical analyses were conducted using Statgraphics V.8 for Windows.

RESULTS AND DISCUSSION

Chichen Itza hybrid showed greater plant height at 35- and 49-days post-transplant (DPT). Plants sown in plastic mulch and growth bags had greater height at 35 DPT, whereas at 49 and 63 DPT this was greater only in plants planted in plastic mulch (Table 1). An interaction effect between hybrid and growing system was observed for plant height at 7 and 84 DPT. At 7 DPT, the height of Megalodon plants grown in plastic mulch and soil was greater than that of Chichen Itza, but similar to that of plants grown in growth bag. At 84 DPT, the height of Megalodon plants cultivated in plastic mulch and growth

Table 1. Plant height of two habanero pepper hybrids in three greenhouse cultivation systems.

Factor	Days post-transplant					
	7	21	35	49	63	84
Hybrid						
Chichen Itza	13.40 b	19.12 a	36.24 a	63.53 a	87.22 a	120.44 b
Megalodon	16.56 a	19.36 a	34.81 a	48.42 b	81.39 b	138.25 a
SEM	0.34	0.39	0.77	1.09	1.31	2.81
MSD	0.97	1.10	2.19	3.08	3.68	7.92
p (α=0.05)	0.00001	0.6630	0.1963	0.00001	0.0010	0.00001
Cultivation system						
Plastic mulch	15.23 ab	19.85 a	38.00 a	61.15 a	93.46 a	144.29 a
Coconut coir growth bag	13.88 b	18.72 a	35.15 ab	54.67 b	78.79 b	125.50 b
Soil	15.83 a	19.14 a	33.42 b	52.10 b	81.42 b	118.25 b
SEM	0.42	0.48	0.95	1.33	1.60	3.44
MSD	1.42	1.62	3.22	4.53	5.42	11.66
p (α=0.05)	0.0053	0.2480	0.0042	0.00001	0.00001	0.00001
Hybrid×Cultivation system						
p (α=0.05)	0.0024	0.0869	0.9437	0.1454	0.4819	0.00001

[a,b] Means with different letters are significantly different according to Tukey's test (p=0.05). SEM: standard error of the mean; MSD: minimum significance difference.

bags was greater than that of Chichen Itza, but similar when cultivated in soil. Likewise, Megalodon plants were taller when planted in plastic mulch compared to soil and growth bag, while Chichen Itza was similar in all three cultivation systems.

Contrary to what was observed in the present study, Llamas *et al.* (2024) reported an inverse behavior in plant height of both hybrids grown in bags with coconut coir substrate, with higher values for the Chichen Itza hybrid. Furthermore, at a similar age, the Megalodon hybrid was shorter (117.2 cm) and the Chichen Itza hybrid taller (136.1 cm) than in the present study (Megalodon 138.75 cm and Chichen Itza 112.25 cm). Regarding cultivation systems, Toscano-Verduzco (2023) observed that the use of shade mesh increased plant height of Megalodon at 60, 75 and 90 DPT by 12.2, 9.5 and 7.4%, respectively. In contrast to the results of this study, he also observed that the use of plastic mulch negatively affected plant growth, and at 90 DPT, plants grown without plastic mulch were 21.8% taller. This author suggested that the use of plastic mulch increases soil temperature and salt concentration, causing abiotic stress in the plants and negatively affects their growth. Another study concluded that plants grown with silver-colored plastic mulch showed greater height and growth index compared to those grown without mulch (Torres *et al.*, 2017).

Other authors have identified various cultural practices that affect the plant height of *C. chinense*. Javier *et al.* (2022) evaluated the use of different fertilization sources and substrates in this crop and observed that, at 199 DPT, plants grown in sand and vermicompost and fertilized with chemical and organic sources, respectively, were taller than those grown in a mixture of vermicompost, soil and gravel fertilized with and an organic source (192.6, 173.4 and 126.43 cm, respectively). Tapia-Vargas *et al.* (2016) evaluated the application of a hormonal compound on the height of chocolate habanero pepper plants and observed greater height in the treated plants due to enhanced cell division and elongation. An advantage of smaller genotypes is the ease and speed with which some agronomic practices such as pruning, defoliation, and harvesting can be performed without the use of stilts, resulting in lower risk and cost for the producer.

At 49 DPT, Megalodon plants showed a greater stem diameter than Chichen Itza plants, while at 49 and 63 DPT, plants grown in plastic mulch and growth bags showed a greater stem diameter than those grown in soil (Table 2). An interaction effect was observed for this variable at 35 and 84 DPT. At 35 days, the stem diameter of both hybrid plants grown in plastic mulch and growth bag was similar, but Megalodon plants had greater stem diameter when grown in soil. At 84 DPT, the stem diameter of Megalodon plants grown in plastic mulch and growth bags were larger than those grown in soil.

At 84 DPT, Megalodon plants grown in growth bags showed a larger stem diameter than Chichen Itza plants (14.54 *vs.* 10.85 mm, respectively). However, Llamas *et al.* (2024) did not observe any difference in stem diameter between the two hybrids. Likewise, these authors reported at 87 DPT a smaller stem diameter for Megalodon plants (12.85 mm) and a larger one for Chichen Itza plants (12.41 mm) than those observed in the present study. According to Toscano-Verduzco (2023), at 90 DPT the use of shade mesh did not affect the stem diameter of Megalodon plants, while at 84 DPT the use of plastic mulch significantly decreased stem diameter, a trend also observed in the present study. As with

Table 2. Stem diameter of two habanero pepper hybrids in three greenhouse cultivation systems.

Factor	Days post-transplant					
	7	21	35	49	63	84
Hybrid						
Chichen Itza	2.47 a	3.92 a	6.21 b	6.92 a	8.42 a	10.48 b
Megalodon	2.57 a	3.78 a	6.84 a	6.56 b	8.74 a	13.40 a
SEM	0.05	0.09	0.13	0.10	0.13	0.18
MSD	0.15	0.26	0.36	0.29	0.36	0.51
p (α=0.05)	0.1605	0.2852	0.0008	0.0124	0.0804	0.00001
Cultivation system						
Plastic mulch	2.50 a	3.86 a	6.60 ab	7.01 a	8.90 a	12.72 a
Coconut coir growth bag	2.58 a	4.01 a	6.85 a	7.14 a	8.97 a	12.69 a
Soil	2.88 a	3.68 a	6.13 b	6.07 b	7.88 b	10.40 b
SEM	0.07	0.11	0.16	0.12	0.16	0.22
MSD	0.22	0.39	0.53	0.42	0.53	0.75
p (α=0.05)	0.4465	0.1365	0.0064	0.00001	0.00001	0.00001
Hybrid×Cultivation system						
p (α=0.05)	0.2794	0.5983	0.0074	0.4340	0.0987	0.0006

[a,b] Means with different letters are significantly different according to Tukey's test (p=0.05). SEM: standard error of the mean; MSD: minimum significance difference.

plant height, this author indicated that abiotic stress caused by increased soil temperature and salt concentration negatively affected plant growth.

Other authors have reported that the source of fertilization and the substrate used affect the stem diameter of *C. chinense* plants. In this regard, Javier *et al.* (2022) recorded larger stem diameters at 199 DPT in plants grown in vermicompost with organic fertilization (19.06 mm) compared to those grown in sand with chemical fertilization (15.95 mm), in a mixture of vermicompost, soil, and gravel with organic fertilization (15.72 mm), and in a mixture of vermicompost, sand, and gravel with organic fertilization (13.46 mm).

Regarding the chlorophyll index, it was higher in Megalodon plants and in plants grown in soil at 7 and 35 DPT, respectively (Table 3). An interaction effect was observed at 21, 49, and 84 DPT.

At 21 and 49 DPT, Chichen Itza plants grown in growth bags showed a lower chlorophyll index than Megalodon plants, while plants of both hybrids grown in plastic mulch and soil showed similar values. At 84 DPT, Chichen Itza plants grown in plastic mulch had a lower chlorophyll index than Megalodon plants, with similar values for both hybrids when grown in growth bags and soil.

The relative chlorophyll index is an indicator of the total chlorophyll content in leaves and allows for the evaluation of the plant's nitrogen status, which is essential to produce amino acids and other molecules and tissues required for plant growth. The chlorophyll index recorded in both hybrids during the growth phase was higher than that observed for these same hybrids by Llamas *et al.* (2024). This difference can be explained by the altitude at which the crops were established, since as elevation increases, the diffusion

Table 3. Chlorophyll index of two habanero pepper hybrids in three greenhouse cultivation systems.

Factor	Days post-transplant					
	7	21	35	49	63	84
Hybrid						
Chichen Itza	124.17 a	196.19 a	203.58 b	363.86 a	354.33 a	323.11 b
Megalodon	127.67 a	189.56 a	238.58 a	327.72 b	360.11 a	367.56 a
SEM	2.77	3.08	6.60	8.64	8.80	5.61
MSD	7.81	8.69	18.64	24.37	24.83	15.85
p (α=0.05)	0.3742	0.1322	0.0004	0.0043	0.6439	0.00001
Cultivation system						
Plastic mulch	115.46 b	183.75 b	210.67 a	359.33 a	370.42 a	371.17 a
Coconut coir growth bag	117.33 b	180.33 b	226.29 a	315.08 b	359.21 a	342.42 b
Soil	144.96 a	214.54 a	226.29 a	362.96 a	342.04 a	322.42 b
SEM	3.39	3.77	8.09	10.58	10.78	6.88
MSD	14.76	12.79	27.43	35.87	36.54	23.32
p (α=0.05)	0.00001	0.00001	0.2948	0.0030	0.1801	0.0000
Hybrid×Cultivation system						
p (α=0.05)	0.5920	0.0119	0.1485	0.0000	0.3340	0.0005

[a,b] Means with different letters are significantly different according to Tukey's test (p=0.05). SEM: standard error of the mean; MSD: minimum significance difference.

of solar radiation decreases, which negatively affects the photosynthetic rate (Montero-Torres, 2022). In this sense, the present study was carried out at an altitude of 33 m.a.s.l., while Llamas *et al.* (2024) established their cultivation at 645 m.a.s.l. Toscano-Verduzco (2023) reported a chlorophyll index of 651.6 at 90 DPT in the Megalodon hybrid grown in a greenhouse under shade mesh. Previous studies have indicated that the use of shading can improve the photosynthetic capacity of pepper leaves (Zhu *et al.*, 2012), as they tend to have a greater number of light-harvesting complexes per unit area to capture as much light as possible (Griffin *et al.*, 2004).

Fruit width of Megalodon was greater than that of Chichen Itza and similar across the three cultivation systems, while an interaction effect was observed for fruit length and weight (Table 4). Fruit length of Chichen Itza was greater than that of Megalodon and did not differ among cultivation systems, whereas Megalodon fruit length was greater when plants were grown in soil. Fruit weight of Megalodon was higher when grown in soil, while that of Chichen Itza was higher when grown in soil and under plastic mulch.

Fruit size and weight of *C. chinense* show great variability and both are key characteristics that determine its quality. Borges-Gómez *et al.* (2010) indicate that the fruit can be classified according to its weight as first grade fruit, when it is greater than 6.5 g, second grade fruit, when it is between 5.5 and 6.4 g, and third grade fruit when it is less than 5.4 g. In the present study, the width and length of the Megalodon fruit were larger and smaller than those of Chichen Itza respectively, and based on their weight, they were classified as first-grade fruits. Average fruit width of both hybrids was greater than that reported by Ramírez *et al.* (2018) and Meneses-Lazo *et al.* (2020), for Jaguar (25-30 mm)

Table 4. Fruit width, length, and weight of two habanero pepper hybrids in three greenhouse cultivation systems.

Factor	Fruit width (mm)	Fruit length (mm)	Fruit weight (g)
Hybrid			
Chichen Itza	32.47 b	45.03 a	10.31 a
Megalodon	35.68 a	34.65 b	9.93 a
SEM	0.39	0.46	0.17
MSD	1.09	1.28	0.48
p (α=0.05)	0.0000	0.0000	0.1160
Cultivation system			
Plastic mulch	34.79 a	39.07 b	10.27 b
Coconut coir growth bag	33.43 a	38.59 b	9.09 c
Soil	34.01 a	41.87 a	11.00 a
SEM	0.48	0.57	0.21
MSD	1.59	1.88	0.70
p (α=0.05)	0.1329	0.0001	0.00001
Hybrid×Cultivation system			
p (α=0.05)	0.1734	0.0215	0.0430

[a,b] Means with different letters are significantly different according to Tukey's test (p=0.05). SEM: standard error of the mean; MSD: minimum significance difference.

and Naranja (29 mm) varieties, but was smaller than that reported by Castillo-Aguilar *et al.* (2019) for Rosita variety (51.20 mm). Regarding fruit length, Chichen Itza was longer than the Rosita (44.10 mm) and Naranja (40.00 mm) varieties (Castillo-Aguilar *et al.*, 2019; Meneses-Lazo *et al.*, 2020), while the length of these varieties was greater than that of Megalodon. Similarly, Ramírez *et al.* (2018) reported a fruit length of 38 to 55 mm for the Jaguar variety, which is also greater than that observed here for Megalodon. The average weight (21.79 g) of the fruit of Rosita variety (Castillo-Aguilar *et al.*, 2019) far exceeded that recorded here for both hybrids, while that reported by Meneses-Lazo *et al.* (2020) for Naranja variety (8 g) was lower. For its part, Jaguar variety has a fruit weight ranging from 6.5 to 10 g (Ramírez *et al.*, 2018), which is lower than that observed for Chichen Itza in the present study.

Chichen Itza hybrid showed a higher number of fruits, production per plant and yield than Megalodon, while the highest number of fruits, production per plant and yield were obtained when both hybrids were grown in plastic mulch and soil (Table 5).

The superior productive performance of the Chichen Itza hybrid compared to Megalodon, contrasts with the results of Llamas *et al.* (2024), who cultivated these two hybrids under greenhouse conditions, in growth bags using a semi-hydroponic system and found no differences in the number fruits and production per plant and yield in t ha^{-1}.

In addition to genotype, the production and yield of *C. chinense* depend on several factors, including the cultivation system and agronomic management. In the present

Table 5. Number of fruits, production per plant and yield of two habanero pepper hybrids in three greenhouse cultivation systems.

Factor	Fruits per plant	Production (kg plant^{-1})	Yield (t ha^{-1})
Hybrid			
Chichen Itza	126.50 a	0.94 a	20.53 a
Megalodon	69.08 b	0.60 b	13.17 b
SEM	3.72	0.03	0.60
MSD	10.50	0.08	1.70
p (α=0.05)	0.00001	0.00001	0.00001
Cultivation system			
Plastic mulch	114.92 a	0.91 a	19.96 a
Coconut coir growth bag	74.67 b	0.55 b	12.15 b
Soil	103.79 a	0.84 a	18.44 a
SEM	4.56	0.03	0.74
MSD	15.45	0.11	2.51
p (α=0.05)	0.00001	0.00001	0.00001
Hybrid×Cultivation system			
p (α=0.05)	0.4906	0.8755	0.8809

[a,b] Means with different letters are significantly different according to Tukey's test (p=0.05). SEM: standard error of the mean; MSD: minimum significance difference.

study, production per plant and yield per hectare were similar when both hybrids were grown in soil with or without plastic mulch, and in both cases higher than when grown in growth bags. These results differ from those reported by Torres *et al.* (2017), who found that the production of *C. chinense* using transparent, silver, and white mulch was 996.9, 824.7, and 725.2 g m^{-2}, respectively, while plants grown without mulch yielded only 353.4 g m^{-2}. Ramírez *et al.* (2018) reported that the Jaguar variety produces around 15 t ha^{-1} when established in irrigated production systems or under favorable rainfed conditions in the south-central regions of Veracruz, Chiapas, and Campeche, reaching yields of 30 t ha^{-1} under drip irrigation and fertigation systems, and up to 43 t ha^{-1} under protected agriculture conditions. Javier-López *et al.* (2022) evaluated the production of the Chichen Itza hybrid in a greenhouse using four substrates and two fertilization sources and observed that cumulative yield per plant was 52.14% higher when grown in vermicompost with organic fertilization than in sand with chemical fertilization (616.41 g *vs.* 405.14 g). One of the factors that most influences yield is fertilization. In this study, a formula of 180-200-220 kg ha^{-1} of N-P-K was applied according to each phenological stage of the crop using a fertigation system. However, Llamas *et al.* (2024), using a semi-hydroponic system in bags with coconut fiber, reported yields of 65.8 and 59.6 t ha^{-1} for Megalodon and Chichen Itza hybrids over 28 harvests, applying nutrient solutions with conductivities of 0.8 to 1.6 mS cm^{-1}, which could explain the lower performance observed with stage-based fertigation.

CONCLUSION

The Megalodon habanero pepper hybrid showed greater plant height and stem diameter, with wider but similarly weighted fruits compared to the Chichen Itza hybrid. The Chichen Itza hybrid produced a higher number of fruits, resulting in greater production per plant and yield per hectare. Regardless of the hybrid, the highest fruit number and total fruit weight per plant and per hectare were obtained with direct sowing in soil, both with and without the use of plastic mulch.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Evaluation of the effect of a whey-based biofertilizer on agronomic and biochemical parameters in sweet potato [*Ipomoea batatas* (L.) Lam.]

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ABSTRACT

Objective: To compare the effects of applying an organic biofertilizer (APIS®), formulated from whey, on agronomic and biochemical variables in sweet potato (*Ipomoea batatas*).

Design/Methodology/Approach: A randomized block design was implemented over a 1.5 ha open-field plot. Treatments included foliar applications of APIS®, Bayfolan Forte® (a commercial fertilizer), and water (as a control) at 35- and 65-days post-transplanting (dpt). Sampling was conducted at 35, 60, and 85 dpt to evaluate morphological parameters; quantification of total bacteria and filamentous fungi; elemental analysis; chlorophyll content; total protein; total polyphenols; soluble sugars; and hydrogen peroxide (H_2O_2) levels.

Results: The organic biofertilizer APIS® led to a higher number of tubers and, consequently, greater yield per hectare 22% and 32% higher compared to Bayfolan Forte® and the control, respectively. APIS® also enhanced microbial load during the second sampling, followed by a reduction in the third. No statistically significant differences were observed between the APIS® and control treatments regarding morphological parameters, chlorophyll content, total protein, polyphenols, soluble sugars, and H_2O_2. However, H_2O_2 concentrations increased in the Bayfolan Forte® treatment compared to the control.

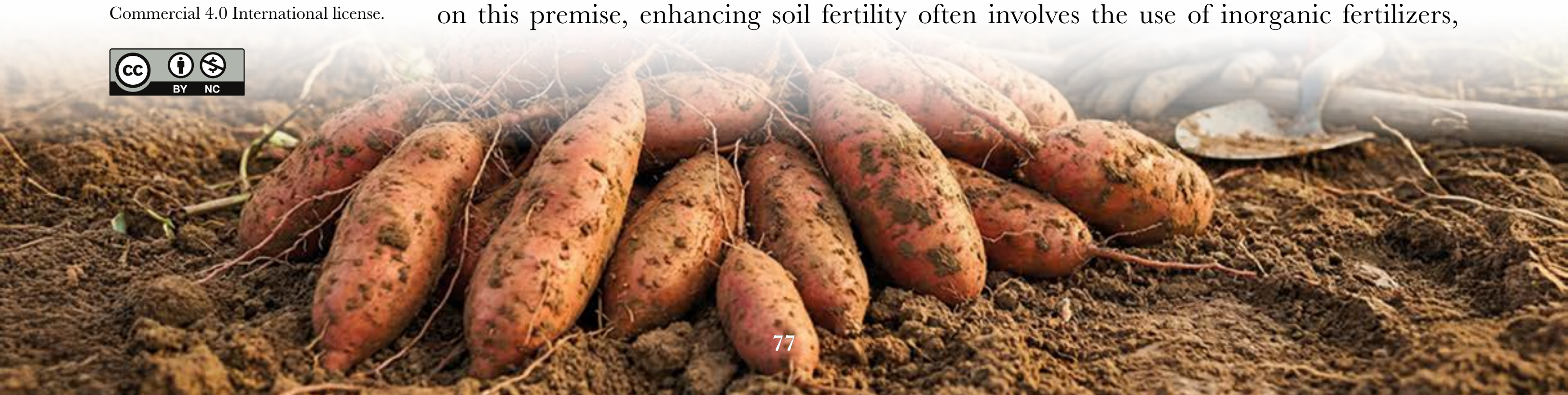
Limitations/Implications: The study was conducted during a single agricultural cycle, which may limit broader generalizations.

Findings/Conclusions: APIS® demonstrates potential as a viable biofertilizer for sweet potato cultivation, showing comparable or superior performance to the commercial fertilizer.

Keywords: Elemental composition, total bacteria, chlorophyll content, filamentous fungi, total polyphenols.

INTRODUCTION

In agricultural production systems, it is essential to develop fertilization strategies that align with soil fertility, crop variety, and the production cycle (Aguilar, 2021). Based on this premise, enhancing soil fertility often involves the use of inorganic fertilizers,



which are the most commonly applied to meet the nutritional requirements of sweet potato crops. For instance, the recommended practice includes the application of 184 kg ha^{-1} of 10-30-10 (Nitrogen, Phosphorus, Potassium: N-P-K) at eight days and again at two months post-planting (Aguilar, 2021). However, the excessive and improper use of synthetic inputs has led to their accumulation in soil, water, and air, resulting in soil salinization and toxicity, disruption of biogeochemical cycles and trophic chains in agricultural zones, and increasing difficulties in pest, disease, and weed management all of which negatively affect agricultural productivity (Chávez-Díaz *et al.*, 2020; García-Galindo *et al.*, 2020; Andrade-Sifuentes *et al.*, 2022; García-De La Paz *et al.*, 2022). As a result, there is a pressing need to develop plant nutrition strategies that reduce reliance on synthetic fertilizers. This has driven interest in the use of organic amendments and biofertilizers, which not only supply essential nutrients for crop growth but also improve soil nutrition and increase organic matter reserves (Arreola *et al.*, 2020).

Biofertilizers are natural fertilizers containing living microorganisms that, when introduced into the soil, enhance nutrient availability to host plants, stimulate their development, and may promote the production of plant growth regulators and biocontrol agents (Nafi'Ah *et al.*, 2021). Recently, various types of biofertilizers have been developed using waste products from different industries. One such by-product, whey, is produced in large quantities by the cheese and yogurt industries and poses a significant environmental challenge due to its high organic load (Lizárraga-Chaidez *et al.*, 2023). Whey contains valuable nutrients for plants, including nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur, as well as lactose, proteins, and minerals (Ahmed *et al.*, 2020). These components, combined with the activity of naturally occurring microorganisms, make whey a promising organic fertilizer for improving crop health and productivity (Akay & Sert, 2020). For example, the application of whey in agricultural soils improves physicochemical properties, enhances structural stability and aggregation, increases yields, and boosts N, P, and K content (Akay, 2020; Ahmed *et al.*, 2020). Furthermore, its content of water, proteins, lactose, fats, and vitamins promotes plant growth (Ahmed *et al.*, 2020). In this regard, the use of hydrolyzed whey in crops such as pea (*Pisum sativum* L.), wheat (*Triticum aestivum* L.), and sweet potato (*Ipomoea batatas* L.) has led to improvements in agronomic traits such as pod length and growth, dry weight, and nutrient uptake, ultimately enhancing crop yield (Sun *et al.*, 2024).

Sweet potato (*I. batatas*) is a high-potential crop with increasing economic relevance. It has been cultivated in Mexico since pre-Hispanic times and is currently grown in 21 states, with Michoacán, Veracruz, Guanajuato, and Chihuahua being the leading producers (Vidal *et al.*, 2018; Córdova *et al.*, 2022; SIAP, 2024). *I. batatas* adapts well to diverse edaphoclimatic conditions, is easily propagated, supports multiple production cycles per year, and serves various industrial purposes, including biofuel, paper, cosmetics, animal feed, and confectionery (Solís & Ruiloba, 2017; Salehin *et al.*, 2020; Montes-Sierra *et al.*, 2023; Gama *et al.*, 2023). Additionally, the tuber contains bioactive compounds with significant health benefits, provides essential nutrients, and exhibits nutraceutical properties (Vidal *et al.*, 2018). In response to the growing need for sustainable agricultural alternatives, a new organic biofertilizer APIS® has been developed using whey, a by-product of the cheese-making industry. This formulation is enhanced with lactic acid bacteria, yeasts, and molasses. APIS® aims to reduce the environmental impact caused by agrochemicals while supporting crop nutrition. The primary aim of this study was to compare the effects of APIS® application on agronomic and biochemical variables in sweet potato plants relative to a commercial fertilizer. It is anticipated that whey will emerge as a viable nutritional alternative for agricultural crops, contributing to its revalorization.

MATERIALS AND METHODS

Field location

The study was conducted on private agricultural plots located in the community of San Nicolás de Los Agustinos (20.251640, −100.961281), in the municipality of Salvatierra, Guanajuato, Mexico.

Treatments

Sweet potato (*I. batatas*) plants were sown in January 2022. A randomized block design was employed over 1.5 hectares. Each experimental unit (EU) consisted of 10 ridges, each 1.6 m wide and 10.5 m long, with 15 cm spacing between plants and 90 cm between rows. Three EUs were used per treatment, at a planting density of 44,300 plants ha^{-1}. Four furrow irrigations were applied. No soil fertilization, insecticides, or fungicides were used. Weed control was performed mechanically and manually. Foliar fertilization was applied using a motorized backpack sprayer according to the manufacturer's recommendations for each of the following treatments: (1) Whey-based biofertilizer (APIS®, Irapuato, Gto.); (2) Commercial inorganic fertilizer Bayfolan Forte® (Bayer, Mexico) and (3) Water only (control).

All treatments included 100 mL ha^{-1} of the adjuvant Break Thru® (BASF de México).

The first foliar application was performed at 35 days post-transplanting (dpt): 4 L ha^{-1} of APIS® in 200 L of water, 4 L ha^{-1} of Bayfolan Forte® in 200 L of water, and 200 L ha^{-1} of water for the control. A second application at 65 dpt consisted of 5 L ha^{-1} of each fertilizer (APIS® and Bayfolan Forte®), while the control group received only water. For sampling, three ridges per treatment were randomly selected, and 21 plants were collected randomly from central rows at 35, 60, and 85 dpt. Samples were flash-frozen in dry ice, transported to the laboratory, and stored at −40.0 °C.

Morphological parameters

The following morphological variables were evaluated: root length (cm), total plant length (cm), number of leaves, fresh weight (g), dry weight (g), and yield (t ha^{-1}). Samples were first cleaned of residual substrate. Root and total length were measured with a measuring tape. Leaf number was counted manually. Fresh weight was recorded using an analytical balance (Sartorius), and samples were dried in a hot-air oven (Ecocell) at 75 °C for 72 hours or until constant weight. Final yield (t ha^{-1}) was reported by the grower at the end of the crop cycle.

Microbiological quantification

Colony-forming units (CFU) were quantified following Girón-Calva *et al.* (2012), with modifications. Five leaves per treatment were collected at each sampling time (30, 60, and 85 dpt), ground with 3 mL of phosphate buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4), and centrifuged at 3,000 rpm for 3 min at room temperature. Serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}) were plated on nutrient agar (NA) and incubated at 35 °C for 24 h to quantify total bacteria (TB). Simultaneously, dilutions were plated on potato dextrose agar (PDA) supplemented with benzylpenicillin (100 mg L^{-1}) and incubated at 28 °C for 7 days to quantify total filamentous fungi (TFF).

Elemental quantification

Carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) were quantified based on Ma *et al.* (2017) and Easa *et al.* (2019), with slight modifications. Leaf samples from all three sampling times (30, 60, and 85 dpt) were dried in a convection oven at 45 ± 3 °C to constant weight. Three milligrams of sample were combined with 10 mg of vanadium oxide (V_2O_5) in tin capsules. Elemental content was measured using a CHNS-O elemental analyzer (Thermo Fisher Scientific Inc., 2009), with a run time of 10 minutes per sample.

Chlorophyll quantification

Following Arnon (1949), with modifications, 1 mL of 96% ethanol was added to 100 mg of fresh leaf tissue. Samples were incubated for 24 h at 4 °C, then centrifuged at 11,000 rpm for 15 min at 4 °C. Two hundred microliters of the supernatant were transferred to a microplate, and absorbance was measured at 645 nm and 663 nm (Multiskan™ Sky, Thermo Scientific).

Soluble protein quantification

Soluble protein content was determined using the Bradford method (Bradford, 1976). Dried leaf samples (100 mg) from each treatment and sampling time were homogenized in 250 μL of Tris-HCl buffer (0.05 M, pH 7.4), vortexed, and incubated at 4 °C for 15 min. Samples were centrifuged at 12,000 rpm for 15 min at 4 °C. In a 96-well microplate, 5 μL of extract, 5 μL of distilled water, and 200 μL of 1:5 diluted Bradford reagent were added per well. After 5 min of incubation at room temperature, absorbance was read at 595 nm. A bovine serum albumin (BSA) standard curve was used for quantification.

Total polyphenol quantification

Total polyphenols were measured in lyophilized and ground leaf tissue using the Folin-Ciocalteu method (Attard, 2013). A 100 mg sample was extracted with 500 μL of 70% methanol under agitation for 12 h, then centrifuged at 12,000 rpm for 5 min. In a microplate, 237 μL distilled water, 45 μL of 15% Na_2CO_3, 3 μL of extract, and 15 μL of 10% Folin-Ciocalteu reagent were mixed and incubated for 30 min at room temperature. Absorbance was read at 760 nm using a gallic acid calibration curve.

Total soluble sugar quantification

Total soluble sugars were quantified following Laurentin and Edwards (2003). One hundred milligrams of dried leaf tissue were extracted with 1.5 mL of 96% ethanol, centrifuged at 3,500 rpm for 10 min at 4 °C. Forty microliters of the supernatant were placed in a 96-well plate, mixed, and incubated at 4 °C for 15 min. Then, 100 μL of anthrone solution (2 g/12 mL H_2SO_4) was added. The plate was incubated in a water bath at 92 °C for 3 min, cooled to room temperature for 5 min, and then incubated at 45 °C for 15 min. Absorbance was read at 630 nm using a microplate spectrophotometer.

Hydrogen peroxide quantification

H_2O_2 concentration was determined using the Peroxide Assay Kit (Sigma-Aldrich®, 2019). A total of 100 mg of leaf tissue was mixed with 1 mL of distilled water, shaken at 1,500 rpm for 10 min, and centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was collected. Quantification reagent (1:100 mixture of Reagents A:B) was prepared. In a 96-well plate, 40 μL of sample and 200 μL of reagent were added per well. After 30 min of incubation at room temperature, absorbance was measured at 585 nm. A standard curve was constructed using H_2O_2.

Statistical analysis

Data were analyzed using one-way ANOVA followed by Tukey's HSD multiple comparison test, both at a 5% significance level. Statistical analyses were performed using Minitab 20.3 software.

RESULTS AND DISCUSSION

Morphological Parameters

There is limited information on the application of biofertilizers in sweet potato plants, and to date, no studies have been reported using formulations based on whey. In this study, sweet potato plants were treated with a foliar application of a whey-based biofertilizer (APIS®) and compared to a commercial fertilizer (Bayfolan Forte®) and an untreated control. The results revealed statistically significant differences ($P \leq 0.05$) at 60 days post-transplant (dpt; second sampling) in the number of leaves between the APIS® and Bayfolan Forte® treatments (Figure 1a), as well as in root length (Figure 1c), stem length (Figure 1c), and number of tubers between the control and APIS® treatments (Figure 1d). Regarding tuber production, the average number of tubers recorded at the second sampling (60 dpt) was 4.6±0.9, 7.4±1.5, and 10.8±2.3 for the control, Bayfolan Forte®, and APIS® treatments, respectively (Figure 1d). A further increase in tuber count was observed in the third sampling (85 dpt) for the APIS® (12.3±0.6 tubers) and control (8±1.0 tubers) treatments. However, no increase was recorded for Bayfolan Forte® (7.3±5 tubers). At 85 dpt, no statistically significant differences ($P \leq 0.05$) were found among treatments in terms of number of leaves, fresh and dry plant weight, root length, stem length, or tuber weight (Figure 1a-e). García *et al.* (2022) evaluated the application of the APIS® biofertilizer in broccoli (*Brassica oleracea* var. *italica*) and reported significant improvements in axial and

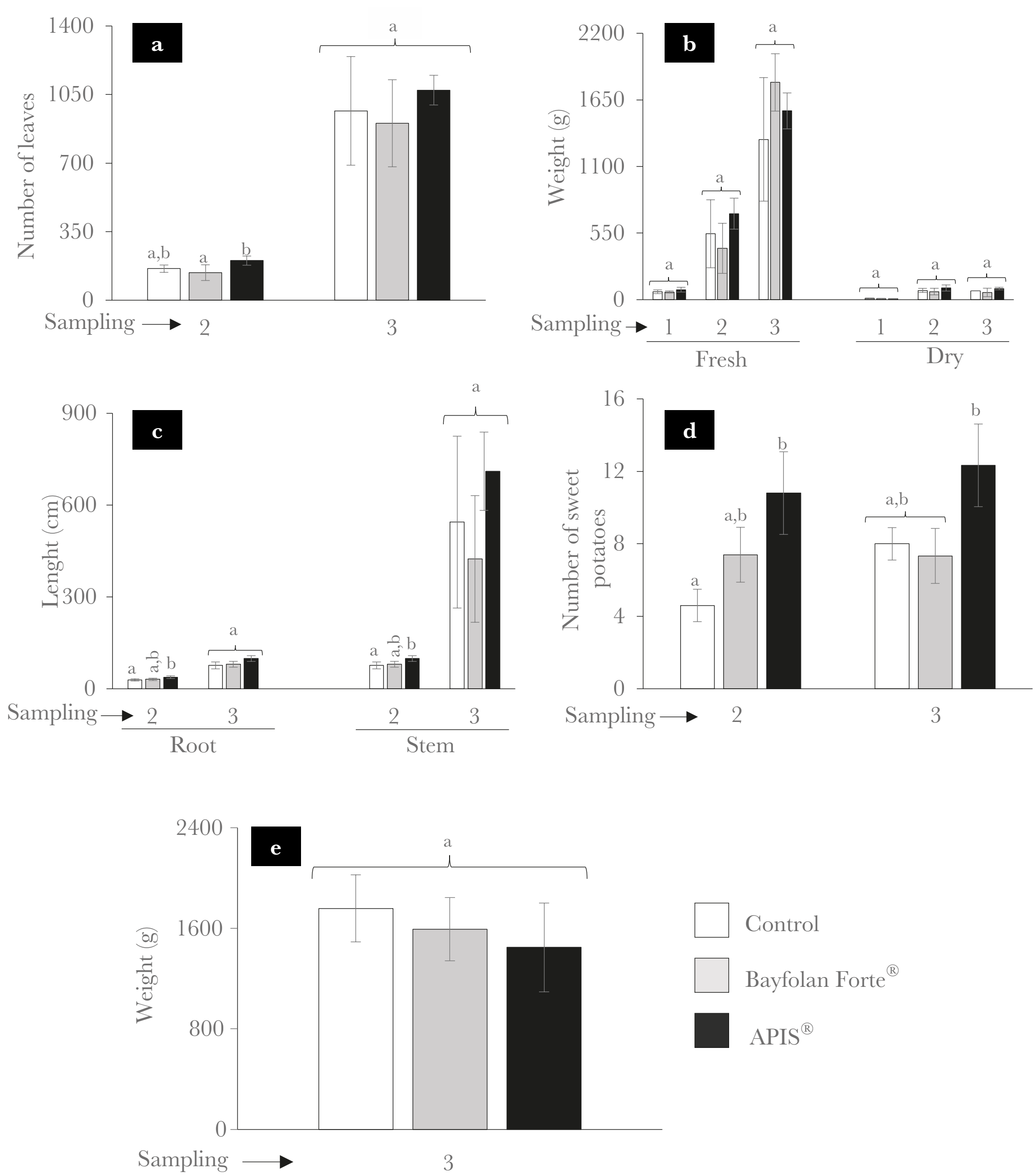


Figure 1. Morphological parameters of sweet potato (*I. batatas*) plants treated with a biofertilizer. (a) Number of leaves; (b) fresh and dry weight; (c) root and stem length; (d) number of tubers per plant; and (e) total tuber weight per plant at different sampling times (35, 60, and 85 days post-transplant, dpt). Bars represent the mean ± standard deviation (n=5). Different letters indicate statistically significant differences according to Tukey's test ($P \leq 0.05$).

longitudinal length, stem length, leaf count, and floret diameter compared to both the control and Bayfolan Forte® treatments.

Although the control treatment produced the lowest number of tubers compared to the APIS® treatment (Figure 1d), the tubers harvested from the control group showed a trend toward a higher average weight, although no statistically significant differences were detected among treatments (Figure 1e). Notably, the tuber weights obtained in this study exceeded those reported by Sakamoto and Suzuki (2020), who evaluated sweet potato production under hydroponic conditions with varying conductivity levels (0.8, 1.4, and 2.6 dS m^{-1}), achieving an average tuber weight of 1,300 g.

Fertilization plays a critical role in sweet potato cultivation, as it can influence either vegetative growth or tuber production (Sakamoto & Suzuki, 2020). In this study, the application of APIS® resulted in a higher number of tubers per plant, translating into a commercial yield of 26.4 t ha^{-1}, compared to 21.6 t ha^{-1} for Bayfolan Forte® and 20 t ha^{-1} for the control (Table 1).

It has been reported that the average yield of sweet potato crops in Mexico is approximately 21.13 t ha^{-1} (SIAP, 2025). In this context, the application of APIS® resulted in a 24.7% increase in yield, while Bayfolan Forte® yielded a 2.3% improvement. Similarly, Rodríguez *et al.* (2023) reported a commercial yield of 26.44 t ha^{-1} using clones derived from stem cuttings of orange-fleshed tuber-producing plants, planted at a density of 50,000 plants ha^{-1} and fertilized with a 60-40-100 N-P-K formulation. Furthermore, Elwaziri *et al.* (2023) documented that foliar application of 0.2% milk protein hydrolysate increased yield from 24 to 32 t ha^{-1} compared to the control. Notably, the combined application of 240 kg ha^{-1} of K_2O with 0.2% milk protein hydrolysate produced yields of up to 36 t ha^{-1}, surpassing the 29 t ha^{-1} obtained with the control treatment. The findings of the present study suggest that applying APIS® could enhance the economic returns for sweet potato producers in Mexico. Based on the minimum market prices reported by the León, Guanajuato central wholesale market on August 11, 2025, projected revenues would be approximately $527,140 MXN ha^{-1} for APIS®, $432,160 MXN ha^{-1} for Bayfolan Forte®, and $399,120 MXN ha^{-1} for the control (SNIIM, 2024). Consequently, the use of APIS® could result in additional earnings of $94,890 MXN compared to Bayfolan Forte® and $128,020 MXN compared to the untreated control.

Microbial load

Regarding microbial load results obtained from the different sweet potato leaf samplings, no statistically significant differences ($P \leq 0.05$) were observed among treatments in the first and third sampling points (Figures 2a and 2b). However, during the second sampling, leaves from plants treated with APIS® exhibited significantly higher levels of both total bacteria and filamentous fungi compared to the control and Bayfolan Forte® treatments ($P \leq 0.05$). This increase may be attributed to the biological nature of APIS®, which is formulated with lactic acid bacteria and yeasts microorganisms that are likely to be isolated and quantified in greater abundance, particularly during the mid-stage of plant development. Additionally, it has been reported that whey contains bioactive peptides with antimicrobial and antioxidant properties, which may play a role in modulating microbial populations (Carrasco & Guerra, 2010). Kalla *et al.* (2021) further observed that exposure of milk to

Table 1. Commercial yield of sweet potato (*I. batatas*) following foliar fertilization treatments.

	Treatment		
	Control	**Bayfolan Forte®**	**APIS®**
Performance (t ha^{-1})	20.0	21.6	26.4

t: tonne; ha: hectare.

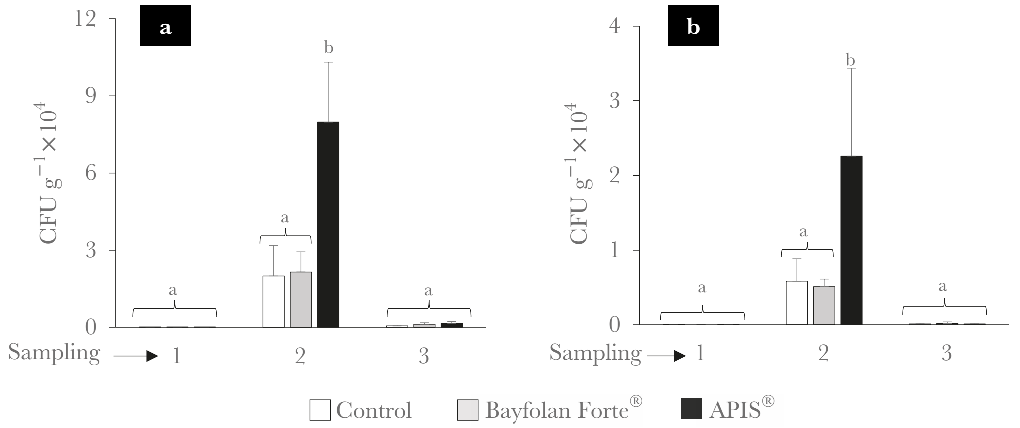


Figure 2. Microbial load in sweet potato leaves. (a) Total bacteria and (b) total fungi at different sampling points (35, 60, and 85 days post-transplanting, dpt). Bars represent the mean ± standard deviation (n=3). Different letters indicate statistically significant differences according to Tukey's test ($P \leq 0.05$).

solar ultraviolet radiation generates superoxide anions $\left(O_2^{-2}\right)$ and reactive oxygen species, which can disrupt the cell membranes of fungal pathogens such as *Phytophthora infestans*. Similarly, Chee *et al.* (2018) reported that the application of anhydrous milk fat effectively controlled downy mildew caused by *Sphaerotheca fuliginea* in various squash cultivars, outperforming commercial fungicides such as Bravo® 720 (Syngenta) and Kumulus®. These findings may explain the subsequent decline in microbial load both bacterial and fungal observed during the third sampling (Figure 2).

Elemental Parameters

Whey is an excellent source of macro- and micronutrients that can be utilized as a biofertilizer to support plant development (Akay & Sert, 2020). The elements carbon, hydrogen, oxygen, nitrogen, and sulfur (CHONS) are essential to all life forms and constitute the fundamental biomolecules required for plant growth, development, and reproduction (Monib *et al.*, 2023). For instance, carbon serves as a structural component in plants and is involved in the biosynthesis of defense-related compounds such as phenols and terpenes (Xing *et al.*, 2021). Nitrogen is vital for physiological processes including photosynthesis and biomolecule synthesis (Xing *et al.*, 2021). Sulfur, although required in smaller quantities, is essential for chlorophyll formation, protein synthesis, oil production, and amino acid biosynthesis (Monib *et al.*, 2023). The analysis of these elemental components and their interactions is critical for understanding plant nutrition and implementing effective crop management practices. In the present study, the concentrations of carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) in sweet potato leaves did not show statistically significant differences ($P \leq 0.05$) among the experimental treatments applied (Figures 3a-d). Carbon and hydrogen levels remained relatively stable across the three sampling periods. In contrast, some variation was observed in nitrogen and sulfur concentrations over time, although these were not statistically significant ($P \leq 0.05$). Garcia *et al.* (2022), in their study applying APIS® to broccoli plants at various phenological stages, reported that C and H concentrations remained constant over time,

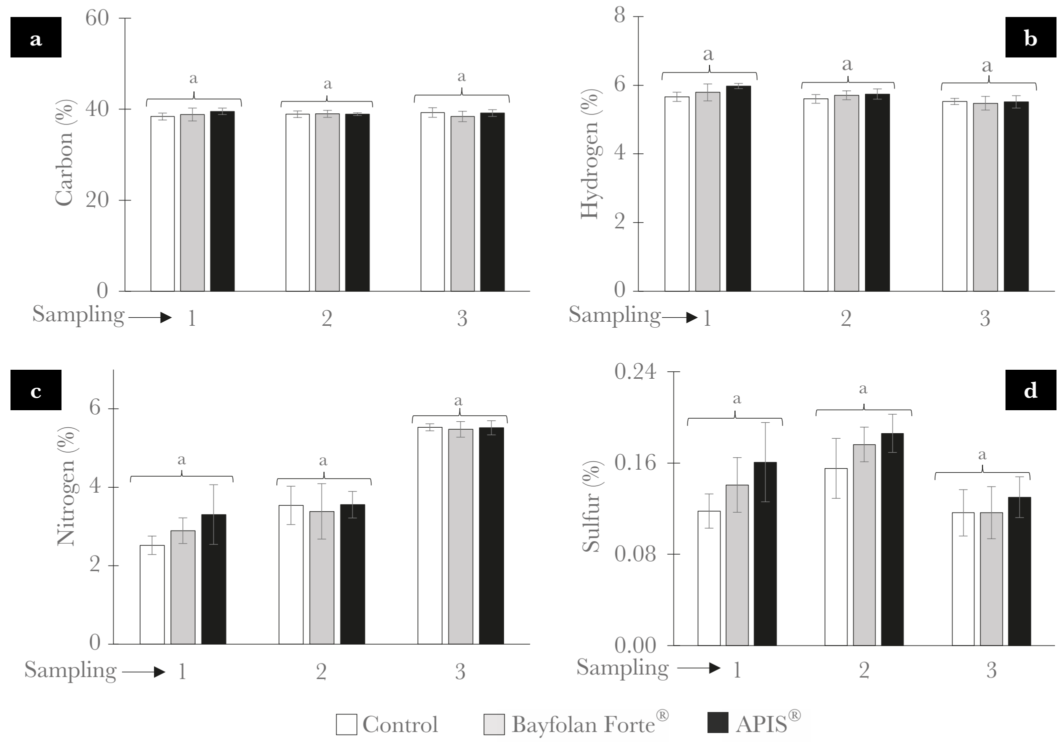


Figure 3. Elemental composition (%) in sweet potato leaves. (a) Carbon, (b) Hydrogen, (c) Nitrogen, and (d) Sulfur at different sampling times (35, 60, and 85 days post-transplanting, dpt). Bars represent the mean ± standard deviation (n=3). Different letters indicate statistically significant differences according to Tukey's test ($P \leq 0.05$).

while statistically significant differences ($P \leq 0.05$) were observed in N and S content across the three sampling periods.

Total chlorophyll and soluble protein

Total chlorophyll content increased over time (Figure 4a), although no statistically significant differences were observed among treatments ($P \leq 0.05$). The APIS® treatment recorded the highest chlorophyll concentration, followed by Bayfolan Forte® and the control, with values of 206.0, 177.8, and 170.2 mg chlorophyll g^{-1} FW, respectively. Variations in chlorophyll levels among plants may be attributed to factors such as cultivar differences, altitude, fertilization type, and the crop›s nutrient assimilation efficiency (Milenković *et al.*, 2024). In contrast, soluble protein concentration declined at 60 dpt following the first sampling but increased again at 85 dpt, with no statistically significant differences among treatments (Figure 4b). Proteins are essential macromolecules composed of amino acids that play key roles in enzymatic activity, structural integrity, storage, photosynthesis, biosynthesis, transport, and in defense mechanisms against pathogens or abiotic stress factors (Rasheed *et al.*, 2020).

Total polyphenols, soluble sugars, and hydrogen peroxide

Previous studies have demonstrated that sweet potato leaves are an excellent source of antioxidant compounds, such as caffeic acid and caffeoylquinic acids, which not only

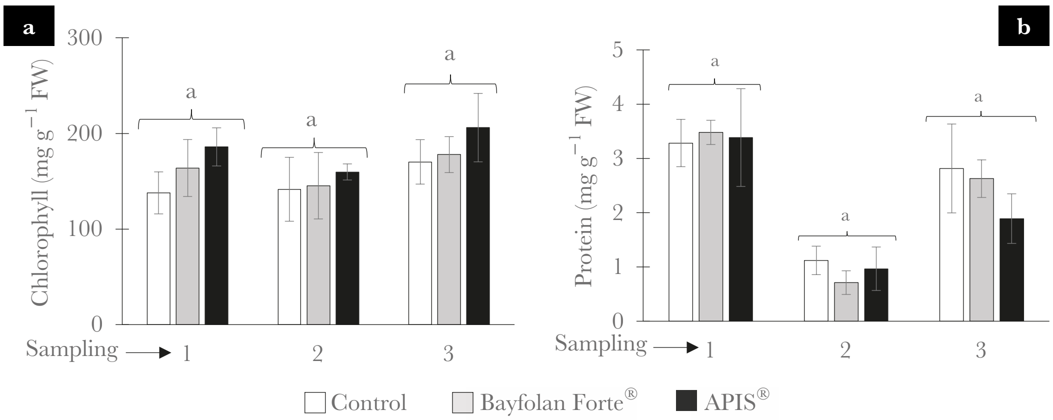


Figure 4. Concentration of biomolecules in sweet potato (*I. batatas*) leaves. (a) Total chlorophyll and (b) total protein at different sampling times (35, 60, and 85 days post-transplanting, dpt). Bars represent the mean ± standard deviation (n=3). Different letters indicate statistically significant differences according to Tukey's test ($P \leq 0.05$).

exhibit strong antioxidant capacity but also possess remarkable bioactive properties including anti-inflammatory, antimicrobial, hepatoprotective, cardioprotective, LDL oxidation-preventive, and neuroprotective effects (Sasaki *et al.*, 2015).

In the present study, the highest concentrations of total polyphenols were observed during the first and third sampling periods (Figure 5a). Notably, at 35 dpt, both nutrient treatments APIS® and Bayfolan Forte® led to significantly higher polyphenol levels compared to the untreated control ($P \leq 0.05$), whereas no significant differences were detected among treatments at 60 and 85 dpt. Ghasemzadeh *et al.* (2012) reported that total polyphenol content in leaves of six *I. batatas* varieties ranged from 4.47 to 8.11 mg gallic acid g^{-1} DW. In contrast, this study recorded higher concentrations, ranging from 9.44 to 20.28 mg gallic acid g^{-1} FW, induced by APIS® and Bayfolan Forte®, respectively.

Another class of biomolecules involved in cellular maintenance are soluble sugars (Figure 5b). These play multiple roles in plants, including energy storage, growth, nutrient signaling, membrane and macromolecule stability, osmotic potential regulation, chlorophyll pigment stabilization, and defense mechanisms, as well as the mitigation of reactive oxygen species (ROS) (Kitayama *et al.*, 2020; Savchenko & Tikhonov, 2021). The highest concentration of soluble sugars was recorded at the third sampling in plants treated with APIS® (24.62 mg g^{-1} FW), which was statistically higher ($P \leq 0.05$) than those of the control and Bayfolan Forte® treatments. Kitayama *et al.* (2020) reported total soluble sugar levels in leaves of *I. batatas* varieties "Japanese Yellow" and "Blackie" at 80 and 20 mg g^{-1} DW, respectively.

Finally, the highest concentration of hydrogen peroxide (H_2O_2) was observed in the third sampling for plants treated with Bayfolan Forte® (503.0 μM g^{-1} FW), followed by the control (402.06 μM g^{-1} FW) and APIS® (381.5 μM g^{-1} FW), though no statistically significant differences were found among treatments (Figure 5c). It is well-established that plant cells continuously generate basal levels of ROS, which are non-toxic and, in coordination with antioxidant molecules, participate in signaling processes that

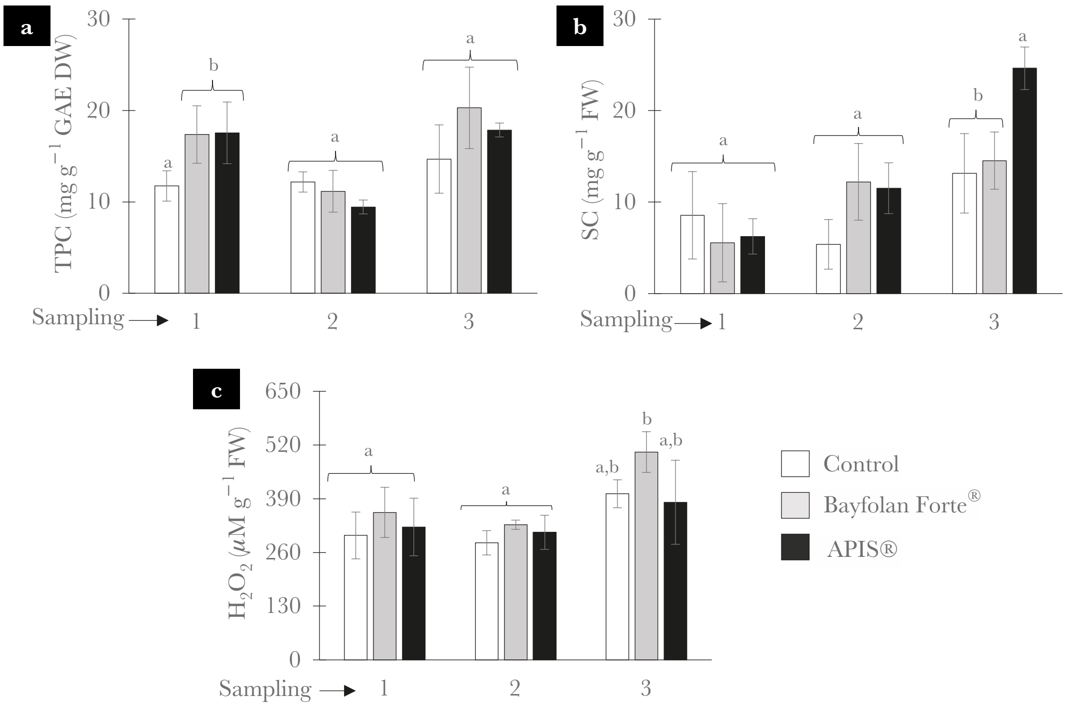


Figure 5. Biochemical stress markers in sweet potato (*I. batatas*). (a) Total polyphenols (TPC), (b) Sugar concentration (SC), and (c) Hydrogen peroxide (H_2O_2) at different sampling times (35, 60, and 85 days post-transplanting, dpt). Bars represent the mean ± standard deviation (n=3). Different letters indicate statistically significant differences according to Tukey's test ($P \leq 0.05$). Total Polyphenols Concentration (TPC) reported as Gallic Acid Equivalents (GAE) in Dry Weight (DW). Sugar Concentration (SC) from Fresh Weight (FW).

regulate gene expression under various biotic and abiotic stress conditions (Sperdouli *et al.*, 2022).

CONCLUSIONS

The whey-based biofertilizer demonstrated comparable effects to the commercial fertilizer Bayfolan Forte® in the morphological variables evaluated during the third sampling. The elemental composition (C, H, N, and S) was statistically similar between treatments with APIS® and Bayfolan Forte®. APIS® application contributed to increased tuber yield, while Bayfolan Forte® enhanced the number of tubers produced. The total bacterial and filamentous fungal load in sweet potato leaves treated with APIS® increased during the second sampling but declined by the third. Total chlorophyll and protein concentrations remained consistent across all treatments. However, polyphenol content was elevated in APIS® and Bayfolan Forte® treatments during the first sampling. The highest soluble sugar concentration was recorded in the APIS® treatment at the third sampling, while H_2O_2 levels remained statistically unchanged among treatments.

The APIS® biofertilizer is a viable alternative for sweet potato (*I. batatas*) cultivation, offering an environmentally friendly option that can contribute to reducing agricultural production costs. Further research is warranted to assess the combined use of whey-based biofertilizer with other organic and biological products.

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Colegio de Postgraduados

Evaluation of different salinities in polyculture of tilapia *Oreochromis* sp., and white shrimp *Litopenaeus vannamei* in a biofloc system

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ABSTRACT

Objective: Polyculture in aquaculture is considered an effective strategy for improving production yields per unit area, and the use of biofloc systems represents a viable alternative for mitigating the negative environmental impacts associated with aquaculture discharges. This study evaluated the effect of different salinities on a polyculture of *Litopenaeus vannamei* post-larvae and *Oreochromis* sp. hatchling in a biofloc system.

Design/Methodology/approach: The experiment lasted 60 days, during which, four different salinities (T1=5 g/L, T2=10 g/L, T3=15 g/L, T4=20 g/L) were tested in triplicate, in addition to a control treatment (TC=2 g/L). The stocking density was 60 tilapia fry and 20 white shrimp post-larvae per treatment. The parameters evaluated included growth (weight and length) and survival of both organisms.

Results: Regarding growth, no significant differences were observed among treatments for any of the species ($p>0.05$). The average growth values for tilapia were 8.51 ± 2.76 g and 5.92 ± 0.85 cm, and for shrimp 1.73 ± 2.55 g and 5.84 ± 1.98 cm, with no differences among treatments. However, significant differences were found in survival. Treatments T1 (5 g/l) and T2 (10 g/l) showed higher survival for tilapia (96.43 ± 1.19%) and shrimp (90.97 ± 1.69%) compared to T3 (15 g/l) and T4 (20 g/l), which showed lower survival (tilapia: 73.10 ± 5.11%, shrimp: 74.47 ± 15.65%). The control treatment (CT) showed similar survival to treatments T1 and T2 for both species ($p<0.05$).

Limitations on study/implications: The scale of the study, conducted in experimental tanks, could limit the direct extrapolation of the results to larger-scale commercial conditions.

Findings/conclusions: The results highlight the effectiveness of biofloc systems in combination with polycultures, especially under low to moderate salinity conditions, as a sustainable, economical, and efficient strategy for aquaculture production.

Keywords: polyculture, biofloc, post-larva, survival.

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INTRODUCTION

Global fisheries and aquaculture production reached 223.2 million tons in 2022, comprising 185.4 million tons of aquatic animals and 37.8 million tons of algae. Notably, for the first time, aquaculture production (94.4 million tons) surpassed that of extractive fisheries (92.3 million tons), accounting for 51% of the total. Approximately 89% of aquatic

animal output is directed toward direct human consumption, underscoring its critical role in global food security. In the same year, around 61.8 million individuals were employed in the fisheries and aquaculture sector, which remains vital to the livelihoods of millions worldwide. According to projections by the Food and Agriculture Organization (FAO, 2024), aquatic animal production is expected to increase by 10% by 2032, reaching 205 million tons, primarily driven by the expansion of aquaculture and the recovery of extractive fisheries. Nonetheless, a key challenge in aquaculture lies in managing water quality within production ponds, where issues are often addressed through routine water replacement. This approach, however, leads to increased operational costs, significant water wastage, and environmental degradation in receiving ecosystems. In light of rising global demand for aquaculture products, there is an urgent need to refine production practices to enhance sustainability, ensure social acceptability, and safeguard food security (CONAPESCA, 2022). Within this framework, polyculture emerges as a strategic approach that enhances the natural productivity of ponds and the water column. Effective implementation, however, necessitates a thorough evaluation of species based on their feeding habits, behavior, environmental adaptability, and pond productivity to identify the most profitable combinations (Luján Monja, 2010). The application of biofloc technology (BFT) has also gained prominence as a sustainable alternative, addressing environmental concerns linked to effluent discharge and the industry's reliance on fishmeal and fish oil (De Schryver *et al.*, 2008). Biofloc systems are composed of aggregates of microalgae, bacteria, protozoa, and other particulate organic matter as feces and uneaten feed which collectively enhance environmental control in production systems and help prevent disease outbreaks in intensive aquaculture (De Schryver *et al.*, 2008). These systems promote the bioconversion of organic matter, thereby improving water quality and fostering a healthier environment for aquatic organisms. In Ecuador, tilapia has demonstrated strong adaptability to brackish water aquaculture due to its robust physiological resilience, enabling trials in polyculture with the white shrimp *Litopenaeus vannamei*. This approach has emerged as a promising strategy to mitigate mortality caused by White Spot Virus (WSV). Tilapia has proven beneficial by enhancing the bioecological conditions of the culture environment and reducing horizontal disease transmission (Massaut & Rodríguez-Grimón, 2004). Prior studies indicate that tilapia, which thrives under euryhaline conditions, exhibits rapid growth and favorable performance in captivity qualities that make it an ideal candidate for shrimp polyculture. Moreover, its omnivorous diet, adaptability to formulated feeds, and growing international market demand further enhance its appeal. This study aims to evaluate the effects of varying salinity levels on the polyculture of tilapia (*Oreochromis* sp.) and white shrimp (*Litopenaeus vannamei*) within a biofloc system, with the objective of identifying optimal conditions to maximize production efficiency and sustainability in aquaculture.

MATERIALS AND METHODS

Study location

This study was conducted at the Boca del Río Technological Institute (IT-BOCA), located in the municipality of Boca del Río, Veracruz, Mexico. Specifically, the research took place in the LIAA Aquaculture Laboratory, situated at 19° 05’ 48.33” N and 96° 06’ 30.20” W, at an elevation of 8.0 meters above sea level.

Experimental design and system construction

A biofloc system was employed to evaluate the polyculture of *Litopenaeus vannamei* post-larvae, with an average total length and weight of 3.2±0.3 cm and 0.277±0.008 g (mean ± SD), respectively, and *Oreochromis* sp. fry, with an average total length and weight of 1.5±0.7 cm and 0.113±0.004 g (mean ± SD), respectively. Four salinity treatments were tested in triplicate (T1=5 g/L, T2=10 g/L, T3=15 g/L, T4=20 g/L), along with a control treatment (TC=2 g/L), using plastic tanks with a working volume of 200 L. Each tank represented an independent experimental unit, enabling full treatment replication. Stocking density was maintained at 60 tilapia fingerlings and 20 shrimp post-larvae per tank. Continuous aeration was provided through a 1.27 cm diameter PVC pipe per tank, connected to a 1.9 cm diameter aeration line with flow control valves, all powered by a 2 HP Pioneer® blower linked to a 3.81 cm diameter PVC main line. Water was sourced from two 10,000 L reservoirs on-site: Reservoir 1 held water at 30 g/L salinity, and Reservoir 2 contained freshwater (0 g/L).

Animal acquisition

Tilapia fry (*Oreochromis* sp.) were obtained from the LIAA laboratory at IT-BOCA (60 individuals), while 20 *L. vannamei* post-larvae were acquired from a commercial shrimp hatchery located along Mexico Highway 150 and the Gulf Coast Highway, in Veracruz-Minatitlán. Specimens were randomly assigned to experimental units. Prior to introduction, animals were acclimated for 20 minutes per replicate, ensuring equal water temperatures between the source and destination environments to avoid thermal shock.

Feeding regime

Three feed particle sizes were utilized throughout the study: powdered feed, 1.0 mm pellets, and 2.0 mm pellets. During the first 30 days, powdered feed and 1.0 mm pellets were offered; in the subsequent 30 days, only 2.0 mm pellets were used. The commercial diets included Purina® powder feed (45% protein, 15% fat) and Bio Fingerling® pellets (35% protein, 15% fat), administered twice daily (09:00 and 17:00 h). A fixed feeding rate of 15% of the estimated biomass was established at the outset, in alignment with the metabolic requirements of the juvenile stages (El-Sayed, 2006). However, the feeding rate was not adjusted during the experiment, and no detailed monitoring of feed intake or waste was conducted.

Molasses supplementation

To maintain a carbon-to-nitrogen (C:N) ratio of 14:1 in the culture water, molasses was added biweekly to all tanks (including controls). The supplementation rate followed the recommendations of Asaduzzaman *et al.* (2008).

Water quality monitoring

Water quality parameters were assessed daily at 09:00 h using a YSI 560 multiparameter instrument (±0.1 °C precision) to measure temperature (°C), salinity (g/L), dissolved oxygen (mg/L), and pH. Concentrations of ammonia (NH_3), nitrite (NO_2^-), and nitrate (NO_3^-) were measured every three days via colorimetric testing. No water exchange was performed during the experiment; only evaporative losses were replenished every five days.

Growth assessment

Growth performance was evaluated biweekly. In each sampling round, 20 tilapia and 10 shrimp were randomly selected per treatment (TC, T1, T2, T3, T4). Specimens were gently dried on a cloth and measured using an ichthyometer (precision ±0.1 mm) and an Ohaus® analytical balance (precision ±0.0001 g). Total length measurements were taken from the rostrum to the telson for shrimp and from the mouth to the caudal fin for tilapia. Sampling was non-destructive, although individual tracking was not possible. Nevertheless, the sample size was statistically representative (Hendrickx, 1995).

Survival analysis

Survival rate (%) was calculated at the conclusion of the study using the formula:

$$Survival(\%) = \left[\left(Initial\ number - Final\ number \right) / \ Inital\ number \right] \times 100$$ (Utne, 1979).

Primary productivity assessment

To analyze biofloc composition, samples were collected biweekly. For phytoplankton analysis, 1 mL samples were observed under an Olympus ZSX50 microscope (100x objective) following Azim & Little (2008). For zooplankton (ciliates, rotifers, nematodes), 10 mL samples were preserved in 5% formalin and analyzed under a stereoscopic microscope. Taxonomic identification was performed to the genus level (Aladro Lubel, 2009). Organism density was estimated using a Neubauer chamber, counting 20 fields of view per sample, and results were expressed as cells/mL.

Determination of settleable solids

Biofloc volume was measured using Imhoff cones. A 1 L homogeneous water sample from each tank was transferred to the cones and allowed to settle for 20 minutes. The settled biofloc volume was then recorded (Avnimelech, 2009).

Health status evaluation

To monitor the health of cultured organisms, five tilapia and three shrimp per tank were collected every 30 days and euthanized via rapid freezing. External and internal examinations were conducted under a 10x stereoscopic microscope (Leica Zoom 2000, Switzerland) to detect melanized lesions or external parasites. Gills and muscle tissues were also examined under a 10x optical microscope (Leica MCE, Switzerland) for signs of necrosis or discoloration indicative of stress or disease (Bruce A, 2002).

Table 1. Summary table of the experimental design (species, treatments, density, replication).

Treatment	Species involved	Stocking density	Replication
T1	*L. vannamei* / *Oreochromis* sp.	60 tilapia+20 shrimp	Triplicate
T2	*L. vannamei* / *Oreochromis* sp.	60 tilapia+20 shrimp	Triplicate
T3	*L. vannamei* / *Oreochromis* sp.	60 tilapia+20 shrimp	Triplicate
T4	*L. vannamei* / *Oreochromis* sp.	60 tilapia+20 shrimp	Triplicate
TC (control)	*L. vannamei* / *Oreochromis* sp.	60 tilapia+20 shrimp	Triplicate

Table 2. Survival rates (%) of *Litopenaeus vannamei* in polyculture with *Oreochromis* sp. (mean ± standard deviation, n=3) under five salinity treatments: 2 g/L (control, TC), 5 g/L (T1), 10 g/L (T2), 15 g/L (T3), and 20 g/L (T4).

Species	TC	T1	T2	T3	T4
L. vannamei	92.00 ± 1.01^{a}	93.65 ± 1.22^{a}	88.30 ± 2.34^{a}	82.30 ± 2.45^{a}	66.6 ± 2.88^{b}
Oreochromis sp.	93.76 ± 1.63^{a}	96.21 ± 1.44^{a}	96.66 ± 1.39^{a}	75.66 ± 2.86^{b}	70.5 ± 2.92^{b}

Means with different superscripts within a row are statistically different ($p<0.05$).

RESULTS

Survival

In *Litopenaeus vannamei*, treatments T1 (5 g/L) and T2 (10 g/L) exhibited the highest survival rates, reaching 90.97±1.69% and 88.45±2.33%, respectively. These values were statistically superior ($p<0.05$) to those observed in treatments T3 (15 g/L), T4 (20 g/L), and the control (TC, 2 g/L), which ranged between 70% and 75% (Figure 1). These results indicate that moderate salinities (5-10 g/L) enhance shrimp viability in polyculture systems operating under biofloc technology. A similar trend was observed in *Oreochromis* sp., where treatments T1 and T2 yielded significantly higher survival rates (96.43±1.19%

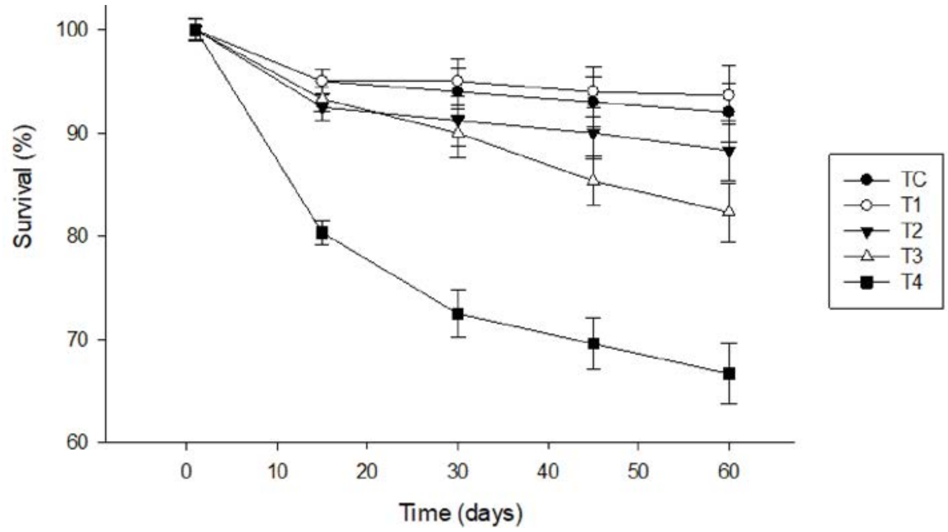

Figure 1. Survival rates (mean ± standard deviation, n=3) of *Litopenaeus vannamei* post-larvae cultured over 60 days in polyculture with *Oreochromis* sp. across five salinity levels: 2 g/L (control, TC), 5 g/L (T1), 10 g/L (T2), 15 g/L (T3), and 20 g/L (T4).

and 94.87±2.01%, respectively) compared to T3, T4, and the control group ($p<0.05$) (Figure 2). This finding suggests that although tilapia is a euryhaline species, salinity levels exceeding 15 g/L may induce osmotic stress, adversely affecting survival under biofloc culture conditions.

Growth

As shown in Tables 3 and 4, no statistically significant differences ($p>0.05$) were observed among treatments for either species. *Oreochromis* sp. attained an average final weight of 8.51±2.76 g and a length of 5.92 cm, while *Litopenaeus vannamei* reached an average weight of 1.73±2.55 g and a length of 5.84±1.98 cm. Although these differences

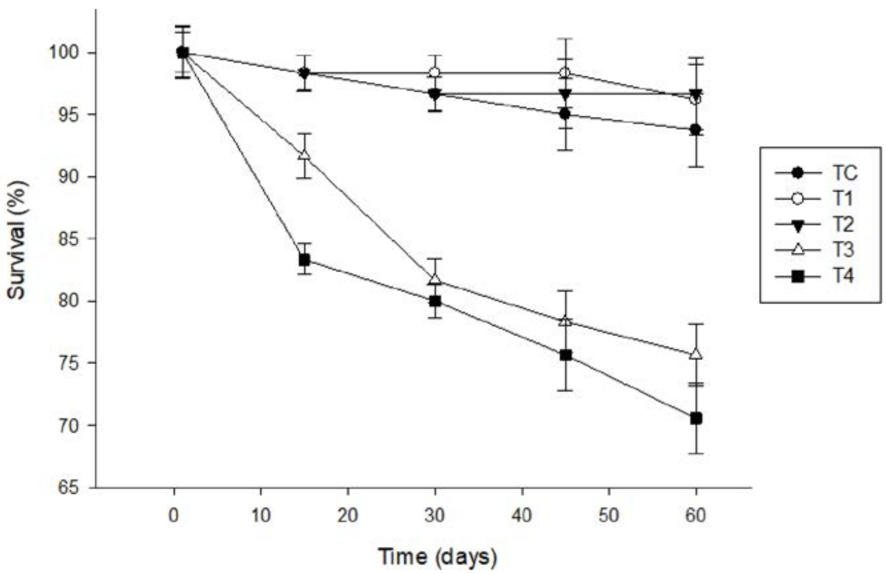

Figure 2. Survival rates (mean ± standard deviation, n=3) of *Oreochromis* sp. fry cultured for 60 days in polyculture with *Litopenaeus vannamei* under five salinity conditions: 2 g/L (control, TC), 5 g/L (T1), 10 g/L (T2), 15 g/L (T3), and 20 g/L (T4).

Table 3. Growth performance of *Oreochromis* sp. (mean ± standard deviation, n=3) cultured under five salinity treatments: 2 g/L (control, TC), 5 g/L (T1), 10 g/L (T2), 15 g/L (T3), and 20 g/L (T4).

	TC	T1	T2	T3	T4
Growth (weight) *Oreochromis* sp.	8.95±1.33[a]	9.91±1.45[a]	8.76±1.29[a]	7.86±1.94[a]	7.07±2.23[a]
Growth (length) *Oreochromis* sp.	6.00±1.54[a]	6.17±1.39[a]	5.98±1.68[a]	5.82±1.88[a]	5.65±2.36[a]

Means with different superscripts within a row are statistically different ($p<0.05$).

Table 4. Growth performance of *Litopenaeus vannamei* (mean ± standard deviation, n=3) cultured under five salinity treatments: 2 g/L (control, TC), 5 g/L (T1), 10 g/L (T2), 15 g/L (T3), and 20 g/L (T4).

	TC	T1	T2	T3	T4
Growth (weight) *L. vannamei*	2.00±1.27[a]	1.87±1.56[a]	1.76±1.43[a]	1.69±2.15[a]	1.35±2.12[a]
Growth (length) *L. vannamei*	6.26±1.83[a]	5.98±1.44[a]	5.92±1.35[a]	5.77±1.79[a]	5.31±1.99[a]

Means with different superscripts within a row are statistically different ($p<0.05$).

were not statistically significant, there was a slight tendency for improved growth in lower salinity treatments (TC, T1, and T2), potentially due to enhanced feed conversion efficiency and reduced osmotic stress. These findings partially support the hypothesis that salinity influences the performance of species cultured in polyculture under biofloc technology. While growth was not significantly affected, survival exhibited a clear salinity-dependent pattern, with optimal outcomes observed at moderate salinities (5-10 g/L). This supports the use of polyculture systems with moderate salinity levels as a sustainable strategy for maximizing productivity in biofloc-based aquaculture.

Water quality

Average water quality parameters recorded throughout the polyculture period are presented in Table 3. These values remained generally stable within each treatment. Primary productivity, particularly of diatoms, rotifers, and cladocerans, was notably higher in treatments T1, T2, and TC. The mean diatom density for these treatments was 77,895.00±6,321.14 cells/mL, and productivity remained consistent throughout the experimental period. The highest diatom density was observed in the control treatment (TC) during the first month, reaching 109,288.00±1,125.12 cells/mL. In contrast, significantly lower diatom densities were recorded in treatments T3 and T4, averaging 31,246.19±5,335.54 cells/mL and 25,575.99±3,791.11 cells/mL, respectively. These values were markedly lower than those in T1 and T2, which registered 74,523.49±3,142.24 cells/mL and 65,879.41±4,238.97 cells/mL, respectively. Throughout the experimental period, water transparency declined significantly across all treatments, as measured using a Secchi disk. Initial transparency levels ranged between 30 and 35 cm; however, by the end of the experiment, values had decreased to between 10 and 15 cm. This reduction in transparency corresponded with an increase in microbial biomass and a visible shift in water coloration from green to brown indicative of elevated suspended solids and active biofloc formation, characteristic of BFT systems.

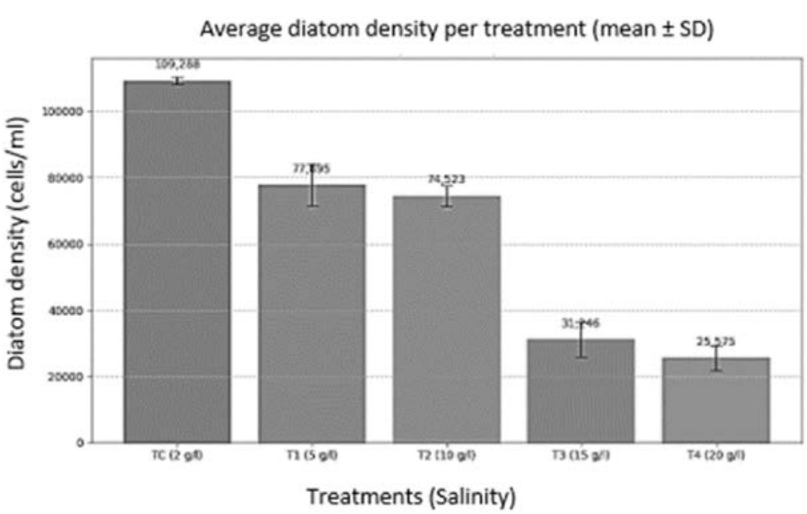

Figure 3. Average diatom density (cells/mL) per treatment during the polyculture of *Litopenaeus vannamei* and *Oreochromis* sp. in a biofloc system.

Table 5. Physicochemical variables (mean ± SD per treatment) evaluated during the study.

Treatment	Temperature (°C)	Salinity (gL^{-1})	O_2 ($mg\ L^{-1}$)	pH	Ammonium ($mg\ L^{-1}$)
TC	28.14 ± 0.26^a	2.98 ± 1.46^b	6.97 ± 1.55^a	8.54 ± 0.34^a	0.1 ± 0.01^a
T1	28.38 ± 0.53^a	5.57 ± 0.27^b	6.88 ± 1.56^a	8.49 ± 0.37^a	0.1 ± 0.01^a
T2	28.26 ± 0.52^a	9.90 ± 1.36^b	6.70 ± 1.53^a	8.62 ± 0.32^a	0.1 ± 0.01^a
T3	28.43 ± 0.59^a	14.52 ± 3.16^b	6.68 ± 1.61^a	8.55 ± 0.36^a	0.1 ± 0.01^a
T4	29.10 ± 0.22^a	18.81 ± 4.85^b	6.47 ± 1.56^a	8.49 ± 0.24^a	0.1 ± 0.01^a
Optimal	28-30 °C	6-9	5-9	6.5-9	<0.1

Means with different superscripts within a row are statistically different ($p < 0.05$).

Although ammonium concentrations remained stable across all treatments (0.10 ± 0.01 $mg\ L^{-1}$), pH values ranged from 8.49 to 8.62. Under these conditions, it is important to consider that total ammonium (NH_4^+) can be converted to un-ionized ammonia (NH_3) in biofloc systems, especially at elevated pH levels. This is particularly relevant in treatments such as T2, where pH reached 8.62, potentially increasing the proportion of toxic NH_3. Despite the low total ammonium concentrations, the interaction between high pH and ammonium could pose a risk of ammonia toxicity. Although no negative effects on growth or survival were observed during this study, this interaction warrants close monitoring in longer-term or higher-density commercial applications.

Health status of the organisms

The results revealed a clear increase in health anomalies at higher salinities, particularly in treatments T3 and T4. Both fish and shrimp exhibited early signs of physiological stress, including gill necrosis and melanized lesions. In contrast, no visible pathologies were observed in treatments TC, T1, and T2, indicating that lower salinities (2-10 $g\ L^{-1}$) are more conducive to maintaining organismal health under biofloc conditions. The increased incidence of external parasites and tissue necrosis in the higher salinity treatments may be attributed to osmotic stress and a consequent reduction in immune response, as previously documented in saline or high-density polyculture systems. These findings underscore the importance of optimizing salinity levels to preserve the physiological integrity and welfare of aquatic species cultured in biofloc systems.

Table 6. BTC=Total harvested biomass; AT=Total feed; TCAA=Apparent feed conversion ratio.

Treatment	*Oreochromis* sp.			*L. vannamei*		
	BTC (kg)	AT (kg)	TCAA	BTC (kg)	AT (kg)	TCAA
TC	1.6 ± 0.21^a	1.5 ± 0.00^a	0.94 ± 0.48^a	0.06 ± 0.27^a	0.18 ± 0.00^a	2.72 ± 0.41^a
T1	1.8 ± 0.25^a	1.5 ± 0.00^a	0.83 ± 0.37^a	0.07 ± 0.23^a	0.18 ± 0.00^a	2.43 ± 0.29^a
T2	1.4 ± 0.33^a	1.5 ± 0.00^a	1.07 ± 0.64^a	0.06 ± 0.33^a	0.18 ± 0.00^a	2.90 ± 0.38^a
T3	0.81 ± 0.38^a	1.5 ± 0.00^a	1.85 ± 0.55^a	0.05 ± 0.22^b	0.18 ± 0.00^a	3.52 ± 0.26^a
T4	0.58 ± 0.29^a	1.5 ± 0.00^a	2.58 ± 0.93^a	0.02 ± 0.31^b	0.18 ± 0.00^a	7.5 ± 0.79^b

Means with different superscripts within a row are statistically different ($p < 0.05$).

This study confirms the viability of polyculture involving *Litopenaeus vannamei* and *Oreochromis* sp. in biofloc systems under low-salinity conditions, demonstrating its potential as a sustainable and efficient aquaculture strategy. The significantly higher survival rates observed at 5 g L^{-1} and 10 g/L (T1 and T2) indicate that these salinity levels provide optimal physiological conditions for both species (Li *et al.*, 2008; Verdegem *et al.*, 2008). Although no statistically significant differences in growth were detected across treatments, marked differences in survival and health were evident, particularly in T3 and T4 (15 and 20 g L^{-1}), where a higher incidence of lesions, tissue necrosis, and reduced phytoplankton density were observed. These findings suggest that elevated salinities may induce sublethal osmotic stress, compromising immune function, as previously reported (Van Wyk & Scarpa, 1999; Verdegem *et al.*, 2008). Additionally, a significant difference in primary productivity was recorded, with higher values in treatments T1, T2, and TC, indicating that moderate salinities promote phytoplankton particularly diatom growth. This enhanced productivity likely contributes to increased availability of natural food, potentially supporting better feed conversion and survival, although these relationships were not directly quantified (Azim & Little, 2008; Martínez-Córdova *et al.*, 2015). Despite consistently optimal levels of dissolved oxygen, temperature, and pH, ammonium concentrations remained low ($<$0.1 mg/L) across all treatments, demonstrating the efficacy of biofloc systems in nutrient remediation (Avnimelech, 2009; De Schryver *et al.*, 2008). This aligns with the findings of Avnimelech (2009), who highlighted the role of microbial communities in metabolizing nitrogenous waste, thereby improving water quality. However, the interaction between pH and ammonium warrants further investigation, particularly given that alkaline conditions ($>$8.4 pH) can favor the formation of toxic un-ionized ammonia (NH_3). The lack of observed toxicity may be attributed to the rapid assimilation of ammonium by heterotrophic bacteria within the biofloc, as previously reported (Crab *et al.*, 2012). The observed differences in health and survival may be linked to the physiological demands imposed by salinity stress. Tilapia expend greater energy to maintain osmotic homeostasis at salinities exceeding 10 g/L, potentially compromising growth and immune function (Li *et al.*, 2008; Suresh & Lin, 1992). Moreover, salinity-induced changes in the microbial composition of the biofloc may have affected the availability and nutritional quality of microbial feed, thus influencing metabolic efficiency (Ekasari *et al.*, 2015). One notable limitation of this study is its small experimental scale (200 L tanks), which, while allowing for controlled conditions, may limit extrapolation to commercial-scale operations, where variables such as water flow, organic loading, and climatic fluctuations can significantly influence biofloc dynamics and species interactions (Martínez-Porchas & Martínez-Córdova, 2012). Additionally, an economic assessment was not included, which would be crucial for evaluating the financial viability of this system under real-world production conditions.

The findings suggest that a salinity level of 5 g L^{-1} is optimal for operating tilapia-shrimp polycultures in biofloc systems. This salinity supports high survival and health in both species (Li *et al.*, 2008; Verdegem *et al.*, 2008), maintains elevated primary productivity (Azim & Little, 2008), and enables efficient biofloc operation without water exchange (Avnimelech, 2009; Ekasari *et al.*, 2015). From an operational standpoint, maintaining salinities around 5 g L^{-1} is feasible in regions with access to both freshwater and brackish water, allowing for salinity adjustment without high salt concentrations thereby reducing both economic and environmental costs. Nonetheless, pilot-scale trials on commercial farms are recommended to validate these findings under production conditions (Crab *et al.*, 2012; De Schryver *et al.*, 2008).

The findings of this study confirm that the polyculture of *Litopenaeus vannamei* and *Oreochromis* sp. in biofloc systems under low to moderate salinity conditions (≤ 10 g L^{-1}) represents a viable and promising strategy for advancing more sustainable aquaculture practices. Elevated survival rates and enhanced primary productivity particularly diatom abundance were observed under these conditions, indicating that salinity directly influences both the physiological resilience of the cultured species and the availability of natural food resources within the system. Nevertheless, it is important to underscore that these results are derived from a pilot-scale experiment (200 L tanks) conducted under controlled laboratory conditions. As such, caution should be exercised in extrapolating the findings to commercial operations without further validation. Future studies should account for variables such as stocking density, environmental fluctuations, and economic feasibility.

For practical application, a salinity range of 5 to 10 g L^{-1} is recommended for polyculture of white shrimp (*L. vannamei*) and tilapia (*Oreochromis* sp.) in biofloc systems. This range appears to optimize survival and maintain water quality without imposing additional stress on the organisms. Routine monitoring of key parameters particularly phytoplankton composition, pH, and ammonium concentrations is essential for maintaining system balance and preventing biological disruptions. Salinities above 15 g L^{-1} are not advisable in systems involving tilapia, as they may compromise health and performance due to osmotic stress.

CONCLUSIONS

From a research and development standpoint, it is imperative to conduct commercial-scale trials that incorporate economic variables, including production costs, feed efficiency, and profitability, to assess the system's viability under real-world farming conditions. Furthermore, a more in-depth evaluation of interactions among key water quality parameters especially pH and ammonium are warranted to fine-tune biofloc management strategies.

Future investigations should also integrate physiological and stress biomarkers (*e.g.*, cortisol levels, gene expression profiles, or hepatic enzyme activity) to establish a direct link between salinity and the health status of cultured organisms. Additionally, exploring the role of biofloc-associated microbiota under varying salinity regimes could yield valuable insights into its influence on host immunity and nutrition, thereby informing strategies to enhance overall system performance and sustainability.

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Colegio de Postgraduados

Method of qualitative analysis of innovation dynamics in the transformation of agroecosystems: Bioeconomy in coffee farming policies in Mexico

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ABSTRACT

Objective: To propose a qualitative method to analyze the impact of the dynamics of innovation generated by innovation agents on the transformation of agroecosystems, and to apply it to the study of public policies that promote innovation in coffee production in Mexico.

Design/Methodology/Approach: A methodological proposal was designed for the qualitative analysis of innovation dynamics in agroecosystem transformation. To do so, first, the elements of circular bio-economics present in the design of public policies were identified. Secondly, the proposal was applied as a case study of public policies for innovation in coffee production in Mexico.

Results: a map of innovation dynamics in the transformation of coffee agroecosystems was generated, identifying public policies that affect the innovation of specific structures within coffee-producing agroecosystems, resulting from interactions between innovation agents and the target population. Then, a matrix analysis of public policies for innovation in agroecosystems was designed, and the barriers to disseminating each policy were identified.

Limitations/Implications of the study: The study is an exploratory phase aimed at understanding a general phenomenon. It is recommended that in-depth case studies be conducted, with specific objectives. Limitations in access to knowledge were also identified, enabling producers to use public policies better to transform the coffee agroecosystems they manage.

Findings/Conclusions: Heterogeneity in the dynamics of innovation in agroecosystems. The transformation in specific structures was identified; therefore, the complementarity potential of public policies to generate innovations at the agroecosystem scale, in its differentiated components. There are also some limitations in access to knowledge, as well as low levels of training for decision-making towards the marketing stage.

Keywords: coffee, development, dissemination, transformation.

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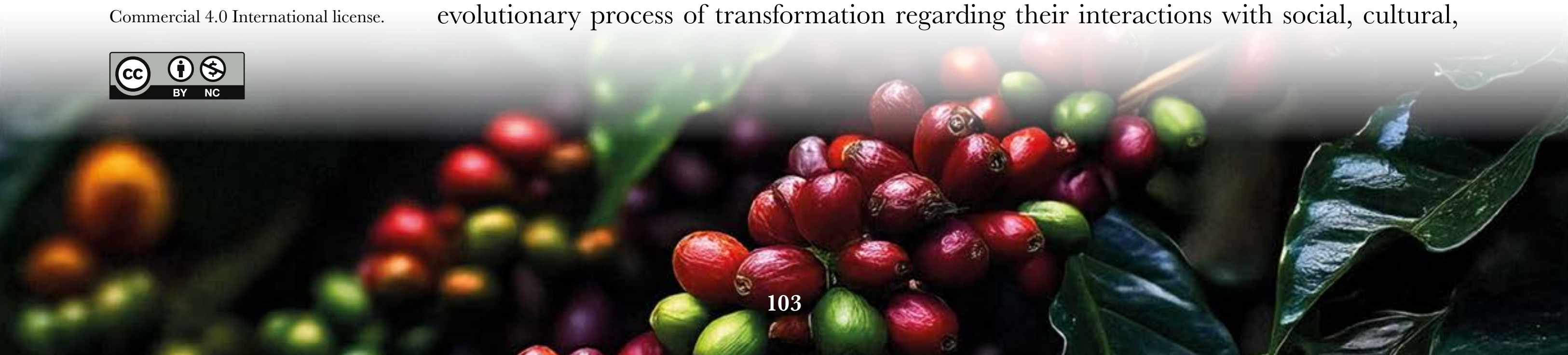
INTRODUCTION

The current structure and functionality of agroecosystems are the result of a historical evolutionary process of transformation regarding their interactions with social, cultural,



political, environmental, and technological elements. Those transformations occur through complex dynamics of innovation, understood as non-linear processes, in which social actors —responsible for generating knowledge, as well as innovation agents and the target population— intervene. To the latter, dissemination strategies aim to advance technological change that improves the quality of life of small producers.

However, the strategies of public policies for agrifood innovation in the public sector are generally oriented towards the inclusion of a certain number of producers with similar characteristics within a given period, to provide knowledge, build technical capacities, subsidize infrastructure, and transfer organizational models. This occurs without considering that the knowledge previously acquired from the producers, as well as the simultaneity with public policies of different levels of government, and the innovation agents from any social organization, institution, or company, generate a collection of information and resources, which explicitly impacts specific components of agroecosystems, not in their entirety.

Consequently, the adoption of innovations in agroecosystems is a social phenomenon in which producers decide to what degree and in what ways the changes offered by the different agents of innovation are implemented. These adoptions depend on the criteria producers use based on their vision, culture, values, and motivations, which result in the adjustments they make. These adjustments are often distant from the expected outcomes of public policies; they generate innovation dynamics that adapt to barriers or limits that are not anticipated in the initial design but emerge in social systems.

This proposal is based on the principles of Ruttan and Hayami's (2011) theory of induced innovation, which postulates that technologies are developed in response to changes in resource availability and societal needs. This study contributes by providing a model and a methodological framework for generating maps of innovation dynamics, facilitating qualitative analysis of the phenomena that motivate innovation adoption in agroecosystems. All of which is done to allow redesigning the factors that interact with agroecosystems, under the paradigm of the transformation.

A case study of bioeconomy in public policies of coffee growing in Mexico is presented. The bioeconomy in agroecosystems is understood as the use of residual biomass from the primary production process, leveraging knowledge to generate new products, processes, and services, aimed at strengthening economic, climatic, and social resiliencies. The objective was to propose a qualitative methodology for analyzing the impact of innovation dynamics generated by innovation agents on agroecosystem transformation, and to apply it to public policies that foster innovation in coffee production in Mexico.

MATERIALS AND METHODS

According to Gülpınar (2024), qualitative studies prioritize experience, addressing research problems determined within the framework of existing theories, handling holistic phenomena and situations in their own singularities in interactions and intersubjective transformation. In addition, the authors behind them make efforts to describe, understand, and interpret, exploring and elucidating factors in depth; to observe intrinsic trends and attitudes, as well as differences and similarities in understanding the phenomena.

A model and methodological framework were developed for the qualitative analysis of the innovation dynamics that influence the transformation of agroecosystems. This proposal focuses on identifying elements of the circular bioeconomy within those processes. A semi-structured interview questionnaire was used for data collection, and thematic analysis was used to identify agents of innovation and the relationships within the transformation dynamics. Thematic analysis is one of the most widely used methods for analyzing qualitative data and offers a structured, flexible framework for identifying, analyzing, and interpreting meaning patterns in datasets (Ahmed *et al.*, 2025). The choice of a qualitative and interpretative approach is justified by the complexity and contextual nature of innovation processes and agroecosystem transformations, which are not easily quantifiable using statistical or numerical methods.

The model and methodological framework thus created were applied to a case study of public policies promoting innovation in coffee production in Mexico, involving beneficiary producers and extensionists. The study was developed from January to June 2024. The non-probabilistic convenience sampling method was used. A matrix of analysis of public policies for innovation in agroecosystems was created to recognize the barriers that limit their dissemination. This allowed strengthening the criteria for the design of public policies of innovation and, finally, comparing the results with the requirements for public policies of bioeconomy in coffee proposed by the International Coffee Organization to evaluate the existence of strategies to strengthen the bioeconomy.

An unstructured survey was administered to 10 producers in Puebla and Veracruz who benefit from public policies for innovation in coffee production to identify elements in the design of these policies that meet the criteria of a circular bioeconomy.

RESULTS AND DISCUSSION

The application of the proposed methodology enabled us to understand the dynamics of induced innovation that have promoted the transformation of the structure and functioning of agroecosystems. It also facilitated the identification of the social and economic conditions that enabled the adoption of new knowledge, thereby generating a map of innovation dynamics. This map shows the transformation in specific components of agroecosystems and identifies barriers or limits to innovation in the territory. All of which facilitate pertinent updates in the process of innovation dynamics, thereby promoting technological change.

The methodological proposal focused, in a first stage, on 1: Proposing a research objective regarding innovation dynamics in the transformation of agroecosystems; 2: Surveying decision-makers about the changes in structure and functioning of their agroecosystems that have occurred over the last generations in their family.

Then, on 3: Identifying the agents of induced innovation that have generated changes in the structure and functioning of agroecosystems, in the processes of production, processing, marketing and consumption; towards 4: Generating a map of induced innovation dynamics that connects the graphs of the innovation agents to those components of the agroecosystems where technological change has been generated (Figure 1).

In the second stage, the model proposes 1: To prepare the analysis matrix of the innovations induced in the agroecosystem studied, which is composed of the following

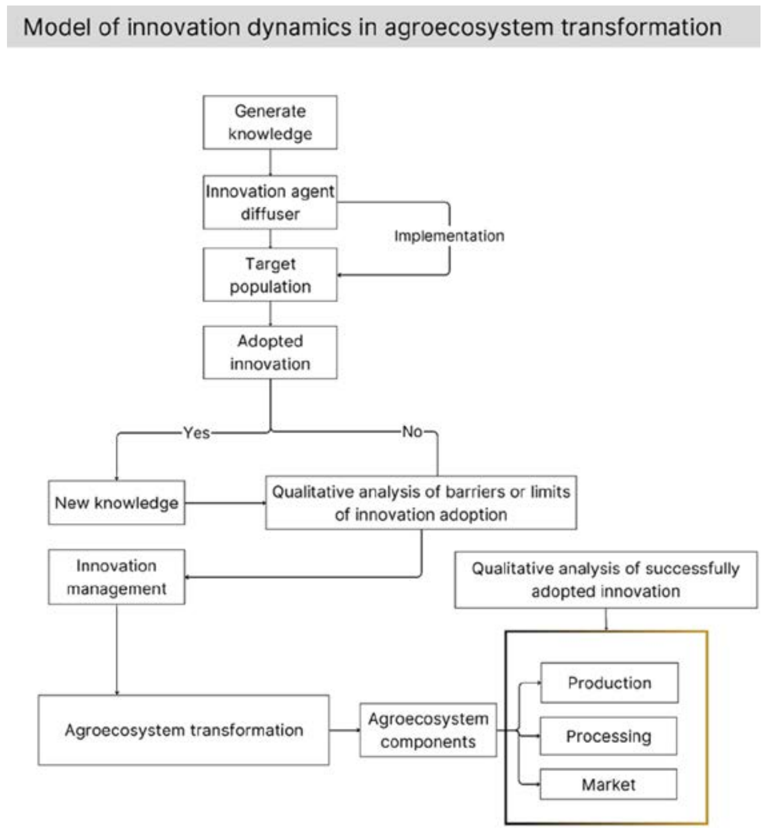


Figure 1. Model of innovation dynamics in the transformation of agroecosystems (prepared by the authors with their own original data).

categories: Innovation agent, induced innovation, transformation of the agroecosystem, social and economic conditions, and barriers or limits. In the matrix, keywords are added that help to understand the phenomenon of induced innovation dynamics. The purpose is to clarify the success and failure factors and to provide innovation agents with valuable information for reformulating dissemination strategies. Subsequently, 2: To indicate, in qualitative terms, the factors identified in the field research, map, and analysis matrix that were effective in the transformation of the agroecosystem as a result of technological change. Also, identifying the factors that caused failures in the adoption of innovations.

The map of innovation dynamics of public policies on coffee growing in agroecosystems shows four public policies in this sector. Two of which are public policies of national scale: "Planting Life" (Sembrando Vida) and "Technical Assistance in Production for People's Welfare" (Producción para el Bienestar Asistencia Técnica). The other two are public policies at the state scale: "Coffee growing recovery in Puebla" (Recuperación de la cafeticultura poblana) and "A Model of Farming Schools in Veracruz" (Modelo de escuelas campesinas en Veracruz) (Figure 2).

"Sembrando Vida" has an impact on innovations in coffee agroecosystem production by promoting the establishment of agroforestry systems and crop diversification. A typology of elderly producers and housewives is identified, as they spend most of their time in their

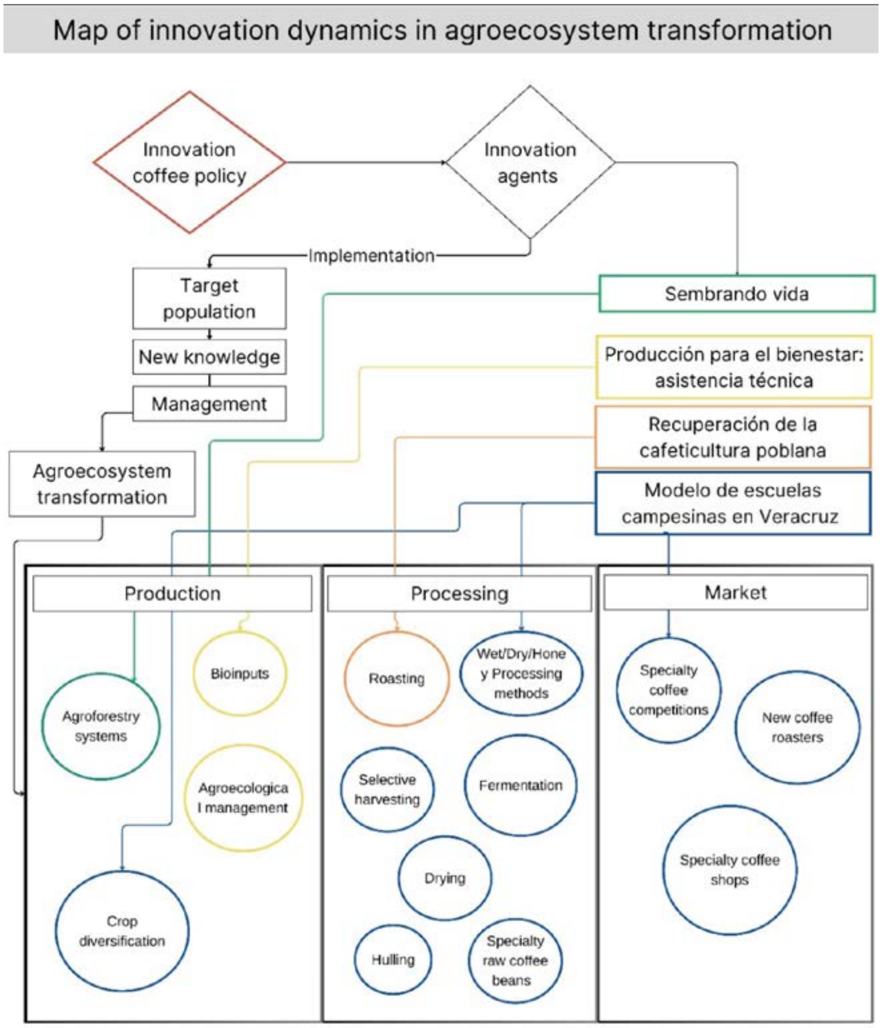


Figure 2. Map of innovation dynamics in the transformation of agroecosystems (prepared by the authors with their own original data).

territories. This program provides a monthly payment to the producer as beneficiary, although production is generally limited.

"Producción para el Bienestar Asistencia Técnica" has a greater reach in beneficiaries per territory. Its focus is on training in agroecological management, through a dissemination program with concise objectives for a defined period. Among those, the generation of bio-inputs that reduce production costs while improving the sustainability of production in environmental terms. However, some producers reject this program, arguing that it demands more effort. Yet they receive the same price for their coffee in local markets because the commercial coffee sales prices govern those in their territory, as it is sold to intermediaries. There is also a linkage with the agroecological tianguis (the traditional street market) of the government initiative, whose purpose is to open markets for the agroecological coffee produced by the program's beneficiaries, as well as to reach new consumers.

The program for the "Recuperación de la cafeticultura poblana" focused on strengthening the acquisition of machinery and equipment to process and add value to coffee. The producers who used the program more were those with greater business knowledge, because they identified differentiated coffee markets. Whereas some of the cherry coffee producers sold the equipment, such as cherry pulpers and roasters, due to lack of interest in integrating more processes and immediate money needs, coupled with a lack of training of the benefited producers in some territories.

Finally, the scope of the public policy "Modelo de escuelas campesinas en Veracruz" was identified, which has a dissemination model similar to "Producción para el Bienestar Asistencia Técnica". It affects innovation in production, processing, and marketing, and trains beneficiary producers to sell their coffee in the specialty coffee market. Producers participate in coffee fairs and quality competitions, where they connect with roaster businesses and entrepreneurs. The main reason for this is that coffee growers are located far from markets in nearby cities or from logistics companies that ship their coffee to customers in other states. Once they find customers, their challenge is to maintain production to meet market demand, as they are mainly small-scale producers.

Coffee farmers acquire knowledge from various innovation agents and make their own decisions about adoption. In designing this method, we investigated producers who have already made verifiable changes to their production systems.

At different stages of the coffee production and marketing processes, a variety of barriers can impede the dissemination of innovation (Gruenhagen & Parker, 2019). To address these barriers, changes are needed at each stage; these should address the specific needs of the production process. To exemplify this, we present the criteria of Bioeconomy for coffee, which should be considered in public policies in the sector (Figure 3).

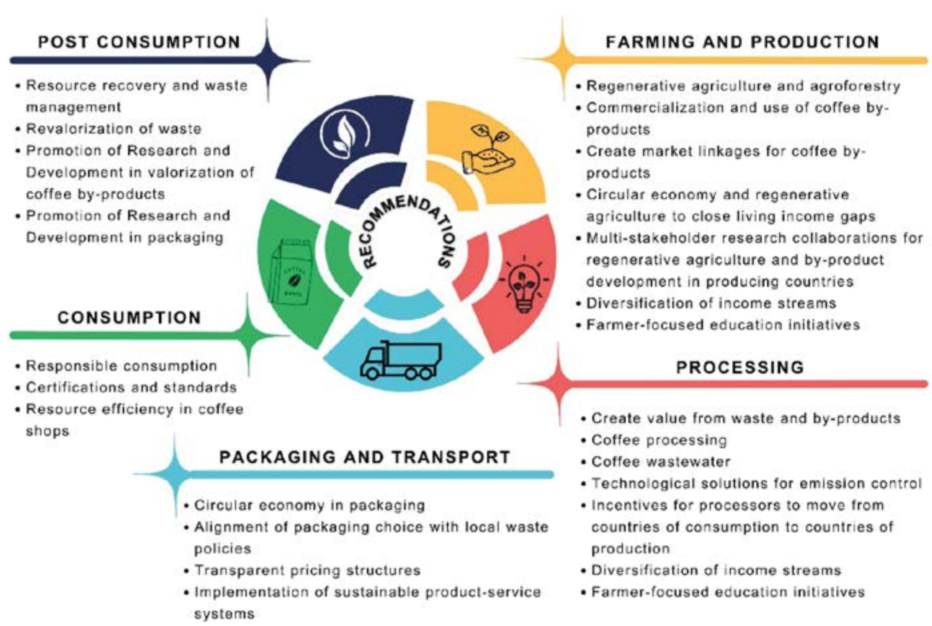


Figure 3. Public policy recommendations based on the criteria of the Bioeconomy in Coffee. Source: adapted by the authors from ICO (2024).

The innovations generated by public policies were identified in the analysis matrix we created (Table 1). The innovations adopted were selected for their specific impact on the agroecosystem's transformation. We also identified the barriers or limits that prevent maximum use; we evaluated the presence or absence of practices in bioeconomy in the design of the public policies analyzed.

Innovations can strengthen the different emerging resilience approaches in coffee farming (Moat *et al.*, 2017). When reductions in rainfall or temperature increases are considered, there is a need to examine the impact of climate change on coffee production (Bracken *et al.*, 2023).

Climate change negatively affects coffee production by impacting quality and yields. Tanner *et al.* (2014) indicated that, although the concept of resilience is increasingly inspiring research and public policy design, the transfer of ecological theory to social systems still leads to a weak commitment to the political, social, and normative aspects of the adaptation to climate change. Such resilience is the ability of all people throughout generations to maintain and improve their opportunities for a better life and welfare, despite environmental, economic, social, and political disturbances. This resilience is supported by human action and empowerment in individual and collective operations, and is reflected in human rights, which are key in the dynamic processes of social transformation.

Each coffee-producing region where agroecosystems are located faces challenges that differ from those in other areas regarding the potential to adopt innovations through public policies that contribute to resilience at the agroecosystem scale. Scientific research should be specific and relevant in identifying actions adaptable to the emerging properties of

Table 1. Matrix of analysis of public policies for innovation in agroecosystems.

Public Policy Programs (Name)	Innovation	Agroecosystem transformation	Barriers or thresholds	Bioeconomical practices
Sembrando vida	Agroforestry production system	Redesign of the agroecosystem	Primarily, the self-consumption scale	Yes
Producción para el bienestar: asistencia técnica	Agroecological practices and the manufacture of bio-inputs	Composting area, planned production, diversification, waste use	Little or no reach to a differentiated market, investment of time in new processes	Yes
Recuperación de la cafeticultura poblana	Coffee processing infrastructure	Vertical integration of production processes, redesign of the agroecosystem, and management	Only market-oriented coffee producers, of varying quality, took advantage of the infrastructure.	No
Modelo de escuelas campesinas en Veracruz	Training for the production of specialty coffee, with agroecological practices	Specialty coffee selection and processing area, roasting, and packaging	Geographically located at long distances from points of sale or logistics or transport companies	No

agroecosystems, and in establishing and monitoring particular lines of action implemented in the territories. To this day, some elements that can be cataloged within bioeconomy initiatives are identified, but in an incipient way, and only in the manufacture of bioinputs with resources available on the coffee farms of the region, in the case of governmental programs "Sembrando Vida" and "Modelo de escuelas campesinas en Veracruz".

According to Torres-Tadeo *et al.* (2025), bioeconomic agroecosystems are those that have undergone a transformation of knowledge in their determinants over time, thereby giving rise to non-linear innovation patterns between innovation agents and producers. Based on this, research on value networks for knowledge is proposed to understand the causes of functionality and the motivations for adopting innovations in agroecosystems. This means exploring what drives producers' interest in adopting diversification strategies to create new products and services that address the effects of climate change on rural households.

Contreras-Medina *et al.* (2020) proposed that knowledge creation is critical to improve sustainable practices in coffee production and supply chains. The innovation dynamics in the transformation of the coffee agroecosystems analyzed show a complementarity where those components pertinent to the production, transformation, and marketing processes are aligned to adopt value-added oriented strategies towards obtaining specialty coffee, which then is supplied in differentiated markets On the other hand, Lachenmeier *et al.* (2024) indicated that the use of coffee by-products has economic, nutritional, and environmental potential.

Regarding the analyzed programs, the bioeconomy paradigm is incipient, yet only from a public policy perspective. Those elements identified still show causal effects of the adoption of agroecological innovations, and they remain far from being a central objective in public discussion of public policies in coffee farming. According to Venus *et al.* (2024), the transition to a circular bioeconomy requires innovation across many sectors, particularly in social aspects. Ubertino *et al.* (2016) observed that social capital is the most critical element in the adoption of innovation by coffee producers. According to Gebru *et al.* (2024), the criteria for the production stage in coffee systems are those that improve producers' welfare.

CONCLUSIONS

Understanding innovation dynamics and their impact on the structural transformation of coffee agroecosystems enables us to identify more specific phenomena in innovation that need to be studied, such as the relevance and potential of expected results over a given period. The structures of agroecosystems are potentially transformable in response to social and functional conditions, which are not always anticipated in the dissemination of public innovation policies.

The acceptance or rejection phenomena identified in the research show the causes of interest for designing new public policies or strengthening those currently in place. Mainly, in determining the interests and socioeconomic possibilities of the different types of producers, to evaluate the degree to which they are prone to adopt or reject specific innovations.

The qualitative approach at the agroecosystem scale facilitates a broad study of poorly understood phenomena in system functioning. For example, actions and consequences stemming from public policies strengthen resilience.

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Ornamental fish culture and trade in Mexico

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ABSTRACT
Objective: To present the situation and context regarding the commercialization of ornamental fishes in Mexico, their principal commercialized species and its impact when extracted from their natural habitat.
Design/methodology/approach: This research was carried out by searching and compiling bibliographic literature, as well as consulting articles in different databases, for subsequent analysis and information processing.
Results: In Mexico, more than 25 million ornamental fish are sold per year, ornamental aquaculture production is mainly concentrated in the state of Morelos, with the Guppy and Japanese as the most produced and consumed fish within the national territory: however, some native species are extracted from natural aquatic systems in Mexico with serious consequences.
Limitations on study/implications: There is scarce information because various producers and extractors of ornate fish do not have permits, so it is difficult to keep a record of their activities, likewise the trade in marine species does not have official records of their catches and sales.
Findings/conclusions: The Poecilidae family is the most cultivated fish in Mexico. The most sold fish in Mexico are the Guppy (*Poecilia reticulata*) and the goldfish (*Carassius auratus*), both produced mainly in the state of Morelos.

Keywords: Ornamental fish, Aquarism, exotic species, aquaculture

INTRODUCTION

Nowadays, the ornamental fish trade has grown considerably, due to its wide acceptance and its good development prospects (Lango-Reynoso *et al.*, 2012; Evers *et al.*, 2019) being one of the markets with the highest profits, which reach 30 billion dollars annually. In Mexico, approximately 45 million freshwater ornamental fish are sold annually, and in recent years, national production has consolidated as a business with

prospects for social and economic growth that is developing satisfactorily, as a result of the growing demand from the aquarium sector (Cedillo *et al.*, 2001; Ramírez-Martínez *et al.*, 2010; Brito, 2018). Ornamental aquaculture activity in Mexico generates around 48 thousand jobs and an economic impact estimated at 800 million pesos, and although it is a relatively recent economic activity, it is projected to have favorable growth in the coming years due to the increase in demand for ornamental fish (Villaseñor-Garrido *et al.*, 2020).

MATERIALS AND METHODS

A bibliographic search was carried out in which the selection of information was carried out by searching different online databases and scientific search engines, such as: Elsevier-Scopus, SCIELO and Google Scholar. Data from the FAO and official pages were also consulted, as well as digital repositories of different universities and book chapters.

RESULTS AND DISCUSSION

Marketing of ornamental fish in Mexico

At the national level, ornamental fish farming has increased its production from three million organisms per year in the nineties to more than 25 million today, which represents a sustained annual growth of 14%, which contributes to the generation of more than 40 thousand direct jobs, making it of great importance to the population (Ruiz, 2022). At the national level, ornamental fish production is concentrated in the states of Morelos, Jalisco, Yucatán, and Veracruz. However, the state where most ornamental fish are produced and bred is Morelos, while Mexico City is considered the distribution center, as it is there that the fish produced in the producing states are sold and from there they are distributed throughout the national territory (Villaseñor-Garrido *et al.*, 2020). Within Mexican territory, ornamental fish production comes from aquaculture farms, most of which, 333, are located in the state of Morelos, where more than 20 million organisms are produced annually (Ramírez-Martínez *et al.*, 2010; Brito, 2018; Villaseñor-Garrido *et al.*, 2020; Ruíz, 2022), and the largest concentration of these aquaculture farms within the state of Morelos are located in the municipality of Ayala (Brito, 2018), in which 54.65% of farms registered for the state are located, this is due, among other things, to the fact that this region has a favorable climate for raising fish in ponds (21-25 °C) and also a factor that influences is the accessibility to markets, since it is located close to communication routes and marketing channels (Brito, 2018; Cortés *et al.*, 2025).

In Mexico there are two main models of ornamental fish production: in ponds and in "breeding rooms", the first corresponds to tanks exposed to environmental conditions, so there is no precise control of them and the breeding rooms, which are indoor facilities in which water quality is controlled for the breeding of more demanding species (Espinosa *et al.*, 2011).

The production and trade of ornamental fish in Mexico focuses mainly on freshwater species, since there are limitations in the marine aquarium industry, which are related to the lack of technological implementation that allows long-term economic viability, since the technology to establish aquaculture farms producing marine species is very expensive and is beyond the reach of national producers, so it is a poorly developed activity, despite having national species with potential in aquariums (Lango-Reynoso *et al.*, 2012).

Traded species

In Mexico, the most sold ornamental fish (Table 1) belong to six main families: Poecilidae with 34 species, Cyprinidae 19, Cichlidae 14, Characinidae 9, and Anabantidae with 6 species, all of them freshwater species (Devezé *et al.*, 2008). However, of all of them, the ones that sustain almost all the sales of specimens are the Poecilidae (Devezé *et al.*, 2004; Maya *et al.*, 2007). With its largest representative, the Guppy, as the most sold and most in-demand fish along with the goldfish, this is attributed to the fact that both species are highly resistant to changes in the quality of the farming water and do not require very sophisticated facilities (Espinosa *et al.*, 2011), Although these fish could be considered cheap at first glance, as their prices are around four pesos; however, their profit is in the sales volume, since 191 tons of guppies and 124 tons of goldfish are sold per year, obtaining greater profits with the goldfish (of about 28%) but higher sales with guppies (Devezé, 2008). In the case of marine fish, there is no precise information on sales or marketing (Dávila Camacho *et al.*, 2019). The main groups of marine fish that have dominated the market belong to the families: Pomacentridae, Acanthuridae, Balistidae, Labridae, Pomacanthidae y Chaetodontidae, Most of which come from Indonesia, the Philippines, Sri Lanka, the Maldives and the Central Pacific islands (Lango-Reynoso *et al.*, 2012; Villaseñor-Garrido *et al.*, 2020).

Extraction of native fish

The extraction of native freshwater ornamental fish in Mexico can primarily cause the deterioration of aquatic systems, resulting in a loss of biodiversity and an imbalance in food chains. This is due to the extraction of fish from their natural habitat. This not only reduces their local populations but also deteriorates natural environments. Many species of ornamental fish have very restricted distribution areas, making their collection a risk factor for extinction. Likewise, the extraction of ornamental fish itself includes inappropriate practices that cause environmental damage, such as the use of cyanide in marine fish

Table 1. Main species of fish marketed in Mexico and their origin.

Common name	Scientific name	Origin
Guppy	*Poecilia reticulata*	Southamerica
Goldfish	*Carassius auratus*	Asia
Angel fish	*Pterophyllum scalare*	Central America and Southamerica
Cebra fish	*Danio rerio*	Asia/India
Betta	*Betta splendens*	Asia
Molly	*Poecilia sphenops*	Native
Platy	*Xiphophorus maculatus*	Native
Sword tail	*Xiphophorus helleri*	Native
Nun fish	*Gymnocorymbus ternetzi*	Southamerica

(Salazar *et al.*, 2008), It also encourages invasive species to replace those extracted (Jelks *et al.*, 2008; Miller *et al.*, 2009). Among the freshwater fish of which there is a record of being extracted for ornamental purposes for national consumption, are cichlids such as the guapote *Trichromis salvini* (Ruíz, 2022), firemouth cichlids (*Thorichthys* spp.), among which stands out *T. helleri*, the genus *Vieja*, and poecilids of the genus *Xiphophorus* (Lorán *et al.*,2006; Ramiréz *et al.*, 2004; Lorán-Núñez *et al.*, 2013 Del Moral-Flores *et al.*, 2018; López-Segovia *et al.*, 2024).

On the other hand, extractions of native fish have been reported in the north of the country, among which the desert pupfish stand out (*Cyprinodon macularis*), the little sardine (*Cyprinella* sp.), the mexican stone roller (*Campostoma ornatum*), the Pacific topote (*Poecilia butleri*), the Del Sonoyta puppy (*Cyprinodon eremus*), the green belly (*Agosia chrysogaster*), These are endemic to the north of the country and are taken abroad, mostly to the United States and France, where they are distributed to the rest of the European countries (Hignette, 2003; Varela-Romero & Hendrickson, 2009).

There are no exact figures regarding the extraction of marine fish from Mexico, as they are not considered a consumer fishery; however, various studies indicate that the main endemic marine fish extracted is the giant meerkat (*Opistognathus rhomaleus*), the fine pointed big mouth (*Opistognathus punctatus*), the blue dotted big mouth (*Opistoganthus rosenblatti*), the angel of Cortés (*Pomacanthus zonipectus*) and the queen angel *Holocanthus passer*, which in recent years has been the subject of various studies focused on achieving its reproduction in captivity, These are caught in the Gulf of California legally, but it is believed that many more species are being extracted illegally because their extraction responds to demand without considering biological or environmental aspects (Gijón-Díaz *et al.*, 2017; Pérez-Velázquez, & González-Félix, 2025).

Ornamental aquaculture as a conservation method

Captive cultivation of ornamental fish can help sustain the aquarium industry and mitigate the impact on the environment by reducing the need to collect specimens from wild native populations, so that in the future it will not be necessary to extract any native fish from their habitat, since as the cultivation and maintenance techniques for each species are adapted and standardized, to meet market demand, With this, the volumes of wild fish extraction will be depleted (Lango-Reynoso *et al.*, 2012; Vivas, 2019), In addition, the goal is for fish from aquaculture to be higher quality specimens and in better health than wild ones, so ornamental aquaculture includes the breeding of species that are difficult to collect or whose natural populations are already low, so aquaculture can be a useful tool in the recovery of populations where they had already been eradicated, all because breeding in captivity allows the production of organisms without depending on direct extraction from the environment (Tlusty, 2002; Lango-Reynoso *et al.*, 2012). Aquaculture is a basic tool for conservation programs for native and endangered species, and even many species used in aquariophilia have been maintained and bred for conservation and preservation purposes (Dykman, 2012) such as the barbo *Puntius tetrazoma* and the pygmy botia (*Botia sidthimunki*) and also in the case of species such as the blue arowana (*Osteoglossum ferreirae*) of which their populations are in serious danger and aquaculture is projected as the best alternative to be able to meet the demand it has in the market, so for native species of Mexico this type of aquaculture focused on conservation would also be of great importance (Tlusty, 2002; Barajas-Pardo *et al.*, 2017), As has been achieved in the populations of the godeid *Zoogoneticus tequila*, because it was extinct in its natural environment and thanks to stocks maintained in captivity, from which breeding stock were obtained, work has been done on repopulation in its natural habitat (Domínguez *et al.*, 2018).

CONCLUSIONS

Mexico is a country with a significant market for ornamental fish, most of which are produced in the central region of the country, where most ornamental fish farms are concentrated. The national market is dominated by two main species: the goldfish (*C. auratus*) and the guppy (*P. reticulata*), which support most of the trade. However, there is also the capture of native fish for ornamental purposes, which leads to serious ecological problems. Therefore, the use of aquaculture is proposed as a tool to avoid overexploitation and conserve native species.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Characterization of shade-grown coffee production in Totonac communities in the Northeastern Sierra of the state of Puebla, Mexico

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ABSTRACT

Objective: to characterize the production of shade-grown coffee (*Coffea arabica* L.) in Huehuetla, Ixtepec and Zongozotla, municipalities with Totonac population in Sierra Nororiental, Puebla, Mexico.

Design/Methodology/Approach: this study was conducted from January to June 2024. To collect data, 93 semi-structured interviews were conducted, consisting of three types of records; 1: general data, 2: socioeconomic and productive aspects; and 3: agronomic management. Among the surveyed participants, 69% were men and 31% were women.

Results: in these municipalities, 12 varieties of coffee are grown, associated with 50 plant species, 16 of which are used for shade. Coffee growers identified coffee rust (*Hemileia vastatrix*) as the main disease, and the coffee-berry borer (*Hypothenemus hampei*) as the primary pest. The yield of parchment coffee in this region is lower than the average in the state of Puebla (460 kg ha^{-1}). The most common practices identified are weed control, pruning of coffee plants, shade management, and soil fertilization.

Limitations/Implications of the study: foliar fertilization, application of insecticides and fungicides are implemented to a lesser extent; which suggest that coffee growers require attention and related training.

Findings/Conclusions: based on the results obtained, we suggest that agricultural activities be increased; while promoting the coffee sector through government agencies. Management plans should be designed in a way that, in addition to considering the biological requirements of coffee cultivation, they also incorporate the socioeconomic and cultural contexts.

Keywords: coffee growing, agroecosystem, diversity, agricultural practices.

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INTRODUCTION

There are more than 100 species of coffee in the world, however, Arabica coffee (*Coffea arabica*) represents 70% of the products in international trade, and Robusta (*Coffea canephora*) only 30%. Coffee is grown in the open air or under shade, mainly associated with native trees and with low use of inputs. These agroecosystems are fundamental because they provide ecosystem services and economic benefits; it is estimated that

coffee growing worldwide involves the participation of at least 25 million small producers (García-Domínguez *et al.*, 2021).

At the national scale in Mexico, *C. arabica* represents 97% of production, with the varieties Típica, Borbón, Caturra, Mundo Novo, Garnica, Catuai, Pluma Hidalgo and Maragogype predominating, while 3% corresponds to *C. canephora* (Barrera-Rodríguez *et al.*, 2021). Mexico ranks eleventh in coffee production worldwide, and represents 0.66% of Mexico's agriculture GDP. The main producing states are Chiapas, Veracruz, and Puebla, the latter ranking third nationally, followed by Oaxaca and others with a smaller contribution. Therefore, at the close of the 2023 agriculture cycle, the state of Puebla produced 225 663.84 tons (Megagrams, Mg) of coffee cherry, which represents 21.36% of Mexico's national production (SIAP, 2025).

In the Northeastern Sierra (Nororiental region) of the state of Puebla (Mexico), coffee growing is primarily done by native peoples, who play a fundamental role in the creation and maintenance of shade forest agroecosystems that house diverse plant strata. These are located in strategic locations for water collection, adjacent and priority areas for biodiversity conservation, and represent important biological corridors. Most coffee plantations are located in tropical and temperate zones (Aguirre-Cadena *et al.*, 2018).

Coffee-growing areas have high levels of poverty, and in highly and very-highly marginalized communities, one characteristic is their lack of productive diversification, which impacts the low incomes of indigenous farmers. Since the elimination of global coffee price regulation, that coincided with the demise of the Mexican Coffee Institute (INMECAFE), the coffee-production sector has suffered significant abandonment, resulting in low yields, phytosanitary problems, and aging plantations. In addition, this crop currently faces uncertainty due to climatic factors, low prices, pests, and diseases, despite its economic, environmental, and social importance. In this region, some varieties are used that were developed and released by INMECAFE (Garnica variety) and the Mexico's National Institute of Research in Forestry, Agriculture, and Livestock-INIFAP (Oro Azteca variety), according to Gómez-Martínez (2019).

For this crop, should the importance be emphasized of agronomic management, environmental conditions, production systems, harvest and post-harvest management to increase yields and improve the quality of coffee products. Therefore, the diversification of agricultural practices in the crop is important. Nowadays, coffee production systems are classified into five types; rustic system, traditional polyculture, commercial polyculture, shade monoculture, and open-air monoculture (Escamilla-Prado, 2015).

In the Sierra Nororiental of Puebla, the rustic system predominates (Benítez-García *et al.*, 2015). The main characteristics of this system are the low use of agrochemicals and that agricultural works are done only to a lesser extent; among those, the most notable are the pruning of coffee plants, and weed control under the canopy. The management of coffee plantation species is an activity in which the producer analyzes and decides what to plant, maintain, or eliminate. Therefore, growers design coffee plantations with specific trees present, this considering biological, economic, and cultural needs (López-Santiago, 2019). The importance of this knowledge is emphasized, for it provides ecosystem services and biodiversity conservation (Espinoza-Guzmán, 2020).

Puebla is the third-largest coffee cherry producer nationwide, with a production of 225 663.84 Mg at the close of the 2024 agriculture cycle. Coffee production is the main source of income for farming families in the Northeastern Sierra. To have a record of those agricultural activities that coffee growers do would help identifying those aspects that require attention. The objective of this study was to characterize the shade-grown coffee (*Coffea arabica* L.) production system in Huehuetla, Ixtepec, and Zongozotla, municipalities with Totonac population in Sierra Nororiental, Puebla, Mexico.

MATERIALS AND METHODS

Location of the study area

The study was developed in Huehuetla, Ixtepec and Zongozotla, all localities belong to the Sierra Nororiental in the state of Puebla, Mexico (Figure 1).

In Huehuetla there is a humid semi-warm climate with rain throughout the year, temperatures range from 18-24 °C with precipitations from 2900 to 3600 mm, Leptosol soil predominates. Ixtepec also has a humid semi-warm climate with rain throughout the year, temperatures range from 18 to 24 °C, precipitations from 2400 to 3600 mm where Leptosol soil predominates. Whereas, Zongozotla has humid semi-warm and humid temperate climates, with rain throughout the year, with temperatures ranging from 16 to 22 °C, precipitations from 1900 to 2100 mm, where luvisol and andosol soils predominate (INEGI, 2025). In regard to socioeconomic characterization, Huehuetla is considered a

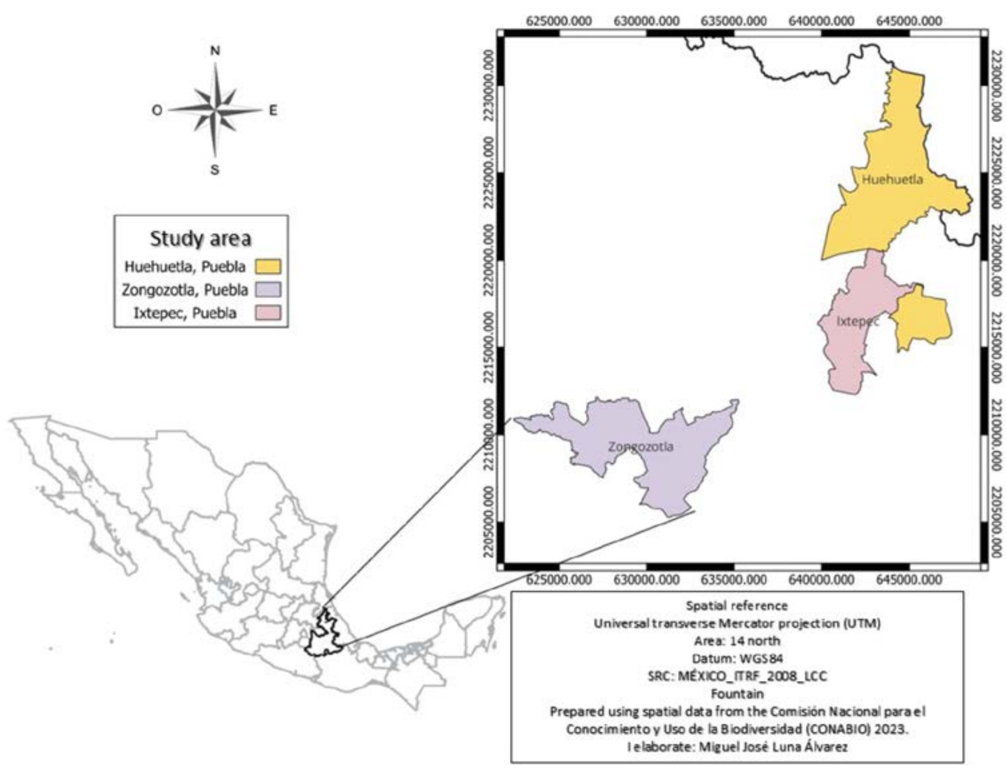

Figure 1. Location of the study area; the municipalities Huehuetla, Ixtepec, and Zongozotla, in the state of Puebla, Mexico. Source: prepared in ArcGIS® 10.5 with vector information from CONABIO.

municipality with very-high marginalization index, while Ixtepec and Zongozotla present a high marginalization index (CONAPO, 2025).

Collaboration with local authorities and organizations

Before beginning field survey activities, meetings were held with representatives of the municipalities of Huehuetla and Ixtepec. In the case of Zongozotla, a meeting was held with representatives of the Cooperative Association "Productora y Procesadora de café CAPERSA S.C. de R.L. de C.V." During those meetings, the objective of this study was discussed, as we requested authorization for its implementation.

Sample selection

Through representatives of the Rural Development Directorate and the representative of the Cooperative Association, the number of coffee growers in each municipality was obtained. There were 596 in Huehuetla, 450 in Ixtepec, and 1200 in Zongozotla. Simple random sampling was used; the sample size was determined using the formula described by Santiago-Hernández *et al.* (2023).

$$n = \frac{NZ^2_{a/2}pq}{Nd^2 + Z^2_{a/2}pq}$$

where, N: total population (2246 coffee producers); $Z^2_{a/2}$, 95% reliability (1.96), p=0.5, q=0.5, d=precision (0.10). Therefore, a sample size of 93 producers was obtained.

Semi-structured interviews

Ninety-three semi-structured interviews were conducted in Huehuetla (24 producers), Ixtepec (19 producers), and Zongozotla (50 producers). The interviews consisted of 20 questions grouped into three sections; 1: general information; 2: socioeconomic and productive aspects; sex, age, occupation, educational level, ethnic group, importance of the crop, coffee varieties grown, other plants in the coffee plantations, shade trees, planting frame and density, age of coffee plants, annual production, presentation, and selling price; 3: agronomic management; implemented agronomic practices and phytosanitary management. Producers who reported owning their property were interviewed; the interviews were conducted in Spanish and in their native language (Totonac). The information was systematized in a database and analyzed using descriptive statistics.

RESULTS AND DISCUSSION

Socioeconomic aspects

The ages of the coffee growers ranged from 19 to 81 years, 69% of those interviewed were men and 31% women. The low participation of women as owners of the coffee plantation is due to the fact that in these communities the role of women focuses on domestic tasks. According to Alvarado-Méndez *et al* . (2006), the tradition in the municipality of Huehuetla is that the head of the family is the man, while women are dedicated to household chores and family care.

The 100% of the producers were Totonac speakers, although some are bilingual and also communicate in Spanish (Figure 2). Their main activity is coffee farming, as they consider it the best productive option and a traditional activity. For this reason, coffee production is widely accepted, although it is sometimes not profitable. In this regard, indigenous farmers represent 51% of the total number of producers in the state of Puebla. Among them, 60% are Nahua, 34% are Totonac, 4% are Mazatec, and 2% are Otomi (Alvarado-Méndez *et al.*, 2006).

Figure 2. Participation of producers in informational meetings on coffee growing in the municipality of Ixtepec, located in the Northeastern Sierra (Nororiental Region) of the state of Puebla.

Through the interviews we found that educational level of coffee growers is low; 43% reported that have finished just primary education, 26% with secondary education, only 8% graduated from high school, while 23% have no education. However, one notable finding is that some producers' children, regardless of sex, are pursuing bachelor's degrees outside of these municipalities. This occurs because in the municipalities of Ixtepec and Zongozotla there are only elementary and secondary education up to high school. Meanwhile, in the municipality of Huehuetla, in addition to elementary and intermediate education, there are three higher education institutions; where the Intercultural University of the State of Puebla is outstanding because they offer undergraduate and graduate programs related to coffee growing (Aguilar-Tlatelpa and Fajardo-Franco, 2020).

Productive aspects

Up to 12 coffee varieties are grown in the study area, ranging from short growth habit, to medium and tall. These varieties include Marsellesa, Oro Azteca, Costa Rica 95, Mundo Novo, Garnica, Tipica, Sarchimor, Caturra, Colombia, Borbon, Obata, and Geisha. In both Huehuetla and Ixtepec, the most common variety mentioned was Costa Rica 95, while in Zongozotla the most mentioned was Oro Azteca (Figure 3).

Damage such as chlorotic to necrotic spots and defoliation due to coffee rust have been reported in the Typica and Garnica varieties in the study area (Fajardo-Franco *et al.*, 2020). The occurrence of diseases in different coffee-growing regions, particularly coffee rust, has led to the use of commercial varieties called catimores for the renewal of coffee plantations.

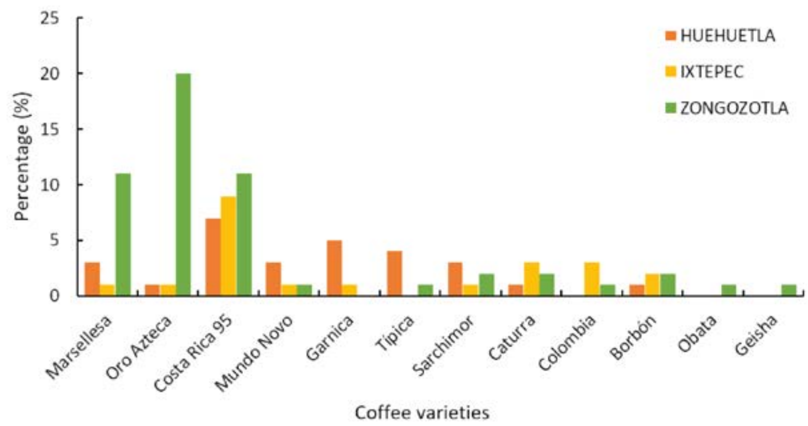

Figure 3. Coffee varieties grown by Totonac producers in municipalities of the Northeastern Sierra (Nororiental Region) of the state of Puebla.

Among these varieties tolerant to that disease are Costa Rica 95, Colombia, and Oro Azteca (Hernández-Martínez and Velázquez-Premio, 2016).

In regard to the age of the coffee plantations, it was found that some of the producers interviewed have plants up to 50 years old. Among those, Garnica outstands with a lifespan from 5 to 50 years; Caturra from 3 to 30 years, Borbón from 3 to 30 years, Tipica 5 to 20 years, Mundo Novo 5 to 15 years. However, an aspect to highlight is that in the last 15 years, varieties resistant to rust have been incorporated. It was found that of the Costa Rica 95 variety there are plantations from 2 to 15 years; of Sarchimor from 2 to 10 years; with Oro Azteca from 1 to 8 years, Marsellesa from 3 to 8 years. Plantations with variety Colombia ranged from 3 to 7 years, in the case of Geisha from 3 to 5 years, and with Obata, only young plantations, 2 years-old, were found. These results suggest a lack of coffee plantation renewal, as some plantations were identified over 20 years old from establishing. According to Díaz-Gutiérrez (2019), a renewal plan should be implemented for older crops to avoid phytosanitary problems that can be detrimental to production capacity.

The average surface area of each producer is 1.35 ha in Huehuetla, 1.25 ha in Ixtepec, and 0.55 ha in Zongozotla. In areas of these sizes, the average production of parchment coffee per hectare (kg ha^{-1}) is 190.9 kg (3.32 quintales) in Huehuetla. One "*quintal*" is equivalent to 57.5 kg of parchment coffee beans, that is the local traditional unit for measuring coffee yield. While in Ixtepec coffee growers obtain 242.7 kg ha^{-1} (4.22 *quintales*), Zongozotla stands out with a production of 460 kg ha^{-1} (8 *quintales*). This coincides with a previous study in Huehuetla and the entire region, that reported coffee producers have small plots ranging from one quarter of hectare to one hectare and their average production is 402.5 kg ha^{-1} (7 *quintales*) of parchment coffee (Alvarado-Méndez *et al.*, 2006).

The yield in parchment coffee (the bean with its softest husk) is smaller than the state (Puebla) average 782 kg (13.6 *quintales*), also smaller than Mexico's national average 368 kg (6.4 *quintales*), except in Zongozotla (SIAP, 2025). Production leaves an annual income per hectare of approximately 7500 Mexican pesos (MXN) in Huehuetla, MXN 9700 in Ixtepec and MXN 20 600 in Zongozotla. However, this income is unstable due to price fluctuations and the very small area per producer. Therefore, these amounts are insufficient facing the level of poverty of the Totonacs in the Northeaster Sierra (Nororiental region) of Puebla (SADER, 2020). In this region, more than 80% of Totonac population live in poverty; Ixtepec and Zongozotla show high marginalization, while Huehuetla is officially recorded as of very high marginalization (CONAPO, 2025).

Given this situation, diversifying coffee agroecosystems is an important strategy to strengthen the resilience of farming families, thus reducing the risk of dependence on a single product. Diversification would increase access to food to improve the family diet; therefore, the implementation of agroforestry systems is suggested (Fonseca-Castillo *et al.*, 2025). The production of each plot is different, as well as the form of sale and price. In this study, 68% of producers sell coffee cherry at a price ranging from 7-10 MXN kg^{-1}, while only 32% of producers handle the wet and dry processing to obtain parchment coffee to sell in a range 3-45 MXN kg^{-1}; On the other hand, green or gold coffee is marketed from 120-180 MXN kg^{-1}, while roasted or ground coffee reaches a value between 140-200 MXN kg^{-1}. The opposite occurs in Pantepec, Chiapas, where Vázquez-López *et al.* (2022) reported that 78.8% of coffee growers sell parchment coffee, 18% coffee cherry, and only 3.3% ground coffee.

Planting frames are also a key factor in coffee production per hectare. In Huehuetla and Ixtepec, the most common distances are 2 m between plants and 2 to 3 m between rows. Whereas, in Zongozotla, the distances are 1.5 to 2 m between plants and rows, resulting in a higher plant density per hectare. The coffee harvest lasts approximately four months; in that period women's participation for that activity is particularly significant (Figure 4).

Figure 4. Women participate in coffee harvesting at the cutting of coffee cherry.

Plants associated with coffee plantations

Seventy-three percent of producers grow coffee under shade, and 27% grow coffee under the sun without any other cover. Shade trees are pruned and used to provide fuel for homes. In other cases, the waste is used to generate organic fertilizer. Shade trees also provide seasonal fruit, which is harvested for personal consumption and local sale. Regarding plant community associated to coffee, those interviewed mentioned up to 50 plant species present in the coffee plantations, of which 16 are used as shade trees.

Totonac families use firewood to cook food, which is obtained from shade trees in coffee plantations such as chalahuite (*Inga vera*), tropical cedar (*Cedrella odorata*), allspice (*Pimenta dioica*), carboncillo (*Ocotea puberula*), sangregado or dragon's blood (*Croton draco*), coffee (*Coffea arabica*), Jamaican nettletree (*Trema micrantha*), ocote (*Pinus montezumae* o *P. teocote*). However, in coffee plantations there are also trees whose sole purpose is to provide shade and habitat for fauna, such as jonote (*Heliocarpus appendiculatus*) know in some countries as majagua, chaca– "gumbo-limbo" (*Bursera simaruba*), Mexican kidneywood (*Eysenhardtia polystachya*), tawaxkatat Totonac for the broadleaf alchornea (*Alchornea latifolia*). There are also some fruit trees such as, orange (*Citrus sinensis*), mango (*Mangifera indica*), pahua– the coyo avocado (*Persea schiedeana*), banana (*Musa paradisiaca*), and mamey (*Pouteria sapota*) (Figure 5).

In the case of sangregado (locally known as, dragon's blood), its importance in the medicinal field is outstanding, as it is used as a wound-healing agent. This use has also been documented in the Totonac municipality of Filomeno Mata, in Veracruz, where it is used to treat wounds (López-Santiago, 2019). Plant community in coffee plantations is a very important aspect for maintaining the cover in the various tree strata, since it promotes an internal microclimate and also favors the diversity of fauna. Libert-Amico & Paz-Pellat (2018) indicated that shade trees in coffee plantations are carbon stores in aboveground and soil biomass, they also provide a variety of environmental services and contribute to the amortization of climatic stress and nutritional imbalance.

In a study conducted in Huehuetla, Basilio-González *et al.* (2022) mentioned that coffee cultivation has better indices of richness, abundance and diversity of species, therefore,

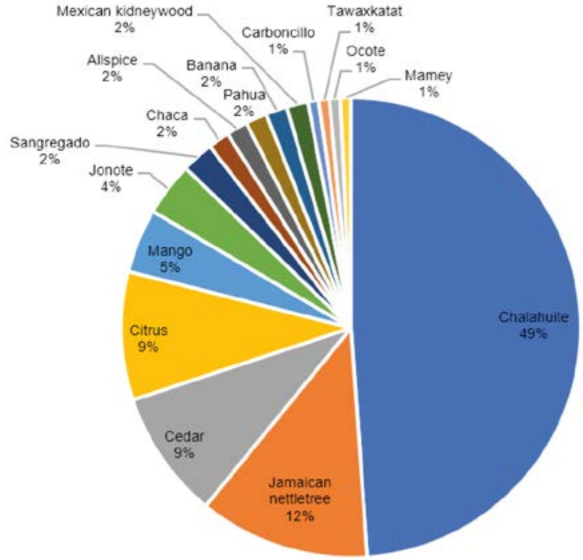

Figure 5. Shade trees in coffee plantations in the Northeastern Sierra (Nororiental region) of the state of Puebla.

it presents better conditions for development compared to other crops such as maize. Producers who cultivate coffee plants using the shade system, they prune the trees from March to May to allow sunlight to reach the coffee plants. They also remove dead or diseased branches, which decompose and generate organic fertilizer that is reintegrated into the soil. Coffee growers observe that coffee plantations with abundant trees have less water erosion because the drops fall on leaves of the trees and not directly onto the soil. The time when they do shade control is from April to June, because agricultural activities are fewer or scarce.

Agronomic management

Producers perform a variety of practices, primarily by hand, with hand-made tools and materials. However, as a result of the recent Governmental program "Recuperación del Campo Poblano", some producers have benefited with chainsaws, brush cutters, and motor-powered sprayers, among other pieces of equipment. The practices with the greatest positive impact on production and pest and disease reduction are the pruning of coffee plants, weed removal, shade control, soil fertilization, and the incorporation of organic matter. On the other hand, those implemented in less than 50% of cases are living fences, deadfalls, terracing, contour planting, associated crops, coffee plantation renovation, '*pepena*' (the local word for the collection of fruits remaining on soil), foliar fertilization, and the application of insecticides and fungicides (Table 1).

These practices coincide with those mentioned by Venegas-Sandoval *et al.* (2021) in a research implemented in a coffee-growing area of the state of Chiapas, Mexico.

Table 1. Agronomic and phytosanitary practices in coffee cultivation in the municipalities of Huehuetla, Ixtepec and Zongozotla, in the Northeastern Sierra of Puebla, Mexico.

Management Practice	Huehuetla		Ixtepec		Zongozotla	
	Y (%)	N (%)	Y (%)	N (%)	Y (%)	N (%)
Pruning of coffee plants	96	4	84	16	100	0
Weed control	100	0	100	0	100	0
Shade management	96	4	100	0	52	48
Soil fertilization	38	62	37	63	100	0
Foliar fertilization	13	87	11	89	10	90
Application of insecticides and fungicides	50	50	16	84	14	86
Level curves	8	92	11	89	4	96
Terraces	17	83	16	84	8	92
Living fences	33	67	21	79	22	78
Dead barriers	29	71	11	89	10	90
Organic matter	96	4	100	0	52	48
Associated crops	17	83	16	84	36	64
Replanting	13	87	16	84	28	72
Gathering of fruits on soil (pepena)	25	75	21	79	36	64

Source: original prepared with data from surveyed participants, 2024 (Huehuetla n=24, Ixtepec n=19, Zongozotla n=50). Y: yes, practice is present; N: no, practice is not present at; Municipalities with Totonac population: Huehuetla, Ixtepec, Zongozotla (Puebla), Mexico.

Weed control

Weed control is made by all the producers (100%), who emphasize its importance because it is complicated to get other activities done if the plot is invaded by weeds and shrubs. This practice consists of cutting the weeds with a long blade (called machete) to a height of 5 to 10 cm from the ground, to prevent erosion in the rainy season, and for the roots to help avoiding the loss of soil; to a lesser extent, the hoe or a brush cutter are used. In coffee plantations in the productive stage, weeding is done 2 to 3 times a year, in January, May and September; whereas, in plantations in growing stage, more than 3 cuttings are made. This coincides with Hernández-Solabac *et al.* (2011) who emphasized that timely weed control is the main practice in coffee-growing communities in Veracruz, because it favors production by preventing the coffee plant from competing for water and nutrients with other species.

Pruning of coffee plants and shade control

Almost all coffee growers (96%) do at least one annual pruning, mainly topping, coppicing and cleaning. Topping stands out, which has the purpose of forming new shoots, thus eliminating unproductive shoots in plants. In second place, coppicing which corresponds to cutting the top-shoots of coffee plants to stop the height growth of the plant. Coffee growers do this at a height determined by each producer (1.60 m is the most usual). According to experience this practice favors the generation of secondary branches and increases production. In terms of cleaning, they cut the damaged "bandolas" (first-order branches bearing leaves and fruits); they do it throughout the crop coppice. Merlín-Uribe et al. (2018) mentioned that pruning strengthens production and decreases the incidence of coffee rust. On the other hand, 100% of producers do shade control in this shade-grown production system.

Fertilization

Up to 71% of farmers fertilize the soil, primarily with urea, while only 10% of them apply foliar fertilization, two to three times a year with chemicals such as Gro Green® or Bayfolan®. In this regard, the municipality of Zongozotla is highlighted because 100% of producers fertilize the soil, applying an average of 300 grams per plant spread over three applications per year. In contrast, only 38% growers fertilize the soil in Huehuetla, and 37% do so in Ixtepec, applying approximately 200 grams per year spread over two to three applications.

Therefore, it is suggested to promote practices that strengthen plant nutrition, not only at the soil level, but also integrating foliar fertilization into agricultural practices, since the latter is not a widespread practice among producers. In the case of Zongozotla, producers apply fertilizers to the foliage 3 to 12 times a year. The most common dose is 50 grams of fertilizer dissolved in a 20L-sprinkler. Seventy-three percent of farmers use organic fertilizers such as compost and pruning waste as alternatives due to the high cost of chemical products. Producers do not have a schedule for fertilizer application, so the frequency of applications depends on their own financial means and on the government programs that provide technology packages to producers.

Pests and diseases

Farmers (75%) also mentioned coffee rust (*Hemileia vastatrix*) as the main disease affecting coffee crops and the coffee-berry borer (*Hypothenemus hampei*) as the most important pest, similar to what was reported by Leguizamo-Sotelo *et al.* (2024) in Amatepec, State of Mexico. Producers recognize and detect the severity of this disease, however, only 23% implement prevention practices or control activities through foliar application of chemicals. Among them, 15% receive fungicides through government agencies, mainly through the head of their Municipality and the State Committee for Plant Health in the state of Puebla (CESAVEP); while others acquire agrochemicals in stores outside their communities.

Locally the sale of agricultural inputs is scarce and it is difficult to find a specific product for a pest or disease for the management of coffee plantations. Fifty percent of Huehuetla producers apply fungicides and insecticides, while only 15% of Ixtepec and Zongozotla producers do so, making it essential to adopt phytosanitary practices. Producers in Huehuetla reported that the highest incidence of rust occurs in December and April, while in Ixtepec they mentioned December. In Zongozotla, they noted that the disease is most common in September and October.

The application of insecticides and fungicides is a very essential practice to maintain the sanitation of crops and is an essential part of obtaining good harvests and economic resources for the sustenance of Totonac families dedicated to coffee production. However, temperature and relative humidity also affect production, as mentioned by Jaramillo-Villanueva *et al.* (2022), the increase in pests and diseases, strong winds, excessive rain and droughts are events that are attributed to climate change, this seriously affects production because it is reduced by up to 30%.

These affectations have been constant since the appearance of rust, an example is what was reported by Escamilla-Prado (2016) who observed that the period between 2012 and 2016 was very complicated for coffee growers, for there is the estimation from the attack of rust that it caused a decrease of up to 50% of the national figure of coffee production in Mexico. Weed control, the pruning of coffee plants, shade control, and fertilization are the main maintenance activities. On the other hand, rust and the coffee-berry borer are the main pathogens that require phytosanitary solutions (Figure 6).

Among other practices that could be strengthened in the Totonac communities is the design of plantation in contour lines, considering that there are steep slopes in the region. Therefore, it is suggested to implement plantations with individual terraces, terraces in furrows, as well as to rehabilitate drainage ditches for each plant. However, living fences and deadfalls should also be encouraged. They serve to delimit plots, act as windbreaks, thus providing crop protection from climatic factors such as hurricanes, droughts, or excessive rainfall.

Deadfalls should be constructed using the existing materials on the plots; stones, branches, and logs should be placed transversely to the slope. This will reduce the velocity of water flow, thus retaining organic matter. In the coffee plantations of the study sites there is a diversity of plants in the cultivation areas; however, the association of crops through a spatial arrangement with which different products are obtained in the same surface unit is essential.

Figure 6. Agricultural activities most commonly implemented by coffee growers in the Northeastern Sierra (Nororiental region) of Puebla, Mexico. A: monitoring damage by the coffee-berry borer, B: monitoring damage by coffee rust, C: manual weed removal with a machete, D: shade control with a chainsaw, E: pruning of coffee plants (shoot reactivation), F: foliar fertilizer application, G: mechanized weed removal with a brush cutter, H: traditional shade control with handy tools, I: pruning of coffee plants, J: fertilization with agrochemicals, K: organic fertilization by compost application, L: shade control and firewood production.

On the other hand, replanting and renewal of coffee plantations is a critical point because in the study area they found plantations that exceed 30 years of production, which require a renewal plan, agronomic and phytosanitary management. This coincides with what was mentioned by Cardeña-Basilio *et al.* (2019) in a study on the production system established in Hueytamalco, (Puebla) Mexico (Figure 7).

Fourteen agricultural practices were identified, the most common is weed control, pruning of coffee plants, shade management, soil fertilization, and use of organic matter. However, gathering fruits on soil, crop association, living fences, foliar fertilization, application of insecticides and fungicides, replanting coffee plants, use of deadfalls, terracing, and planting on contour lines are implemented to a lesser extent. Therefore, it is necessary to strengthen and increase government support programs for coffee growing, based on the design of inclusive public policies in accordance with the linguistic, cultural, economic, and geographic contexts of the Northeastern Sierra. In addition, phytosanitary and soil fertility diagnoses should be done, in order to design management and technical support plans that allow determining the most appropriate agricultural activities for each production unit.

Figure 7. Less common practices that could be implemented in coffee plantations in the Northeastern Sierra (Nororiental region) of the state of Puebla, Mexico. A: furrow terraces; B: furrow layout on contour lines using the "A" frame level; C: individual draining ditch construction; D: individual terraces; E: coffee plantation renovation.

CONCLUSIONS

The age of the coffee growers ranged from 19 to 81 years; their predominant native language is Totonac. Coffee growing in Huehuetla, Ixtepec, and Zongozotla is established in diversified agroecosystems where coffee plants coexist other with fruit, medicinal, food, fuel, construction, and shade tree species. Among those, the Mexican chalahuite (*Inga* sp.) is predominant. Twelve varieties of coffee are grown in these municipalities; the varieties Costa Rica 95, Oro Azteca, and Marsellesa are the most prominent, established in young plantations with less than 15 years old. Although there are other plantations, with varieties Garnica, Caturra, and Borbón that were established over 30 years ago.

Thus, regarding phytosanitary aspects, the presence of coffee rust (*Hemileia vastatrix*) and the coffee-berry borer (*Hypothenemus hampei*) are the main problems. Average cultivation surface is one hectare per producer; where they obtain in average, 297.85 kg (5.18 quintales) of parchment coffee. These results can be considered for decision-making and the design of actions in the sustainable management of the coffee agroecosystem in the region.

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Colegio de Postgraduados

Anthocyanins in local, healthy and specialty agrifood from the Sierra Nevada of Puebla

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ABSTRACT

Objective: to quantify anthocyanins in blue maize, raspberry, and blackberry fruit, grown in family systems in three localities of the Sierra Nevada in Puebla, in order to know the local perception and valuation of those compounds.

Design/Methodology/Approach: during 2024-2025, a mixed methodology was applied in the selected localities (San Mateo Ozolco, San Miguel Tianguizolco and San Diego Buenavista, in the state of Puebla, Mexico). Anthocyanins were quantified in five samples per culture using Ultraviolet-Visible spectrophotometry, analysis of variance and Tukey ($p \leq 0.05$). To evaluate perception, 45 surveys were applied following a non-probabilistic convenience sampling in local agrifood fairs.

Results: high anthocyanin contents were recorded; blackberry fruit (5733.6 mg kg^{-1}), raspberry (923.9 mg kg^{-1}), and blue maize (673.5-893.7 mg kg^{-1}); all above what is recommended for nutraceutical effects. A 76% of partially informed consumers was identified, with a willingness to pay a premium for agrifood, proven it is rich in anthocyanins.

Limitations/Implications of the study: it is necessary to expand this analysis to more regions and to consider agronomic variables, processing and bioavailability; in addition to promoting socialization strategies that translate scientific evidence into socioeconomic and food value, strengthening the rural economy and consumption.

Findings/Conclusions: blue maize and berries grown in family systems have high nutraceutical and socioeconomic value that can be used as an agri-bio-cultural heritage in rural farming economy, through sustainable rural and indigenous production schemes.

Keywords: functional agrifood, nutraceuticals, cyanidin-3-glucoside (C3G), local perception, socioeconomic value.

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INTRODUCTION

The hegemonic global agrifood system has transformed production and consumption, displacing local agrifood with ultra-processed products. These latter are harmful to health, environment, culture and local economies; as they have high content of refined sugars, salt,

fats and additives. This transition has weakened farming systems, crop diversity, peasant artisanal processes, and marketing; in addition to affecting food culture and aggravating food insecurity (Torres & Rojas, 2024).

The problem is aggravated in rural and indigenous areas that, despite the production of healthy agrifood, have seen its consumption displaced, which have generated food and health problems. In Mexico, 55.5% of households live in food insecurity, with Puebla among the most affected entities according to Mexico's Consejo Nacional de Evaluación de la Política de Desarrollo Social (CONEVAL, 2020). In view of this, there is a growing interest in agrifood such as blue maize and berries known as red fruits or just, berries. They are recognized for their anthocyanins content, which are pigments of the flavonoid group that give reddish, purple and bluish tones, and have nutraceutical properties.

In rural and indigenous communities of the Sierra Nevada in Puebla, berries cultivation and consumption strengthen food security and local economy through sustainable systems and community social participation (Xochipa *et al.*, 2024). In San Diego Buenavista (state of Puebla, Mexico), the second largest locality as producer of berries in the state, more than 11 million Mexican pesos (MMXN) are generated by the selling of 383 tons (Megagrams, Mg) raspberries, and 2.6 MMXN per 103 Mg of blackberry fruits, according to Mexico's Agri-Food and Fisheries Information Service (SIAP, 2024).

In San Miguel Tianguizolco and San Mateo Ozolco, native blue maize represents cultural and linguistic identity (Nahuatl), linked to the ancient farming worldviews (Xochipa-Morante *et al.*, 2024). Although these practices are invisible in official statistics, they yield annually 6825 Mg of berries in the state, cultivated in 1606 hectares (ha) with a value of more than 43 Mmxn according to Mexico's Agri-Food Information System Database under the Secretariat of Agriculture (SIACON, 2023).

Family systems with traditional, transitional, or agroecological management have a direct impact on the concentration of anthocyanins that determine the functional and nutraceutical quality of berries and blue maize. Their sale as raw material, and processed artisanal or semi-industrial products in short-selling circuits (SSC) strengthens direct sales, proximity, trust and exchange of knowledge between producers and consumers. These dynamics promote fair trade, solidarity economy, and access to agrifood with bioactive compounds beneficial to health (Xochipa-Morante *et al.*, 2021). Among those compounds is cyanidin-3-glucoside (C3G), an anthocyanin present in blue maize, raspberry and blackberry fruits.

However, most studies focus on commercial cultivars (Vázquez-Carrillo *et al.*, 2025), leaving aside native and wild species in rural and indigenous contexts. This omission reduces the understanding of the local perception and valuation of these compounds, limiting scientific knowledge, the revaluation of biodiversity, and their incorporation into rural development policies aimed at food security and sovereignty. This security understood as the continuous access to sufficient, nutritious and quality food; and sovereignty, as the character that defends the right of people to decide their own local and sustainable food systems (Xochipa *et al.*, 2024). The problem addressed is the lack of knowledge about the anthocyanin content in those crops, as well as the local perception and valuation of these compounds and their effects on food and health.

This lack of information limits the utilization of crops with high socioeconomic, environmental, and health value. Therefore, the objective is to quantify the anthocyanin content (C3G) in blue maize, blackberry, and raspberry cultivated in three locations of the Sierra Nevada, and to assess the local perception and valuation of these compounds and their consumption.

The hypothesis posits that these agri-foods possess functional and socio-productive properties that can be harnessed within agrobiocultural heritage and Short Food Supply Chains (SFSC).

The study's relevance lies in highlighting the nutraceutical and socioeconomic value of these agri-foods grown in family farming systems, recovering traditional and agroecological practices, and reducing the use of GMOs and agrochemicals. Overlooking them would continue to marginalize knowledge, crops, and territories that are fundamental for transitioning toward healthy, sustainable, and culturally appropriate food systems.

MATERIALS AND METHODS

This study was designed as of mixed type, with both quantitative and qualitative characteristics, in an exploratory and analytical-descriptive approach. It was developed in two stages; the first one was the chemical analysis of native and wild fruit samples. And the second stage was the exploration of perception and valuation on the part of consumers, about agrifood with anthocyanins.

Study area

The study was implemented in three localities of the Sierra Nevada in Puebla (Mexico). San Mateo Ozolco belongs to the municipality of Calpan, while San Miguel Tianguizolco and San Diego Buenavista are in the municipality of Huejotzingo (Figure 1).

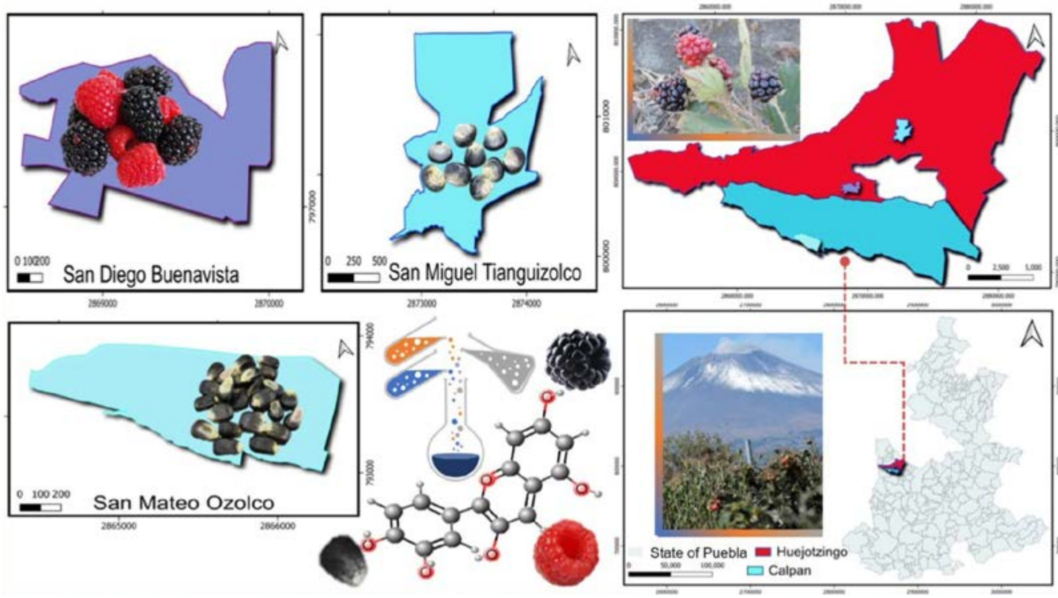

Figure 1. Geographical location of the study area. Source: prepared by the authors in QGIS.

These localities are between 2200 and 2600 m altitude, in a temperate subhumid climate, with fertile volcanic soils and ideal conditions for the cultivation of blue maize (*Zea mays* L.) and berries such as blackberry fruit (*Rubus fruticocus*) and raspberry (*Rubus idaeus*). Production systems are family-based on traditional practices, agroecological practices, or developing in a transitional process between both.

The socio-territorial context includes elements such as migration, marginalization, indigenous population, high levels of food insecurity, and health problems under the criteria of Mexico's National Council for the Evaluation of Social Development Policy (CONEVAL, 2020) and Mexico's National Institute of Statistics and Geography (INEGI, 2020).

Quantification of anthocyanin content

Experimental design. A completely randomized design was applied with a single factor (community) and three replicates per type of crop. In total, 15 samples were analyzed; five native blue maize, five blackberry fruits (Maravilla variety), and five raspberry (Tuppy variety).

Genetic material and sample collection. During the harvest season (November 2024 and May 2025), 200 grams of samples of blue grains and blackberry and raspberry fruits in an optimal state of ripeness were collected for consumption. Harvest was made directly in the plots of the farmers.

Sample preparation and anthocyanin extraction. These procedures were done in the Laboratorio de Instrumentacion de Alimentos, under the Universidad Tecnológica de Huejotzingo (UTH), following the methodology of Mendoza-Mendoza *et al.* (2017).

Blue maize. Sampled grains were dried at a temperature of 70 °C in a Felisa® stove, then pulverized in a Wiley® stainless steel mill, with a 2-mm mesh test sieve, until a homogeneous flour was obtained.

Berries. The fruits were dried at 65 °C in a Felisa® oven, then ground to a particle size of 1 mm in diameter for preservation.

Extraction. It was made using 70% acidified methanol with 1% hydrochloric acid (HCl), at room temperature for 15 minutes. The solutions were then vacuum filtered with Whatman No. 1 filter paper. The residue was washed with 10 mL of acidified methanol and measured at 50 mL with buffer solutions of potassium chloride (KCl) pH 1 and sodium acetate (CH_3-COONa) pH 4.5, then the corresponding dilutions were carried out ensuring that the dilution for both samples had an absorbance lower than 1.0 and in the range of 0.4 to 0.6. The samples were stored at −20 °C until analysis. The reagents and solvents used were Sigma-Aldrich® and JT Baker® analytical grades.

Spectrophotometric quantification, calculations and statistical analyses. The differential pH method proposed by Wolstrad (1976) was used, spectral measurements were made on a single-beam UV-Visible spectrophotometer (CARY 50 Conc) at 515 nm and 700 nm in triplicate.

The total anthocyanins concentration (TAC) was calculated as cyanidin 3-glucoside mg kg^{-1} of dry matter (DM) of grain or fruit flour, according to the formula of Mendoza-Mendoza *et al.* (2017). A descriptive analysis was performed, followed by an analysis of variance to compare differences between communities by crop and the Tukey test to identify those means that were significant ($p \leq 0.05$). All analyses were executed in SPSS© v.26.

Local perception on anthocyanins and consumption valuation

During 2024-2025, 45 surveys (15 per crop) were applied to frequent consumers of blue maize, raspberries and blackberries in short selling circuits (SSC) of agrifood. The sampling was non-probabilistic for convenience, applied in representative local fairs: the berry fair (Buenavista), the maize fair (Tianguizolco) and the pulque fair (Ozolco), the latter with a strong presence of blue maize.

The questionnaire included questions on knowledge of the term 'anthocyanins' and assessments associated with its consumption. Data were captured in Microsoft™ Excel® and analyzed with descriptive statistics (frequencies, means, standard deviations) with SPSS© v.26. Analysis results were represented in graphics, and data frequencies, in word clouds.

RESULTS AND DISCUSSION

Anthocyanin content

We recorded concentrations of cyanidine 3-glucoside (C3G) in blue maize, raspberry and blackberry fruit from the Sierra Nevada in Puebla (Mexico). Our results showed that blackberry fruit had the highest anthocyaninis content value (5733.6 mg kg^{-1} DM), followed by that of raspberry (923.9 mg kg^{-1} DM), and blue maize (673.5-893.7mg kg^{-1} DM) in third place (Table 1).

These findings coincide with those of Castañeda-Cardona *et al.* (2024), with 7780 mg kg^{-1} DM in blackberries and 880 mg kg^{-1} in raspberries. Also they coincide with those of Kim *et al.* (2021), who reported 11 500 and 2500 mg kg^{-1} DM in blackberries and raspberries, respectively. This confirms the high accumulation capacity of anthocyanins in blackberry fruit. In this study, the varieties Tuppy and Maravilla, together with the production system in agroecological transition, were able to influence the levels recorded in raspberry and blackberry fruit.

In blue maize, in Tianguizolco we found a higher concentration (893.7 mg kg^{-1}) than in Ozolco (673.5 mg kg^{-1}). These differences can be attributed to factors such as altitude,

Table 1. Concentration of 3-glucoside cyanidine (C3G) in agrifood, per locality and per crop, in three localities of Puebla (Mexico).

Crop	Locality	Mean (mg C3G kg^{-1} DW)	Range (mg C3G kg^{-1} DW)	Production System
Blue maize	Ozolco	673.46±73.80 B	551.8-726.4	Agroecological
	Tianguizolco	893.66±13.33 A	728.5-1002.4	Traditional
Raspberry	Buenavista	923.88±133.75 B	789.0-1123.0	In agroecological transition
Blackberry fruit		5733.56±539.63 A	5070.2-6487.5	

Values are expressed as mean ± standard deviation (n=5). DW: dry weight (biomass). Means with different letters indicate significant difference (Tukey; $p \leq 0.05$).

local variety, agricultural practices, soil management, and especially to the availability of nitrogen and phosphate, both of which are critical in anthocyanin synthesis.

The grain of the traditional system presented higher C3G than the agroecological grain of Ozolco, possibly due to the use of mineral fertilizers (N-P-K), DAP (diammonium phosphate), UREA and Triple 18, which stimulate the PAL, F3H, DFR enzymatic pathways, associated with the synthesis of anthocyanins (Feng *et al.*, 2024).

In agroecological systems with the application of organic fertilizers (compost, manure) and foliar fertilizers, nitrogen is released slowly, levels may be suboptimal, which does not allow to achieve similar values of accumulation of anthocyanins. Nonetheless, these results show that both family-based systems, traditional and agroecological, can generate functional agrifood with high nutraceutical value.

Local perception about anthocyanins and consumption valuation

The surveyed participants were mostly women (62%), with an average age 40.6±10.8 years old (range, 21-66 years), their level of education is predominantly a bachelor degree. Among the products valued are those with native blue maize in value-added presentations such as tostadas, tortilla chips and baked nachos, and freshly made tlacoyos. As for berries, their consumption is mainly fresh and seasonal, with purchases made in short cycles, either weekly, monthly, or occasionally.

Local knowledge of the term 'anthocyanins'

Of the interviewees, 47% have heard the term 'anthocyanins', this indicates an initial but significant knowledge about these compounds. The other half of participants is unaware of this concept, thus reflecting there is a gap between scientific knowledge and everyday knowledge. So, consumers' profile feature is defined as 'partially informed' (Figure 2).

This lack of knowledge contrasts with the scientific relevance of anthocyanins, since the impact of these substances on health is widely documented. It also reinforces the need for accessible dissemination strategies that bring scientific evidence closer to local contexts (urban and rural).

When we asked which word they associate anthocyanins with, the most frequent answers were 'antioxidants' in the case of berries, and 'pigments' in blue maize. Other

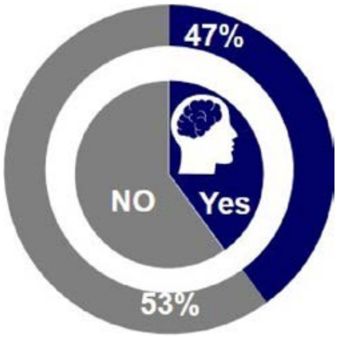

Figure 2. Knowledge of the participants on the term, by answering the question "Have you heard of anthocyanins?"

general terms, such as 'healthy' and 'nutraceuticals' were also recorded, although their specific functions were not clearly identified by the participants (Figure 3).

These associations reflect a basic, largely intuitive and non-technical understanding. Although there is valuable local knowledge, it is fragmentary and empirically linked to well-being and healthy food. This perception represents an important basis to be strengthened, through food education actions, as well as through diversification of products and territorial development (Xochipa *et al.*, 2024).

Criteria for traditional valuation associated with consumption

Consumption of this agrifood occurs mainly in SSCs (specialty niches, healthy markets, agrifood fairs, traditional cuisines and direct sales on the farm), which strengthens the link between origin, perceived quality and consumer confidence. This dynamic facilitates direct communication between producer and consumer, also creating strategic opportunities to introduce precise knowledge on concepts such as 'anthocyanins' and the health benefits with which they contribute to human health, as 'functional foods'.

We summarize here the evaluation criteria recorded with a higher frequency in the responses of surveyed participants for the evaluated agrifood.

The main reasons for consumption are organoleptic properties —taste, color, texture, consistency, flexibility, freshness— (80%); followed by nutritional properties (11%), perceived health benefits, origin (native creole maize); finally, because of tradition, artisanal production, and freshness. This combination reveals that there is a tangible appreciation of functional qualities, even without formal knowledge about its bioactive compounds.

It is worth noticing that 76% of consumers declared to be willing to pay a premium between 5% and 30%, if it is guaranteed that these products contain anthocyanins beneficial to health, while the remaining 24% expressed economic limitations or mistrust. This willingness-to-pay represents an opportunity to differentiate products, especially those made with blue grains rich in anthocyanins. For this reason, it is key to quantify their

Figure 3. Words associated with the concept 'anthocyanins' in berries and blue maize. Source: word clouds created in atlas.ti, with data from the field survey.

Table 2. Valuation associated with the consumption of blue maize and berries in San Mateo Ozolco, San Miguel Tianguizolco and San Diego Buenavista (Puebla) Mexico.

Variable	Blue maize from Ozolco	Blue maize from Tianguizolco	Berries from Buenavista
Site of purchase	Specialty niches (healthy markets & stores, organic & gourmet) (40%) Production unit (40%) Fairs (13%) Traditional cuisines	Urban markets and street markets 87% Production unit: 13%	Supply market (33%) Direct sales on farm (27%) Healthy store (27%) Fairs (13%)
Reason for consumption	Organoleptic properties (45%) Beneficial to health (33%)	Organoleptic properties (60%) Creole maize (origin), artisanal tradition.	Organoleptic properties (75%) Beneficial to health (25%)
Are you willing to pay more for the benefit of agrifood rich in anthocyanins?	Yes (80%) No (20%)	Yes (73%) No (27%)	Yes (73%) No (27%)
Additonal payment	17.5±7.2% (5-30%)	11.0±7.4% (3-30%)	14.6±4.2% (10-20%)

content after processing, to communicate it then through innovative labeling, since these compounds provide competitive value in specialty niches interested in functional foods and nutraceuticals.

CONCLUSIONS

Anthocyanin content [cyanidin, 3-glucoside (C3G)] in blackberry fruit, raspberry and blue maize grown in the Sierra Nevada in Puebla (Mexico), reached levels with nutraceutical effects. This confirms potential as functional and nutraceutical agrifood with strategic value. The variations observed reflect the influence of local production systems, variety and local agro-environmental condition.

The importance was highlighted of integrating the traditional knowledge of rural farmers and agroecological approaches in the use of these compounds. Partially informed consumers were identified, with willingness-to-pay a premium for products with anthocyanins in short-selling circuits. A diversification of products requires for attractive labeling strategies, food education and health prevention processes to strengthen the linkage between producer and consumer.

Blue maize and berries are key resources for productive diversification, to the strengthening of agro-bio-cultural heritage and development of solidarity economies for rural farmers. It is recommended to research stability and bioavailability of anthocyanins, also, on healthy food strategies with a territorial approach. It is required as well, to translate scientific knowledge into practical and communal benefits towards creating fair, sustainable and culturally relevant agrifood systems.

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Colegio de Postgraduados

Evaluation of adherents to coat maize seeds (*Zea mays* L.) with vegetable flours and their effect on germination

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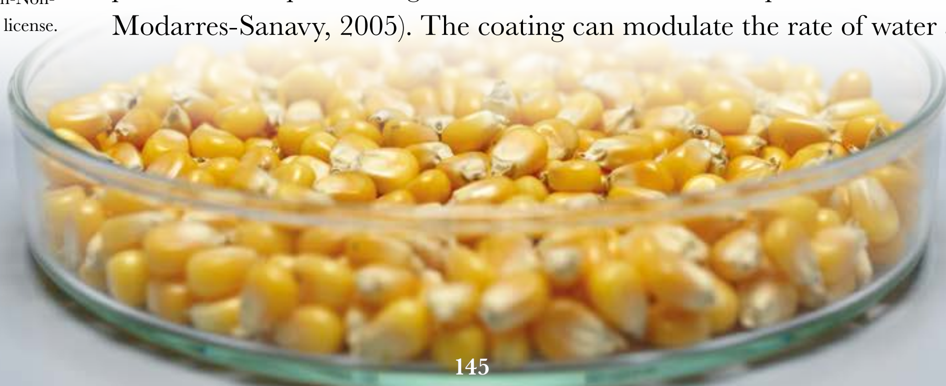
ABSTRACT

Objective: to evaluate the adhesion capacity of different materials in the coating of seeds with vegetable flours from broccoli shears as biostimulant extracts.
Design/Methodology/Approach: the percentage of seed coverage treated with different adherents was evaluated; commercial, polyterpene, carboxymethyl, starch and casein, previously standardized for application in doses of 1 mL in 100 seeds. The percentage of germination, growth and some biochemical variables of the seeds treated with adherents with and without vegetable flour was evaluated, with the application of 1.5 g in 100 seeds.
Results: the adherents with the highest coverage were commercial and polyterpene, with coverage percentages higher than 98%. Germination was not modified by the treatments. There were non-significant differences in the other variables in relation to treatments without HV. Except the application of HV that reduced plumule length by 7%, and fresh weight by 12%. In biochemical variables, the application of adherent and vegetable flours favored increasing in the content of phenols, total proteins and pigments.
Limitations/Implications of the study: in regard to adherents, similar results were found for most variables; this is, there were non-significant differences among adherents. Thus suggesting that some variations can be introduced in the composition or concentrations of adherents in order to amplify the test.
Findings/Conclusions: the information from this study can serve as a model for applying vegetable flours as bio-stimulant extracts in seeds or plants, with a sustainable approach.

Keywords: bio-stimulants, seed coating, germination, bio-compounds.

INTRODUCTION

Seed coating is a technique that consists of covering the surface with materials that protect and improve the germination and initial development of the seedling (Ehsanfar & Modarres-Sanavy, 2005). The coating can modulate the rate of water absorption and gas



exchange; promote metabolic and enzymatic activation; protect the seed from pathogens; facilitate mechanical manipulation; and mainly, coating can act as a carrier of compounds that stimulate or protect the seedling (Su *et al.*, 2017). In addition, the size, shape, weight and texture of the seeds can be modified.

In seed coating techniques, a layer of adherent material is applied that acts as a vehicle for the adhesion of the materials. This allows incorporating not only nutrients and chemical protectants, but also bio-inputs of plant origin, such as extracts, vegetable flours, essential oils or beneficial microorganisms, in a direct and localized way (Piri *et al.*, 2019). The adhesion and homogeneous distribution on the seeds are determined by the physical and chemical characteristics of the adherent, as well as by its concentration. These adherents, depending on the purpose of the coating, are related to the improvement in water retention capacity; they are easy to degrade, provide adhesion strength and compatibility with nutrients and other additives (Montoya *et al.*, 2021).

In sustainable agriculture, vegetable flours and plant extracts are a source of bioactive compounds with biostimulant and biofungicidal potential (Sanfilippo & Patti, 2022). On the other hand, vegetable flours can also serve as soil improvers, biofertilizers, and a source of carbon (Arámbula-Castillo *et al.*, 2023). Species of the genus *Brassica*, such as broccoli (*Brassica oleracea* var. *italica*), contain secondary metabolites such as glucosinolates, isothiocyanates and phenols in their tissues, which have shown activity in stimulating plant growth, defending against pests and diseases, and improving nutriments absorption (Oulad El Majdoub *et al.*, 2020). In particular, flour obtained from dried and ground broccoli leaves represents a stable, easy-to-store and easy-to-apply form that retains a good part of these functional compounds (Waisen *et al.*, 2022; Peñas de la Corte, 2025).

That is why, the objective in this study was to evaluate the adhesion capacity of different materials in the coating of seeds with vegetable flours made of broccoli shears as biostimulant extracts.

MATERIALS AND METHODS

This study was done in the Department of Horticulture of the Autonomous Agrarian University Antonio Narro (UAAAN, Coahuila, Mexico), in the plant physiology laboratory. Seeds of the maize variety Tuxpeno were used; with various adherents in different concentration that were standardized before being applied to the seeds (Table 1). Distilled water was used as the zero control (T0) and a commercial adherent (pegoDel™, AgroDelta, Mexico) as a commercial control (COM). For the application of treatments, the indications on the label was used (Table 1).

Broccoli shears were used, leaves dried at ambient temperature, macerated and sifted in mesh number 100, in the form of vegetable flour (HV) as a biostimulant extract; of which we analyzed and characterized its biochemical composition (Table 2).

For conditioning, 200 maize seeds were used per treatment, previously disinfected with 5% sodium hypochlorite and dried in the environment. The application dose of the adherents was 1 mL in 100 seeds.

Table 1. Standardization of adherents.

Adherent	Concentration (%)	Temperature (°C)	Agitation (minutes)
T0	100	Ambiente	1
COM	100	Ambiente	1
P100	100	Ambiente	5
P75	75		
C0.5	0.5	45	20
C0.25	0.25		
AL	20	50	30
CS	20	45	40

T0: zero control; COM: commercial adherent; P100: 100% polyterpene; P75: 75% polyterpene; C0.5: carboxymethyl-cellulose 0.5; C0.25: carboxymethyl-cellulose 0.25; AL: maize starch; CS: sodium caseinate (Casein).

Table 2. Biochemical characterization of vegetable flour (HV) applied to seeds as biostimulant extracts.

Total Phenols mg GAE 100 g^{-1}	Flavonoids mg CE 100 g^{-1}	Glutathione mg 100 g^{-1}	Antioxidant capacity mg AAE100 g^{-1}	Total proteins mg AE 100 g^{-1} DW
501.157	7.040	498.134	40.44	882.928

GAEs: gallic acid equivalents; CE: catechin equivalents; AAE: azinobis acid equivalents; AE: albumin equivalents; DW: dry weight.

They were shaken for 1 minute and allowed to dry for 5 minutes. Then, 1.5 g of HV was added, to those treatments that included it; and mixed slowly with circular movements until homogenized (Figure 1).

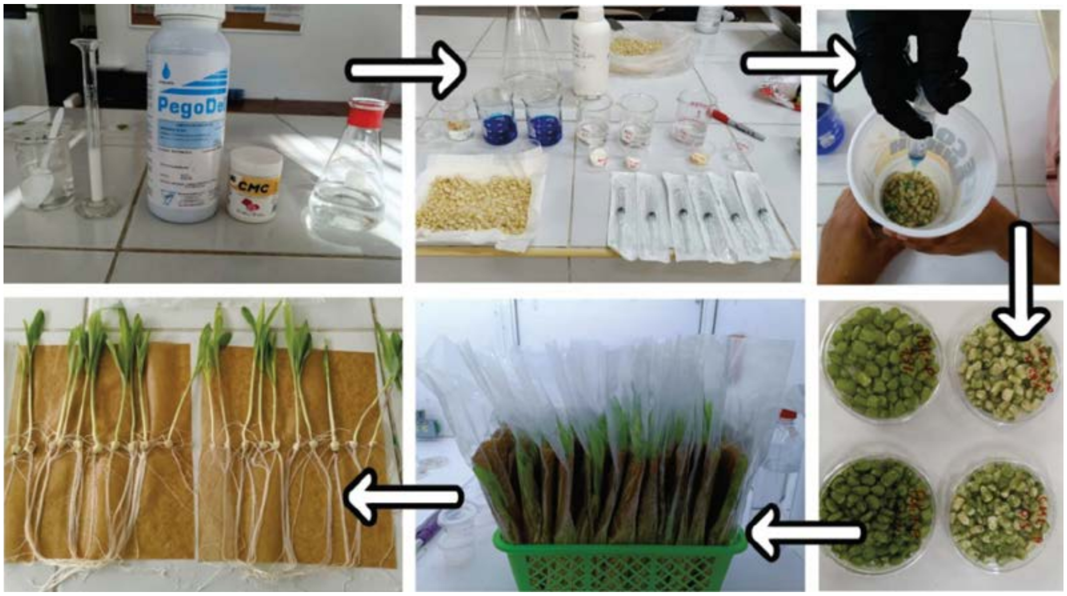


Figure 1. Process of preparation, application and evaluation of treatments on maize seeds.

Seed coating variables

Coverage percentage. Photographs were taken with a (Nikon Coolpix L23) digital camera coupled to a (NOVA XTD 20) stereoscope. The coverage percentage (based on surface area) was determined using Image-Pro Plus version 6.0 (Media Cybernetics, Rockville, MD, USA).

Coating structure. A (JEOL JSM-IT510, InTouchScope) scanning electron microscope (SEM) was used with an integrated (Philips) digital camera. The samples were coated with Palladium by means of sputtering equipment (Sputter Coater LITH-SD80).

Physiological variables

Germination. Twenty-five seeds were sown on Anchor paper sheets with 4 replicates, placed in plastic bags in a semi-automatic germination chamber at a temperature of 28 °C for 7 days. The results are expressed as a germination percentage.

Length of plumule and radicle. The plumule was measured from the coleoptyl to the apex of the primary leaf with a digital vernier (Caliper, 0.001”). The radicle was measured from the junction with the coleoptyl to the tip of the taproot.

Fresh and dry matter. The endosperm was separated in 10 seedlings to assemble the experimental unit, with four replicates, they were weighed on an analytical balance (OHAUS). Then, those were dried in an oven (Memmert UF75) at a temperature of 75 °C for 72 hours, afterwards they were weighed.

Biochemical variables in seedlings

Frozen samples (Thermo Scientific-40086FA) and lyophilized samples (Labconco FreeZone) were used to determine biochemical variables.

Pigments. Determined as proposed by (Wellburn, 1994), 30 mg of the sample with 2000 μL of pure methanol. Quantification was performed in a UV-Vis spectrophotometer (Genesis 10s Uv-Vis, Thermo Scientific, USA) at 666, 653 and 470 nm.

Phenols. Determined by colorimetry with the Folin Ciocalteu method proposed by Singleton *et al*. (1999); 10 mg of the sample with 1000 μL of pure methanol for extraction. Absorbance was measured at 760 nm.

Glutathione. Determined according to Xue *et al*. (2001); DTNB (5,5-dithiobis-2 benzoic nitro acid) was used for the staining reaction. Absorbance was measured at 412 nm.

Flavonoids. Determined according to Zhishen *et al*. (1999); in a 100-mg sample with 2 mL of pure methanol for extraction. Absorbance was measured at 510 nm.

Total proteins. Determined by Bradford spectrophotometric; in 100 mg of the sample and 10 mg of polyvinylpyrrolidone (PVP) with 2 mL of 0.1 M phosphate buffer (pH 7-7.2). Absorbance was measured at 595 nm.

Antioxidant compounds. Determined according to Re *et al*. (1999), with the discoloration test of the radical cation ABTS [2,2’-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid]. Absorbance was measured at 754 nm.

Experimental design

A completely randomized design with factorial arrangement was used. Factor 1 was the type of adherent and Factor 2, HV added or not-added. A Pearson correlation analysis was performed for physiological and growth variables, and a univariate analysis for the other variables. We arranged 10 seedlings as the experimental unit with four replicates per treatment. Once the assumptions of normality and homogeneity were met, an analysis of variance and the Fisher mean test ($p \leq 0.05$) were performed. All statistical procedures were performed with Statistica® v8.0 (TIBCO Software, Palo Alto, USA) and the graphs were generated with SigmaPlot© (v12.0).

RESULTS AND DISCUSSION

Seed coating with adherents and vegetable flour

The adherents that had the highest adherence capacity were those with vegetable flour (HV) that showed the highest coverage percentage on the seeds (Figure 2). The adherents Polyterpene, at concentrations 100% and 75%, and the commercial COM were the highest, with coverage percentages greater than 98% of the total surface area of the seed ($p \leq 0.05$). The adherents with the lowest coverage percentage were the zero control (9.44%), carboxymethyl in the two tested concentrations (5.56%), maize starch (9.90%) and casein (2.69%).

The comparison is shown of an uncoated seed with seeds treated with adherents and HV (Figure 3). There, the statistical results of coverage percentage discussed above (Figure 2) are visually confirmed. This information is of great importance since it allows us to make a selection of the adherents that can be used most effectively, when conditioning seeds with products in the form of flours or powders. Using the correct adherent in seed conditioning minimizes product losses during application, which makes the process more efficient and reduces costs (Job *et al.*, 2018).

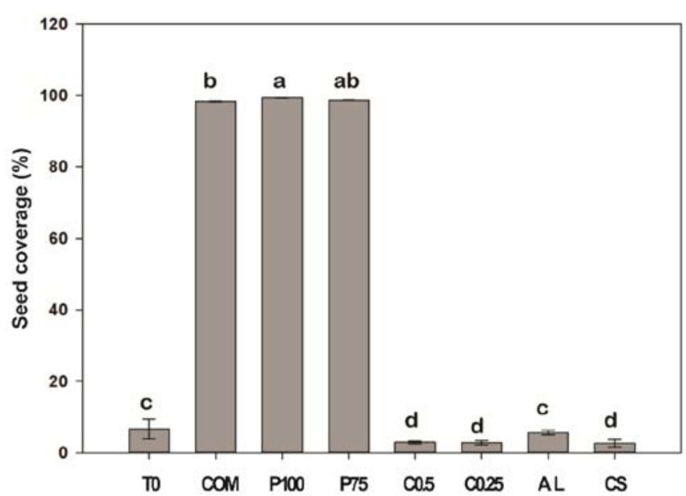


Figure 2. Coverage percentage of maize seeds conditioned with the different adherents and vegetable flour (HV). Different letters indicate statistical difference (Fisher, $p \leq 0.05$). T0: zero control; COM: commercial adherent; P100: 100% polyterpene; P75: 75% polyterpene; C0.5: carboxymethyl-cellulose 0.5; C0.25: carboxymethyl-cellulose 0.25; AL: maize starch; CS: sodium caseinate (Casein).

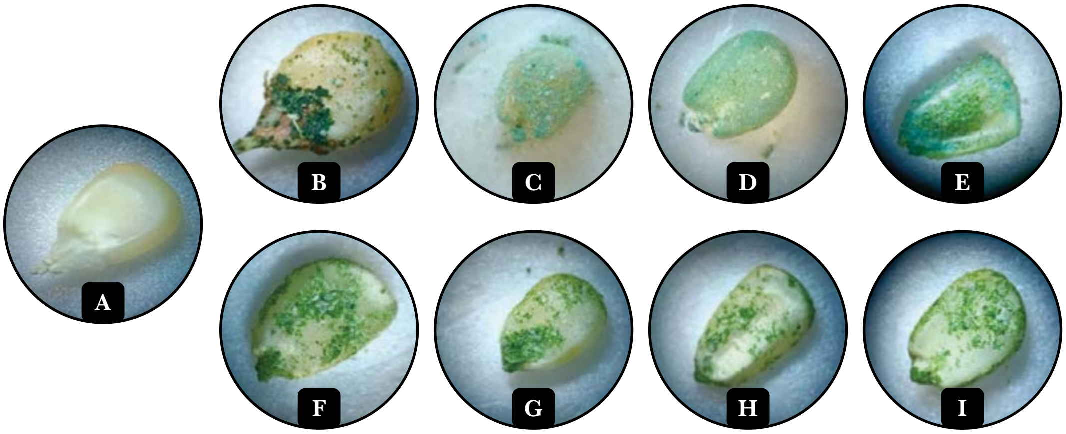


Figure 3. Maize seeds conditioned with the different adherents and vegetable flour (HV). A: unconditioned seed; B: zero control (water); C: commercial adherent; D: 100% polyterpene; E: 75% polyterpene; F: 0.5% carboxymethyl; G: carboxymethyl 0.25%, H: maize starch; I: Casein.

Figure 4 shows the images captured under a scanning electron microscope (SEM) of the surface of the seeds coated with the different adherents and their distribution. The commercial adherent was the most homogeneous (Figure 4A), followed by the polyterpene in its two concentrations (Figure 4B). Those were different from carboxymethyl-cellulose, maize starch, and casein (Figure 4C, Figure 4D and Figure 4E), which showed a low presence of the adherent.

Although the seed surface distribution of the polyterpene was visibly lower compared to the commercial adherent, the latter was statistically lower than the 100% polyterpene, but equal to the 75% polyterpene concentration. The characteristics that must be met may be related to physical processes, such as fixing the material of interest without it being detached during handling or storage, it must not be toxic so that physiological processes are carried out properly, it must not react with the compounds of interest that are applied, so that they do not interfere with biostimulant activity (Zhang *et al*., 2022).

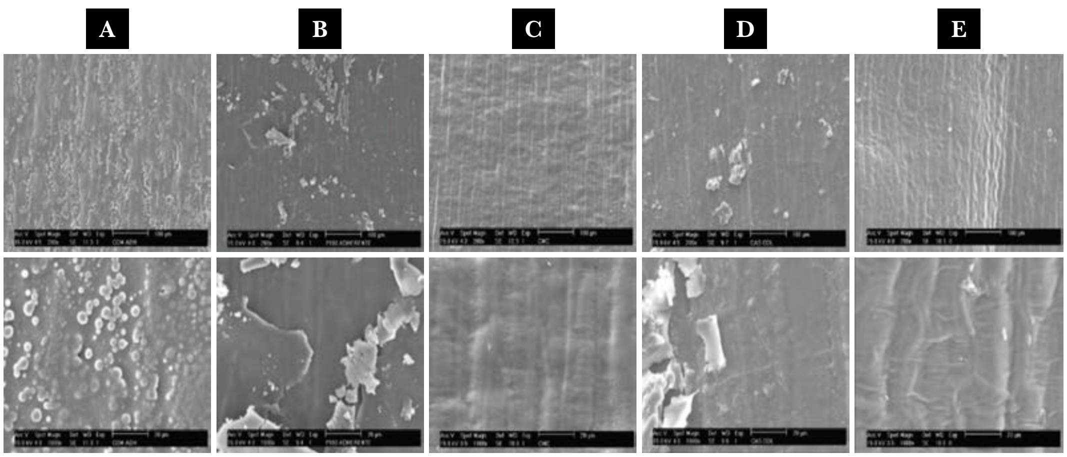


Figure 4. Views through scanning electron microscopy (SEM) of the coatings, with lenses 200X (top images) and 1000X (bottom images), on the surface of the seeds, with the different adherents. A: commercial adherent; B: polyterpene; C: carboxymethyl; D: maize starch; E: casein.

The SEM visualizations of the seed conditioned with the commercial adherent and the vegetable flour are presented (Figure 5). We can observe a homogeneous coating on the surface of the seed (Figure 5, A1), and when making a cross-section of the seed, it is observed how the HV forms volumes of different sizes on the surface (Figure 5, A2). The same is true for seeds conditioned with 100% Polyterpene (Figure 5, B1 and B2). An adequate coating with the biostimulant facilitates to handle the seed and allows an adequate release in the immediate environment to germination. The choice of adherent depends on the ability to keep these products attached to the surface of the seed (Sohail *et al.*, 2022).

Physiological and biochemical indicators

There were non-significant differences in the germination percentage between seed lots treated with HV or without HV (Figure 6), which indicates that germination was not influenced by the application of HV. Regarding only the adherent factors, there were statistical differences ($p \leq 0.05$) in the treatment with maize starch without HV (86% $\pm$15) which was the lowest and similar to the commercial adherent (92% $\pm$5), compared to the others, polyterpene (93% $\pm$9) and casein (94% $\pm$5) with HV. In regard to the treatments with adherents with HV (control, carboxymethyl and casein) and without HV (control, polyterpene, carboxymethyl and casein), the germination percentages were higher than 99%, with non-significant difference among them.

In their study Gorim & Asch (2012) determined that there is a strong influence of seed coat thickness and composition on the germination rate and mobilization efficiency of reserves stored in the endosperm. Those authors used hydrophilic adherents with humic acid and biplanto (a commercial biostimulant); with those the coating significantly reduced

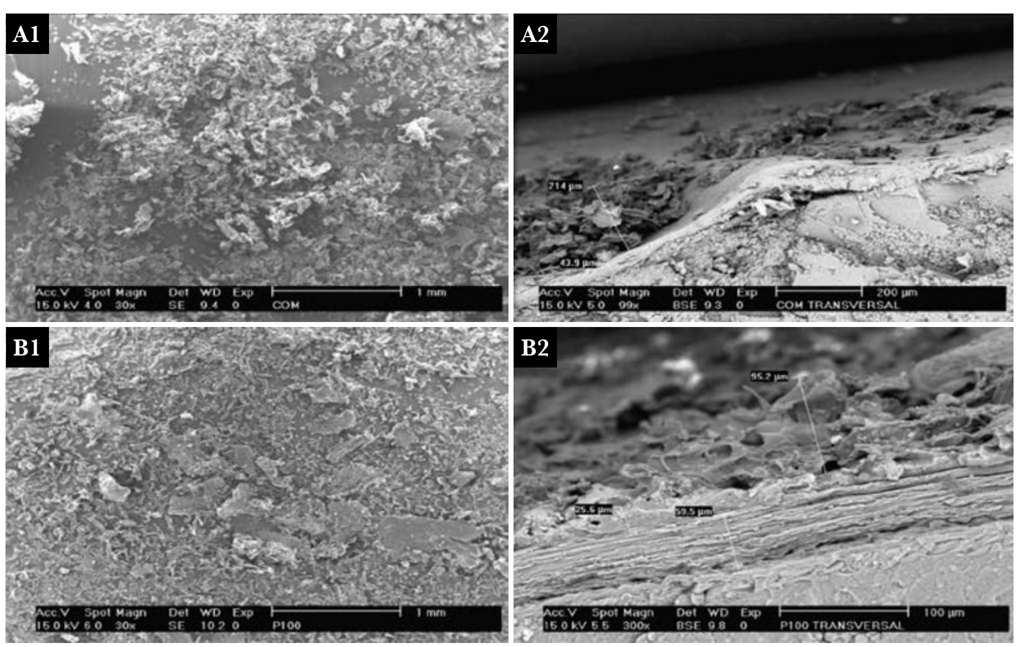


Figure 5. Surface area of maize seeds viewed in a scanning electron microscope (SEM). A1: commercial adherent with HV; B1: 100% polyterpene with HV [both, 30X objective lens]. Cross-sectional views of seeds with A2: commercial adherent plus HV and B2: 100% polyterpene plus HV [99X and 300X objective lens].

the total germination of barley, rye and wheat, compared to uncoated seeds. Germination is a useful parameter to measure the effect of a product with biostimulant action, since this latter either can or cannot improve the metabolic processes in the initial stages of the seedling (Kola & Carvalho, 2023).

In this study, the application of HV as biostimulant extract did not have an effect on germination. However, when evaluated together with the different adherents, it was observed that the adherents with the highest percentage of coverage (Figure 2) were statistically equal to the lowest value of germination without HV of the maize starch (Figure 6). The reduction in the percentage of germination with this latter treatment may be due to its physical properties, since it forms a thick layer that can act as a physical barrier that hinders the absorption of water and gas exchange, which delays or prevents imbibition in seeds.

The combined effect of maize seed coating with the different adherents and the application or not of HV on plumule length and radicle length is presented in Figure 7. Statistically significant differences ($p \leq 0.05$) were observed, between applying or not applying HV, in the plumule length, but not in radicle length (Figure 7). Plumule growth was reduced by 7% in HV treatments compared to non-HV treatments. These results suggest that the biostimulant activity of HV was not positive on the initial development of the seedling. On the other hand, type of adherent did have a variable behavior with or without HV (Figure 7).

In the variable plumule length, the zero control with HV presented the lowest values compared to most treatments with or without HV. Although it was statistically equal ($p > 0.05$) to the maize starch adherent (with or without HV), and to the commercial adherent with HV. Regarding the radicle length variable, most of the adherent treatments

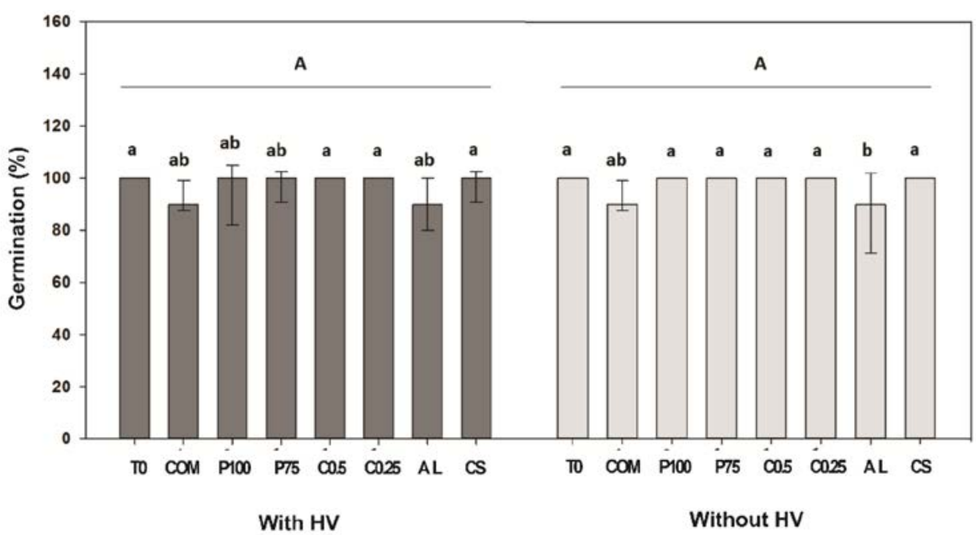


Figure 6. Germination percentage in maize seeds conditioned with different adherents and vegetable flour (HV). T0: zero control; COM: commercial adherent; P100: 100% polyterpene; P75: 75% polyterpene; C0.5: carboxymethyl-cellulose 0.5; C0.25: carboxymethyl-cellulose 0.25; AL: maize starch; CS: sodium caseinate (Casein). Letters in uppercase correspond to Factor 2 (with or without HV), letters in lowercase correspond to Factor 1 (type of adherent). Means with different letters indicate statistical difference (Fisher, $p \leq 0.05$).

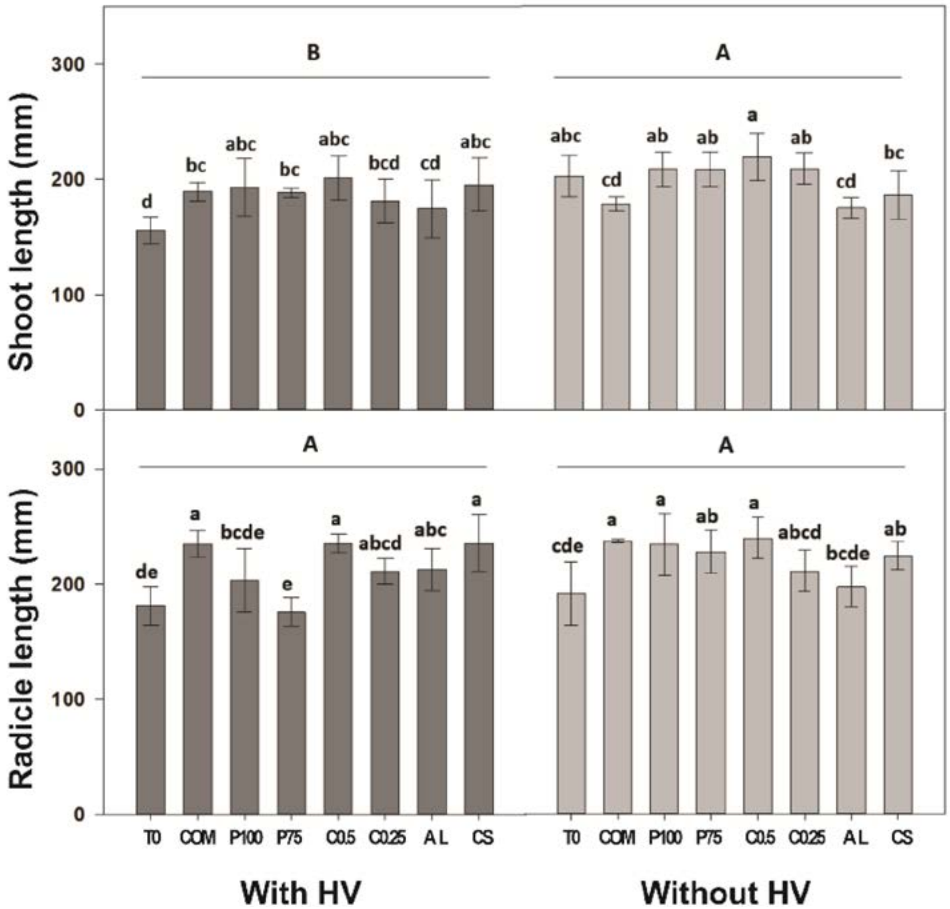


Figure 7. Plumule length and radicle length of maize seedlings with different adherent treatments with or without vegetable flour (HV). T0: zero control; COM: commercial adherent; P100: 100% polyterpene; P75: 75% polyterpene; C0.5: carboxymethyl-cellulose 0.5; C0.25: carboxymethyl-cellulose 0.25; AL: maize starch; CS: sodium caseinate (Casein). Letters in uppercase correspond to Factor 2 (with or without HV), letters in lowercase correspond to Factor 1 (type of adherent). Means with different letters indicate statistical difference (Fisher, $p \leq 0.05$).

(with or without HV) were statistically equal, except the zero control and polyterpene in its two concentrations with HV. This is of great importance since the greatest coverage with HV was with commercial adherent and polyterpene (Figure 2). These same treatments without HV showed the highest values in radicle length.

Results indicated that seeds with greater coverage percentage and with HV had a similar development in plumule length, compared to seeds without HV. In contrast, regarding radicle length, the lowest values were present in seeds treated with HV, compared to treatments without HV. This, although there were non-significant differences ($p>0.05$), indicates there could be an effect between the adherent and the HV. Our results coincide with those obtained by Qiu *et al*. (2020), who evaluated the seed coating with a mixture of soybean flour, diatomaceous earth, and vermicompost extracts, obtaining positive results in germination rate and growth of red clover plants, but a reduction on these same variables in seeds of perennial grass (Ray grass).

In a study developed by Qiu *et al.* (2020), those authors proposed a method for the application of bio-based biostimulants as seed coatings. Those authors used different sources of liquid and powder forms of vermicompost and soybean flour, and the formulations did not decrease germination parameters and were non-significantly different from those of the control. The efficiency of biostimulants, applied as a coating on seeds, may be influenced by the adherent material. These may or may not favor the solubility and release of the bioactive compounds present in biostimulants, moreover, adherents can even interfere with the biostimulant activity (Ayed *et al.*, 2022). As an example, adherents with hydrophilic properties can promote the release of the compounds during imbibition, whereas more compact or hydrophobic adherents can delay that process (Tweddell *et al.*, 2000).

On the other hand, the use of biostimulant extracts can modulate different physiological and metabolic processes since they contain biocompounds such as phenols and flavonoids. Therefore, it is important to consider the application doses of those biocompounds, because in high doses they can generate toxicity (Van Duijn, 2024).

Figure 8 shows fresh weight and dry weight of the maize seedlings treated with seed coatings. It can be observed that there were significant differences ($p \leq 0.05$) in fresh weight, but there were non-significant differences in dry weight with or without HV (Figure 8). The application of HV reduced fresh weight by 12.02% in relation to treatment without HV. As for adherents, the highest values were found in fresh weight with adherents without HV, although statistically equal with only the maize starch adherent with HV (Figure 8). The opposite case was observed in dry weight; the highest value (maize starch 102.34 mg$\pm$4.34) and the lowest value (casein 56.83 mg$\pm$9.43) were found with adherents plus HV.

Fresh weight and dry weight in seedlings are important indicators of water processes and physiological development, because they are related to structural biomass and to the yield as growth of functional structures. The application of biostimulants has a positive effect, in a generalized way, on fresh and dry biomass, this effect is closely related to the application dose. In a study where the application of a plant-based biostimulant in forest seeds was evaluated, positive results were obtained in some variables, such as biomass and photosynthetic capacity (Huang *et al.*, 2019). Overall, these results highlight the importance of considering carefully the product to be used, the application dose, and the interaction with the adherent selected to apply to seeds.

Regarding the results obtained in the biochemical variables, those only highlighted the seedlings from seeds treated with the adherents that had the highest coverage percentage (Figure 2) and coated with HV. Table 3 presents the results obtained, where we can observe statistically significant differences ($p \leq 0.05$) in the content of phenols, total proteins and pigments, but not in glutathione or flavonoids contents. In the case of phenols, the treatment with commercial adherent and 100% polyterpene had a higher content (46.87% and 54.41%), compared to the control with HV; also 20.25% and 26.42% more compared to the control without HV. For total proteins, the commercial adherent had the highest values, and was statistically equal to the control without HV; indicating that the increase cannot be attributed to HV application. In pigments (chlorophylls and carotenoids) the control without HV had the lowest contents in relation to HV treatments.

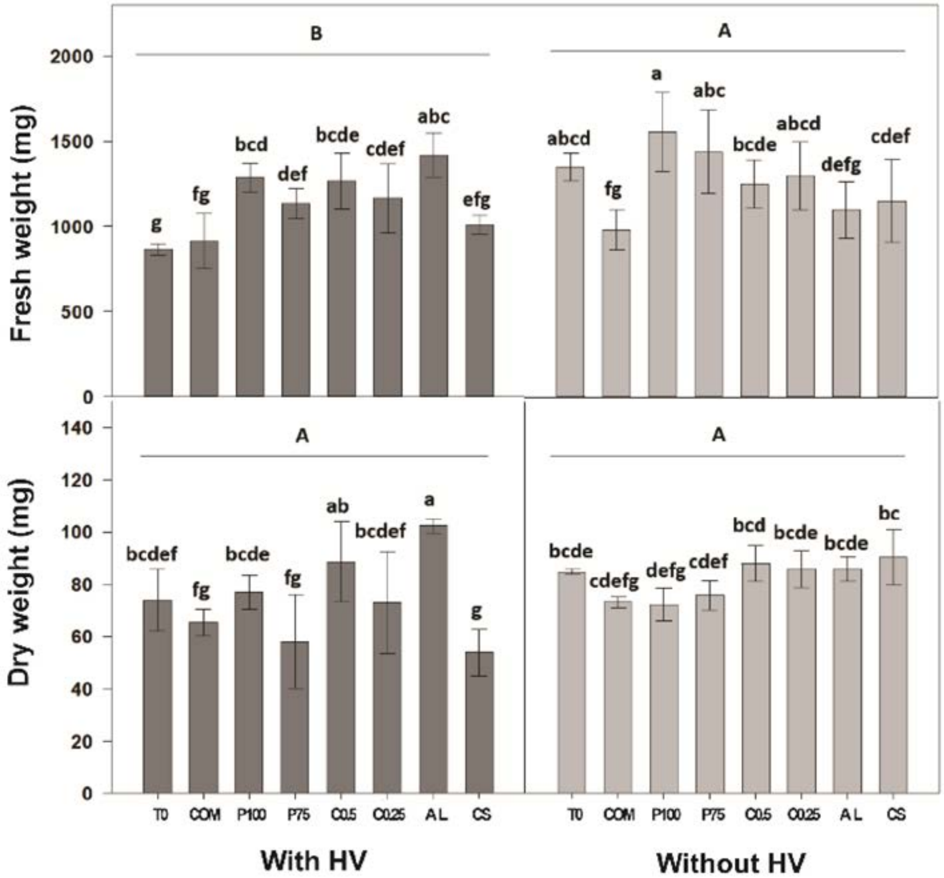


Figure 8. Fresh weight and dry weight of maize seedlings with the different adherent treatments with or without vegetable flour (HV). T0: zero control; COM: commercial adherent; P100: 100% polyterpene; P75: 75% polyterpene; C0.5: carboxymethyl-cellulose 0.5; C0.25: carboxymethyl-cellulose 0.25; AL: maize starch; CS: sodium caseinate (Casein). Letters in uppercase correspond to Factor 2 (with or without HV), letters in lowercase correspond to Factor 1 (type of adherent). Means with different letters indicate statistical difference (Fisher, $p \leq 0.05$).

Table 3. Biochemical variables of maize seedlings with different seed coating treatments done with those adherents with the highest coverage percentage.

	T0 without HV	T0 with HV	COM with HV	P75 with HV	CV
Glutathione mg 100 g^{-1} DW	580.07±60 a	593.98±80 a	632.25±44 a	623.989±52 a	7.03
Phenols mg GAE 100 g^{-1} DW	1200.23±101 b	982.63±148 c	1443.28±74 a	1517.36±108 a	8.63
Flavonoids mg CE 100 g^{-1} DW	5.43±0.98 a	5.91±0.97 a	5.98±0.85 a	4.97±0.83 a	17.87
Total proteins mg AE 100 g^{-1} DW	1055.67±165 ab	686.45±90 c	1184.14±150 a	853.12±133 bc	14.54
Total chlorophyll mg 100 g^{-1} DW	1810.88±162 b	3133.14±430 a	3412.095±565 a	2513.78±355 ab	18.44
Carotenoids mg 100 g^{-1} DW	36.45±9.45 c	196.70±62 b	310.90±27 a	202.39±37 b	20.9

Means with different letters indicate statistical difference (Fisher, $p \leq 0.05$). CV: coefficient of variation; GAEs: gallic acid equivalents; CE: catechin equivalents; AE: albumin equivalents. DW: dry weight. T0: zero control; COM: commercial adherent; P75: 75% polyterpene.

The increase in phenol content could be associated with the activation of antioxidant and plant defense metabolic pathways, generated by stress. Stress can be identified in physiological variables such as fresh weight and plumule length, for which HV treatments had lower results compared to non-HV treatments. Biostimulants have the ability to potentiate the production of biocompounds in plants, because they regulate biochemical mechanisms and promote tolerance to oxidative stress (Mir & Khah, 2024). Other studies conclude that the application of biostimulants, in the early stages of the plant and in adequate doses, improves antioxidant production, stimulates enzymatic and non-enzymatic activity, improves cellular homeostasis and adaptation to stress (Johnson & Puthur, 2022).

CONCLUSIONS

Results obtained in this study indicated that the adherents with the best adhesion capacity were the commercial compound and the proposed polyterpene as a viable alternative. The application of vegetable flour did not have a significant impact compared to treatments without it. In most of the variables evaluated, those were statistically equal, except in plumule length and fresh weight, where they were even lower.

Seeds treated with adherents showed non-significant differences among treatments. Biochemical variables did have a positive effect with the application of adherents and vegetable flour, compared to the zero control with and without it. The information obtained in this study can serve as a model for the application of vegetable flours, as extracts in solid form with biostimulant activity in seeds or plants, towards a sustainable approach to crop conditioning and management. Additional studies are also necessary to evaluate and propose other application rates for vegetable flours and their effect on the entire plant cycle.

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Colegio de Postgraduados

Agro-industrial residues: a link in the circular economy chain

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ABSTRACT

Objective: to compile, through documentary research, the potential of agro-industrial residues in different agriculture sectors, through their use as raw material towards generating other economically viable products.

Design/Methodology/Approach: documentary research in different primary and secondary sources of current consultation and databases of Mexico's Government Secretariats (Agriculture-SADER, Economy, and Natural Resources-SEMARNAT), to analyze the importance of agro-industrial waste as a source of raw material in processes that generate value-added products. The analysis of the information focused on highlighting the importance of the current use and transformation of residues and waste generated in various sectors of the agri-food industry. Also, in the identification of the challenges faced by this industry to achieve a circular economy. Emphasis was placed on innovative strategies and research aimed at converting these residues into value-added products.

Results: according to the sources consulted, about 35% on average of the raw materials used in food production end up as waste. Many of these residues have bioactive, nutritional or functional properties that make them suitable for alternative applications. In recent years, agro-industrial by-products and residual fluxes have attracted the attention of the scientific community due to their potential for valorization. A synthesis of information on current food products was obtained that give a guideline to generate usable waste and with easily extracted active compounds. Certain components of residues, nowadays wasted, can be reintegrated into the production cycle as raw materials for new processes, thus reducing environmental impact and promoting a circular economy.

Limitations/Implications of the study: only databases with official information were consulted; without discrimination for specific or extensive documentary sources by subsector or product. However, innovation was identified and emphasized in those by-products identified with the greatest potential for transformation.

Conclusions: despite the interest in sustainability and minimizing environmental damage, the environmental problem and non-sustainability of the food industry that often affects the country occurs because the industry continues to be aligned with a linear economic model of extraction, production and disposal that generates a considerable amount of waste. Limited infrastructure for waste recovery in new value chains is a barrier to the large-scale implementation of circular economics principles, which is another crucial step in achieving long-term sustainability.

Keywords: food industry, valorization, zero waste, sustainability.

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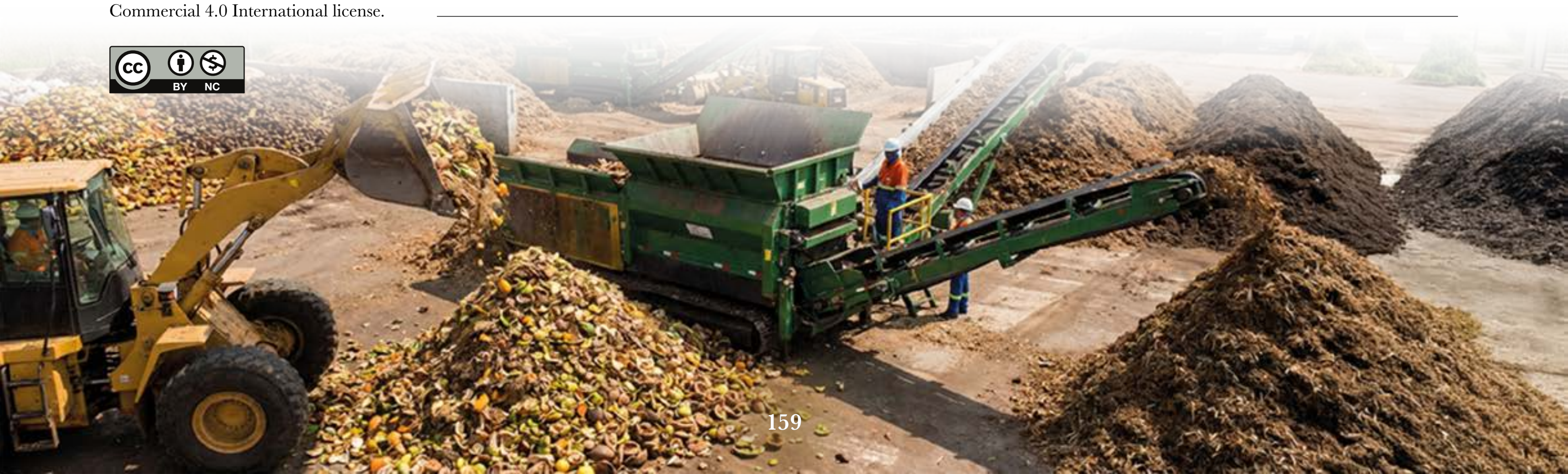

INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO, 2020) defines agribusiness as the set of economic activities that favor the transformation of products from agriculture, livestock, fishing and forestry into more valuable final goods. That is, the production of raw materials and intermediate products derived from the primary sector, which creates employment opportunities and contributes to global economic development.

On the other hand, according to the Secretariat of Agriculture and Rural Development (SADER, 2016), there are five types of agribusiness: food, non-food, supplier of raw materials, consumer of raw materials and artisanal. Food agribusiness comprises raw materials from the agricultural, livestock, aquaculture and forestry sectors, and is aimed exclusively at obtaining food. Meanwhile, non-food agribusiness is responsible for the transformation of agricultural products into non-food products such as wood, flowers, tobacco, fibers, dyes, among other very diverse products.

In a similar and complementary way, the raw material supplier industry is engaged in the initial processing of agricultural products such as milling, tanning, pressing, sawing and canning. While the raw material consuming industry is responsible for the manufacture of articles based on intermediate products derived from agricultural materials, such as the manufacture of paper, fabrics, clothing, footwear or rubber manufactures. In each of the aforementioned agro-industries, residues and waste materials are generated, biomass that is not fully used. These agro-industrial residues can constitute a real pollution problem in some parts of the world due to the increase in the demand for food consumption, which forces a constant increase in food production.

The main negative impacts on agribusiness are related to the contamination of soils and aquifers, as well as the emission of gases that contribute to the greenhouse effect. This is due to the elimination of solid, liquid and semi-liquid waste with a high concentration of organic load, chemical oxygen demand (COD) and biochemical oxygen demand (BOD) that require adequate sanitation to minimize their environmental impact and excessive expenses that this industry sometimes fails to cover. However, agro-industrial waste can be an important and rapidly developing source of accessible raw material with low cost of production. Because they are derived from a main process, the industry accounts for expenses in the production of that main product and not the by-product. In addition, any agro-industrial residue can be converted into various by-products, which generates added value and less environmental pollution.

To explore various options, the objective of this review was to compile, through documentary research, the potential of agro-industrial residues in different agriculture sectors, through their use as raw material towards generating other economically viable products.

MATERIALS AND METHODS

A database search and documentary review of recent scientific articles was done on official websites of the Secretariat of Agriculture and Rural Development (SADER), Secretariat of Economy (SE), and Secretariat of Environment and Natural Resources

(SEMARNAT), among others. The keywords considered important information related to food production and waste generation from the food industry.

The criterion for determining the objects of analysis comes from Mexico's General Law for the Prevention and Comprehensive Management of Waste (PROFEPA, 2023); according to which, waste is "those materials or products whose owner or possessor discards and which are in a solid or semi-solid, liquid or gaseous state, are contained in containers or deposits and may be susceptible to recovery or require treatment or final disposal in accordance with the provisions of the same Law". Although their disposal is intended to avoid health or environmental problems, the scarcity of some raw materials or the depletion of their sources means that residues can be reused or included as a constituent part of other processes.

The analysis was emphasized on agro-industrial waste, which is waste, by-products, or leftover solid or liquid materials of organic origin that are generated during the processes of reception, production and processing, packaging, and even storage of agricultural, livestock, and food products (Romero-Sáez, 2022). The by-products identified by the analysis come from the livestock, poultry, and cattle and sheep breeding sectors; from the food industry (production of dairy, meat, fruits, vegetables, bakery, or additives); from the beverage industry (juices, wines, beers); from the sugar sector (bagasse, molasses, and blackstrap molasses); as well as from the confectionery industry and oil industry.

RESULTS AND DISCUSSION

Agro-industries that generate waste

Food agro-industries includes the production, processing and marketing of products for consumption as human food. This industrial sector includes sugar production; the non-alcoholic beverage industry, and the beer or table wine industry; the dairy industry, with the production of cheese, yogurt, and packaged liquid milk; the meat industry, which produces cuttings of meat and sausages; the bakery industry (bread, pasta); the canning industry (such as dressings, sauces and dips); as well as the confectionery industry.

Each of the agro-industries described above generates between 35-40% of residues throughout the production chain, due to the natural or induced deterioration of food, or as part of its preparation. Residues from the food agro-industry can be classified into agri-waste (agriculture debris, such as stems, leaves, or roots discarded in the harvesting process) or by-products. Agriculture debris are also known as waste, because in most cases they are only discarded and disposed, because they are not recognized as valuable to be used in other production processes. Although there are some examples reported of agricultural residues used in composting, energy generation (Razia *et al.*, 2024); construction materials (Vieira *et al.*, 2025); fertilizer production (Mahish *et al.*, 2025) and in the extraction of bioactive compounds (Panda *et al.*, 2025).

On the other hand, there are processing residues or by-products (pulp, bagasse, seeds, fibers, bones, serums, wastewater, among others). Also known as discarded products, which are materials that are generated during processing, but are not the main products. These are disposed as well because they are not considered valuable to be used in other production processes. As by-products are included, the harvest remains of fruits and vegetables, peels or husks, and seeds from agriculture use; and from livestock, blood, bones, skin, fat, as well as those products that do not meet quality standards for sale to the consumer.

Food industries must implement good waste management, separating residues, classifying them not to be mixed with other stuff or chemicals, to avoid the risk of turning them into hazardous waste that cannot be used again, such is the case of some chemical waste derived from transformation processes. The consumer also discards packaging that is a mixture of plastic, metal and paper which are difficult to separate, among others. Each food industry has specific process residues or by-products that depend on the type of main product that is generated. Some have the potential to be used, through being reprocessed to become raw materials for other products. So, those can generate other forms of marketing that minimize pollution; examples are described in the next section.

Sugar industry

The sugar industry is engaged in the production, processing and marketing of sugar or sucrose from sugarcane (*Saccharum officinarum*). In Mexico, 55 589 515 tons (Megrams, Mg) of sugar approximately are produced per year, which position this country in the eighth place as sugar producer worldwide (SADER, 2024). The general flow chart for obtaining sugar shows the most important stages of the process; and it is worth mentioning that each mill or industry has clear specifications for its individual or peripheral processes (Figure 1).

In general, once the sugar cane is harvested, it is transferred to the mill to wash it and obtain the juice by crushing and squeezing the cane. In this part of the process, the first residue, called bagasse, is obtained. Later, the juice goes through a process of evaporation of water by heating and in this way the sugar is concentrated.

This results in a denser and darker syrup due to enzymatic browning, and the obtained residues are the molasses, which are the most important fraction in the process to obtain sugar. Finally, the sugar crystals are separated from the remaining liquid by centrifugation;

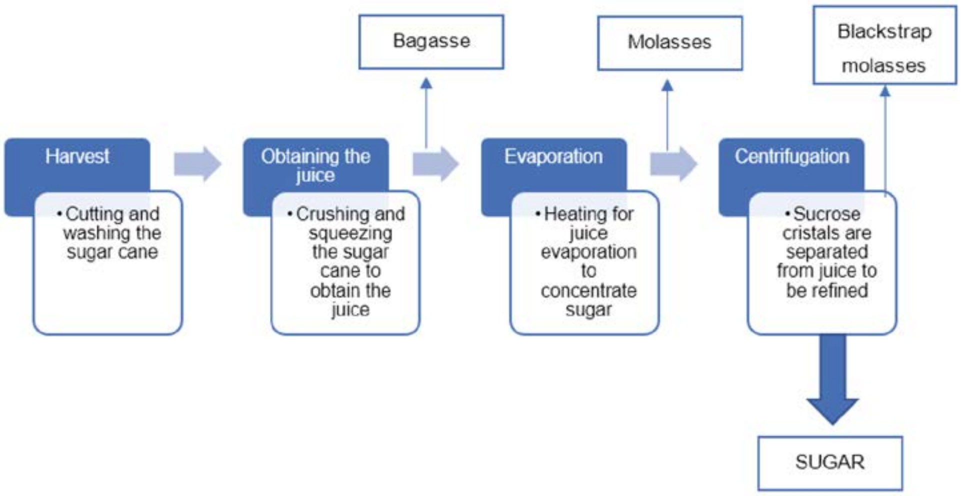

Figure 1. General diagram of sugar production, as obtained from sugar cane (*Saccharum officinarum*). Source: original prepared by the authors.

and they are refined to obtain brown or white sugar. The darkest and densest waste obtained here is another by-product called blackstrap molasses.

Sugarcane bagasse is a fibrous waste composite of cellulose, hemicellulose and lignin, and is used as a material for construction, energy production, and bioplastics production (Phiri *et al.*, 2024). Whereas, molasses are dense and dark liquids that contains non-crystallized carbohydrates, minerals and vitamins, whose uses are mainly as a sweetener (Camelo-Rangel *et al.*, 2025) and for citric acid production (Dementev *et al.*, 2024). Blackstrap is a jellified and dark by-product formed from the sediments of tanks that contain water, impurities, waxes, soil particles; hydrocarbons and residual dissolved sugars from the sugar cane. Blackstrap molasses is used as fertilizer (Singh *et al.*, 2025).

Currently, waste derived from the sugar industry is part of an area of opportunity to produce other viable compounds of economic interest that could even be reused by the sugar industry itself. Such is the case of biogas production (as fuel in the mills), or the return of some residues to be used as fertilizers in sugarcane cultivation.

Beverage industry

In Mexico, according to unofficial sources, more than 35 billion liters (L) of sugar beverages were produced to be consumed in 2024. This figure corresponds only to the non-alcoholic beverage industry, which includes soft drinks, juices, nectars, functional drinks, among others. On the other hand, alcoholic beverages are represented by the beer industry and the wine industry. In Mexico, the beer industry is paramount because of a great recent growth; just in 2023, 13.64 billion L were produced, representing an economic contribution to the country equivalent to 1.6% of the national Gross Domestic Product (GDP) (SADER, 2024).

Then there is the wine industry, Mexico produces around 39.6 million L of grape wine per year, some of which are of world quality, which makes the country to have an impact and to be characterized by the flavor and texture of its wines. Both the non-alcoholic beverage industry and the industries that produce beer and table wines generate residues with opportunities to be used as raw materials that can be transformed into other important products. Examples are, in the brewing industry, beer bagasse, residual brewer yeast and filtration residues (diatomaceous earth) which are considered as waste, but can be mainly used in bio-digesters for biogas production (Lozano-Tever, 2024).

Dairy industry

Mexico is in fourteenth place in milk production worldwide (CANILEC, 2024). The dairy industry in this country produced 13.3 billion L of milk, with Durango, Coahuila and Jalisco as the states with the highest production in 2023. Among the main products, cheeses, cream and yogurt were obtained; 1.4 billion L of milk were needed to produce them in that year, and 617 726 Mg of cheeses were produced, among which fresh cheese, double cream cheese, and Panela, Chihuahua, Manchego and Oaxaca are the most important types. These processes in turn originated around 984 million L of whey, the main waste generated by the dairy industry, which represents a danger of contamination if it is thrown directly into streams or other bodies of water, due to the high concentrations of organic matter it contains.

Among the main evidence of whey use is the extraction of enzymes, such as lipases and peptidases (Sumny & Kempka, 2025); the extraction of organic acids, such as gluconic acid and lactic acid (Mukherjee *et al.*, 2023); and the generation of biofuels, as a bio-digestion substrate for bioethanol and bioenergy production (Kumar *et al.*, 2023).

Meat industry

The meat industry produces chicken, beef, and pork, which represent the most consumed animal species in Mexico, with a production of around 7.8 million Mg in 2023; in this production, about 40% of the inputs are waste (SADER, 2024). The main waste generated by the meat industry during the slaughter, processing and packaging of meat includes organic waste such as skin, blood, feathers, bones, fat, gastrointestinal contents and other inedible materials. But packaging materials, including paper, plastic and metal, are also discarded in processing and consumption. This, not to mention the waste that is generated by rearing and breeding these animals.

Among the alternative uses reported for this waste, in recent years its use is worth noticing for composting and its application as fertilizer; also, to generate biogas via anaerobic digestion (Samuel & Oluwoye, 2024); bones and viscera used for animal feed (Ogava *et al.*, 2024); leather for bags and footwear; and gelatin production (Demaman *et al.*, 2025).

As can be seen in the previous options, most of the viable alternative uses for processing waste or by-products are in composting and fertilization. But also, in the generation of energy (biogas or biofuel); and in the extraction of bioactive compounds such as phenols, for food or pharmaceutical applications. This reflects the great potential that waste processing has, for promoting the generation of alternative sources of commercialization; which are necessary, in many cases, to support the approach of circular economy.

CIRCULAR ECONOMY

The circular economy is an economic system designed to eliminate waste and keep resources in use for as long as possible. According to the definition of the FAO (2020), it is an economic approach "that aims to reduce the use of raw materials in the production chain, as well as the waste generated in it". The circular economy is based on the principles of sustainable production, through the reuse of waste to create new products; and with the reduction of pollution or the environmental impact of production, to allow the regeneration of natural systems.

The implementation of a circular economy that makes use of agro-industrial waste directly connects the environmental, economic and social parts of the principles of sustainable management. For example, in the environmental field, it minimizes waste and garbage generation, as well as pollution; fewer greenhouse gases are generated and less dependence on non-renewable resources such as oil or agrochemicals is generated. Likewise, alternative energies such as the generation of biofuels are used; or biological fertilizers are produced that have the same characteristics of a chemical or synthetic fertilizer, with the difference of less damage to the soil microbiome (Shieh *et al.*, 2025).

In the economic sphere, new products are developed that generate new sources of employment, open new markets, and savings in production costs due to the alternative use of the by-products generated. In addition to favoring the local reuse of resources that improve sustainability and self-sufficiency, to achieve resilience in the supply chain.

In the social sphere, the formation of development, research and innovation pathways with a circular approach and zero waste are promoted, where everything is taken care of and progress is made towards the sustainability dictated several decades ago. The circular economy can be put into practice in all agribusinesses, but its application depends on the type of agro-industry, the nature of the waste it generates, and the technology and infrastructure available.

The key principle of the circular economy is to close resource cycles, which is particularly compatible with bio-based sectors such as agriculture. Some examples of circular economic generation, not cited among the examples already mentioned, were added in Table 1.

Challenges and limitations of the circular economy

This section discusses some implications and challenges in the exercise of the circular economy.

Economic viability. Some waste recovery processes require a high initial investment or complex technology that may be unaffordable for smallholder farmers or developing regions.

Lack of infrastructure. Collection, processing and distribution systems for waste recycling may be non-existent or underdeveloped, especially in rural areas.

Gaps in policies and regulations. Inconsistent waste management policies, lack of incentives, or regulatory issues can impede the implementation of circular economy practices.

Technical restrictions. Some types of agricultural waste are more difficult to process, or less suitable for current reuse technologies, for example, mixed or contaminated waste. In other words, the circular economy also implies a correct and efficient separation and classification of waste.

Market demand. Even if products such as compost, bio-charcoal or bioplastics are produced, there must be a market willing to pay for them. Current markets, however, often

Table 1. Examples of agrifood industries that apply the circular economy approach.

Agrifood industry	Type of waste	Circular economy-based solution	Source
Milling of cereals and grains	Peel, bran and powder	Biomass to generate biofuel; dietary fiber; Packaging Material	Gari *et al.*, 2024
Coffee/cocoa	Peels, pulp	Composting; cosmetics; substrate for fungal growth	Kongor *et al.*, 2024
Palm oil	Clusters of empty fruits; husks	Bio-charcoal; composting; biogas production	Singh *et al.*, 2025

do not take risks, because consumers prefer to buy what they know, without recognizing the environmental or economic implications of obtaining the products that are the most commercialized.

CONCLUSIONS

Creating a circular economy with agro-industrial waste is not just about reducing environmental damage, but about a strategic opportunity to add value, drive innovation, and build more sustainable food systems. It is a move from a linear model of "extract-make-dispose" to a closed-loop system where waste becomes a resource.

The circular economy approach is adaptable to all agribusinesses and agro-industries, but its success depends on adapting solutions to local conditions. As well as investment in enabling economic systems, and the promotion of a change in mentality, which starts from the elimination of waste to the optimization of resources.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Production of *Agave* spp. and Mezcal

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ABSTRACT

Objective: To generate information on the production systems of *Agave* spp. and mezcal in the state of Oaxaca.
Design/methodology/approach: Surveys were conducted with *Agave* spp. and mezcal producers in the Central Valleys and Sierra Sur regions of Oaxaca, which are key areas for this activity. The municipalities with the largest cultivated areas and highest production levels were selected. The survey, consisting of 40 questions, covered producer information, production systems, product characteristics, and main challenges.
Results: Agave and mezcal producers in Oaxaca represent a wide range of ages, from young to older adults. The cultivated area varies considerably, with an average of 4.96 hectares per producer. Although at least seven *Agave* species are used, mezcal production is primarily concentrated on four: *A. angustifolia*, *A. potatorum*, *A. karwinskii*, and *A. americana* var. *oaxacensis*. Notably, more than 83% of mezcal is produced through artisanal methods, while the remainder is obtained using ancestral techniques.
Limitations of the study/implications: The findings underscore the need for improved planning of *Agave* cultivation, greater genetic diversification of plants, and strengthened phytosanitary control. Investment in infrastructure and capacity building is also necessary to enhance mezcal production while preserving its traditional identity.
Findings/conclusions: In the Central Valleys and Sierra Sur regions of Oaxaca, Agave and mezcal producers differ in age and educational background. Intensive and poorly planned cultivation practices generate economic uncertainty. Traditional mezcal production faces limitations related to infrastructure, pests, diseases, and loss of genetic diversity, all of which affect the sustainability of the production system.

Keywords: *A. americana* var. *oaxacensis*, ancestral, artisanal, genetic diversity, productivity.

INTRODUCTION

The genus *Agave* L., commonly known as maguey, belongs to the family Asparagaceae, subfamily Agavoideae (APG III, 2009; APG IV, 2016). It is native to the American continent, with a distribution range extending from the southern United States to Colombia and Venezuela (García-Mendoza, 2007). This genus comprises approximately 210 species, of which 159 are found in Mexico (García-Mendoza *et al.*, 2019). In Mexico, *Agave* species occur in more than 75% of the national territory, at elevations ranging from sea level to 3,400 m a.s.l., although they are most common between 1,000 and 2,000 m a.s.l. (García-Mendoza, 2007).

Mexico is recognized as the geographical center of origin and diversification of maguey plants, as these species were among the first to be utilized by Mesoamerican civilizations (García-Mendoza, 2007).

Since ancient times, *Agave* species have represented an invaluable resource and will continue to do so due to the wide range of benefits they offer and the diversity of products derived from them (Altieri and Nicholls, 2008). For these reasons, they are considered keystone species (Hernández-Hernández *et al.*, 2014), with both economic and cultural importance for past and present societies in Mexico (Arzaba-Villalba *et al.*, 2023).

Maguey exhibits a remarkable diversity of uses, including human and animal food, beverage production, medicinal applications, fuel, ornamental purposes, fiber extraction, fertilizers, and construction materials (García-Mendoza, 2007). It is also employed for soil stabilization, erosion prevention, and combating desertification (García-Moya *et al.*, 2011), and it has the capacity to adsorb lead (Romero-González *et al.*, 2007). From a chemical perspective, maguey is notable for the variety of compounds it contains, with saponins and sapogenins being particularly important (Jean-François *et al.*, 2024).

Agave species are of industrial importance, as they are used to produce distilled beverages such as mezcal (Zárate-Martínez *et al.*, 2024), although they are more widely known for tequila production (Jean-François *et al.*, 2024). In the state of Oaxaca, Mexico, at least ten *Agave* species are used for mezcal production (García-Mendoza, 2018). Mezcal is a traditional beverage obtained from the sugars contained in the *Agave* stem, known as the "piña," which is harvested when the plant reaches its maximum weight and size (Torres *et al.*, 2013). The organoleptic characteristics of mezcal are primarily determined by the *Agave* species, the production process, and the region of origin (Hernández, 2017).

The main *Agave* species used for mezcal production include: *Agave angustifolia* Haw. (cultivated), *Agave rhodacantha* Trel. (semi-cultivated), *Agave potatorum* Zucc. (semi-cultivated), *Agave seemanniana* Jacobi (wild), *Agave marmorata* Roezl (wild), *Agave karwinskii* Zucc. (wild), *Agave americana* L. var. *americana* (semi-cultivated), and *Agave americana* L. var. *oaxacensis* Gentry (wild) (García-Mendoza, 2010).

Oaxaca hosts the greatest diversity of *Agave* species in Mexico, with 38 species, 13 of which are endemic (García-Mendoza, 2018). These species occur under different management conditions: some are cultivated, others semi-cultivated, and others remain in the wild (García-Mendoza, 2010). Despite the biological, cultural, and economic importance of *Agave* in the region, there are currently no studies detailing the area dedicated to the production of each species. The Agricultural and Fisheries Information Service (SIAP, for its acronym in Spanish) reported that in 2024, 11,774 ha in Oaxaca were allocated to *Agave* production, without specifying the species (SIAP, 2024). Meanwhile, the Mexican Mezcal Regulatory Council (COMERCAM) reported that in 2024, mezcal with 45% Alc. Vol. reached a production volume of 10,305,729 L, of which 97.01% corresponded to the artisanal category and 85.25% was produced using maguey espadín (*Agave angustifolia*) (COMERCAM, 2025). It is important to note that the data provided by COMERCAM do not include producers not affiliated with the organization.

The study hypothesized that there are no significant differences in management, use, or cultivated area among the different *Agave* species in Oaxaca. Accordingly, the objective of this study was to generate information on the production systems of *Agave* and mezcal in Oaxaca.

MATERIALS AND METHODS

Study Area

The study was conducted in the state of Oaxaca, Mexico, one of the regions with the highest diversity of *Agave* species in the country. The research focused on two key regions: the Central Valleys and the Sierra Sur, due to their importance in *Agave* and mezcal production.

Identification of Representative Municipalities

In 2022, surveys were conducted with *Agave* and mezcal producers in Oaxaca. Municipalities were selected based on those with the largest areas allocated to *Agave* cultivation, according to SIAP (2021), and those with the highest mezcal production, according to Martínez (2017) (Table 1).

Table 1. Municipalities with the highest *Agave* and mezcal production in the state of Oaxaca, Mexico.

Region	District	Municipality	Surveys Conducted
Valles Centrales	Tlacolula	San Pedro Quiatoni	10
Valles Centrales	Tlacolula	Tlacolula de Matamoros	9
Valles Centrales	Tlacolula	San Donisio Ocotepec	4
Valles Centrales	Tlacolula	San Lorenzo Albarradas	4
Valles Centrales	Tlacolula	Santiago Matatlán	4
Valles Centrales	Tlacolula	San Juan Guelavia	2
Valles Centrales	Ejutla	San Agustín Amatengo	5
Valles Centrales	Ejutla	Heroica Ciudad de Ejutla de Crespo	4
Valles Centrales	Ejutla	La Compañía	3
Valles Centrales	Ocotlán de Morelos	San Baltazar Chichicápam	3
Sierra Sur	Miahuatlán	San Luis Amatlán	10
Sierra Sur	Miahuatlán	San Francisco Logueche	2
Sierra Sur	Miahuatlán	Sitio de Xitlapehua	1
Sierra Sur	Yautepec	Nejapa de Madero	9
Sierra Sur	Sola de Vega	Villa Sola de Vega	3

Sampling

In each municipality, the sample size was determined using the methodologies proposed by Aguilar-Barojas (2005) and Rojas (2013), applying Equation 1. As a result, a sample of 73 producers was obtained, and surveys were administered across 15 different municipalities.

$$n = \frac{\frac{Z^2 p_n q}{d^2}}{\frac{1 + Z^2 p_n q}{N d^2}}$$

Where: Z=Confidence level (95%); d=Precision level (10%); p_n=Proportion of the population belonging to the group of interest (0.8); $q=(1-p_n)=0.2$; N=Population size; n=Sample size.

Structure and Content of the Survey Administered to *Agave* and Mezcal Producers

The survey administered to *Agave* and mezcal producers consisted of 40 questions, organized into four sections: (a) general information of the producer, (b) description of production systems, (c) product characteristics, and (d) main issues in production systems. The questions included in each section were as follows:

a) **General information of the producer**: age, education level, and family members.
b) **Description of production systems**: land area (ha), number of plants per producer, planting density (plants ha^{-1}), and number of species in production.
c) **Product characteristics**: *Agave* weight (kg), *Agave* age (years), sugar content (°Brix), number of plants per producer, sale price of *Agave* in 2021 (MXN \$ kg^{-1}), mezcal presentation, whether the mezcal is certified, annual mezcal production volume, sale price of mezcal in 2021 (MXN \$ L^{-1}), and market.
d) **Main issues in production systems**: problems in *Agave* production and problems in mezcal production.

Data Analysis

A database was created using Excel® version 16. For the statistical analysis, a descriptive analysis was conducted, and the following were calculated: a) Measures of central tendency: mean (the average value of a set of numerical data), median (the middle value of an ordered dataset from smallest to largest), and mode (the value that occurs most frequently within a dataset). b) Measures of dispersion: range (the difference between the largest and smallest values), variance, standard deviation (SD), and coefficient of variation (CV).

Variance was calculated using Equation 2.

$$S^2 = \frac{\sum (x_i - \bar{x})^2}{n-1}$$

Where: S^2=variance; $\sum$=summation; x_i=value of the *i*-th observation of variable *x*; $\bar{x}$ =mean value of variable *x*; *n*=sample size.

The standard deviation (SD) was determined using Equation 3.

$$S = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$$

Where: *S*=SD.

The coefficient of variation (CV) was calculated using Equation 4.

$$CV = \frac{S}{\bar{x}} * 100$$

Where: *S*=SD; $\bar{x}$ =mean value of variable *x*.

RESULTS AND DISCUSSION

General Information of Producers

The measures of central tendency for the general information of *Agave* and mezcal producers indicate that the mean age of the producers was 48.3 years, the median was 48 years, and the mode was 50 years. Regarding education, the mean was 9.11 years, corresponding to the completion of secondary school. The mean family size was 3.98 members, with a mode of 5 members (Table 2).

The measures of dispersion for the general information of producers indicate that the age range was 53 years, with a standard deviation (SD) of 12.15 years and a coefficient of variation (CV) of 25.16%. Regarding education, the range was 24 years with a CV of 52.51%. The family size ranged by 6 members, with a CV of 37.95% (Table 2).

Agave and mezcal producers belong to different age groups, ranging from young adults (18-44 years), through middle-aged adults (45-59 years), to older adults (60-74 years). The average age of the producers interviewed in this study aligns with the findings of Cuevas *et*

Table 2. Measures of central tendency and dispersion for the general information of producers.

Variable	Mean	Median	Mode	Range	SD	CV
Age (years)	48.3	48	50	53	12.15	25.16
Education (years)	9.11	9	9	24	4.78	52.51
Family members	3.98	4	5	6	1.51	37.95

SD=Standard deviation; CV=Coefficient of variation.

al. (2019), who surveyed three different groups of *Agave* producers in Oaxaca and reported mean ages of 47.7, 51.1, and 60.7 years. Similarly, Reyes-Terrazas *et al*. (2023) conducted a study in Cuautlacingo, Otumba, State of Mexico, where they interviewed nopal producers and found mean ages of 41 and 50 years for two groups of producers. These results reflect consistency in the ages of agricultural producers across the country. This is further supported by the coefficient of variation for age (25.16%), indicating high homogeneity among *Agave* and mezcal producers.

Regarding education, the mean, median, and mode indicate that the producers have completed secondary school; however, the CV of 52.51% indicates a high level of heterogeneity.

Authors such as Antonio and Ramírez (2008) interviewed small *Agave* producers in the state of Oaxaca and reported a mean education level of 5.2 years (incomplete primary education), which is 3.8 years lower than that reported in this study. The age and educational level of producers are key factors that influence their cultivation methods and their capacity to adopt new technologies (Borja-Bravo *et al*., 2016). A higher level of education facilitates access to updated information and the incorporation of technological innovations into production practices (Chilonda and Van Huylenbroeck, 2001).

Academic training influences not only the efficiency of production processes but also the willingness of producers to adopt new techniques and tools that contribute to improving the quality and sustainability of production. Characterizing producers and their educational level allows for a better understanding of production systems and is key for designing policies and strategies that promote the improvement and adaptation of their practices in response to sectoral challenges (Cuevas *et al*., 2019).

The average number of family members refers to those who directly participate in activities related to *Agave* and mezcal production. In this study, this average was lower than that reported by Hernández *et al*. (2022), who found an average of five members. This factor is crucial, as family labor is primarily responsible for productive tasks. Antonio *et al*. (2015) noted that most *Agave* producers in the region do not hire external labor, relying instead on family workforce. Analyzing this indicator is essential for understanding labor dynamics within production units and for designing strategies to optimize the use of family resources in *Agave* and mezcal production.

Description of Production Systems

The area dedicated to *Agave* production varies among producers, with a mean of 4.96 ha, a median of 3.0 ha, and a mode of 3.0 ha. Planting density depends on factors such as the species cultivated, land slope, and crop management practices; however, in this study, the mean density was 1,744.52 plants ha^{-1}, the median was 1,225 plants ha^{-1}, and the mode was 1,000 plants ha^{-1}. Producers reported having plants of different ages, and considering all age classes, the mean number of plants per producer was 6,977.78, with a mode of 2,000 plants. The number of *Agave* species managed by producers varied, with a mean of 2.69 species, although the mode was only one species (Table 3).

According to the measures of dispersion, the range of area dedicated to *Agave* production was 19 ha, with a CV of 93.96%, indicating high variability. The range of planting density was 3,666 plants ha^{-1}, with a CV of 67.99%, reflecting heterogeneity in these data. The range of plants per producer was 37,000, with a CV of 128.89%, indicating high variability in the number of plants per producer. Regarding the number of species, the range was 4 species, with a CV of 60.45%, indicating heterogeneity in these data. This is further evidenced by the mode, median, and mean, which were 1, 2, and 2.69 species, respectively (Table 3).

Production systems in the state of Oaxaca are heterogeneous. The maguey-mezcal production system in Oaxaca identified four types of *Agave* producers in 2013: (a) subsistence producers, with up to 3 ha under cultivation; (b) small-scale producers, cultivating between 3 and 6 ha; (c) medium-scale producers, with 6 to 22 ha under cultivation; and (d) large-scale producers, managing more than 22 ha (Hernández *et al.*, 2022). Years later, Martínez (2017) reported the percentages of each producer category: subsistence producers accounted for 68.2%, small-scale producers for 19.7%, medium-scale producers for 10.8%, and large-scale producers for 1.4%. In this study, 70.37% of *Agave* producers manage 3 ha or less, which aligns with Martínez (2017) and confirms that the majority of *Agave* producers are subsistence farmers. However, it is important to note the ongoing need to increase the area dedicated to *Agave* production (Zárate-Martínez and Rodríguez-Hernández, 2022) due to growing demand for raw material.

Planting density in *Agave* production is highly variable and depends on factors such as the species cultivated, the type of system (monoculture or polyculture), the use of machinery, land slope, and the intended purpose of the production. In Oaxaca, up to 94.3% of producers practice intercropping, combining *Agave* with maize and beans (Cuevas *et al.*, 2019). Cruz *et al.* (2013) reported that the planting density of *A. angustifolia* ranges from 1,500 to 2,200 plants ha^{-1}, which is consistent with the mean of 1,744.52 plants ha^{-1} found in this study. However, given that the range was 3,666 plants ha^{-1}, the high heterogeneity of *Agave* production systems in Oaxaca can be inferred.

In the municipality of Villa Sola de Vega, Oaxaca, 60% of producers collect, propagate, or plant more than one *Agave* species (Ríos-Colín *et al.*, 2022); in other words, most producers manage more than one species. This aligns with the findings of this study, in which the mean number of species per producer was 2.69, with a range of 4 species. However, Hernández *et al.* (2022) concluded that most *Agave* producers in Oaxaca cultivate only a single species. This was common prior to the mezcal boom; subsequently, some producers sought to diversify and cultivate more than one *Agave* species. The producers interviewed in this study utilize at least seven *Agave* species (Table 4).

Table 3. Measures of central tendency and dispersion for the characteristics of *Agave* production systems.

Variable	Mean	Median	Mode	Range	SD	CV
Size of land (ha)	4.96	3	3	19	4.66	93.96
Density (plants ha^{-1})	1,744.52	1,225	1,000	3,666	1,186.12	67.99
Plants per producer	6,977.78	3,000	2,000	37,000	8,993.95	128.89
Number of species	2.69	2	1	4	1.62	60.45

SD=Standard deviation; CV=Coefficient of variation.

Hernández *et al.* (2022) identified the main *Agave* species cultivated in Oaxaca as *Agave angustifolia*, *A. rhodacantha*, *A. potatorum*, *A. karwinskii*, *A. marmorata*, and *A. convallis*, which aligns with the results of this study. Among these, *A. angustifolia* is the most preferred by producers due to its agronomic advantages. According to Cuevas *et al.* (2019), this species has a short growth cycle, produces large piñas, and generates a high number of pups or hijuelos. The producers interviewed highlighted that the size of the piñas allows for higher yields and, consequently, greater income.

Few producers utilize *A. lyobaa* due to its restricted distribution of less than 10,000 km^2, classifying it as a microendemic species. In addition, its medium size, with stems approximately 80 cm in height (García-Mendoza *et al.*, 2019), limits its use. In Oaxaca, small *Agave* producers have experienced changes in their cultivation and marketing practices driven by the increased consumption of mezcal (González *et al.*, 2023).

In 2019, production was expanding, with the reactivation of land and adaptation of traditional techniques to meet growing demand. However, the current scenario shows an oversupply of *Agave* relative to demand, which could create imbalances in the *Agave*-mezcal production chain.

Product Characteristics

When comparing the weight of the piñas of *A. americana* var. *oaxacensis*, *A. angustifolia*, *A. karwinskii*, and *A. potatorum*, it was observed that *A. americana* var. *oaxacensis* had the heaviest piñas, with a mean weight of 130 kg. This was followed by *A. angustifolia* at 83.75 kg, while *A. potatorum* had the lightest piñas, with a mean weight of 44.17 kg (Table 5).

Agave angustifolia is harvested at an average age of 7.19 years, when it reaches a sugar content of 33.09 °Brix, and in 2021 its price was MXN \$9.37 kg^{-1}. For *A. karwinskii*, producers harvest at 8.63 years, with an average sugar content of 22.67 °Brix. *A. potatorum* is harvested at 8.33 years, reaching 25 °Brix. Finally, *A. americana* var. *oaxacensis* is harvested at 8.8 years, although producers did not report sugar content data.

Agave angustifolia is the most widely cultivated species in the state of Oaxaca, as all surveyed producers grow it, with a mean of 28,855 plants per producer. This is followed by *A. potatorum*, with a mean of 10,683 plants, and *A. americana* var. *oaxacensis*, cultivated by 18.6% of producers, with a mean of 6,400 plants. Finally, *A. karwinskii* has a mean of 4,875 plants per producer.

Table 4. Main *Agave* species utilized by producers.

Species	%	Species	%
A. angustifolia	100	*A. karwinskii*	35.6
		A. potatorum	23.7
		A. americana var. *oaxacensis*	18.6
		A. rhodacantha	11.9
		A. marmorata	6.8
		A. lyobaa	3.4

According to the coefficients of variation, the variables evaluated for different *Agave* species show varying levels of dispersion. For *A. angustifolia*, the CV for piña weight was 29.68, for harvest age 19.50, for sugar content 16.36, and for sale price 16.92, indicating relative homogeneity of the data and representative means. In *A. karwinskii*, piña weight and harvest age had CVs of 28.59 and 26.91, respectively, also suggesting that the means are representative. Sugar content showed lower variability (CV=10.83) with a range of 5 °Brix.

In contrast, for *A. potatorum*, both sugar content (CV=34.64) and harvest age (CV=36.13) show high dispersion, indicating heterogeneous data and a non-representative mean. Finally, *A. americana* var. *oaxacensis* exhibited a CV of 24.77 for harvest age, suggesting homogeneity and a reliable mean (Table 5).

A. angustifolia exhibits notable morphological diversity, evidenced by the wide variation in piña weight. According to Cruz *et al.* (2013), piñas can weigh between 31 and 133 kg, with a mean of 80.87 kg and an average total reducing sugar (TRS) content of 21.16%±3.15. Despite this variability, producers do not consider it problematic, as they prioritize piñas with high sugar content, typically those older than six years. For mezcal production, piñas with sugar concentrations above 35 °Brix are preferred, ensuring the

Table 5. Measures of central tendency and dispersion for the characteristics of *Agave* piñas marketed.

Variable	Mean	Median	Mode	Range	SD	CV
A. angustifolia						
Weight (kg)	83.75	80	60	90	24.85	29.68
Age (years)	7.19	7	7	6	1.40	19.50
Sugar content (°Brix)	33.09	35	35	17	5.41	16.36
Plants per producer	28855	11000	3000	198000	41124.17	142.52
Price 2021 ($ kg^{-1})	9.37	9	10	8	1.58	16.92
A. karwinskii						
Weight (kg)	75.63	80	80	75	21.62	28.59
Age (years)	8.63	9.50	6	6	2.33	26.97
Sugar content (°Brix)	26.67	25	25	5	2.89	10.83
Plants per producer	4875	4000	2000	8000	3399.05	69.72
A. potatorum						
Weight (kg)	44.17	35	35	70	27.46	62.18
Age (years)	8.33	7.5	12	7	3.01	36.13
Sugar content (°Brix)	25	30	30	15	8.66	34.64
Plants per producer	10683	5750	3000	39400	14804.11	138.57
A. americana var. *oaxacensis*						
Weight (kg)	130	100	100	230	73.45	56.50
Age (years)	8.8	8	8	2	2.18	24.77
Sugar content (°Brix)	S/D	S/D	S/D	S/I	S/I	S/I
Plants per producer	6400	2000	2000	19000	10311.86	161.12

SD=Standard deviation; CV=Coefficient of variation.

desired quality and product profile. *A. angustifolia* is the most commonly commercialized species for this purpose.

Agave prices are highly variable, primarily due to a lack of planning in its production. Producers often establish their plantations based on market prices, creating cycles of oversupply and scarcity. For example, the average price of *Agave* decreased from MXN \$9.37 kg^{-1} in 2021 to only MXN \$3.00 kg^{-1} in 2024. This price volatility is particularly significant, as producers must wait up to six years to recover their investment and achieve profits.

To reduce the risk associated with purchasing raw material, local buyers often personally select the piñas, ensuring they meet the required maturity and quality standards. According to Vega and Pérez (2017), this practice is key to guaranteeing a high-quality final product and fair compensation for producers. In this context, market dynamics and personalized purchasing highlight the need for strategic planning in both production and marketing of *Agave*. The ability to adapt to market fluctuations and secure high-quality raw material is fundamental for the sustainability and profitability of the sector.

Cruz *et al.* (2013) reported that the piñas of *A. karwinskii* weighed between 8 and 57 kg, values lower than those obtained in this study, where the mean weight was 75.63 kg. Various authors have highlighted the cultural and ecological importance of this species, as well as its notable morphological diversity, reflected in the complex traditional nomenclature used to describe its variants (Vázquez-Pérez *et al.*, 2020). According to Martínez-Jiménez *et al.* (2019), *A. karwinskii* piñas reach weights between 35.39 and 57 kg over approximately 12 years, with total reducing sugar (TRS) concentrations ranging from 19.20% to 27.29%.

A. potatorum is highly valued by the mezcal industry due to its high sugar content, being even more profitable than *A. angustifolia* (Hernández *et al.*, 2022). Although it is a small wild species, with piñas of approximately 9.27 kg that reach maturity in 6.33 years and have a TRS content of 20.10% (Martínez-Ramírez *et al.*, 2013), the data from this study differ from these reports, as producers indicated a higher mean weight of 44.17 kg and maturity reached at 8.33 years.

Regarding *A. americana* var. *americana*, Lira *et al.* (2009) reported piñas weighing 49.67 kg, reaching maturity at 5 years, with a TRS content of 25.87%. However, in this study, a much higher mean weight of 130 kg was observed. Additionally, Guerrero *et al.* (2023) documented an anomaly in this species, consisting of a double floral stalk, possibly caused by an autosomal mutation or apical damage from insects or pathogens. This condition was not observed among the producers interviewed.

In Oaxaca, mezcal is produced from *A. marmorata*; however, none of the producers interviewed in this study reported using this species, possibly due to its long life cycle. Jiménez-Valdés *et al.* (2010) noted that *A. marmorata* is common in small-scale rural economies because of its large size and the possibility of obtaining up to 5 L of mezcal per plant. Nevertheless, its maturation can take up to 35 years, making it a highly long-lived species (Nogales, 2017). This information could not be confirmed in the present study.

Agave production is carried out primarily using traditional methods and knowledge transmitted across generations (González *et al.*, 2023). The recent increase in demand has raised prices, attracting new actors, particularly young people interested in cultivation as an economic opportunity. According to González *et al.* (2023), this interest aims not only to capitalize on the market but also to prevent potential shortages. However, an oversupply of *Agave* is currently observed, creating imbalances in production systems and challenging the resilience of producers, defined as their capacity to adapt to change without losing their structure or identity (Cinner and Barnes, 2019).

In the Miahuatlán district of Oaxaca, *Agave* production is closely linked to mezcal production (Cuevas *et al.*, 2019). Although this relationship has been common throughout the state, in recent years external buyers outside the *Agave*-mezcal chain have emerged, encouraging the entry of new producers and the expansion of cultivated areas. This dynamic has created an imbalance between supply and demand, resulting in overproduction, price drops, and social conflicts, including job losses. The rapid expansion of the mezcal market, both nationally and internationally, has put pressure on the adaptive capacity of the production system and small-scale producers. González *et al.* (2023) also emphasize that these changes pose a significant challenge to the resilience of the *Agave* sector in Oaxaca.

Throughout Oaxaca, there has been a notable increase in the area dedicated to *Agave* cultivation, with plantations now visible even in non-traditional zones, such as along road margins. According to SIAP data (2024), between 2018 and 2023, the cultivated area increased by 44.88%, from 8,100 to 11,736 ha. During the same period, production rose by 162%, from 97,777 to 256,549 t. This growth suggests improvements in production systems, reflected in more efficient land use, higher planting densities, better yields, and increased investment.

The limited financial capacity of producers hinders the expansion and intensification of cultivation to meet growing demand (González *et al.*, 2023). Nevertheless, over the past five years, the average yield has increased by 12.88% (COMERCAM, 2024), and more plantations than necessary to meet market demand have been established. Toledo *et al.* (2020) caution about the uncertainty in the adaptive capacity of the *Agave*-mezcal value chain in Oaxaca and the risk of raw material shortages, which could negatively affect mezcal producers. Currently, the first effects of oversupply are already being observed; however, producers are learning to interpret market signals and rely on their experience, allowing them to anticipate changes and adapt to the new context (Young *et al.*, 2006).

Eighty-four percent of producers sell their mezcal in bulk, 10% in bottles, and 6% offer both options depending on customer demand. Regarding certification under NOM-070-SCFI-2016 (2017), 68% indicated that their mezcal is not certified, as their market is local and customers value flavor over certification; only 32% of producers hold certification. Currently, five recognized certification bodies operate in the mezcal sector: the Asociación de Maguey y Mezcal Artesanal A.C. (AMMA), the Centro de Innovación y Desarrollo Agroalimentario de Michoacán A.C. (CIDAM), Certificación Mexicana S.C. (CMX), the Consejo Mexicano Regulador de la Calidad del Mezcal A.C. (COMERCAM), and Verificación y Certificación PAMFA A.C. (PAMFA). Historically, COMERCAM was the sole certifying body, playing a key role in standardizing and regulating the sector. The emergence of new certifiers has diversified and enhanced the process, providing more specialized and comprehensive evaluations that help ensure the authenticity and quality of mezcal.

Most producers sell their mezcal in bulk and are not motivated to obtain certification, as they do not perceive clear benefits. Furthermore, older producers often reject the idea of engaging with external organizations for certification. Certification faces several challenges, as it is viewed more as an expense than an investment, increasing production costs. Toledo *et al.* (2020) note that producers perceive COMERCAM more as a revenue-collecting entity than as an agent that promotes development, supervises compliance with NOM-070-SCFI-2016, or contributes to value creation.

Mezcal is primarily produced from four species: *A. angustifolia*, *A. potatorum*, *A. karwinskii*, and *A. americana* var. *oaxacensis* (Table 6). More than 83% is produced using artisanal methods, while the remainder is made using ancestral techniques. The average annual production per producer is 2,359 L for *A. angustifolia*, 537 L for *A. potatorum*, 456 L for *A. karwinskii*, and 322 L for *A. americana* var. *oaxacensis*. Mezcal from *A. potatorum* commands the highest price, with an average of MXN \$248 L^{-1}, followed by *A. karwinskii* at MXN \$241 L^{-1}, while the most affordable is from *A. angustifolia* at MXN \$214 L^{-1}.

Measures of dispersion reveal high variability in annual mezcal production among producers. The production ranges by species were as follows: *A. angustifolia* (29,940 L), *A. potatorum* (1,900 L), *A. karwinskii* (4,940 L), and *A. americana* var. *oaxacensis* (950 L) (Table 7), with CVs of 253.27%, 112.92%, 219.01%, and 205.55%, respectively, indicating the high variability in mezcal production capacity among the producers surveyed.

Mezcal production in Oaxaca involves at least eight species and seventeen varieties of *Agave*, both wild and cultivated (Cuevas *et al.*, 2019). However, *A. angustifolia* accounts for the majority of production, despite not being endemic to the region (Vega and Pérez, 2017). Beyond its economic value, *Agave* and mezcal production represents a practice deeply rooted in cultural identity, passed down from generation to generation (Antonio and Terán, 2008).

In 2023, 86.31% of mezcal produced in Mexico was made from *A. angustifolia*, followed by *A. potatorum* at 2.42%, data that align with the results of this study (COMERCAM, 2024). Mezcal from *A. potatorum* is highly valued by consumers, and its price can be up to 300% higher than that of other species (Martínez-Ramírez *et al.*, 2013). The flavor of mezcal varies depending on the species used, due to differences in volatile aromatic compounds (Vera-Guzmán *et al.*, 2010).

Most of the producers interviewed indicated that mezcal is primarily marketed at the local and regional levels, together accounting for 66.7% of sales (40% local and 26.7%

Table 6. Measures of central tendency for mezcal production and sale prices.

Species	Type of mezcal: arte-sanal/ancestral (%)	Production (L/year)	Price 2021 (\$/L)
A. angustifolia	96.5 / 3.5	2,359 / 500 / 500	21.21 / 200 / 200
A. potatorum	89 / 11	537 / 350 / 300	248.49 / 250 / 250
A. karwinskii	100 / 0	456 / 200 / 100	239.62 / 250 / 200
A. americana var. *oaxacensis*	83 / 17	322 / 200 / 250	216.04 / 200 /200

Mean / Median / Mode.

Table 7. Measures of dispersion for mezcal production and sale prices.

Species	Mezcal: artesanal/ ancestral (%)	Variable	Range	Variance	SD	CV
A. angustifolia	96.5 / 3.5	Producción*	29,940	35,706,266	5,975	253.3
		Precio+	200	1,949.54	44.15	482.9
A. potatorum	89 / 11	Producción	1,900	368,392.86	606.95	112.9
		Precio	200	2,007.30	44.8	554.6
A. karwinskii	100 / 0	Producción	4,940	999,107.33	999.55	219
		Precio	180	2,205.62	46.96	510.2
A. americana var. *oaxacensis*	83 / 17	Producción	950	438,054.44	661.86	205.6
		Precio	170	892.13	29.86	723.3

*=L year-1, +=$ L^{-1}, SD=Standard deviation, CV=Coefficient of variation.

regional). Only 16.7% is sold nationally, and a small proportion is exported. Regarding Agave, 90% is destined for the local market and 10% for the regional market. *Agave* buyers are mainly from the region and primarily act as intermediaries to distribute it to other states in the country. In Oaxaca, the *Agave*-mezcal value chain is key due to its impact on employment generation. In 2023, national mezcal production reached 12.2 million liters, of which 90.5% (11.07 million liters) was produced in Oaxaca. Of this amount, 8.32 million liters were bottled, and approximately 5.75 million liters were destined for export (COMERCAM, 2024).

Main problems in production systems

Thirty-nine point six percent of the producers interviewed reported that the primary issue in *Agave* production is pest control, particularly the agave weevil (*Scyphophorus acupunctatus* Gyllenhal). Most producers combat this pest primarily using fipronil. In second place, 38.4% identified diseases as a significant concern (Figure 1). *S. acupunctatus* is considered the most severe and important pest affecting commercial *Agave* species (Arista-Carmona *et al.*, 2023), as it substantially limits production (González *et al.*, 2007).

Agave distilled product production in Mexico has grown exponentially in recent years (Arellano-Plaza *et al.*, 2022), creating pressure on wild populations, loss of natural vegetation, and intensification of agricultural management (Lira *et al.*, 2022). Among the producers interviewed, intensive production and asexual reproduction through pups or hijuelos are commonly used to shorten the cultivation cycle. However, clonal propagation carries risks such as the accumulation of deleterious mutations, loss of genetic diversity, and an increased pathogen load (Lian *et al.*, 2019).

Although some studies have identified a certain degree of genetic variability in asexual reproduction (García-Mendoza, 2007), Agave species generally combine sexual and asexual reproduction, a strategy that favors their survival in harsh environments (Arizaga and Ezcurra, 2002). Genetic diversity is fundamental for biodiversity at all levels (Frankham *et al.*, 2010), and in agaves, hybridization, polyploidy, and vegetative reproduction are key evolutionary mechanisms (García-Mendoza, 2007). However, the

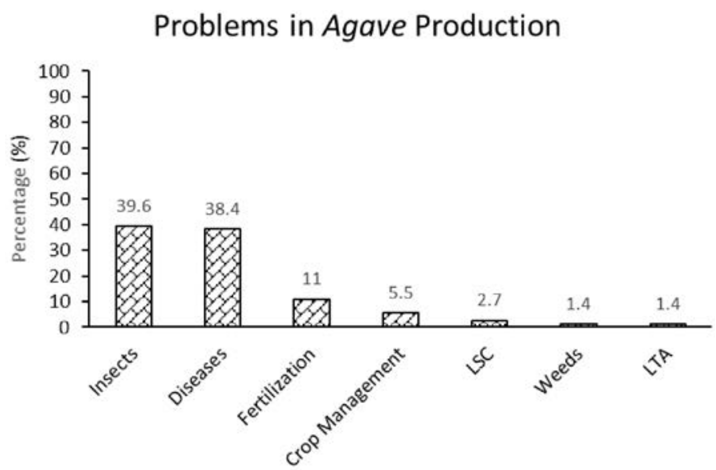

Figure 1. Main problems affecting Agave production. LSC: Low Sugar Content; FdAT LTA: Lack of Technical Assistance.

producers interviewed reported that they do not use seeds to establish new plantations, relying solely on pups or hijuelos from their existing crops.

The mode of reproduction in *Agave*, whether sexual or asexual, directly influences its genetic diversity. In the case of *A. angustifolia*, a key species in mezcal production in Oaxaca, understanding its genetic variation and population structure is essential for its conservation, sustainable management, and assessment of the impact of domestication (Klimova *et al.*, 2023). However, due to high demand, many plants do not reach the flowering stage, thereby limiting sexual reproduction. *A. angustifolia* is a diploid, self-incompatible species capable of both sexual and asexual reproduction and is pollinated by bats (Leptonycteris), birds, and bees (Rivera-Lugo *et al.*, 2018; Molina-Freaner and Eguiarte, 2003). However, the producers interviewed indicated that asexual reproduction via pups or hijuelos is the only method employed, suggesting low genetic and morphological diversity. This reduction is common in domesticated species, where artificial selection and genetic drift decrease variability (Doebley *et al.*, 2006; Khoury *et al.*, 2021).

In this context, it is necessary to implement conservation and genetic improvement strategies. Some producers reported the extraction and theft of wild *Agave*, which exacerbates the loss of diversity. This situation is particularly critical for species such as *A. potatorum*, which reproduces only by seed and whose flowering is often interrupted before completion, with no conservation measures in place (Ríos-Colín *et al.*, 2022). Genetic diversity, in both wild and cultivated species, is essential for the future of cultivation, adaptation to climate change, and pest management (Allendorf *et al.*, 2022; Swarup *et al.*, 2021).

The development of a sustainable mezcal industry requires the active participation of local communities, informed management based on data from both wild and cultivated *Agave*, and, eventually, the implementation of environmental regulations (Klimova *et al.*, 2023). Currently, the absence of a sustainable management plan places several *Agave* species at risk (Zárate-Martínez *et al.*, 2024). To conserve genetic resources, it is essential to promote both *in situ* and *ex situ* preservation and to encourage the use of germplasm collections (seeds or nurseries), taking into account local cultural, ecological, and climatic factors. The use of a limited genetic pool increases the vulnerability of cultivation to diseases, pests, and climate change (Klimova *et al.*, 2023).

The growing demand for *Agave* has led producers to expand cultivation areas, integrate into other stages of the agave-mezcal value chain, form new organizations, adopt modern communication methods, and enter into lease agreements for communal lands (González *et al.*, 2023). As early as 2015, it was reported that several wild species, such as tepeztate (*A. marmorata*), arroqueño (*A. americana* var. *oaxacensis*), coyote, and sierrudo (*A. americana*), were at risk due to overexploitation (Ríos-Colín *et al.*, 2022). A total of 67.6% of producers indicated that the main limitation in mezcal production is the lack of infrastructure, while 29.4% identified low production capacity, primarily attributed to difficulties in adapting to climatic conditions. These factors are closely related, as insufficient infrastructure is often the direct cause of limited production capacity (Figure 2).

Mezcal production is experiencing an unprecedented boom, driven primarily by the growth of the international market, with a sustained increase of nearly 40% (Zárate-Martínez and Rodríguez-Hernández, 2022). However, producers acknowledge that their infrastructure is insufficient to meet current demand. This rapid growth has generated significant environmental consequences, including the overexploitation of wild Agave, a reduction in the genetic diversity of cultivated species (Klimova *et al.*, 2023), and increasing ecological pressure on ecosystems (Zárate-Martínez *et al.*, 2024). Additionally, the production process requires large amounts of firewood, contributing to deforestation (Valiente-Banuet, 2023).

The industry also faces technical challenges, such as inefficient cooking, incomplete fermentation, limited control during distillation, and high generation of agroindustrial residues (Acosta-García *et al.*, 2023). Nevertheless, traditional agricultural systems have demonstrated a high capacity for resilience in the face of disturbances, suggesting that producers can adapt, innovate, and improve their socioeconomic conditions without losing their identity or ancestral practices (Barrientos-Rivera *et al.*, 2020).

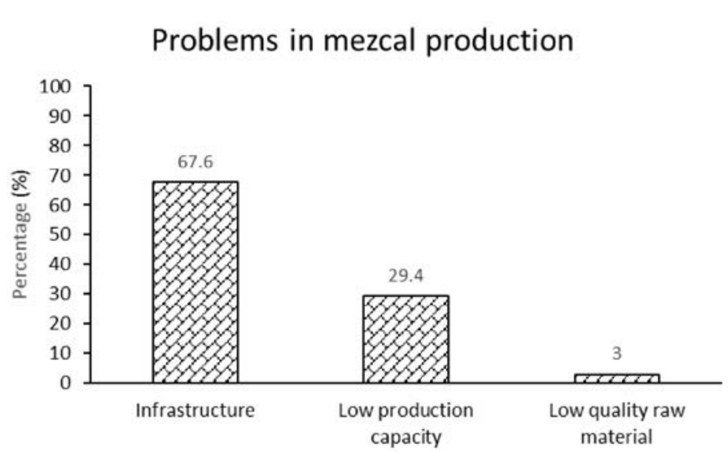

Figure 2. Main issues affecting mezcal production.

CONCLUSIONS

In the Central Valleys and Southern Sierra regions of Oaxaca, agave and mezcal producers exhibit a wide range of ages and educational levels. Agave production is carried out intensively, predominantly as monoculture, and without strategic planning, primarily responding to market fluctuations. This lack of foresight generates uncertainty regarding selling prices and affects the economic stability of producers.

Mezcal production in the region is largely carried out using traditional methods oriented toward the national market. These practices, transmitted from generation to generation, contribute to the authenticity and quality of the product, although they also face limitations due to increasing demand and insufficient infrastructure. Among the main challenges in agave production are pest and insect management, the spread of diseases, and the loss of genetic diversity associated with the extensive use of asexual reproduction and the expansion of monocultures. These factors reduce the resilience of the crop under adverse environmental conditions.

Regarding mezcal production, the main challenge is the limited production capacity of many producers, who are not always able to meet market demands in terms of volume and continuity.

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Colegio de Postgraduados

Physicochemical characterization and antioxidant activity of *Tamarindus indica* L. seed and shell flours

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ABSTRACT

Objective: To determine the physicochemical characteristics and antioxidant capacity of tamarind seed (*Tamarindus indica* L.) and peel flours.

Design/methodology/approach: To this end, tamarind pods were collected from San Antonio Sahcabchén, Calkiní, Campeche. They were subsequently pulped manually, and the peels and seeds were dried and ground to produce flour. To determine the physicochemical composition, several parameters were measured, including pH, titratable acidity (Aw), water solubility, moisture, protein, fat, ash, and fiber. In addition, antioxidant capacity was evaluated by determining total polyphenols and using DPPH and ABTS+ assays.

Results: There are differences ($P<0.05$) between flour types for all the physicochemical characteristics evaluated. The seed flour showed higher values for aw (0.34), water solubility (12.40%), moisture (6.96%), protein (11.67%), fat (4.72%), and carbohydrates (52.65%). Meanwhile, the shell flour showed higher values for pH (5.66), titratable acidity (2.46%), ash (4.63%), and crude fiber (53.82%). In addition, greater ($P<0.05$) antioxidant capacity was observed in the seed flour than in the tamarind shell flour.

Findings/conclusions: This information is useful in the search for alternatives for the use of these tamarind by-products, thus reducing their waste.

Keywords: Antioxidant activity, DPPH, total polyphenols.

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INTRODUCTION

Tamarind (*Tamarindus indica* L.) is a fruit tree native to tropical Africa. Currently, it is cultivated in regions of the world with a dry tropical climate. The most important producing countries are India, Thailand, Mexico, Indonesia, the Philippines, Brazil, Guatemala, Costa Rica, Nicaragua, among others (Anchundia-Salazar, 2021). In 2023, Mexico had a total tamarind production of 52,694.16 tons; the main producing states were Jalisco (43.4%), Colima (30.7%), Guerrero (14.4%), and Michoacán (7.27%) (SIAP, 2023). It is an economically valuable and multipurpose plant, since almost all of its parts can be used

(Limsangouan *et al.*, 2019). However, it is mostly known for its fruit, which is used for direct consumption or to produce various by-products, such as beverages, juices, jams, syrups, traditional sweets and culinary ingredients (Viveros-García *et al.*, 2012).

Tamarind contains 30% pulp, 40% seeds, and 30% peel relative to the total fruit weight (Rao *et al.*, 2015). The fruit is a rich source of protein (15-18%); it contains between 30 and 40% sugars and up to 11% organic acids, such as citric, acetic, ascorbic (vitamin C), and mainly tartaric, pectin, vitamins, and minerals (Montañez-Valdez *et al.*, 2023). Furthermore, the seeds, leaves, flowers, branches, bark, and roots are used as aphrodisiacs and for other medicinal purposes. Specifically, the seed has been reported to contain secondary compounds such as alkaloids, saponins, total phenolic compounds, and tannins, highlighting its antioxidant activity (Viveros-García *et al.*, 2012; Montañez-Valdez *et al.*, 2023). However, during peak tamarind production periods, much of the fruit remains in the orchards due to the low selling price or the product does not meet the quality standards required by the company, making its harvest economically unviable (Montañez-Valdez *et al.*, 2023). Traditionally, producers only sell the pulp and simply do not use the remaining seeds or peels (Viveros-García *et al.*, 2012). If these parts of the tamarind fruit are not managed properly, they end up as waste with no economic or other benefits.

An interesting strategy for managing these residues is to convert them into useful by-products that can facilitate their utilization, such as flour. Studies have been reported describing the advantages and disadvantages of utilizing tamarind residues, such as seeds and shell, in different industries (Rao *et al.*, 2015; Mansingh *et al.*, 2021; Montañez-Valdez *et al.*, 2023; Rahmat *et al.*, 2023). A recent study explored the chemical composition and in situ degradability of the whole tamarind fruit, demonstrating its potential as a feed resource for ruminants (Montañez-Valdez *et al.*, 2023). Other authors showed that tamarind seeds could be used to enrich local cereals, such as corn, to produce complementary foods with high nutritional quality, locally available at low cost (Oluseyi & Temitayo, 2015). In this regard, Uthai & Chetyakamin (2020) evaluated the chemical composition, mineral and phenol content, antioxidant activity, and sensory acceptance of different noodle pasta formulations made from the partial replacement of wheat flour with tamarind seed flour. The authors concluded that replacing up to 10% of wheat flour with tamarind seed flour could be successfully used for pasta production, increasing its nutritional value without negatively affecting its acceptability. Despite this research, the use of tamarind seeds and peel flours remains underexplored, largely due to the scarcity of information on their nutritional profile. Therefore, it is necessary to generate information on their nutritional values so that they can be considered an alternative in the food industry. Therefore, the objective of this study was to determine the physicochemical characteristics and antioxidant capacity of tamarind (*Tamarindus indica* L.) seed and peel flours.

MATERIALS AND METHODS

Sample Collection and Processing

Tamarind (*Tamarindus indica* L.) fruit was collected in the community of San Antonio Sahcabchén, located northeast of the municipality of Calkiní, Campeche (19° 50' 16.91" N and 90° 31' 39.72" W), at an altitude of 10 masl. The region has a warm subhumid climate with summer rainfall (Aw). The average annual temperature is 26.4 °C, and the total annual rainfall is 1,253.8 mm (INEGI, 2024).

The tamarind samples were transferred to the Chemical Biological Analysis Laboratory of the Calkiní Higher Technological Institute in the State of Campeche for processing. Initially, impurities and dust were removed from the tamarind pods by manual washing with distilled water. The pods were then dried in a convection oven (Isotemp Premium Oven, Fisher Scientific®) at 50 °C for 40 h. The average pod weight was 9.90±4.45 g, with a length between 4 and 15 cm. The pulp, seed, and husk were separated manually. The dried husks were ground using an electric mill (GoldFruit® 300W Electric). The dried seeds were ground using a conventional grain mill (Estrella®, Mexico), followed by fine grinding using a commercial blender (Osterizer®, USA). The ground material, both husk and seed, was sieved through a #40 mesh sieve (W.S. Tyler®, USA), with a particle size of 0.45 mm, until the flours were obtained. The flours were stored in Ziplot® airtight bags in a cool, dry place to prevent moisture absorption until further use.

Physicochemical Analysis

The pH measurement was performed using a digital potentiometer (Science Med®, mod. SM-3BW) according to AOAC method 943.02 (Lane, 1995). 10 g of flour was weighed and placed in an Erlenmeyer flask, where 50 mL of distilled water at room temperature was added. The contents were subsequently mixed in an electric stirrer for 30 min. The contents were placed in a beaker, allowed to stand for 10 min, and the pH was measured (Natukunda *et al.*, 2016). Buffer solutions of pH 4.0 and 7.0 were used for potentiometer calibration. pH measurements were performed in triplicate.

Total titratable acidity was determined by titration according to the AOAC (1999). 1.5 g sample was weighed and placed in an Erlenmeyer flask, where 20 mL of distilled water was added. The mixture was then mixed on a hot plate at 40 °C for 60 min. The resulting mixture was filtered, and 10 mL of the filtered solution was taken, to which 3 drops of 1% phenolphthalein were added as an indicator. 0.1 N sodium hydroxide was used for titration until a faint pink color was observed or until the solution reached a pH of 8.5 (Araujo *et al.*, 2016). The results were expressed as % tartaric acid, using the molar mass of tartaric acid as the equivalent weight of acid (Natukunda *et al.*, 2016).

Water activity (a_w) was determined using the LabStart-aw kit (NOVASINA®, Lachen, Switzerland). To determine water solubility, 0.5 g of sample was weighed and mixed with 20 mL of distilled water. Soluble particles were separated by centrifugation (3,500 rpm) using a refrigerated centrifuge (Centrifuge 5702R, Eppendorf®) at 25 °C for 10 min. The supernatant was then transferred to a crucible and dried in a digital oven (Thermolyne®) at 105 °C for 5 h. Measurements were performed in triplicate.

Proximate Analysis

The proximate composition of tamarind shell and seed flours was determined using standard methods (AOAC, 2019). Moisture content was determined by oven drying at 105 °C to constant weight (AOAC 925.10). Ash content was determined by ashing in a muffle furnace (BL Barnslead/Thermolyne, Thermo Fisher Scientific MX®) at 550 °C

(AOAC 923.03). Crude protein content was calculated using the Kjeldahl method with a nitrogen-to-protein conversion factor of 6.25 (AOAC 984.13). Crude fat was extracted using the Soxhlet method using petroleum ether as a solvent (AOAC 920.39). Crude fiber was determined using the Weende method, which involves digesting the sample in acidic and alkaline solutions (AOAC 978.10). Carbohydrate content was determined by difference (Geethalaxmi *et al.*, 2024). Carbohydrate (%)=100−(%moisture + %fat + %protein + %crude fiber). All analyses were performed in triplicate, and the results were expressed as dry weight.

Total Polyphenol Quantification

Total polyphenols were quantified using the Folin-Ciocalteau method (Blainski *et al.*, 2013). First, 50 μL of the sample was taken and placed in 15 mL Falcon tubes, followed by the addition of 3 mL of distilled water and 250 μL of Folin-Ciocalteau reagent. The contents were homogenized and left to stand for 8 min in complete darkness. After this time, 750 μL of 20% sodium carbonate ($NaCO_3$) and 950 μL of distilled water were added. The solution was then homogenized and kept in complete darkness at room temperature for 2 h, and the absorbance was measured at a wavelength of 765 nm using a PerkinElmer® UV-Vis spectrophotometer. An external calibration was performed using gallic acid as a polyphenol reference.

Determination of Antioxidant Capacity by DPPH

Antioxidant capacity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, following the methodology described by Brand-Williams *et al.* (1995), with some modifications. 3800 μL of the DPPH solution (0.1 mM) was added to 15 mL Falcon tubes, followed by 200 μL of the extract, and the solution was homogenized by vortexing. The extracts were allowed to stand for 30 min to react with the radical. Subsequently, the absorbance at 515 nm was measured using a Perkin Elmer® UV-Vis spectrophotometer, and the % DPPH inhibition was calculated for each sample. The calibration curve was prepared using Trolox at different concentrations as a reference standard.

Determination of Antioxidant Capacity by ABTS+

Antioxidant capacity was determined using the ABTS following the methodology of Nenadis *et al.* (2004), with some modifications. 2970 μL of the ABTS solution was added to a 15 mL Falcon tube, followed by 30 μL of antioxidant extract, and the solution was homogenized by vortexing. The sample was allowed to stand for 6 min, and the absorbances were read on a spectrophotometer at 784 nm to calculate the % ABTS inhibition for each sample. The calibration curve was prepared using Trolox at different concentrations as an antioxidant reference.

Statistical Analysis

Data processing consisted of exploratory analysis, descriptive statistics, and one-way analysis of variance (ANOVA) with a significance level of 0.05%. Means between flour types were compared using Tukey test ($P \leq 0.05$). Results were expressed as mean ± standard deviation. All analyses were performed using IBM® SPSS® version 21.0 (Armonk, NY, USA).

RESULTS AND DISCUSSION

Physicochemical composition

One-way ANOVA showed significant differences ($P<0.05$) between flour types for all physicochemical characteristics evaluated (Table 1). Both pH and titratable acidity were higher in shell flour compared to tamarind seed flour. pH is a chemical parameter that measures the acidity or alkalinity of the solution and is used to evaluate titratable acidity, which indicates the concentration of organic acids present in the flour. Therefore, these variables are important indicators in evaluating the conservation status of a food product. In addition, they influence flavor, consistency, texture, and aroma, which are fundamental attributes in determining the degree of acceptability by consumers (Bernal-Morales *et al.*, 2024). pH values below 4.5 can prevent the growth of microorganisms (Santos *et al.*, 2014). The pH value found for tamarind seed flour was close to 4.5; therefore, it could be considered a slightly acidic flour that offers antimicrobial characteristics. Similar results were reported by Silva *et al.* (2022), who observed pH values of 2.9 and 5.8 in flours from the shell and seed of tamarind grown in Brazil, respectively.

On the other hand, it was observed that tamarind seed flour presented higher ($P<0.05$) aw and water solubility compared to shell flour. Specifically, a water solubility of 12.40 and 7.02% was observed for seed and shell flour, respectively. According to Osei-Tutu *et al.* (2024), this functional characteristic indicates the % of soluble solids, being essential in food systems such as pastry, since flours with high solubility can produce a less cohesive dough. These authors also mention that the variation in the % water solubility in vegetable flours is due to starch concentration, the amylose/amylopectin ratio and proteins, as well as the degree of interaction with water.

Proximate analysis provides essential information for characterizing tamarind seed and shell flour. It was observed that the seed flour had higher ($P<0.05$) moisture content

Table 1. Physicochemical characteristics of seed and shell flours of *Tamarindus indica* L.

Item	Seed flour	Shell flour	P-value
pH	3.69 ± 0.02^{b}	5.66 ± 0.18^{a}	0.001
Titrable acidity (%)	0.13 ± 0.03^{b}	2.46 ± 0.04^{a}	0.001
a_w	0.34 ± 0.01^{a}	0.22 ± 0.02^{b}	0.001
Water solubility (%)	12.40 ± 1.14^{a}	7.02 ± 0.19^{b}	0.023
Moisture (%)	6.96 ± 0.31^{a}	3.27 ± 0.32^{b}	0.001
Protein (%)	11.67 ± 1.09^{a}	3.77 ± 1.07^{b}	0.001
Fat (%)	4.72 ± 0.62^{a}	1.26 ± 0.08^{b}	0.001
Ash (%)	4.25 ± 0.04^{b}	4.63 ± 0.02^{a}	0.001
Crude fiber (%)	23.37 ± 3.53^{b}	53.82 ± 0.73^{a}	0.001
Carbohydrates (%)	52.65 ± 6.05^{a}	33.21 ± 1.14^{b}	0.005

[ab] Different letters in the same row indicate a statistically significant difference ($P<0.05$).

(6.96%), protein (11.67%), and fat (4.72%), but lower ash (4.25%) and fiber (23.37%) contents than shell flour. Regarding the moisture content, this is of great relevance because moisture contents greater than 13-14% favor damage caused by the presence of fungi and other microorganisms during prolonged storage, consequently affecting the quality of flours in the manufacture of food products (Rodríguez-Pérez *et al.*, 2023). The moisture content obtained in tamarind flours is less than 13%, which indicates its storage stability.

On the other hand, high protein percentages can provide good nutritional value due to the presence of essential amino acids (Bernal-Morales *et al.*, 2024). Legume seeds are known to contain 10-30% protein, which is confirmed by our findings. Thus, tamarind seed flour can be considered a useful functional ingredient in the production of soups, pastas, and baked goods, due to its protein content. However, Geethalaxmi *et al.* (2024) recently observed that roasting tamarind seed significantly reduced the protein content of the flour, but improved the ash, carbohydrate, fat, and flavonoid contents.

In this regard, ash content is a parameter related to the amount of minerals present in foods, which is essential for determining their nutritional value. Low ash percentages are favorable because they can provide a greater amount of minerals for food. On the contrary, high ash percentages are particularly undesirable because they darken the flours. The ash concentration in flours for food use is expected to be less than 2% (Rodríguez-Pérez *et al.*, 2023). Although the results showed that the flours evaluated in the present study had an ash content greater than 2%, they are consistent with those observed by Silva *et al.* (2022), who reported ash values of 5.0 and 2.3 g.100 g^{-1} in tamarind shell and seed flours, respectively.

The results regarding fat content showed significant differences among the flours evaluated, which has important implications for the nutritional profile and functional quality of the products made from them. Tamarind seed flour had the highest fat content (4.72%), while shell flour had the lowest (1.26%). The variability in fat content among tamarind flours can be explained by the differential concentration of lipids, which can be beneficial, including essential unsaturated fatty acids, which characterize legume seeds (Moo-Huchin *et al.*, 2025). Similar results were reported by Silva *et al.* (2022), who reported mean values of 0.6 and 4.3 g.100 g^{-1} in shell and seed of tamarind grown in Brazil. For their part, Yusuf *et al.* (2007) observed that the lipid value in raw seeds of *T. indica* was 6.94%, while in whole seeds it was 11.43%.

Crude fiber is composed mainly of cellulose (60-80%) and lignin (4-6%); additionally, by minerals (Madhu *et al.*, 2017). In the present study, the crude fiber content ranged between 23.37% and 53.82% for tamarind seed flour and shell, respectively. The shell flour presented a high crude fiber content ($P<0.05$) compared to the seed flour, this is because the tamarind shell is mainly made up of cellulose, hemicellulose and lignin (Cruz-Montesinos *et al.*, 2024). Likewise, the high crude fiber values in the seed flour could be related to the lack of separation of the coat and endosperm. Fiber has a positive impact on human health, reducing blood cholesterol levels, the risk of cardiovascular diseases and helping to prevent colon cancer (Sheikh *et al.*, 2019). In this sense, both flours evaluated could be a potential source of crude fiber for food formulation. The carbohydrate content of the flours ranged from 33.21 to 52.65%. Flour made from tamarind seeds had a higher carbohydrate content

(P<0.05) compared to the data obtained for shell flour. Similar results were reported by Kumar & Bhattacharya (2008) where they indicated that a considerable amount of carbohydrates (50-57%) are found in tamarind seeds. In accordance with other studies, carbohydrate contents ranging from 42.76 to 71.11% have been reported for tamarind seed flour, noting that factors such as temperature and roasting time significantly influence the final carbohydrate content (Geethalaxmi *et al.*, 2024).

Determination of antioxidant capacity

Figure 1(A) shows the concentration of total polyphenols in the seed and shell flours of *Tamarindus indica* L. These results demonstrate that, of both flours analyzed, the flour from the seed had the highest concentration of these compounds (127.8±9.5 mg gallic acid/g of sample). Meanwhile, the flour from the shell had a concentration of 82.4±9.1 mg gallic acid/g of sample. This behavior is consistent with the findings reported by Sudjaro *et al.* (2005), who indicated that the seeds of *Tamarindus indica* L. have a significantly higher concentration of total polyphenols (6.54 g kg^{-1}) compared to the pericarp (2.82 g kg^{-1}). The results indicate that the seed has a high potential as a raw material for obtaining bioactive compounds. This statement is supported by the study carried out by Aengwanich & Suttajit (2013), who reported that tamarind seeds constitute a significant source of polyphenols. These compounds are of great importance for the agri-food sector focused on the production of functional foods, because they have been shown to have relevant biological effects, such as the reduction of oxidative damage and the inhibition of lipid peroxidation, both *in vitro* and *in vivo* studies (Aengwanich & Suttajit 2009).

On the other hand, the results corresponding to the antioxidant capacity determined by the DPPH radical assay are presented in Figure 1(B). It can be observed that the seed flour was the one that presented the highest antioxidant activity (128.98±3.2 mg of Trolox/g

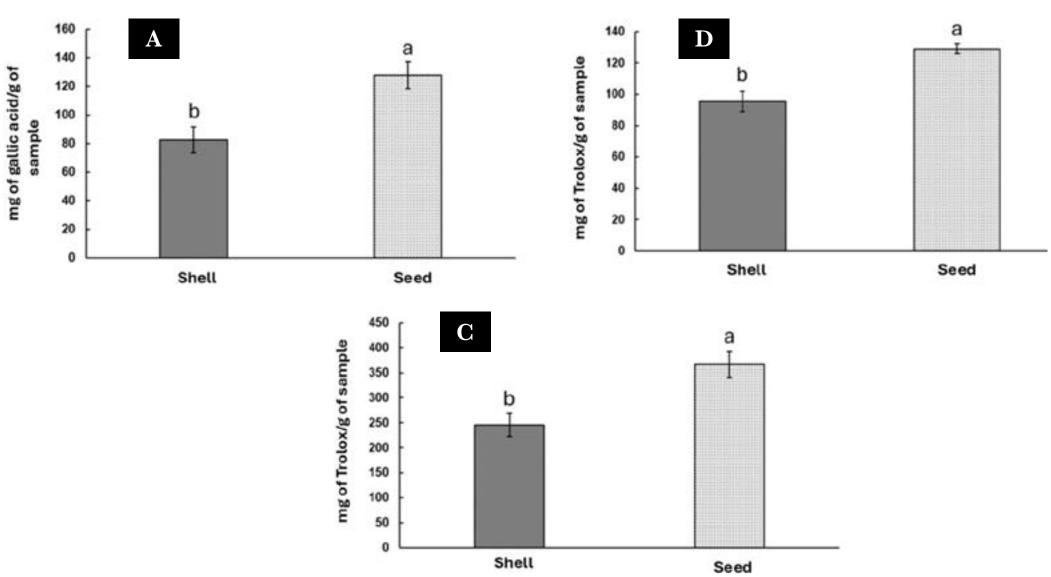

Figure 1. Quantification of antioxidant capacity in seed and shell flours of *Tamarindus indica* L. A) total polyphenols, B) DPPH, and C) ABTS+.

of sample). While the shell flour presented an antioxidant activity of 95.45±6.6 mg of Trolox/g of sample. The study carried out by Farooq *et al.* (2022) agrees that the antioxidant activity, determined by the DPPH technique, was significantly higher in the tamarind seed (74.1±0.8%) compared to the pulp (72.2±0.4%).

While the antioxidant capacity determined with the ABTS+ technique (Figure 1(C)) showed the same behavior, the *Tamarindus indica* L. seed flour presented a higher antioxidant activity (366.7±25.9 mg of Trolox/g of sample) compared to the shell flour (245.2±23.4 mg of Trolox/g of sample). This corroborates that the seed flour has a higher concentration of compounds with antioxidant activity compared to the shell flour. Farooq *et al.* (2022) also evaluated the antioxidant activity of the tamarind seed using the ABTS technique, comparing their results with those obtained in the pulp. Their findings coincide in that the seed presented significantly higher values of antioxidant capacity (79.2±0.4%) compared to the pulp (75.7±0.2%), which suggests a higher concentration of bioactive compounds responsible for this activity.

These results are due to the fact that *Tamarindus indica* L. seeds are a rich source of polyphenols, flavonoids, and other secondary metabolites with recognized antioxidant capacity, particularly due to their effectiveness in neutralizing free radicals. This phytochemical composition could explain the high levels of antioxidant activity observed using the DPPH and ABTS methods. Furthermore, the dense structure and lower water content of the seed matrix, compared to the pulp, could favor the accumulation and stability of bioactive compounds.

CONCLUSIONS

Tamarind seed flour stood out in parameters such as moisture, protein, fat, and carbohydrates. It also exhibited greater antioxidant activity compared to tamarind shell flour. This information is useful for industries interested in utilizing tamarind products, allowing them to reduce waste and adding value to the fruit. Future studies on the determination and quantification of antinutritional compounds are needed to understand its true nutritional value.

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Colegio de Postgraduados

Effectiveness and phytotoxicity of selective post-emergent herbicides in the sugarcane variety Mex 68-1345

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ABSTRACT

Objective: This study assessed the effectiveness and phytotoxicity of selective post-emergent herbicides for managing major weed species in a tropical agroecosystem.

Design/methodology/approach: A randomised complete block design with four replicates was implemented, testing nine herbicide treatments and one untreated control on the Mex 68-1345 variety. Weed control and crop phytotoxicity were assessed visually at 7, 14, and 21 days after application (DAA) using the EWRS scale. In addition, SPAD chlorophyll index readings were recorded as an indicator of foliar phytotoxicity. The herbicide combinations Ametryn+Atrazine (AMT+ATZ), Ametryn+Atrazine+Diuron (AMT+ATZ+DIU), and Ametryn+2,4-D (AMT+2,4-D) achieved "adequate control" (87.5-93%) with mild and transient phytotoxicity (<3.5%).

Results: A significant negative correlation ($P<0.0001$) was observed between herbicide effectiveness and SPAD index values, indicating a physiological cost associated with weed suppression and short-term stress in sugarcane. Environmental conditions particularly low soil moisture (<50%) and high temperatures (>34 °C) negatively affected herbicide efficacy and favoured weed regrowth.

Limitations on study/implications: The study was conducted under specific environmental conditions and assessed only short-term herbicide effects (up to 21 DAA), without assessing yield or economic return. Species-specific resistance was not confirmed by bioassays. Despite these limitations, the findings provide practical insight into herbicide performance under field conditions and highlight the need for integrated weed management strategies that include environmental monitoring, resistance awareness, and longer-term assessments.

Findings/conclusions: These findings support the strategic use of post-emergent herbicides as a component of integrated weed management (IWM) programmes aimed at sustainable sugarcane production.

Keywords: Sugarcane (*Saccharum officinarum* L.); post-emergent herbicides; phytotoxicity.

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INTRODUCTION

Impact of weeds on sugarcane productivity

Sugarcane (*Saccharum officinarum* L.) is a major crop in the agricultural economy of Mexico, due to both its extensive farming system and its socioeconomic relevance. The sugarcane agro-industry is one of the country's primary agricultural sectors, covering over 800,000 hectares across 15 states and generating approximately 440,000 direct and 2.2 million indirect jobs (CONADESUCA, 2023). However, sugarcane productivity can be significantly reduced by weed competition for essential resources such as sunlight, water, macro- and micronutrients, and space (Suganthi *et al.*, 2019). Under conditions of high infestation, yield losses can range from 10 to 70% (Rathika *et al.*, 2023). In large-scale production systems, weed infestation is among the leading causes of low yields. Moreover, the continuous and indiscriminate use of herbicides contributes to environmental pollution. Therefore, it is essential to identify herbicides that provide effective weed control with fewer agrochemical applications, aiming to mitigate ecological impacts (Rodríguez-Tassé *et al.*, 2024).

Invasive flora and predominant weed species in sugarcane

In Mexico, approximately 700 non-native wild plant species have been recorded, representing 2.8% of the estimated 23,000 native flora. Of these, around 80% are considered naturalised, and between 58 and 180 species have been classified as invasive weeds with potential ecological and socio-economic impacts (Espinosa-García and Villaseñor, 2017). Studies have shown that floristic variability is influenced by factors such as soil type, rainfall regime and distribution, agricultural production systems, and weed management history highlighting the need for localised chemical and integrated weed control strategies (Aekrathok *et al.*, 2021).

Weed control practices have evolved from manual removal and rudimentary tools to mechanisation and herbicide-based approaches. Although various control methods are available, chemical weed management remains essential due to the vast cultivated areas, limited labour availability, and the high cost of manual operations (Rodríguez-Tassé *et al.*, 2024). The effectiveness of integrated weed management is positively influenced by the maintenance of a current inventory of dominant weed species within cultivated areas (Martínez-Ramírez *et al.*, 2024).

In tropical agroecosystems such as the Huasteca region, high infestations of both narrow- and broadleaf weed species have been documented, many of which exhibit traits that favour persistence under conventional agricultural management practices. The most common grasses include *Sorghum halepense* (Johnson grass), *Panicum maximum* (Guinea grass), *Cyperus* spp. (nutgrass), *Cynodon dactylon* (Bermudagrass) (Wright *et al.*, 2025), *Setaria viridis* (green foxtail), *Brachiaria plantaginea* (Alexandergrass), *Eleusine indica* (goosegrass), and *Digitaria* spp. (crabgrass), all noted for their capacity to regenerate following herbicide application and for their tolerance to drought conditions.

Among broadleaf species, *Convolvulus arvensis* (field bindweed), *Tithonia tubaeformis*, *Euphorbia heterophylla* (wild poinsettia), *Caperonia hirtus*, *Amaranthus hybridus* (pigweed), *Portulaca oleracea* (purslane), *Taraxacum officinale* (dandelion), *Raphanus raphanistrum* (wild radish), *Physalis angulata*, *Rumex crispus* (curled dock), *Mimosa pudica* (sensitive plant), *Melilotus indicus* (yellow sweet clover), *Solanum nigrum* (black nightshade), and *Cucumis anguria* (bur gherkin) are among the dominant species. Other weeds of concern include *Bidens pilosa* (Spanish needle), *Xanthium strumarium* (cocklebur), and several locally recognised species such as "valpichichi", "amargoso", and "frijolillo" (CONABIO, 2025). Weed management systems are a fundamental component of agricultural production and can be implemented through various strategies. The key lies in achieving effective control using methods that are both economically viable and operationally practical (Monteiro and Santos, 2022).

Critical stages of weed management: sprouting and tillering

Sugarcane canopy closure plays a crucial role in the competitive dynamics between the crop and weeds during the early stages of development. It is defined as the overlapping of leaf blades between adjacent rows, which helps reduce weed infestation and moisture loss through evaporation (CIDCA, 2025). Regarding sugarcane, canopy closure typically occurs between 90 and 120 days after planting, coinciding with the phenological stages of bud emergence and tillering (Leon and Odero, 2018; TNAU, 2025). The speed of canopy closure is influenced by several factors, including cultivar, planting density, production system, and edaphoclimatic conditions (Ali *et al.*, 2017; Muhammad Zafar *et al.*, 2010). This period represents the critical window for weed management, as it is characterized by high infestations of grasses and broadleaf species (Aekrathok *et al.*, 2021; Yirefu *et al.*, 2012). Weed competition during this phase reduces shoot emergence, tillering, and the development of millable stalks, ultimately decreasing stalk population, yield, and sucrose concentration (TNAU, 2025). Yield losses can exceed 50%, with no potential for later recovery (Chauhan, 2020; Yirefu *et al.*, 2012).

The application of selective herbicides in sugarcane cultivation constitutes an effective strategy for weed control. According to Espinosa-García and Villaseñor (2017), pre-emergent herbicides exhibit high efficacy when applied within 8 to 10 days after planting, under adequate soil moisture conditions and prior to bud emergence. On the other hand, post-emergent herbicides have demonstrated "very good control" when applied to weeds at a height of ≤10 cm and when their coverage exceeds 40% of the cultivated area (Servín-Niz *et al.*, 2018).

An ideal weed management programme in sugarcane comprises the use of a pre-emergent herbicide at planting, followed by shallow mechanical cultivation and a post-emergent treatment during the critical growth stage. However, implementation under field conditions may be hindered by asynchronous weed emergence, environmental variability, limited availability of agrochemicals, and logistical constraints (Chauhan, 2020). Moreover, the recurrent use of herbicides has been associated with environmental pollution, including a decline in soil biodiversity and the degradation of soil fertility (Polanco-Rodríguez *et al.*, 2019). Within this context, the aim of this study was to evaluate the effectiveness and phytotoxicity of selective post-emergent herbicides in sugarcane, with a focus on the agronomic management of the predominant weed species in the region.

MATERIALS AND METHODS

Location and experimental plot establishment

The experiment was conducted in López Rayón, within the municipality of González, Tamaulipas, Mexico, located at an altitude of 25 m above sea level, at geographical coordinates 22° 29' 5.33" N and 98° 28' 59.08" W. The study was carried out between April and July 2025. Soil preparation involved ploughing, harrowing, and ridging, with a row spacing of 1.6 m. The sugarcane variety Mex 68-1345 was sown in single rows; this cultivar was selected for its regional adaptability and late maturity, making it suitable for mechanical harvesting during the final third of the sugarcane industrialisation period. Surface irrigation was applied to promote uniform crop emergence. Following herbicide application, a cumulative rainfall of 350 mm was recorded during the evaluation period. Herbicide treatment was performed 60 days after planting, under soil moisture conditions of approximately 45%, with an estimated plant density of 44,000 plants ha^{-1}.

Herbicide selection and application

Based on previous assessments, nine post-emergence herbicides were applied to 60-day-old sugarcane (Table 1). These herbicides have demonstrated good overall weed control and caused only mild and short-term phytotoxicity symptoms in the CP 72-2086 sugarcane variety. Applications were carried out using a tractor-mounted boom sprayer equipped with 22 flat-fan nozzles (F110-02 type). The operating pressure was set at 160 psi, and the

Table 1. Active ingredients, acronyms, application rates, chemical families, target weed types, and modes of action of the post-emergence herbicides evaluated in sugarcane.

Herbicide (active ingredient)	Acronym	Dose (ha^{-1})	Chemical family	Target weed type	Mode of action
No herbicide	Control	-	-	-	-
Ametryn (80%)	AMT80	2.5 kg	Triazines	Broadleaf and grasses	PSII
Atrazine (90%)	ATZ90	2.0 L	Triazines	Broadleaf and grasses	PSII
Diuron (80%)	DIU80	2.25 L	Urea-derived compound	Broadleaf and grasses	PSII
Ametryn (23.8%) + 2,4-D (16.36%)	AMT+2,4-D	5.0 L	Triazines + Phenoxyacetic acid	Broadleaf and grasses	AAS
Ametryn (25.5%) + Acid 2,4-D (16.4%)	AMT+Acid 2,4-D	6.5 L	Triazines + Phenoxyacetic acid	Broadleaf	PSII + AAS
Ametryn (38.2%) + Atrazine (38.2%)	AMT+ATZ	2.5 kg	Triazines	Broadleaf and grasses	PSII
Clomazone (19.61%) + Ametryn (29.42%)	CLZ+AMT	4.0 L	Isoxazolidinone + Triazines	Broadleaf and grasses	ISC + PSII
Diuron (44.49%) + Hexazinone (5.59%)	DIU+HEX	3.0 L	Substituted urea + Triazinones	Broadleaf and grasses	PSII
Ametryn (38.2%) + Atrazine (38.2%) + Diuron (38.2%)	AMT+ATZ+DIU	4.5 kg	Triazines + Substituted urea	Broadleaf and grasses	PSII

Note: The evaluated herbicides act through the inhibition of Photosystem II (PSII), synthetic auxin activity (AAS; Beffa *et al*., 2019), or the inhibition of carotenoid biosynthesis (ISC; Laborde, 2024).

spray volume was 200 L ha^{-1}. To reduce water pH, Phase® was added at a concentration of 1 mL per litre of herbicide solution, adjusting the final pH to approximately 5.5. In addition, a surfactant (ADH®) was added at a dose of 1 mL L^{-1} to enhance herbicide adherence and efficacy.

Weed infestation and evaluation of herbicide efficacy and phytotoxicity

The predominant weed species identified in this experiment were *Cynodon dactylon* (Bermudagrass), *Convolvulus arvensis* (field bindweed), *Tithonia tubaeformis*, *Euphorbia heterophylla* (wild poinsettia), *Caperonia hirtus*, *Bidens pilosa* (Spanish needle), *Cucumis anguria* (bur gherkin), as well as locally recognised species such as "amargoso" and "frijolillo". The effectiveness of herbicides for weed control and their phytotoxic effects on sugarcane were evaluated at 7, 14, and 21 days after application (DAA). The effectiveness of herbicides for weed control and their phytotoxic effects on sugarcane were evaluated at 7, 14, and 21 days after application (DAA). Each plot covered an area of 1,250 m^2 and was treated as a replicate. Overall weed control, species-specific control, and sugarcane phytotoxicity were assessed using the visual scale proposed by the European Weed Research Society (EWRS, EWRS, 2025) following the methodology described by Flores *et al.* (2005), (Figure 1).

Relative chlorophyll content measurement: SPAD index

Relative chlorophyll content was measured using SPAD index readings (Soil Plant Analysis Development) with a portable SPAD-502Plus meter (Konica Minolta, Japan). The SPAD index was used as an indicator of phytotoxicity in sugarcane leaves. Three readings were taken per plant in each replicate, directly on the leaf lamina. Measurements were consistently recorded from fully expanded leaf blades of the sugarcane plants.

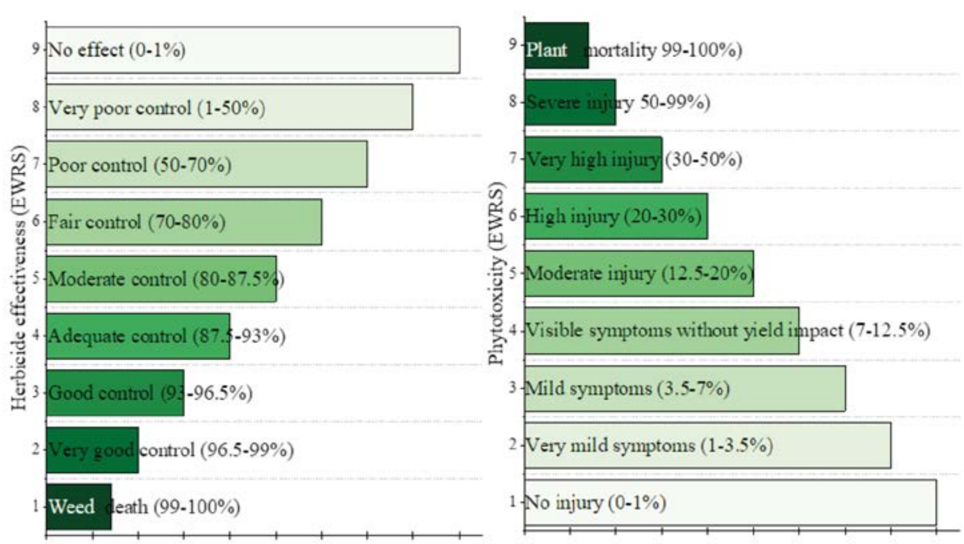

Figure 1. Adapted EWRS scale for herbicide effectiveness (EWRS, 2025) and phytotoxicity in sugarcane (Flores *et al.*, 2005).

Experimental design and statistical analysis

A randomised complete block design (RCBD) with four replications was employed. Each experimental unit covered an area of 1,250 m^2. Data on general herbicide effectiveness, species-specific weed control, and sugarcane phytotoxicity at 7, 14, and 21 DAA were subjected to statistical analysis. A one-way analysis of variance (ANOVA) was performed for each variable and sampling time, with herbicide treatment considered as the source of variation. When significant differences were detected ($P<0.05$), Tukey's multiple comparison test was applied ($\alpha=0.05$). All statistical analyses were conducted using SAS software (SAS, 2013) and Python (McKinney, 2010).

RESULTS AND DISCUSSION

Overall weed control effectiveness and sugarcane phytotoxicity

Significant differences in weed control effectiveness were observed among herbicide treatments (Tukey, $P\leq0.05$; Figure 2). According to the threshold proposed by Flores *et al.* (2005), an acceptable level of weed control corresponds to a score of 4 on the visual scale, equivalent to 87.5-93%. Based on this criterion, three treatments achieved "good control" at 7, 14, and 21 DAA: AMT+ATZ), AMT+ATZ+DIU, and AMT+2,4-D.

Atrazine, included in two of the most effective herbicide combinations in this study, is among the most widely used herbicides globally. While its broad-spectrum weed control efficacy is well established, its persistence, leaching potential, and frequent detection in surface and groundwater have raised environmental concerns (Hansen *et al.*, 2013). Atrazine has also been associated with chronic toxicity in aquatic organisms and is considered a recalcitrant compound, resulting in restrictions in the United States and bans in several European Union countries (Bethsass and Colangelo, 2006).

In contrast, atrazine remains widely used in Mexico, where it is not subject to specific regulation (Lagunas-Basave *et al.*, 2022). Its environmental mobility depends on factors such as soil texture, organic matter content, pH, and application rate (Hansen *et al.*, 2013). Although natural attenuation mechanisms such as adsorption and microbial degradation

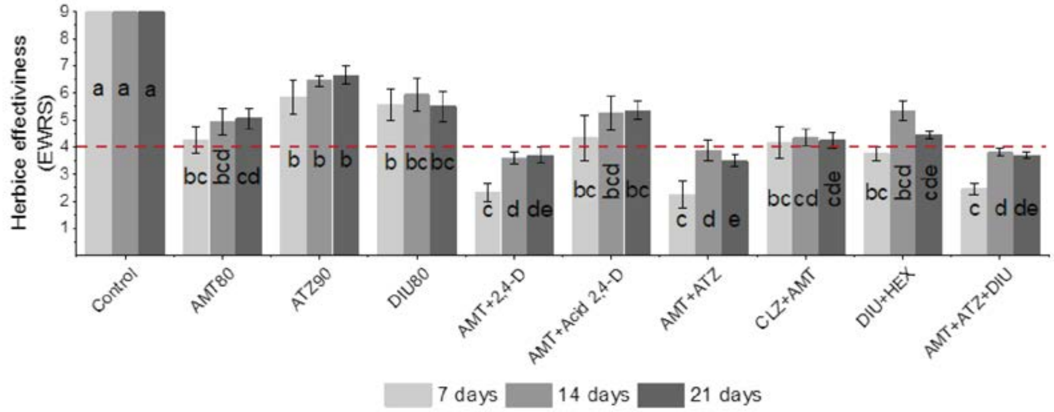

Figure 2. Weed control effectiveness of evaluated herbicides. Note: Control scale based on the European Weed Research Society (EWRS), where 1=total control and 9=no control. Control is considered adequate when EWRS ≤ 4 (87.5-93 % efficacy, red dashed line). Error bars represent significant differences among herbicides (Tukey test, P≤0.05).

can mitigate its impact, risks increase under high rainfall or in soils with low organic matter. These considerations emphasise the need to balance agronomic effectiveness with environmental sustainability. Despite its strong performance in this trial, the potential ecological risks associated with atrazine highlight the importance of integrated weed management and the exploration of environmentally safer alternatives.

Weed control effectiveness and species-specific responses

The evaluated herbicides demonstrated effectiveness classified from "very good control" to "weed death" (≤96.5%) against predominant broadleaf weed species in this study, including *Tithonia tubaeformis*, *Caperonia hirtus*, and *Cucumis anguria*. These outcomes indicate high sensitivity of these species to the active ingredients applied. However, Bidens pilosa exhibited a distinct response. The DIU80 treatment achieved only "fair control" (70-80%), whereas other treatments reached 93-96.5%, categorised as "good control". This diminished effectiveness may be attributed to specific resistance mechanisms in *B. pilosa*, a species widely recognised for herbicide resistance (Alcántara-de la Cruz *et al.*, 2019). To date, more than 214 weed species with confirmed herbicide resistance have been documented, including *B. pilosa*, which may explain the limited effectiveness of diuron (Muniz *et al.*, 2019). These findings underscore the importance of considering herbicide-use history and resistance potential when selecting chemical control strategies.

The species, *Convolvulus arvensis*, was effectively controlled by AMT+2,4-D, AMT+ATZ+DIU, and DIU+HEX treatments, with control levels ranging from "adequate" to "very good" across the 7-21 DAA period. *Phaseolus lathyroides* showed a similar pattern in response to AMT+ATZ, AMT+ATZ+DIU, AMT+2,4-D, and DIU+HEX treatments. Regarding *Euphorbia heterophylla*, AMT+2,4-D and AMT+Acid 2,4-D achieved 87.5-96.5% control, also falling within the "adequate" to "very good" categories. These outcomes are consistent with Ferreira *et al.* (2016), who reported effective suppression of several *Euphorbia* species using hexazinone + diuron combinations, even under bagasse-mulched conditions of sugarcane and long-term dry periods. For the perennial grass *Cynodon dactylon*, AMT+ATZ and AMT+ATZ+DIU treatments achieved "sufficient" to "very good" control levels (Figure 3). *Euphorbia hirta* was completely controlled (100%) by both herbicide combinations evaluated. These findings highlight the species-specific performance of the herbicides and reinforce the need to tailor herbicide mixtures to the local weed spectrum.

Notably, *Convolvulus arvensis* and *Euphorbia heterophylla* showed regrowth potential, suggesting partial resistance and the need for a second herbicide application, ideally in combination with mechanical inter-row cultivation. Ametryn 80%+2,4-D amine (58%) has also been reported as effective in the CoM0265 sugarcane variety (Patil and Pachunde, 2024), which supports the present findings. On average, the treatments AMT+ATZ, AMT+ATZ+DIU, and AMT+2,4-D achieved "adequate control" (87.5-93.0%) at 7, 14, and 21 DAA, with an estimated application cost ranging between \$1,260 and \$2,000 MXN ha^{-1}.

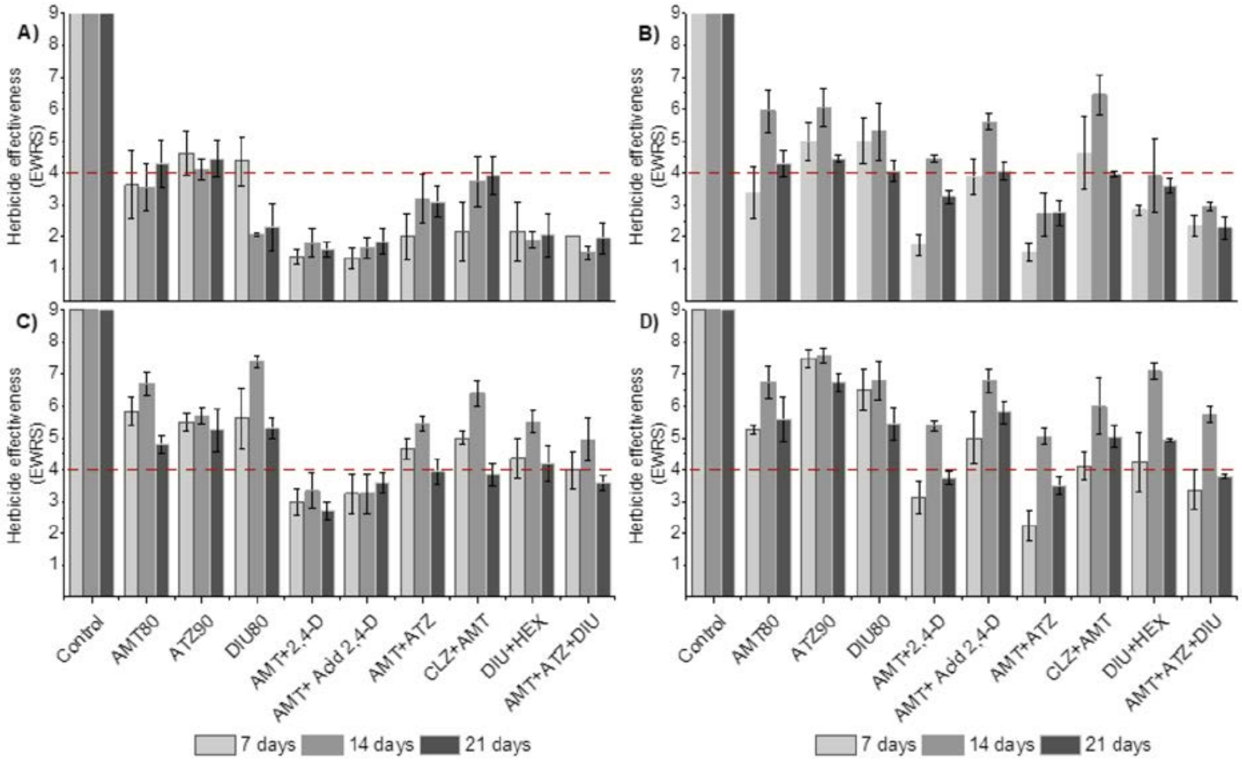

Figure 3. Herbicide efficacy in the control of *Convolvulus arvensis* L. (bindweed, A), frijolillo (B), *Euphorbia heterophylla* L. (wild poinsettia, C), and *Cynodon dactylon* (Bermuda grass, D). Error bars represent standard errors. Red dashed lines indicate the threshold for adequate control (87.5-93 %). Significant differences between herbicides were determined using Tukey's test ($P \leq 0.05$).

Environmental and edaphic influences on herbicide effectiveness

Environmental and edaphic factors may have limited herbicide effectiveness by affecting the uptake and translocation of active ingredients. A critical factor is photodegradation under low humidity, which reduces herbicide persistence on leaf surfaces (Regíl Lux, 2024). Under prolonged drought stress, weeds tend to develop thicker cuticles, leading to lower metabolic activity and restricted systemic herbicide movement. Microclimatic conditions such as elevated temperatures and wind speed, further contribute to herbicide volatilisation. According to Regíl Lux (2024), optimal application conditions are defined as temperatures below 35 °C, relative humidity of at least 60%, and wind speeds below 10 km/h. In addition, high colloid and organic matter content in clay-rich soils can increase herbicide sorption. This effect is particularly relevant in residual soils typical of grass crops, where excessive binding limits the availability of herbicides for root absorption.

Phytotoxicity responses in sugarcane

Significant differences in sugarcane phytotoxicity were observed among herbicide treatments according to the EWRS scale (Figure 4). Atrazine 90% (ATZ90) exhibited the lowest phytotoxicity, classified as "mild symptoms" (3.5-7%) and associated with "fair" weed control (70-80%). In contrast, the AMT+2,4-D, AMT+ATZ+DIU, and AMT+ATZ treatments, which achieved higher levels of weed control, induced "visible symptoms

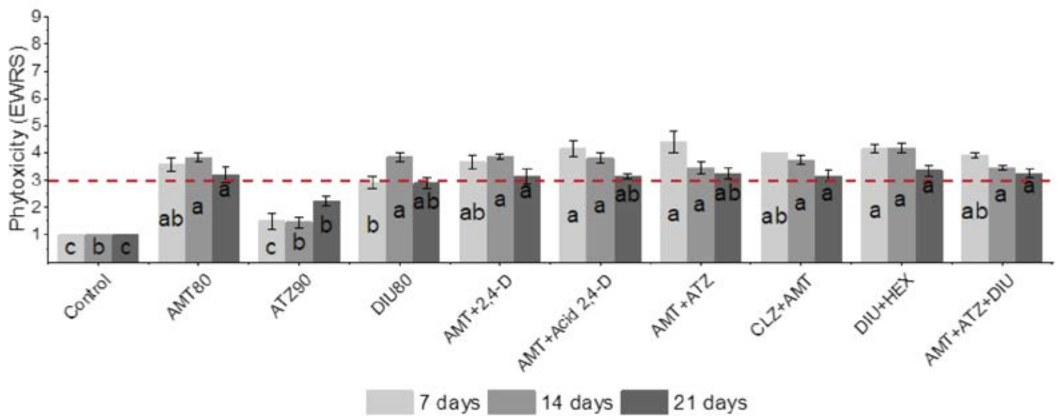

Figure 4. Phytotoxicity in sugarcane caused by the evaluated herbicides. Note: Phytotoxicity was assessed using the European Weed Research Society (EWRS) scale, where 1=no effect and 9=plant death. "Very mild symptoms" correspond to EWRS=3 (3.5-7% toxicity, red dashed line). Error bars represent standard errors. Significant differences between herbicides were determined using Tukey's test ($P \leq 0.05$).

without yield impact" (7-12.5%) at 7 days after application (DAA), which declined to "mild symptoms" (1-3.5%) by 21 DAA. This trend indicates progressive physiological recovery, with an estimated recovery period of approximately three weeks, and aligns with the findings reported by Aekrathok *et al.* (2021).

SPAD index and phytotoxicity expression

There was a significant negative correlation ($P<0.0001$) between herbicide effectiveness and SPAD index values, suggesting that less effective weed control corresponded to higher SPAD readings. This may be attributed to reduced exposure to phytotoxic compounds, which helped preserve foliar chlorophyll content. The expression of phytotoxicity varies depending on genotype, the type of active ingredient, crop growth stage, and the plant's nutritional status (da Silva *et al.*, 2014). Herbicides can induce subvisual symptoms or physiological disorders such as chlorosis, necrosis, or cell death, depending on their mode of action and the sensitivity of the crop (Sakadzo *et al.*, 2018).

Soil moisture effects on weed control

Effectiveness weed control in sugarcane typically requires a three-step management approach: pre-emergent herbicide application, mechanical cultivation, and post-emergent herbicide treatment (Sutthiwaree *et al.*, 2010). In this study, differences in herbicide effectiveness were associated with soil moisture at the time of application. The first herbicide treatment was applied under conditions of less than 50% soil moisture, which may have limited the performance of some active ingredients. Species-specific responses were observed under these suboptimal conditions: *P. lathyroides* showed minimal regrowth, *E. heterophylla* and *C. arvensis* showed moderate regrowth, and *C. dactylon* exhibited high regrowth potential. These findings agree with Aekrathok *et al.* (2021), who reported significant reductions in herbicide efficacy under low soil moisture conditions. The assessments for herbicide effectiveness at 14 and 21 DAA indicated that elevated soil moisture levels (>90%) with high temperatures (34-37 °C) adversely affected the performance of certain treatments. According to Hess (2018), waterlogged soils can interfere with herbicide uptake by limiting root absorption, reducing mobility, and impairing translocation, ultimately decreasing herbicide efficacy and persistence. These edaphoclimatic fluctuations may explain the observed variability in both phytotoxicity and weed control following the initial application. Therefore, effective and timely weed management should consider not only the type and timing of herbicide application but also prevailing environmental and soil conditions.

CONCLUSIONS

Selective post-emergent herbicides differed significantly in their effectiveness and phytotoxic impact on sugarcane. The combinations AMT+ATZ, AMT+ATZ+DIU, and AMT+2,4-D provided "adequate control" (87.5-93%) of major weed species such as *Cynodon dactylon*, *Euphorbia heterophylla*, and *Convolvulus arvensis*, while inducing only minimal phytotoxicity with no observable yield impact. The negative correlation between herbicide effectiveness and SPAD index values indicates that increased weed suppression may be temporarily associated with reduced chlorophyll content. Moreover, soil moisture and temperature fluctuations influenced both the efficacy of treatments and the regrowth capacity of weed species. These findings highlight the importance of integrating timely chemical applications with environmental monitoring and species-specific herbicide selection. Such strategies can enhance weed control, minimise crop stress, and contribute to the long-term sustainability and productivity of sugarcane-based cropping systems.

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Yellow berry in wheat: genetics, impact on grain protein content, technological quality, and value-added applications

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ABSTRACT

Objective: To highlight the importance of wheat grain quality in the food industry, with emphasis on the presence of the disorder known as yellow berry (YB).

Design/Methodology/Approach: This review analyzes the importance of wheat grain quality, focusing on YB, its physiological and nutritional causes, and its relationship with nitrogen availability. It also addresses advances in genetics and breeding aimed at increasing the stability of grain protein content, along with potential value-added applications for affected wheat.

Results: Wheat (*Triticum* spp.) is a staple crop in the human diet and one of the most consumed cereals. Grain quality, especially its protein content, is a key attribute for nutrition and food security. However, the presence of YB represents a significant limitation, as it is associated with a reduction in protein concentration and an increase in starch content. This disorder has been primarily linked to nitrogen deficiencies in the soil. Affected grains exhibit a floury, opaque, chalky-looking endosperm, accompanied by a heterogeneous protein matrix.

Study Limitations/Implications: Genetic improvement strategies are needed to mitigate the effects of YB; nevertheless, studies on this topic are scarce.

Findings/Conclusions: Optimizing management practices, such as nitrogen fertilization, is essential to balance crop yield and grain quality. This contributes to ensuring economic viability and promoting environmental sustainability in wheat production.

Keywords: protein, starch, bread, durum, nitrogen.

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INTRODUCTION

Wheat (*Triticum* L.), as the predominant staple crop, is essential for global food security, supplying more than 20% of calories and protein, and contributing more than 30% of the energy consumed by humans (Wan *et al.*, 2023), in addition, it is one of the main cereals in the global economy due to its high productive potential and technological value. The most widely cultivated variety is *Triticum aestivum*, known as common wheat (Biel *et al.*, 2021), considered one of the most consumed cereals in the world, along with rice

and maize (Nardino *et al.*, 2022). On the other hand, durum wheat (*Triticum turgidum* L. var. *durum*) is the tenth most prominent and cultivated cereal globally, with an average annual production of 40 million tons. Wheat plays a significant role in nutrition, as today wheat grains contribute greatly to the human diet by providing major nutrients such as carbohydrates, proteins, dietary fiber, minerals, vitamins and as well as phenolic acids known for their antioxidant potential (Mariem *et al.*, 2020).

Nonetheless, the quality of this important food can be compromised by various factors, and sometimes, the harvested grain may exhibit quality deviations, primarily due to a high incidence of the physiological defect known as yellow berry (YB), which prevents it from meeting industry standards (Solís Moya & Díaz De León Tobías, 2001), considered a serious disorder in the grains of bread wheat, durum wheat, and triticale. This problem arises mainly due to nitrogen (N) deficiency in the soil (Zárate Márquez *et al.*, 2015) and is manifested by the appearance of areas with starchy spots in generally vitreous grains, which results in a less compact structure, with numerous open spaces and a physically variable protein matrix in the grains (Taranto *et al.*, 2023); so that this character is closely associated with a reduction in the protein content in the grain (Rodríguez *et al.*, 2011) significantly affecting the quality of flour in bread wheat and pasta in durum wheat (Zárate Márquez *et al.*, 2015).

Although YB has been detected in both durum wheat and bread wheat; in the case of the manufacture of durum wheat products, large, vitreous grains with high protein content, yellow pigment, and strong gluten are needed. The physiological alterations associated with YB extend beyond the grain level; the reduction in vitreousness and protein content directly comprises both technological performance and commercial value. The proportion of vitreous grains is an indicator of quality, as it is related to semolina yield and protein content. In contrast, an increase in "YB grains" deteriorates quality, reducing protein content by up to 1% for every 10% of these grains (Solís *et al.*, 2009). In this context, YB grain is characterized by its opacity and floury texture, which reduces the vitreous properties of durum wheat and results in a higher commercial penalty compared to bread wheat (Castro, 2022). In this regard, the amount of wheat grain protein sets up the price paid to producers and is subject to the amount of N in the canopy at anthesis and the absorption capacity of available N during grain filling (Longmire *et al.*, 2023).

The technological and nutritional quality of durum wheat semolina fundamentally depends on the type of proteins that constitute gluten and their quantity (Graziano *et al.*, 2019); the rheological properties of gluten are indispensable not only for the manufacture of bread but also for a wider variety of foods that are made exclusively from wheat, such as noodles, pasta, breads, cakes, cookies and other food products (Žilić *et al.*, 2011). Quality is therefore a key factor in wheat improvement, as its genetic origin is less affected by the environment and has a significant impact on the commercial value of a cultivar. Consequently, if a cultivar has a specific combination of alleles at important loci, it is likely to present valuable qualitative traits with respect to the quality of the final product and this applies to both durum wheat and bread wheat germplasm (Varzakas *et al.*, 2014).

On the other hand, alternatives have recently been proposed for the potential use of wheat grain with YB, over its high starch content and low protein concentration.

Although this imbalance is undesirable in other industries, it can be unbelievably valuable in beer brewing, since starch is an essential substrate to produce of this beverage (García *et al.*, 2023).

Concerning the above-mentioned aspects, the content of this review aims to highlight the importance of wheat grain quality in the food industry, with special emphasis on the physiological defect YB and its impact on wheat grain quality, including protein content, technological properties, and nutritional properties. It synthesizes current knowledge on the genetic, physiological, and agronomic determinants of YB and outlines their implications for quality improvement and industrial valorization.

Importance of wheat crop

Wheat is the most widely cultivated cereal in the world in terms of grain yield and cultivated area. Today, the most important wheat varieties are hexaploid soft wheat (*Triticum aestivum* L.) and tetraploid durum wheat (*Triticum durum* Desf.) (Rossi *et al.*, 2024), which are two species strongly related with potentially different adaptations and few different technological properties that make semolina and wheat flour destined to produce of pasta and bakery products, respectively (Mastrangelo & Cattivelli, 2021). Around 80 million farmers depend on wheat for their livelihood; between 90% and 95% of the wheat grown worldwide is common or bread wheat, which is used for the production of refined or whole wheat flours in the production of a wide variety of bakery products (Giraldo *et al.*, 2019). Of the total wheat production, 5% is represented by durum wheat, with a planting area of 16 million hectares worldwide (Beres *et al.*, 2020). Foods made from durum wheat vary greatly between producing countries; although pasta is the most recognized product worldwide and an icon of Italian cuisine, couscous is the most common durum wheat-based food in North Africa. In southern Italy, Spain, Turkey, and the Mid-East Mediterranean regions, durum wheat breads have traditional importance (Laddomada *et al.*, 2021).

Nutritional value of wheat

Wheat grain is essentially made up of starch, proteins, and cell wall polysaccharides, constituting approximately 90% of its dry weight. In addition, it contains other components in smaller quantities, such as lipids, terpenoids, phenolic compounds, B-complex vitamins, and minerals (Shewry *et al.*, 2013). The Table 1 shows the main nutritional components of wheat.

Wheat is a carbohydrate-rich food composed mainly of starch, a main source of energy to maintain metabolic balance, which involves a complex linkage of several factors, such as nutrient intake, hormonal regulation, and metabolic processes (El Houssni *et al.*, 2024). Starch granules hold approximately 30% linear amylose molecules and 70% branched amylopectin molecules. In these granules, amylopectin adopts a double helix structure, which gives the system a high capacity to retain water and a rapid expansion capacity (Hidalgo *et al.*, 2016). Likewise, cereals are a rich source of dietary fiber, such as non-starch polysaccharides, with the predominant components of wheat grain being arabinoxylans and β-glucans (De Santis *et al.*, 2018).

Table 1. Nutritional composition of wheat and nutrient reference values.

Macronutrients	Content (%)	Mineral	Content (mg/100 g)	NRVs-R (mg/100 g)	Vitamins	Content (μg/ g)	NRVs-R (mg/100 g)
Carbohydrates	70-75	Ca	15-34	1000	Niacin (B3)	0.86	15
Proteins	10-18	K	141-431	-	Riboflavin (B2)	~1.0	1.2
Lipids	1.5-2.5	Mg	13-42	310	Thiamin (B1)	1.0-4.0	1.2
Fiber	~1.6	Fe	3.6-6.8	22	Pyridoxine (B6)	1.89	1.3
Minerals	~1.6	Se	33.9-70.7	60	Folate (B9)	0.56	400
		Na	2-3.8	-			
		Zn	0.7-4.2	14			

NRVs-R, nutrient reference values-requirements.
Source: FAO & WHO, 2011; Khalid *et al.*, 2023; Marcotuli *et al.*, 2020; Shewry *et al.*, 2013; Wieser *et al.*, 2020.

Proteins are compounds of vital importance for the functioning of all cells (Sallam *et al.*, 2019). Wheat grain proteins are divided into two categories: prolamins and non-prolamins. Prolamins comprise gliadins and glutenins that make up approximately 75% of all grain proteins, while non-prolamins, such as albumin and globulin, represent about 25%. Albumins and globulins are soluble proteins that include various enzymes and inhibitors, playing essential structural and metabolic roles during the grain filling process (B. Zheng *et al.*, 2021). On the other hand, the prolamin content and endosperm composition influence the quality of wheat flour (Khalid *et al.*, 2023), in this context, gliadins contribute to the viscosity of the dough, while glutenins are responsible for providing elasticity and resistance (X. Wang & Liu, 2021), both proteins interact to form a viscoelastic gluten network in the dough, which is essential for determining the baking quality of products made from wheat flour (B. Zheng *et al.*, 2021). Proteins are present throughout the grain, but their concentrations vary considerably between different compartments. The germ has approximately 7.5% of the total proteins, the aleurone layer contains 14.2%, the pericarp and the testa 3.8%, and the starchy endosperm presents 74.5%; considering the distribution of these compartments, the largest amount of proteins is located in the starchy endosperm (Wieser *et al.*, 2020).

Regarding lipid content, wheat germ is one of the main byproducts of wheat milling, with an oil content that varies between 5.2% and 15.5%. This oil is an abundant source of health-beneficial compounds, such as essential fatty acids and unsaponifiable lipids, including tocopherols, phytosterols, and policosanols (Harrabi *et al.*, 2021).

Yellow berry in wheat grain

Yellow berry is a physiological disorder in the wheat grain that is characterized by having a high starch content, significantly affecting the protein content, which results in inferior quality bakery products and pasta production, generating penalties for wheat farmers (Rodríguez *et al.*, 2014). Grains with YB are distinguished by a smooth (Serranti *et al.*, 2013) and mottled appearance due to spots that range from white to yellowish-white (Figure 1), depending on the color of the outer layers of the seed (Solís & Díaz De León, 2001). An insufficient amount of protein in the grain causes areas where the starch granules

Figure 1. Grains of three durum wheat genotypes with YB expression.
Source: Own elaboration.

are not properly cohesive. These areas with low protein are composed of gaps empty of air that appear as clear and mottled patches (Hare, 2017), and in grains normally these spaces are filled with a protein matrix. The gaps in the endosperm reduce translucency, which gives starchy grains an opaque appearance (López-Ahumada *et al.*, 2010). This reduces the vitreous appearance of durum wheat grain, causing it to be more penalized in the market compared to bread wheat (Castro, 2022).

According to the specifications of the Official Mexican Standard NOM-FF-36-1984, YB grains are those grains that have at least a quarter of their surface with a floury appearance (DOF, 1984). In durum wheat, vitreous grains have a higher protein content and therefore higher grain quality than YB grains (Bnejdi & El Gazzah, 2008). In this regard, the minimum content of hard and vitreous grains in the marketing of durum wheat is a key parameter. YB grains are also known as non-vitreous kernels whose protein content is lower than that of vitreous kernels (Dexter & D'Egidio, 2012). Farmers do not typically obtain higher prices for high protein content in grains, but do face lower prices when more than 15-20% of durum wheat grains are affected by YB (Grahmann *et al.*, 2014), because it can reduce the protein content in the grain between 2% and 4% (Ibarra *et al.*, 2023).

Factors that contribute to the development of YB in wheat grain

Among the main biological processes associated with wheat yield is grain development, considered a complex process that is characterized by three fundamental stages: cellularization/differentiation, grain filling, and maturation. The main transformation in grain development occurs when essential storage components such as starch and protein begin to accumulate (Kaushik *et al.*, 2024). During this stage, defects in commercial quality may arise due to environmental conditions, such as relatively low temperatures, low radiation, and high humidity, causing the formation of grains with YB, which are also influenced by genetic factors and low availability of N (Simón, 2022).

It has long been observed that YB grain disorder is mainly related to a limited presence of nitrates in the soil. Furthermore, N stress in the grain in its initial phase of development reduces the concentration of grain protein, and especially the proportion of gliadins (López-Ahumada *et al.*, 2010); likewise, a significant reduction in N leads to a decrease in the number of grains per ear, and consequently affects grain yield in wheat (Zi-meng *et al.*, 2025). For that reason, increasing the availability and improving the efficiency of N uptake is considered crucial to increase both wheat grain yield and protein content. However, one of the challenges in wheat production and breeding is to increase grain yield without compromising its protein content (Derebe *et al.*, 2022).

Impact of N on yellow berry expression and protein content in wheat grain

The photosynthetic capacity of plants is essential during the growth of crops. Concerning N fertilizers, they are a key factor, since N is a critical component of plant amino acids, proteins, and chlorophyll in the photosynthesis process (Xiao *et al.*, 2023). N yield comes from two sources: N is stored in the vegetative parts before flowering and N is absorbed during the grain filling period (X. Zheng *et al.*, 2020). Grain N is produced through two pathways: N remobilized from the canopy (leaves and stems) and up to 50% from the soil after anthesis (Zörb *et al.*, 2018). Applying N during reproductive development increases protein synthesis and storage in the grain, while excessive delay in fertilization can limit the amount of N that is converted into quality protein (Blandino *et al.*, 2015).

Generally, durum wheat is more sensitive to N availability; its protein accumulation exhibits a stronger physiological response to N supply compared with bread wheat (Giunta *et al.*, 2019). On the other hand, there is a negative correlation between grain protein content and grain yield because high transpiration limits the rate of N mineralization (Govta *et al.*, 2022), since a high number of grains contributes to high performance and can establish an imbalance between the N source represented by the amount of N absorbed during anthesis and the N sink represented by the number of fixed grains (Giunta *et al.*, 2019). Additionally, a high yield reflects the starch content and, in the case of wheat, the gluten protein content. In this regard, starch represents approximately 80% of the grain, so protein content is expected to decrease with increasing yield, which is often attributed to yield dilution. Furthermore, mineral micronutrients, such as iron and zinc, may decrease because of yield dilution (Lovegrove *et al.*, 2020). Therefore, as yield increases, there is a higher concentration of starch, consequently, it is possible to consider that YB does not affect crop yield (Ibarra *et al.*, 2023).

The limited availability of N is the main cause that affects the prevalence of grains with YB (Akman, 2013; Sissons *et al.*, 2012); so, by appropriately increasing the N fertilization rate, it can increase wheat production (Ma *et al.*, 2015), improve grain protein content and alter wheat protein composition (Trevisan *et al.*, 2022). Nevertheless, after applying increasing amounts of N fertilizer, the protein content in the grain reaches a maximum peak and then stabilizes, without the crop increasing the absorption or redistribution of N, which reduces the efficiency of fertilizer use (Barneix, 2007), in addition to increasing production costs and causing contamination of groundwater as N leaches into the soil (Ramírez-Wong *et al.*, 2014).

Some studies have proved the effect of N on the expression of YB. Rodríguez *et al.* (2014) investigated three wheat genotypes and found that one of them presented a higher percentage of YB (2.31%); this phenomenon was attributed to the fact that said genotype also showed a higher grain yield compared to the other two; becoming to its tall habit, this genotype had higher N uptake demands, that resulted in a higher YB content and a lower protein content compared to the other genotypes. Ibarra *et al.* (2023) reported that the presence of YB grains in durum wheat was only affected by fertilization doses, with the highest percentage (31%) observed in the lowest N doses of 0 kg N ha^{-1}, while at a high dose of 250 kg N ha^{-1} obtained results of 0%. Mon *et al.* (2016) performed two years of experiments to evaluate the effects of N fertilizers on certain parameters in durum wheat, including YB. The results revealed that, in 2013, YB was expressed in 54 and 7% with N doses of 84 and 168 kg N ha^{-1}, respectively; in 2014, YB occurred in 71 and 37% with N doses of 84 and 168 kg N ha^{-1}, respectively; while at N doses of 252 kg N ha^{-1} or more the YB content was insignificant. Furthermore, it has been shown that applying N at 150 kg N ha^{-1} has reduced the YB content in wheat grain by up to 1% (Ramírez-Wong *et al.*, 2014; Rodríguez-Félix *et al.*, 2014).

Increasing the level of N fertilization from 150 to 200 kg ha^{-1} significantly affects the increase in yield and certain grain quality parameters, such as total protein (8.0% increase), gluten content (9.9%), Zeleny sedimentation indices (15.9%), grain vitreous (7.3%) and falling number by 14.7% (Jańczak-Pieniążek *et al.*, 2020). The Table 2 shows studies in various regions of the world that have focused on the application of different doses of N fertilization (0, 30, 50, 100-300 kg N ha^{-1}), with the aim of increasing the protein content in the grain, which have demonstrated a direct relationship: by increasing the doses of N, both grain yield and protein concentration increase. Hence, the application of N is essential as one of the main management factors that affect wheat production (Zhong *et al.*, 2018), in the yield components (Wang *et al.*, 2022), in the protein storage and the technological quality of the grain (Blandino *et al.*, 2015).

Table 2. Influence of different N levels on the yield and protein content of wheat grain.

N rates (kg ha^{-1})	Grain yield (t ha^{-1})	Grain protein (%)	Reference
0 - 288	1.69 - 9.08	9.35 - 14.08	(Godfrey *et al.*, 2010)
100	-	10.43	(Chope *et al.*, 2014)
350	-	13.34	
0 - 270	0.69 - 3.80	7.50 - 11.50	(Assefa *et al.*, 2023)
30 - 120	3.40 - 4.25	12.40 - 13.09	(Haile *et al.*, 2012)
50 - 150	5.64	11.20 - 14.00	(Rossini *et al.*, 2025)
0 - 270	4.88 - 9.13	9.92 - 15.05	(Meng *et al.*, 2024)
120 (application in four split doses)	5.06	14.90	(Muhammad *et al.*, 2018)
130 (application in two split doses)	6.10 - 6.20	13.30 - 14.40	(Blandino *et al.*, 2016)
0 - 300	7.73 - 8.65	12.00 - 14.40	(Zheng *et al.*, 2020)
170	>7.00	<11.50	(Zheng *et al.*, 2021)
0 - 240	-	14.90 - 16.20	(Gerba *et al.*, 2013)
75 - 250	-	14.28	(Rodríguez *et al.*, 2014)
75 - 250	-	12.02 - 15.22	(Ramírez-Wong *et al.*, 2014)
60 - 110	6.42 - 6.90	12.10 - 12.50	(Tsvey *et al.*, 2021)

Genetics of yellow berry disorder and grain protein content

Although several studies have focused on improving grain quality, particularly protein content, research aimed at identifying genes associated with the expression of the physiological disorder YB remains limited. In this context, Ammiraju *et al.* (2002) reported the association of two microsatellite markers, Xgwm174 and Xgwm190 (located on chromosome 5D). They also identified an inter-simple sequence repeat (ISSR) marker, $UBC842_{600}$, and a randomly amplified polymorphic DNA (RAPD) marker, $OPR8_{1000}$, on chromosome 6B, all linked to YB tolerance. Prashant *et al.* (2011) mapped a major gene associated with YB tolerance to the short arm of chromosome 5D and also identified a minor locus on chromosome 2D that explained 6.9% of the phenotypic variance for this trait.

More recently, Taranto *et al.* (2023) evaluated 123 durum wheat samples by genome-wide association study (GWAS) and revealed 28 reliable quantitative trait nucleotides (QTNs) related to plant morphological characteristics and grain-related traits, highlighting a strong association of YB on chromosome 6A (Q.Yb-6A), in a region containing the NADH-ubiquinone oxidoreductase subunit, a gene involved in starch metabolism.

Grain protein content, in particular, is determined by a complex genetic system and is strongly influenced by environmental factors and agronomic practices (Govta *et al.*, 2022). Accordingly, several studies have focused on identifying molecular markers associated with grain quality traits, especially protein content.

Krystkowiak *et al.* (2017) examined the interaction between alleles at the Glu-1 and Glu-3 loci, identifying several quantitative trait loci (QTL) associated with key quality attributes in bread wheat. Specifically, the Glu-D1 locus was found to influence protein content, thousand-kernel weight, starch content, wet gluten, the Zeleny sedimentation index, the alveograph parameter W, and grain hardness. In contrast, the Glu-B1 locus was associated only with protein content, the Zeleny index, and wet gluten content; while the most important marker-trait associations were found on chromosomes 1D and 5D.

In addition, seven QTLs influencing protein concentration have been reported on the chromosome arms 4BS, 5AL, 6AS (two loci), 6BS, 7AS, and 7BS (Blanco *et al.*, 2002). Complementarily, Suprayogi *et al.* (2009) identified a QTL on chromosome 7A associated with increased protein content, while Gonzalez-Hernandez *et al.* (2004) reported three QTL with similar effects, all in durum wheat.

In a more recent study, Tian *et al.* (2025) conducted a GWAS on 341 bread wheat accessions, identifying 97 significant and stable single-nucleotide polymorphisms (SNPs) distributed across 43 loci associated with protein quality. The most relevant associations were found on chromosomes 1A, 1B, and 1D. The Figure 2 illustrates the most relevant genomic regions, facilitating an integrated representation that synthesizes the findings of some of the cited studies.

The identification of loci and molecular markers associated with these traits enables a more targeted selection approach aimed not only at increasing protein content but also at reducing the incidence of YB, a disorder that adversely affects the industrial quality of the grain.

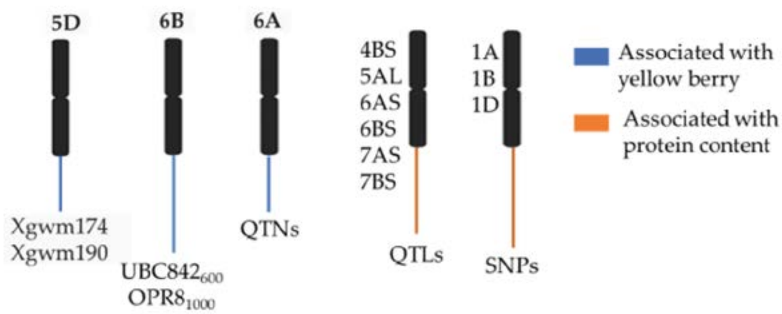

Figure 2. Genomic distribution of QTLs, QTNs, and genes associated with yellow berry and grain protein content in wheat. Source: Ammiraju *et al*., 2002; Taranto *et al*., 2023; Blanco *et al*., 2002; Suprayogi *et al*., 2009; Tian *et al*., 2025.

Impact of wheat quality on the food industry

Over time, rapid population growth and progressive decline in arable land have contributed to pressure on researchers to increase wheat productivity. High concentrations of chemical fertilizer application are generally used to promote wheat yield (Xu *et al*., 2018). However, often, increases in wheat production are related to a decrease in grain quality (Mariem *et al*., 2020).

Wheat grain quality, especially protein content and quality for human nutrition, is considered a fundamental aspect of food security, it has received limited attention, and it is often neglected in the need to improve crop production (Hu *et al*., 2022). Recently the market has paid special attention to the protein content of wheat grain, as farmers receive a higher price when the protein level exceeds 13%, equivalent to the 12% protein in ground semolina. Low protein content can result in significant economic losses for producers, given that protein is a key quality factor in the market (Melash & Ábrahám, 2022).

Wheat quality varies widely due to differences in physiology, growing conditions, crop management practices, and grain storage; for this reason, grain quality control is of utmost importance throughout the world to guarantee a stable quality of wheat for marketing (Jayas *et al*., 2016), in such a way that it is possible to define it through its physical, chemical attributes, and technological. Wheat quality is quite complex, so it can be divided into processing quality including milling, flour yield, grain weight, grain hardness, flour whiteness, flour quality, dough quality, baking quality, baking speed, dough formation time, stability time, settling value, softening degree, etc.; edible quality referring to the flavor of the products during the baking, cooking and frying processes; nutritional quality and phytosanitary quality (Y. Wang *et al*., 2024).

Influence of yellow berry on grain composition and processing quality of wheat

Physical characteristics such as vitreousness, kernel weight, and test weight have a significant impact on the rheological properties and processing of wheat; as for the chemical characteristics, they include the content of proteins, ash, lipids, and gluten, all of which are determinants for the quality of the final product (Manai-Djebali *et al*., 2021). Several factors influence the quality of durum wheat, nonetheless, the three most crucial in determining its commercial value are the proportion of hard vitreous grain, protein content (Xiao Fu *et al.*, 2017), and the concentration of carotenoids, which are responsible for the yellowish color of semolina (Giannetti *et al.*, 2023), and characteristics of the final product, such as color, aroma, and flavor (Menga *et al.*, 2023), and also contribute to good human health on account of its antioxidant properties (Ma *et al.*, 2021; Sai Prasad *et al.*, 2022). Likewise, in the world market, wheat must meet certain quality indicators, such as sedimentation value, percentage of yellow berry (YB), β-carotene content, etc. (Ozberk *et al.*, 2005).

Kernel vitreousness is the primary determinant of milling performance in durum wheat because it reflects protein-rich and compact endosperm structure (Wang *et al.*, 2023). Vitreous kernels generate higher semolina extraction rates and better cooking quality (Molfese *et al.*, 2017), whereas non-vitreous (YB) kernels contain air spaces between starch granules, resulting in softer texture and lower semolina yield (Venora *et al.*, 2009). To ensure adequate protein (12.5%) and acceptable extraction rates, industry standards typically limit YB content to less than 15% (Grahmann *et al.*, 2014). Because YB grains also contain less protein, their presence reduces dough strength and compromises pasta firmness and resistance to breakage (Dexter & D'Egidio, 2012).

The content and composition of proteins in the grain not only defines the nutritional properties, but also the rheological characteristics (Nuttall *et al.*, 2017) and the technological characteristics of the dough, impacting the quality of products such as pasta (De Vita & Taranto, 2019) and baking (Geisslitz *et al.*, 2018). So, they can influence the properties of wheat flour during kneading (such as the water absorption rate), the formation of the gluten network, the characteristics of the dough (hardness, viscosity, elasticity, extensibility, plasticity, water retention, among others) and in cooking qualities (Lin *et al.*, 2019). In this context, a higher protein content is linked to a higher proportion of gliadins and glutenins (Trevisan *et al.*, 2022); when flour is mixed with water, a protein bond is formed because of the precipitation of these two proteins; on the one hand, glutenin polymerizes forming an elastic union, while gliadin is incorporated into an extensible sticky dough, entering the gluten nexus (Varzakas, 2016), so that the properties of these proteins provide viscoelasticity and extensibility to the dough. Furthermore, quantity plays a crucial role in determining its nutritional quality (Yiğit, 2023), given that high protein content, and consequently gluten, are parameters associated with the firmness of the pasta and limited cooking (Menga *et al.*, 2023). Consequently, by decreasing the protein content due to the presence of YB, the cooking quality is also affected (Samson *et al.*, 2005); because the proteins favor the hydration of the semolina particles during the mixing process and provide structure to the fresh or dry pasta. Furthermore, the union and resistance of the protein matrix that is formed during extrusion is essential to determine the texture properties in the cooked pasta, therefore, a high protein content is the essential requirement to obtain a high quality of pasta cooking (Fu *et al.*, 2017).

Impact of yellow berry on wheat processing quality

Milling forms the initial phase of wheat processing. This process constitutes continuous crushing and sieving operations to separate the starchy endosperm from the external layers and the germ (Lullien, 2020). The starchy endosperm of the mature wheat grain is made up of three classes of main cells, that include sub-aleurone cells, prismatic cells, and central cells, which differ in terms of their concentration of functional components: gluten proteins, starch, cell wall polysaccharides, and lipids (Shewry *et al.*, 2020). Semolina consists of a coarse fraction of the endosperm, which results from the first breaking down of the wheat grain before the particle size is reduced to obtain fine flour. It is mainly used for the preparation of various forms of pasta, for which hard semolina is preferred, generally obtained from durum wheat rich in proteins (Miskelly & Suter, 2017), although it can also be produced from bread wheat (Bustos *et al.*, 2015), nevertheless, pasta made from durum wheat exhibits a more intense yellow coloration, the texture of the pasta is denser and firmer and shows more elastic properties than pasta made from bread wheat (Nilusha *et al.*, 2019). In this regard, durum wheat is more resistant to crushing, the endosperm structure is quite compact, the flour is coarse textured with more damaged starchy and with the capacity to absorb more water than soft wheat flour (He *et al.*, 2023). In this sense, during the milling of durum wheat when it has YB there is an increase in the starch content (Prashant *et al.*, 2011), disintegrating into smaller particles, which are incorporated into the finer flour fraction of lower value (Hare, 2017). On the other hand, it is important to mention that in durum wheat, sedimentation values have been positively correlated with the yellow pigment content, percentage of YB, thousand-grain weight, glume color, color, and grain yield (Hailu & Merker, 2008).

Value-added applications of yellow berry affected wheat grain

Given the growing interest in soft wheat in the food industry, a viable alternative for the use of YB grains in this sector is their use as raw materials in the production of cookies and cakes. Specifically, grains with a low protein and gluten content are valued, as these characteristics are essential for obtaining high-quality products, especially in cookies production (Yang *et al.*, 2022). This is because the amount of protein in the grain, especially that stored in the seed, significantly influences the quality of the biscuits. Wheat with low gluten content and a poorly developed gluten network forms doughs that expand more easily, which favors the production of cookies with a wider diameter after baking (Wu *et al.*, 2022).

Furthermore, as previously noted, YB grains have a higher starch content, a compound that plays a key role in the textural properties of numerous foods. Starch is widely used in both the food industry and in industrial applications, where it acts as a thickener, colloidal stabilizer, gelling agent, bulking agent, and water retention agent (Lafiandra *et al.*, 2022). Starch also has significant promise for the development of films aimed at preserving fresh fruits and vegetables. As a biodegradable, non-toxic, and edible material, it represents a sustainable alternative to conventional plastics, reducing both environmental impact and health risks associated with traditional packaging (Karnwal *et al.*, 2025). In addition, starch presents considerable potential in the pharmaceutical industry for the production of nanofibers through the electrospinning process. Starch nanofibers obtained using this technique find applications in areas such as drug delivery, tissue engineering, and the development of wound dressings (Huang *et al.*, 2021).

High protein content is advantageous for farmers and bakers but problematic for the brewery industry, as it causes long filtration periods, fermentation issues, and reduced flavor stability (Faltermaier *et al.*, 2014). The protein content is essential in cereal malting (Padilla *et al.*, 2022); however, durum wheat contains more protein than barley (9.5-10.5% in barley *vs.* 11-13% in durum wheat), which may hinder fermentation and generate undesirable flavors (García *et al.*, 2023). Thus, low protein levels are preferred for malting. The presence of YB in wheat decreases the protein content and increases starch; for this reason, YB grains could have the potential to produce brewing malts (Padilla-Torres *et al.*, 2022).

On the other hand, ethanol production is influenced by the supply of starch-rich crops, among which wheat is one of the main contributors, on account of its price, availability, and conflicts in the use of crops as food or fuel. In the case of wheat, it has a starch content of 62-75% and is mainly used for food, while for the production of ethanol, is produced especially in the United States, China, and Canada (Li *et al.*, 2022). In this sense, it is possible to consider starch-rich YB wheat as raw material to produce ethanol, nonetheless, further studies are still needed in this regard.

CONCLUSION

Nitrogen nutrition plays a central role in wheat quality, as deficiencies promote YB expression by reducing protein and vitreosity while increasing starch, negatively affecting both the nutritional properties and the rheological and technological characteristics of wheat flour. This highlights the need for genetic improvement strategies aimed at enhancing tolerance to YB and stabilizing quality under variable N conditions. At the same time, efficient N management remains essential to balance yield, grain quality, and environmental sustainability. YB-affected grains, with their elevated starch content, offer emerging industrial opportunities in brewing, biofilm, pharmaceuticals, and biofuel production. Future research should prioritize genetic mapping and agronomic optimization to transform YB-affected wheat into a value-added bioresource.

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DATA AVAILABILITY STATEMENT

The data collected in this research are available from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

Espitia-Hernández: Writing – original draft, writing – review & editing manuscript, investigation, conceptualization. Velasco-López: Writing – review & editing manuscript, validation, conceptualization. Ruiz-Torres: Writing – review & editing, validation, conceptualization. Ruelas-Chacón: Writing – review, and editing manuscript, conceptualization. Lozano-del Río: Writing – review & editing, conceptualization. All authors read and approved the final manuscript.

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AGRO PRODUCTIVIDAD

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Nutritional analysis of two corn hybrids (*Zea mays* L.) for forage production

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ABSTRACT

Objective: To characterize and compare the yield and bromatological composition —such as fresh matter, crude protein, and other nutritional indicators— of two corn (*Zea mays* L.) hybrids, developed under fertigation conditions, at three different plant densities.

Design/Methodology/Approach: The experiment was conducted using a factorial experimental design with two factors (hybrids) and three levels (densities).

Results: The CEBU hybrid recorded the highest fresh and dry matter yield. According to the bromatological analysis, it also obtained the highest crude protein (CP) values and total ash content. Meanwhile, the A7573 hybrid reached a higher total carbohydrate content.

Study Limitations/Implications: The low temperatures recorded during the experiment impacted both the corn plant and crop development cycles.

Findings/Conclusions: The agronomic characteristics and nutritional value of evaluated hybrids recorded significant differences. CEBU had a more efficient forage and biomass production in all the evaluated sowing densities (50,000, 62,500, and 83,333 plant ha^{-1}). In the bromatological analysis, CEBU obtained a higher crude protein and ash content than A7573. However, A7573 recorded a higher total carbohydrate content than CEBU.

Keywords: Bromatological composition, forage, plant density, fertigation, and corn.

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INTRODUCTION

Corn (*Zea mays* L.) is the most important crop in Mexico from a food, political, economic, and social perspectives. Domestic production has reached over 27 million tons, grown in more than 6 million hectares. The main corn producers are Sinaloa, Michoacán, the State of Mexico, Guanajuato, and Chihuahua (Secretaría de Agricultura y Desarrollo

Rural [SADER], 2023). Corn has multiple uses, including forage to feed animals. Corn forage has high nutritional value, and its high dry matter yield per ha results in a high productivity. Nevertheless, forage quality cannot be determined solely by the amount of production, but fundamentally by its nutrient composition —*i.e.*, the type and content of its nutritional components, such as dry matter (DM), crude protein (CP), ashes, fats, neutral detergent fiber (NDF), acid detergent fiber (ADF), non-fiber carbohydrates, and minerals (Ramírez *et al.*, 2024; Maguiña-Maza *et al.*, 2021).

The selection of a corn variety or hybrid as forage depends on its dry matter yield (DMY), which is related to the morphological components of the plant, such as leaves and stems (Rivas *et al.*, 2020). To achieve successful forage production, corn must be established under the right environmental and nutritional conditions that favor its appropriate growth and development (Zulueta-Rodríguez *et al.*, 2020). Corn must receive macro and micronutrients to meet its nutritional requirements. However, the plant usually receives these nutrients through chemical fertilization, due to the low fertility of the soils in the semiarid regions where it is grown (Lagunes-Domínguez, 2018). In this context, fertigation is an efficient alternative that enables the simultaneous application of water and fertilizers, optimizing resources and maintaining productivity in environments with limited water availability (Kalpana and Fanish, 2014).

In their study on fresh corn forage production, Gutiérrez-Guzmán *et al.* (2022) recorded a 55.8 t ha^{-1} yield with the early-cycle Syngenta 8285 variety. The sowing density was 100,000 plants ha^{-1}. The plants were sown with a 0.76 m separation between furrows and were fertilized with a 280-80-00 formula. In addition, in their study about the nutritional composition of the PM 213 hybrid forage, Silva *et al.* (2018) reported the following results: 22.45% DM, 8.47% CP, 24.71% fiber, 42.40% ADF, 53.46% NDF, and 4,366.40 kcal of gross energy.

Therefore, the objective of this study was to characterize two corn (*Zea mays* L.) hybrids and compare their bromatological composition, based on nutrient parameters such as fresh and dry matter, crude protein, carbohydrates, and other nutrient quality indicators. In addition, the forage yield was measured under fertigation conditions with three plant densities to provide a feasible forage production alternative to regional producers. This production could be used to feed cattle during the winter or dry season.

MATERIALS AND METHODS

Study Area

The research was conducted from August 16 to November 15, 2024, in the "Los Chavales" property, located at 22° 35' 07.4" N and 101° 43' 20.3" W, in the municipal seat of Salinas, San Luis Potosí, Mexico.

Hybrid Selection

Two corn hybrids (CEBU and A7573) were selected for the experiment. These hybrids have interesting agronomic characteristics such as resistance to corn stalk lodging, adaptability, and yield potential. The seeds were purchased from ASGROW (Mexico).

Experimental Design

A completely randomized experimental design with a 2×3 factorial arrangement was used. The factors were the two corn hybrids (CEBU and A7573), and the levels were the three plant densities (50,000, 62,500, and 83,333 plants ha^{-1}).

The experiment had six treatments (factor interaction). Each treatment was established in 40 m^2 (4 m wide × 10 m long) plots. Each plot consisted of five 10-m rows with a 0.80 m separation between them. One-meter-wide corridors separated the plots, which resulted in a total useful area of 240 m^2.

Establishment of the Experiment

An organic-mineral fertilizer was prepared with 5 t of dry, raw bovine manure mixture, previously sieved with a 5-mm sieve, to increase the contact area with the mineral fertilizers. Subsequently, the manure was combined with a mineral fertilization formula of 60 N:60 P_2O_5:60 K_2O kg ha^{-1}.

The following quantities were used in the total area of the experiment: 120 kg of manure, 7.0 kg of ammonium sulfate, 6.5 kg of single super phosphate, and 2.4 kg of potassium chloride. The mixture was homogenized and applied as background fertilization, incorporating it into the soil at a ≈30 cm depth. Afterwards, it was covered with soil.

Fertigation was used to supply nutrients to the crops, using the 280 N: 200 P_2O_5: 200 K_2O and 40 MgO kg ha^{-1} formula. Table 1 includes the sources and quantity of fertilizer applied through the irrigation water from September to November 2024.

The nutrient solution was applied twelve times during September and October, and three times in November. The pH was adjusted with phosphoric acid to reach 6.5.

Yield Determination and Biomass Percentage

Plants from each treatment were collected within the useful area of each plot to determine fresh and dry matter yield.

The samples were weighted to obtain the average fresh weight per plant (kg) and subsequently the fresh matter yield (FMY) per ha was calculated (t ha^{-1}) with the following equation:

Table 1. Fertilization doses and water volume applied during the monthly scheduled irrigation to corn grown under fertigation (September-November, 2024).

Soluble fertilizer	Months (2024)		
	September	October	November
Macronutrients	Kilograms per irrigation		
Monoammonium phosphate	0.35	0.22	0.17
Potassium nitrate	0.25	0.50	0.40
Phosphonitrate	0.35	0.55	0.60
Magnesium nitrate	0.20	0.25	0.25
Micronutrients	Grams per irrigation		
Carboxy micros	30.00	30.00	30.00
Water volume applied by irrigation (m^3)	0.90	1.35	1.35

$$FMY = \frac{fresh\,weight\ per\ plant \times Plant\ density\ ha^{-1}}{1000}$$

The samples were placed in a convection drying oven at 65 °C until they reached a constant weight. Subsequently, they were weighed and the dry weight was used to calculate dry matter yield (DMY) (t ha^{-1}) with the following equation:

$$DMY = \frac{dry\,weight\ per\ plant \times Plant\ density\ ha^{-1}}{1000}$$

Dry matter percentage (%) was determined following the methodology described by Gutiérrez-Guzmán *et al.* (2022).

Nutritional Analysis

The nutritional analysis was conducted in the Laboratorio de Química y Bioquímica of the Coordinación Académica Región Altiplano Oeste of the Universidad Autónoma de San Luis Potosí (UASLP).

Crude Protein

Crude protein (CP%) content was determined with the micro-Kjeldahl method, following the AOAC standard (2005) that includes three stages: digestion, distillation, and titration. To determine digestion, 0.25 g of the crushed sample was weighed and subsequently placed in a 100-mL Kjeldahl flask. Afterwards, 1.0 g of catalyst (potassium sulphate and copper sulphate) and 3.5 mL of H_2SO_4 (96%) were added to the flask. The samples were placed in a digester, at 400 °C for approximately two hours, until the mixture changed from black to transparent.

Subsequently, the RapidStill I (Labconco Corp., Kansas City, MO, USA) system was used to distill the mixture, adding 10 mL of distilled water at 40 °C and 10 mL of a 60% NaOH solution. The distillate was collected in an Erlenmeyer flask, mixing 5 mL of boric acid saturated solution and three drops of methyl red as indicator. Distillation stopped when a 50 mL final volume was obtained. Finally, titration was conducted with a HCl 0.09 N standard solution until it turned from green to pink. The crude protein percentage was calculated with the following equation, using the 6.25 factor.

$$CP\% = \frac{Vol.of\ HCl \times N\ of\ HCl \times Meq\ N \times Food\ Factor}{Grams\,of\ sample} \times 100$$

Total Ash Content

The total ash percentage (TA%) was determined following the methodology proposed by Kirk *et al.* (1996). A 3 g sample was crushed and sieved; afterwards, it was briefly ashed over a burner until all visible vapors faded away. Subsequently, the samples were placed in a muffle furnace at 550 °C until white or slightly grey ashes were obtained. The percentage was calculated based on the ratio of ash weight and dry matter initial weight.

Total Carbohydrates

Carbohydrate content was determined with the methodology described by Ammar *et al.* (2013). A small sample (0.05 g) was taken from each treatment and soaked in 20 mL of distilled water. Samples were centrifuged at 4,000 rpm for ten minutes and the extracts (supernatants) were used in the colorimetry reaction. An aliquot (3 mL) of H_2SO_4 (96%) was added to 1 mL extract from each sample. Subsequently, the mixture was stirred for 30 seconds and incubated into an ice bath for 2 minutes. The absorbance was determined at 315 nm in a spectrophotometer, using distilled water as a blank.

Statistical Analysis

The data obtained were analyzed with STATISCA v. 7.0 and Sigma Plot v. 10.0 software. The mean and its corresponding standard error $(\bar{x} \pm SE)$ were the central tendency and dispersion measurements used in the analysis. They were also used to the error bar of figures. An analysis of variance (ANOVA) was used for the contrasts. The ANOVA included a completely randomized design with a 2×3 factorial arrangement, using the CEBU and A7573 hybrids and sowing density (50,000, 62,500, and 83,333 plants ha^{-1}) as factors. Tukey's post-hoc test ($p \leq 0.05$) was used to compare means and to identify significant differences between treatments.

RESULTS AND DISCUSSION

Fresh Forage Yield

In this study, the maximum yields of fresh forage reached 49.08±0.92 and 48.02±2.04 t ha^{-1} (Table 2). These results are partially similar to those reported by Rodríguez-Montalvo *et al.* (2021), who recorded 53.10 and 51.94 t ha^{-1} for the H-564C and HE-3B hybrids, respectively. These hybrids were grown under a high sowing density and with intensive fertilization. Although the results of this study are slightly lower than the results obtained by those authors, they show a similar trend, particularly when the efficient use of the available water resources is considered. Meanwhile, Zaragoza-Esparza *et al.* (2019) reported higher yields with the PUMA 1181 and BUHO hybrids, reaching 58 and 74 t ha^{-1}, respectively. These differences could be the result of genetic factors, favorable edaphoclimatic conditions, or a more intensive agronomic management than those used in this experiment, particularly the delay of crop development due to low temperatures. In contrast, Gutiérrez-Guzmán *et al.* (2022) reported a 32.4 t ha^{-1} yield at a 47.2 cm irrigation depth. This result is similar to the lowest yield recorded in this study (28.56±0.43 t ha^{-1}). During this experiment, the irrigation volume (1,200 m^3 ha^{-1}) was complemented with accumulated precipitation (970 m^3 ha^{-1}). These results confirm the findings of Gutiérrez-Guzmán *et al.* (2022) regarding the importance of water management in the production of forage corn. Overall, the results show that —although fresh forage yield can change depending on the agronomic management, environmental conditions, and

Table 2. Fresh and dry forage yield of two corn hybrids, resulting from the hybrid and sowing density factors and their interaction.

Main factor	**Forage yield (t ha^{-1})**	
Hybrids	**Fresh** $\bar{x}$ ± ***SE***	**Dry** $\bar{x}$ ± ***SE***
CEBU	43.72±2.38b	10.33±0.71a
A7573	38.45±3.01a	9.96±0.57a
Density		
50,000	31.59±1.42b	7.87±0.21b
62,500	43.12±2.54a	10.72±0.46a
83,333	48.55±1.03a	11.85±0.28a
Interaction	**CEBU**	
50,000	34.61±0.89bc	7.65±0.38bc
62,500	47.48±1.84a	11.40±0.51a
83,333	49.08±0.92a	11.96±0.39a
	A7573	
50,000	28.56±0.43c	8.09±0.18c
62,500	38.76±3.14b	10.04±0.57b
83,333	48.02±2.04a	11.74±0.46a

Different letters in the same column indicate significant differences, based on Tukey's HSD test ($P<0.05$).

genetic material— the irrigation volume and timing is a determining factor in fresh forage production.

Dry Forage Yield

Dry matter yield showed significant differences between the sowing densities evaluated. The density of 50,000 plants ha^{-1} recorded the lowest value with the CEBU hybrid (7.65 t ha^{-1}); it was statistically different from the other two densities ($F_{5,\ 0.05}=18.76$, $P<0.05$). On the contrary, the densities of 62,500 and 83,333 plants ha^{-1} recorded the highest yields with CEBU (11.40 and 11.96 t ha^{-1}), while the 83,333 plants ha^{-1} density with A7573 reached 11.74 t ha^{-1} (Table 2). No significant differences were found between both treatments. These results suggest that increasing the sowing density of CEBU up to 62,500 plants ha^{-1} can improve dry matter yield, without productive benefits with an additional increase.

Table 2 shows that the maximum dry forage yield (11.96 t ha^{-1}) was lower than the yield (28.1 and 30.3 t ha^{-1}) reported by Ramírez *et al.* (2024) for the DK 4018, Noble, Antílope, and XR-49 hybrids. Their experiment was carried out during the spring-summer agricultural cycle, harvesting from 121 to 142 days after sowing (DAS). This difference could be attributed to the time of the harvest: the highest values were recorded during more advanced phenological stages (approximate grain maturity at R2, R3, R4, and R5), when more dry matter is accumulated. Meanwhile, this study was carried out during the summer-autumn agricultural cycle, harvesting 91 days after emergence, during earlier phenological stages (VT to R1). This condition could account for the lower yield observed. For their part, Maguiña-Maza *et al.* (2021) recorded an average yield of 22.22 t ha^{-1} at 110 DAS, using four corn genotypes sown at a 60,000 plants ha^{-1} density. Although that result is higher than the maximum value recorded in this study, both fall within a complementary production range that can be subjected to comparisons, strengthening the validity of the findings and confirming the joint influence of sowing density, harvest time, and agroclimatic conditions on the dry matter productivity of forage corn.

Dry Matter Content

Table 3 shows that the dry matter content (%) recorded differences between hybrids at the end of the crop cycle ($F_{1,\ 0.05}$=8.31, $P<0.05$). A7573 hybrid had a higher dry matter percentage (26.27±0.66%) than CEBU (23.56±0.82%). No significant differences ($F_{2,\ 0.05}$=0.15, P=0.85) were detected through the analysis of sowing densities; however, the density of 62,500 plants ha^{-1} recorded the highest average value (25.09±1.04%).

Figure 1 shows that in the combined analysis of hybrids and densities there were not significant interaction effects ($F_{5,\ 0.05}$=3.27, $P>0.05$). Values ranged from 22.13% to

Table 3. Nutritional analysis of corn forage regarding the hybrid and plant density per hectare factors.

Main factor	**Nutritional analysis**			
Hybrid	**DM (%)**	**CP (%)**	**TAC (%)**	**CARBS**
CEBU	23.56±0.82a	9.39±1.02a	22.41±0.27a	19.27±1.74a
A 7573	26.27±0.66b	8.59±0.69a	21.48±1.66a	22.50±0.89b
Density				
50,000	25.24±1.56a	8.93±1.11a	21.28±1.87a	19.33±0.70a
62,500	25.09±1.04a	7.82±1.09a	23.22±1.52a	23.46±1.10a
83,333	24.41±0.41a	10.22±0.87a	21.33±2.06a	19.86±2.65a

Dry matter (DM); crude protein (CP); total ash content (TAC); and total carbohydrates (CARBS). Different letters in the same column indicate statistically significant differences, according to Tukey's HSD test ($P<0.05$).

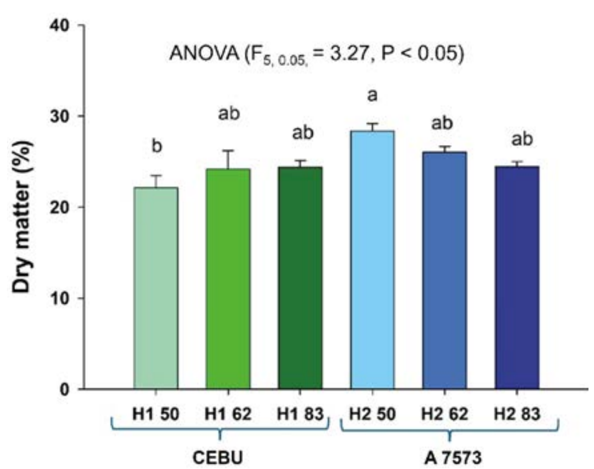

Figure 1. Dry matter content comparison between two forage corn hybrids. Letters on top of the bars indicate statistically significant differences, according to Tukey's test ($P<0.05$).

28.34%; the highest values were obtained with A7573 at a density of 50,000 plants ha^{-1}. Significant differences between hybrids were observed only with respect to density, with A7573 exhibiting a higher dry matter content than CEBU.

The results of this study are lower than those reported by Zaragoza-Esparza *et al.* (2019), who recorded 31.5% and 35.2% DM for the PUMA 1165 and 1185 hybrids, respectively; both hybrids were grown under irrigation conditions and at a density of 70,000 plants ha^{-1}. Similarly, Gutiérrez-Guzmán *et al.* (2022) obtained 22.42% DM in forage corn, under subsurface drip irrigation (SDI), at a 53 cm irrigation depth and a density of 100,000 plants ha^{-1}. Likewise, Rivas *et al.* (2018) reported 26.71% DM with the single-cross HT-6 forage hybrid, sown at a density of 90,000 plants ha^{-1}. The dry matter in this study ranged from 22.13% to 28.34%, indicating a lower dry biomass accumulation compared to the highest values reported in the literature. Nevertheless, these results are consistent with those obtained under similar management conditions and high sowing densities, in which an increased competition between plants can limit the accumulation of dry matter in plant tissue.

Crude protein

Table 3 shows that the analysis of the crude protein (CP%) content at the end of the crop cycle did not show any significant differences between both hybrids ($F_{1, 0.05}$=0.55, P=0.47>0.05) and between sowing densities ($F_{2, 0.05}$=1.35, P=0.29). The combined analysis of hybrids and densities also showed no significant interaction effects. Values ranged from 6.53% to 10.83%; the latter was recorded by CEBU at a density of 50,000 plants ha^{-1} (Figure 2). The maximum crude protein value recorded in this study fell within the range reported by García-Chávez *et al.* (2022), who documented 4.4% and 14.9% contents, under various management conditions.

Meanwhile, Gutiérrez-Guzmán *et al.* (2022) recorded a 10.36% CP with the Syngenta 8285 hybrid, sown at a density of 100,000 plants ha^{-1} and with a subsurface irrigation system at a 93.5 cm depth. Similarly, Zaragoza-Esparza *et al.* (2019) reported CP values from 8.0 to 9.0% in the PUMA 1167 and BUHO hybrids, grown under irrigation conditions

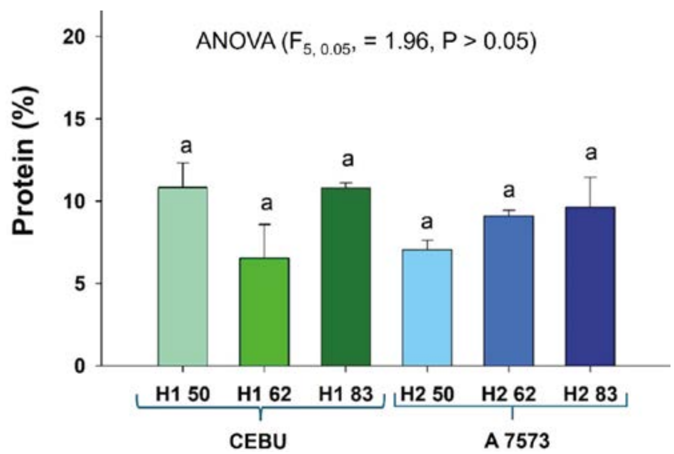

Figure 2. Crude protein content of two corn forage hybrids at three sowing densities. Mean values in the bars do not have significant differences, according to Tukey's test (P<0.05).

and at a density of 70,000 plants ha^{-1}. Finally, Ramírez *et al.* (2024) recorded an average of 8.0% CP in four corn hybrids sown at a density of 93,211 plants ha^{-1}, under irrigation conditions, harvested at 121 DAS.

Within this context, the highest value of this study (10.83%) falls within the upper range of previously reported data, suggesting a favorable performance of the genetic material or the management conditions. This result could be associated with such factors as sowing density, harvest time, or the water regime, which, according to previous studies, have a direct influence on the accumulation of crude protein.

Total Ash Content

Table 3 shows that, at the end of the crop cycle, the evaluated hybrids did not exhibit significant differences ($F_{1,\ 0.05}$=0.25; P=0.62) in total ash content (%). Likewise, Table 3 and Figure 3 show no significant differences between sowing densities ($F_{2,\ 0.05}$=0.36; P=0.70>0.05) and the hybrid × density interaction ($F_{5,\ 0.05}$=1.81; P>0.05), respectively. Values fluctuated between 18.55% and 26.30%. The latter value was recorded by A7573, at a density of 62,500 plants ha^{-1}.

These results are higher than the total ash content reported by Ramírez *et al.* (2024) and Solís and Castaño (2022), who registered 6.6% and 13.29%, respectively. Discrepancies in the ash content could be attributed to differences in the phenological stage of the harvest (135 days after emergence), post-silage processes (60-day fermentation), and nitrogen fertilization levels (180 kg N ha^{-1}). These factors can modify the mineral concentration of the plant tissue and consequently its ash content.

Total Carbohydrates

Table 3 shows that the total carbohydrate content (mg g^{-1} DM) at the end of the crop cycle displayed significant differences between the evaluated hybrids ($F_{1,\ 0.05}$=44.91, P<0.01). A7573 had a higher total carbohydrate content (22.50±0.89 mg g^{-1} DM on average) than CEBU (19.27±1.74 mg g^{-1} DM).

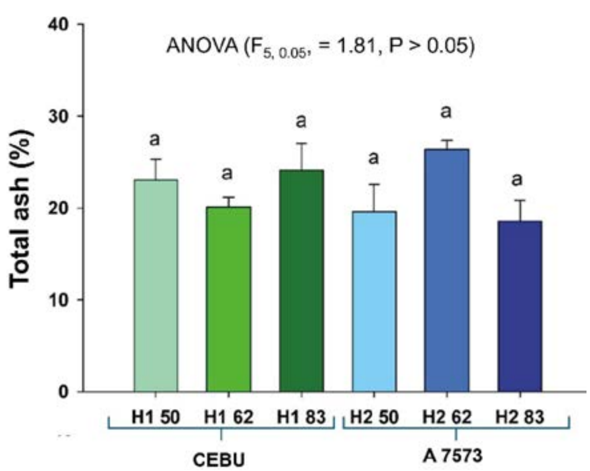

Figure 3. Total ash content (%) in the dry matter (DM) of whole plants from two corn hybrids, harvested at 91 days after emergence. Mean values in the bars do not have significant differences, according to Tukey's test (P<0.05).

Table 3 shows no significant differences ($F_{2, 0.05}=1.72$, $P=0.21>0.05$) for the effect of sowing densities. Nevertheless, the highest value (23.46 ± 1.10 mg g^{-1} DM) was recorded at the density of 62,500 plants ha^{-1}. Figure 4 shows statistically significant differences ($F_{5, 0.05}=59.54$, $P<0.05$) in the hybrid × density interaction, indicating a differential response of hybrids to population density variations. CEBU was statistically different from the other treatments and recorded the lowest carbohydrate content at the density of 83,333 plants ha^{-1}.

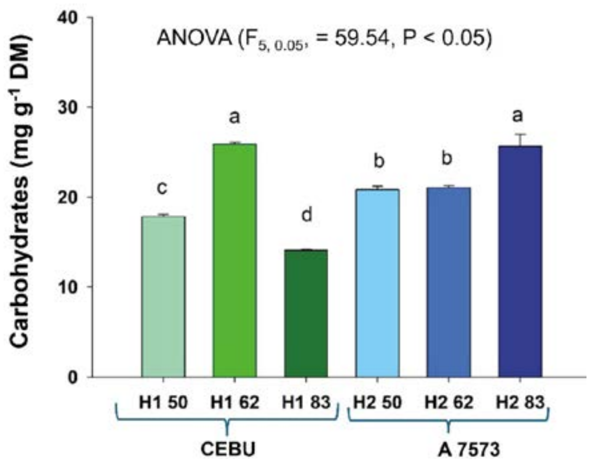

Figure 4. Total carbohydrate content based on hybrid and corn sowing density (%). Letters on top of the bars indicate statistically significant differences, based on Tukey's test ($P<0.05$).

The carbohydrate content values ranged from 14.07 to 25.88 mg g^{-1} DM. The highest value was recorded by CEBU, sown at a density of 62,500 plants ha^{-1}. In the case of A7573, the highest carbohydrate content was reported at a 83,333 plants ha^{-1} density (Figure 4). These results differ from the 87.0 mg g^{-1} DM total carbohydrate content reported by Singh *et al.* (2024). This difference could be attributed to the diverse sampling conditions, as they harvested 135 days after emergence and the matter was subsequently subjected to a silage process. The sample was collected 60 days later. Variations in both post-harvest and sampling management could have significantly influenced carbohydrate levels.

CONCLUSIONS

The results of this research show significant differences between the corn hybrids evaluated, both in their agricultural performance and their nutritional value. The CEBU hybrid had a higher yield in both fresh and dry matter across all sowing densities. According to the nutritional analysis, the CEBU hybrid had a high crude protein and total ash content. However, the A7573 hybrid had the highest total carbohydrate values. The analysis realized in this study allowed to describe and compare the bromatological composition of these two corns (*Zea mays* L.) hybrids, thus meeting the objective of the research. Consequently, it is possible to suggest to regional producers the use of the CEBU hybrid for forage production, with fertigation during the autumn-winter agricultural cycle.

Producers should sow corn at the end of June to prevent a reduction in yield as a result of weather conditions. This recommendation would allow producers to have high-quality and nutritious forage for their cattle during the dry season.

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Colegio de Postgraduados

Morphological characterization and genetic evaluation using RAPD molecular markers of *Agave maximiliana* Baker

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ABSTRACT
Objective: To study perform morphological characterization and use RAPD molecular markers in individuals of the species *Agave maximiliana* to detect its genetic variation.
Design/methodology/approach: This study was conducted with 40 samples from individuals collected in four locations in the municipalities of Mixtlán and Mascota, Jalisco. Morphological characterization of the selected individuals was performed by measuring seven foliar variables. Genetic characterization was performed using the RAPD technique, using primers OPA02 and OPA03 to obtain amplicon patterns. Cluster analysis, AMOVA, PERMANOVA, and Mantel analysis were performed based on the data obtained.
Results: The first three principal components explain 99.64% of the observed variability. Additionally, molecular variables show greater genetic variation within populations (96%) than between populations (4%). These values yield a p-value of 0.2717 and a PhiPT of 0.052, indicating a moderate level of genetic differentiation among the studied populations. The PERMANOVA showed a p-value of 0.0793, and the Mantel test resulted in a p-value of 0.3305.
Limitations on study/implications: The samples of *A. maximiliana* correspond only to an area called the "mountainous raicilla zone" in Jalisco, Mexico, and the results of this study are exclusive to that region.
Findings/conclusions: The analysis of morphological diversity showed no significance among the sampling populations and the individuals were not separated into groups defined by geographical origin. Furthermore, molecular analysis showed that there is more variability within local populations than among sampled populations.

Keywords: *Agave* sp., raicilla, morphological characterization, population variability, RAPD.

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INTRODUCTION

Agaves comprise more than 200 species worldwide, and Mexico has approximately three-quarters of them; some are endemic, although their distribution throughout the country is uneven (Delgado-Lemus *et al.*, 2014; López-Pujol *et al.*, 2016; Eguiarte *et al.*,

2021). They are used as raw materials in the production of various industrialized products. Mexico is the source of agave species and also a center of domestication. It is believed that their historical domestication began 9,000 years ago, and they were used as food, beverages, clothing, and other uses (Arellanes *et al.*, 1997; Bellon *et al.*, 2009; Trejo *et al.*, 2018; Pérez-Zavala *et al.*, 2020). Currently, 53 species of agave have been registered that are used for the production of distilled beverages (Torres *et al.*, 2015), to mention such as *A. tequilana* Weber, *A. angustifolia* Haw., *A. karwinski* Zucc., *A. marmorata* Roezl, *A. potatorum* Zucc., *A. americana* L. var. *oaxacensis* Gentry, *A. cupreata* Trel. and Berger, *A. rhodacantha* Trel., *A. salmiana* Otto ssp. *crassispina* (Trel.) Gentry, *A. wocomahi* Gentry, *A. durangensis* Gentry and *A. maximiliana* Baker (García-Mendoza, 1998; Colunga-GarcíaMarín *et al.*, 2017).

Among the most profitable agave alcoholic products are distilled spirits such as tequila, mezcal, bacanora, and raicilla (Carrillo-Trueba, 2007; Lucio-López, 2022). In the case of raicilla, it is mainly consumed in Mexico and, to an incipient extent, it is exported to the USA. It is produced in a specific region of Mexico using mainly the species *A. maximiliana* and *A. angustifolia* and to a lesser extent the species *A. inaequidens*, *A. rhodacantha*, and *A. valenciana*. This region is defined by the municipalities of Mascota, Mixtlán, Cabo Corrientes, and Talpa de Allende in the state of Jalisco and the municipality of Bahía de Banderas in the state of Nayarit (IIEG, 2024a; IIEG, 2024b).

Distilled beverages are made from adult individuals before they complete flowering (Zizumbo-Villarreal *et al.*, 2013), which impairs the reproduction of this species, reduces its populations and reduces genetic variability (Aguirre & Eguiarte, 2013). This leads to a loss of diversity, limiting its adaptation to environmental changes and increasing its risk of extinction (Bourguiba *et al.*, 2012). In these cases, it is important to conduct genetic diversity studies, focusing on actions for the sustainable use of economically valuable species (Huang *et al.*, 2009; Zizumbo-Villarreal *et al.*, 2013). To achieve this, it is necessary to evaluate genetic variation using molecular techniques to distinguish plant types and their origins (Alfaro *et al.*, 2007; Rodríguez-Garay *et al.*, 2008; Torres-Morán *et al.*, 2010).

Agave maximiliana is found in the states of Sonora, Chihuahua, Durango, and Sinaloa, at an altitude of 930 to 1850 meters above sea level in pine and oak forests (Eggli & Nyffeler, 2020). It is a species whose stems with detached leaves ("pineapple", or the heart of the agave plant) are used in the production of the distilled raicilla, in the mountainous area of the state of Jalisco (Colunga-GarcíaMarín *et al.*, 2007; Vázquez *et al.*, 2007; Cabrera *et al.*, 2020); individuals present wide morphological variability. Furthermore, in the mountainous area of the raicilla region of Jalisco, the species *A. maximiliana* exhibits high morphological variability. Therefore, the presence of ecotypes (Cachúa-Torres *et al.*, 2025) was observed, which could indicate high genetic variability in this species. Therefore, the objective of this study was to perform morphological characterization and use RAPD molecular markers to establish variation among populations of *Agave maximiliana* in this region. Although it is assumed that for farmers this species is the same throughout the raicilla region, its morphological variability would be expected to correspond to its genetic variability measured with molecular techniques in *Agave maximiliana*.

MATERIALS AND METHODS

Plant Material

In situ, morphological measurements were made, and leaf samples were collected from 40 individuals identified by raicilla producers as the *Agave maximiliana* species. This work was carried out in four locations within two municipalities in the state of Jalisco: one location in Mixtlán and three in Mascota (Table 1). Collections of each site were considered for this study as separate populations. Sampling in the agricultural fields was entirely randomized based on the material each producer allowed us to collect, since most of the selected plants are grown for commercial purposes, thus minimizing the impact on owners' incomes. Although there is no physical distance that guarantees that the *A. maximiliana* used by farmers are not related, given that some pollinators (bats and insects) can travel great distances, the Mixtlán site was considered sufficiently distant in a straight line from the three Mascota sampling sites (33.6-42.2 km). With these distances, it was expected that the sampled specimens would not have any related kinship between site 1 and sites 2-4.

Morphological Characterization

Individuals were described, measuring seven morphological variables in centimeters: leaf length, leaf width (maximum), leaf thickness, terminal spine length, lateral spine length, and lateral spine width; the number of lateral spines was measured in the middle of the leaf (considering 10 cm of leaf length). These data were used to calculate principal components and scatter plots. Furthermore, a dendrogram was also created using Paired group algorithm (UPGMA) and the analyses were performed using the statistical software PAST 4.12. The variables were standardized to perform the principal components analysis (PCA). PCA loadings plot for principal components 1 and 2 were included as a visualization that shows how original variables contribute to the principal components (PCs) by plotting them as vectors. Also the PCA loading matrix is included in this work.

Genetic characterization

DNA extraction

Approximately 10 cm of the terminal part of the leaf adjacent to the bud was taken from the 40 plants. These samples were placed in a cooler, transported to the laboratory

Table 1. Locations, number of samples per location and type of population.

Municipality	Geographic coordinates	Number of samples	Population type (key)
Mixtlán	20° 33' 14.1" N 104° 28' 37.8" W	12	Cultivated (MXC)
Mascota I	20° 31' 16.9" N 104° 51' 08.3" W	13	Cultivated (MTC)
Mascota S	20° 31' 03.6" N 104° 51' 13.8" W	10	Wild (MTS)
Mascota II	20° 33' 05.4" N 104° 53' 06.7" W	5	Cultivated (MSC)

of the TecNM ITEL Llano Aguascalientes, and stored at −20 °C. DNA extraction was carried out using the method of Coelho *et al.*, (2009) with modifications. PVP was added to the extraction buffer, where it was added to the sample when it was ground with liquid nitrogen. The samples were incubated at 65 °C for 30 minutes, the sample was fractionated twice with phenol-chloroform-isoamyl alcohol mixture (25:24:1), and centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was recovered and combined with 700 μL of cold isopropanol and incubated at −20 °C for 30 minutes to precipitate the DNA. It was centrifuged at 10,000 rpm for 10 minutes and the resulting pellet was resuspended with 300 μL of TE buffer plus 150 μL of 5 M NaCl and 800 μL of absolute ethanol and incubated at −20 °C for 30 minutes. It was centrifuged again at 10,000 rpm for 10 minutes. The supernatant was discarded, the pellet was washed with 70% ethanol and centrifuged at 10,000 rpm for 5 minutes. The pellet was dried at room temperature and resuspended in 50 μL of TE buffer. This standardized DNA extraction method used by our work group has allowed us to obtain high-purity DNA in sufficient quantities for agaves and other plant species, as verified by PCR analysis. In this study the extracted DNAs were previously tested by positive amplification of a 610 bp fragment of the b-actin gene. This was used to validate that the PCR reaction was accurate and that there were no inhibitors in the sample that could affect the reaction.

The PCR conditions were: 1.5 μl of DNA resuspended in TE 1X buffer, 2.5 μl of primers (100 μM), 6.25 μl of PlatinumTM PCR SuperMix High Fidelity (Invitrogen), and 14.75 μl of water were mixed in each PCR tube. For PCR, a Techne TC-512 gradient thermocycler was used, with a program consisting of an initial heating at 94 °C for 5 minutes and 40 cycles, as follows: denaturation (1 min, 94 °C), annealing (1 min, 51 °C) and extension (3 min, 72 °C). The final extension was 10 min at 72 °C.

RAPD Molecular Marker Analysis

Primers 1 to 20 of the OPA series (Operon Technologies, Alameda, CA, USA) were tested. Primers OPA02 and OPA03 were selected because they presented more bands and greater polymorphism in a previous analysis. The molecular weight of the bands was determined using GelAnalyzer 19.1 software (Lazar and Lazar, 2023) using the molecular weight marker (GeneRuler 1 kb DNA Ladder, SM0311, ThermoFisher) as a reference, which was included in the gels. The amplification products were visualized on 1.2% agarose gels in 1X TBE buffer at 60 Volts for two hours and stained with ethidium bromide for 15 minutes.

Statistical analysis

For morphological characters measurements, mean and standard deviation was calculated. ANOVA was also performed on these measurements. Means were compared whenever significance was observed ($p<0.05$) with Tukey's test using Minitab 17®.

For banding patterns from RAPDs, the presence (1) or absence (0) of each band was recorded visually, captured in a binomial data matrix in Excel, and analyzed using the Paired Group Algorithm (UPGMA) to construct the dendrogram with the statistical program PAST 4.12 (Hammer *et al.*, 2001) and the AMOVA analysis was performed with GenAlEx 6.503 (Peakall & Smouse, 2012). In addition, a Principal Coordinate Analysis (PCoA), PERMANOVA, and the Mantell test were performed comparing the four populations, also using PAST 4.12 software. The distances between sampling sites in kilometers were determined in a straight line using Google Maps software, using the geographic coordinates of each site as a reference point.

RESULTS AND DISCUSSION

Morphological Analysis

Most of the characteristics measured in the sampled specimens had means that were statistically similar, with the exception of leaf thickness and leaf length, which presented $p<0.05$ (Table 2). Tukey's test on leaf thickness showed two groups (Table 3). However, the Tukey test for leaf length showed no differences, generating a single group ($\alpha=0.05$).

Principal component (PC) analysis performed on the seven variables showed that the first three components: PC1, PC2 and PC3, presented eigenvalues greater than 1.0, which together explained 99.64% of the variance in the original data (Table 4). More detail of the most influential PC is shown on the loadings matrix (Table 5). The study revealed noticeable phenotypic diversity among morphological variants of A. maximiliana, collected in plots and wild areas in the mountainous area of the state of Jalisco, Mexico. Other studies confirm that there are individuals of *A. maximiliana* in the mountainous raicilla zone showing wide morphological variations in their "pineapple" sizes, leaves, and spines. These cases were considered "ecotypes" that produce different contents of reducing sugars, ranging from 32% to 83% (Cachúa-Torres *et al.*, 2025).

Table 2. Counts and measurements in cm (mean and standard deviation) per site and observed significance (*p*) for each measured variable.

Variable	MXC	MTC	MTS	MSC	p
Lateral spines/10 cm	9.33±6.08	7.00±3.58	7.20±4.21	13.40±9.18	0.1345
Lateral spine length	0.63±0.26	0.75±0.46	0.86±0.3	0.92±0.37	0.3796
Lateral spine width	1.12±0.59	1.03±0.47	0.87±0.21	0.68±0.19	0.2457
Leaf thickness	2.04±0.65	2.23±0.63	1.45±0.36	1.90±0.54	0.0216
Leaf length	74.58±20.74	83.84±22.33	84.00±15.60	56.80±9.53	0.0491
Leaf width	15.83±4.21	19.76±5.91	17.80±4.70	16.20±1.48	0.2105
Terminal spine length	3.08±1.04	3.84±1.40	2.80±0.53	2.80±0.27	0.0765

Table 3. Tukey's comparisons for leaf thickness.

Factor	N	Mean	Grouping
MTC	13	2.231	a
MXC	12	2.042	ab
MSC	5	1.900	ab
MTS	10	1.450	b

Means that do not share a letter are significantly different.

Table 4. Principal components (PC) of seven morphological variables of *A. maximiliana* from the root zone.

Principal component	Eigenvalue	% Variance
1	433.256	90.345
2	28.007	5.8402
3	16.5709	3.4555
4	1.13957	0.23763
5	0.359115	0.074884
6	0.161083	0.03359
7	0.0656927	0.013699

Table 5. Matrix loadings for principal components of morphological features of *A. maximiliana*.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
Spines in 10 cm	−0.1	0.97	0.2	0.02	0.01	0.03	−0.01
Terminal spine length	0	−0.02	0.01	0.03	0.09	0.68	0.72
Lateral spine width	0.01	−0.03	0.01	0.11	0.25	0.68	−0.68
Leaf thickness	−0.01	−0.01	0.02	0.22	0.93	−0.26	0.11
Leaf length	0.99	0.12	−0.11	−0.01	0.01	0	0
Leaf width	0.13	−0.19	0.97	−0.02	−0.02	−0.02	0

Bartlett's test is to verify that different groups within a dataset exhibit similar levels of variability. The test for determinant matrix was very close to zero (1.49E-16), suggesting that the correlation matrices are not singular and that there are significant correlations between the variables (Table 6).

Furthermore, the p-value indicates a statistically significant difference from the null hypothesis that the correlation matrix is an identity matrix. Therefore, the null hypothesis is rejected, and it is concluded that the data are sufficiently correlated to proceed with multivariate analyses

The values obtained in our work are higher than those reported in wild populations of *Agave angustifolia* from Sonora, Mexico, which with the same number of PCs obtained 51.9% of the variance (Fragoso *et al.*, 2021). Morphological differences were observed, between and within plots and wild areas, in the characteristics of the measured agaves, the variables with the greatest weight were: leaf length in cm (PC1) and lateral spines in 10 cm (PC2) (Figure 1). Similarly, García-Núñez *et al.* (2020) also obtained 74% morphological

Table 6. Bartletts's sphericity test.

Determinant	1.49E-16
Chi^2	1364.8
df	27
p (spherical)	6.91E-271

Figure 1. Lateral spines of *A. maximiliana* leaves collected in the municipality of Mixtlán.

variability using only these two variables, which allowed them to more accurately distinguish the species and variants of "pulquero" agave in the states of Hidalgo and Mexico.

On the dispersion graph (scatter plot), the first two principal components were taken; in the results obtained, this graph did not show defined groups according to the collection site (Figure 2). These results differ from those reported by Esparza-Ibarra *et al.* (2015), this is because they worked with different species of "mezcal" agaves in the Potosino and Zacatecas plateau; in our work, only one of these species was considered.

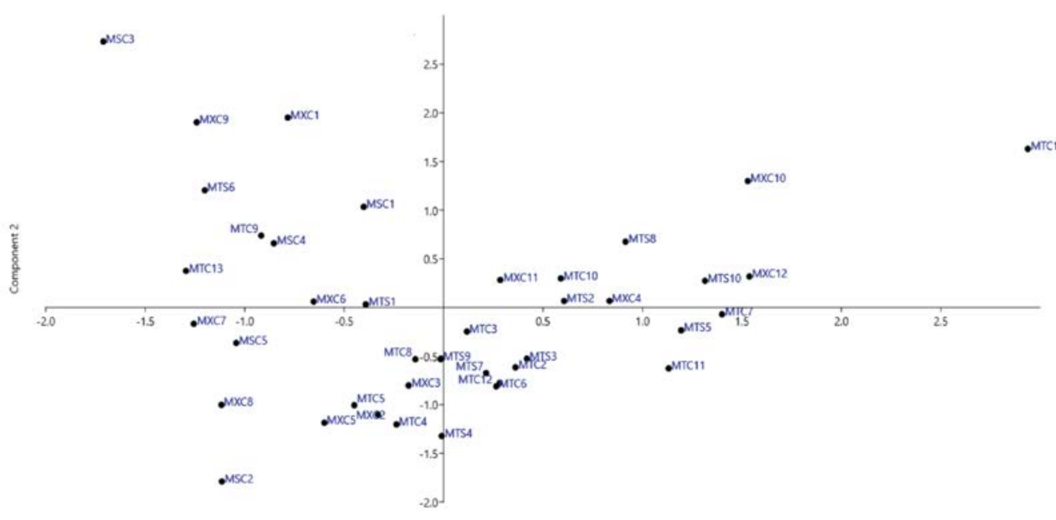

Figure 2. Scatter plot from the first two PCs in the analysis of seven variables measured in *A. maximiliana*, both wild and cultivated. Tags: MXC=Mixtlán cultivated, MTC=Mascota cultivated I, MTS=Mascota wild plants, MSC=Mascota cultivated II. The number at the end of the tag indicates the sample number. The units of the axes are indicated in eigenvalues.

In the cluster analysis (dendrogram) two defined groups were found that were composed of samples from more than two of the four sites (Figure 3). Sample MTC1 appeared separated because it was from the individual with the largest leaves, this variation can be explained according to the comments from more experienced producers, that agaves tend to cross with other agaves, and in this particular case, greater robustness was obtained in the plant.

Genetic Analysis

Of the 20 primers tested in our lab, the majority generated amplified products in all gels. The primers that showed the greatest band amplification were OPA02 (Figure 4), OPA03, OPA04, OPA09, and OPA10. We focused on analyzing the samples based on the amplifications obtained with primers OPA02 and OPA03. Primer OPA2 generated 17 bands ranging from 260 to 2350 bp, 10 of which were considered polymorphic (59%). Primer OPA3 generated 13 bands ranging from 370 to 2400 bp, 10 of which were considered polymorphic (77%). The polymorphic information content (PIC) were estimated as ranges: OPA2 PIC 0.095-0.499 and OPA3 PIC 0.049-0.480. These values ranged from moderately informative (0.25-0.5) to less informative (<0.25).

Alfaro *et al.* (2007) also used the same 20 OPA primers, and this study also agreed on the efficiency of using primer OPA02 instead of OPA20. They evaluated genetic diversity between and within populations in 10-year-old agaves, representative of variants of *Agave salmiana* and *A. mapisaga* from the northeast of the state of Mexico, using molecular markers and morphological characteristics, to obtain basic information for the development of conservation and genetic improvement strategies.

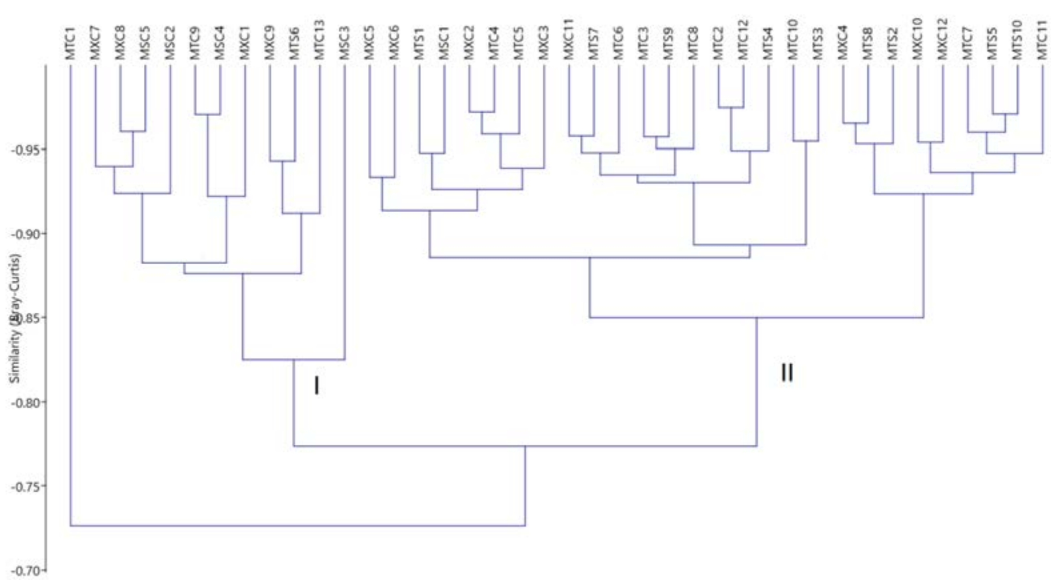

Figure 3. Dendrogram generated from the analysis of seven morphological variables measured in A. maximiliana. Dendrogram constructed using the Paired group algorithm (UPGMA) with the Bray-Curtis similarity index. Cophenetic Correlation Coefficient=0.742. Labels: MXC=Mixtlán, MTC=Mascota I, MTS=Mascota wild plants, MSC=Mascota II. The number at the end of the labels indicates the sample number.

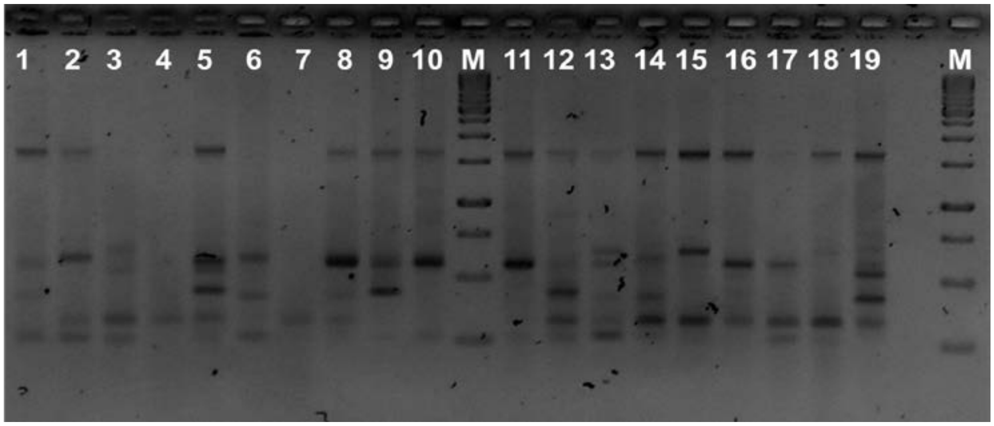

Figure 4. Amplicons were obtained from PCR using the OPA02 primer in individuals of *A. maximiliana* collected in the municipalities of Mixtlán (1 to 12) and Mascota (13 to 19). M indicates the molecular marker.

In the dendrogram, two main groups were formed (Figure 5). Genotypes from all of the four sampling sites were intermingled; despite being geographically distant, the genetic analysis indicates that they are genetically similar individuals. This is because part of the materials are shared between both locations, being the origin the wild areas and nurseries of institutions and among the same producers. For their part, Martínez-Velasco & Arzate-Fernández (2022) in their research using the RAPD primers (Y24 and Y4) and the ASSR (ASSR 20 and ASSR 29) obtained the necessary products to estimate the genetic integrity of *A. salmiana* and *A. marmorata* seedlings regenerated in vitro from leaf tissue of seedlings.

The PCoA scatter plot shows the first two principal coordinates as the x and y axes, showing a visual representation of how similar or dissimilar the samples are (Figure 6). As is evident in that figure, samples of the four sampling sites are dispersed and mixed without

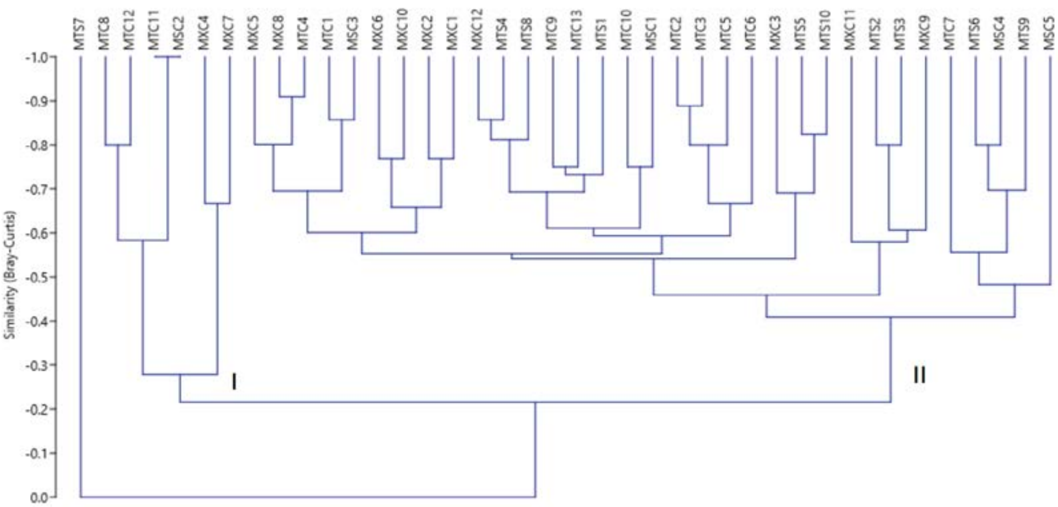

Figure 5. Dendrogram generated from the analysis of primers OPA02 and OPA03 in *A. maximiliana*. Dendrogram constructed using the Paired group algorithm (UPGMA) with the Bray-Curtis similarity index. Cophenetic Correlation Coefficient = 0.819. Labels: MXC=Mixtlán, MTC=Mascota I, MTS=Mascota wild, MSC=Mascota II. The number at the end of each label is the sample number.

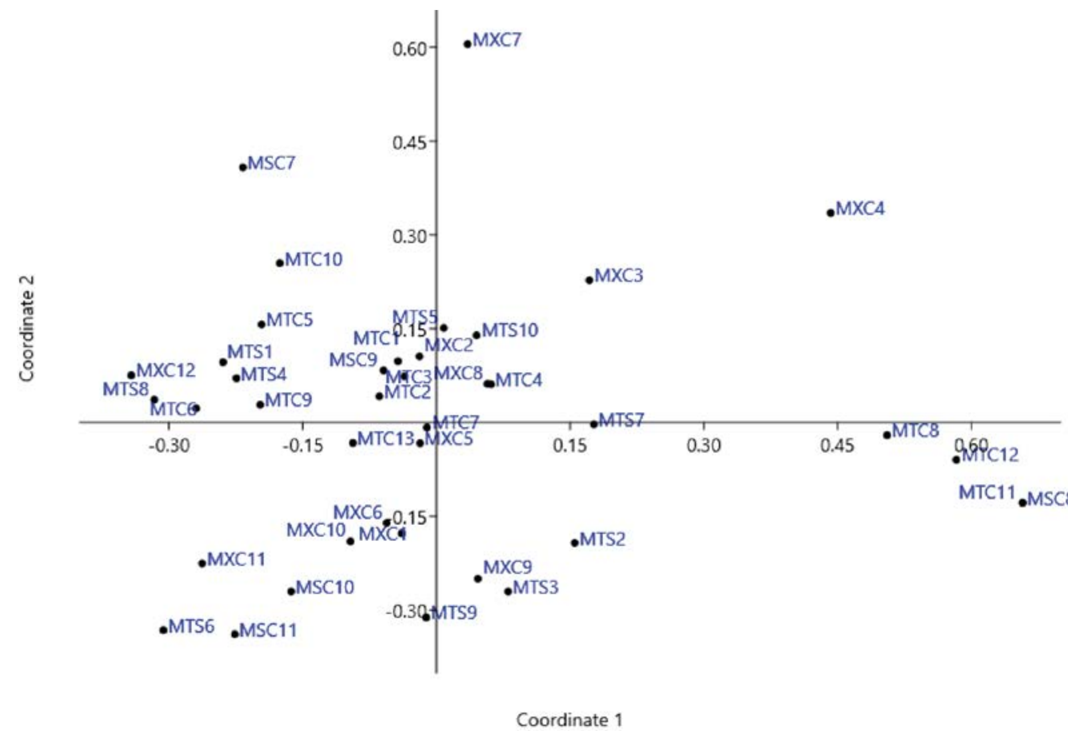

Figure 6. PCoA scatter plot from binary data obtained from RAPD amplicons of *A. maximiliana* using primers OPA02 and OPA03.

generating clear group conglomerates and showing that in general samples are genetically variable similar among genotypes even though these came from different sites. Values for the PCoA (Eigenvalues and Percent) per axis are shown on Table 7.

The analysis of molecular variance (AMOVA with 999 permutations) was performed considering the four populations according to the site of collection. The highest percentage of variation was detected within populations defined by the sampling site (96%), in contrast to the variation between populations (4%) (Table 8), p=0.2717 with a PhiPT=0.035 (PhiPT=AP/(WP+AP)=AP/TOT; AP=Est. var. among pops, WP=Est. var. within pops) (Figure 5). This PhiPT of 0.035 is a very low value, indicating little genetic differentiation among the populations being compared. Therefore, the genetic variation is mostly found within the populations rather than among them. Should be noticed that unbalanced sample sizes across populations may have induced bias by causing a systematic overestimation or underestimation of population parameters, making it difficult to draw accurate conclusions for all groups. Larger groups may bias the results, while smaller groups are more likely to have their characteristics measured less accurately and their specific outcomes underrepresented.

Considering that the traditional propagation system of the species is through seeds, this value reflects the variability that exists within each population and its degree of isolation. This result is very similar to the results obtained for *A. potatorum*, of 90% and 80% for *A. cupreata* among wild populations, reported by Aguirre & Eguiarte (2013). Félix-Valdez *et al*. (2016) also obtained lower values for *A. potatorum*, of 61%, due to the extraction of

Table 7. Table of values for the PCoA (Bray-Curtis similarity index).

Axis	Eigenvalue	Percent
1	23.05	18.32
2	18.66	14.82
3	13.62	10.82
4	12.2	9.69
5	10.62	8.44
6	9.09	7.22
7	7.17	5.7
8	6.81	5.41
9	5.21	4.14
10	4.25	3.38
11	4.23	3.36
12	2.76	2.19
13	2.29	1.82
14	1.69	1.34
15	1.23	0.98
16	0.94	0.75
17	0.78	0.62
18	0.6	0.47
19	0.43	0.34
20	0.25	0.20

Table 8. AMOVA for the four populations of *A. maximiliana*.

Source	df	SS	MS	Est. Var.	%
Among Pops	3	12.781	4.260	0.116	4%
Within Pops	36	113.094	3.141	3.141	96%
Total	39	125.875		3.257	100%

individuals for mezcal production. Finally, it is worth mentioning that Cabrera-Toledo *et al*. (2020) in their study of populations of *A. maximiliana* collected in municipalities of Jalisco where raicilla is produced, in the states of Zacatecas, Nayarit, Sinaloa, and Durango, in their results they obtained little diversity between and within the morphological variables and in the AMOVA analysis 61% within populations and 38% between populations.

The PERMANOVA analysis across groups showed an overall p-value of 0.0793 (Table 9). When comparing sampling sites, only a significant p-value ($p=0.0403$) was found between the MXC and MTS data, which included cultivated and wild genotypes, respectively (Table 10). The Mantel Test showed an $R=0.5122$ value that indicates a moderate positive correlation between the two matrices being compared (genotype group *vs*. geographical distance among groups). Finally, a $p=0.3305$ for the Mantel test indicates that there is no statistically significant correlation between the two distance matrices previously mentioned (Table 11).

Table 9. PERMANOVA for the four populations of *A. maximiliana*.

Permutation N	9999
Total sum of squares	125.9
Within-group sum of squares	113.1
F	1.356
p (same)	0.0793

Table 10. PERMANOVA p-values matrix for the four sampling sites of *A. maximiliana*.

	MXC	MTC	MTS	MSC
MXC		0.0548	0.0403	0.4958
MTC	0.0548		0.2636	0.2081
MTS	0.0403	0.2636		0.5065
MSC	0.4958	0.2081	0.5065	

Table 11. Mantel Test for the four populations of *A. maximiliana*.

Permutation	N=9999
Correlation	R=0.5122
p (uncorr; onetailed):	p=0.3305

CONCLUSIONS

Briefly, it was found that both the morphological analysis and the RAPD analysis show that there is more variability among individuals within the same group than between groups.

More in detail, comparison of morphological traits showed that almost all of these variables were statistically identical, with the exception of lateral spine width; however, only two locations differed. Furthermore, it was found that all genotypes were intermixed when placed on a two-dimensional principal component plane, with PC1 and PC2 contributing the most to the characteristics of leaf length and lateral spines present in 10 cm.

According to the morphological study, a 99.64% variability index observed in *A. maximiliana* in the raicilla region was explained solely by three principal components obtained from foliage measurements such as leaf length, lateral spines/10 cm and leaf width, that are related to the plant's overall appearance. Bartlett's analysis showed that the matrices used were not singular and that multivariate analysis of the data could proceed without problems.

The dendrogram on morphological characteristics only formed two groups, which included mixtures of genotypes from all sampling sites, indicating that relative similarity was found between most of the genotypes.

The molecular results showed that the RAPD primers OPA02 and OPA03 used in this work were effective in differentiating the studied individuals, as observed in *A. maximiliana* from the state of Jalisco. When analyzing RAPDs in clusters as a dendrogram, two groups were found, one of which included genotypes from all four sites. Furthermore, the PCoA scatter plot showed the samples to be dispersed, indicating dissimilarity without generating clear clusters. AMOVA analysis showed little genetic differentiation between the compared populations ($p=0.2717$, $PhiPT=0.035$). The AMOVA analysis of the RAPD data revealed that the greatest diversity was found among individuals within the populations of the selected localities, but only slight diversity was found among populations. PERMANOVA showed a non-significant p-value ($p=0.0793$), and the Mantel test showed an $R=0.5122$ indicating a moderate positive relationship between the distance matrices and genetics.

Finally, it can be concluded that the variability from both the cultivated and wild genotypes collected in the raicilla region are relatively homogeneous among sites.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effects of conservation agriculture and bioinoculation on the microbiota of a soil cultivated with maize

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ABSTRACT

Objective: To evaluate the influence of conservation agriculture practices and bioinoculation on the soil microbiota in maize cultivation systems from the Frailesca region, Chiapas, Mexico, using 16S rRNA gene amplicon sequencing.

Design/methodology/approach: Microbial diversity was characterized in soils cultivated with maize white grain (*Zea mays* L.) under the influence of conservation agriculture (CA) and seed bioinoculation. Two experimental fields (A and B) were selected, each divided into two plots (P). Field A contained plot P1 (control) and P2 (CA management), while field B contained plots P3 (seed bioinoculation) and P4 (CA + bioinoculation). Soil samples were collected four months into the crop cycle to characterize microbial communities using 16S rRNA gene amplicon metabarcoding.

Results: The results showed changes in the microbial composition of the soil. The beta diversity analysis (PCoA-Bray-Curtis) showed differences in the microbial communities between the plots, where a separation of P4 (CA + bioinoculation) from P1, P2 and P3 was observed. The soil microbiota was dominated by the genera *Candidatus Koribacter* (P3>P4>P2>P1), *Gemmatimonas* (P4>P1>P2>P3) and *Candidatus Solibacter* (P4>P1>P3>P2), which are characteristic of the soil, with the ability to metabolize a wide range of simple and complex carbohydrates.

Limitations on study/implications: This study was conducted in a specific region and agricultural cycle; future research could consider seasonal variations and other crops to broaden our understanding of these microbial patterns.

Findings/conclusions: These results suggest that the combination of conservation agriculture and bioinoculation can promote specialized microbial communities, contributing to the maintenance of relevant ecological functions in agricultural soils.

Keywords: Biofertilizer; microbial diversity; regenerative agriculture.

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INTRODUCTION

A vast area of the Mexican state of Chiapas is used for the cultivation of corn (56% of the planted area). In the Frailesca region, 16% of the total production of Chiapas is reported,

while the municipality of Villaflores contributes with 40% of the region's production (SIAP, 2023). However, the intensive use of agrochemicals (particularly nitrochemicals) for the cultivation of corn has caused a significant loss of soil fertility. This has led to a growing interest in agroecological methods, such as conservation agriculture (CA) (minimal movement of the soil, retention of residues from the previous crop on the surface of the land, and rotation / diversification of crops) and the bioinoculation of corn seeds with Azospirillum and mycorrhizae (Rodríguez Larramendi *et al.*, 2017; Goddard *et al.*, 2022; Mutuku, Kassam and Mkomwa, 2020).

There is evidence that CA and bioinoculation are agroecological techniques that can improve crop yields, although little is known on their effect over soil microbial communities. While there are no studies regarding this specific subject in Chiapas, López Báez *et al.* (2019) evaluated the physical-chemical characteristics of soils cultivated with corn in Villaflores and concluded that it is necessary to increase the quality of these soils. To achieve this quality increase, it is first required to study the soil microbiota and to determine how it is affected by different agroecological techniques.

Soil microbes play important roles in biogeochemical cycles (Challacombre *et al.*, 2011; Emmerling *et al.*, 2002) such as the recycling of nutrients, the degradation of organic matter (Ye *et al.*, 2016) and xenobiotic compounds (Tejeda-Agredano *et al.*, 2013), carbon sequestration (Lian *et al.*, 2017), and prevention of diseases in crops (Tao *et al.*, 2015; Lim *et al.*, 2013). Moreover, agricultural practices influence soil microbial diversity, thus proper agricultural management could promote the settling of microbial communities that improve soil quality and increase plant productivity (Hartmann *et al.*, 2015; Liao *et al.*, 2019; Shrestha *et al.*, 2020).

The objective of this study was to evaluate the influence of conservation agriculture practices and bioinoculation on the soil microbiota in maize cultivation systems from the Frailesca region, Chiapas, Mexico, using 16S rRNA gene amplicon sequencing.

MATERIALS AND METHODS

Description of the study area and soil sampling

The experimental fields were established on Frailesca region at the southeast of the state of Chiapas, Mexico at the common land Las Graditas (MasAgro module), located in Ejido Calzada Larga, in the Villaflores municipality (16° 20' 30.46453" N and 93° 19' 4.02390" W) (Figure 1). The predominant soil is luvisol, with acidic conditions (pH 4.9-6.6).

Two experimental fields (A and B) were established and divided into plots (P), where white grain maize (*Zea mays* L., PIONEER4082) was grown under different conditions: Field A: P1, control; P2, conservation agriculture (CA). Field B: P3, seed bioinoculation; P4, CA + seed bioinoculation.

Inoculation consisted of applying biofertilizers based on Azospirillum and commercial arbuscular mycorrhizal fungi (Azofer® + Mycorrhizafer®) to the seeds prior to sowing following the manufacturer's instructions.

After four months of cultivation, soil samples were collected at a depth of 10-15 cm (the region closest to the plant roots) using a soil core sampler (5 cm in diameter). For each plot, soil samples were collected at five subsites, one at each corner and one in the center,

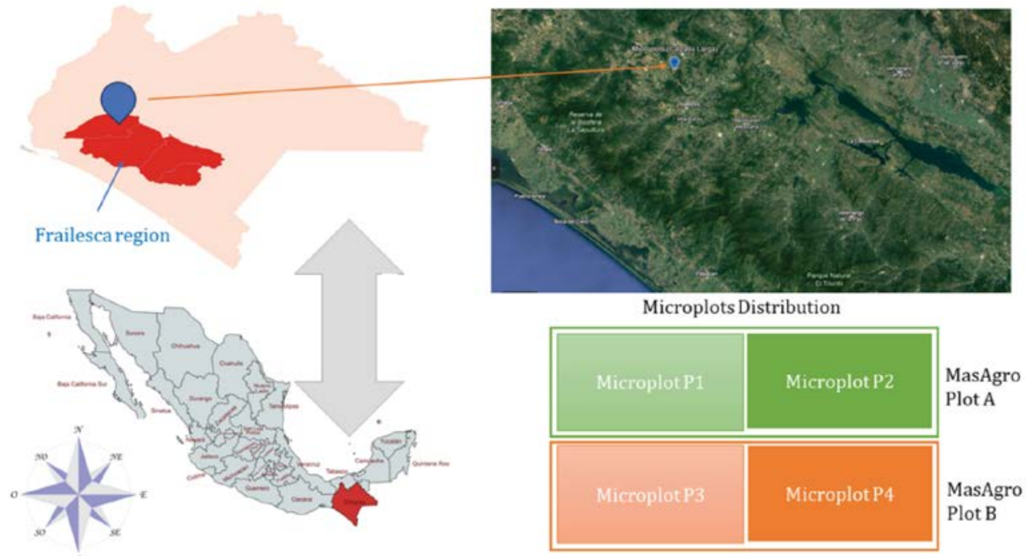

Figure 1. Location of the sampling area. Map prepared with picture Google Earth app.

to generate a composite sample for each plot (n=4). The samples were placed in sterile 50 ml tubes (Falcon®) and stored at 4 °C until processing at the Ecogenomics Laboratory (UNAM-Yucatán).

Characterization of microbial communities: 16S rRNA gene amplicon sequencing

To characterize microbial communities, total DNA was extracted using the methodology described by Rojas-Herrera *et al.* (2008) with some modifications, such as the addition of lysozyme without prior washing. The obtained DNA was used to amplify the V3-V4 regions of 16S rRNA gene using the primers S-D-Bact-0341-b-S-17 (5'-CCTACGGGNGGCWGCAG-3') and R: S-D-Bact-0785-a-A-21 (5´-GACTACHVGGGTATCTA ATCC-3´). The PCR conditions were carried out according to reported by Klindworth *et al.* (2013). The DNA quality was verified by agarose gel (1%) electrophoresis and quantified with a fluorometer (Quantus™ Promega). The PCR products were purified and paired-end sequenced in an Illumina MiSeq platform (2x250) (Illumina, San Diego, CA, USA) at CINVESTAV-Mérida.

16S rRNA gene sequence analyses

The bioinformatic analysis was performed in the MG-RAST platform. Low-quality sequences were removed to achieve a 99% accurate identification.

The diversity of each plot was analyzed in terms of alpha and beta diversity. Alpha diversity was assessed in terms of species richness (S) and the Margalef index, and diversity structure was evaluated using equity indices (Shannon-Wiener index (H) and Pielou index (J) and dominance indices (Simpson index (Λ), and Simpson-based diversity index (1-λ). Beta diversity was evaluated using the Bray-Curtis index.

Statistical analyses

The statistical and diversity analyses were carried out using the ecological statistics program PRIMER-e v7 (Plymouth Routines in Multivariate Ecological Research, version 7). The beta diversity analysis was performed using a principal coordinate analysis (PCO) and a CLUSTER hierarchical analysis (Group average and LINKTREE) (Marti, Gorley y Clarke 2008; Clarke, Somerfield y Gorley 2008). The SIMPROF (similarity profile, which consisted of 999 permutations at 95% confidence) and SIMPER (percentage of similarity and dissimilarity of one way, with only higher-contribution variables cut-off 70% at 95% confidence) analyses were conducted to assess the richness and abundance of each Operational Taxonomic Unit (OTU). Both statistical tests are based on Bray-Curtis dissimilarity metrics.

RESULTS AND DISCUSSION

Microbial diversity

A total of 207,329 16S rRNA gene reads were obtained after quality checks and were grouped in 2,004 OTUs: 481 in P1, 511 in P2, 471 in P3 and 541 in P4. The alpha diversity indicators revealed slight variations among the plots (P1-P4). Species richness (S) ranged from 194 to 227, with the highest value observed in P4, indicating a greater number of taxa in this plot (Table 1). The OTU distribution (N) was highest in P4 (11,021), suggesting a larger community size. The dominance indices (D and Λ) were relatively low (0.097-0.12), while the Simpson's diversity index ($1-\lambda$) remained high (0.87-0.90), reflecting a balanced community structure with no strong dominance of a few taxa. Shannon's index (H') showed similar values across treatments (3.06-3.20). The Pielou's evenness (J') ranged from 0.58 to 0.60, this index ranges from 0 to 1, with 0 representing an absence of evenness in abundances and 1 indicating that all species (in this case, genera) are equally abundant (Pumasupa Banda *et al.*, 2021), suggesting moderate evenness in species distribution. Considering all indices together, the combination of conservation agriculture and bioinoculation (P4) exhibited the highest richness and total abundance, along with the highest diversity, indicating a slightly more diverse and evenly distributed community (Table 1).

The remaining indices demonstrated a high degree of uniformity in the distribution of species within a community (Table 1). Some authors suggest that AC such as crop rotation and the utilization of biofertilizers influenced the microbial biomass, rather than the composition or richness of the communities (Song *et al.*, 2022). An equity index must

Table 1. Microplot diversity indices.

Microplots	S	N	D	J'	H'	Λ	$1-\lambda$
P1	194	8389	21.36	0.60	3.19	0.10	0.89
P2	217	7179	24.32	0.58	3.16	0.11	0.88
P3	194	7855	21.51	0.58	3.06	0.12	0.87
P4	227	11021	24.28	0.59	3.20	0.097	0.90

S: Total OTUs; N: Total reads; D: Margalef index; J': Pielou uniformity index; J':Shannon-Wiener (log) index; Λ: Simpson dominance index, $1-\lambda$: Simpson-based diversity index.

be independent of richness. The sensitivity of each index is of great importance in the evaluation of changes in the environment. Among the equity indices evaluated here, the most sensitive is Pielou (Barona-Narváez , 2021).

More than 50% of the dominance was attributed to the first four bacterial genera, including the unidentified genera, as illustrated in Figure 2. Additionally, the plot reveals that the representative genera of each microplot are comprised of the first 20 OTUs, which can be seen on the horizontal axis of Figure 2.

Beta diversity

By performing the principal coordinates analysis, the Beta diversity of the samples taken from the four microplots was characterized. The first important result is that two new variables or components (PCO1 and PCO2) were formed, which were able to explain 88.5% of the total variance. In this case, PCO1 made the largest contribution with 56.8% of the total variance (Figure 3). Beta diversity analysis showed a similarity of 74% between microplots, with P4 being the most distant (Figure 3).

Microbial communities composition

The dominant phyla were Acidobacteria, Actinobacteria, Proteobacteria, Verrucomicrobia, and Firmicutes, along with a significant proportion of unclassified bacteria that varied between microplots. Jeanbille *et al.* (2016) reported that Acidobacteria

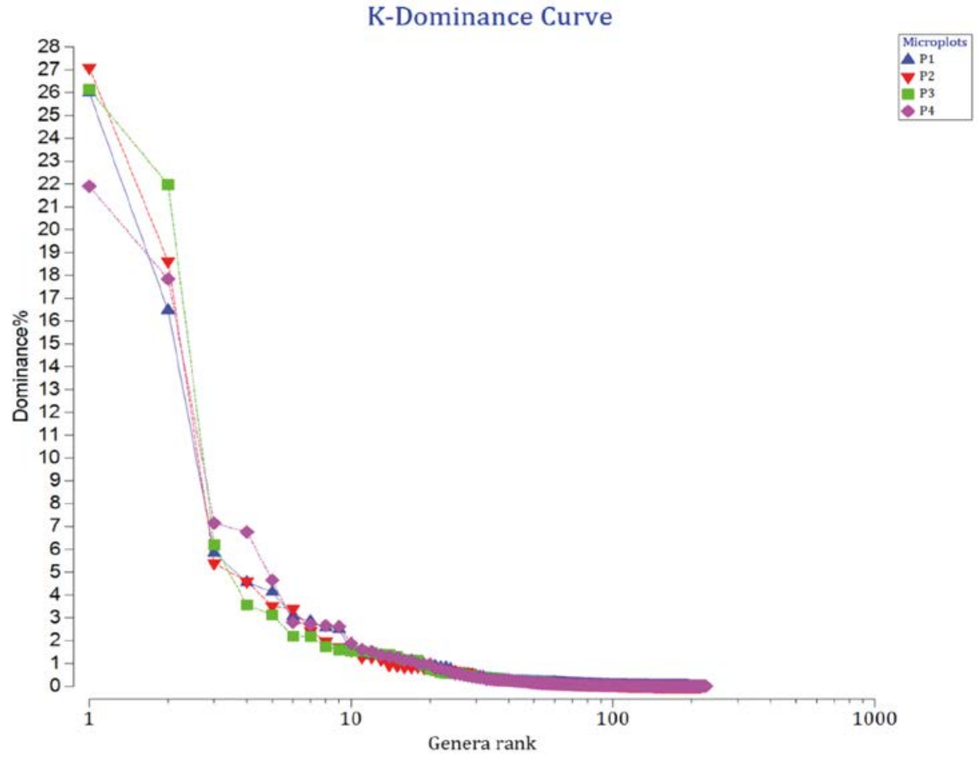

Figure 2. Dominance curve of the genera identified in each plot. P1=Witness, P2=First year of implementing Conservation Agriculture (CA) practices, P3=Bioinoculation in seed (by six year) and stop CA practices (it was done for five years) and P4=CA with biofertilizer (both for six years).

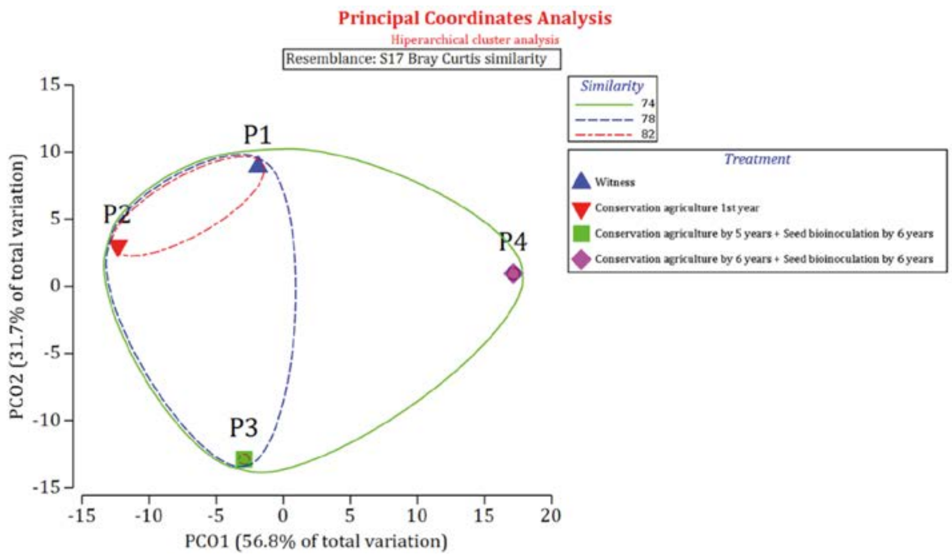

Figure 3. Principal coordinate analysis of the results of the microbiota in soils. The colored lines represent similarity percentages of diversity (based on Bray-Curtis). The figures and colors represent the treatments of the soils.

and Proteobacteria are more abundant in acidic and nutrient-poor soils, characteristics of our study area. Wang *et al.* (2022) concluded that Actinobacteria and Proteobacteria are the dominant phyla in subtropical soils, usually acidic. This is further evidence that the pH of a soil can entail an important effect on the structure of soil microbial communities. Rivera-Rivera and Cuevas (2020) reported that the dominant bacteria in a tropical soil were Proteobacteria and Actinobacteria, followed by Bacteroidetes, only to change during the dry season, when the presence of Actinobacteria increased significantly, while that of Proteobacteria and Bacteroidetes decreased.

The SIMPER analysis at the taxonomic family level revealed a 76.3% similarity between plots P1 and P3 (without CA practices) and a similarity of 67.5% between P2 and P4 (with CA practices). There was a dissimilarity of 24.0% between CA and non-CA plots.

The bacterial families with the highest abundance in CA plots included Solibacter, Verrucomicrobia sub 3, Bacillaceae, Acidobacteriaceae, Planctomycetaceae, Thermomonosporaceae, Clostridiaceae, Nitrospiraceae, and Ktedonobacteriaceae. Non-CA plots showed higher abundance of Pseudomonadaceae, Conexibacteraceae, Nitrosomonadales (unclassified), and Nocardioidaceae

The relative abundance of the dominant bacterial genera varied among the four microplots (Figure 4). *Candidatus Koribacter* was the most abundant taxon across all samples, ranging from 16.47% in P1 to 26.15% in P3. On the other hand, *Candidatus Solibacter* was the second most abundant genus, reaching its highest value in P4 (6.76%), while lower proportions were observed in the other plots (3.40-4.14%). Both genera belong to the phylum Acidobacteria, indicating that this group dominated the microbial communities across all sites. This phylum is abundant in soil samples (Paz and Menjivar, 2019). Candidatus Koribacter is a carbon-monoxide oxidizing bacterium, and C. Solibacter can degrade complex compounds such as hemicellulose, cellulose, and others. Both species

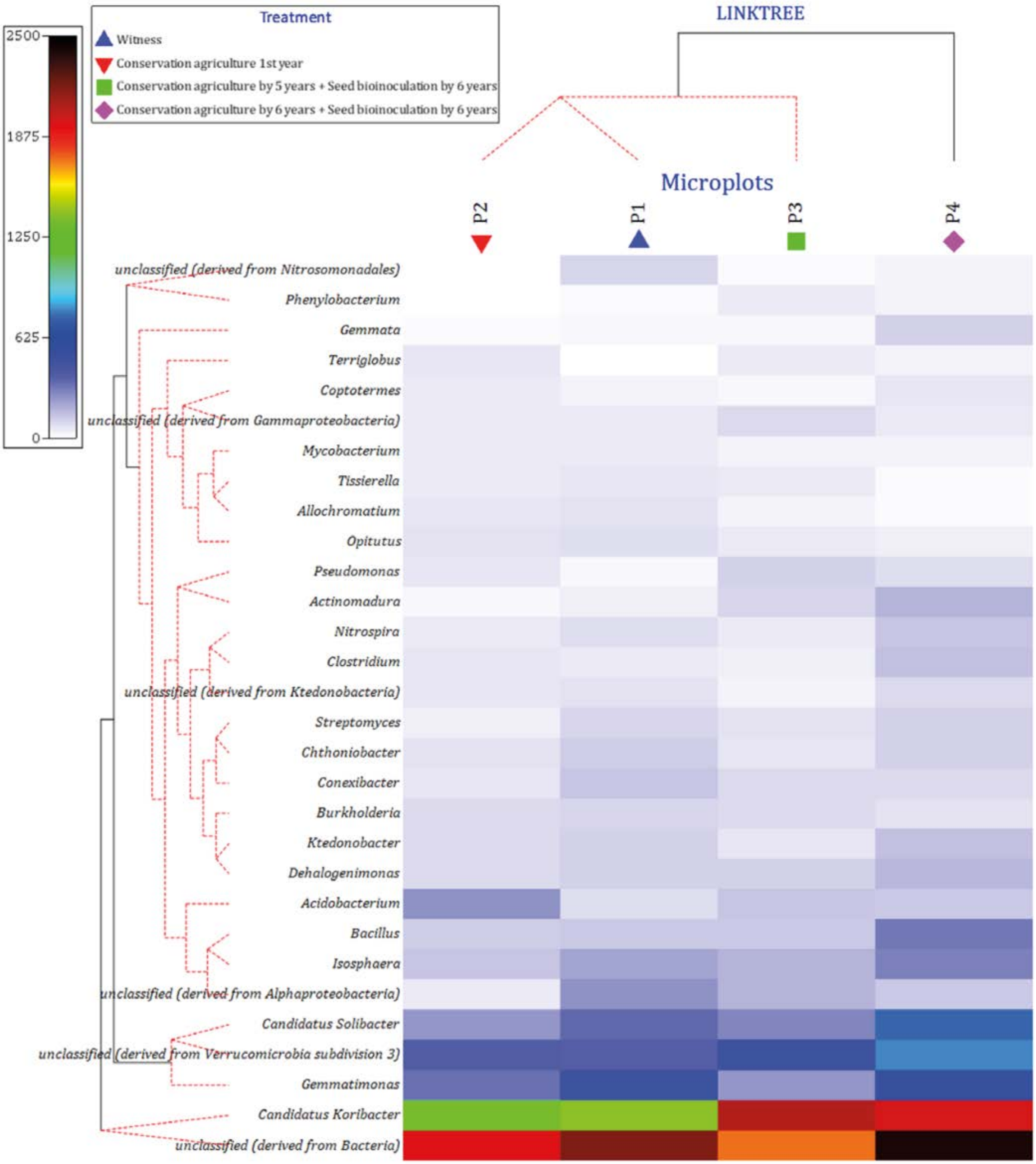

Figure 4. Shade plot of the samples (columns) according to the genera identified (30 most abundant). The depth of the spectrum shading is linearly proportional to an abundance of reads. The dendrograms correspond to the groupings by similarity of abundances of the biotic variables according to the CLUSTER LINKTREE analysis. Red lines correspond to the statistical grouping according to the SIMPROF analysis.

account with resistance potential against desiccation (Challacombre *et al.*, 2011), which could significantly influence the structure of microbial soil communities. Microplots P3 and P4 are where there is the greatest abundance of them, and they are where AC practices have been applied for the longest time.

Among the remaining taxa Verrucomicrobia-bacterium showed a gradual increase from P1 (4.55%) to a maximum in P4 (7.15%), whereas the *Bacillus* genus reached its highest abundance in P4 (2.80%), highlighting it as another prominent taxon in this plot. Regarding *Gemmatimonas* genus, displayed high relative abundance in P1 (5.85%) and P4 (4.65%), while *Isosphaera* reached its maximum in P4 (2.66%) (Figure 5). The *Gemmatimonas* genus is related to the reduction of N_2O, a compound that is stored in soils (Oshiki *et al.*, 2022). Moreover, *Isosphaera* is usually found in aquatic habitats and wastewater. However, some studies have reported both genera in soils and compost leachates (Wang *et al.*, 2002).

Other genera, including *Acidobacterium*, *Burkholderia*, *Conexibacter*, *Dehalogenimonas*, and *Ktedonobacter*, were present at lower abundances (<2-3%), showing no clear pattern across plots. In turn the genus *Streptomyces* remained relatively stable (~1%) across all plots (Figure 5).

Overall, microplot P4 was characterized by higher relative abundances of *Candidatus Solibacter*, *Verrucomicrobia*-bacterium, *Bacillus*, and *Isosphaera*, suggesting microenvironmental or management conditions that may specifically favor these taxa and distinguish P4 from the other plots in terms of microbial community composition (Figure 5).

Unique and Shared Soil Microbial Phylotypes Among Agricultural Treatments

A Venn diagram showed the distribution of unique phylotypes among the four plots (P1, P2, P3, and P4) (Figure 6). Plot P4 exhibited the highest number of exclusive phylotypes (56; 15%), followed by P2 with 47 (12%), P3 with 36 (9%), and P1 with 35 (9%). In addition, 92 phylotypes were shared among all four plots (24%), indicating the presence of a common core microbiota, along with a differentiated fraction associated with each management

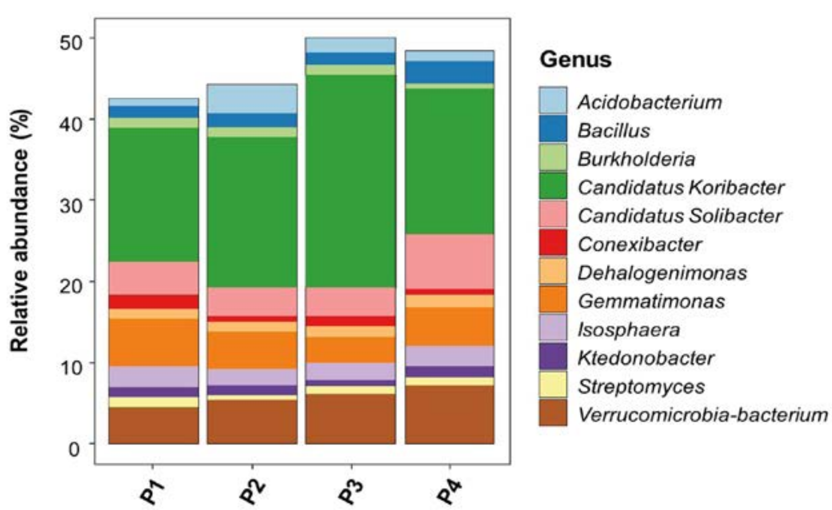

Figure 5. Relative abundance of dominant bacterial genera across microplots.

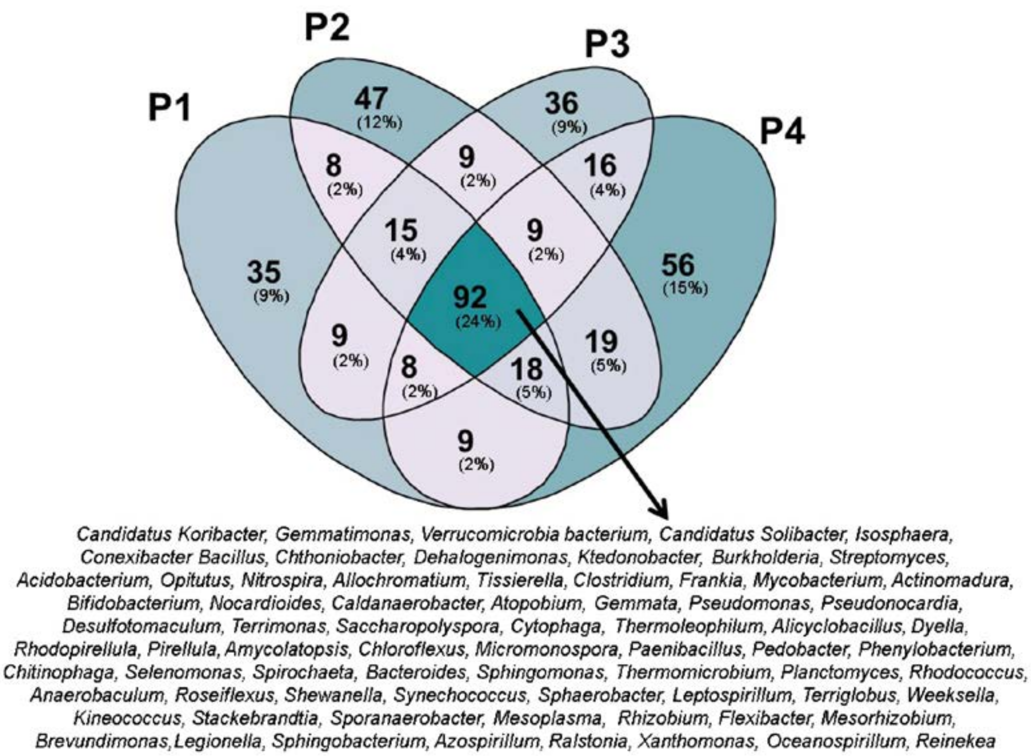

Figure 6. Distribution of unique and shared phylotypes among soil samples from plots. P1, P2, P3, and P4.

practice (Figure 6). These findings suggest that agricultural practices, particularly conservation agriculture and bioinoculation, influence the uniqueness and composition of soil microbial communities.

The shared bacterial genera represent the set of microbial taxa that remain stable and persistent across different environmental conditions or agricultural practices. These microorganisms are associated with the maintenance of the basal functioning of soil, acting as key components of microbial stability and resilience (Custer *et al.*, 2022). Their consistent presence suggests their involvement in essential microbial processes, such as nutrient cycling, organic matter decomposition, and the regulation of plant-microbe interactions. In this context, the core microbiota constitutes the functional backbone of the soil ecosystem, ensuring the stability of biogeochemical processes even under changes in agricultural management (Custer *et al.*, 2023).

Some bacterial genera shared among the four plots are related to plant growth promoting activities. Such is the case of the genera *Bacillus*, *Burkholderia*, and *Pseudomonas*, include some opportunistic pathogenic species of great importance to public health, species with bioremediation potential, and species that have been used as growth promoters (Hashem *et al.*, 2019; Madigan *et al.*, 2015). Regarding *Azospirillum* genus is a cosmopolitan genus found in soil samples and associated with roots in tropical, subtropical, and temperate regions. Some species of this genus have been used as bioinculants due to their plant growth-promoting capabilities, such as nitrogen fixation, hormone production, phosphorus solubilization, and siderophore production (Pedraza *et al.*, 2020).

Another bacterial genera with agricultural importance identified in this study included *Frankia*, *Paenibacillus*, *Rhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Azorhizobium*, *Clostridium* and *Pseudomonas*, among others (De Souza *et al*., 2015; Madigan *et al*., 2015), corresponded to bacterial genera with potential as biofertilizers, which are widely used in agriculture. It has been shown that the use of conservation agriculture techniques can have a beneficial effect on soil microbial activity, thereby increasing the diversity of soil microbial populations. The combination of bioinoculation practices with conservation agriculture can have an even more beneficial effect, although proper selection of micro-organisms is required to achieve the expected effects. However, further research is needed to determine the stability of the soil microbiota in relation to the practices used, the microorganisms used as bioinoculants and the timing of their application to the soil.

CONCLUSIONS

The combination of conservation agriculture and bioinoculation appears to promote a more diverse and balanced soil microbial community, as evidenced by both alpha and beta diversity patterns. This suggests that management practices can play a key role in shaping microbial assemblages, potentially enhancing soil ecosystem functionality and resilience. These results highlight the potential of integrating bioinoculants with sustainable agricultural practices to support soil health.

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Colegio de Postgraduados

In vitro efficacy of fungicides for the control of *Fusarium* spp., the causal agent of root rot in common bean (*Phaseolus vulgaris* L.)

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ABSTRACT

Objective: Currently, common bean cultivation takes place in several regions with high population demand. However, production has been affected by vascular fusariosis, one of the main limiting factors. Chemical control is considered one of the primary alternatives for managing this disease.

Design/Methodology/Approach: Four fungicides at different concentrations: Verango Prime, Fungi Mix, Vibrance Beans, and Cobrethane WP were evaluated to quantify the percentage of mycelial growth inhibition (MGI) in three *Fusarium* species: *Fusarium nygamai*, *Fusarium verticillioides*, and *Fusarium falciforme* under *in vitro* conditions. A completely randomized design with a factorial arrangement and three replications was established. The effectiveness of each fungicide was evaluated based on the percentage of mycelial growth inhibition (MGI) 25 days after inoculating the strain in the culture medium.

Results: Tukey's multiple comparison test ($\alpha = 0.05$) was performed.

Conclusions: The fungicide Verango Prime achieved the highest MGI for the three *Fusarium* species, with inhibition values of 67.5%, 37%, and 42.5% for *F. nygamai*, *F. verticillioides*, and *F. falciforme*, respectively.

Keywords: Chemical control, mycelial diameter, *Fusarium nygamai*, inhibition percentage.

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the staple crops in the Mexican diet. In Mexico, it is among the most important agricultural commodities from nutritional, economic, and social perspectives. Bean production occurs throughout the Mexican territory. During the 2022-2023 agricultural cycle, 761,316.72 hectares of beans were harvested, yielding a total production of 723,642.32 tons. The state of Sinaloa ranks as the leading bean producer in the country, with a production volume of 172,161.73 tons and an average yield of 1.97 t ha^{-1}, representing 23.7% of the total national production (SIAP, 2024).

Low grain yields are attributed to both biotic factors, such as pests, weeds, and diseases, and abiotic factors, mainly water scarcity. Regarding diseases, phytopathogens including fungi, bacteria, viruses, and nematodes cause damage to the roots, stems, leaves, and

pods of common bean, thereby affecting grain quality, germination, yield, and ultimately the profitability of the crop. One of the most recent reports for this crop describes wilt symptoms in the Culiacán Valley, Sinaloa, caused by the fungus *Fusarium nygamai*, which induces wilting and yellowing symptoms that can easily be mistaken for nutrient deficiencies or infections caused by other soilborne pathogens (Salgado *et al*., 2013).

The *Fusarium* genus comprises a wide variety of phytopathogenic species with broad distribution and occurrence, highlighting their toxicological capacity and the problems they pose to crops of major agricultural and economic importance, such as common bean. It is essential to conduct phytosanitary inspections of crops throughout their developmental stages in order to establish accurate diagnoses and implement appropriate control measures (Salgado *et al*., 2013).

Given this problem, it is necessary to implement appropriate measures for the control of this disease. Among these measures is the application of chemical compounds such as fungicides, which help prevent and promote the rapid recovery from fungal diseases. Chemical control is also one of the most widely used strategies, being applied in more than 90% of crops (Salgado *et al*., 2013). The main objective of the present study was to determine the effect of chemical control using fungicides *in vitro* at different concentrations on the percentage of mycelial growth inhibition in three *Fusarium* species: *Fusarium nygamai*, *F. verticillioides*, and *F. falciforme*.

MATERIALS AND METHODS

Study location

The research was conducted in the Culiacán Valley, Sinaloa, Mexico, at the Phytoprotection Laboratory of the Faculty of Agronomy (Figure 1), Autonomous University of Sinaloa (FA-UAS), located at Carretera Culiacán-Eldorado km 17.5, Sinaloa, Mexico.

Figure 2. Research facilities of the Faculty of Agronomy.

Strain selection

The *Fusarium nygamai* strain identified as FNTL6P7CULSIN in the NCBI GenBank (access number OK491917) was selected; it was isolated from common bean plants. Additionally, *Fusarium verticillioides* FVTL6P6CULSIN (access number OK491916) and *Fusarium falciforme* FFTL6P5CULSIN (access number OK491915) were included. All strains were obtained from the Phytoprotection Laboratory of the Research Center at the Faculty of Agronomy, Autonomous University of Sinaloa. The isolation of *Fusarium nygamai* was cultured on PDA medium and previously identified both morphologically and molecularly. This isolate has been reported as the causal agent of root rot and wilt in common bean crops in Sinaloa, Mexico (Vega-Gutiérrez *et al.*, 2022).

Selection of chemical products for the *in vitro* control of *Fusarium nygamai* Four commercial chemical fungicides were selected, as shown in Table 1. These fungicides are used to combat damage caused by fungal diseases in various horticultural crops and are approved by the Federal Commission for the Protection against Sanitary Risks (COFEPRIS), the Secretariat of Environment and Natural Resources (SEMARNAT), and the Secretariat of Agriculture and Rural Development (SADER) (COFEPRIS, 2024).

Experiment setup

The isolate was subjected to an *in vitro* sensitivity test using different fungicides at concentrations of 1, 10, 100, 1,000, and 10,000 ppm on potato dextrose agar (PDA) medium (Gálvez *et al.*, 2016), following the poisoned food technique. This involved preparing the culture medium in sterilized Erlenmeyer flasks at 120 °C for 15 minutes in an autoclave. The medium was then allowed to cool to 40 °C before adding the fungicides at the corresponding concentrations in 9-cm diameter Petri dishes (Dingran & Sinclair, 1985).

The pathogen isolate was grown for 7 days at 28 °C in the dark and was then used to inoculate the different treatments by placing a 0.5 cm diameter disc. The plates were incubated at 28 °C for the first 7 days, at which point the control treatment reached 100% of the Petri dish surface (Muiño *et al.*, 2010). A control treatment without fungicide was included to compare mycelial growth against the growth observed in the fungicide-treated plates.

Experiment evaluation

The colony diameter was measured in two perpendicular directions using a digital caliper. The growth of the pathogen colonies was compared with that of the control treatment, following the methodology described by the radial colony growth method (FAO,

Table 1. Description of fungicides used.

Fungicides	Chemical group
Vibrance Beans	Pirazolanilidas
Fungi Mix	Orgánico
Verango Prime	Etil Benzamidas
Cobrethane Wp	Ditiocarbamatos

1982). The effectiveness of the treatments was evaluated using the percentage of mycelial growth inhibition (PMGI), calculated according to the formula proposed by Pandey *et al.* (1982).

$$PMGI(\%) = \left[(RC - RT) / RC \right] \times 100$$

where: *PMGI*: Percentage of mycelial growth inhibition. *RT*: Radial growth of the fungus in each treatment (mm). *RC*: Radial growth of the fungus in the control (distilled water) (mm).

Experimental design

A completely randomized design with a factorial arrangement was established, comprising 60 treatments and an untreated control without fungicide. Each treatment was replicated three times. An analysis of variance (ANOVA) and Tukey's multiple comparison test were performed on the PMGI values obtained from the fungicide treatments. This analysis was conducted separately for each Fusarium species. Subsequently, ANOVA and Tukey's test were carried out to evaluate the effectiveness of the fungicides using the SAS statistical software (version 9.0), considering the concentration of each fungicide and the *Fusarium* species separately.

RESULTS AND DISCUSSION

Mycelial diameter

The results of mycelial growth showed that treatments 35 and 45, which consisted of the fungicide Verango Prime, completely inhibited the mycelial growth of Fusarium nygamai, with a diameter of 0.000 mm. This represented a significant difference compared to the other fungicides, as shown in Figure 2. These findings are consistent with those reported by Yossen and Conles (2014), where all evaluated fungicides achieved over 94% mycelial growth inhibition, with benzimidazoles being the most effective, reaching over 99%. Similarly, Srivastava *et al.* (2011) reported that the radial growth of *F. oxysporum* was

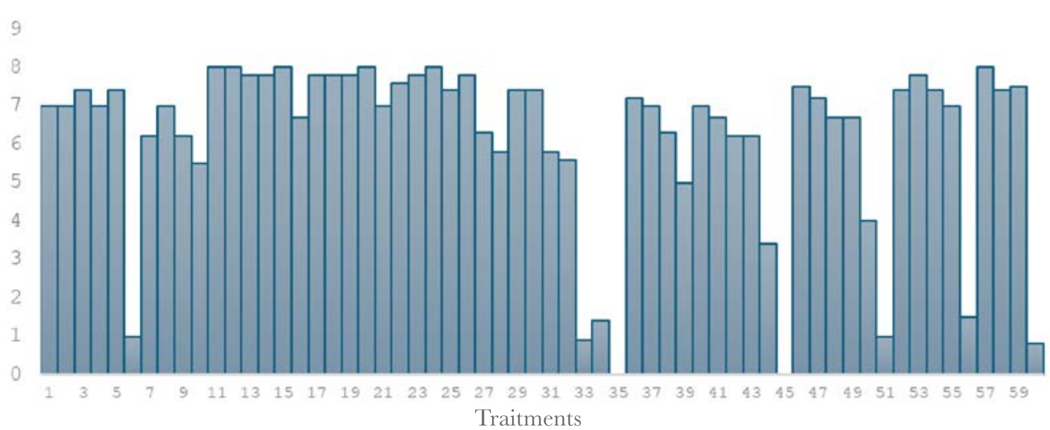

Figure 2. Mycelial diameter of *Fusarium nygamai*, *Fusarium verticillioides*, and *Fusarium falciforme* at different fungicide concentrations, expressed in millimeters (mm).

inhibited by carbendazim at different concentrations in *in vitro* assays. However, all high fungicide concentrations reduced the mycelial diameter across the three *Fusarium* species evaluated. These results indicate that the effectiveness of fungicides decreases at very high concentrations. Overall, these findings have significant social, economic, and health implications.

Percentage of mycelial growth inhibition (PMGI)

The fungicide that exhibited the highest percentage of mycelial growth inhibition for the three *Fusarium* species was Verango Prime, with values of 67.5%, 37%, and 42.5% for *F. nygamai*, *F. verticillioides*, and *F. falciforme*, respectively. Conversely, *F. falciforme* showed very low inhibition levels when treated with Vibrance Beans, with a value of 1.3% (Figure 3).

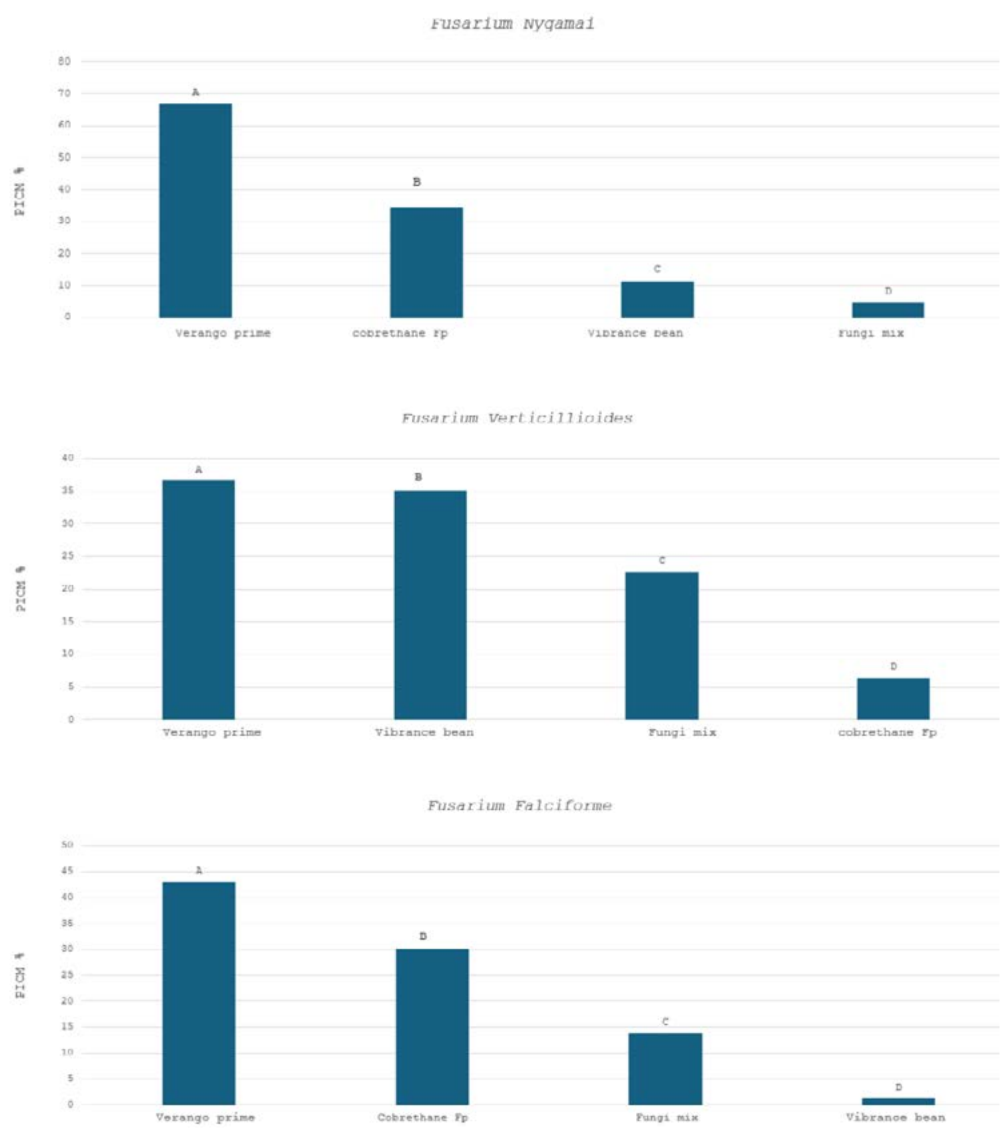

Figure 3. Percentage of mycelial growth inhibition (PMGI) in *F. nygamai*, *F. verticillioides*, and *F. falciforme*. PMGI is expressed as a percentage, and Tukey's multiple comparison test was performed ($p \leq 0.05$).

This is attributed to the fact that the fungicide Vibrance Beans is not recommended for *Fusarium* species (Syngenta Global AG, 2018). However, the product showed slightly higher inhibition percentages at a concentration of 1 ppm. The inhibition percentages reported by Ochoa *et al.* (2012) are consistent with those obtained in this study, as they evaluated *F. oxysporum*, *F. culmorum*, and *F. solani*, obtaining 43.15%, 31.81%, and 45.58%, respectively. Similarly, the values obtained in this study are in agreement with those reported by Guédez *et al.* (2012), who observed mycelial growth inhibition percentages of 80%, 72%, and 71% against the three pathogenic fungi *F. oxysporum*, *R. solani*, and *S. rolfsii*, respectively.

When evaluating the three *Fusarium* species at different fungicide concentrations, it was observed that higher fungicide concentrations tended to increase PMGI. *F. falciforme* at 10,000 ppm showed the highest PMGI, with a value of 55.6%, as shown in Figure 4. It is important to consider that high fungicide concentrations can increase pathogen resistance, production costs, and contamination of production systems (Chin *et al.*, 2001). Contact or systemic fungicides are essential in the management of fungal diseases, but they increase economic costs and environmental risks. Therefore, a resistance management program should be implemented to reduce the number of applications. In Mexico, the most commonly used fungicides are mancozeb and chlorothalonil (preventive), as well as systemic active ingredients from the benzimidazole, triazole, strobilurin, and anilopyrimidine groups (Martínez *et al.*, 2012).

Authors such as Deepak and Lal (2009) observed in their study that the fungicides thiabendazole and thiophanate-methyl showed intermediate performance, with values close to 80%, while mancozeb exhibited the lowest control efficacy among all applied treatments, with less than 60% PMGI. Tebuconazole has been reported as one of the most effective fungicides for controlling *Fusarium* spp. in cereal grains. On the other hand, carbendazim has shown a good preventive and curative effect against *F. oxysporum* in tomato crops.

Some authors agree that this fungicide, as well as other benzimidazoles, shows high efficacy in controlling various *Fusarium* species across different crops (Jahanshir and Dzhalilov, 2010). Kopacki and Wagner (2006) reported that mancozeb exhibited the lowest efficacy for controlling *F. avenaceum*, with PMGI values ranging from 24% to 74%. Despite

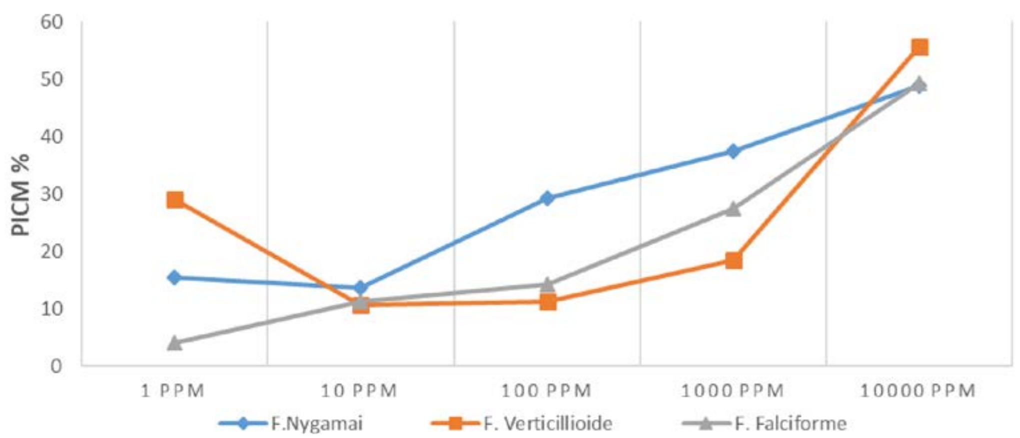

Figure 4. Percentage of mycelial growth inhibition (PMGI) at different parts-per-million concentrations in *F. nygamai*, *F. verticillioides*, and *F. falciforme*.

these results, this fungicide is considered a useful tool for reducing the development of pathogen resistance, as its mode of action affects multiple sites within fungal cells and disrupts numerous biochemical processes, significantly decreasing the likelihood that pathogens will develop resistance.

CONCLUSIONS

All the fungicides evaluated have been used to control diseases affecting the root system of common bean crops. The fungicide Verango Prime exhibited the highest mycelial growth inhibition (PMGI) value (67.5%) at a concentration of 10,000 ppm against *Fusarium nygamai*. Applying this fungicide dose is recommended to reduce the damage caused by this species in common bean cultivation. For *F. verticillioides*, Verango Prime also provided the most effective control, and the same product showed the best control of mycelial growth in *F. falciforme*.

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Colegio de Postgraduados

Production of chile de árbol (*Capsicum annuum* L.) seedling with designation of origin in Yahualica, Jalisco

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ABSTRACT

Objective: Different chile de árbol (*Capsicum annuum* L.) seedling production systems were evaluated and compared with traditional systems to identify the effects of new techniques.

Design/Methodology/Approach: Five chili varieties from different regions were evaluated (S1O, S2B, S3L, S4J, and S5A). The methods used in the experiment were greenhouse germination trays (GT) and open field nurseries (OFN). In addition, traditional (T), conventional (C), and alternative (A) fertilization systems were used. The variables evaluated were plant height (PH), stem diameter (SD), and number of leaves (NL).

Results: The GT+A fertilization treatment increased the PH and NL of the seedlings. The OFN+T combination stood out during the last stages, recording a higher PH. Meanwhile, the GT+C seedlings achieved a regular growth, obtaining good ST and NL results during the intermediate stages. At 30 and 45 days, S2B+GT+T recorded the highest PH and NL, while, at 60 days, S2B+GT+A obtained the highest SD and PH. At 90 days, S2B+OFN+T recorded the highest PH.

Study Limitations/Implications: The seedling production of the open field nurseries was 15 days behind the 60-day greenhouse germination tray system (GT) with peat moss.

Findings/Conclusions: The traditional method recorded the most homogenous plants. The OFN+A treatment and the OFN+T treatment had good performance and adaptability. The root analysis showed that the A system promoted a better root development. Management systems did not impact the health quality of the crops.

Keywords: Chili crops, *Capsicum annuum* L., alternative, conventional, and traditional agriculture.

INTRODUCTION

The cultural and natural richness of Mexico has enabled the development to highly valued products worldwide. These products are protected under the Designation of Origin (DO) standard. Currently, the country has 18 DO certificates, granted by the Instituto Mexicano de la Propiedad Industrial (IMPI) (Villaseñor, 2020). The Yahualica chile de

árbol stands out among these products. Its DO certification was granted in 2018 to 11 municipalities: nine in Jalisco and two in Zacatecas (Economía, 2018). The DO certificate guarantees the authenticity and origin of this chili, protecting traditional techniques and strengthening the cultural identity of the producer communities (SIAP, 2024).

Chile de árbol production is fundamental for the local economy in the Altos Norte region of Jalisco and the southern region of Zacatecas, providing income for 400 producers, who produce 1,440 t in ≈1,200 ha every year (Saavedra, 2022). However, this crop faces challenges, including phytosanitary issues and adverse climate conditions, that impact the first stages of its development, particularly, during the seedling production stage (SIAP, 2021).

The quality of the seedlings is fundamental for a successful chili production, because it directly influences crop yield and resistance to diseases and stress conditions (Martínez *et al.*, 2019). Height, stem diameter, number of leaves, root development, and plant health are some of the characteristics that define a good quality seedling (López-Pérez *et al.*, 2020). A vigorous and healthy seedling is easier to establish in the field, maximizing productive potential (Gómez *et al.*, 2018).

Seedling production systems include open field nurseries (OFN) and greenhouse germination trays (GT). Each system has specific costs, management, and quality advantages and challenges (Rodríguez *et al.*, 2021). Adopting modern technologies and sustainable agricultural practices can significantly improve traditional systems and, consequently, the quality of Yahualica *chile de árbol* seedlings, increasing its competitivity in the market (Hernández and Ramírez, 2022). Therefore, different seedling production systems were evaluated in order to identify the effect of the implementation of new technologies and to compare them with the traditional system. The objective was to optimize the quality, innocuousness, and yield of *chile de árbol* crops, guaranteeing their long-term sustainability in a region with great cultural and economic importance.

MATERIALS AND METHODS

Location of the Study Area

The research was conducted from January to May 2024, in the facilities of Invernaderos Icuata, located 3 km away from the municipal seat of Nochistlán de Mejía, Zacatecas, México (21° 21’ 23” N and 102° 52’ 30” W, at 1,899 m.a.s.l.) (Figure 1) (INEGI, 2020). The area has a monsoon influenced humid subtropical climate (Cwa), with a 14-20 °C annual temperature and a 700-1,000 mm annual rainfall (García, 2004). The main soils of the region are phaeozem and regosol (INEGI, 2018).

Plant Material

This research evaluated the performance of five varieties of chile de árbol seeds in three production systems: traditional (T), conventional (C), and alternative (A). The seeds came from municipalities within the designation of origin region. These systems were established based on: (a) sowing system, consisting of germination trays (GT) with substrate in a protected environment and open field nurseries (OFN); and (b) source of the fertilizer. Seeds from five varieties of *chile de árbol* were extracted from dry mature

Figure 1. Location of the study area.

fruits. One of the five varieties was an endemic regional plant, known as the "original plant." This variety has been preserved in backyards. The other four seed varieties are native to the region and were collected in three communities by local producers (Table 1): Benjamín Santos de La Portilla, Nochistlán; Leandro Yáñez, Apulco, Apulco; José Sandoval, El Salto Verde, and Yahualica, and Luis Antonio Plascencia, Manalisco, Yahualica. The seeds were identified as S1O, S2B, S3L, S4J, and S5A, respectively. All the seeds were treated with 10% trisodium phosphate to reduce their seedborne pathogen load (Díaz *et al.*, 2019).

Experimental Design

Two sowing patterns were used for each type of seed: greenhouse germination trays (GT) and open field nurseries (OFN). Three fertilization arrangements were applied: traditional (T), conventional (C), and alternative (A) (Table 2). The experiment was developed using a randomized complete block design, with three repetitions per treatment. The experimental units in the greenhouse consisted of 15 rows with three

Table 1. Description of the *chile de árbol* factors evaluated.

Factors	Level	Identifier
Seed type of sowing fertilization system	Original	S1O
	Benjamín	S2B
	Leandro	S3L
	José	S4J
	Antonio	S5A
	Tray	Ch
	Seed bed	Al
	Traditional	T
	Conventional	C
	Alternative	A

Table 2. Fertilization arrangements of Yahualica *chile de árbol* seedlings in different systems.

System	Product	Content
Traditional	Ammonium sulfate $(NH_4)_2SO_4$	21-0-0-24S
Conventional	Various sources	38-52-80-16S
	Micronutrients	0.1%B, 0.05%Cu, 0.1%Fe, 0.05%Mn, 0.01%Mo, 0.1%Zn
Alternative	*Bacillus subtilis*	1×10^{10} UFC/mL

columns of 200 cavity trays, established 0.1 m apart from each other. The open field seeds were sown in nine rows, with four columns, in 1×1.20 m nurseries, 0.50 m apart from each other.

Sowing and Management

The germination trays were filled with a 75% peat and 25% coconut fiber substrate. One seed was sown per cavity. The seeds were irrigated at field capacity, covered with a black plastic, and stored in a warehouse for 10 days. After the seeds sprouted, they were placed inside a greenhouse with a 720-caliper polyethylene cover, above a 0.65 m base made up of plastic washbasins. The seedlings were sprinkled every day and each tray received 1 L of water.

Meanwhile, the nurseries were established at ground level. Dry cow manure was burnt on top of the soil (cow horn manure method), following the traditional fertilization method and using 19 L of water. Sowing was conducted above the ashes.

Before the sowing, the alternative system was treated with a liquid inoculant with 99.98 % mycorrhizae (*Bacillus subtilis*) (8×10^{10} spore/mL of water). In the conventional system, the seeds were directly sown in the soil. Two-hundred and fifty seeds were used in all the systems. All the nurseries were covered with a 720-caliper polyethylene cover for 22 days. After the seeds sprouted, they were only covered during the night. The cover was completely removed approximately 45 days after the sowing.

After their germination, seedlings were irrigated every third day, using the waterlogging method. Fertilization was conducted 20 days after the sowing and, subsequently, it was performed weekly in both sowing patterns, depending on each system. For its part, the traditional system was focused on a rich N and S source. As a result of the application of the 30% nutritive solution proposed by Hewit and Smith (1952) and modified by Gómez *et al*. (2019), the N, P, K, and S content in the soil of the conventional system was more balanced. Finally, 1×10^{10} UFC/mL *Bacillus subtilis* was used for the soils of the alternative system, in order to release its nutrients (Table 2).

Response Variables

Five seedlings from each nursery and five seedlings from each tray were randomly selected and subjected to a growth analysis. The measurements were carried out at 30, 45, 60, and 90 days after sowing.

Plant Height (PH - cm): measured from the base of the stem to the apex of the seedling, with a flexometer (cm/mm), following the recommendations of López *et al*. (2018).

Stem Diameter (SD - mm): measured in the center of the stem, between the soil or substrate and the first knot, with a Vernier or a caliper (mm), following the recommendations of González and Pérez (2019).

Number of Leaves (NL): every pair of leaves was counted. The leaves located after the knot were considered as a single leaf, following the recommendations of Hernández (2020).

Root Length (RL - cm): measured from the base of the stem to the point of the main root, with a 50×50 cm horizontally and vertically graduated wood board (Ramírez *et al.*, 2017).

Root Volume (RV - ml): determined using the water displacement method, in a 100 mL measuring cylinder. The whole root was immersed and the displaced water volume was measured, following the recommendations of López and Sánchez (2019).

Rot Health (RH): evaluated through the observation of the color of the root. Completely white roots were considered healthy, while brownish or yellowish roots were classified as sick (Hernández *et al.* 2020).

Statistical Analysis

The ST, PH, and NL response variables were subjected to an analysis of variance (ANOVA) to establish differences that could have been influenced by the following factors: origin of the seed, sowing type, and fertilization system. In addition, Tukey Honestly Significant Difference (HSD) ($P \leq 0.05$) was used to compare and evaluate the differences between treatments. Both evaluations were conducted on sampling days 30, 45, 60, and 90. RL and RV were subjected to an analysis of variance (ANOVA), including interactions between the seed, sowing type, and fertilization system factors. This evaluation was conducted to establish their impact on the development of the root. In addition, the results were compared using the Tukey's method for multiple comparisons. Contrasts were applied to compare fertilizations systems. Meanwhile, the normality of the residuals was verified through statistical tests and graphs. Finally, boxplots were developed to visualize the distribution and the correlation between length and volume was calculated. The analysis was carried out with the SAS Studio v.3.81 software.

RESULTS AND DISCUSSION

Plant Height (PH)

The model was significant during sampling days 30, 45, and 60. However, no significant effects were recorded during day 90. This situation suggested that the differences between treatments were sharper during the intermediate stages (30-60 days) (Table 3).

Differences ($P \leq 0.05$) were recorded in the average PH between treatments. On day 30, S2B+GT+T recorded the highest development (2.5 cm), followed by S3L+GT+C (2.1 cm). On day 45, S2B+OFN+T obtained the highest result (3.0 cm), followed by S4J+OFN+A (2.8 cm). These results showed the first differences between sowing systems. On day 60, S2B+GT+T stood out again (3.5 cm), but all the combinations recorded an overall PH increase.

Meanwhile, on day 90, S2B+OFN+T still recorded the highest results (4.0 cm), followed by S4J+OFN+A (3.9 cm), as the difference between treatments stabilized (Figure 2). Over

Table 3. Factors and interactions that had a significant impact on the height of *chile de árbol* seedling during each sampling.

Sampling	Effects Significant	Interations	Combination Outstanding
Día 30	Seed ($p<0.05$) System ($p<0.01$)	Variety×System ($p=0.0039$)	S2B+Ch+T
Día 45	Seed ($p<0.01$) System ($p<0.05$) Type ($p<0.05$)	Variety×System ($p<0.0001$) Variety×Type ($p<0.0001$)	S2B+Al+T
Día 60	System ($p<0.01$) Seed ($p<0.01$)	Variety×System×Type ($p=0.0311$)	S4J+Ch+A
Día 90	Seed ($p=0.5402$)	Variety×System (not significant)	S2B+Al+T

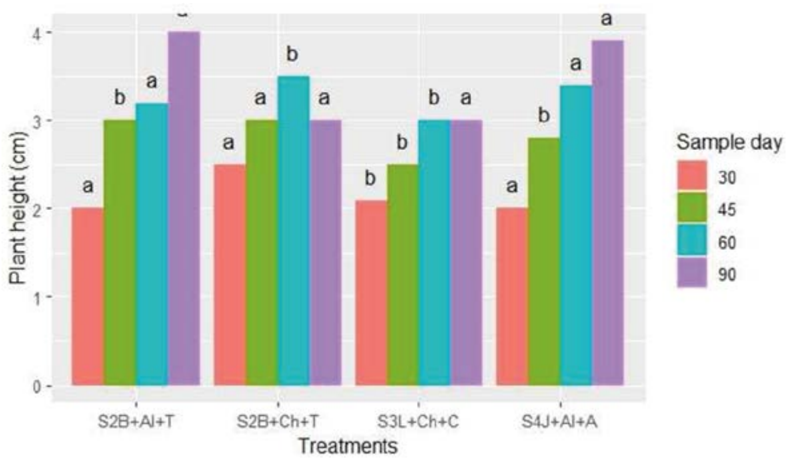

Figure 2. Evolution of the height of *chile de árbol* seedlings at 30 and 90 days after the sowing in treatments with the best performance.

time, the alternative system (A) favored PH growth, particularly during the intermediate stages (45 and 60 days), while the traditional system (T) recorded the lowest growth in all the stages, especially during the first days of the experiment. Meanwhile, the conventional system (C) showed a medium growth, particularly during the final stages. Regarding sowing type, GT favored a faster growth during the first stages (30 and 45 days), while OFN recorded a more competitive growth during the last stages (60 and 90 days), showing a greater PH during the last measurements.

Stem Diameter (SD)

Significant SD differences were recorded on sampling days 30, 45, and 60. On day 90, seed had a significant effect; however, no significant interactions were found. The SD differences were more noticeable during the early development stages; however, they stabilized towards the end of the growth cycle (Table 4).

Significant differences were recorded between treatments; S2B stood out with the highest ST average in all the stages. On day 30, S2B+GT+T recorded the highest ST (2.10 mm), while S3L+GT+C obtained the lowest ST (1.80 mm). On day 45, S2B+OFN+T

Table 4. Factors and interactions that had a significant impact on the stem diameter of *chile de árbol* seedling during each sampling.

Sampling (day)	Effect Significant	Interaction	Outstanding
30	Seed ($p<0.05$) System ($p<0.01$)	Seed×System ($p<0.01$)	S2B+Ch+T
45	Seed ($p<0.01$), System ($p<0.05$) Type ($p<0.05$)	Seed×System ($p<0.0001$) Seed×Type ($p<0.0001$)	S2B+Al+T
Da 60	System ($p<0.01$) Seed ($p<0.01$)	Seed×System×Type ($p=0.0311$)	S2B+Ch+A
90	Seed ($p=0.01$)	Seed×System (not significant)	S2B+Al+T

and S2B+OFN+A obtained the highest ST (2.5 mm), followed by S4J+OFN+A (2.40 mm). On day 60, the ST was more homogenous, with S2B+GT+T standing out once again (2.80 mm).

On day 90, the differences stabilized, with S2B+OFN+T reaching a 3.00 mm SD and showing a steady growth (Figure 3). The traditional (T) and alternative (A) systems produced higher ST than the conventional (C) system in all stages. OFN recorded a higher ST during the final stages, while GT was more competitive during the intermediate stages. These results highlighted the significant influence of the factors and their interactions in the development of the SD.

Number of Leaves (NL)

The results of the ANOVA indicated that this variable had an impact ($p<0.05$) during all sampling days, except for day 90. The results suggest that NL was influenced by the variety, sowing type, and fertilization system factors, recording significant differences throughout the experiment (Table 5).

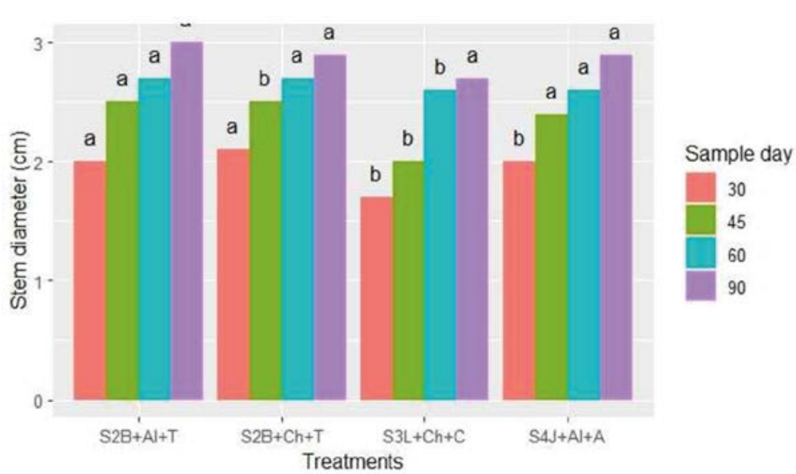

Figure 3. Evolution of the stem diameter of *chile de árbol* seedlings at 30 and 90 days after the sowing in the treatments with the best performance.

Table 5. Factors and interactions that had a significant impact on the number of leaves of *chile de árbol* seedling during each sampling.

Sampling (day)	Effect Significant	Interaction	Combination Outstanding
30	Seed ($p<0.05$) System ($p<0.01$)	Seed×System ($p<0.01$)	S2B+Ch+T
45	Seed ($p<0.01$) System ($p<0.05$) Type ($p<0.05$)	Seed×System ($p<0.0001$) Seed×Type ($p<0.0001$)	S2B+Al+T
60	System ($p<0.01$) Seed ($p<0.01$)	Seed×System×Tipo ($p=0.0311$)	S4J+Ch+T
90	System ($p=0.305$)	Seed×System (not significant)	S2B+Al+T

On day 30, S2B+GT+T recorded the highest NL average (6.8). The differences were particularly marked between fertilization systems. On day 45, S2B+OFN+T reached the highest growth (8.5 leaves), followed by S3L+GT+C (7.2 leaves), recording major differences between sowing types. On day 60, S4J+OFN+A obtained the highest NL (10.2 leaves), followed by S3L+GT+A (9.1 leaves). These results indicated an overall NL increase in all the treatments. Finally, on day 90, S2B+OFN+T reached the highest yield (12 leaves), followed by S4J+OFN+A (11.8 leaves) (Figure 4).

Over time, the alternative system (A) showed a steeper NL, particularly during the intermediate stages (45 and 60 days). The traditional system (T) recorded the lowest NL during all the sampling stages, with a slight increase towards the end. Meanwhile, the conventional system (C) had a medium performance, although it stood out during the final stages (60 and 90 days). Regarding sowing type, GT favored a higher NL during the first stages (30 and 45 days), while OFN was more effective during the final stages (60 and 90 days).

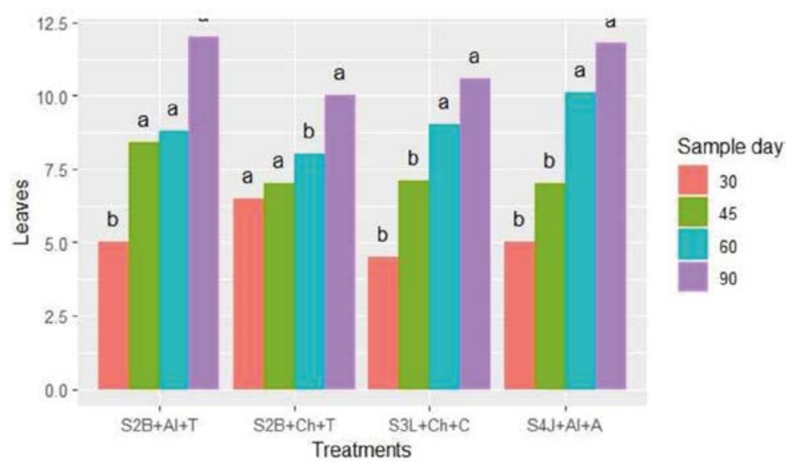

Figure 4. Evolution of the number of leaves of chile de árbol seedlings at 30 and 90 days after the sowing in the treatments with the best performance.

Root Length

The GLM analysis for the root length indicated that the model was highly significant, recording a high F-value and a <0.0001 p-value. These results suggest that the explicative variables significantly impacted root length.

The root length contrast between the traditional and conventional systems and the alternative system recorded a significant difference: a high F-value and a <0.0001 p-value. These results suggest that the traditional system had a different effect in root length than the other two systems. The length normality test of the residuals suggested that they were normally distributed. Clear mean differences were recorded between the systems (Figure 5), indicating that some production systems resulted in longer roots than the other systems. Possible atypical values suggest a variability within the systems.

Root Volume

The GLM model also showed a significant root volume, recording a high F-value and a <0.0001 p-value. These results indicate the strong influence of the variables in root volume. The root volume contrast between the traditional and the conventional system *vs.* the alternative system had significant differences: a high F-value and a <0.0001 p-value.

The normality test of the residuals suggested that they were normally distributed, strengthening the validity of the model. Furthermore, the length-volume correlation showed a significant positive ratio: root volume increased along with root length.

Just like in the case of length, differences were recorded regarding mean and data dispersion. In average, some systems resulted in a larger root volume (Figure 6). Atypical values were also clear, reflecting the response variability of root volume within the systems.

Root Health

All the evaluated seedlings had a white or cream and firm and homogeneous roots. This situation suggests that all the roots were healthy in all the treatments.

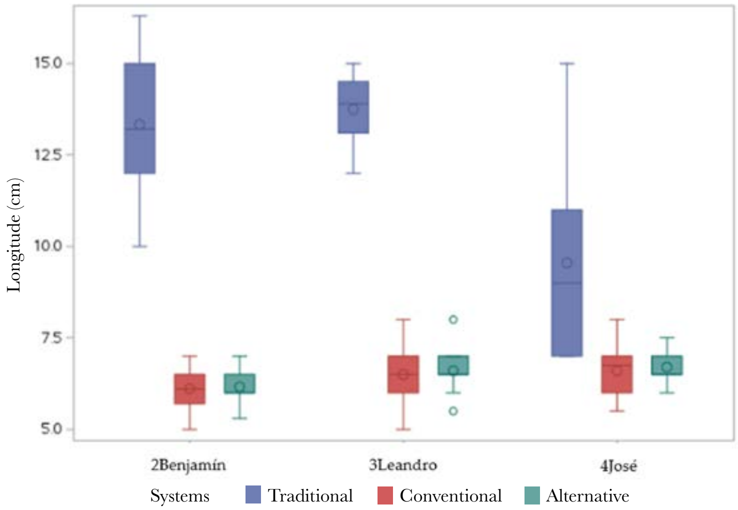

Figure 5. Root length comparison of different *chile de árbol* seeds grown under different systems.

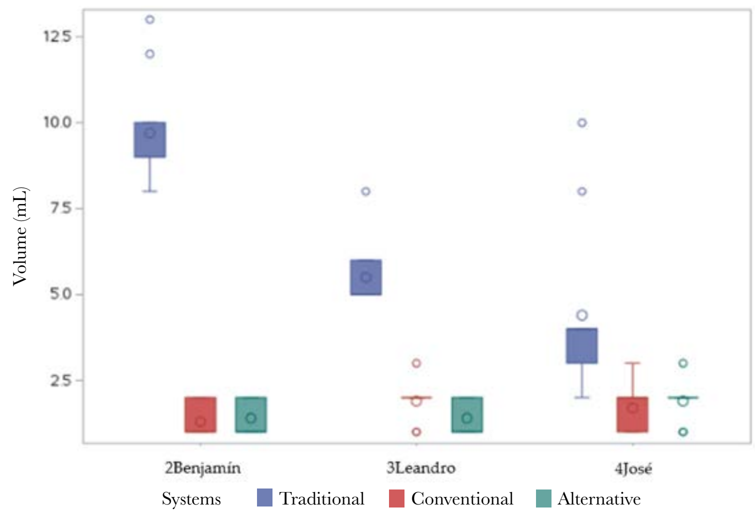

Figure 6. Comparison of root volume of different *chile de árbol* seeds grown under different systems.

The interaction between the genetic material effect and the agronomic management significantly influenced the growth of chili seedlings. The S2B seed stood out in PH, SD, and NL under the traditional fertilization system. For its part, S4J stood out during the final stages and in less conventional systems (*e.g.*, OFN+A), while S3L had a limited performance in most scenarios.

According to Bosland and Votava (2012), this response shows the genetic capacity to efficiently use nutrients and genetic plasticity to adapt to changeable environments. Selecting genotypes adapted to specific sowing and fertilization practices optimizes the genetic-environment interaction, promoting a more consistent and strong crop development (Rodríguez-Burruezo and Prohens, 2005). Meanwhile, the performance of the seeds could had been impacted by factors out of the control of the research team, such as the method used to dry them. Moo-Muños *et al.* (2016) pointed out that the drying method could impact the characteristics and growth of seedlings.

Based on Bautista and Sánchez (2017), the results of this study showed that, depending on the growing stage of the crop, the GT and OFN systems had a distinguished influence on the growth of chile the árbol seedlings. During the first stages, GT favored a vigorous initial growth thanks to the controlled conditions of the greenhouse that optimized water and nutrient availability. Such was the case of S2B+GT+T, which recorded the highest PH, SD, and NL at day 30. In contrast, OFN showed significant advantages during the final stages, particularly S2B+OFN+T and S4J+OFN+A, at day 90.

Meanwhile, environmental conditions significantly influenced the growth of chili seedlings, particularly SD and NL (Medina-Lara and Martínez-Damián, 2020). During the initial stages, the controlled conditions and protected environment of the GT system favored a homogenous growth: S2B stood out with a 2.1 mm SD and 6.8 leaves at day 30. Nevertheless, during the final stages, the OFN system promoted a stronger structural development: S2B+OFN+T reached a 3.00 mm diameter and had 12 leaves at day 90.

Meanwhile, the high temperatures within the greenhouse during certain days (>50 °C) reduced the growth of the GT seedlings. These results match the findings of Rosmaina *et al.* (2021), who pointed out that chili seedlings subjected to high temperatures (≥35 °C) reduced both crown and root growth.

Fertilization System

The results indicated that the traditional fertilization system was more effective for the growth of chili seedlings, particularly during the initial and intermediate stages. This system —based on local practices and the balanced management, N, P, K, and other nutrients— favored a homogenous early growth and a strong structural development (Ochoa-Velasco and Cruz-Valenzuela 2016). For example, S2B recorded the highest PH, SD, and NL, at 30 and 60 days. The impact of the alternative fertilization system showed more variability; however, the seedlings showed a good development during the final stages (genotype 4J). These results match the findings of Castillo-Aguilar *et al.* (2017), who reported similar data for *Capsicum chinense* Jacq. seeds, inoculated with rhizobacteria that positively impacted PH and SD growth. In addition, Alori *et al.* (2017) pointed out that the use of microbial inoculum improves the soil capacity to provide N, P, and K to the crops, favoring soil structural stability and mitigating plant stress, caused by draught, soil pollution, and salinity.

These findings highlight the importance of combining traditional practices with complementary approaches, adjusting them to genotype and specific crop stages, in order to maximize chili plant growth throughout their development cycle. Organic fertilization in both the alternative and traditional systems improved soil quality and favored a sustainable growth; however, this type of fertilization took longer to provide nutrients, impacting the yield of certain genotypes. For its part, the chemical fertilization used in the conventional system drove initial growth, but it was less sustainable (Sánchez and Osuna, 2019).

Production System

A production system is a set of agricultural practices applied to the crop, such as sowing methods, soil management, irrigation, fertilization, and pest control. The results obtained in this study highlight the importance of these production systems in the development of roots, particularly root length and volume. These findings were consistent with the reports of Smith *et al.* (2020), who pointed out that production systems have a significant influence on the morphological characteristics of roots, impacting both their length and volume. The adoption of alternative systems has shown different effects, suggesting that crop management optimization could improve root development.

CONCLUSIONS

Benjamín seed (S2B)+GT+nutrient intake, under the traditional system, was the most efficient combination during the initial stage, recording the highest PH, SD, and NL. Their growth was driven by the protected environmental conditions and the nutrient intake within the greenhouse. During the final stages, the Benjamín (S2B)+OFN+Traditional combination stood out, showing the strongest and homogenous development. In addition, José (S4J)+OFN+Alternative and Leandro (S3L)+OFN+Traditional had an outstanding adaptability to open field conditions and to sustainable fertilization systems during the final stages of the crop. Meanwhile, the alternative system promoted a better root development. The positive root length-volume correlation highlighted a direct ratio between both parameters. Finally, the optimal health of all the treatments showed that neither system impacted the health quality of the crops.

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Colegio de Postgraduados

Sustainability assessment of mixed farming systems among farmer field school participants

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ABSTRACT

Objective: To assess the sustainability of mixed farming systems among producers participating in Farmer Field Schools in ten municipalities of Zacatecas, Mexico, and identify strengths and areas needing intervention.

Design/Methodology/Approach: A structured, participatory survey was conducted with 58 producers. The survey covered five sustainability dimensions: economic, environmental, social, technical-productive, and climate resilience. Data analysis included descriptive statistics, principal component analysis (PCA), and ordinal logistic regression.

Results: The findings indicated that 50% of producers reached a medium sustainability level, 36% high, and 14% low. The environmental dimension scored the highest, while technical-productive and climate resilience showed the greatest weaknesses. Agricultural surface area was the only significant predictor of sustainability ($p<0.05$), suggesting that land access supports adopting agroecological practices.

Limitations/Implications of the study: The cross-sectional design restricts the ability to track changes over time. Future longitudinal studies are recommended to evaluate the long-term effects of technical assistance and training programs over time.

Findings/Conclusions: Access to productive resources and strengthening technical and organizational capacity are crucial for moving toward resilient, sustainable mixed farming systems in semi-arid areas.

Keywords: Agroecology; Farmer Field Schools; climate resilience; mixed farming systems; rural sustainability.

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INTRODUCTION

Mixed farming systems, characterized by the integration of crop and livestock activities within the same production unit, represent a traditional and resilient strategy used by many rural producers in semi-arid regions like Zacatecas, Mexico. These systems enable risk diversification, efficient use of available resources (such as water, soil, and organic residues), and income generation from multiple sources, which is crucial for the food security of smallholder families (FAO, 2001; Herrero *et al.*, 2010). In Latin America, crop-livestock integration has been shown to enhance the overall agroecological performance of farming systems (Stark *et al.*, 2018).

Despite these benefits, mixed systems face multiple challenges that threaten their long-term sustainability. Increasing climate variability, natural resource degradation, rising production costs, and limited access to technical and extension services have jeopardized their economic, environmental, and social viability (Altieri & Nicholls, 2017; Gliessman, 2015). Given these challenges, there is a need for participatory approaches that strengthen farmers' technical, organizational, and adaptive capacities while promoting sustainable production practices. Farmer Field Schools (FFS) represent one such approach, providing a platform for hands-on learning and peer-to-peer exchange tailored to local agroecological conditions. Assessing the sustainability of mixed farming systems among FFS participants can therefore provide valuable insights into their effectiveness and identify priority areas for intervention. In this context, it is important to strengthen producers' abilities to handle these challenges through participatory learning and local adaptation processes.

Farmer Field Schools are a training approach centered on practical learning, knowledge sharing, and collective problem-solving. Their implementation has shown positive effects in strengthening technical and organizational skills and promoting the adoption of sustainable practices in various countries around the world (Braun *et al.*, 2006; van den Berg *et al.*, 2021). In Mexico, FFS have been integrated into public rural development programs; however, there is a lack of systematic evaluations of their impact on the sustainability of production systems, especially in mixed systems of semi-arid regions.

Sustainability, defined as the ability of productive systems to maintain their function over time without depleting natural resources or harming the well-being of future generations, must be evaluated holistically. This includes considering economic, environmental, social, technical-productive, and climate resilience aspects (Pretty, 2008). Assessing these aspects helps identify opportunities, guide improvement actions, and generate evidence to support decision-making at both producer and public policy levels.

This study evaluated the sustainability of mixed farming systems among producers involved in Farmer Field Schools across ten municipalities in Zacatecas. Using a structured survey with a participatory approach, the five dimensions of sustainability —economic, environmental, social, technical-productive, and climatic— were thoroughly analyzed, highlighting strengths and key areas for improvement. The findings serve as a foundation for developing technical assistance strategies that encourage sustainable practices tailored to rural settings. Therefore, the objective of this study was to assess the sustainability of mixed farming systems among Farmer Field School participants in Zacatecas, Mexico, and to identify their main strengths and areas requiring intervention.

MATERIALS AND METHODS

Study type and study area

A cross-sectional, quantitative study was conducted in the first quarter of 2025 in the state of Zacatecas, Mexico. The influence area included ten municipalities selected for their participation in agricultural training programs through Farmer Field Schools: Jerez, Valparaíso, Zacatecas, Villanueva, Villa de Cos, Fresnillo, Vetagrande, General Enrique Estrada, Susticacán, and Tepetongo. These municipalities are situated in steppe dry climate zones and share similar agroecological characteristics (INEGI, 2022).

Population and sample

The target population consisted of producers actively participating in Farmer Field Schools promoted by the Secretariat of Agriculture and Rural Development (SADER, 2023). A non-probabilistic convenience sampling method was used, selecting 58 producers who voluntarily attended training sessions during which the survey was administered. The non-probabilistic sampling limits the generalization of results to the broader population of mixed farming producers in Zacatecas; however, it provides relevant insights into the targeted group participating in Farmer Field Schools.

Assessment instrument

A structured survey specifically designed for this study was used, based on principles of sustainable agriculture and participatory evaluation criteria (Pretty, 2008; Gliessman, 2015). The survey included a series of direct sociocultural questions aimed at characterizing the producers' profiles. These questions addressed aspects such as gender, age, education level, type of producer (family or commercial), experience in the production system, livestock type, land tenure, and available surface area. The survey instrument was validated through expert review and a pilot test with 10 producers to ensure clarity and relevance.

Additionally, the instrument included 26 dichotomous-response items (Yes=1, No=0) spread across five sustainability dimensions: economic (5 items), environmental (6 items), social (5 items), technical-productive (5 items), and climate resilience (5 items). Each dimension featured specific questions aimed at capturing producers' practices, resources, and perceptions. For example, the economic dimension asked about income diversification, access to credit, and marketing; the social dimension covered participation in organizations, generational renewal, and perceptions of well-being; the environmental dimension examined the use of agrochemicals, soil conservation, and waste management; the technical-productive dimension focused on record-keeping, management planning, and crop or forage rotation; finally, the climate adaptation dimension considered practices like adjusting agricultural or livestock calendars and implementing adaptive technologies.

The maximum score a producer could achieve was 26 points. Based on the total score, three sustainability levels were defined: low (0-10 points), medium (11-17 points), and high (18-26 points), using criteria adapted from previous agroecological assessment models (Astier *et al.*, 2012). This approach allowed for capturing both observable practices and decision-making processes relevant to the sustainable performance of mixed systems.

All questions were designed around technical criteria for good agricultural and livestock practices, as well as principles of agroecology and rural sustainability. The survey was administered directly by researchers during field sessions to ensure participants' understanding. All participants provided informed consent prior to the survey. The study adhered to ethical research principles.

Data processing and statistical analysis

A descriptive analysis of frequencies, means, and standard deviations was performed for both quantitative and categorical variables. Additionally, bivariate analyses were conducted to examine associations between sociodemographic variables (gender, education level, livestock type) and sustainability levels using the Chi-square test (χ^2). Statistical analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA), with a significance level of $\alpha=0.05$.

Multivariate principal component analysis (PCA) was used to reduce the dimensionality of the five sustainability dimensions and visualize clustering patterns among producers. The first two principal components explained 66.3% of the total variance. Ordinal logistic regression was performed to identify variables that predict sustainability levels (low, medium, high). Variables included age, gender, education, livestock type, agricultural surface area, technical support, and climate change adaptations. The dependent variable was ordered, and the model was estimated using maximum likelihood estimation.

RESULTS AND DISCUSSION

Sociocultural and productive profile

The average age of the surveyed producers was 45.8 years, ranging from 18 to 83 years. Disaggregated by gender, men had an average age of 46.2 years (range: 18 to 83 years), while women averaged 43.2 years (range: 22 to 70 years). These data reflect the active participation of an adult population, mostly within productive age, in the Farmer Field Schools of mixed agricultural systems. The producers' age may influence the sustainability of production systems both positively and negatively. An older average age may be linked to greater experience in agricultural management, while a younger age is often associated with higher openness to technological change (Fertő *et al.*, 2025). Generational renewal in agriculture is a global challenge, constrained by factors such as low motivation among young people and limited access to land (Borda *et al.*, 2023). The slight age difference between men and women may relate to different paths of entry into farming, as women often become more actively involved at later stages, for instance, as household heads or in response to male migration (FAO, 2017). Evidence suggests that younger farmers, particularly women, may show greater adoption of sustainable and innovative practices (Fertő *et al.*, 2025). This finding highlights the importance of designing training strategies with a generational and gender perspective that recognizes and values age diversity as a strength for rural territorial sustainability.

The analysis showed that 72% of the producers were men and 28% women, highlighting a notable level of women's participation in Farmer Field Schools. Despite their significant involvement, women in agriculture across Latin America have historically been under-represented and face barriers in access to resources and education, often accounting for only about 20% of the agricultural labor force and encountering disparities in land ownership and technology access (Gibbons *et al.*, 2022). Regarding education, most respondents had completed primary (39%) and secondary (29%) education, while only 8% had achieved professional or higher education levels. Those proportions mirror broader trends in rural areas, where limited opportunities for technical and higher education —especially for women— constrain their advancement (FAO, 2011). Additionally, 24% reported having technical or vocational training. Concerning the type of producer, 65% identified as family producers and 35% as commercial producers. This aligns with the predominance of family farming in the region: globally, family farms constitute over 98% of agricultural establishments and manage more than half of the agricultural land, often operating under small- or medium-sized production structures with strong family foundations (Graeub *et al.*, 2016). These figures indicate that most participants are involved in small- or medium-sized production structures with a strong family foundation.

The analysis of livestock types showed diversity among participating producers. The most common category was cattle (61%), followed by those combining cattle with small ruminants (sheep and goats) at 31%. The remaining included specialized systems like sheep, beekeeping, pigs, or combinations of these. These results reflect a widespread trend toward diversified livestock systems, a typical trait of family-based mixed systems in rural areas, which is crucial for the sustainability of production units in regions with high climatic and economic variability (Altieri & Nicholls, 2012; FAO, 2018). Recent studies indicate that functional interactions among components of multi-species systems enhance the technical and environmental performance of production units (Steinmetz *et al.*, 2021). The diversification observed in these production systems aligns with experiences reported in other parts of Latin America, where crop-livestock integration has shown benefits for productivity, resilience, and efficient resource use (Stark *et al.*, 2018).

This trend toward diversification aligns with the agroecological approach of resilient systems, where functional biodiversity reduces risks, enhances nutrient cycling, and provides complementary income for rural households. Agroecological principles emphasize diversity, synergy, and economic diversification as core strategies for sustainable transformation (Wezel *et al.*, 2020). Additionally, the presence of minor species such as bees, pigs, or poultry, although less reported, reflects a multifunctional view of the production system that combines economic, food security, and ecological goals. Agricultural diversification has been shown to increase long-term financial profitability, biodiversity, and ecosystem services —sometimes by over twenty-fold— while maintaining stable yields (Raveloaritiana & Wanger, 2024). The high number of producers who rely on cattle as their main species may also be influenced by cultural factors, access to basic infrastructure, and market demand. However, cattle production requires more feed, water, and health management, presenting a challenge to overall sustainability if not supported by training and technical assistance.

The average area dedicated to livestock activities among surveyed producers was 30.8 hectares, with a range from small backyard systems (0 ha) to large operations covering up to 300 ha. This variability highlights the coexistence of family production units with limited land access and larger systems in rural areas. The wide variation in livestock area reflects the structural diversity of mixed agricultural systems in Zacatecas. Small-scale units, including backyard setups, are common in peri-urban or mountainous regions and are often linked to subsistence economies, self-consumption, and the multifunctionality of rural households (Altieri & Toledo, 2011). In contrast, extensive units exceeding 100 hectares make up a smaller yet significant portion that may have greater capacity to invest in technology, infrastructure, and strategic forage management. This duality indicates the need for tailored policies that recognize the specific needs of both groups: some require technical assistance and access to basic resources, while others could benefit from certification schemes, differentiated marketing, and advanced training to move toward sustainable livestock models.

Sustainability level of mixed production systems

The assessment of sustainability levels in the studied mixed agricultural systems showed that 50% of producers were categorized as medium, 36% as high, and 14% as low. This suggests that, although many producers have adopted sustainable practices, there are still technical and structural gaps in different parts of the production system.

When analyzing the average scores by dimension, the environmental dimension had the highest mean value (3.83±1.51), followed by the economic (3.52±1.13) and social (3.50± 1.10) dimensions. In contrast, the technical-productive (2.52±1.42) and climate resilience (2.36±1.37) dimensions scored the lowest, indicating weaknesses in livestock planning, the use of appropriate technologies, and adaptation strategies for extreme climatic events.

The economic dimension had an average score of 3.52 points, with scores ranging from 0 to 5, while the social dimension reached the full 5 points with a similar average of 3.50. The technical-productive dimension, however, showed more variability among producers, likely due to differences in access to resources and basic infrastructure. Climate resilience performed particularly poorly, highlighting an urgent need to enhance capacities in drought management, water harvesting, and climate change adaptation.

These results clearly highlight areas for improvement that should be tackled through technical support strategies, building local capacities, and tailored policy support for mixed agricultural systems in the region.

Figure 1 displays the distribution of the sustainability level achieved by producers based on their gender. It shows that both men and women are present across all three levels (low, medium, and high); however, women tend to be more concentrated in the medium and high levels, while the low level is more commonly associated with men.

This pattern indicates a potential positive impact of women's involvement in training and organizational activities within the Farmer Field Schools. Several studies have shown that rural women, when actively engaged in resource management, tend to adopt agroecological practices more effectively, especially those related to water efficiency, waste management, and organizing productive activities at the family level (Altieri & Toledo, 2011; Van den Berg *et al.*, 2020).

Tabla 1. Descriptive statistics by sustainability dimension

Dimension	Media	DE	Minimum	Maximum
Economic	3.52	1.13	0	5
Environmental	3.83	1.51	0	6
Social	3.5	1.10	1	5
Technical-productive	2.52	1.42	0	5
Climate resilience	2.36	1.37	0	5

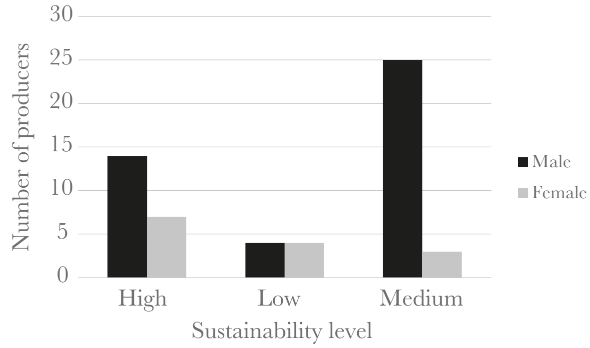

Figure 1. Sustainability level by gender of mixed farming system producers.

Furthermore, the observed trend aligns with research recognizing the strategic role of women as agents of change in diverse agricultural systems, especially when they participate in collective learning spaces and technical training. Their holistic approach to production —which combines economic, social, and environmental factors— may explain the improved performance in sustainability indicators.

Although the sample size does not allow for establishing causal relationships, these findings emphasize the importance of promoting greater inclusion and empowerment of women in rural extension programs, recognizing their potential to contribute to more sustainable and resilient systems.

Figure 2 illustrates the relationship between the producers' education level and the sustainability level achieved in their production systems. A positive trend is evident: the higher the education level, the larger the proportion of producers in medium and high sustainability categories. Conversely, lower sustainability levels tend to be concentrated among producers with only basic education or no formal schooling.

This finding aligns with previous studies that identify human capital as a crucial factor in adopting innovations, acquiring technical knowledge, and adapting to adverse conditions (Pretty, 2008; Astier *et al.*, 2012). Higher education levels improve access to, understanding

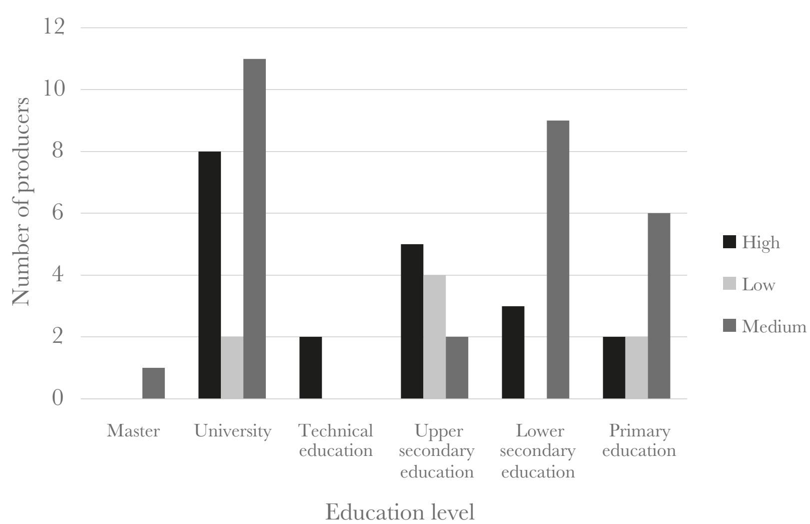

Figure 2. Sustainability level by education level of the producers.

of, and application of concepts related to good agricultural practices, technical planning, and climate adaptation strategies.

Furthermore, producers with higher levels of education often have better access to information, support networks, and effective management tools, which leads to more informed and efficient decisions. In the context of Farmer Field Schools, this may indicate a greater willingness to participate actively in training sessions and a better understanding of the content presented.

These results emphasize the importance of creating tailored training strategies that consider the existing educational gaps in rural areas. Modifying teaching methods to different levels of understanding can help reduce disparities in adopting sustainable practices and boost the effectiveness of interventions in communities with limited access to formal education.

Multivariate analisis

Figure 3 presents the Principal Component Analysis (PCA) ordination of the production units, showing an overall separation among sustainability levels (high, medium, and low) across the first two components.

Logistic regression model

The model showed that agricultural land area was the only statistically significant predictor variable ($p<0.05$), with a positive coefficient indicating that larger land areas increase the likelihood that the producer would be classified at a higher level of sustainability. This could be due to greater resource availability, allowing for the implementation of agroecological practices, crop diversification, or access to financing. The goodness-of-fit statistics indicated that the model had a McFadden's pseudo-R^2 of 0.21 and an Akaike Information Criterion (AIC) value of 154.67, suggesting an acceptable fit and explanatory power for the data.

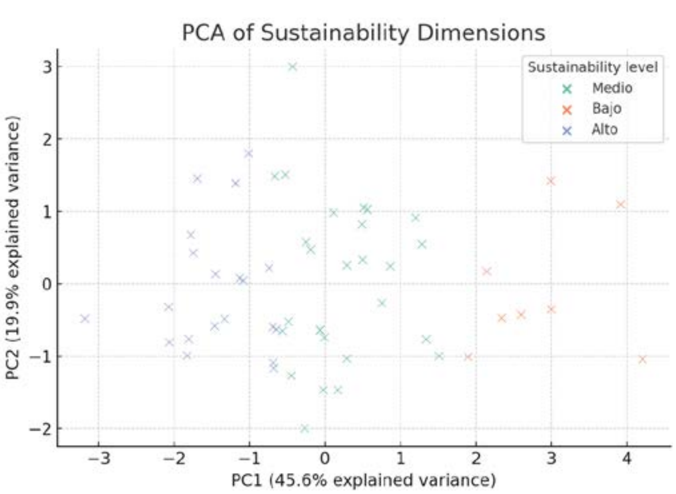

Figure 3. Principal Component Analysis (PCA) of sustainability dimensions, showing the distribution of production units according to sustainability level (high, medium, low). The first two components explain 45.6% (PC1) and 19.9% (PC2) of the total variance.

Although variables such as education level, technical support, and climate change adaptations were not statistically significant, their coefficients were positive, indicating a potential favorable link with sustainability. These findings align with previous research emphasizing the importance of human capital and access to extension services in supporting the shift toward sustainable systems.

Producer age and livestock type did not have significant effects, indicating that other structural and contextual factors might play a more crucial role in determining the sustainability level of mixed-system producers.

The findings of this study emphasize the complexity of the factors affecting the sustainability of mixed farming systems in rural areas. The importance of agricultural land size aligns with previous studies showing that access to larger land parcels enables farmers to diversify crops, adopt good agricultural practices, and implement more sustainable technologies (Astier *et al*., 2012; Altieri *et al*., 2015).

On the other hand, although variables such as education level, technical assistance, and climate change adaptations were not statistically significant, their positive effects support the literature highlighting the role of human capital and technical support in enhancing resilience and sustainability in rural systems (Pretty, 2008; Gliessman, 2014).

Despite their theoretical relevance, variables such as education level and climate change adaptation practices were not statistically significant, possibly due to limited sample size or heterogeneity in their implementation across producers.

The lack of significance of the "livestock type" variable may stem from the high diversity of species and management practices among the surveyed producers. Similarly, producer age —usually linked to experience— did not clearly influence sustainability, indicating that other institutional or market factors might be affecting its impact. Several studies have demonstrated that diversification in mixed systems not only enhances environmental resilience but also boosts food production and improves system performance. At the farm level, effectively integrating crops and livestock has been shown to improve the balance between productivity and sustainability (Puech & Stark, 2023).

These results indicate that strategies to improve the sustainability of mixed systems should focus on both increasing access to productive resources and enhancing the technical and organizational skills of producers. Additionally, they highlight the importance of further analyzing contextual factors that either hinder or support the shift toward more resilient and sustainable systems.

Tabla 2. Results of the ordinal logistic regression model

Variable	Coefficient	p-value	Significance
Farmer's age	−0.034	0.158	Not significant
Sex	1.004	0.321	Not significant
Education level	0.195	0.380	Not significant
Agricultural área (ha)	0.052	0.038	Significant ($p<0.05$)
Type of livestock	−0.013	0.906	Not significant
Climate change adaptation practices	0.644	0.477	Not significant
Technical assitance for adaptation	0.656	0.382	Not significant

CONCLUSIONS

This study offers a detailed analysis of the sustainability of mixed farming systems in Zacatecas, Mexico, based on a structured survey of producers involved in Farmer Field Schools. Agricultural land area emerged as the most significant positive factor influencing sustainability, emphasizing the key role of access to productive resources in rural areas. Although variables like education level, technical assistance, and climate adaptation strategies were not statistically significant, their positive trends suggest that they should be considered in future efforts.

These findings can inform public policies focused on strengthening rural extension services, technical assistance, and agricultural resource management, especially for producers participating in training programs like Farmer Field Schools. Additionally, longitudinal studies are advised to track the evolution of sustainability over time and evaluate the effects of different intervention strategies.

The results of this study can be used to develop effective support models and training strategies customized to local contexts, helping to promote a sustainable and resilient transition of mixed farming systems. These findings are relevant not only for Zacatecas but also for other semi-arid regions in Latin America facing similar constraints, and can guide targeted interventions to strengthen mixed farming systems.

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