

# AGRO PRODUCTIVIDAD



Año 18 • Volumen 18 • Número 9 • suplemento, 2025

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

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# Traffic Light System Methodology (TLSM): a versatile methodology for the diagnosis, monitoring, and auditing of the agroecological transition of oil palm (*Elaeis guineensis* Jacq.) agroecosystems in Mexico

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## ABSTRACT

**Objective:** To describe and identify the relationships of Good Agricultural Practices (GAP) and verify the versatility of the Traffic Light System Methodology (TLSM) for the diagnosis, monitoring, and auditing of 13 agroecological objectives, as well as its scales of application at different levels for the agroecological transition.

**Design/methodology/approach:** The process included the results of various field practices carried out during the First International Diploma in Agroecological Oil Palm Production. Document analysis and group discussions were conducted to identify the transition of agricultural and food systems and the development of agricultural resilience at three scales of application and four out of the five levels for the transition to sustainable food systems.

**Results:** The versatility of the TLSM in oil palm agroecosystems was confirmed for the diagnosis, monitoring, and auditing of the implementation of agroecological principles, across its three scales of application and at least four of the five levels of the agroecological transition.

**Limitations of the study/implications:** The application of the methodology in another agroecological palm system.

**Findings/conclusions:** The 13 pillars of GAP analyzed through the TLSM address 12 of the 13 agroecological principles necessary for the transition of agricultural and food systems to achieve global food and nutrition security and to develop agricultural resilience through climate change adaptation, along with their corresponding application scales and four of the five levels for the transition to sustainable food systems. The TLSM proves to be suitable for diagnosing, monitoring, and auditing the implementation of agroecological principles in oil palm agroecosystems.

**Keywords:** Good agricultural practices, agroecological principles, application scales, transition levels, oil palm.



## INTRODUCTION

Agroecological approaches combined with various innovations have gained prominence in scientific, agricultural, and political discourse in recent years, suggesting pathways to transform agricultural and food systems that address environmental degradation and biodiversity loss. A profound transformation is thus needed to confront the challenges of persistent malnutrition and rural poverty, further exacerbated by the growing consequences of climate change (CSA 2025; GIZ 2023; Wezel *et al.*, 2020; HLPE, 2019). Pohlen *et al.* (2023) propose that Good Agricultural Practices (GAP) in oil palm (*Elaeis guineensis* Jacq.) agroecosystems should lead to an agroecological transition of these systems, based on strategic planning. These authors summarize that the Traffic Light System Methodology (TLSM) is a transparent and precise tool aimed at all actors in the oil palm production sector, whether smallholders, medium, or large producers. Its purpose is to diagnose, monitor, and audit the state of the art of GAP as a key component in agricultural enterprise management. This methodology comprises 13 pillars, which are described and analyzed in Chapter III of the Manual for Oil Palm Producers in Mexico (Marroquín *et al.*, 2023). A total of 13 principles were defined by Wezel *et al.* (2020), based on literature that views agroecology as a science, a set of practices, and a social movement (HLPE, 2019). These principles align well and are complementary to the 10 elements of agroecology developed by FAO (2018). The first seven principles correspond to the agroecosystem scale and the first three levels of the transition toward sustainable food systems proposed by Gliessman (2007), while principles 9 through 13 relate to the food system, with principle eight serving as the central axis. The dialectical interactions between the Sustainable Development Goals, the 2030 Agenda, planetary boundaries, and GAP outcomes in oil palm agroecosystems through the TLSM are analyzed in Marroquín *et al.* (2024) and Salazar *et al.* (2023). This qualitative research aims to describe and identify the relationships between the results of the 13 pillars of GAP analyzed through the TLSM during the First International Diploma in Agroecological Oil Palm Production in Soconusco, Chiapas, as presented in the Manual for Oil Palm Producers in Mexico, and the 13 agroecological principles for the transition of agricultural and food systems to achieve global food and nutrition security and to develop agricultural resilience through climate change adaptation, with their respective application scales and the five levels of transition toward sustainable food systems. Another objective is to confirm the versatility of the Traffic Light System Methodology for diagnosing, monitoring, or auditing the 13 agroecological objectives and their application scales across the different levels of the agroecological transition.

## MATERIALS AND METHODS

This study complements the one published by Marroquín *et al.* (2024), which originated from the preparation of various classes and sessions during the “International Diploma in Agroecological Oil Palm Production in Soconusco, Chiapas, Mexico,” where the state of Chiapas, the history of agriculture in the Soconusco region, and the TLSM were described.

### Methodological Approach, Scope, and Design

The methodological approach consisted of utilizing the results of various field practices conducted during the “First International Diploma in Agroecological Oil Palm Production,” which enabled a qualitative and participatory analysis of the 13 pillars of the TLSM and their relationship with the five levels for the transition to sustainable food systems and the 13 agroecological principles for the transformation of agricultural and food systems toward achieving global food and nutrition security and developing agricultural resilience through climate change adaptation.

The methodological perspective is qualitative and was based on document analysis and discussion groups. Four focus group discussions were held with the 42 producers who participated in the international course on agroecological oil palm management conducted at the Autonomous University of Chiapas. The scope of this qualitative methodological perspective is correlational, with a non-experimental design.

### Pillars and Components of the Traffic Light System Methodology in Oil Palm Agroecosystems

The identification and utilization of the 13 pillars and their 55 components provide a scientific-practical analysis of the processes involved in the agroecological transition of oil palm agroecosystems (Table 1).

**Table 1.** Pillars of the TLSM in oil palm agroecosystems with their respective components (Marroquín *et al.*, 2023).

Pillar	Component
<b>Pillar 1.</b> History and organization of the agroecosystem by plot or lot	Lack of mapping or georeferencing
	Absence of soil analysis for heavy metals
	Climate and carbon footprint
	Topography or location
	Infrastructure
	Management of the stages of the oil palm production life cycle
<b>Pillar 2.</b> Origin of seeds and cultivars	Non-certified or non-elite seeds and/or plants
	Insufficient quality of pre-germinated seeds
	Bag size and substrate quality
	Cultivars
<b>Pillar 3.</b> Establishment of the nursery and quality of the seedlings	Presence of pests and diseases
	Pruning the root system
	Suitable plants for transplanting
	Well-nourished plants
<b>Pillar 4.</b> Agroecological soil management	Irrigation and drainage for the pre-nursery and nursery
	Slopes greater than 15 degrees
	Erosion exists
	Runoff prevention and good drainage
	Accumulation of organic matter (OM) and carbon within the soil

**Table 1.** Continues...

Pillar	Component
<b>Pillar 5.</b> Oil palm transplantation and crop establishment systems	Establish strip palm grove systems and combine them with intercropping strips
	Take advantage of different existing strata and their diversity
	Oil palm transplantation
<b>Pillar 6.</b> Management of oil palm systems and associated crops	Forest felling, monoculture, and ecological footprint
	The use of protective equipment is not guaranteed
	Management of systems with associated crops
<b>Pillar 7.</b> Weed management	Herbicides restricted by importing countries
	The use of protective equipment is not guaranteed
	Over-application rates
	Number of applications per year
<b>Pillar 8.</b> Nutrition or fertilization management	Inadequate storage conditions
	Use of dirty containers
	Relationship between fertilizer type and Need = Knowledge of the phenology of the oil palm
	Application rates
<b>Pillar 9.</b> Pest and disease management	Inadequate storage conditions
	Use of dirty containers
	Products not permitted by importing countries
	Lack of guaranteed use of protective equipment
	Over-application rates
<b>Pillar 10.</b> Irrigation, water footprint, drainage, associated crops and diversification	Irrigation with contaminated water
	Absence of strategies to reduce the water footprint
	Agroforestry systems
	Diversification of production and services in the short, medium, and long term
<b>Pillar 11.</b> Management of sanitation pruning and cleaning of oil palm	Lack of guaranteed use of protective equipment
	Sanitary and weed control in the oil palm
	Recycling of pruning waste
<b>Pillar 12.</b> Management of Harvest and Primary Transport of Fresh Fruit Bunches (FFB)	Inadequate means of transportation
	Inadequate tools and techniques for harvesting bunches
	Inadequate timing for transporting bunches to the field
	Organization and pace of harvesting bunches
<b>Pillar 13.</b> Social and Corporate Responsibility (SCR): social support, training and capacity building, agricultural and accounting records	Minors allowed to work
	Absence of agricultural and accounting records
	Poor food and housing quality
	Social security and safety are not guaranteed Labor
	Bonuses, Transportation, and Tours
Training and Training	

### Description of the 13 agroecological principles for the transition of agricultural and food systems

The 13 agroecological principles proposed by Wezel *et al.* (2020), along with their scales of application, are summarized by plot (P), agroecosystem (A), and food system (FS). Details are presented in Table 2.

### Description of the levels of agroecological transition

The following is a summary of the five levels for the transition toward sustainable food systems proposed by Gliessman (2007):

**Table 2.** Principles and scale of application of the 13 proposed agroecological principles (according to Wezel *et al.*, 2020).

Principle	Scale of application
1. <b>Recycling:</b> Preferably use locally renewable resources and close nutrient and biomass resource cycles as much as possible.	P and A
2. <b>Input reduction:</b> Reduce or eliminate dependence on purchased inputs and increase self-sufficiency.	A and FS
3. <b>Soil health:</b> Ensure and improve soil health and function to enhance plant growth, particularly through organic matter management and improving soil biological activity.	P
4. <b>Animal health:</b> Ensure animal health and well-being.	P and A
5. <b>Biodiversity:</b> Maintain and enhance species diversity, functional diversity, and genetic resources, and thus maintain the overall biodiversity of the agroecosystem over time and space at the field, farm, and landscape scales.	P and A
6. <b>Synergy:</b> Enhance positive ecological interactions, synergies, integration, and complementarity among agroecosystem elements (animals, crops, trees, soil, and water).	P and A
7. <b>Economic diversification:</b> Diversify agricultural incomes by ensuring farmers have greater financial independence and opportunities to add value, while enabling them to respond to consumer demand.	A and FS
8. <b>Knowledge co-creation:</b> Enhance co-creation and horizontal knowledge sharing, including local and scientific innovation, especially through farmer-to-farmer exchanges.	A and FS
9. <b>Social values and diets:</b> Build food systems based on the culture, identity, tradition, and social and gender equity of local communities that provide healthy, diversified, seasonally and culturally appropriate diets.	A and FS
10. <b>Justice:</b> Support decent and robust livelihoods for all actors involved in food systems, especially small-scale food producers, based on fair trade, fair employment, and fair treatment of intellectual property rights.	A and FS
11. <b>Connectivity:</b> Ensure proximity and trust between producers and consumers by promoting fair and short distribution networks and reintegrating food systems into local economies.	A
12. <b>Land and natural resource governance:</b> Strengthen institutional arrangements for improvements, including the recognition and support of family farmers, smallholder farmers, and peasant food producers as sustainable stewards of natural and genetic resources.	A and FS
13. <b>Participation:</b> Promote social organization and greater participation in decision-making by food producers and consumers to support decentralized governance and adaptive local management of agricultural and food systems.	FS

1. Increase input use efficiency to reduce the reliance on costly and environmentally harmful inputs.
2. Substitute conventional inputs and practices with agroecological alternatives.
3. Redesign: diversification through integrated plant and animal systems.
4. Re-establish a more direct relationship between food producers and consumers.
5. Build a new global food system based on equity, participation, and justice to enable the transformation of agroecosystem

### **Presentation and analysis of results**

The results are presented in a matrix or double-entry table that associates or relates each TLSM pillar with the 13 agroecological objectives (D: Directly addressed, I: Indirectly addressed, : Not addressed), along with their corresponding scale of application (P: Plot, A: Agroecosystem, and FS: Food System at the family, local, and international levels) and the levels of agroecological transition (1, 2, 3, 4, and 5).

### **RESULTS AND DISCUSSION**

The 13 Pillars for the Diagnosis, Monitoring, and Auditing of GAP in Oil Palm Agroecosystems through the TLSM (Table 3) address 12 of the 13 agroecological principles proposed by Wezel *et al.* (2020), representing 92.3% of these principles. Principle 12, related to “land and natural resource governance” (Tables 2 and 3), is the only agroecological principle not covered by the TLSM pillars in oil palm agroecosystems in Soconusco, Chiapas, Mexico, corresponding to 7.7%. Based on these results, it is essential that Pillar 13 (Social and Corporate Responsibility) of the TLSM (Table 1) includes an additional component to diagnose, monitor, and audit “the recognition and/or support of family farmers, smallholders, and peasant food producers as sustainable managers of natural and genetic resources,” which certifies the diagnosis, monitoring, and auditing of the social, corporate, and environmental responsibility of oil palm growers. With the inclusion of this component in Pillar 13 of the TLSM, all 13 agroecological principles proposed by Wezel *et al.* (2020) will be addressed, directly and/or indirectly, which are vital for the transition of agricultural and food systems, achieving global food and nutrition security, and developing agricultural resilience through climate change adaptation, as they are connected to the five levels for the transition to sustainable food systems proposed by Gliessman (2007). The TLSM pillars most strongly related or associated, directly and/or indirectly (Table 3), with the 13 agroecological principles are Pillar 1 (history and zoning of the agroecosystem by plot or lot), Pillar 10 (irrigation, water footprint, drainage, agroforestry systems, and diversification), Pillar 6 (management of oil palm systems and associated crops), Pillar 4 (agroecological soil management), Pillar 5 (transplant systems for oil palm and establishment of associated or intercropped crops), Pillar 7 (weed management), Pillar 8 (nutrient or fertilization management), and Pillar 11 (pruning management and sanitation of oil palm), which cover 7, 7, 6, 5, 5, 5, 5, and 5 agroecological principles respectively. These eight TLSM pillars represent 61.5% of the total, and collectively address, either directly or indirectly, 11 agroecological principles, which equates to 84.6% of the total (Table 3). From this, it can be inferred that these eight TLSM pillars contribute most significantly to the

diagnosis, monitoring, and auditing of the implementation of agroecological principles in the transition of oil palm agroecosystems in Soconusco, Chiapas, Mexico.

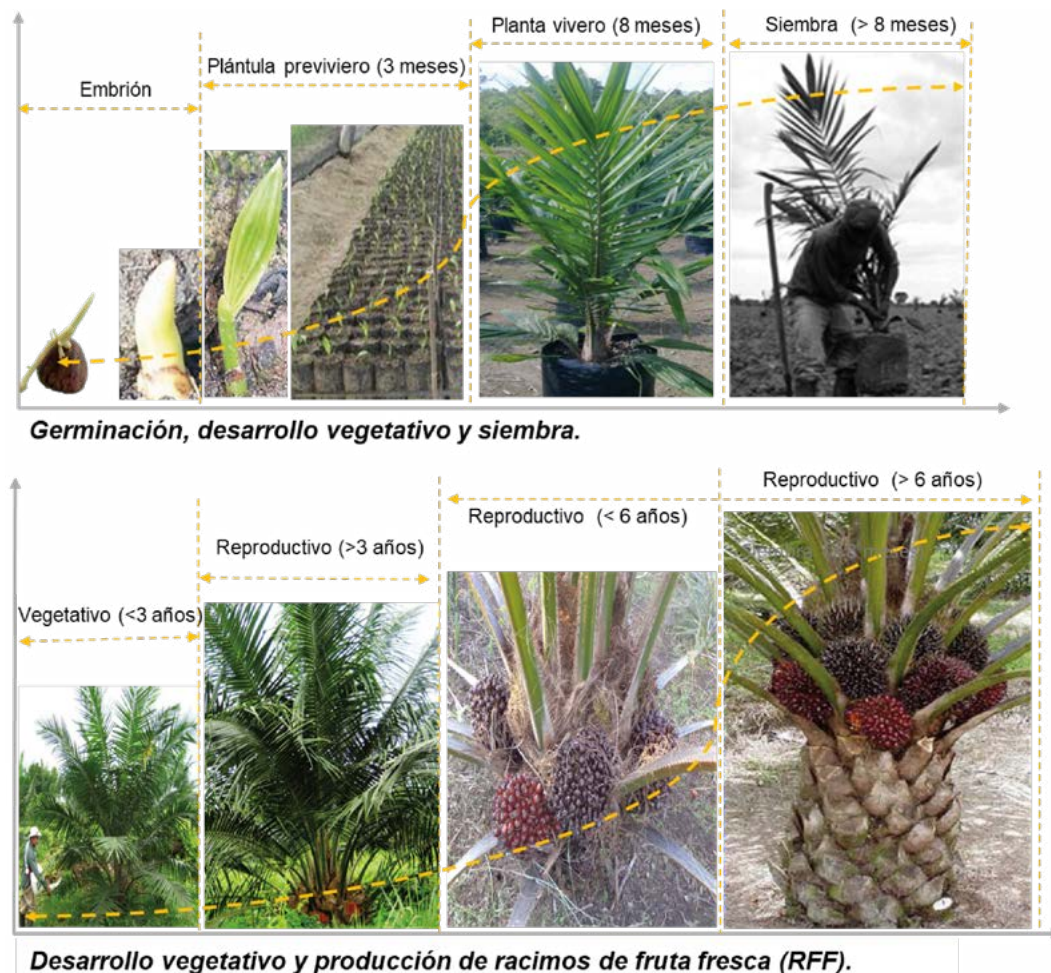
The pillars 2 (origin of seeds and cultivars), 9 (pest and disease management), and 13 (social and corporate responsibility) each include, directly and/or indirectly, four agroecological principles (Tables 1, 2, and 3), while Pillar 3 (nursery establishment and seedling quality) encompasses three agroecological principles (Figure 1). The only pillar that includes, indirectly, two agroecological principles is Pillar 12 (harvest management and primary transport of fresh fruit bunches).

The most represented agroecological principles, directly and/or indirectly, within the TLSM pillars are Principle 5 (biodiversity), Principle 6 (synergy), and Principle 3 (soil health), found in 11, 11, and 9 TLSM pillars respectively (Tables 1, 2, and 3). These three principles significantly enhance the resilience of agroecosystems (HLPE, 2019; Tittonell, 2019). Additionally, the findings confirm that soil health is closely linked to biodiversity and species synergies, which are manifested in various forms of symbiosis (mutualism, commensalism, parasitism) interactions between two or more biological species. This relationship fosters the rehabilitation and improvement of soil health and quality in oil palm agroecosystems, making it feasible to reduce inputs (Principle 2) and promote social values and diets (Principle 9) that support healthy food systems at family, local, and international levels. These two principles are addressed, directly or indirectly, in seven and eight TLSM pillars respectively (Tables 1, 2, and 3). Recycling (Principle 1) reusing local renewable resources and closing nutrient and biomass cycles as much as possible is addressed, directly

**Table 3.** Pillars of the Traffic Light System Methodology (TLSM), Agroecological Principles Proposed by Wezel *et al.* (2020), and the Five Levels for the Transition Toward Sustainable Food Systems Proposed by Gliessman (2007).

Pillars TLSM	Agroecological principles														D/T ratio	Scale of application	Level for agroecological transition
	1	2	3	4	5	6	7	8	9	10	11	12	13				
Pillar 1	-	-	I	-	I	I	-	D	I	D	D	-	-	3/7	A	1 and 4	
Pillar 2	-	I	-	-	D	I	-	-	I	-	-	-	-	1/4	P, A and FS	1 and 3	
Pillar 3	-	-	-	-	I	I	-	-	D	-	-	-	-	1/3	P and A	1	
Pillar 4	D	I	D	-	D	I	-	-	-	-	-	-	-	3/5	P and A	1	
Pillar 5	-	-	I	-	D	I	D	-	D	-	-	-	-	3/5	P, A and FS	1 and 3	
Pillar 6	I	I	I	-	D	I	I	-	-	-	-	-	-	1/6	P, A and FS	1 and 3	
Pillar 7	-	I	I	-	D	I	-	-	D	-	-	-	-	2/5	P, A and FS	1	
Pillar 8	-	I	I	-	D	I	-	-	D	-	-	-	-	2/5	P and A	1	
Pillar 9	-	-	I	-	D	I	-	-	D	-	-	-	-	2/4	P, A and FS	1	
Pillar 10	I	I	D	I	D	D	D	-	-	-	-	-	-	4/7	P, A and FS	1 and 3	
Pillar 11	D	I	D	-	I	I	-	-	-	-	-	-	-	2/5	P and A	-	
Pillar 12	-	-	-	-	-	-	-	-	-	-	I	-	I	0/2	FS	1	
Pillar 13	-	-	-	-	-	-	I	-	D	D	-	-	D	3/4	P, A and FS	1 and 5	
D/T ratio	2/4	0/7	3/9	0/1	7/11	1/11	2/4	1/1	6/8	2/2	1/2	0/0	1/2				

D: Directly addressed, I: Indirectly addressed, -: Not addressed, T: Total, P: plot or field; A: Agroecosystem, FS: Food system; levels of agroecological transition (1, 2, 3, 4, and 5).



**Figure 1.** Stages of the vegetative and reproductive life cycle of oil palm plants (own elaboration).

or indirectly, in four TLSM pillars (Tables 1 and 3): Pillars 4, 6, 10, and 11. Similarly, Principle 7, linked to economic diversification to enable oil palm producers to respond to consumer demand, is addressed in Pillars 5, 6, 10, and 13. Animal health (Principle 4) is indirectly addressed in Pillar 10 (irrigation, water footprint, agroforestry systems, and diversification), particularly in systems integrating poultry and small livestock (oxen, buffalo, pigs, and sheep). Co-creation of knowledge (Principle 8) is directly addressed only in Pillar 1 (history and zoning of the agroecosystem by plot), which includes infrastructure components to ensure basic services and connectivity that enable active interaction across social networks, platforms, and information and communication technologies among producers, agricultural collaborators, and oil palm product consumers at local, national, and international levels. These results suggest that the primary levels of application of the 13 TLSM pillars are at the agroecosystem (A) and/or plot (P) scale, since the agroecological principle of justice, connectivity, and participation mainly refers to the food system (FS) level. Thus, the three scales of application of the 13 agroecological principles proposed by Wezel et al. (2020) are covered by the TLSM pillars (Table 3). 84.6% and 92.3% of the

TLSM pillars correspond to the plot (P) and agroecosystem (A) levels respectively (Table 3), reaffirming this inference. The food system (FS) level at local or international scales is diagnosed, monitored, and audited in 61.5% of TLSM pillars.

Four out of the five levels for agroecological transition proposed by Gliessman (2007) are included in the 13 TLSM pillars (Table 3). Notably, Level 1 (increase input efficiency to reduce reliance on costly and harmful inputs) is the most represented, with 92.3% of pillars addressing it (Table 3). Only Pillar 11 (management of pruning and sanitation in oil palm) does not address any transition level.

The only transition level not directly addressed by the 13 TLSM pillars is Level 2 (replace conventional inputs and practices with agroecological alternatives). However, oil palm farmers who produce their own biostimulants, organic fertilizers (solid and/or liquid), and plant-based products and incorporate them in agroecological soil management (Pillar 4), fertilization (Pillar 8), and pest and disease control (Pillar 9) fulfill Level 2 criteria.

Level 3 (Redesign) is represented in 30.7% of TLSM pillars: Pillar 2 (nursery establishment and seedling quality), Pillar 5 (transplant systems and establishment of associated/intercropped crops), Pillar 6 (oil palm and intercropped systems), and Pillar 10 (irrigation, water footprint, agroforestry systems, and diversification).

Levels 4 (reconnecting producers and consumers) and 5 (building a new global food system based on equity, participation, and justice) are included in Pillar 1 (history and zoning of the agroecosystem) and Pillar 13 (social and corporate responsibility), respectively.

The 13 TLSM pillars and their components reaffirm the proposal by Pohlen et al. (2023) that Good Agricultural Practices (GAP) in oil palm agroecosystems (*Elaeis guineensis* Jacq.) must lead to an agroecological transition rooted in strategic planning (mission, vision, objectives, principles, and actions), including:

- An economic plan covering labor, administrative activities, and risk management.
- An agro-environmental plan with practices, technologies, management methods, and scheduling to regenerate and improve the environment (recarbonization, restoration of soil health, internal and external biodiversity promotion, and nutrient cycles C, N, P, K).
- A compliance plan ensuring adherence to laws, human and labor rights, training activities, and risk management.

In summary, the application of the TLSM in oil palm agroecosystems additionally supports the diagnosis, monitoring, and auditing of the implementation of agroecological principles across all three application scales and addresses at least four of the five levels for agroecological transition, thereby confirming the versatility of this methodology.

## CONCLUSIONS

The 13 pillars of GAP analyzed through the TLSM in oil palm agroecosystems in Soconusco, Chiapas, Mexico, address 12 of the 13 agroecological principles essential for the transition of agricultural and food systems toward achieving global food and nutrition security and developing agricultural resilience through climate change adaptation,

including their respective scales of application and four of the five levels for the transition to sustainable food systems. The versatility of the TLSM in oil palm agroecosystems was confirmed for the diagnosis, monitoring, and auditing of the implementation of agroecological principles, across its three application scales and within the five levels of agroecological transition for these agroecosystems.

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# Fungicides obtained from plant extracts for the management of *Moniliophthora roreri* Cif. in *Theobroma cacao* L.

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## ABSTRACT

**Objective:** The goal of this research is to develop plant extracts and determine their characteristics with effectiveness in the regulation of *M. roreri*, which can be integrated into a management plan for cocoa production in Mexico

**Design/Methodology/Approach:** The regulatory effect of extracts of: *Pimenta dioica*, *Zingiber officinale*, *Syzygium aromaticum*, *Origanum vulgare*, *Tradescantia spathacea* and *Cinnamomum zeylanicum* on *M. roreri*, using four forms of extraction, carrying out effectiveness tests in the laboratory, and the best extracts were evaluated under field conditions.

**Results:** The hydrolates of *S. aromaticum* and *C. zeylanicum* at 20% (v/v) were established to be efficient, recording incidences of *M. roreri* in cocoa fruits of 1.18% and 1.08% respectively, with 69.6% in the absolute control. In the *C. zeylanicum* hydrolate, 17 compounds were identified. The hydrolates of *S. aromaticum*, and *C. zeylanicum* at 20% are efficient in the management of cocoa moniliasis *M. roreri*.

**Study Limitations/Implications:** The production of extracts has specific technical needs, requires investment in producing the extracts in volume and must be made more accessible to producers.

**Findings/Conclusions:** The use of these extracts on *M. roreri* in traditional or organic production systems is technically and economically feasible.

**Keywords:** *Cocoa moniliasis*, Cocoa, Plant extracts, Disease management.

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## INTRODUCTION

Cocoa (*Theobroma cacao* L.) is a tropical tree from whose fruits we obtain the seeds from which chocolate is made, as well as the cocoa butter used in the pharmaceutical and food industries. It is estimated that worldwide more than 20 million people depend directly on this crop for subsistence and 90% of the production is harvested from smallholdings (less than 5 hectares). It is mainly grown in 13 countries, of which Ivory Coast, Cameroon,



Ghana, Malaysia, Indonesia, and Brazil obtain 80% of the production. For Mexico, this crop is of great historical, cultural, social, productive, and environmental importance, since it is grown in biodiverse agroforestry systems, which provide multiple ecosystem benefits. Currently, 52,449 hectares are planted in Mexico, located in Tabasco, Chiapas, and Oaxaca, generating more than eight million wages per year, with a contribution of 28,119 tons (SIAP, 2023). Cocoa moniliasis caused by the fungus *Moniliophthora roreri* affects all stages of fruit development. This disease has its first damage reports in Colombia and to date has affected cocoa plantations in 11 different countries in South and Central America. It entered Mexico in 2005, causing production losses, destruction and abandonment of the crop by thousands of producers, causing negative effects on the residents who depended on this crop, a shortage of cocoa, and adverse effects on the ecosystems, since the destruction of biodiverse plantations caused changes in land use, mainly for livestock, sugarcane, African palm, mango, among other monoculture systems (Phillips & Wikinson, 2007; Ramírez, 2008). The production statistics indicate that in 2006, one year after the entry of *M. roreri*, production reached a historical decrease of 50.95% and currently there is a reduction in production and planted hectares of 36% compared to the year prior to its entry to Mexico (SIAP, 2023). Various research works have shown that the management of moniliasis requires precise cultural management, and that the applications of chemically synthesized fungicides can allow a certain degree of control of the pathogen, but with a considerable increase in production costs and this causes environmental deterioration (Ochoa *et al.*, 2017). The use of plant extracts has been shown in various research works to be a highly effective input to manage various phytosanitary problems (Ranasinghe, 2002; Matan & Matan, 2007; Gupta *et al.*, 2008; Tamayo *et al.*, 2016), but it is necessary carry out further research that generates efficient and economically viable alternatives that can be easily carried out by the producer, as well as carry out studies to determine the secondary metabolites that exert activity on phytopathogenic microorganisms and that contribute to the advancement of knowledge of this control alternative (Ramírez *et al.*, 2006). This work aimed to develop and characterize plant extracts that are effective in the management of *M. roreri* which can be incorporated into an organic cocoa production system, for which it was proposed to establish the regulatory effect on *M. roreri* of six plant extracts: *Pimenta dioica* L., *Zingiber officinale* Roscoe, *Syzygium aromaticum* L., *Origanum vulgare* L., *Tradescantia spathacea* Swartz, and *Cinnamomum zeylanicum* Nees.

## MATERIALS AND METHODS

**Location of the study area.** For the development of the research, three phases were proposed: the first in the laboratory, to carry out the selection and determination of the minimum inhibitory concentration of the extracts obtained from six plants with four forms of extraction. The second phase was developed in the field in a monoclonal cocoa plantation in Tapachula, Chiapas, Mexico, where the best extracts selected in the first phase were evaluated and an economic analysis was carried out. In a third phase, the possible components of the extracts that showed the best action against the pathogen were determined, using gas chromatography coupled to mass spectrometry (GC-MS).

**Biological material.** *Cinnamomum zeylanicum* Nees (leaf and bark), *Syzygium aromaticum* L. (Leaf and flowers), *Pimenta dioica* L. (Seed and Leaf) from the municipality of Pichucalco, Chiapas, Mexico, *Origanum vulgare* L. (Aerial part), *Tradescantia spathacea* Swartz (Leaves), and *Zingiber officinale* Roscoe (Rhizome) from Tapachula, Chiapas. Pathogen evaluated: *Moniliophthora roreri* 'Pichucalco' strain, isolated by the Biotechnology Laboratory of the AUDES Cacao/UNACH.

### Extraction methods

Every plant was used in the following four extraction methods:

- **Distillation:** a distiller adapted to obtain extracts was used, for which the plant material was chopped to 1 cm. together with the solvent, they were placed in the distiller kettle and the system was hermetically closed so that the continuous and constant extraction process could be carried out by applying heat and pressure up to 15 psi. With a cooling system, a liquid called hydrolate was obtained.
- **Pressurized:** A kettle with a pressure indicator was used, inside which the 1 cm-chopped plant material was placed together with the solvent and they were subjected to temperature and pressure. Upon reaching 15 psi it was maintained for 10 min, and once it cooled down, it was filtered and the liquid was obtained.
- **Aerobic fermentation:** The 1 cm-chopped plant material was placed in a glass container adapted as a biofermenter and a liter of sterile distilled water was added. It was stirred every day to induce oxygenation and fermentation. On day 14, it was filtered to obtain the liquid.
- **Anaerobic fermentation:** A hermetically closed glass container was used, adapted for the biofermentation process with a safety valve for gas outlet; the 1 cm-chopped plant material was introduced along with sterile distilled water. The fermentation process lasted 14 days; the extract was later obtained by filtration of the mixture.
- **Pathogen strain:** The strain was isolated by the Biotechnology Laboratory of the AUDES Cacao/UNACH, from infected material from the municipality of Pichucalco, Chiapas. The strain was reactivated under laboratory conditions, in Petri dishes with culture medium composed of Difco® brand potato-dextrose-agar (PDA) at  $23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ .

### Phase one. Three effectiveness evaluation methods were used

**Agar diffusion method. Test setup at 50% concentration.** Each extract was added individually to the culture medium at a concentration of 50% (volume/volume). Once the medium with the extract was prepared, a disk of the fungus was inoculated in the center of the box. They were maintained in a culture chamber under controlled conditions of  $23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ . The following were included as controls: an absolute control in which the fungus was grown in the PDA medium without any control and another mineral control (10% calcium polysulfide). The total number of treatments was 38. The inhibitory effect was quantified by: the growth of the pathogen by measuring the diameter of the mycelium within each Petri dish every 24 hours, for 12 days, and the production of spores with the support of a

Neubauer chamber (methodology described by Ramírez, 2011). The experimental unit was a Petri dish, and the treatments were distributed in a completely randomized design with five replicates. To determine the effects of the 38 treatments studied, an analysis of variance and Tukey's comparison of means test (5%) were performed on the data obtained.

**Determination of the minimum inhibitory concentration:** The extracts that showed total inhibition of the growth and development of the pathogen from the previous test at 50% were determined for the minimum inhibitory concentration; this was done by evaluating concentrations of 40, 30, 20, and 10% (volume/volume). The culture medium was prepared with PDA to which each of the extracts was added at the concentrations to be studied, then the inoculation of the pathogen was carried out. The variables evaluated were the same as those of the previous trial.

**Paper disc method.** It is a qualitative test; 100-mm Petri dishes with PDA medium were used. A disk of the fungus previously grown in PDA medium and with an incubation time of eight days was sown in the center of each box. At a distance of three cm from the disc with the fungus, 9 mm thick and 7 mm in diameter cellulose discs, previously impregnated with each of the 36 extracts, were placed. The four extraction methods for each plant and a chemical control were placed in each Petri dish. They were left to incubate in a culture chamber under controlled conditions of  $23\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  and the inhibition zones that appeared around the paper discs containing the samples were read. Four replicates were done per treatment. The chemical control with i.a. zinc dithiocarbamate, 7 mm diameter Whatman No. 2 paper discs and 50 mm Petri dishes were used.

**Kirby-Bauer paper disc method:** The PDA culture medium was prepared and kept in a water bath at  $45\text{ }^{\circ}\text{C}$ , the homogenized solution of the fungus *M. roreri* was inoculated and 15 mL was poured into 100 mm Petri dishes. Once the medium had solidified, 9 mm thick and 7 mm in diameter cellulose discs impregnated with the extracts were placed on the medium at a radius of 30 mm from the center of the dish. The chemical control was i.a. zinc dithiocarbamate. The samples were subsequently incubated at  $23\text{ }^{\circ}\text{C}$ ; after 24 hours of incubation each dish was evaluated. For the interpretation of the results regarding the activity of the extract (proposed by Monks *et al.*, 2002), the following interpretive categories are established for the diameters of the inhibition zones: (–) No activity, (+) Mild or weak activity (diameter between 7-11 mm), (++) Moderate activity (diameter between 11-16mm), (+++) Strong activity (diameter greater than 16 mm).

**Work under field conditions:** The tests were carried out on a monoclonal (Clone UNACH 130) cocoa plantation, located at  $14^{\circ} 52' 33.4''$  NL and  $92^{\circ} 21' 28.8''$  WL, at an altitude of 47 masl, in Tapachula, Chiapas, Mexico.

**Effect of extracts on germination inhibition.** In order to obtain fruits of the same age and without incidence of monilia, artificial pollination was carried out on flowers of Clone UNACH 130 and then a plastic device was placed to isolate the flowers and waited for their development. Subsequently, the inoculum of *M. roreri* was prepared and each of the extracts and calcium polysulfide were sprayed on 70-day-old fruits and covered with a polyethylene bag and a damp towel was placed in each for three days. Then, they were uncovered and inoculated with *M. roreri*, using dry conidia attached to the tip of a dissecting needle ( $9 \times 10^4$  conidia/mL), which were deposited in an area of two square

centimeters of the area previously marked with enamel and moistened with sterile water. After inoculation, the young fruits were protected in a humid chamber (according to the methodology described by Merchán, 1991). Another group of fruits was inoculated with *M. rozeri* with the same methodology and one day later the treatments were sprayed, keeping the chamber humid.

**Treatments:** The extracts obtained in the form of hydrolates of *C. zeylanicum* (bark), *Pimenta dioica* (seeds), *S. aromaticum* (flowers), and calcium polysulfide were tested, applied before and after inoculation of the fungus; an uninoculated control and one inoculated with *M. rozeri* were also included.

**Evaluation:** To finish the test, 80 days after inoculation with *M. rozeri*, destructive sampling of the ears of each treatment was carried out in order to measure the following variables: incidence, external severity (ES), and internal severity (IS).

- **Incidence:** percentage of diseased fruits in relation to the total of inoculated fruits.
- **External severity:** based on the external appearance of the fruit and the signs of the pathogen, using the scale: grade 0=healthy fruit, 1=humpback, 2=mosaic, 3=spot (necrosis), 4=mycelium up to 25% of the spot, 5=mycelium in more than 25% of the spot (adapted from Brenes, 2003).
- **Internal severity:** it is the percentage of internal necrosis observed in the fruit when it is cut longitudinally and measured with a scale developed by Sánchez *et al.* (1982).

**Experimental design:** A completely randomized design was used with four treatments and 10 replicates, with the experimental unit being one fruit for a total of 40 fruits for the test.

**Statistical analysis:** The Internal Severity Index (ISI) and External Severity Index (ESI) data were transformed using the formula  $(value + 0.5)^{1/2}$ . To determine if there were significant differences in each of the treatments, the analysis of variance was performed and Tukey's mean comparison test was applied at the 5% significance level, when a significant difference existed. The data were processed in the SAS program for Windows 9.0.

### Effect of extracts on the development of the disease in cocoa trees

**Conditioning of the Experimental site:** With the best extracts obtained from the laboratory phase, field tests were carried out in a commercial plantation (Clone UNACH 130) and in the organic certification process, the conditioning of the trees was carried out with cleaning, pruning work, elimination of fruits diseased by moniliasis and other diseases, and layout of the experimental plot.

**Application of extracts:** Each of the extracts was mixed with water to complete the volume corresponding to the concentration of each hydrolate. The applications were done every 15 days with a manual sprayer to all the trees in each of the plots, in the morning hours.

**Treatments:** The best extracts from Phase 1 were evaluated, hydrolates of: *S. aromaticum* 20% (v/v), *C. zeylanicum* (30% (v/v), and *P. dioica* 30% (v/v), and calcium polysulfide (10%

as chemical control. The fruits were sprayed every 15 days using spray pumps intended exclusively for this test; two controls were included: cultural management and absolute control to which no handling was done in order to appreciate the natural behavior of the disease in the test plot.

**Experimental design:** A completely randomized design was used with five replicates per treatment and six treatments. The experimental unit was one tree for a total of 30 trees per treatment throughout the evaluation period.

**Evaluation:** Evaluations were taken every 15 days from each of the trees in each treatment. The variables quantified were:

- **Incidence of the disease:** evaluating: number of: healthy young fruits, young fruits diseased by *M. rozeri*, healthy ears, ears diseased by *M. rozeri*.
- **External severity (ESI) and Internal severity (ISI):** the scale developed by Sánchez *et al.* (1982) was used.
- **Production:** every eight days, the number and total weight of the healthy fruits harvested and the dry weight of the grains were determined.

**Statistical analysis:** The incidence data obtained were transformed using the arcsine formula  $(percentage / 100)^{1/2}$  and ISI and ESI using the formula  $(value + 0.5)^{1/2}$ . An analysis of variance and Tukey's mean comparison test at 5% were performed. The data were processed in the SAS program for Windows 9.0. An economic analysis was carried out establishing the cost of production and the value of the sale of cocoa.

**Phytochemical characterization of the extracts:** Using gas chromatography coupled to mass spectrometry (GC-MS), the extracts were analyzed, characterizing their compounds, as well as their contents. The analyses were carried out at the National Laboratory for Prevention and Control of Doping-CONADE-Mexico. For the GC-MS study, a Gas Chromatograph (model 6890 N) coupled to a Mass Spectrometer (Model 5973N) was used, both from Agilent Technologies, manufactured in China, equipped with a split/splitless (12:1) injection port. Agilent 19091A-002 column, Methyl Siloxane, capillary, with the following characteristics: length 25 m, diameter 200.0  $\mu\text{m}$  with a particle size of 0.11  $\mu\text{m}$ , carrier gas, Helium, with initial flow of 1 mL/min and then constant flow. Injection volume 1  $\mu\text{L}$ . Initial temperature 60 °C, final temperature 325°C, Run time 114.67 min. For the identification of the compounds, they were compared with the NIST MS 2.0 database.

## RESULTS AND DISCUSSION

The results show that all the plants evaluated contain metabolites that are capable of inhibiting the development of the pathogen to a greater or lesser extent. *In vitro*, the most effective were the hydrolates of the flower buds of *S. aromaticum*, leaves and bark of *C. zeylanicum*, and leaves of *P. dioica* at 20% (V/V), as well as the pressurized flower buds of *S. aromaticum*, followed by hydrolates of seeds from *P. dioica*, *C. zeylanicum* bark, and *Z. officinale*, and the aerobic fermentation of *S. aromaticum* flower buds at 30% (V/V). Regarding the best way to obtain extracts, hydrolate turned out to be the method that showed the

best regulatory effect on *M. royeri*, followed by pressurization and aerobic fermentation. Of the three methods evaluated (agar diffusion, modified paper discs, and Kirby-Bauer) to determine the in vitro effectiveness of the extracts on *M. royeri*, the hydrolates of *S. aromaticum*, *C. zeylanicum*, and *P. dioica* were the extracts that showed the greatest inhibition under the three methods evaluated. Table 1 shows the main results.

Under field conditions, both for the artificial inoculation test and for application in a monoclonal cocoa plantation, the hydrolates of *S. aromaticum* and *C. zeylanicum* at 20% showed their high effectiveness in the management of cocoa moniliasis, since even with a high natural incidence of *M. royeri* (69.6%), the reduction values in the disease's impact when applying the hydrolates of *S. aromaticum* and *C. zeylanicum* in the field was 98% (Table 2), and cocoa production increased between 800 and 1000% (Table 3) with respect to the control. This allows obtaining a Benefit/Cost ratio greater than 2 and a profitability of 127.95% and 138% for the hydrolates of *S. aromaticum* and *C. zeylanicum* respectively (Table 4), indicating a high technical and economic feasibility of these alternatives within a management plan for cocoa moniliasis, whether in a traditional system or with organic management.

Regarding the *S. aromaticum* hydrolate, the phytochemical analysis recorded six peaks, of which peak four and six are not natural products, being considered impurities. The majority compound being Eugenol Acetate, the percentage of this compound removing the impurities would be 58.95%, the next compound under these same circumstances would correspond to Eugenol with 15.96%, 2-propenal, 3-(4-hydroxy-3-methoxyphenyl) with 14.84%, and finally peak two corresponding to vanilla with 10.23%. These compounds have been reported by several authors as components of the essential oil of *S. aromaticum*, although in different percentages since they report eugenol as the majority compound with 70 to 85%, and other compounds such as  $\alpha$ - and  $\beta$ -caryophyllenes, isoeugen, acetate eugenol, and small amounts of esters, ketones, and alcohols. There are also reports that clove essential oil contains 85 - 95% eugenol and acetyleneugenol (Mazzafera, 2003; Matan & Matan, 2007; Omidbeygi, 2007; Padrón, 2010; Kumar *et al.*, 2012). Achimon *et al.* (2021) reported only 9 constituents for the essential oil, eugenol being the main component (88.70%), followed by  $\beta$ -caryophyllene (6.55%).

The effect of the *S. aromaticum* hydrolate on *M. royeri* may be due to eugenol, which is a phenolic compound with antiseptic action with a recognized inhibitory effect on various organisms. Cai and Wu (1996) verified the antimicrobial potential of the non-polar extracts of *S. aromaticum* against Gram-negative oral pathogens, bacteria such as *E. coli*, and *Salmonella* strains (Di Pascua *et al.*, 2006). Dorman and Deans (2000) established its effect on 25 gram-positive and gram-negative pathogenic bacteria in cultures and humans. Mazzafera (2003) also reported its effect on nematodes and insects, and as an antiviral.

Padrón (2010) found that fungi were the most susceptible microorganisms to the fraction with antimicrobial activity of the hexane extract of *S. aromaticum*, whose main constituent was eugenol. It was also observed that the most susceptible fungi were the filamentous ones, mainly *Trichophyton tonsurans* and *Sporotrix schenckii*. These antifungal qualities of *S. aromaticum* on filamentous strains have also been described

**Table 1.** Comparison of three methods for evaluating the regulatory effectiveness of plant extracts and chemical synthesis products on the growth of *M. royeri*.

Extract	Dilution method		Paper Disc Methods (Degrees of inhibition)	
	50 %	CMI	Modified Kirby-Bauer	Paper discs
<i>C. zeylanicum</i> leaf hydrolate	I	20	–	+++
<i>C. zeylanicum</i> leaf pressurized	I	40	–	+
<i>C. zeylanicum</i> leaf Aerobic F.	I	50	–	+++
<i>C. zeylanicum</i> leaf Anaerobic F.	C	50	–	++
<i>C. zeylanicum</i> bark hydrolate	I	30	++	++
<i>C. zeylanicum</i> bark pressurized	C		–	+
<i>C. zeylanicum</i> bark Aerobic F.	C		–	–
<i>C. zeylanicum</i> bark Anaerobic F.	C		–	–
<i>P. dioica</i> leaf hydrolate	I	20	–	–
<i>P. dioica</i> leaf pressurized	I	50	–	–
<i>P. dioica</i> leaf Aerobic F.	C		–	–
<i>P. dioica</i> leaf Anaerobic F.	C		–	–
<i>P. dioica</i> seed hydrolate	I	30	+	++
<i>P. dioica</i> seed pressurized	I	40	–	
<i>P. dioica</i> seed Aerobic F.	C		–	–
<i>P. dioica</i> seed Anaerobic F.	C		–	–
<i>S. aromaticum</i> leaf hydrolate	I	50	–	–
<i>S. aromaticum</i> leaf pressurized	C		–	–
<i>S. aromaticum</i> leaf Aerobic F.	C		–	–
<i>S. aromaticum</i> leaf Anaerobic F.	C		–	–
<i>S. aromaticum</i> seed hydrolate	I	20	+	+++
<i>S. aromaticum</i> seed pressurized	I	20	–	++
<i>S. aromaticum</i> seed Aerobic F.	I	30	–	+
<i>S. aromaticum</i> seed Anaerobic F.	I	40	–	++
<i>O. vulgare</i> hydrolate	I	50	–	–
<i>O. vulgare</i> pressurized	C		–	–
<i>O. vulgare</i> Aerobic F.	C		–	–
<i>O. vulgare</i> Anaerobic F.	C		–	–
<i>T. spathacea</i> hydrolate	I	50	–	–
<i>T. spathacea</i> pressurized	C		–	–
<i>T. spathacea</i> Aerobic F.	C		–	–
<i>T. spathacea</i> Anaerobic F.	C		–	–
<i>Z. officinale</i> hydrolate	I	30	–	–
<i>Z. officinale</i> pressurized	C		–	–
<i>Z. officinale</i> Aerobic F.	C		–	–
<i>Z. officinale</i> Anaerobic F.	C		–	–
CHEMICAL				
i.a, Zn dithiocarbamate 5 g/L	I		++	+

C=Pathogen growth I=Growth inhibition + Light inhibition ++ Moderate +++ Strong

**Table 2.** Comparison of means by Tukey's Multiple Range test of the treatment effect of *S. aromaticum*, *C. zeylanicum*, and *P. dioica* hydrolates on the incidence of *M. royeri* in a cocoa plantation.

Treatment	% Total incidence	Tukey*	% Incidence in young fruit	Tukey*	% Incidence in ears	Tukey*
Calcium polysulfide	0.5375	a	0.0763	a	0.4612	a
<i>C. zeylanicum</i> Hydrolate	1.0841	b	0	a	1.0841	b
<i>P. dioica</i> Hydrolate	1.1593	b	0.2637	b	0.8956	b
<i>S. aromaticum</i> Hydrolate	1.1852	b	0.0806	a	1.1046	b
Cultural control	21.023	c	5.2295	c	15.7939	c
Absolute control	69.641	d	19.1607	d	50.4805	d

\* Means with the same letter are not statistically different.

**Table 3.** Comparison of means by Tukey's Multiple Range test of the effect on dry cocoa production of the application of hydrolates of *S. aromaticum*, *C. zeylanicum*, and *P. dioica* for the management of *M. royeri*, in a commercial plantation.

Treatment	Kg ha <sup>-1</sup>	Tukey*
Absolute control	89.05	a
Cultural control	280.91	a
<i>P. dioica</i> Hydrolate	760.35	b
<i>S. aromaticum</i> Hydrolate	780.90	b
<i>C. zeylanicum</i> Hydrolate	917.90	b
Calcium polysulfide	928.35	b

\* Means with the same letter are not statistically different.

**Table 4.** Economic analysis of each of the treatments applied to Clone UNACH 130.

Treatment	Total costs USD	Total Income USD	B/C	Net benefit	Profitability %
<i>C. zeylanicum</i> Hydrolate	1887.50	4302.66	2.3	2415.16	127.95
<i>S. aromaticum</i> Hydrolate	1537.50	3660.47	2.4	2122.97	138.1
<i>P. dioica</i> Hydrolate	1887.50	3564.14	1.9	1676.64	88.8
Calcium polysulfide control	1337.50	4351.64	3.3	3014.14	225.4
Cultural control	868.75	1316.48	1.5	447.73	51.5
Absolute control	543.75	417.42	0.8	-126.32	-23.2

B/C=Benefit:Cost ratio.

by authors such as Amiri *et al.* (2008), who mention eugenol as a possible active agent in formulations to prevent the development of post-harvest diseases of fruits such as apples. Likewise, Costa (2011) reports the effect of *S. aromaticum* on *F. oxysporum*, and *R. solani*, and also cites Amaral & Bara (2005) who report an effect on the reduction of mycelial growth of the essential oil of *S. aromaticum* on *Rhizopus stolonifer*. Ranasinghe *et al.* (2002) demonstrate the inhibitory action of *S. aromaticum* on *Lasioidiplodia theobromae*,

*C. musae*, and *C. proliferatum*, *Fusarium*, and *Colletotrichum* in banana. Acedo-Zegarra *et al.* (2020), demonstrated 100% total antifungal activity against *Fusarium oxysporum* for a 5% concentration of *S. aromaticum* extract.

Gómez-López *et al.* (2020) report the *in vitro* effect of total inhibition of the growth of *M. roseri* from bio-oils at 500  $\mu\text{L L}^{-1}$  of *O. vulgare*, *S. aromaticum*, *T. vulgaris*, and *C. verum*, results similar to those of Tamayo *et al.* (2016). However, in the present work, greater activity was found both *in vitro* (using three analysis methods: agar diffusion, modified paper discs, and Kirby-Bauer) and in the field of *S. aromaticum*, *C. zeylanicum*, and *P. dioica* hydrolates, which managed to reduce the incidence of *M. roseri* by more than 98% and increase production and thus profitability, thus being the first reports of the regulatory effect in the laboratory and in the field of the use of vegetable extracts in the control of *M. roseri*.

The antifungal activity of the *S. aromaticum* essential oil is related to its hydrophobicity, which allows it to interact with the lipid wall, the cell membrane, and the permeability of the mitochondria, altering them and causing disturbances in these structures. Oil components can bind to ions and molecules (hormones) of other cells. Dorman & Deans (2000) report that natural antifungals cause damage to the cell membrane of cells exposed to them, leaving them extremely soluble and fractures that ultimately expose the cellular contents, including the nucleus. According to Padrón (2010), essential oils have strong antibacterial properties because they contain a high percentage of phenolic compounds such as eugenol. The above suggests that its mechanism of action is similar to that of other phenolic compounds, by altering the cytoplasmic membrane, interrupting the proton motive force (PMF), the flow of electrons, active transport, and coagulation of cellular contents. It has been found that sublethal concentrations of eugenol inhibit the production of amylase and proteases of *B. cereus*, deteriorating the cell wall, which causes cell lysis. The hydroxyl group of eugenol, to which certain proteins bind, is believed to prevent enzymatic action in *E. aerogenes*.

The effectiveness of the hydrolates of *S. aromaticum*, *C. zeylanicum*, and *P. dioica* is possibly due to their preventive effect by inhibiting the germination and multiplication of conidia, as well as the fungicide effect by destroying the conidia of *M. roseri*, which together causes the reduction of the incidence and external and internal severity of the disease. For the *C. zeylanicum* hydrolate, 17 compounds were identified, the majority being cinnamic aldehyde with 74.08%, eugenol with 6.8%, and cinnamyl acetate with 5.18%. Meanwhile for the *S. aromaticum* hydrolate, the majority compounds are eugenol acetate (58.95%) and eugenol (15.96%), which have reports of activity on various organisms, and these compounds may be the ones that exert inhibitory action on *M. roseri*.

## CONCLUSIONS

The hydrolates of *S. aromaticum*, and *C. zeylanicum* at 20% are efficient in the management of cocoa moniliasis *M. roseri*; these are technically and economically viable to incorporate into a *T. cacao* production system in Mexico.

The best way to obtain the extracts is the hydrolate, since in the evaluated plants, *P. dioica*, *Z. officinale*, *S. aromaticum*, *O. vulgare*, *T. spathacea*, and *C. zeylanicum*, this turned

out to be the method that showed the best regulatory effect on *M. royeri*, followed by pressurization and aerobic fermentation.

Even with a high natural incidence of *M. royeri* (69.6%) in a monoclonal cocoa plantation, the reduction values in the disease's impact when applying the hydrolates of *S. aromaticum*, *C. zeylanicum*, and *P. dioica*, and calcium polysulfide in the field allowed an increase in cocoa production and with a Benefit/Cost ratio greater than 2. This indicates a high technical and economic feasibility of these alternatives within a management plan for cocoa moniliasis.

The effectiveness of hydrolates from *S. aromaticum*, *C. zeylanicum*, and *P. dioica* is possibly due to the preventive effect by inhibiting the germination and multiplication of conidia, as well as the fungicide effect by destroying the conidia of *M. royeri*, which together causes the reduction of the incidence and external and internal severity of the disease.

In the case of the hydrolate, 17 compounds were identified, the majority being cinnamic aldehyde, eugenol, and cinnamyl acetate; while for the hydrolate of *S. aromaticum*, they were eugenol acetate and eugenol, which have reports of activity on various organisms, and these compounds may be the ones that exert inhibitory action on *M. royeri*.

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# Ecological condition of the riverbanks of the Cacaluta river, Chiapas, México

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## ABSTRACT

**Objective** To evaluate the bank quality of the river Cacaluta, Chiapas, Mexico, using a structural and functional approach applying the RQI index.

**Design/methodology/approach:** Twenty-six sites were evaluated based on their characteristics and location in the watershed. Surveys were performed using line transects between 180 and 550 m, and approximately 2-3 km apart. Overall.

**Results:** show a river with important degradation signs, three sites show good quality, while twenty-two sites show moderate quality, ten sites show poor quality, and one site has bad quality. The most affected attributes were width of the riverbank, longitudinal continuity, vegetation structure and composition, and vegetation diversity and regeneration. The study sites far from rural areas and of difficult access show better quality and conserved riverbank. The presence of human settlements, livestock, agriculture, and river canalization and dredging are the influential activities that result in poor quality sites.

**Limitations on study/implications:** No limitations were identified; however, further evaluations are needed.

**Findings/conclusions:** the development model implemented in the Cacaluta river basin does not represent any guarantee of sustainability for ecological functioning of the river, since the change and use of land adjacent to the riverbanks and the uncontrolled population growth implies significant pressure on riverbanks.

**Keywords:** fluvial system, riparian forest, watershed

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## INTRODUCTION

The natural value of riverbanks goes beyond physiognomic and taxonomic aspects, as they are considered important elements of landscape diversification and, above all, as biological and stabilizing components of fluvial ecosystems (Jimeno *et al.*, 2025; Corenblit *et al.*, 2015; Suárez and Vidal-Abarca, 2000).

Riparian vegetation in good condition provides multiple environmental services to fluvial landscapes, including bank stabilization, generation of aquatic and terrestrial habitats, nutrient filtering, CO<sub>2</sub> sinks, entry of food sources into the channel, regulation

of water temperature and luminosity, acting as a barrier to flood waters and generation of ecological corridors (Corenblit *et al.*, 2024; Ortiz, 2019; Palma *et al.*, 2009; Rico *et al.*, 2006).

However, riparian areas are not immune to anthropic pressure on rivers, where changes in land use for agricultural and livestock areas, channeling of the stretches on which they are located, construction of hydraulic infrastructure, and pollution by invasive species have affected their structure and functioning and, consequently, a reduction in the environmental services they provide at local and global levels (Ortiz *et al.*, 2024; Riis *et al.*, 2020; Millennium Ecosystem Assessment, 2005; Cecon, 2003). Therefore, the environmental assessment of riverbanks is nowadays a topic of great interest, and methods have been developed for a rapid and effective evaluation of the ecological state of riverbanks (Escalona-Domenech *et al.*, 2021; Feio *et al.*, 2021; González del Tánago and García de Jalón, 2006). There are several methods to estimate the quality of riparian vegetation and evaluation of the ecological state of riverbanks, such as the river habitat index (IHF) (Pardo *et al.*, 2002), the riparian forest quality index (QBR) (Munné *et al.*, 2003), Riparian Quality Index (RQI) (González del Tánago and García de Jalón, 2011), Riparian Forest Evaluation (RVF) (Magdaleno *et al.*, 2010), among others.

The RQI index collects quantitative information by evaluating seven attributes related to the structure and functioning of riparian systems, which are strongly determined by hydromorphological river dynamics (González del Tánago and García de Jalón, 2006). With the information generated by applying the RQI index, elements are provided allowing the competent authorities, civil organizations or the public to build integrated riparian vegetation management plans or schemes, which in some countries are nonexistent (Ortiz, 2019; Rodríguez-Téllez *et al.*, 2012; González del Tánago and García de Jalón, 2011).

Given its location, the Cacaluta River is of high ecological importance (CONAGUA, 2011), where the geomorphology of the area and the plant formations found in the watershed provide a high ecosystemic diversity, which is why this basin is part of the polygons of two natural protected areas (NPAs): El Triunfo Biosphere Reserve (REBITRI) and La Encrucijada Biosphere Reserve (REBIEN) with RAMSAR category (The Nature Conservancy, 2010). However, this river exhibits a significant alteration in its ecological functioning due to agricultural production practices, the conversion of natural ecosystems into productive areas and canalization works, as well as the lack of knowledge and interest shown by governments or agencies responsible for addressing this environmental situation (The Nature Conservancy, 2010; Tovilla, 2005). Therefore, the Cacaluta River, like most of the rivers of the Chiapas coast, is currently one of the most threatened ecosystems (Tovilla, 2005). The objective of this study was to evaluate the quality of the Cacaluta riverbank from a structural and functional point of view by applying the RQI index. The following hypotheses were proposed: 1) the ecological functioning of the Cacaluta River is expected to be better in areas where the riparian vegetation has not been altered than in areas where it has been, and 2) the banks of the Cacaluta River exhibit a good quality in areas within and adjacent to the natural protected areas in the upper and lower parts of the basin compared to those areas where land has been changed for use.

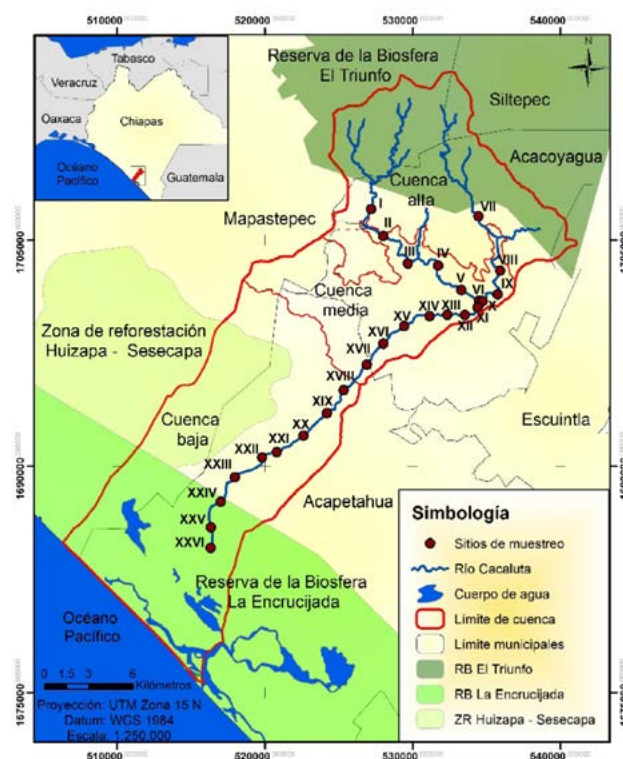
## MATERIALS AND METHODS

### Study area

The Cacaluta hydrological basin (where the river of the same name is located) belongs to hydrological region number 23: Chiapas coast. It is in the eastern part of the Sierra Madre de Chiapas and the Pacific Coastal Plain, within the coordinates 15° 32' 24" and 15° 10' 36" north latitude and 92° 56' 24" and 92° 37' 36" west longitude, with a surface area of 462.24 km<sup>2</sup> (CONAGUA, 2010) (Figure 1).

The Cacaluta River flows 51 km, with an average flow of 144.2 m<sup>3</sup>/s. From its river source in El Triunfo Biosphere Reserve (at an altitude of 2436 m.a.s.l.), it flows southwest into the wetlands of La Encrucijada Biosphere Reserve. The predominant climate in the basin according to Köppen's classification is warm humid with abundant rainfall in summer (Am(w)). The average annual rainfall is 2,911 mm with an average rainfall of 3,600 mm in the rainiest years for the period from May to October with an average temperature of 27 °C (CONAGUA, 2010).

The basin is located mainly in socioeconomic regions IX Istmo-Costa and X Soconusco, covering part of the territories of five municipalities: Acacoyagua, Acapetahua, Escuintla, Mapastepec and Siltepec, with a total of 214 localities scattered along the Cacaluta River. In total, the population density along the river is estimated to be 16,043 inhabitants according to the Population and Housing Census (INEGI, 2020). The Cacaluta river basin has remnants of mesophilic forest in the upper part along with medium sub evergreen forest that is distributed to the middle part and the presence of



**Figure 1.** Cacaluta river watershed, Chiapas. The twenty-six study sites are shown in red circles.

hydrophilic vegetation in the lower part, where the red mangrove (*Rhizophora mangle*) is the dominant species (Vásquez, 2008). In terms of land use, the watershed has a variety of crops such as African palm, mango, coffee, banana, seasonal agriculture, livestock, among others. Grasslands represent the largest extension in the basin with an area of 14131.76 ha.

### **Selection of sampling sites**

In choosing the number of sampling sites, the length of the river under study and the desired detail were considered, provided that the distance between sampling sites did not exceed 10 km, since at greater distances the aspects described would not have continuity and the resulting values would be very varied and a large part of the existing riparian vegetation would remain unevaluated (Rodríguez-Téllez *et al.*, 2012; Acosta *et al.*, 2009). As a result, 26 sampling sites were selected at an approximate equidistance of two to three kilometers, with accessibility to the sites being very important. Therefore, site VII was taken as the reference site for the REBITRI area (Figure 1). The study section was determined according to the riverbank width plus the sum of 20 m of contiguous width to the riverbed (10 m on each side) in accordance with Article 3, section XLVII of the National Water Act. The sum of both values was multiplied by five according to the Mexican standard for ecological flow in hydrological watersheds, NMX-AA-159-SCF1-2012 (SE, 2012), thus obtaining the length of the study section, which was divided in two to determine the distance to be traveled both upstream and downstream.

### **Application of RQI index**

To evaluate the ecological quality of the riverbank, the RQI index (González del Tánago and García de Jalón, 2011) was used, which integrates attributes related to the riverbank structure and functioning. The structure is characterized by the longitudinal continuity of vegetation, lateral dimensions (width) of river space containing natural riparian vegetation and composition and structure of riparian plant communities. Riparian forest functioning is reflected through the natural regeneration rate of riparian woody species, the condition of the banks, the lateral connectivity of the channel with its banks and the permeability of riparian soils.

These seven attributes were applied to the study sections (distance ranged from 180 to 550 m) throughout February and March 2014. Each of the attributes was evaluated on a scale of 1 to 15, according to its frame of reference, where the higher the score, the better the ecological condition of the riverbanks. Attributes related to riverbank structure were evaluated separately for each riverbank while attributes related to riverbank functioning were evaluated together for both banks. Each attribute was evaluated on a quality scale from 1 to 15, being very good (15-13), good (12-10), moderate (9-7), poor (6-4) and bad (3-1). Upon completion, the value of each attribute was added to determine overall assessment of riverbank quality of study section based on index ranges: very good (150-130), good (129-100), moderate (99-70), poor (69-40), bad (39-10) and very bad (<10) (González del Tánago and García de Jalón, 2011).

## RESULTS

Four of the six quality ranges for the RQI index were found in the 26 sampled sites. Stations V, VII, and VIII exhibited good bank condition (103-121 points), while site XVII exhibited a poor bank condition (39 points), mainly due to the presence of human settlements, a gabion structure for flood control, the removal of riparian vegetation to introduce crops such as oil palm, and the silting of the riverbed due to the lack of communication between the river and its floodplain (Figure 2). The other two quality ranges present were moderate and poor, the former being the one with the highest presence with 12 sites (Table 1 and Figure 3).

Regarding the attributes of the riverbank structure, the bank width exhibited significant alterations due to the change and use of adjacent land, with values ranging from 4 to 7 points mainly, with only sites V, VII and VIII showing a bank width in good condition (10-12) (Table 1). The longitudinal continuity showed a fragmentation by human actions, compromising its function as a corridor, since most of the sites exhibited a poor to moderate quality (4 to 8 points) while the composition and structure is composed by the dispersion of trees of the native or original vegetation such as *Sideroxylon portoricense* Urb., *Dendropanax arboreus* (L.) Decne. & Planch., *Calophyllum brasiliense* Cambess., *Vatairea lundellii* (Standl.) Killip ex Record, *Plumeria rubra* L., *Aspidosperma megalocarpon* Müll. Arg., among others, with an abundance in open spaces of shrub and herbaceous species with riparian preferences such as *Euphorbia graminea* Jacq., *Acalypha macrostachya* Jacq., *Boehmeria ulmifolia* Wedd., *Amaranthus spinosus* L., *Indigofera suffruticosa* Mill., *Costus pulverulentus* C. Presl, *Casearia sylvestris* Sw and *Panicum maximum* Jacq. In terms of attributes related to functioning, age and regeneration diversity of sites I, V, VII and VII exhibited all age classes (seedlings, juveniles and adults) of the main woody species mentioned above, while sites XVI to XXVI exhibited poor quality (4 to 6 points) due to channelization of the river, preventing the natural regeneration of woody species such as *Coccoloba barbadensis* Jacq., *Tabebuia rosea* (Bertol.) A. DC., *Bursera simaruba* (L.) Sarg., *Diphysa americana* (Mill.) M. Sousa, *Ficus obtusifolia* Kunth, *Platymiscium dimorphandrum* Donn. Sm., *Swietenia humilis* L., to name a few.

The condition of banks in most sites from I to XIII (except for VII) exhibited good quality (10-12) with dead wood and vegetal detritus on the sides of the riverbed, while the



**Figure 2.** From left to right: diversion of water with a barrier of sandbags, silted channel, laminar flow over a sandy bed surrounded by oil palm crops and effects of river channeling in the lower reaches. Photographs taken in the field by the author Jorge Hernández Hernández.

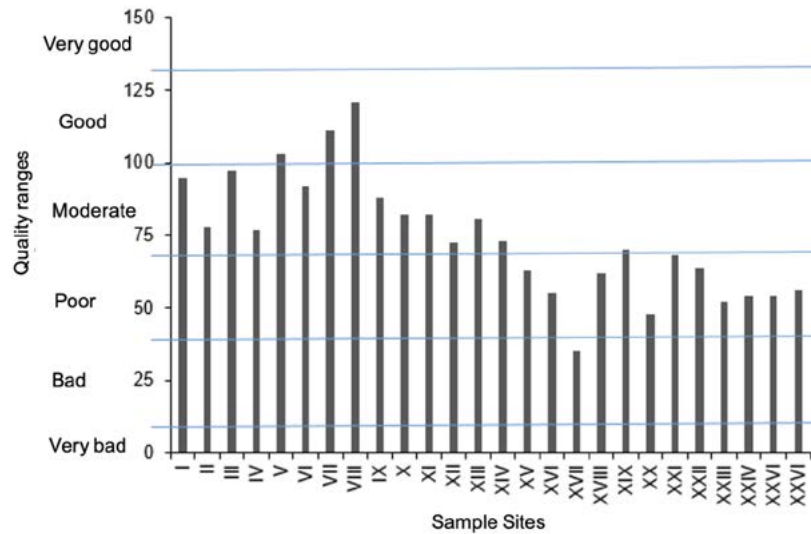
**Tabla 1.** Value for each attribute of the RQI index for the sampling sites of the Cacaluta river, Chiapas.

Sites	Attributes							Total	Basin	ANP
	AER*	CLV*	CEV*	RN	CO	CLC	PS			
I	15 (8.7)	15 (8.7)	16 (9.7)	11	12	13	13	95	High	No
II	12 (7.5)	13 (7.6)	13 (7.6)	7	10	11	12	78	Medium	No
III	15 (6.9)	19 (8.11)	20 (7.13)	9	11	12	11	97	Medium	No
IV	10 (5.5)	13 (6.7)	13 (6.7)	6	10	13	12	77	Medium	No
V	19 (13.6)	20 (13.7)	20 (13.7)	10	10	13	11	103	Medium	No
VI	13 (10.3)	16 (11.5)	17 (12.5)	8	11	15	12	91	Medium	No
VII	21 (8.13)	22 (9.13)	21(8.3)	10	11	13	13	111	High	Yes
VIII	23 (11.12)	24 (12.12)	24 (12.12)	12	12	13	12	120	Medium	No
IX	16 (8.8)	16 (8.8)	14 (7.7)	8	10	12	12	88	Medium	No
X	13 (7.6)	14 (7.7)	14 (7.7)	7	7	15	12	82	Medium	No
XI	12 (6.6)	12 (6.6)	12 (6.6)	5	13	15	13	82	Medium	No
XII	15 (7.8)	15 (7.8)	13 (6.7)	7	10	11	11	72	Medium	No
XIII	12 (6.6)	14 (7.7)	14 (7.7)	8	11	11	11	81	Medium	No
XIV	10 (5.5)	11 (6.5)	12 (7.5)	8	7	6	9	73	Medium	No
XV	8 (4.4)	15 (8.7)	10 (5.5)	8	7	6	9	63	Medium	No
XVI	8 (5.3)	11 (7.4)	8 (5.3)	4	8	6	10	55	Medium	No
XVII	7 (4.3)	6 (3.3)	6 (3.3)	3	3	4	7	36	Medium	No
XVIII	7 (4.3)	9 (5.4)	11 (5.6)	5	9	11	10	62	Low	No
XIX	13 (6.7)	13 (6.7)	12 (6.6)	8	9	5	10	70	Low	No
XX	9 (4.5)	8 (4.4)	8 (4.4)	3	7	8	5	48	Low	No
XXI	9 (3.9)	14 (6.8)	11 (4.7)	6	9	9	10	68	Low	No
XXII	8 (5.3)	12 (7.5)	12 (6.6)	6	9	8	9	64	Low	No
XXIII	9 (6.3)	9 (6.3)	9 (6.3)	5	5	5	10	52	Low	No
XXIV	6 (3.3)	12 (6.6)	8 (4.4)	4	10	4	10	54	Low	Yes
XXV	11 (6.5)	10 (5.5)	10 (5.5)	4	7	5	7	54	Low	Yes
XXVI	9 (4.5)	11 (5.6)	9 (4.5)	4	5	10	8	56	Low	Yes

Note. \* Sum of the values of both margins; AER: Width of riparian space; CLV: longitudinal continuity of vegetation; CEV: composition and structure of riparian vegetation; RN: natural regeneration rate of riparian woody species; CO: condition of banks; CLC: lateral connectivity of the channel with its banks and PS: permeability of riparian soils; ANP: Location within Natural Protected Areas.

rest of the sites (XIV to XXVI) exhibited a moderate to poor quality (5 to 7 points). The lateral connectivity and flooding of sites I to XIII exhibited the best conditions allowing flooding to the alluvial plain while the rest of the sites were cataloged with a moderate to poor quality (5 to 7 points) (Table 1), because they exhibit a significant reduction in the magnitude and frequency of natural flooding due to the channelization of the riverbed, with only overflow occurring with large floods. In relation to the substrate and vertical connectivity, most sites have a good infiltration quality (10-12 points) because there are no paved areas or significant extraction of stone minerals (sand and gravel).

Based on the previous paragraphs, a significant alteration was observed in four of the seven attributes of the RQI index, namely width, longitudinal continuity, composition,

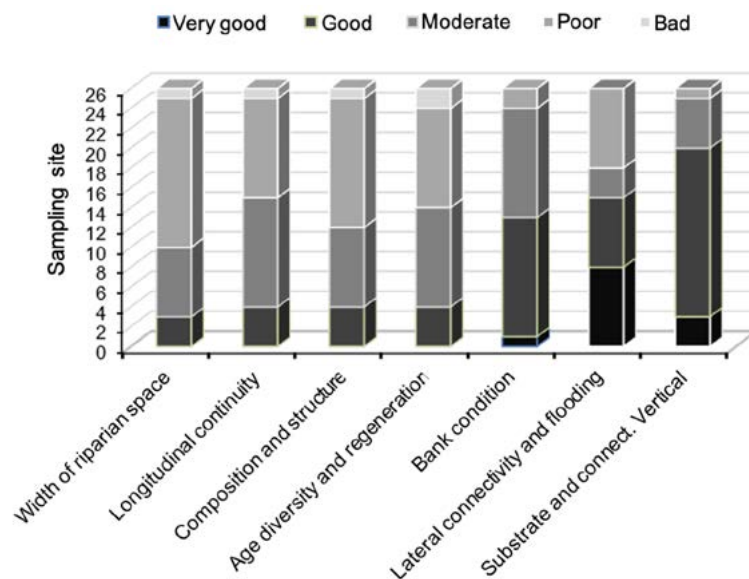


**Figure 3.** Histogram for RQI values obtained at each sampling site of the Cacaluta river.

structure, age and regeneration diversity of the riparian space. The range of quality for these attributes was from moderate to poor (Figure 4).

**DISCUSSION**

Site VII, located in the upper part of the basin and as a reference point for the REBITRI, had a good quality range (111 points), as did sites V (103 points) and VIII (121 points) located in the middle part of the basin, related to places difficult to access and areas far from population centers. As one begins to descend towards the mouth of the Cacaluta river, site quality begins to vary towards the moderate to poor ranges, like what is described by Suárez-Alonso and Vidal-Abarca (2000) for the Segura River in Spain; Fernández *et*



**Figure 4.** Quality stage of the studied attributes using the RQI index in the 26 study sites of the Cacaluta river.

*al.* (2009) for the Maullín river in Chile, Rodríguez-Téllez *et al.* (2016) for the La Sauceda river in México and Escalona-Domenech *et al.* (2021) for the Margarita river in Chiapas, México. With this statement, the hypothesis that the sites located within natural protected areas exhibit better riverbank quality with respect to the middle of the watershed where there has been a change in land use is partially fulfilled, since sites XXIV, XXV and XXVI located in the REBIEN exhibited poor riverbank quality, so it is ruled out that the presence of NPAs in the area are a conditioning factor for conserving riverbanks.

The most affected site was XVII with a poor quality due to its proximity to two population centers with the highest population, Hidalgo (1343 inhabitants) and Jiquilpan (1097 inhabitants), like what was reported by Kutschker *et al.* (2009) and Escalona-Domenech *et al.* (2021), where the areas rated with the worst quality corresponded to those closest to population centers. With respect to the hypothesis that the ecological functioning of the Cacaluta River is better in areas where the riparian vegetation has not been altered than in areas that were modified, this is completely fulfilled, since sites V, VII and VIII, which showed a range of good quality, consequently exhibited values of very good to good quality (10 to 13 points) in all attributes (see Table 1), as shown Mendoza-Cariño *et al.*, 2023.

Different degradation effects were observed in the Cacaluta riverbank due to some human activities such as deforestation, grazing, agriculture, dredging and canalization, these being the conditioning factors that determined the low scores in most of the sampling sites as reported by Diaz-Pascacio *et al.* (2018), Ortiz (2019); and Escalona-Domenech *et al.* (2021). The structure attributes of the sites located in the middle and lower reaches of the Cacaluta River exhibited a poor quality, which implies that the riverbank suffers from loss, fragmentation or replacement of natural vegetation and thus reduces the possibility of the riverbank acting as a biological corridor between the REBITRI and the REBIEN. The application of the RQI index to evaluate the structure and functioning of riparian vegetation gave a clear idea of the current conditions of this ecosystem, proving to be a simple tool that can be quickly applied and provides a great deal of hydromorphological information on river systems (González del Tánago *et al.*, 2006).

## CONCLUSIONS

Given the results obtained in this study, the development model implemented in the Cacaluta river basin does not represent any guarantee of sustainability for ecological functioning of the river, since the change and use of land adjacent to the riverbed and the uncontrolled population growth implies significant pressure on riverbanks. Even though in Mexico, Article 34 (section III) of the General Climate Change Act proposes that policies and actions must be designed and carried out to protect, conserve and restore riparian vegetation in the use, development and exploitation of riverbanks or federal zones, to date no action has been taken by competent agencies for the Cacaluta River. Therefore, it is essential to develop short- and medium-term management plans for riverbanks using restoration and rehabilitation measures and to reduce pressures and impacts as much as possible to ensure the ecological integrity of the Cacaluta River and at the same time ecological connectivity between the REBITRI and the REBIEN. Faced with this scenario,

there is an alternative to improve the Cacaluta riverbank situation in the medium term, given that CONAGUA (2011) states that this river has a medium feasibility for establishing a potential water reserve, through the detection of an ecological flow.

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# Growth of *Brachiaria decumbens* Stapf biofertilized with *Rhizophagus intraradices*, *Azospirillum brasilense* and foliar application of homobrassinolide in nursery

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## ABSTRACT

**Objective:** To identify the growth of *Brachiaria decumbens* by biofertilizing the seed with *Rhizophagus intraradices* and/or *Azospirillum brasilense* in combination with exogenous applications of brassinosteroids

**Design/methodology/approach:** In this study, nursery research combining biofertilization of *B. decumbens* with *R. intraradices* and/or *A. brasilense* in combination with exogenous brassinosteroid applications, was carried out to study their effects on plant growth.

**Results:** The results indicate an increase in morphological and physiological components of *B. decumbens* with Hbr applications alone and/or in combination with *R. intraradices*. Mycorrhizal colonization favoured and increased N and P content with Hbr applications.

**Limitations on study/implications:** Dry matter allocation may change if *B. decumbens* is biofertilized with other biofertilizer collections.

**Findings/conclusions:** We conclude that Hbr not only favoured growth, but also colonization with *R. intraradices* and consequently biomass production, and N and P content in plant tissue. The growth response and nutrient exchange suggest interdependence between brassinosteroids and the endomycorrhizal fungus.

**Keywords:** Biofertilizers, Homobrassinolide, Yield components.

## INTRODUCTION

Cattle production systems in southern Mexico are mainly based on the grazing of introduced grasses of the genera *Cynodon*, *Panicum*, *Digitaria* and lately the genus *Brachiaria*, and within this genus, *B. decumbens* Stapf is one of the preferred grasses in this region.

In general, grasslands are maintained with the minimum of inputs and over time they degrade due to the effects of seasonal distribution of precipitation, high temperatures and soil acidity. In addition, the absence of sustainable practices generates seasonal biomass production, with changes in the allocation of dry matter in the yield components and an increase in undesirable plants (Ramírez *et al.*, 2009).

These abiotic factors, in combination with poor grassland management, become a threat to the sustainability of the system. In this regard, there is currently an increased interest in some mechanisms of action of various rhizosphere microorganisms in the sustainable management of grasslands, such as endomycorrhizal fungi (Redecker *et al.*, 2000) that establish symbiosis with the root system of plants in almost all ecosystems (Strack *et al.*, 2003), favour the transport of nutrients and water (Aroca *et al.*, 2011) and have been shown to increase crop yields through nutritional effects and the regulation of plant hormones (Dohroo, 2024).

The symbiosis presents preference between some species and different endomycorrhizal fungi (Aguirre-Medina *et al.*, 2020), however, *B. decumbens* presents high percentage of radical colonization and biomass in-growth in different environments (Prieto *et al.*, 2011; Lozano *et al.*, 2013).

In the case of brassinosteroids, which are essential steroid hormones for plant growth, they exert regulation of various processes in crop growth and yield, such as cell expansion and division, vascular differentiation (Fariduddin *et al.*, 2014; Zhu *et al.*, 2015), pollen and stamen development (Zhu *et al.*, 2015), and homeostasis and signalling (Zebosi *et al.*, 2024). In addition, they have been shown to positively affect symbiotic interaction with changes in plant hormone levels when symbiosis is established (Hause *et al.*, 2007), as in solanaceae (Habsch *et al.*, 2020). They also interact with plants in the presence of biotic and abiotic stresses (Fariduddin *et al.*, 2014; Zhu *et al.*, 2015).

Therefore, in order to improve the production and quality of biomass in *Brachiaria decumbens* Stapf, it was biofertilized with *R. intraradices* and/or *A. brasilense* in interaction with a homobrassinolide in Chiapas México.

## MATERIALS AND METHODS

### Study areas location

The present investigation was carried out from March to July 2023 under nursery conditions in Pichucalco Experimental Station, (Latitude 17° 30' N, Longitude 93° 07' W and 36 m above sea level) of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP).

### Edaphoclimatic conditions of the study area

The climate of the region is type Af (García 2004) warm humid with rain all year round, average annual precipitation of 3996 mm and average temperature of 25 °C.

The soil belongs to the Acrisol group, the substrate was made with the soil plus 50% washed river sand and it was solarized for 72 h with the following physical-chemical characteristics: Sandy crumb texture, sand 62.84%, silt 19.36% and clay 17.80% (Bayoucus), 3.72% organic matter (Walkley-Black), pH 5.58 (1:2 H<sub>2</sub>O), Apparent density 1.47 g mL<sup>-1</sup>,

0.16% N total (%) (Kjeldahl), P 57.7 mg kg<sup>-1</sup> (colorimetry), 84 K<sup>+</sup> int. (mg kg<sup>-1</sup>), (atomic spectrophotometry), 0.3 mg kg<sup>-1</sup> de Na<sup>++</sup>, 284.0 mg kg<sup>-1</sup> de Ca<sup>++</sup>, 1.5 Meq 100 g<sup>-1</sup> cation exchange capacity (CEC) and 0.05 ds m<sup>-1</sup> electrical conductivity (Conductometer). Plastic bags (25 × 35 cm) with a capacity of 5.0 kg were filled with the substrate, previously perforated at the bottom to promote drainage. The bags were placed on wooden structures to avoid contact with the ground.

### **Biofertilizers and homobrassinolide**

The endomycorrhizal fungus *Rhizophagus intraradices* (Schenk et Sm) Walker et Schuessler, was reproduced in sterile soil in the root system of *Sorghum bicolor* L. At the time of packaging there were 40 spores per gram of soil plus propagules, and the level of colonization in the root system was 95% (Micorriza INIFAP<sup>®</sup> Rosario Izapa, Chiapas, México (Data indicated on the product).

*Azospirillum brasilense* Tarrand, Krieg et Döbereiner, was produced by the company Biofabrica Siglo XXI in Xochitepec, Morelos, México, under the trade name AzoFer Plus, having a concentration of 500 × 10<sup>6</sup> bacteria·g<sup>-1</sup> (Data indicated on the product).

Brassinosteroid CIDEF-4 (HBr) is a product of Natura del. Desierto, S.A. de C.V. in Mexico with 80% steroidal content, and 10% active ingredient. Its soluble presentation is not toxic and it is compatible for its application with agrochemicals.

### **Experiment setup and application of microorganisms and homobrassinolide**

The seeds of *Brachiaria decumbens* Stapf were sown in each treatment and the microorganisms were adhered to 4% of the weight of the seed with carboxymethyl cellulose. The inoculated seeds were sown 3 cm deep.

Homobrassinosteroid CIDEF-4 (HBr) was used at 2 mgL<sup>-1</sup>, and the first foliar application of Hbr was carried out 12 days after sowing (das), the time in which the emission of the first true leaves occurred in all treatments, subsequently the application foliar treatment of homobrassinolide was carried out every 28 days.

The treatments were: 1) Control, 2) *Rhizophagus intraradices*, 3) *Azospirillum brasilense*, 4) *R. intraradices* + *A. brasilense*, 5) Homobrassinolide CIDEF-4 (Hbr), 6) Hbr + *R. intraradices*, 7) Hbr + *A. brasilense*, 8) Hbr + *R. intraradices* + *A. brasilense*. In each treatment, there were five repetitions, and they were distributed in a completely randomized design. The experimental unit was one container with one plant. Plants were irrigated with water drawn from a deep well.

### **Variables**

Destructive sampling of the plants was carried out at 28, 56, 84 and 112 (das). In addition, morphological variables (plant height, number of leaves, diameter of stem and number of stems) and physiological variables (dry weight of aerial and root components and leaf area) were recorded. The percentage of mycorrhizal colonization was determined in the root at 28 das using the technique (Phillips and Hayman 1970) and the N and P content in the plant tissue. N was determined by microkjeldahl and phosphorus content with an Olsen/spectrophotometer (Thermo Fisher Scientific Mod-el 400 ¼) at the soil

and water laboratory of the Agricultural Sciences Faculty of the UNACH in Huehuetan, Chiapas, Mexico

The physiological yield components of aerial and root were weighed on a semi-analytical balance (Ohaus Adventurer Pro, USA) after drying in a forced air oven at 60-75 °C to constant weight. Leaf area (cm<sup>2</sup>) was obtained using a leaf area integrator (LICOR, LI 3000<sup>a</sup>, USA).

The Relative Growth Rate (RGR) (Milthorpe and Moorby 1982) was determined.

### Statistical analysis

A completely randomized design was used, performing an ANOVA analysis of variance, using the SAS System for Windows Ver. 8.1 (1999-2000) (SAS 2000), When the ANOVA was significant, and the parameters were compared by Tukey test ( $p \leq 0.05$ ). The data, expressed in percentages, were transformed by the formula  $\text{arc.sin}$ , before the ANOVA and the data were plotted using Sigma Plot version 11.0.

## RESULTS AND DISCUSSION

### Morphological and physiological yield components

The average height of plants in interaction with the microorganisms and the homobrassinolide, alone or combined, increased 53.9% more, compared to the control at 28 das and at the end of the evaluation, the increase represented 16.6% ( $p \leq 0.05$ ).

The maximum average height reached with the treatments where Hbr was applied was 8.8% compared to the biofertilized treatments alone. On the other hand, the biofertilized treatments were 11.2% taller than the control. The most contrasting effect was between the treatments with Hbr and the control with a difference of 20.9 cm in height (Table 1).

In contrast, the ratio was 24% when measuring the height of plants with Hbr compared to biofertilized plants alone. The most contrasting difference in leaf number was at 56 and 84 das and was between the control treatments and the application of Hbr alone or in combination with the microorganisms. This represented an increase of 90.9% (56 das) and 112% (84 das). The average of the four samples in the treatments biofertilized with *R. intraradices* and/or *A. brasilense* was 35% higher than the control. When applying Hbr in combination with the microorganisms, the number of leaves increased in different percentages compared to the same treatments without Hbr. The initial value at 28 das, the difference when applying Hbr was 8%, at 56 das 40.7%, and at 84 and 112 das it was 16%. In all samplings, there were statistical differences ( $p \leq 0.05$ ), the first statistical group was formed by the treatments where Hbr was applied alone and when biofertilization was also applied with *R. intraradices*.

The number of stems per plant showed significant statistical differences ( $p < 0.05$ ) in favour of the treatments applied only with Hbr and in combination with biofertilization of *R. intraradices*, as well as biofertilization alone of *R. intraradices* from the first sampling. The most notable difference between treatments occurred at 84 and 112 das. At 84 das, the number of stems increased 41% in the treatments with the biofertilization of the microorganisms and Hbr compared to the same treatments with only the microorganisms. At 112 das, the Hbr treatment increased the number of stems by 92% in relation to the control,

**Table 1.** Morphological yield components of *Brachiaria decumbens* Stapf. biofertilized with *R. intraradices*, *A. brasilense* and foliar application of homobrassinolide in nursery.

Time (days)	Treatment	Height (cm.plant <sup>-1</sup> )	(Number.plant <sup>-1</sup> )		Stem diameter*** (mm.plant <sup>-1</sup> )
			Leaves	Stems	
28	Control	14.2 c**	3.8 c	2.4 b	2.0 ab
	<i>R. intraradices</i>	15.8 bc	4.6 abc	3.2 ab	2.0 ab
	<i>A. brasilense</i>	15.0 bc	4.8 ab	2.8 b	1.9 ab
	<i>R. intraradices</i> + <i>A. brasilense</i>	16.8 ab	4.4 bc	3.0 b	2.0 ab
	Hbr*	15.8 bc	5.4 a	3.8 a	2.2 a
	Hbr + <i>R. intraradices</i>	18.4 a	5.4 a	3.0 ab	2.3 a
	Hbr + <i>A. brasilense</i>	18.2 a	4.8 ab	2.8 b	1.7 b
	Hbr + <i>R. intraradices</i> + <i>A. brasilense</i>	15.2 bc	5.0 ab	2.6 b	2.0 ab
	CV (%)	5.5	9.9	14.1	8.7
56	Control	36.6 e	14.4 f	3.8 bc	2.4 a
	<i>R. intraradices</i>	47.2 d	21.0 d	3.6 c	2.5 a
	<i>A. brasilense</i>	49.6 bcd	16.2 ef	3.8 bc	2.4 a
	<i>R. intraradices</i> + <i>A. brasilense</i>	46.2 d	18.0 e	4.0 bc	2.5 a
	Hbr	47.6 cd	32.2 a	5.0 a	2.6 a
	Hbr + <i>R. intraradices</i>	58.4 a	29.2 b	4.6 ab	2.5 a
	Hbr + <i>A. brasilense</i>	55.2 abc	23.8 c	4.2 abc	2.6 a
	Hbr + <i>R. intraradices</i> + <i>A. brasilense</i>	56.4 ab	24.8 c	3.8 c	2.6 a
	CV (%)	7.7	4.5	11.8	7.0
84	Control	61.6 c	29.6 d	3.8 d	2.5 b
	<i>R. intraradices</i>	66.8 b	47.0 c	4.6 cd	2.6 b
	<i>A. brasilense</i>	68.8 b	55.2 bc	4.6 cd	2.5 b
	<i>R. intraradices</i> + <i>A. brasilense</i>	67.6 b	53.6 bc	5.4 bc	2.5 b
	Hbr	72.6 a	70.2 a	6.8 a	2.6 b
	Hbr + <i>R. intraradices</i>	69.4 b	68.8 a	5.6 bc	2.9 a
	Hbr + <i>A. brasilense</i>	69.4 b	57.6 b	6.4 ab	2.7 b
	Hbr + <i>R. intraradices</i> + <i>A. brasilense</i>	72.6 a	55.0 cb	6.0 ab	2.9 a
	CV (%)	2.2	7.9	10.1	2.9
112	Control	65.4 d	82.0 c	5.0 d	2.6 b
	<i>R. intraradices</i>	71.8 c	98.4 b	5.8 cd	2.6 ab
	<i>A. brasilense</i>	73.6 c	99.2 b	5.6 cd	2.7 ab
	<i>R. intraradices</i> + <i>A. brasilense</i>	76.2 bc	94.0 b	6.0 bcd	2.6 b
	Hbr	82.4 a	114.8 a	9.6 a	2.8 ab
	Hbr + <i>R. intraradices</i>	81.6 a	124.4 a	8.8 a	2.9 a
	Hbr + <i>A. brasilense</i>	82.6 a	95.4 b	6.6 bc	2.7 ab
	Hbr + <i>R. intraradices</i> + <i>A. brasilense</i>	80.8 ab	122.0a.6	7.2 b	2.8 ab
	CV (%)	3.4	5.2	8.9	5.2

\*Hbr=Homobrassinolide. \*\*Values with the same letter within each factor and column are equal according to Tukey's test at p≤0.05. CV=coefficient of variation (%). \*\*\* Average of three stems per plant.

65% in relation to the treatments with microorganisms and 29% with microorganisms and Hbr. Stem thickness is more consistent when combining Hbr and *R. intraradices* from the beginning of the evaluation. The greatest increase occurred at 28, 84 and 112 das with the Hbr plus *R. intraradices* treatment and was statistically superior ( $p \leq 0.05$ ) to the rest of the treatments. The difference represented 13% more in the thickness of the stem for the treatment *R. intraradices* plus Hbr compared to the control.

In relation to the physiological components of yield (Table 2), the greatest increase in root biomass after 28 das occurred in the treatment where Hbr was applied and was statistically different from the other treatments ( $p < 0.05$ ). At this time, the lowest value was presented with the control.

On the other hand, at 56 and 84 das, the interaction of the application with Hbr and the microorganisms induced increases in root biomass of 19 and 28% respectively in comparison with the biofertilized treatments but without Hbr. In the last evaluation at 112 das, the root biomass of the biofertilized treatments alone increased 43% more in comparison with the same treatments plus Hbr.

Leaf biomass presented the greatest increase with biofertilization of *R. intraradices* plus Hbr at 28, 84 and 112 das, and was statistically different ( $p < 0.05$ ). The treatment with Hbr alone at 56 and 112 das was included in the same statistical group.

Stem dry weight presented the highest values in the first samples at 28 and 56 das with the Hbr alone treatment and statistically different ( $p < 0.05$ ). At 84 das, the individual biofertilization of *R. intraradices* and *A. brasilense* was included in the same statistical group. At the end of the study, the highest biomass allocation to stems was found with the Hbr treatment plus the two biofertilizers.

Leaf area presented differential induction in the response to biofertilization of microorganisms, alone or associated through time and in interaction with Hbr. In the first sampling at 28 das, there was a statistical difference and the greatest leaf area, at this time was in the treatments with Hbr and when biofertilized with the endomycorrhizal fungus. On the other hand, at 56 das, the greatest leaf area was found in plants biofertilized with the endomycorrhizal fungus and nitrogen-fixing bacteria separately. In the third sampling, at 84 das, the greatest increase occurred in the treatment with the biofertilization of *R. intraradices* plus the application of Hbr. At the end of the evaluation, the greatest increase in leaf area and statistically different ( $p \leq 0.05$ ) was in the treatments where Hbr was applied alone, in interaction with the biofertilization with *R. intraradices*, and *A. brasilense* (Table 2).

### **Biomass allocation in shoot and root**

The highest biomass allocation to the root system in relation to the shoot was found in the initial sampling with the symbiosis of the two microorganisms in the plant and when Hbr was included in the same treatment (Figure 1).

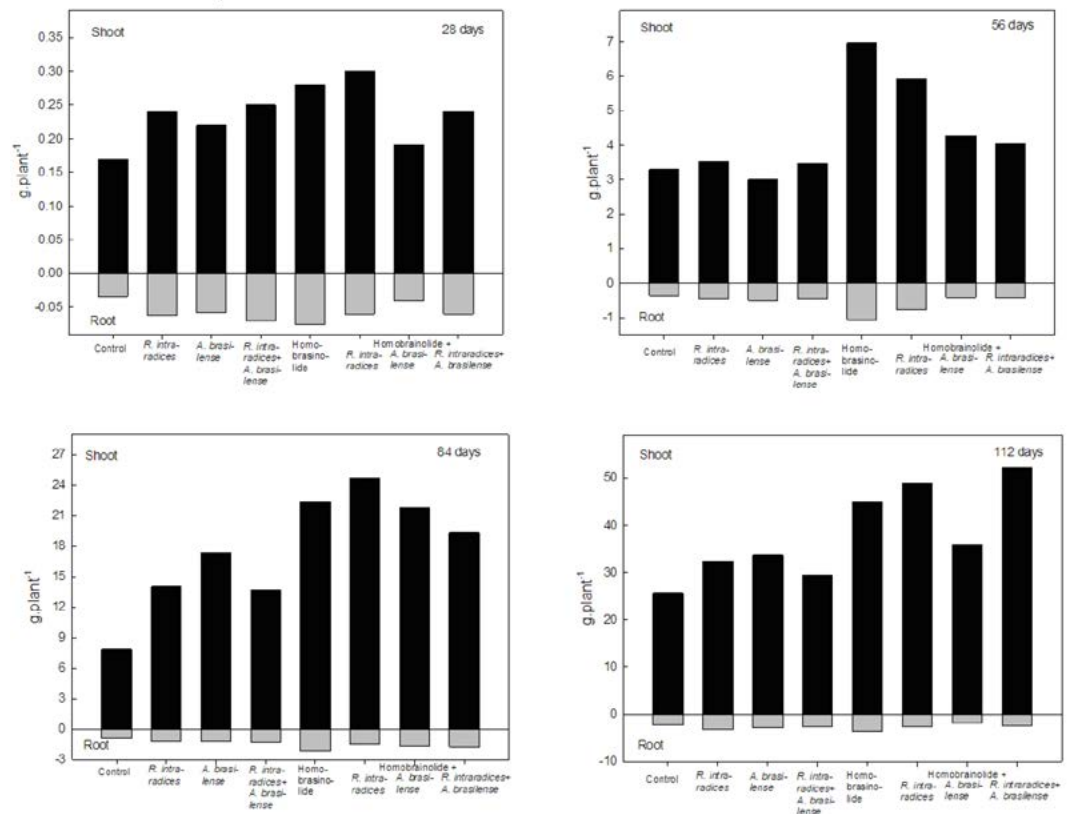
This same effect was found in the plants only treated with Hbr. In the following samples, the highest value was found in the plants where Hbr was included.

The greatest initial increase in biomass allocation to the aerial part of *B. decumbens* Stapf occurred with the treatments biofertilized with *R. intraradices*, alone or together with

**Table 2.** Physiological yield components of *Brachiaria decumbens* Stapf. biofertilized with *R. intraradices*, *A. brasilense* and foliar application of homobrasinolide in nursery.

Time (days)	Treatment	dry weight (g.plant <sup>-1</sup> )			Leaf area (cm <sup>2</sup> .plant <sup>-1</sup> )
		Root	Leaves	Stem	
28	Control	0.034 c**	0.092 d	0.044 e	27.2 c
	<i>R. intraradices</i>	0.062 b	0.122 b	0.068 abc	38.6 b
	<i>A. brasilense</i>	0.064 b	0.104 cd	0.054 de	41.3 b
	<i>R. intraradices</i> + <i>A. brasilense</i>	0.070 ab	0.120 cd	0.066 bc	41.6 b
	Hbr*	0.076 a	0.128 b	0.078 a	53.8 a
	Hbr + <i>R. intraradices</i>	0.060 b	0.154 a	0.076 ab	61.3 a
	Hbr + <i>A. brasilense</i>	0.040 c	0.090 d	0.064 cd	35.3 b
	Hbr + <i>R. intraradices</i> + <i>A. brasilense</i>	0.060 b	0.112 bc	0.068 abc	27.2 c
	CV (%)	9.7	7.1	8.8	8.9
56	Control	0.35 d	1.92 de	1.37 d	285.2 d
	<i>R. intraradices</i>	0.40 cd	2.00 cde	1.50 cd	301.8 d
	<i>A. brasilense</i>	0.49 c	1.65 e	1.34 d	537.4 a
	<i>R. intraradices</i> + <i>A. brasilense</i>	0.46 c	2.00 cde	1.45 cd	581.0 a
	Hbr	1.06 a	3.91 a	3.02 a	469.9 b
	Hbr + <i>R. intraradices</i>	0.77 b	3.12 b	2.61 b	445.2 bc
	Hbr + <i>A. brasilense</i>	0.42 cd	2.53 c	1.73 c	456.7 bc
	Hbr + <i>R. intraradices</i> + <i>A. brasilense</i>	0.42 cd	2.38 cd	1.67 cd	399.1 c
	CV (%)	8.1	11.1	9.8	6.9
84	Control	0.88 e	4.18 e	3.65 d	537.38 f
	<i>R. intraradices</i>	1.23 d	7.07 d	6.93 c	621.13 ef
	<i>A. brasilense</i>	1.24 d	8.69 c	8.63 b	928.17 d
	<i>R. intraradices</i> + <i>A. brasilense</i>	1.32 cd	7.06 e	6.64 c	719.11 e
	Hbr	2.15 a	10.00 b	11.55 a	1055.43 c
	Hbr + <i>R. intraradices</i>	1.52 bcd	12.14 a	12.52 a	1427.05 a
	Hbr + <i>A. brasilense</i>	1.63 bc	10.51 b	11.29 a	1254.99 b
	Hbr + <i>R. intraradices</i> + <i>A. brasilense</i>	1.75 b	10.01 b	9.28 b	1093.19 c
	CV (%)	11.3	6.0	9.2	5.7
112	Control	2.20 e	10.73 c	14.94 ed	151.18 c
	<i>R. intraradices</i>	3.21 b	12.92 b	19.47 d	174.73 c
	<i>A. brasilense</i>	2.86 c	13.41 b	20.26 cd	169.85 c
	<i>R. intraradices</i> + <i>A. brasilense</i>	2.61 cd	12.71 bc	16.79 e	171.86 c
	Hbr	3.64 a	18.26 a	26.63 b	288.16 a
	Hbr + <i>R. intraradices</i>	2.69 cd	19.85 a	28.92 b	274.60 a
	Hbr + <i>A. brasilense</i>	1.80 f	13.58 b	22.26 c	206.41 c
	Hbr + <i>R. intraradices</i> + <i>A. brasilense</i>	2.44 de	19.32 a	32.79 a	2812.44 a
	CV (%)	6.0	6.4	4.9	6.4

\*Hbr=Homobrasinolide. \*\*Values with the same letter within each factor and column are equal according to Tukey's test at  $p \leq 0.05$ . CV=coefficient of variation (%).



**Figure 1.** Root and aerial biomass of *B. decumbens* Stapf biofertilized with *R. intraradices*, *A. brasilense* in interaction with an homobrassinolide. The values are averages of five repetitions per treatment.

*A. brasilense* or when Hbr was included. The application of Hbr alone showed the greatest biomass accumulation in the four samples.

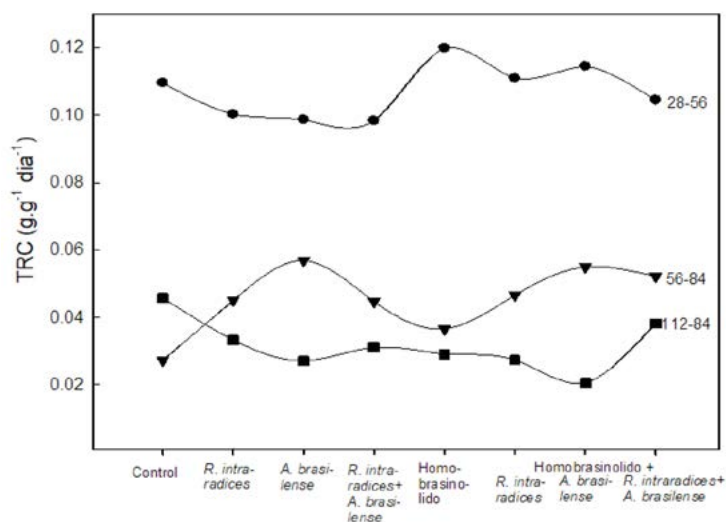
In the aerial part, at 28, 56 and 84 das the highest biomass allocation was presented with the application of Hbr alone and in the treatment with biofertilization of *R. intraradices* plus Hbr. At 112 days, in addition to the previous treatments, the aerial biomass was higher with the biofertilization of *R. intraradices* plus *A. brasilense* and the application of Hbr.

### Relative growth rate

Microorganisms alone or together induce differential effect on the relative growth rate of *B. decumbens* Stapf. There are fluctuations in it over time, and it increases during the first evaluation period when Hbr is included (Figure 2).

In the period from 56 to 84 das, there was a significant increase in the relative growth rate with the biofertilization of *A. brasilense*, which is similar to the growth achieved when Hbr is included in the biofertilization. On the other hand, in the third period, there was a significant increase in the control, same as the induction of the growth rate of the treatment with the symbiosis of the microorganisms plus Hbr.

In general, after the initial high growth, its effect decreases in the following period or at the end of the evaluation. It should be noted that the most consistent relative growth rate was presented in the treatment with Hbr and the symbiosis of the two microorganisms.



**Figure 2.** Relative growth rate of *B. decumbens* Stapf plants biofertilized with *A. brasilense* and/or *R. intraradices* alone and/or combined in interaction with a homobrasinólido. Values are averages of five plants per treatment and sampling.

In the case of *A. brasilense*, the effect is opposite to the growth induced by *R. intraradices*, which was in this case at the beginning, and decreases notably in the last two months of the evaluation. *A. brasilense* increases in the second period and decreases in the third period. The biofertilization of the two microorganisms plus Hbr caused a slight initial decrease compared to the microorganisms alone plus Hbr, but it was higher in the second and third evaluation periods.

### Mycorrhizal colonization

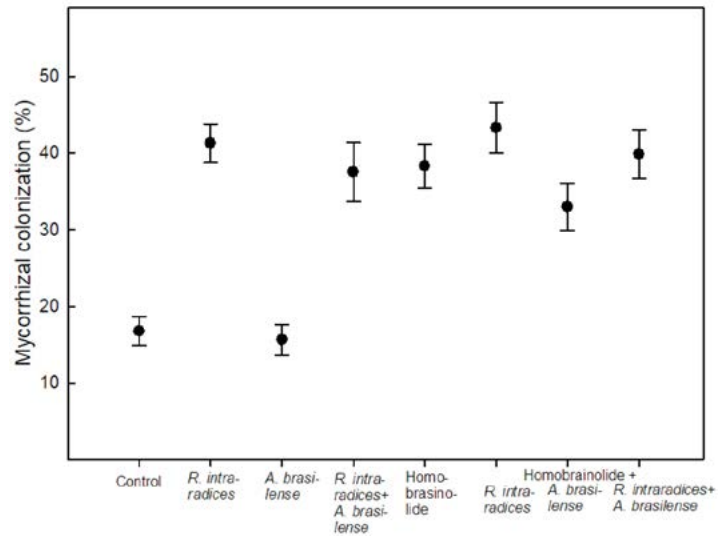
Mycorrhizal colonization increased 42% more with *R. intraradices* alone or together with *A. brasilense* compared to the control and *A. brasilense* (Figure 3).

The application of Hbr alone was 37% and with *R. intraradices* 43%. Hbr in combination with *A. brasilense* showed colonization of 33%. The highest value of 39.8% was with the biofertilization of the two microorganisms plus Hbr and represented 58% more in relation to the control.

### Content of N and P in plant tissue

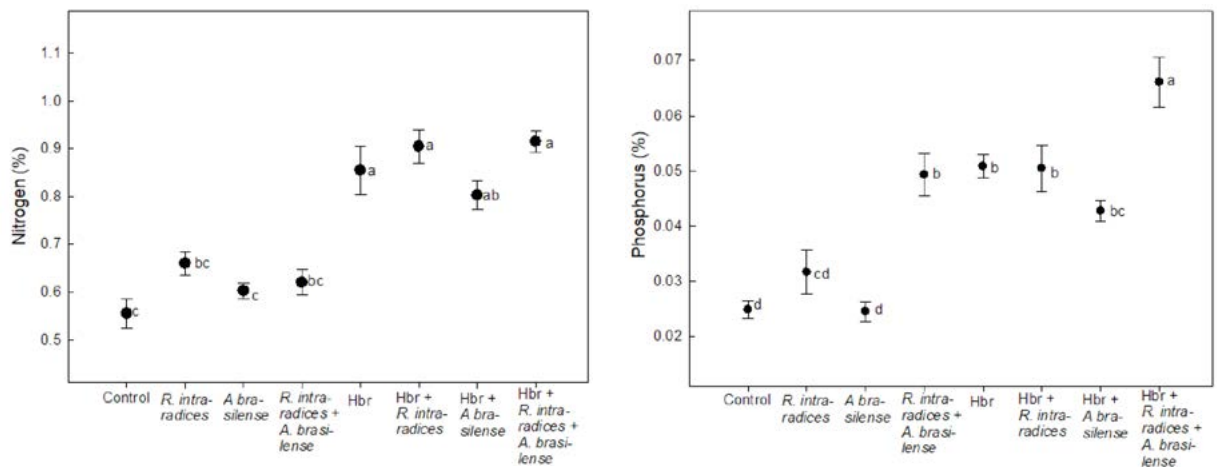
The N content in plant tissue of *B. decumbens* Stapf increased significantly with biofertilizers plus Hbr applications (Figure 4).

The increase in morphological components of *B. decumbens* Stapf biofertilized with *R. intraradices* and/or *A. brasilense* alone or together, indicates compatibility between the two, even though it is possible to find differences in the induction of host plant growth depending on the microorganisms. The development of biofertilized plants at the beginning of root colonization seems to be related to photosynthate demand of the root system. This same effect was higher in the same treatments with the biofertilizers plus Hbr applications. The previous higher expression was when combining biofertilization of *R. intraradices* and exogenous Hbr applications.



**Figure 3.** Mycorrhizal colonization on *B. decumbens* Stapf biofertilized with *A. brasilense* and/or *R. intraradices* alone and/or combined in interaction with a homobrassinolide at 28 das. Values are averages of four samples per treatment.

The positive effect on plant height when applying Hbr alone or combined with *R. intraradices* biofertilization compared to *A. brasilense* has been demonstrated in solanaceae (Hansch *et al.*, 2020), tobacco, tomato (von Sivers *et al.*, 2019) and in wheat where synthetic Br foliar was applied and mycorrhization was increased (Tofighi *et al.*, 2017). In addition, *in vitro* plants of great dwarf banana (*Musa* sp.) also increased the height of shoots with the application of Hbr (Cidef-4) (Herrera *et al.*, 2017). In green-house, plant height was greater with the application of Hbr (Biobras 6) alone, in banana FHIA-18 subjected to high temperatures (González *et al.*, 2005) and in field conditions biofertilization alone of *R. intraradices* induced more height of *B. decumbens* (Zamora *et al.* 2013).



**Figure 4.** Variation in nitrogen and phosphorus content in the plant tissue of *B. decumbens* Stapf biofertilized with *R. intraradices*, *A. brasilense* in interaction with homobrassinolide. Different letters indicate statistically significant differences according to the Tukey test ( $P \leq 0.05$ ). The vertical bars indicate the standard error of the mean ( $n=4$ ). Coefficient of variation = 14.9%N y 15.1%P.

The separate contrasting effects of Hbr and endomycorrhizal fungi in inducing differential response when applied may be due, in the case of endomycorrhizal fungi, to the stimulation of host plant growth through the transport of nutrients (Leigh *et al.*, 2009) and water (Posta and Duc, 2020) and with the application of Hbr, the benefit in the improvement of plants is attributed to its influence on various growth processes (Fariduddin *et al.*, 2014), in addition to improving their permanence in environments with stress, biotic or abiotic (González *et al.*, 2008).

In the case of *R. intraradices*, the contrasting effect between the number of leaves from the first sampling coincides with high mycorrhization (40%) compared to the control (19%). This suggests efficiency of symbiosis with *R. intraradices*. This fact has been cited in *B. decumbens* Stapf (Howeler *et al.*, 1987), and in *B. brizantha* (Hochst. ex A. Rich) cv insurgent with *R. intraradices* (Lozano *et al.*, 2013). In other crops biofertilized with *R. intraradices*, leaf number has also been increased as in *Coffea canephora* (Pierre) ex Froehner (Ibarra *et al.*, 2014).

The application of brassinosteroid alone in various crops, such as *in vitro* plants, induces a positive response in the number of leaves, as in banana clone FHIA-18 with the addition of Hbr (Biobras-6) (Izquierdo *et al.*, 2012), in *Lactuca sativa* L. with the foliar spraying of Biobras-16 (Terry *et al.*, 2011). In contrast, in *Stevia rebaudiana* Bert the highest number of leaves ( $p < 0.05$ ) was found with the mixture of *R. intraradices* and Hbr (Aguirre-Medina *et al.*, 2018). The above response may be related to the extension of the mycelium of the fungus which allows it to act as an extension of the root absorption surface (Leigh *et al.*, 2009) and favours the persistence of the host plant in adverse environmental conditions (Doubková *et al.*, 2013), such as drought (Posta and Duc, 2020) and in general, the concomitant increase in photosynthetic activity and growth (Cruz *et al.*, 2020).

Additionally, these results with positive effects on plant growth also occur under biotic or abiotic stress conditions (Gonzalez *et al.*, 2008; Fariduddin *et al.*, 2014).

In potato (*Solanum tuberosum* L.), *in vitro* plants transplanted in greenhouse, Hbr (Biobras-6) was applied to the foliage and an increase number of minitubers per plant was observed (Jiménez *et al.*, 2002), and banana clone FHIA-18 (Izquierdo *et al.*, 2017) reported an increase in the diameter of the pseudostem, to apply the Hbr (Biobras-6) by immersion and foliar spraying and when applying CIDEF 4 (hbr), the shoot height of *Musa* spp. cv great dwarf is increased *in vitro* by growing in two culture media (Herrera *et al.*, 2017).

In the case of *A. brasilense*, there is a tendency for morphological variables to increase when it is associated with the endomycorrhizal fungus. It is likely that the contributions of radical exudates of the bacterium, such as indole acetic acid (Fonseca y Calderon, 2021), favour communication with soil microorganisms (Singh *et al.*, 2017). Phytohormones have been considered as signalling compounds for endomycorrhizal fungi that can influence hyphal growth and root colonization (Larose *et al.*, 2002).

The differential response in the growth of the different organs of *B. decumbens* Stapf, as a consequence of biofertilization with the microorganisms, suggests compatibility between the two.

### Physiological components

The increase in dry matter allocation to the different physiological components of yield in most samples and plant structures, when Hbr was applied alone, and when combining the same treatment with *R. intraradices*, suggests interdependence between the two. The above response is expressed in the growth of mycorrhizal plants, through the increase in nutrient content of the host and biomass production (Ruiz *et al.*, 2012). In this regard, Tofighi *et al.* (2017) when epibrassinolide was applied to wheat and rice, reported an increase in the growth of both mycorrhizal species established under salt stress conditions. Similar results were reported in cotton when exogenous applications of brassinosteroids were applied, with an increase in yield and greater accumulation of biomass (Lou *et al.*, 2024).

The above response seems to be related to various phytohormones, such as auxins, ethylene, jasmonic acid, brassinosteroid and strigolactones involved in the growth and development of root hairs (Hause *et al.*, 2007; Zhang *et al.*, 2016) as happens when biofertilizing trifoliolate orange under drought stress (Liu *et al.*, 2018).

In other crops, brassinosteroids are also cited as positively affecting the mycorrhizal symbiosis of tobacco and tomato plants (von Sivers *et al.*, 2019) and auxins, brassinosteroids and strigolactones are responsible for the establishment of mycorrhizal associations (Dhiman *et al.*, 2022). In general, microbial communities are attracted by some signaling factors in the form of exudates released by plant roots (Liao *et al.*, 2018) and lead to improved plant growth (Rao and Tak, 2002).

Hormonal homeostasis (in response to fungal associations) leads to signalling and induces various physiological changes to the benefit of the plant (Dhiman *et al.*, 2022), such as the transport of nutrients and water through the hypha, and the signal transmission through AM hyphal network after being stressed by the pathogen (Han Song *et al.*, 2023).

Root colonization is high from the beginning (40%) and the benefits of the symbiosis are reflected in this variable. It is likely that the initial establishment of the mycorrhizal symbiosis and its effect on the number of leaves favoured the transport of nutrients to the plant (Powell and Rillig, 2018).

The increase in root biomass of all biofertilized treatments alone or combined seems to be influenced by the amount of root exudates that favour root growth and this condition allows them to explore more soil surface (Altomare and Tringovska, 2011). The lowest root growth was in the control, possibly due to the low levels of native mycorrhization compared to the functioning of the *R. intraradices*-*B. decumbens* symbiosis that has been cited as effective in improving their productivity (Kanno *et al.*, 2006).

Higher root biomass allocation in response to Hbr applications is supposed to (Mathur *et al.*, 2018) stimulate the intrinsic potential of plants and consequently promote growth and yield (Otic *et al.*, 2022). Brassinosteroids have now been shown to play an important role in yield increase (Lou *et al.*, 2024). The increase in vegetative and reproductive development has been reported in different annual crops biofertilized with endomycorrhizal fungi and *A. brasilense* (Aguirre-Medina, 2006).

The effect of *R. intraradices* in inducing greater biomass in the stem of biofertilized plants from the beginning of the evaluation is due to the nutrient and water supply to the host

plant (Devi *et al.*, 2021), and consequently changes in its structures are induced (França *et al.*, 2016). In *Coffea canephora* (Pierre) ex Froehner (Ibarra *et al.*, 2014) and *Tabebuia donnell-smithii* Rose, the same response occurs when biofertilized with *R. intraradices* (Aguirre-Medina *et al.*, 2014).

Foliar lamina biomass also increases. The above may be related with the establishment of the symbiosis, where the fungus receives photosynthates and the plant increases its access to mineral nutrition and water (Jacott *et al.*, 2017). In general, biofertilization with endomycorrhizal fungi significantly increases the allocation of dry matter to the different morphological and physiological yield components, compared to the control.

The importance of the interaction of rhizosphere microbiota with plant root exudates such as indole acetic acid (Fonseca y Calderon, 2021), cytokinins and gibberellins (Spaepen *et al.*, 2007) has been demonstrated. In addition, it has been shown that the interaction of endomycorrhizal fungi with plants producing specialized metabolites such as flavonoids (Devi *et al.*, 2021), which establish communication with other soil microorganisms (Singh *et al.*, 2017) and are considered signaling compounds for endomycorrhizal fungi that can influence spore germination (Larose *et al.*, 2002).

In addition to the above, plant growth is influenced by various environmental factors, which can be reflected in differential growth regulated by genetic traits with little variation in phenotypic plasticity through modular growth (Collado-Vides, 1997).

In the case of *B. decumbens* Stapf, its utilization depends on the proportion of leaves, stems and roots that are generated and these components result in forage yield. Knowledge of the influence of environmental seasonality on the growth and forage production of species of interest allows the identification of forage availability and, consequently, the adoption of differential management strategies for each species.

### **Mycorrhizal colonization**

Colonization was observed in all *B. decumbens* plants, including the control (possible native strain), and was greater in plants biofertilized with *R. intraradices* compared to plants without biofertilization. The above confirms the presence of endomycorrhizal fungi in the regional substrate; however, they have a lower capacity for colonization and growth induction, even though *B. decumbens* has been considered to have high mycorrhizal dependence (Prieto *et al.*, 2011; Lozano *et al.*, 2013). The average percentage of colonization in the control was 16% and with *R. intraradices* 41%. The above may be related to the supplementation of carbon sources from the host plant to the fungus (Adolfsson *et al.*, 2015). The high colonization capacity of *R. intraradices* has been cited in other crops, annuals and perennials (Aguirre-Medina, 2006). It should be noted that mycorrhizal colonization of plants is influenced, in addition to environmental factors, by variations in the host genotype (Baum *et al.*, 2009).

In general, the results showed that the increase in physiological variables coincides with the increase in colonization levels.

The phosphorus and nitrogen content increased in all treatments with the microorganisms alone or together, and they statistically surpassed the control ( $P \leq 0.05$ ). The preference of endomycorrhizal fungi to transport phosphorus has been cited (Tang *et*

*al.*, 2022), in addition to raising the phosphorus content, iron and zinc are increased (von Sivers *et al.*, 2019) and the transport of nitrogen (Tang *et al.*, 2022).

## CONCLUSIONS

The exogenous applications of Hbr favored the growth of *B. decumbens* in the morphological and physiological components of the yield and the effect was synergistic when combined with the biofertilization of *R. intraradices*. In addition to the production of biomass, the content of N and P in the tissue of the plant. The response in growth and nutrient exchange suggests interdependence between brassinosteroids and endomycorrhizal fungi.

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# Demographic analysis of the immature stages of the parasitoid *Diachasmimorpha longicaudata* under mass rearing conditions

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## ABSTRACT

**Objective:** To analyze the mortality of the immature stages of the parasitoid *D. longicaudata* using demographic parameters from a life table.

**Design/methodology/approach:** Evaluations were carried out with three cohorts, each with a different level of superparasitism: puparia from 1 to 5, 6 to 10, and 11 to 15 scars (oviposition or attempted oviposition). The analysis of data was performed by life table parameters.

**Results:** Mortality was very noticeable in the egg stage and the first larval stage. Mortality was higher in hosts with high levels of superparasitism. After the second stage, mortality was reduced. The parameters of mortality in one day in proportion to the number of the cohort (dx) and mortality per day (qx), had their highest values between the 3<sup>rd</sup> and 4<sup>th</sup> of parasitoid development. Life expectancy at baseline was 7, 5.3 and 4.9 days at the three corresponding levels of superparasitism. After the second instar, life expectancy increased, with a steady drop until the 15<sup>th</sup> day of development. The highest emergence of adults was obtained at the average level of superparasitism (58.33%) and the lowest when the levels of superparasitism were higher (44.66%).

**Findings/conclusions:** Intraspecific competition, host mortality due to trauma and possibly infections can be considered as the main causes of mortality in immature stages in proportions that may explain the emergence percentages obtained in mass rearing.

**Keywords:** Life table, immature stages, insect mortality, immature stage mortality, intraspecific competence.

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## INTRODUCTION

Evaluation of mortality in immature stages of parasitoids is usually a problem, because, mainly to a few indications are presented, also normally they are protected by the the host (Xu *et al.*, 2007; Weinersmith, 2019). This situation is more complicated in endoparasitoids, in which develop is inside the host, so mortality evaluations of immature stages are scarce.

However, during this phase, parasitoids carry out one of the most important parts of their development, which manifests itself in a basic and fundamental feeding activity based on intrinsic physiological interactions (Uçkan & Ergin, 2002; Saini *et al.*, 2019). In most cases, adult parasitoids are considered to emerge with a basic food supplement for their reproduction and even for their survival



(Harvey & Malcicka, 2016; Farahani *et al.*, 2016). This leads to the assumption that during the development of immature stages, the parasitoids receive complete or adequate nutrition for its subsequent adult performance. In the adult state, it has been reported that adult parasitoids, unlike other insects, do not have the capacity for lipogenesis or proteogenesis (Visser & Ellers, 2008; Visser *et al.*, 2023). They can be considered to emerge with a very complete and special load of food. In this regard, in adults, the requirements focus on carbohydrates as a basic resource to extend survival, although with very little contribution to increase or extend fecundity (Kishinevsky & Keasar, 2021; Cavallini *et al.*, 2023).

Considering the influence of immature parasitoid stages, is important to know more about their development in order to make inferences or analysis in the biological potential of adults. In this work we report the results of an analysis with life table parameters in immature stages of the parasitoid of fruit flies (Diptera: Tephritidae), *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). This parasitoid has been used by augmentative releases for the suppression and control of populations of different pest species of fruit flies (Montoya *et al.*, 2000; Harbi *et al.*, 2019; Suárez *et al.*, 2024). According to Mensah Agboka *et al.* (2023), the releases of this insect promotes its establishment and can provide a benefit in a 9:1 ratio. Due the importance of this parasitoid in the biological control, mass rearing has been established in which the adult emergence is a crucial indicator to meet the quality. Adult emergence varied and depend mainly on the quality of the host, this ranges from 30 to 80% (Cai *et al.*, 2018; Cruz *et al.*, 2018; Carta Gadea *et al.*, 2020; Cancino *et al.*, 2023). This raises an initial question: What are the levels of mortality that occur during the development of *D. longicaudata*? In this regard, few references are known, from the classic evaluations that were carried out with immature stages of Opiinae: Braconidae parasitoids reported by Pemeberton and Willard (1918) and in recent years a very complete work of the immature stages description of *D. longicaudata* was introduced by Carabajal-Paladino *et al.* (2010).

The usefulness of knowing the levels of mortality in *D. longicaudata* in immature stages may be important for mass rearing where is a basic need to reinforce the establishment of quality parameters, where emergence is a central indicator (Van Nieuwenhove *et al.*, 2012; Harbi *et al.*, 2016; Suárez *et al.*, 2019). However, it can also be useful to be able to infer the mortality results of hosts in the field where percentages of parasitism are usually obtained based on emergence (Montoya *et al.*, 2000; Cancino *et al.*, 2019).

Mortality data from the immature stages of development of *D. longicaudata* were processed using different demographic parameters (Vargas *et al.*, 2002; Carabajal-Paladino *et al.*, 2010; Estrada-Marroquín *et al.*, 2022). Within these evaluations, superparasitism was considered as an influential factor in egg mortality and the first stage. Based on the above, the objective was to analyze the mortality of the immature stages of the parasitoid *D. longicaudata* using demographic parameters from a life table.

## MATERIALS AND METHODS

Biological material. Eggs-larvae-pupae of *D. longicaudata* hosted into puparia of *Anastrepha ludens* (Loew), were sampled from larvae previously exposed to parasitization in the mass rearing of *D. longicaudata* parasitoids of the Moscafrut Plant. Cohorts were

formed with samples of larvae recently exposed to parasitization (24 h after exposure) to *D. longicaudata*.

### Host sampling

A total of 4500 larvae recent exposed to parasitization were randomly sampled. The total was the cumulative of five samples, each one considered as the same cohort. When the formation of the puparium began (sclerotization covering the host), 24 h after parasitization, three groups were formed in each sample. In each one, observations of the number of oviposition scars were carried out. These groups were formed under the assumption that superparasitism is very frequent in the oviposition of *D. longicaudata* under rearing conditions. The three groups, with different ranges of scars were: puparia of 1 to 5, 6 to 10 and 11 to 15 scars (result of oviposition or attempted oviposition of the parasitoid female) on the sclerotized cuticle. On the first four days, 15 puparia/day of each scar range were taken for dissection, and from the fifth day until day 15, 30 puparia/day were taken for dissection. Of the total sample, the groups were formed by 900 puparia, a total of 2970 puparia were analyzed with dissection.

### Mortality assessment

Immediately after the groups were formed, one day after exposure to parasitization, dissection of the puparia began. Daily, from 1st to 15th day (time of immature develop of *D. longicaudata*), randomly was taken the sample of puparia. With the use of forceps and dissection needles, the central part of the puparium was opened, observing the immature stages of the parasitoid with a microscope (CarlZeiss® Stemi 305, Jena, Germany). In the first four days, the microscope was calibrated to 4.0 X to observe eggs on the first two days and to 2.5 X to observe first-instar larvae, in the third and fourth days. From the fifth day, observations were carried out at 1.5 X. Due to the constant presence of supernumeraries as a result of the superparasitic activity of *D. longicaudata* under mass rearing conditions, the number of eggs and larvae of the first instar present per puparium was counted in the first four days. From the fifth day onwards, larvae of the second to fourth instar or pupae of individual parasitoids per puparium were usually present. In each dissection, the number of live and dead immature parasitoids was counted.

Photos were taken using a digital camera (Nikon® D550, Minato, Tokyo, Japan), coupled to a stereo microscope (Nikon® SMZ-U Zoom 1:10, Tokyo, Japan) and digitized. The images were stacked in Helicon Focus and edited in Adobe Photoshop, to morphologically characterize each of the states and stages in the development of *D. longicaudata*. During the 15 days of development of the immature stages of the parasitoid, the samples of puparia were in cylindrical plastic containers (5 cm in diameter by 7 cm high, 150 ml capacity) with moist vermiculite maintained into a bioclimatic laboratory (Memmert® ICH110L GmbH, Co. KG, Germany), at a temperature of  $26 \pm 1$  °C and 60-70% RH.

### Emergence and sexual proportion

An additional sample of 100 puparia from each group was kept separately in a plastic container (5 cm in diameter by 7 cm high, 150 ml capacity) with a thin layer of vermiculite.

They were kept into the bioclimatic previously mentioned. After 15 days the adults began emerged and the number and sex of adult parasitoids were counted.

### Data analysis

Due to the presence of supernumerary individuals per pupary, mortality in the first four days of each group was analyzed using the average per puparia. From the fifth day onwards, mortality was counted individually per puparium. Based on living and dead individual data, the following parameters were obtained: the average number of living individuals per day,  $lx$  (No. of living individuals/No. of individuals from the initial cohort);  $dx$ , fraction of the original cohort dead in one day  $lx-lx+1$ ;  $qx$ , fraction of living individuals who died in a day  $(1-(lx+1/lx))$ ;  $px$ , fraction of living individuals in a day  $(lx+1/lx)$  and  $Ex$ , individual average of days to live  $(1/2+(lx+1+lx+2... \infty/lx))$  (Carey, 2001). The survival curves from day 1 to day 15 (egg-pupal development) and from day 5 to day 15 (development of individual larva from second instar to pupa) were compared using a log-rank analysis (JMP<sup>®</sup> Version 16. SAS Institute Inc., Cary, NC, 1989-2023). We obtained the averages of total emergence and sex proportion.

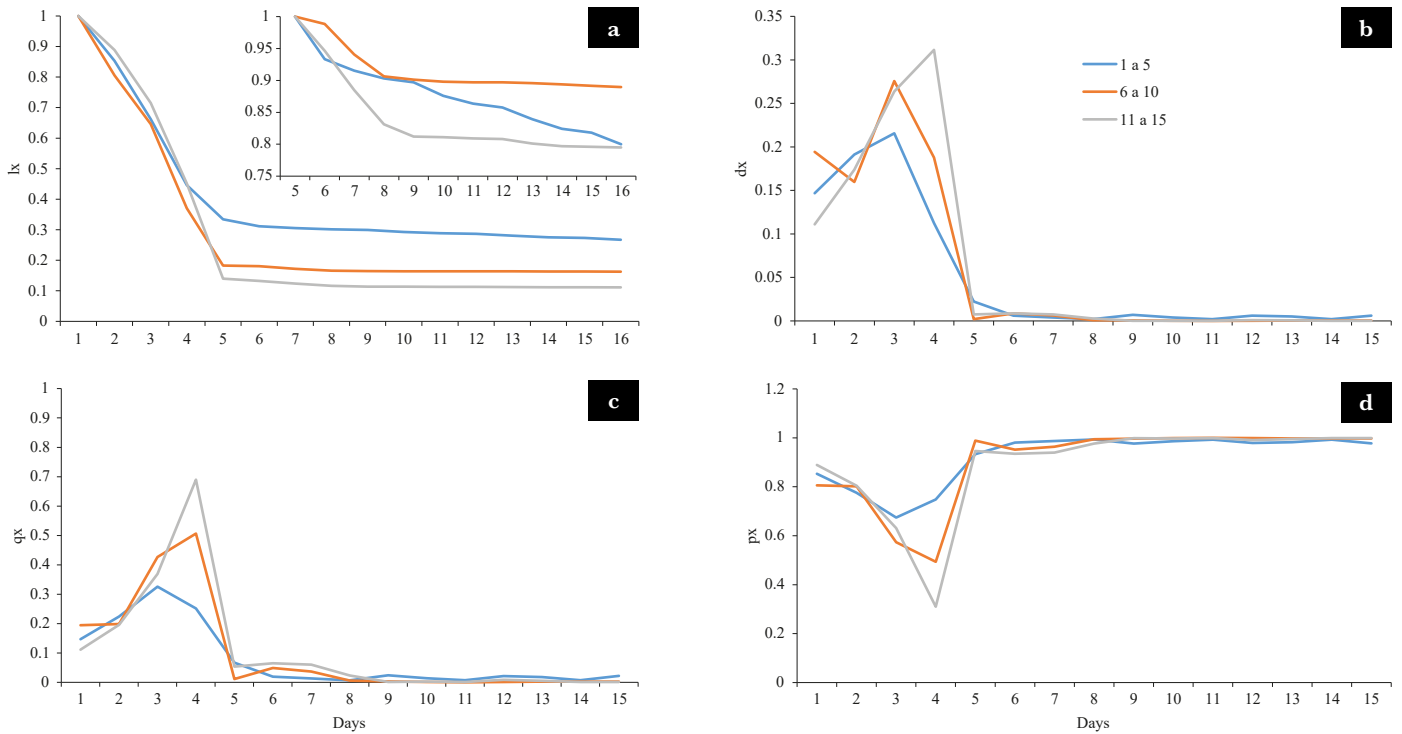
## RESULTS AND DISCUSSION

### Mortality assessment

In the comparison of survival curves, two important things stand out: firstly, the mortality was more accentuated in eggs and larvae of the first instar; and secondly, that mortality in this initial period of development was higher in puparia with a greater number of scars and superparasitism ( $\chi^2=52.16$ , d.f.=2,  $P<0.0001$ ) (Figure 1a).

A direct relationship was obtained between the number of scars and the number of supernumeraries per puparium, with a correlation of 75% ( $r^2=0.75$ ). When parasitoids reached the second stage of development (usually from the fifth day onwards) mortality decreased, keeping survival more constant. However, the mortality continued higher in parasitoids developed from superparasitism range from 11 to 15 scars/puparium. The comparison of partial survival curves, from the second larval stage to the end of pupal development, it was obtained that individuals in the range of 6 to 10 scars were the longest-lived, followed by individuals in the range of 1 to 5 scars, and those that developed in puparia with a range of 11 to 15 scars remained the least long-lived ( $\chi^2=16.54$ , d.f.=2,  $P<0.0003$ ) (Figure into Figure 1a).

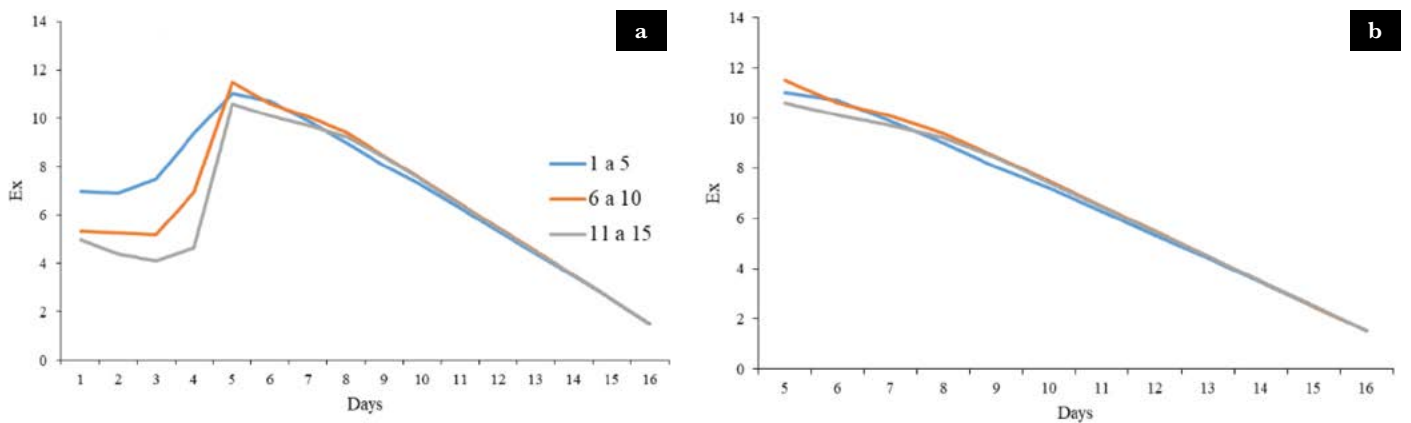
The number of deaths per day considering the original cohort size ( $dx$ ) was more prominent between day 3 and day 4. The puparia with the highest range of scars experienced the highest mortality rates within the original cohort on day 4 (Figure 1b). The mortality of individuals observed per day ( $qx$ ) shown that only in puparia containing 1 to 5 scars is where the highest mortality occurred on day 3, while for puparia with the highest number of scars, the mortality was higher on day 4 (Figure 1c). Puparia with the highest scar range experienced the lowest survival fraction per day ( $px$ ) on the day 4, while puparia within the lower scar range (1 to 5 scars) experienced the lowest survival fraction on day 3 (Figure 1d). Life expectancy ( $Ex$ ) was about 7 days for individuals with 1 to 5 scars, but it was reduced



**Figure 1.** Curves of: a) survivorship ( $l_x$ ), b) mortality in the original cohort ( $dx$ ), c) mortality per day ( $qx$ ) and d) survivors per day ( $px$ ), during the development of immature stages of the parasitoid wasp, *D. longicaudata*, 24 hours after oviposition to one day prior to adult emergence, categorized into three levels based on the number of scars per host puparium (superparasitism).

to 5.3 days for individuals with 6 to 10 scars and to 4.9 days for individuals containing from 11 to 15 scars (Figure 2a, Table 1).

However, life expectancy remains very similar when the analysis was partial, it begins with individuals developed from the second stage, 5 days old and finalizing in the 15<sup>th</sup> day. In this case, the average life expectancy was between 10.59 and 11.49 days, with the



**Figure 2.** Survivorship curve ( $Ex$ ) for the different immature stage of development in the *D. longicaudata*, categorized into three levels base on the number of scars on the host pupae (superparasitism), a) complete immature development, b) as of the 5<sup>th</sup> day, beginning of second instar larva to one day before adult emergence.

**Table 1.** Averages of initial ( $E_0$ ) and partial ( $E_5$ ) life expectative in immature stages, emergence of adults and sex ratio of adults of the parasitoid *D. longicaudata* considering three ranges of scars per puparium from the host.

Scars/puparium	Life expectative Initial ( $E_0$ )	Partial ( $E_5$ )	Percentage of emergence	Sex ratio ♀:♂
1 a 5	6.97	11.02	53.00	1.52
6 a 10	5.33	11.49	58.33	2.10
11 a 15	4.96	11.59	44.66	4.01

highest value being individuals developed in puparia into a range of 6 to 10 scars (Figure 2b, Table 1). Figure 2a shows that life expectancy in individuals from 1 to 4 days begins with low values, then increases on day 5 to have a constant drop until day 15. When the  $E_x$  was graphed from the 5<sup>th</sup> day of age, a constant steady declining trend is observed (Figure 2b). The images gathered throughout the development of *D. longicaudata* allowed to characterize the morphologies changes that occur during each stage (Figure 3 and Figure 4).

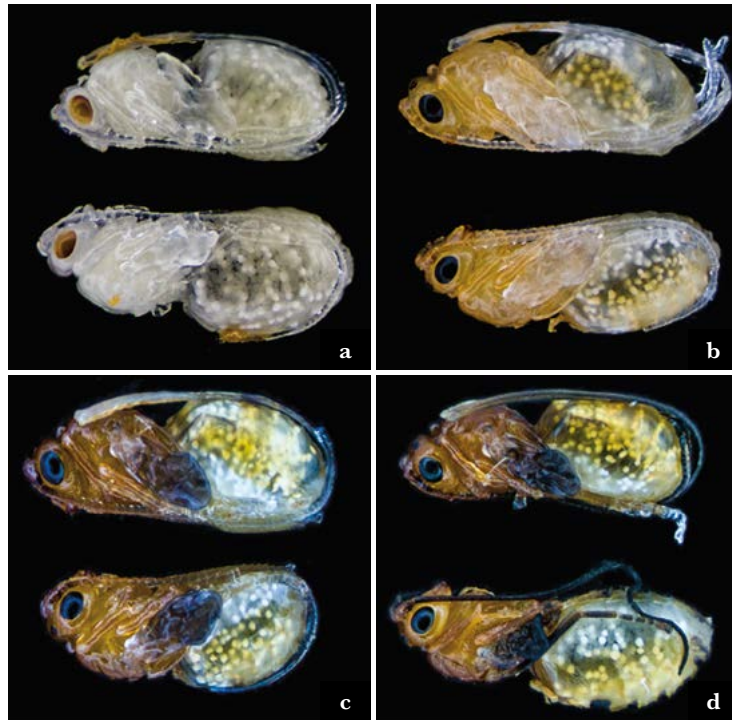
### Emergence and sexual proportion

The highest emergence rate occurred in individuals developed in puparia containing 6 to 10 scars, while the lowest emergence was in individuals developed in puparia with the highest number of scars. The sex ratio in all cases was biased to females, with differences between each range, with the proportion of females increasing as the number of scars per puparium increased (Table 1).

In the first four days, the mortality rates for eggs and first instar larvae of *D. longicaudata* were significantly high. Mean mortality rates of 30% were obtained for the original cohort (dx). In a single day, the mortality rate was 70% (qx) when the number of supernumerary puparia ranged from 11 to 15 scars per puparium. Even in the lowest range, from 1 to 5



**Figure 3.** Immature stages in the development of the parasitoid *D. longicaudata* (a) egg; (b) first larval instar; (c) early second stage; (d) late second stage; (e) third stage; (f) fourth stage-Prepupa.



**Figure 4.** Immature stages of development of the parasitoid *D. longicaudata*, pupa (a, b, c) and pharate (d): female (up) and male (down).

scars per puparium, there was a reduction of up to 20% compared to the original cohort and a 30% mortality rate in a single day.

The increase in egg and first instar larval mortality is likely due to increased competition among the high density of supernumerary parasitoid. Since *D. longicaudata* is a solitary parasitoid (only one adult parasitoid emerges from a single host (Ovruski *et al.*, 2003; Harvey *et al.*, 2012)). The superparasitism in *D. longicaudata* is a very common event (Montoya *et al.*, 2012; Altafini *et al.*, 2013; Devescovi *et al.*, 2017). Therefore, the presence of supernumerary individuals within a host implies intraspecific competition that is expected to intensify when density increases within a single host (Devescovi *et al.*, 2017). The observations focused on quantifying the number of live eggs and first-stage larvae daily, but did not include detailed analysis of mortality causes.

In this regard, two causes of mortality have been proposed, with which the supernumeraries of the host are excluded each other. One is engaging in direct competition in their early stages using physical means like strong mandibles for exclusion or even cannibalism (Lawrence, 1988a; Strand, 2002; Brodeur and Boivin, 2004). Estrada-Marroquin *et al.* (2023) in detailed observations with the braconid parasitoid of fruit flies, *Utetes anastrephae* (Viereck) observed that cannibalism is an exclusive activity among its own species. Another is exclusion due to biochemical changes in the hemolymph of the superparasitized host, where, in addition to a limitation of resources, the environment can negatively impact the survival of individuals who are not well-suited to it which can lead to mortality (Brodeur & Boivin, 2004; Cusumano *et al.*, 2016). It is uncertain whether

mortality is a combination of the two causes or a single one, and there is a limited number of studies on this subject (Carbajal-Paladino *et al.*, 2010; Devescovi *et al.*, 2017).

The initial life expectancy (Ex) of the parasitoid was reduced from around 7 days to around 5 days when parasitoids entered puparia with more than 5 scars (indicator of superparasitism). However, after a period of high competition, where a parasitoid remains a distinct individual within its host, the average life expectancy increased to 11 days across three groups. Mortality decreased by about 80% starting from day 5, coinciding with the onset of the second stage of the parasitoid's development.

In the partial analysis of parasitoid survival, it was found that the mortality rate from the second larval stage to before adult emergence was reduced by only 20%. This likely explains why the surviving parasitoid, after a period of competition with other parasitoids, exhibits a high survival rate (above 80%). However, this high survival rate varied greatly across the different groups of puparia based on the number of scars. Parasitoids with the longest-lifespans were individuals developing into puparia with 6 to 10 scars, with a longevity exceeding those with fewer scars, while in puparia with a high number of scars exhibited the shortest lifespans. One explanation for the higher relative survival rates observed in the mid-stage scar/pupal stage are likely associated with the increased proportion of females emerging with the presence of a moderate range of scars. Adult female *D. longicaudata* wasps typically live longer than adult male. In the range of greater number of scars, the sexual proportion favored females, but increased competition can lead to a reduction in survival (competition for food, reduction of vitality due to competition, etc.) (Montoya *et al.*, 2012). In different studies it has been reported that moderate levels of superparasitism ( $\sim < 10$  immature/puparia) can be beneficial for the mass rearing, because they can lead to a female-biased sex ratio and may not significantly impact offspring survival. This is sometimes considered an adaptive advantage (González *et al.*, 2010).

The high mortality rate experienced by *D. longicaudata* during its immature development can be explained in two ways: firstly, it consists of a very high mortality rate experienced by eggs and early larval stages, dominated by intrinsic and intraspecific competition, plus by a possible immune response from the host. Secondly, the mortality can be viewed as relatively stable and is caused by other factors (possibly more related to environmental conditions, nutrition, or disease prevalence, etc.), which presents an apparently constant rate from the second stage onwards.

With respect to the hosts, in mass rearing conditions, for the *A. ludens*-*D. longicaudata* relationship, between 80-90% were parasitized. This implies that between 10-20% of hosts can emerge as adult flies, however, the emergence of flies is null due to the use of irradiated host larvae in the mass rearing (Cai *et al.*, 2018; Suárez *et al.*, 2019). Furthermore, an additional 9.4% of parasitized hosts experience initial mortality, without a clear cause (oviposition trauma, microbial infection, etc.). An additional 10-20% mortality occurs during the developmental stage, probably due to the aforementioned causes.

Combining values, about 30-50% non-emergence by mortality could be due to the following causes: parasitization is prevented by radiation (flies not emerged due to radiation), initial mortality (competition as a dominant effect) and mortality caused by

different causes present during oviposition or development. The most influential causes of mortality of parasitoids at each age are difficult to determine, they can include, among others, excessive superparasitism, host quality, nutritional deficiency due to excessive competition (Lawrence, 1988 b; Silva-Torres *et al.*, 2009; Couchoux & van Nouhuys, 2014). However, there are also a series of other causes related with the rearing process such as: mass management, environmental conditions or quality of ingredients or the larval diet of the host's development.

This information is key to understanding the behavior of mortality during the development of *D. longicaudata* within the host. The characterization of mortality in the initial period and the period towards adult emergence. Also, this information helps understand the emergence capacity of parasitoids in mass rearing conditions and can prove useful to consider technical measures and reduce mortality during development. Studies on the causes of mortality in the initial phase, during high competition due to superparasitism or during later development could lead to more clearly defined proposals to increase emergence in mass rearing.

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# The rate of fermentation and the *in vitro* degradability of palm kernel meal from *Elaeis guineensis* Jacq. when included in sheep diets

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## ABSTRACT

**Objective:** Measure the biogas production, degradability and fermentation kinetics of diets containing different levels of palm kernel meal.

**Design/methodology/approach:** The treatments were 0%, 15%, 30% and 45% palm kernel meal (PKM). The nutritional and chemical content was determined, including dry matter, total protein, ethereal extract; ash; neutral and acid detergent fiber fractions. Culture vials (120 ml) were used, to which 0.5 g of the experimental diets were added, followed by 50 ml of rumen inoculum under continuous CO<sub>2</sub> flow. The vials were then sealed with neoprene plugs and aluminum rings and incubated at 39 °C for 72 hours. Biogas displacement was measured at 3, 6, 9, 12, 24, 48 and 72 hours.

**Results:** The treatment that produced less biogas ( $p < 0.05$ ) at 6 and 9 h of incubation was the one with 45% inclusion of palm kernel meal. The diet with 45% inclusion of PKM presented the lowest maximum gas volume (291.35) of the four treatments evaluated ( $p < 0.05$ ).

**Study limitations/implications:** Results may vary when using higher and lower PKM inclusions in experimental diets, or when combining them with one or two cereals. The fermentative and digestible behavior of the ingredients may also change.

**Findings/conclusions:** Under *in vitro* conditions, the inclusion of 45% palm kernel meal reduces biogas production. PKM represents an alternative use for inclusion in diets.

**Key words:** Palm kernel meal, biogas, degradability, diet, *in vitro*.

## INTRODUCTION

Palm kernel meal (PKM) is a by-product of the *Elaeis guineensis* Jacq palm oil industry. In Mexico this crop is cultivated in the states of Chiapas, Tabasco, Veracruz and

Campeche. The national total production of fresh fruit bunches (FFB) is 1,194,210.19. In the state of Chiapas in particular, the cultivated area is 45,435.53 hectares, of which an area of 38,580.03 hectares are harvested, producing 554,519.03 tons of FFB (SIAP, 2022).

It is estimated that out of 100% of a FFB the PKM production rate is 2% to 3% (García and Yáñez 2010). This suggests an estimated annual production of 16, 635.57 tons of FFB in the state of Chiapas.

The PKM is a by-product that has been evaluated in several species of zootechnical interest such as poultry, swine, sheep, goats, cattle and fish (Zumbado *et al.*, 1992, Kperegbeyi and Ikperite, 2011; Ebrahimi *et al.*, 2012; Oladokun *et al.*, 2016; Freitas *et al.*, 2017; Mazón *et al.*, 2018).

Sheep farming now faces the dilemma of enteric fermentation, which generates methane ( $\text{CH}_4$ ) emissions with a high global warming potential (IPCC, 2021).

Various feeding strategies have been implemented to reduce gas emissions from cattle and sheep, addressing this situation. One strategy has been to use agro-industrial waste, such as palm kernel cake (PKC), derived from pressing the fruit of *Elaeis guineensis* Jacq after oil extraction.

The inclusion of palm kernel meal (PKM) in cattle diets has been studied as a strategy to mitigate the production of enteric methane, which is one of the main components of biogas generated in the rumen of ruminants.

Palm kernel meal is a viable source for animal feed due to its high crude protein (CP) content (10.55%), ether extract (EE) content (7.27%), neutral detergent fiber (NDF) content (76.56%), and acid detergent fiber (ADF) content (57.20%) (España-Escobar, 2023). Including it in the diets of cattle and sheep is beneficial not only from a nutritional standpoint, but also for its potential to reduce enteric methane emissions.

However, it should be noted that including PKM in cattle rations can modify rumen fermentation and reduce methane production (Tan *et al.*, 2011). Similarly, its lipid content has a deflating effect on protozoa that are symbiotically related to methane-producing Archaea (Machmüller *et al.*, 2003).

Soltan *et al.* (2018) conducted a meta-analysis on this topic and reported that including 15% palm kernel meal in cattle diets can reduce methane emissions by 10-25%. However, the use of palm kernel meal must be balanced since excessive amounts can affect fiber digestibility and alter rumen pH.

In this regard, to evaluate the energy efficiency of a diet, the production of total biogas and methane ( $\text{CH}_4$ ), which represents between 2% and 15% of the energy consumed by the animal, must be estimated (Herrera-Pérez *et al.*, 2018; Carrillo-Hernández *et al.*, 2021).

One technique for estimating the digestibility and rumen fermentation of dry matter (DM) under a kinetic model is *in vitro* gas production (IVGP), which can help establish biogas and methane production and understand the effects of a substrate under study on the total diet (Crosby-Galván & Ramírez-Mella, 2018; Sánchez-Santillán *et al.*, 2020).

Therefore, the objective of this study was to measure the biogas production, degradability, and fermentation kinetics of diets containing different levels of PKM.

## MATERIALS AND METHODS

### Location of the study

The experiment was carried out in the animal nutrition laboratory of the postgraduate program in Genetic Resources and Productivity-Livestock of the Colegio de Postgraduados, Campus Montecillo, Texcoco, State of Mexico.

It is located at kilometer 36.5 of the Mexico-Texcoco Highway at an altitude of 2,240 meters above sea level (asl). All procedures in this study related to the handling of cannulated animals are governed by the Regulations for the Use and Care of Animals in Research at the Colegio de Postgraduados (Reglamento para el Uso de Animales en Investigación, 2016).

### Treatments and chemical analysis

Four diets were formulated to meet the nutritional requirements of fattening lambs with estimated weight gains of  $250 \text{ g d}^{-1}$  (NRC, 2007) (Table 1). The PKM was considered as a coarse ingredient and was replaced by oat hay at levels of 0%, 25%, 50% and 75%.

With this modification, the diets present a total of 0%, 15%, 30% and 45% DM.

To obtain a bromatological analysis of the mixtures, a sample of the experimental diets was collected. The sample was ground in a Thomas Wiley brand hammer mill (Model 4, Arthur H. Thomas Company, Philadelphia, PA, USA), with a 1 mm mesh size.

The dry matter (DM), total protein (TP), ethereal extract (EE) and ash contents were determined in the laboratory according to the methodology described by the AOAC

**Table 1.** Nutritional content of experimental diets with different levels of inclusion of palm kernel meal.

Treatments				
Ingredients	Control	15% PKM	30% PKM	45% PKM
Yellow Corn	16.5	18.5	21	23
Soybean Meal	14	12	9.5	7.5
Palm Kernel Meal	0	15	30	45
Oat Hay	60	45	30	15
Molasses	6	6	6	6
Urea	1.5	1.5	1.5	1.5
Mineral Premix <sup>†</sup>	2	2	2	2
Chemical composition (%)				
Dry Matter	90.44	90.82	90.91	84.67
Crude Protein	15.24	16.32	15.10	13.41
Ethereal Extract	2.35	3.21	4.08	4.94
Neutral Detergent Fiber	41.39	43.54	45.33	51.99
Acid detergent Fiber	19.48	23.07	25.71	30.97
Lignin Acid Detergent	2.25	5.33	7.27	7.47
Ash	9.96	9.55	7.82	6.04
Metabolizable Energy ( $\text{Mcal kg}^{-1}$ )	2.51	2.54	2.59	3.17

PKM=Palm Kernel Meal. <sup>†</sup> Vitasal for sheep: Calcium 20%; Magnesium, 2%; Zinc, 5000 mg; Selenium, 30 mg; Moisture, 5%; and Ash, 95%. Metabolizable energy, calculated using JAVA Feed Tag tool, from the University of California, Davis, Department of Animal Science.

(AOAC., 2005). Neutral Detergent Fiber and Acid Detergent Fiber (NDF and ADF) according to the methodology described by (Van Soest *et al.*, 1991). Metabolizable energy (ME, Mcal kg<sup>-1</sup>) was calculated with data from the chemical analysis of each of the experimental diets and crude fiber intake (calculated from tables), and with the support of the JAVA tool (Feed Tag) from the University of California, Davis, Department of Animal Science (Feed Tag: JAVA, 2021).

### **Anaerobic culture medium**

Rumen fluid was extracted from fistulated Holstein bulls and cannulated in the rumen and filtered with a double blanket. It was then immediately mixed with a reduced mineral solution at a dilution of 1 to 1 (v/v; rumen fluid/mineral solution).

The ACM solution contained 75 mL of mineral solution I [K<sub>2</sub>HPO<sub>4</sub> (6 g L<sup>-1</sup> of water)]; 75 mL of mineral solution II [KH<sub>2</sub>PO<sub>4</sub> (6 g), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (6 g), NaCl (12 g), MgSO<sub>4</sub> (2.45 g) and CaCl<sub>2</sub>-H<sub>2</sub>O (1.6 g L<sup>-1</sup> of water)], 50 mL of 8% sodium carbonate solution [Na<sub>2</sub>CO<sub>3</sub> (8 g/100 mL of water)], 20 mL of reduction solution [L-cysteine (2.5 g) at pH 10 with NaOH 2N (15 mL L<sup>-1</sup>), Na<sub>2</sub>S (2.5 g) and 0.01% resazurin (2 drops of resazurin)] 100 mL of rumen fluid.

The prepared ACM was placed in a water bath at 39 °C with a continuous flow of CO<sub>2</sub> (Miranda-Romero *et al.*, 2020).

### **Biodigesters**

Six 120-mL culture vials per treatment were used, to which 0.5 g of sample (experimental diets) was added, followed by 50 mL of ACM under continuous CO flow. The vials were hermetically sealed with neoprene stoppers and an aluminum ring, then incubated at 39 °C for 72 hours. One vial was considered one experimental unit (Sánchez-Santillán *et al.*, 2019).

### **In vitro gas production**

The gas production technique described by (Miranda-Romero *et al.*, 2020) was used. The biogas produced was captured in a glass syringe (50 mL; BD Yale<sup>®</sup>, Brazil) for measurement. The maximum displacement of the embolus indicated the completion of biogas production (Sánchez-Santillán *et al.*, 2022).

The produced CH<sub>4</sub> was captured in salt traps consisting of serological vials with 90 mL of NaOH solution (2N). This solution contained 80 g of NaOH (Merk<sup>®</sup>) diluted in 1,000 mL of distilled water (Torres-Salado *et al.*, (2018).

### **Biogas production kinetics**

The estimation parameters of the in vitro gas production kinetics were obtained using the accumulated biogas production data and the Gompertz model (Cañaverl-Martínez *et al.*, 2020).

$$Y = A * \left\{ \exp \left[ -b * \exp(-\kappa * t) \right] \right\}$$

For which it is described,  $Y$  = biogas volume at time  $t$  ( $\text{mL g}^{-1}$  de DM);  $A$  = total biogas potential when  $t = \infty$  ( $\text{mL g}^{-1}$  of DM);  $b$  = at the constant rate of biogas production of the potentially degradable material ( $\text{mL h}^{-1}$ );  $\kappa$  = at time lag (h), constant factor of microbial efficiency, defined as the intercept of the time axis of the tangent line at the inflection point;  $t$  = incubation time.

### ***In vitro* degradability**

The *in vitro* degradability of dry matter (IVDDM) test was performed at the end of the biodigester incubation test (72 h). Then, the culture medium was filtered through porcelain crucibles containing 125 mm diameter Whatman 541 filter paper, which was connected to a vacuum pump (EVAR<sup>®</sup>, Model EV-40). The constant dry weight of the filter papers was previously determined and identified with respect to the corresponding biodigester that was filtered. Then, the filtered samples were dried at 60 °C for 48 hours to determine the IVDDM, using the following formula:

$$IVDDM(\%) = ((DSW - (RDW - BW)) / DSW) \times 100$$

Where *IVDDM* = *In Vitro* Dry Matter Degradability expressed in percentage, *DSW* = Dry Sample Weight before fermentation, *RDW* = Residual Dry Weight after fermentation, *BW* = Blank Weight samples.

### **Statistical analysis**

Partial biogas production data, as well as *in vitro* fermentation kinetics and *in vitro* dry matter digestibility, were analyzed under a completely randomized experimental design, with four trials and six replicates (one replicate equal to one biodigester). The analysis was performed with the GLM procedure (SAS, Inc., 2011), for the difference of means the Tukey mean test was used ( $p \leq 0.05$ ).

## **RESULTS AND DISCUSSION**

### **Biogas production**

Gas production with statistically different values ( $p > 0.05$ ) was presented after 6 hours of incubation (Table 2).

The treatment with the highest PKM value (45%) registered the lowest biogas values ( $p < 0.05$ ) during the whole study. On the other hand, the control presented the highest biogas values during incubation, and they were statistically different ( $p < 0.05$ ).

The response obtained in the treatment with the highest PKM value could have decreased biogas production, due to the greater increase in oil residues (Escobar-España *et al.*, 2022), which could have decreased biogas production. Portela *et al.* (2022) report that the presence of palm oil in diets can decrease  $\text{CH}_4$  production *in vitro*.

In general, a high fiber content (particularly of neutral detergent fiber and lignin, see Table 1), and a moderate proportion of fat favor rumen fermentation and concomitantly can reduce methane emissions. In fattening steers, Silva *et al.* (2016) report that including

**Table 2.** *In vitro* cumulative biogas production of diets with different levels of palm kernel meal inclusion.

<b><i>In vitro</i> biogas production</b>	<b>Time (hours)</b>	<b>Control</b>	<b>15% PKM</b>	<b>30% PKM</b>	<b>45% PKM</b>
(ml g <sup>-1</sup> MS)	3	29.48	33.93	36.52	31.56
	6	84.90 a	79.90 a	79.19 a	63.83 a
	9	132.15 a	126.22 a	121.14 a	102.39 b
	12	172.30 a	165.81 ab	158.71 b	139.55 c
	24	263.30 a	259.50 a	237.33 ab	216.66 b
	48	342.47 a	300.15 a	300.15 ab	281.86 b
	72	372.66 a	318.75 bc	318.75 bc	300.78 c

Average values with different letters in the same column indicate significant differences ( $p < 0.05$ ).

PKM in diets decreased dry matter digestibility and microbial protein synthesis, both of which affect rumen gas production.

In other ruminants such as buffaloes, Amaral *et al.* (2008) cite decreased methane gas production when fed palm kernel cake. They attribute this effect to the modification of ruminant fermentation and a decrease in hydrogen as a precursor of methane.

The decrease in gas production is related to the high fiber content in the PKM, which limits the production of volatile fatty acids (Patra, 2017).

Conversely, Jenkins *et al.* (2008) suggest that the fat content in HP may be toxic or inhibitory to the microorganisms in the rumen that produce methane.

In general, PKM modifies rumen microbial populations and can induce decreases in methanogenic microorganisms (Belanche *et al.*, 2015).

The gas production rate shows little variation among the treatments evaluated treatments and no statistical difference. This response is due to the availability of structural and non-structural carbohydrates for microorganisms during fermentation (Elghandour *et al.*, 2016).

Regarding the lag or delay time of the gas production rate, there were no statistically significant differences ( $p > 0.05$ ) between the treatments.

The IVDMD showed significant statistical differences ( $p < 0.05$ ) among the treatments. The diet with 45% PKM inclusion (63.11%) had the lowest percentage of IVDMD at 72 hours.

**Table 3.** Biogas production kinetics in diets with different levels of palm kernel meal inclusion.

<b>Variable</b>	<b>Treatments</b>			
	<b>Control</b>	<b>15% PKM</b>	<b>30% PKM</b>	<b>45% PKM</b>
A (mL g <sup>-1</sup> MS)	356.79 <sup>a</sup>	340.69 <sup>ab</sup>	331.47 <sup>b</sup>	291.35 <sup>c</sup>
K (h)	2.86	2.90	2.89	2.88
B (h <sup>-1</sup> )	0.110	0.108	0.106	0.105
DIVMS	75.83 <sup>a</sup>	70.08 <sup>b</sup>	67.35 <sup>c</sup>	63.11 <sup>d</sup>

A, maximum gas volume; K, gas production rate; B, Lag time; IVDDM, *in vitro* degradability of dry matter in % at 72 hours.

The ratio of structural to non-structural carbohydrates is linked to biogas production. The diet with 0% PKM inclusion should have a higher fermentation volume than diets including PKM due to the level of structural carbohydrates (NDF and lignin) (Sánchez-Santillán *et al.*, 2019). Estimators of fermentation kinetics demonstrate the tendency of fermentation related to the NDF and lignin content in the diet, which affects digestibility (Gómez-Trinidad *et al.*, 2023).

## CONCLUSIONS

Under *in vitro* conditions, the inclusion of 45% palm kernel meal reduces biogas production. PKM represents an alternative use for its inclusion in diets.

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# Mayan herbal medicine: an option to improve respiratory health in Campeche and Chiapas, Mexico

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## ABSTRACT

**Objective:** To document the medicinal plants used by Mayans from Campeche and Chiapas to alleviate respiratory ailments.

**Design/methodology/approach:** Ethnographic and ethnobotanical data were collected through the application of a semi-structured questionnaire with traditional healers and individuals who use medicinal plants for respiratory relief.

**Results:** A total of 28 medicinal plants were recorded, primarily sourced from agricultural fields and home gardens. These plants belong to 19 botanical families: six were registered in the village of Bolonchén de Rejón, 12 in Mazapa de Madero, and 14 in the Motozintla de Mendoza area.

**Limitations/implications:** The study was conducted during a single season; results may vary in other periods.

**Findings/conclusions:** Teas prepared mainly from leaves are used by 43% of respondents, followed by extracts and toasted plant parts.

**Keywords:** traditional medicine, Southern México, local knowledge, mayans.

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## INTRODUCTION

In Mexico, Indigenous communities and those residing in rural areas are considered vulnerable groups due to the social inequities they face daily. These challenges reduce their capacity to respond to a range of problems, including lack of transportation, electricity, poor food distribution, and limited or non-existent healthcare services. This inequity worsened with a 16.2% increase (15.6 million people) in the population lacking access to healthcare services from 2019 to 2020 (CONEVAL, 2021). Given the urgent need for medical services to address various illnesses, these communities turn to medicinal plants as substitutes for pharmaceutical drugs (Gallegos-Zurita, 2016), a practice that remains both periodic and effective across different localities.

It is important to note that in Mexico, medicinal flora is part of the cultural, herbal, and traditional knowledge heritage that still persists among the population. These plants continue to play a vital role in meeting basic healthcare needs and are a crucial link within traditional medical systems for maintaining health (WHO, 2013). The use of these plants as medicines is so valuable that current studies investigate their biological activities and antibacterial properties to explain their mechanisms of action in detail, with the goal of

gaining a deeper understanding of the Mayan ethnopharmacopoeia (Ankli *et al.*, 2002; Sharma *et al.*, 2017).

Ethnobotanical studies on the use of medicinal plants have been conducted in various parts of Mexico, such as Papantla, Veracruz with 101 plants (Lara *et al.*, 2019a); Acanceh, Cantamayec, Tibolón, and Yaxcabá in Yucatán with 96, 123, 53, and 109 species, respectively (Méndez-González *et al.*, 2014); Hopelchén and Calakmul, Campeche with 69 plants (Cahuich-Campos, 2018); and Monterrey in the municipality of Villa de Corzo, Chiapas with 73 species (Campos-Saldaña *et al.*, 2018). In Chiapas herbariums, 16 species from the Labiatae family with medicinal potential have been documented (Domínguez-Vázquez and Castro-Ramírez *et al.*, 2002).

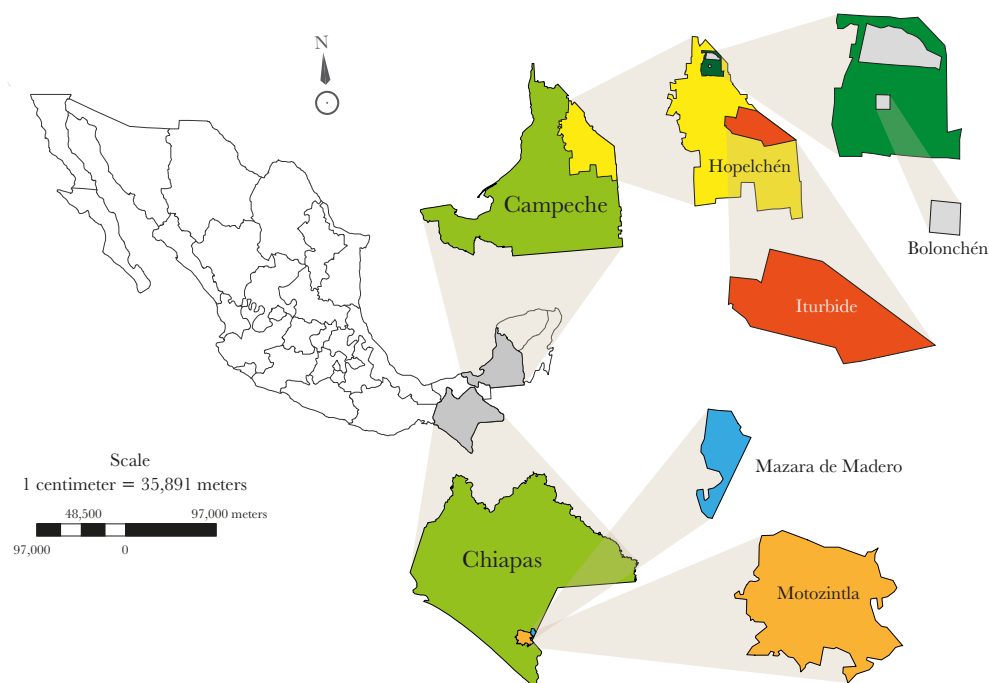
These plants are used to treat various conditions, including climacteric symptoms (Cahuich-Campos *et al.*, 2018), venomous bites, gastrointestinal disorders, infectious diseases (Lara *et al.*, 2019b), nervous system disorders (Castañeda *et al.*, 2022), dermatological conditions, culturally-associated illnesses, musculoskeletal issues, urinary system problems, cardiovascular diseases, female reproductive issues, emotional disorders, pain relief, nervous system conditions (Casanova-Pérez *et al.*, 2022), and kidney-related ailments (Castañeda *et al.*, 2022). However, there are few records of plants specifically used to treat respiratory diseases. Therefore, the objective of this study was to document the medicinal plants used by Mayans in Campeche and Chiapas to address respiratory ailments.

## MATERIALS AND METHODS

**Study Area.** The research was conducted from February to June 2022 in the locality of Bolonchén de Rejón, in the state of Campeche, located at 20° 00' 17.18" N and 89° 44' 51.54" W, at an altitude of 120 masl, with an average temperature of 32 °C and an average annual rainfall of 1,044 mm. It also took place in two municipalities of the state of Chiapas: Motozintla de Mendoza, the municipal seat located at 15° 21' 48.36" N and 91° 14' 52.73" W, at an altitude of 1,300 masl, with an average temperature of 19 °C and annual rainfall ranging from 1,260 to 2,000 mm (INEGI, 2010); and Mazapa de Madero, located at 15° 23' 18.26" N and 92° 11' 72.96" W, with the municipal seat at an altitude of 1,100 masl, an average temperature of 22 °C, and annual rainfall between 800 and 1,080 mm (Figure 1).

**Data Collection.** Ethnographic and ethnobotanical methods were used in this research, employing directed interviews and participant observation as data collection techniques (Kawulich, 2005). For the interviews, a non-probabilistic "snowball" sampling method (Babbie, 1999) was used to identify traditional healers or individuals who use plants to improve health, reaching a total of 10 families per locality.

A questionnaire was then applied covering the following aspects: a) Sociocultural; b) Management and Conservation of medicinal plants in agroecosystems (AES) and ecosystems; and c) Uses of medicinal plants. Participant observation an essential tool in qualitative research, particularly in anthropological and sociological studies as suggested by Kawulich (2005) was employed to foster interaction with involved actors and gain a more holistic understanding of the phenomenon under study. This approach



**Figure 1.** Mayan localities: Bolonchén de Rejón in Campeche, and Mazapa de Madero and Motozintla de Mendoza in Chiapas, Mexico.

allowed the researcher to immerse themselves in the lifestyles of the ethnic groups to learn about their organization, social structure, educational background, and the value they place on medicinal plants in various settings (Alexiades, 1996; Kawulich, 2005; Musante & DeWalt, 2010). Finally, the data were analyzed using descriptive statistics in Excel<sup>®</sup>.

## RESULTS AND DISCUSSION

A total of 27 medicinal plants belonging to 19 families were recorded as being used for bronchial and respiratory ailments. The scientific names, common names, and families are listed in Table 1. The most frequently used plant was bougainvillea (*Bougainvillea glabra* Choisy.) with a usage frequency of 8.5%, followed by lime (*Citrus × aurantifolia* Christm. Swingle) and lemongrass (*Cymbopogon citratus* (DC.) Stapf), each with 7% usage frequency (Table 1). In other regions of Mexico, similar records have been made. In Actopan, Hidalgo, 19 plants were mentioned (Villanueva-Solís *et al.*, 2020); in Nacajuca, Tabasco, 22 species belonging to 15 families were reported (Magaña *et al.*, 2021); and in Julián Blanco, Guerrero, Sotelo-Leyva *et al.* (2022) reported 16 plants used for respiratory issues, including basil (*Ocimum basilicum* L.), arnica (*Heterotheca inuloides* Cass.), bougainvillea (*Bougainvillea spectabilis* Willd.), cinnamon (*Cinnamomum verum* J. Presl.), guapinol (*Hymenaea courbaril* L.), snake herb (*Zornia thymifolia* Kunth.), wild ash (*Fraxinus uhdei* Wenz. Lingelsh.), spearmint (*Mentha spicata* L.), wormwood (*Artemisia ludoviciana* Nutt.), lemon (*Citrus × limon*), muicle (*Justicia spicigera* Schltdl.), mango (*Mangifera indica* L.), oregano (*Origanum vulgare* L.), capote (*Xanthosoma robustum* Schott.),

purple sage (*Lippia alba* Mill.), and broadleaf oregano (*Plectranthus hadiensis* Forssk.). Of the plants reported by those authors, four species were also recorded in the present study, highlighting their common use in treating diseases in Mexico.

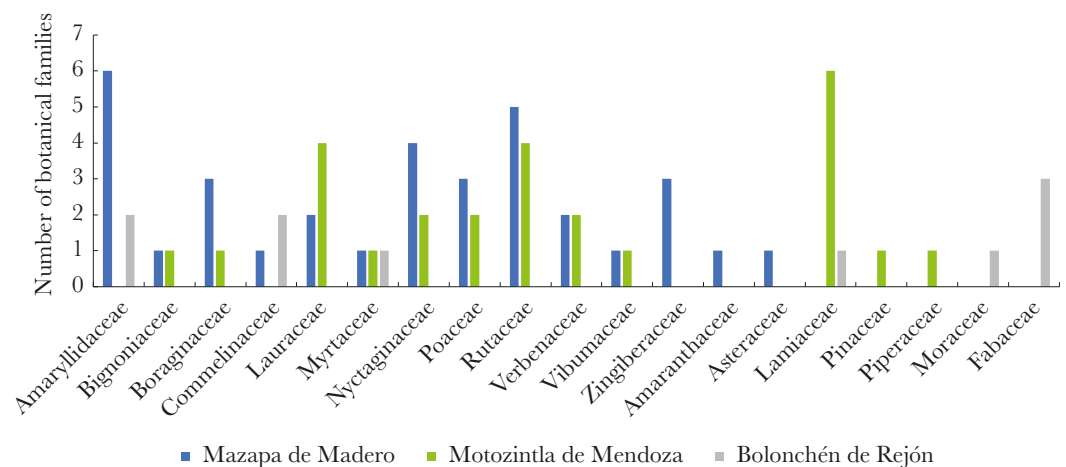
In regions closer to Bolonchén de Rejón, similar findings were reported. For example, the Mayan community of Yaxcabá, Yucatán, recorded 16 plants, with the most commonly used species being arnica (*Tithonia diversifolia*) and purple maguey (*Tradescantia spathacea* Sw.) (Méndez-González *et al.*, 2014). In Xmejía, Hopelchén, Campeche, Cahuich-Campos *et al.* (2014) documented 10 plants: iresine (*Iresine celosia* L.), soursop (*Annona muricata* L.), henequen (*Agave fourcroydes* Lem.), barbasco (*Piscidia piscipula* L.), calabash tree (*Crescentia cujete* L.), purple maguey (*Tradescantia spathacea* Sw.), wild oregano (*Lantana hirta*), oak (*Ehretia tinifolia* L.), sugar apple (*Annona squamosa*), and tobacco (*Nicotiana tabacum* L.). In the state of Chiapas, Domínguez-Vázquez and Castro-

**Table 1.** Medicinal plants reported by the Mayans of Campeche and Chiapas, Mexico.

Scientific name	Common name	Family
<i>Allium cepa</i> L.	Red onion	Amaryllidaceae
<i>Allium sativum</i> L.	Garlic	Amaryllidaceae
<i>Allium tuberosum</i> Rottler ex Spreng.	Chives	Amaryllidaceae
<i>Artemisia absinthium</i> L.	Wormwood	Asteraceae
<i>Borago officinalis</i> L.	Borage	Boraginaceae
<i>Bougainvillea glabra</i> Choisy.	Bougainvillea	Nyctaginaceae
<i>Brosimum alicastrum</i> Sw.	Ramón	Moraceae
<i>Cinnamomum verum</i> J.	Cinnamon	Lauraceae
<i>Citrus × sinensis</i>	Orange	Rutaceae
<i>Citrus × aurantifolia</i> (Christm.) Swingle	Lemon	Rutaceae
<i>Crescentia alata</i> Kunth	El morro	Bignoniaceae
<i>Cymbopogon citratus</i> (DC.) Stapf	Lemon tea	Poaceae
<i>Dysphania ambrosioides</i> (L.) Mosyakin & clemants	Epazote	Amaranthaceae
<i>Eucalyptus globulus</i> Labill.	Eucalyptus	Myrtaceae
<i>Laurus nobilis</i> L.	Bay	Lauraceae
<i>Lippia dulcis</i> Trevir.	Orozu	Verbenaceae
<i>Parmentiera aculeata</i> (Kunth) Seem.	Cuajilote	Bignoniaceae
<i>Pinus ayacahuite</i> C. Ehrenb. ex Schltld.	Pine	Pinaceae
<i>Piper auritum</i> Kunth	Herb santa	Piperaceae
<i>Plectranthus amboinicus</i> (Lour.) Spreng.	Oregano	Lamiaceae
<i>Plectranthus hadiensis</i> (Forssk.) Schweinf. ex Sprenger	Vaporub	Lamiaceae
<i>Psidium guajava</i> L.	Guava	Myrtaceae
<i>Sambucus nigra</i> L.	Elderberry	Viburnaceae
<i>Senna occidentalis</i> (L.) Link	Little bean	Fabaceae
<i>Thymus</i> L.	Thyme	Lamiaceae
<i>Tradescantia spathacea</i> Sw	Purple agave	Commelinaceae
<i>Zingiber officinale</i> Rosc.	Ginger	Zingiberaceae

Ramírez (2002), through a literature and herbarium review of the medicinal uses of the Labiatae family for respiratory conditions, described 16 plants, including: liniment (*Catopheria chiapanensis*), *Hyptis urticoides* Kunth, cancer herb (*Lepechinia schiediana* (Schltdl.)), basil (*Ocimum basilicum*), wild basil (*Ocimum micranthum* Willd.), field anise (*Ocimum selloi*), lavender (*Salvia lavanduloides* Kunth.), rosehip (*Rosa rubiginosa* L.), cock's herb (*Salvia tiliaefolia* Vahl), maltansi (*Satureja brownei* (Sw.) Briq.), pennyroyal (*Satureja mexicana* (Benth.) Briq.), myrtle (*Stachys coccinea* Jacq.), mugwort (*Leonurus sibiricus* L.), self-heal (*Prunella vulgaris* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia* sp.), and verbena (*Teucrium vesicarium* Miller). Likewise, Lara *et al.* (2019b) identified 11 plants in the highlands of Chiapas: chamomile (*Matricaria chamomilla* L.), marigold (*Tagetes erecta* L.), dandelion (*Taraxacum officinale* L.), copal (*Bursera jorullensis* (Kunth) Engl.), elderberry (*Sambucus mexicana* C. Presl ex DC.), lavender (*Salvia lavanduloides* Kunth), mint (*Mentha sativa* L.), bougainvillea (*Bougainvillea glabra* Choisy), camphor flower (*Cinnamomum camphora* (L.) J. Presl), cinnamon tree (*Cinnamomum zeylanicum* J. Presl), and mountain laurel (*Litsea glaucescens* Kunth). Finally, in seven localities in Chiapas —Emiliano Zapata, Libertad, Salto de Agua, Catazajá, Palenque, Chilón, and Benemérito— only three plants were reported for treating respiratory ailments: broadleaf oregano (*Plectranthus amboinicus* (Lour.) Spreng.), spearmint (*Mentha spicata* L.), and guaco (*Mikania laevigata* (Sch. Bip. ex-Baker)) (Martínez *et al.*, 2023).

Of the total medicinal plant families, six were found in the community of Bolonchén de Rejón, 12 were recorded in Mazapa de Madero, and 14 were documented in Motozintla de Mendoza (Figure 2). In Mazapa de Madero, the most representative families were Amaryllidaceae, Rutaceae, and Nyctaginaceae, followed by Boraginaceae, Poaceae, and Zingiberaceae. In Motozintla de Mendoza, the most prominent families were Lamiaceae, Rutaceae, and Lauraceae, followed by Nyctaginaceae, Poaceae, and Verbenaceae. In Bolonchén de Rejón, the most representative family was Fabaceae, followed by Amaryllidaceae and Commelinaceae.



**Figure 2.** Representative botanical families used for respiratory ailments in Mayan communities of Campeche and Chiapas, Mexico.

The botanical families observed in Motozintla align with those recorded in Actopan, Hidalgo (Villanueva-Solís *et al.*, 2020), and Julián Blanco, Guerrero (Sotelo-Leyva *et al.*, 2022), with Lamiaceae being the most abundant family. However, these differ from findings in Loma Alta, Nevado de Toluca, Mexico; Yaxcabá, Yucatán; and the Highlands of Chiapas, where the most representative botanical family is Asteraceae (Méndez-González *et al.*, 2014; Sotero-García *et al.*, 2016; Lara *et al.*, 2019b).

Among the respiratory ailments treated with medicinal plants, interviewees primarily identified nine conditions, many of which are associated with the SARS-CoV-2 virus (COVID-19) (Table 2). These ailments include cough, flu, cold, tonsillitis, and phlegm accumulation (Sotero-García, 2016; Sotelo-Leyva *et al.*, 2022), as well as asthma and bronchitis (Méndez-González *et al.*, 2014), nasal congestion, chills, sore throat, pneumonia, colds, and hoarseness (Lara *et al.*, 2019a; Villanueva-Solís *et al.*, 2020). In Nacajuca, Tabasco, of the medicinal species reported for respiratory issues, 20 are used to treat cough, 15 for flu, 13 for asthma, nine for colds, and one for “chichimeca” (severe coughing fits accompanied by fever and suffocation) (Magaña *et al.*, 2021). To prepare remedies for these respiratory conditions, the most commonly used plant part is the leaf (43%), followed by branches (14%) and flowers (13%) (Table 2), similar to findings in Julián Blanco, Guerrero, where leaves are used in 46% of cases (Sotelo-Leyva *et al.*, 2022). However, among the Tzotzil people in the Highlands of Chiapas, usage patterns differ: they use the entire plant in 31.3% of cases, stem and leaf in 25%, flower in 12.5%, and bark in 6.3% (Lara *et al.*, 2019b).

Regarding the preparation methods of remedies using medicinal plants, three main forms were recorded. Tea (infusion) is the primary method of consumption, followed to a lesser extent by extracts and roasted preparations (Figure 3). It has been observed that tea or infusion is among the most commonly used methods for consuming medicinal plants, with usage ranging from 70% to 100% in various localities (Lara *et al.*, 2019a; Villanueva-Solís *et al.*, 2020; Sotelo-Leyva *et al.*, 2022). Notably, extract and roasted forms are used individually and only in certain communities for instance, the roasted form is preferred in Bolonchén, whereas in Chiapas communities, extracts are used more frequently than roasted preparations (Figure 3).

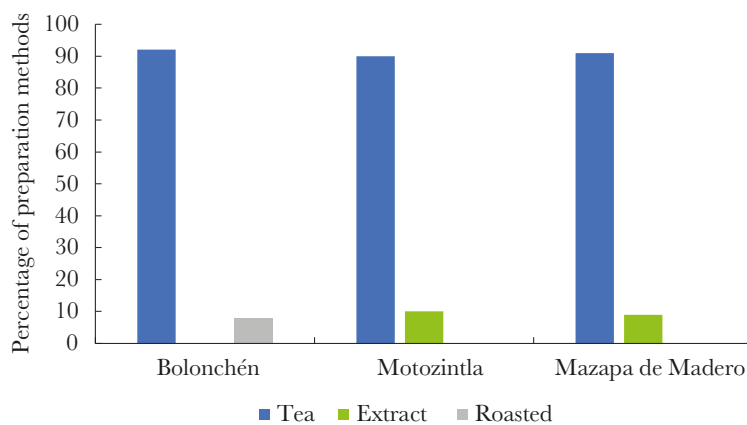
The plants used in the Mayan communities of Bolonchén de Rejón, Mazapa de Madero, and Motozintla de Mendoza, Chiapas, are mainly obtained from agricultural plots and home gardens/backyards (Figure 4). This aligns with findings by Magaña *et al.* (2021), who reported that, of the 22 species used in Nacajuca, Tabasco, 14 are cultivated in home gardens (backyards) and orchards (*C. aurantium*, *C. citratus*, and *P. amboinicus*), six are wild (collected in the field), and only two are purchased. Similarly, in Xmejía, Hopelchén, Campeche, most plants are sourced from gardens or household plots, followed by secondary and mature forests (monte) (Cahuich-Campos *et al.*, 2014). Likewise, Martínez *et al.* (2023) noted that in the communities of Emiliano Zapata, Libertad, Salto de Agua, Catazajá, Palenque, Chilón, and Benemérito in Chiapas, the highest percentage of medicinal plants come from backyards. These plants often coexist with edible species, contributing to food security in terms of access, utilization, and availability.

**Table 2.** Parts of medicinal plants used for respiratory conditions in the Mayan communities of Campeche and Chiapas.

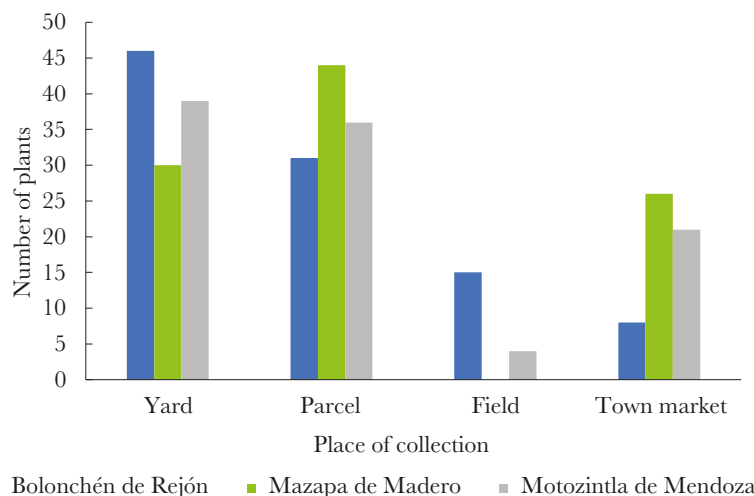
Mayan localities	Ailments	Common name	Part of the plant
Bolonchén de Rejón	Bronchitis	Green Onion	Leaf
	Flu Cough	Purple Maguey	Branch
		Bean	Leaf
		Oregano	Leaf
		Green Onion	Branch
	Sick	Bean	Leaf
		Guava	Leaf
		Ramón	Leaf
	Shortness of breath	Purple Maguey	Leaf
		Bean	Leaf
Mazapa de Madero	Bronchitis	Orozo	Leaf
	Flu Throat infection Lung infection Cold	Eucalyptus	Leaf
		Cinnamon	Stem
		Lemon tea	Leaf
		Orange (leaf)	Leaf
		Lemon	Fruit
		Bougainvillea	Flower
		Elderberry	Flower
	Cough Bronchitis Flu Throat infection	Borage	Branch
		Bougainvillea	Flower
		Garlic	Bulb
		Red onion	Bulb
		Ginger	Rhizome
	Lung infection Cold	Borage	Branch
		Bougainvillea	Flower
		Garlic	Bulb
		Red onion	Bulb
		Ginger	Rhizome
	Bronchitis	Lemon tea	Leaf
	Flu	Matazano	Leaf
Lemon Tea		Leaf	
Lemon		Flower	
Ginger		Rhizome	
Cinnamon		Stem	
Bougainvillea		Flower	
Purple Maguey		Leaf	
Borage		Branch	
Garlic		Bulb	
Red Onion		Bulb	
Orozo		Leaf	
Cuajilote		Fruit	
Orange	Leaf		

**Table 2.** Continues...

Mayan localities	Ailments	Common name	Part of the plant
Motozintla de Mendoza	Dry throat	Lemon	Fruit
	Flu Throat infection Hoarseness Dry throat Flu	Orozo	Leaf
		Lemon Tea	Leaf
		Vaporub	Leaf
		Lemon Bay	Leaf
		Cinnamon	Fruit
		Bougainvillea	Stem
		Orange	Flower
		Thyme	Fruit
		Pine	Branch
	Throat infection	Epazote	Resin
		Thyme	Branch
	Hoarseness	Hierbasanta	Branch
	Cough	Thyme	Leaf
		Wormwood	Branch
		Borage	Leaf
		El Morro	Branch
		Bay	Fruit
		Vaporub	Leaf
		Oregano	Leaf
		Eucalyptus	Leaf
		Orozo	Leaf
		Elderberry	Flower
Bougainvillea (flower)		Flower	
Cinnamon		Stem	
Lemon		Fruit	
Lemon Tea		Leaf	
Lemon Tea	Leaf		



**Figure 3.** Preparation methods for medicinal plants used in the Mayan communities of Campeche and Chiapas, Mexico.



**Figure 4.** Location of medicinal plants obtained to treat respiratory conditions reported by Mayans from Campeche and Chiapas, Mexico.

## CONCLUSIONS

In the Mayan community of Motozintla de Mendoza, Chiapas, a greater number of medicinal plants are used to alleviate respiratory illnesses, followed by Mazapa de Madero, Chiapas with 14 species, and Bolonchén de Rejón, Campeche, where only six plants were recorded. The most commonly used plants across the three localities are bougainvillea and lime, primarily consumed as tea (infusion). The most frequently used medicinal plants are sourced from home gardens and agricultural plots.

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# Bioactive Species Associated with Rambutan (*Nephelium lappaceum* L.) and their Influence on Soil Chemical and Microbiological Properties

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## ABSTRACT

**Objective:** To know the influence of the bioactive plants *Ruta graveolens* L., *Ocimum basilicum* L., *Stachytarpheta jamaicensis* and *Chenopodium ambrosioides* L., on the chemical and microbiological properties of the soil associated with *Nephelium lappaceum* L.

**Design/methodology/approach:** Cuttings of *R. graveolens* L., *O. basilicum* L., *S. jamaicensis* (L.) Vahl and *C. ambrosioides* L. were collected and five treatments with six replications each were established, using a randomized complete block design. At the time of planting the medicinal plants and one year later, a soil sample was collected at a depth of 30 cm for physicochemical (UNACH-FCA) and microbiological analysis of the soil (MASTERLAB S. A. de C. V.) to record variables.

**Results:** The chemical analyses results showed changes in the content of most nutrients between sampling years, with the exception of N. Microorganism populations also exhibited contrasting values between the two sampling periods.

**Limitations on study/implications:** The answer may vary depending on plant density and time of year.

**Findings/conclusions:** The association of bioactive plants induces changes in soil nutrients, with year-to-year fluctuations. Furthermore, it promotes differential growth among the populations of beneficial and harmful microorganisms. Beneficial microorganisms increased, and the presence of pathogens in low populations did not result in plant damage. Beneficial microorganisms such as *Pseudomonas fluorescens* and *Bacillus* spp. were present with *S. jamaicensis*, and *Trichoderma* and *Aspergillus* with *R. graveolens* and *O. basilicum*, suggesting a dependence on exudates.

**Keywords:** rambutan-medicinal plant association; nutrients; rhizosphere; microorganisms; agroecology.

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## INTRODUCTION

*Nephelium lappaceum* L. is a species native to Malaysia and Indonesia, introduced to Chiapas in the 1960s (Castillo-Vera *et al.*, 2017) as an alternative to traditional crops such as coffee (*Coffea arabica* L.), cacao (*Theobroma cacao* L.), banana (*Musa paradisiaca* L.), and mango (*Mangifera indica* L.). Currently, it covers more than 2,500 hectares under production (Osorio-Espinoza *et al.*, 2019) and has become an economically important crop in the Soconusco region (Avenidaño-Arrazate *et al.*, 2011; Flores-Trejo *et al.*, 2016). However,



its productivity has declined, which has been attributed to various environmental and management-related factors.

Previous agronomic issues have been addressed through the use of agrochemicals belonging to various toxicological categories, which has led to ecological imbalances and adverse effects on human health (Ramírez-Montoya *et al.*, 2013), as well as increased production costs. As an alternative, agroecological systems have been implemented using medicinal plants such as *Ruta graveolens* L., *Ocimum basilicum* L., *Stachytarpheta jamaicensis*, and *Chenopodium ambrosioides* L. When associated with crops, these species provide benefits to the soil and, through various mechanisms of action, help reduce pests and diseases (Marroquín-Agreda *et al.*, 2019).

From a systemic perspective, polycultures have a positive impact on insect, fungal, and weed populations by enhancing biological balance and improving nutrient availability (Rodríguez-González *et al.*, 2008). The association of perennial crops with bioactive plants can generate various forms of interdependence, such as synergism, antagonism, symbiosis, allelopathy, or insect attraction within the agroecological system. These interactions are considered to influence nutrient and energy recycling, the replacement of external inputs, the increase of organic matter and soil biological activity, and the diversification of plant species and genetic resources (Osorio-Espinoza *et al.*, 2019). In this context, the objective of the present study was to associate four traditional bioactive plant species from the Soconusco region of Chiapas with a four-year-old *Nephelium lappaceum* L. crop, and to identify their influence on the chemical and microbiological properties of the soil.

## MATERIALS AND METHODS

The research was conducted from June 25, 2021, to June 25, 2022, in a four-year-old agroecologically managed *Nephelium lappaceum* L. (rambutan) plot, located in the municipality of Metapa de Domínguez, Chiapas, at coordinates 14° 50' N and 92° 11' W, with a humid warm climate, an altitude of 100 m, an annual average temperature of 27 °C, and annual precipitation of 2,165 mm (García, 1973). The experimental area trees were selected for a height of 2 meters and non-dense crowns.

**Plantation management.** Vermicompost is incorporated once a year into the mid-drip zone of the trees, along with the foliar application of humic substances, and during the dry season, irrigation is carried out using micro-sprinklers. The average fruit yield ranges between 50 and 60 kg per tree.

**Treatments and experimental design.** Five treatments were established: 1) *Ruta graveolens* + *Nephelium lappaceum*, 2) *Ocimum basilicum* + *Nephelium lappaceum*, 3) *Stachytarpheta jamaicensis* + *Nephelium lappaceum*, 4) *Chenopodium ambrosioides* + *Nephelium lappaceum*, and 5) *Nephelium lappaceum* without medicinal plants (control), under a randomized complete block design. Each block represented a replication and consisted of five rambutan trees, with each tree considered an experimental unit. In each unit, four medicinal plants of a single species were associated according to the assigned treatment. They were planted in a cross shape in the mid-drip zone, for a total of 35 *Nephelium lappaceum* trees and 120 bioactive plants.

### Experiment establishment

Bioactive plants were established around the selected trees; these were obtained from backyard gardens in Tapachula, Chiapas. The plants were propagated by cuttings and initially grown in Acrisol soil inside polyethylene bags. Transplanting to the field was carried out on June 25, 2021, when the plants reached an average height of 15 cm, placing them in the mid-drip zone. The planting area was delimited by tracing a circle using a plastic string and a stake. Then, four points were marked in a cross pattern, and holes of 30×30 cm were dug. From each hole, a soil sample was taken, and the spatula was sterilized by flaming with 96% ethyl alcohol. The soil sample was placed in a labeled transparent plastic bag and stored on ice in a plastic container. Physical, chemical, and microbiological analyses were performed on these soil samples.

### Physicochemical and microbiological properties of the soil

These were analyzed at the beginning and end of the experiment. Soil color was determined using the Munsell Soil Color Chart (GretagMacbeth, 2000 edition, USA). Soil texture was determined using the method proposed by Bouyoucos (1962), based on Stokes' law. Textural classes were identified using the soil texture triangle (Gee and Bauder, 1986). The percentage of porosity (p%) was calculated using the formula proposed by Mandelbrot (1982):

$$\text{Porosity (\%)} = \frac{Rd - Bd}{Bd} \times 100$$

where  $Rd$  = real density and  $Bd$  = bulk density.

Bulk density ( $Bd$ ) was determined using the cylinder method, and real density ( $Rd$ ) was calculated by dividing the total weight of oven-dried soil by the volume occupied by the solids. Soil weight for both density determinations was measured using an analytical balance (Ohaus Adventure Pro AV4101, China). Soil reaction (pH) was measured with a potentiometer at a 1:2 ratio (soil: deionized water) (Thermo Orion model 230A+, USA), and electrical conductivity (E.C.) was measured using a conductivity meter (Thermo Orion 145A+, USA) at a 1:5 ratio (soil: deionized water). Organic matter percentage (O.M.) was determined using the Walkley and Black method (1934). Organic carbon (C) content in the organic residue was also measured. For both O.M. and C determinations, a magnetic stirrer with a lamp was used (Thermolyne Stir Light, USA).

The percentage of nitrogen (N) was determined using the micro Kjeldahl method (Kjeldahl, 1883), and phosphorus using the Olsen method (Olsen *et al.*, 1954) with a mechanical reciprocal shaker (Eberbach 6000, USA). Both determinations were carried out with the help of a spectrophotometer (Metach, model UV-6000 UV/VIS Spectrophotometer, China). Potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) were determined using the 1N CH COONH extraction method at pH 7, quantified by atomic absorption with an analytical balance (Ohaus Adventure, China), a mechanical reciprocal shaker (Eberbach 6000, USA), and an atomic absorption spectrophotometer (GBC Scientific Equipment SensAA, Australia). Sulfur (S) and boron (B) were obtained

using a shaker (Eberbach 6000, USA) and a spectrophotometer (Metach, model UV-6000 UV/VIS Spectrophotometer, China), using the turbidimetric and Azomethine H colorimetric methods, respectively. Cation exchange capacity (CEC) was determined using the sum of exchangeable cations method.

Microbiological analyses were carried out by counting colony-forming units (CFU) of bacteria, fungi, and actinomycetes at the beginning and end of the experiment through dilution and Petri dish plating, isolation for 144 hours, and identification of beneficial microorganisms in semi-selective media (Masterlab SA de CV). Fungi were quantified on PDA-AL medium, bacteria on PDA, King B, and Elmar media, and actinomycetes on ELMAR medium. Identification was performed by macroscopic and microscopic observation of morphology (actinomycetes and fungi), as well as staining and UV light exposure (bacteria).

For nematode detection, the Baermann funnel-sieving technique was used. Pathogenic bacteria (*Clavibacter* sp., *Ralstonia* sp., *Pseudomonas* sp., and *Erwinia* sp.) were identified through dilution and Petri dish counting on PDA, King B, and CTT culture media, with incubation at 28 °C. Additionally, worm cast counts were conducted in the treatments of each block every 15 days during the rainy season. Statistical analysis. Data were entered into a Microsoft Excel<sup>®</sup> spreadsheet, and an analysis of variance (ANOVA) was performed using R<sup>®</sup> software. Significant differences were analyzed with Tukey's test at  $P \leq 0.05\%$ .

## RESULTS AND DISCUSSION

The soil showed a brown color with a clay loam texture. The bulk density (Bd) of the treatments ranged between 1.2 and 1.3 g mL<sup>-1</sup>, and the particle density (Rd) between 1.7 and 1.9 g mL<sup>-1</sup>. It showed low compaction and good moisture retention. The porosity percentage ranged from 31.11 to 33.25% (Table 1).

The chemical properties of the soil showed variations with statistically significant differences ( $p \leq 0.05$ ) in the concentrations of some nutrients; however, a decrease in these nutrients was observed in the second analysis during 2022 (Figures 1 and 2).

The total nitrogen percentage showed little variation between treatments with medicinal plants associated with rambutan during 2021. In the sampling conducted during 2022, this nutrient increased in the soil where *O. basilicum*, *S. jamaicensis*, and *C. ambrosoides* were established, with the lowest value recorded where *R. graveolens* was associated. In contrast, the control treatment without medicinal plants showed the highest total nitrogen percentage in the soil. This same trend was observed in the assimilable nitrogen content.

The phosphorus content in the soil showed contrasting variations during the first sampling in 2021, and in 2022, the values were very similar between treatments.

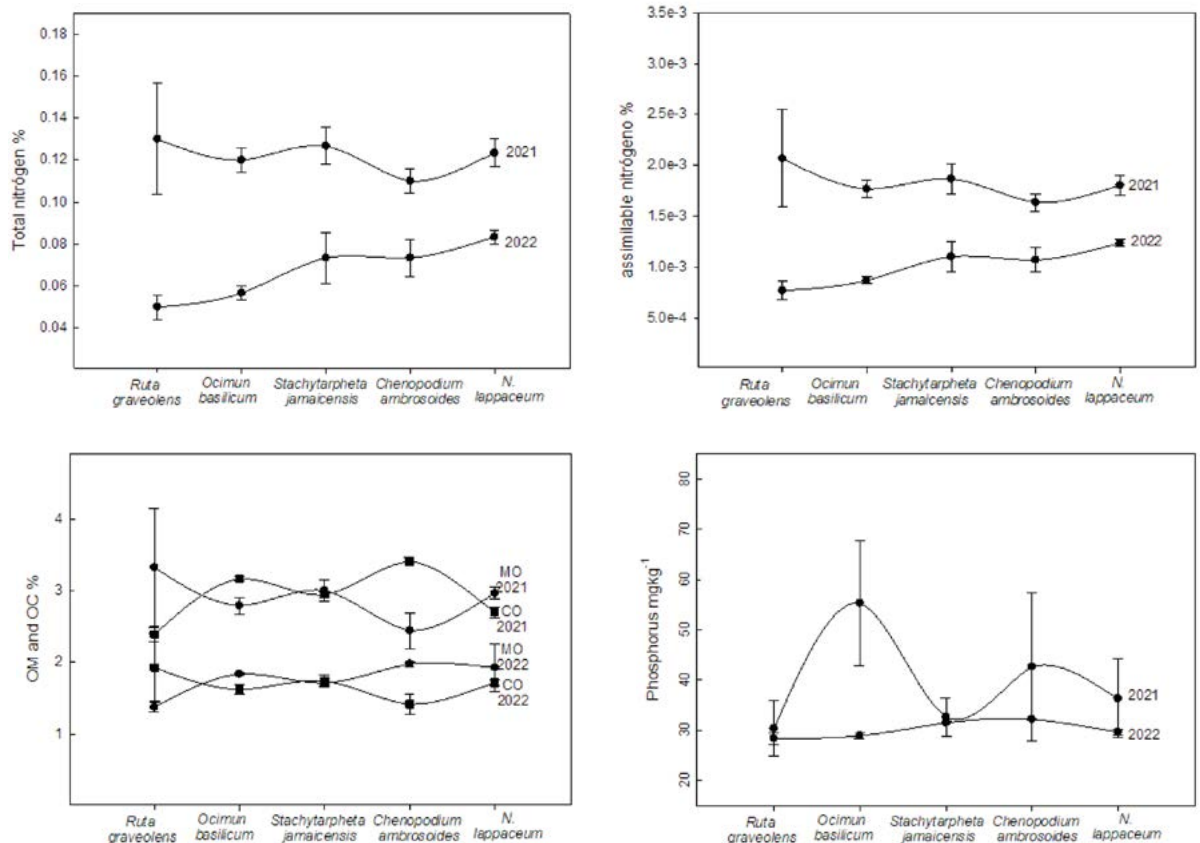
K showed little variation during the first year of sampling. In this case, when *C. ambrosoides* was associated, the content was lower compared to the other treatments, but in 2022, the highest value was recorded. In the second year of sampling, the control treatment showed the lowest value for this nutrient.

In contrast, magnesium content increased in the soil where *S. jamaicensis* was established during 2021 and decreased in 2022. The same effect was observed in the control treatment.

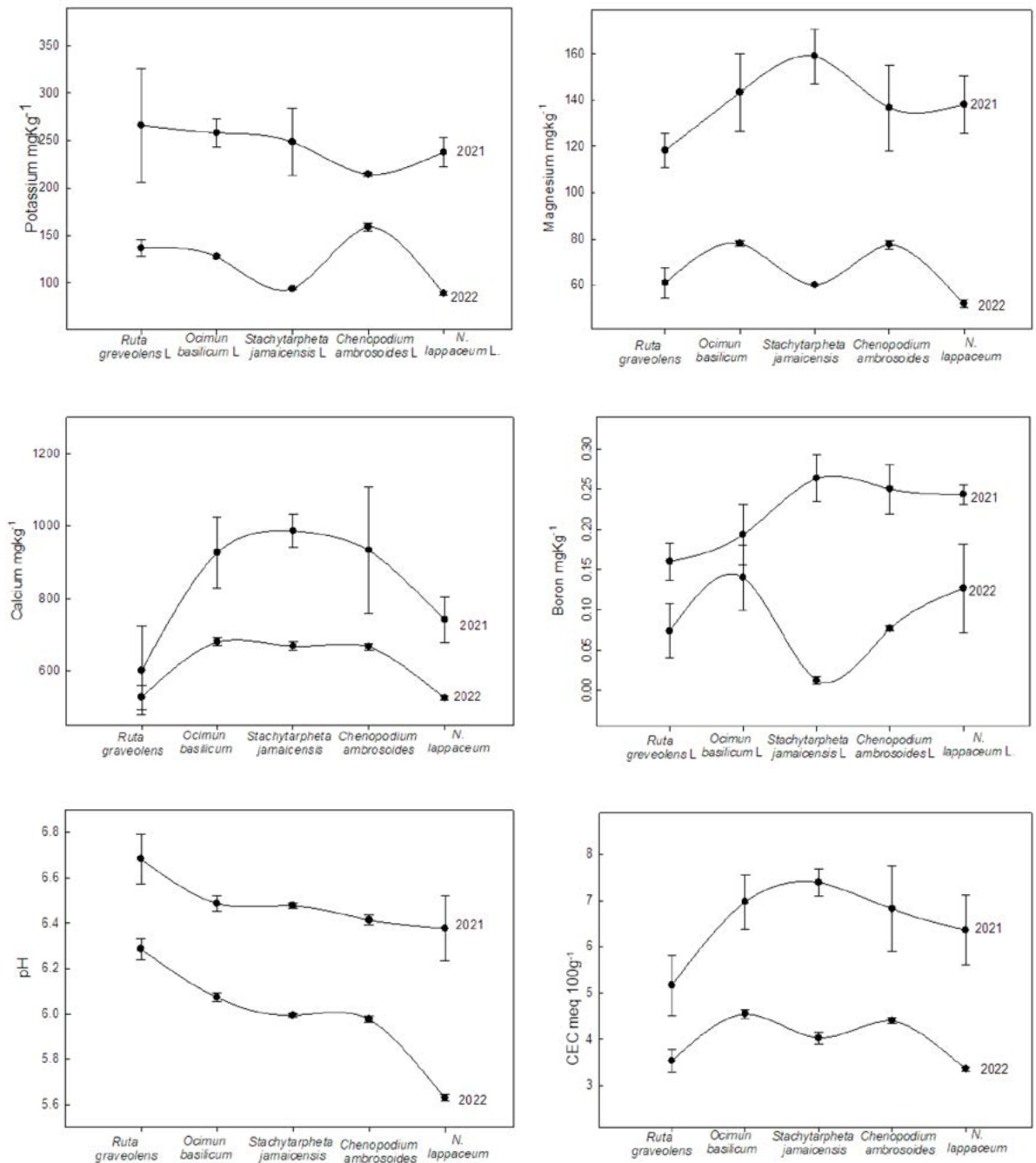
**Table 1.** Physical properties of the soil in the association of different medicinal plants with *N. lappaceum* L in Soconusco, Chiapas.

Component	<i>R. greveolens</i>	<i>O. basilicum</i>	<i>S. jamaicensis</i>	<i>C. ambrosoides</i>	<i>N. lappaceum</i> (Testigo)	CV (%)**
% Sand (2021)*	43.2±3.0	38.2±0.8	38.7±0.5	37.8±0.0	36.5±1.8	7.4 (NS)
(2022)**	40.8±0.6 ab	36.8±0.6 c	41.2±1.0 a	38.2±0.0 bc	30.2±0.0 d	2.8
% Silt (2021)	24.6±4.9	29.2±1.2	24.8±0.9	28.3±0.0	26.0±2.9	17.4(NS)
(2022)	29.0±0.6	30.6±0.8	28.6±0.8	31.6±0.6	30.0±1.4	5.8 (NS)
% Clay (2021)	34.7±0.3	33.1±1.6	36.4±1.5	33.7±0.3	37.4±2.0	6.8 (NS)
(2022)	29.4±1.1	32.4±0.5	32.1±0.6	30.1±0.6	31.7±1.4	5.6 (NS)
Ad g mL <sup>-1</sup> (2021)	1.2±0.03	1.2±0.03	1.3±0.01	1.2±0.01	1.2±0.03	3.7 (NS)
(2022)	1.2±0.01 ab	1.2±0.01 b	1.3±0.02 a	1.2±0.01 ab	1.2±0.006 b	2.0
Dr g mL <sup>-1</sup> (2021)	1.8±0.03	1.8±0.02	1.9±0.01	1.8±0.02	1.9±0.05	3.1 (NS)
(2022)	1.9 ±0.03 a	1.7±0.02 b	1.8 ±0.04 ab	1.8±0.06 ab	1.8±0.02 ab	3.8
% Porosity (2021)	31.1±3.0	31.6±2.4	31.5±0.2	31.9±4.0	33.2±0.4	13.7(NS)
(2022)	35.1±2.0	30.5±0.7	28.4±1.9	30.9±2.6	33.2±0.4	9.6
Color (2021)	7.5YR4/4 brown	7.5YR4/4 brown	7.5YR3/4 brown	7.5YR3/4 brown	7.5YR4/4 brown	
(2022)	7.5YR4/2 brown	7.5YR4/3 brown	7.5YR4/3 brown	7.5YR4/2 brown	7.5YR4/4 brown	

Clay-loam textura. Values are means of three replicates ± standard error. Different letters between lines indicate statistically significant differences (p<0.05). CV=Coefficient of Variation. \* Start of study \*\* End of study.



**Figure 1.** Total and assimilable nitrogen and phosphorus content of soils where *N. lappaceum* was associated with various medicinal plants in Soconusco, Chiapas. Values are means of four replicates ± standard error.



**Figure 2.** Potassium, magnesium, calcium, and boron content, pH, and cation exchange capacity (CEC) in a soil from Soconusco, Chiapas, planted with *N. lappaceum* L. and associated with medicinal plants. Values are averages of four replicates  $\pm$  standard error.

However, with *C. ambrosoides* and *O. basilicum*, the magnesium content in the soil was low during 2021 and increased in 2022.

The calcium content in the soil of the control treatment and where *R. graveolens* was associated showed the lowest values compared to the other treatments during both years of

sampling. In the treatments with the other medicinal plants associated with rambutan, the changes observed occurred between years.

The boron content in the soil increased during 2021 where *S. jamaicensis*, *C. ambrosoides*, and the control treatment were associated. In contrast, during 2022, the lowest value was found when *S. jamaicensis* was associated.

The microorganisms quantified in the soil, fungi and bacteria, also showed contrasting populations between the years of assessment. The differences in bacterial populations of *Pseudomonas fluorescens* and *Bacillus* spp. among the established species were variable. In the case of *P. fluorescens*, the highest increase occurred in the second year of evaluation, as was also the case with *Bacillus* spp. In the treatments where *R. graveolens*, *O. basilicum*, and *C. ambrosioides* were established, the amount of *P. fluorescens* was low, and in *S. jamaicensis* it was initially undetected but reached 100,000 CFU by the end. In the control treatment (*N. lappaceum* without medicinal plants), neither *P. fluorescens* nor *Bacillus* spp. were found. Among both bacteria, *Bacillus* spp. showed the highest populations (Figure 3a).

In the initial sampling, the soil where *R. graveolens* was established showed 426,667.00 CFUs, and in *O. basilicum*, 766,667.00 CFUs. These values increased in the second sampling to 419,333.00 and 3,640,000.00 CFUs, respectively. An increase of this microorganism was also recorded in the control treatment.

It is worth noting that the soil where *S. jamaicensis* was established showed the highest amounts of both bacteria.

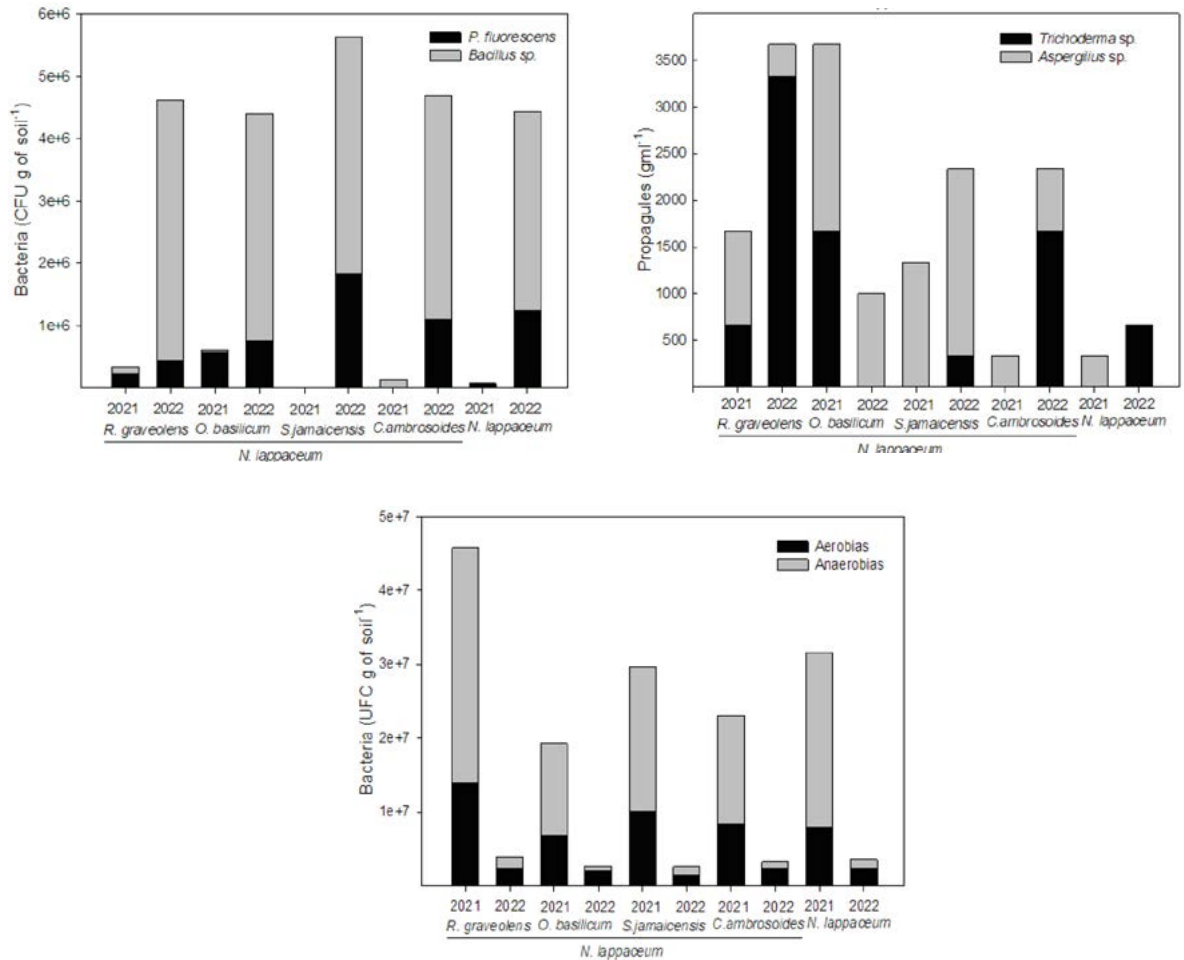
The presence of *Trichoderma* sp. and *Aspergillus* sp. exhibited contrasting variations between years and the species associated with rambutan. In the case of *Trichoderma*, the population increased in the soil where *R. graveolens* was established during the second year of sampling. The same trend was observed when *C. ambrosoides* was associated in 2022.

*Aspergillus* sp. appeared in both soil samplings when medicinal plants were associated with rambutan. In the case of the control treatment, no presence of *Aspergillus* sp. was recorded in the second sampling. The most abundant presence occurred in 2021 with *O. basilicum* and in 2022 with *S. jamaicensis*.

Regarding aerobic and anaerobic bacteria, contrasting values were observed between the two samplings conducted. The highest increase in both types of bacteria occurred during the first sampling (2021) when the medicinal plants were not associated with the rambutan. During this same year, the presence of anaerobic bacteria predominated, whereas in the second year, aerobic bacteria were more abundant.

The highest population of both types of bacteria among the medicinal plants was found in *R. graveolens*, while the lowest was observed in *O. basilicum*. In contrast, actinomycetes decreased in *R. graveolens*, *O. basilicum*, and *S. jamaicensis* but increased in *C. ambrosoides* and *N. lappaceum*.

Fungi of the genus *Fusarium* sp. were abundant in 2022 in the treatments with bioactive plants, but not in *N. lappaceum*. Nematodes such as *Rotylenchulus* sp. were present during the first year of evaluation but were not detected in the second year. In 2022, *Aphelenchus* sp. was found in the treatments with *R. graveolens*, *S. jamaicensis*, and *N. lappaceum*.



**Figure 3.** Bacteria and fungi (colony forming units per g of soil) present in a soil from Soconusco Chiapas established with *N. lappaceum* L. and associated with medicinal plants.

There were no significant differences in the number of worm castings among the treatments. However, visual observations indicated a greater presence of castings in *S. jamaicensis* and *R. graveolens*, with lower visibility in *C. ambrosoides* and *N. lappaceum*.

The higher nutrient content in the soil during the first sampling is considered to be associated with the phenological stage of rambutan, as this sampling coincided with the vegetative stage. The second sampling was conducted during the fruiting stage and was characterized by a decrease in soil nutrients. In this regard, Sosa-Rodríguez and García-Vivas (2020) reported that *N. lappaceum* extracts high concentrations of nutrients throughout the year, with a greater demand for N (41%), Ca (20%), Mg (5%), and S (4%) for the formation of leaves and branches. In contrast, P (6%) is primarily required for seed development, while K is mainly concentrated in the rambutan peel (24%). However, the nutrient content in the different structures of the trees may vary depending on agroclimatic conditions and tree age. Reyes-Moreno *et al.* (2020) further note that nutrient deficiencies in the soil may occur after fruit production.

The high nitrogen (N) demand during fruiting is associated with its role in the photosynthesis process (Combatt-Caballero *et al.*, 2020) and as a component of various organic acids (Reyes-Moreno *et al.*, 2020).

In the case of medicinal plants, limited information is available regarding their nutrient requirements. However, it has been reported that *O. basilicum* requires high amounts of nitrogen (ranging from 100 to 190 kg ha<sup>-1</sup>) and potassium, between 125 and 235 kg ha<sup>-1</sup> (Combatt-Caballero *et al.*, 2020), while *C. ambrosoides* demands higher amounts of nitrogen compared to other nutrients (Aguilar-Carpio *et al.*, 2021).

Regarding beneficial microorganisms, it has been demonstrated that they exert both direct and indirect effects on plants. They release compounds, minerals, and phytohormones, and can modify soil structure by participating in the mineralization of certain nutrients and acting as biological control agents against pathogenic microorganisms (Pedraza *et al.*, 2010).

Regarding microbial populations, their interactions may have influenced the development of other microbial communities. It has been documented that *Trichoderma* sp. is capable of controlling *Phytophthora nicotianae*, *P. aphanidermatum*, *P. parasitica*, *P. capsici*, *Rhizoctonia solani*, and *Pythium* spp. (Companion-González *et al.*, 2019), and the population of this microorganism increased in the presence of *R. graveolens*. *Aspergillus* sp. was also present in the soil of medicinal plants associated with rambutan. Its importance lies in its role in the decomposition of organic matter and in host defense against pathogenic microorganisms (Sacheri-Viteri *et al.*, 2022), such as *Fusarium*, which causes wilting or stem rot (Villa-Martínez *et al.*, 2015).

Nitrifying bacteria such as *Pseudomonas* sp. and *Bacillus* spp. are important in nitrogen mineralization (Másmela-Mendoza *et al.*, 2019) and can also control various pathogens (Pedraza *et al.*, 2020). The abundance of *Bacillus* has also been reported in soils cultivated with medicinal plants such as *Matricaria chamomilla* L., *Calendula officinalis* L., and *Solanum distichum* Schumach. & Thonn., with positive effects on promoting plant growth and increasing flavonoid content (Solaiman and Anawar, 2015). There is a close relationship between vegetation and the rhizospheric microbiota, as rhizosphere bacteria can act as signaling molecules and produce chemical substances that may trigger physiological and morphological changes in plants (Montaño-Arias *et al.*, 2006).

In our case, the highest populations of *S. jamaicensis* favored the abundance of *P. fluorescens* and *Aspergillus* sp. Likewise, the greatest amount of earthworm excreta was found in this soil. This may be related to the presence of arbuscular mycorrhizal fungi. It has been established that bacterial populations increase in the presence of arbuscular mycorrhizal fungi, and this plant is known to be colonized by mycorrhizae of the genus *Glomus* spp. (Aggangan *et al.*, 2015). Mycorrhizae, a symbiotic association between higher plants and microorganisms, are commonly found in nature. In this symbiotic relationship, the host plant provides carbohydrates to the mycorrhizal fungi through photosynthesis, while the fungi provide nutrients in return. It has been demonstrated that a large number of bacteria inhabit the zone around mycorrhizae (Frey-Klett and Garbaye, 2005). Arbuscular mycorrhizal fungi and bacteria known as mycorrhizal helper bacteria (MHB)

are interdependent; the mycorrhizal fungi provide nutrients for bacterial growth through the release of secretions, and the bacteria supply low molecular weight nutrients (Frey *et al.*, 1997; Rangel-Castro *et al.*, 2002). The pathogenic microorganisms were not uniformly found in the soil, but their presence in certain treatments may indicate competition among the increased populations of microorganisms associated with the exudates of medicinal plants. In the case of aerobic bacteria, their high presence indicates that the soil is not saturated and has good aeration, meaning the soil's porosity allows for proper development of the plant root system.

## CONCLUSIONS

The association of bioactive plants results in changes in the chemical properties of the soil, with fluctuations in nutrient contents identified over the two years of evaluation, both with and without the association of medicinal plants. The association of medicinal plants and rambután interferes with the population growth of both beneficial and harmful microorganisms. The diversity of beneficial microorganisms increases, and the presence of pathogens in low populations did not result in damage to the plants. The contrasting presence of beneficial microorganisms in the soil during the evaluation years suggests dependency on exudates from the medicinal plants, such as *P. fluorescens* and *Bacillus* spp. with *S. jamaicensis* and *Trichoderma* and *Aspergillus* with *R. graveolens* and *O. basilicum*.

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# The Importance of Fruit and Vegetable Production in Chiapas: Challenges and Opportunities

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## ABSTRACT

**Objective:** To analyze the importance of growing fruits and vegetables in Chiapas, as well as the conservation of ancestral knowledge, and its contributions to food security and the economy. This analysis will discuss the challenges and opportunities of this sector.

**Design/methodology/approach:** A review of literature related to the diversity of native and exotic species of commercial importance and self-consumption was conducted, emphasizing information that illustrates the challenges and opportunities in agricultural sectors.

**Results:** A high diversity of native and exotic fruit and vegetable species was identified. Most of these species are used for self-consumption, which is related to Mexico's self-sufficiency economy, biocultural richness, and ecosystem services. These species are distributed in diverse ecosystems, including backyards, commercial orchards, and urban areas. Products of national commercial importance and export quality are scarce and face technological, social, and commercial challenges.

**Study limitations/implications:** The results are relevant to species diversity, consumption, and commercialization, but these considerations may change if other environmental and technological variables are taken into account.

**Findings/conclusions:** The diversity of edible vegetables in Chiapas is high and includes native and introduced species highly linked to society. Agricultural diversification strengthens food security and sovereignty in the state. There are species with marketing potential, but innovation is needed in production and processing to increase income through value added. There is a need to reduce food waste and innovate in terms of waste and the circular economy to reduce environmental impact. The strengthening of this sector requires the integration of the community, government and research.

**Keywords:** food security, horticulture and fruit growing, traditional knowledge, biocultural.

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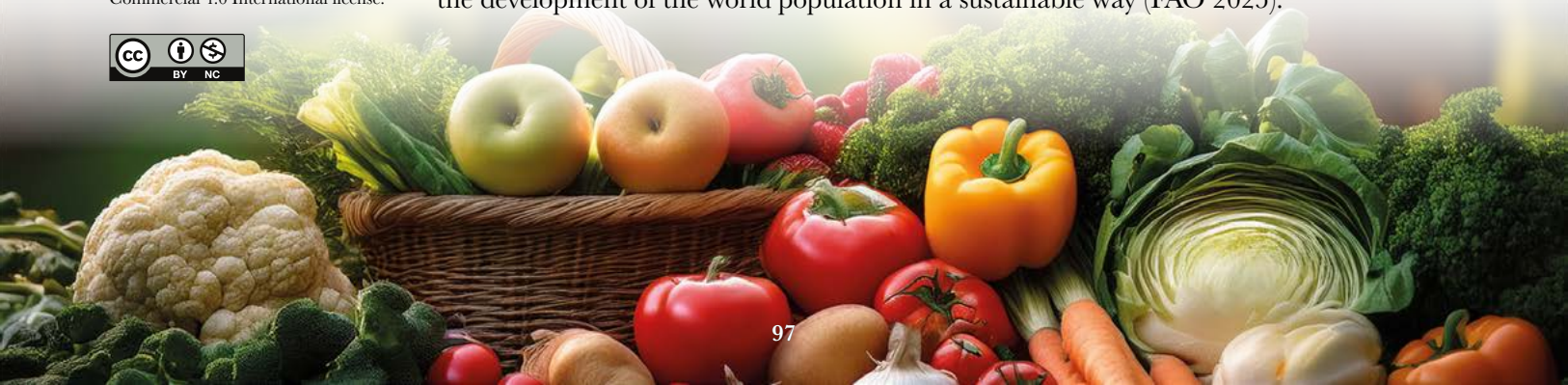
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## INTRODUCTION

The world population increase, the current food insufficiency, together with the increase of diseases related to unhealthy diets, make food demand a priority issue to contribute to the development of the world population in a sustainable way (FAO 2025).



Fruits and vegetables are among the foods recommended for a healthy and sustainable lifestyle, mainly because of their contribution to nutrition as sources of vitamins and minerals. Consumption of 400 g per person/day is recommended with wide variability of colors and flavors to increase the variability of nutrients (WHO 2018; Ibarrola-Rivas *et al.*, 2022).

In addition to the direct benefits to food production, the production and consumption chain generates economic income from fresh products and their derivatives. This contributes to achieving sustainable development objectives (FAO, 2021).

Mexico has enough arable land to supply a healthy diet based on fruits and vegetables. Currently, however, cultivated species do not meet the demand for food, which is insufficient when combined with the high consumption of processed foods that induce health problems (Ramírez-Juárez, 2022; Torres-Rojas, 2018).

Chiapas is favored for its variety of ecosystems, ample diversity, and richness of vegetables, which have been linked to Mayan ethnic groups (Segura *et al.*, 2018). These vegetables are part of the state's culinary richness.

Currently, cultivated species selected for anthropocentric purposes contribute to food security, biocultural heritage, and Mexico's economy (Ubiergo-Corvalán *et al.*, 2020; SIAP, 2024).

Despite the favorable conditions in the Mesoamerican ecosystem for the wide availability of vegetables, there are high rates of poverty, malnutrition, and poor health. This calls for various actions to increase the efficiency of this sector.

This paper presents a list of fruits and vegetables that grow and are produced in Chiapas. Its biocultural importance is identified, as well as its economic importance for including export quality species. It concentrates information on the problems of this sector and presents a panorama of the need for attention through innovation in the productive sectors and the addition of added value to the products.

## MATERIALS AND METHODS

A review was carried out of sources related to fruit and vegetable production, as well as sources related to floristic records that include these vegetables. Statistical records were obtained from the Agrifood and Fisheries Information Service.

The information was obtained through a literature review using the search engines Google Scholar and SciELO. The data are presented in a table that includes citation information from the literature. Additionally, the economic contributions of the main cash crops by economic region in Chiapas were plotted.

### Agro-productive regions of Chiapas

The state of Chiapas is located in southeastern Mexico (14° 32' 00" to 17° 59' 00" north latitude and meridians 90° 22' 00" and 94° 14' 00" west longitude), comprising an area of 73,311 Km<sup>2</sup>.

The predominant climate is represented by two climatic groups: warm-humid and temperate-humid. It has 10 hydrological basins; with 6 main reliefs identified as Mountain Range, Rolling hills, Plain, Plateau, Valley and Tacana Volcano. It is made up of 125

municipalities distributed in 15 economic regions differentiated by physio-geographical regions, altitude, soil, vegetation and land use. Forty-three and a half percent of the land is used for agriculture and livestock, and 53.6% is covered by natural vegetation (CEIEG, 2025) (Figure 1).

Agricultural production is an important activity in the state registering in 2018 sown area was 1,333,510 ha with 1,283,130 ha harvested and increased to 1,367,045 ha sown and 1,342,685 harvested by 2023 (CEIEG, 2025).

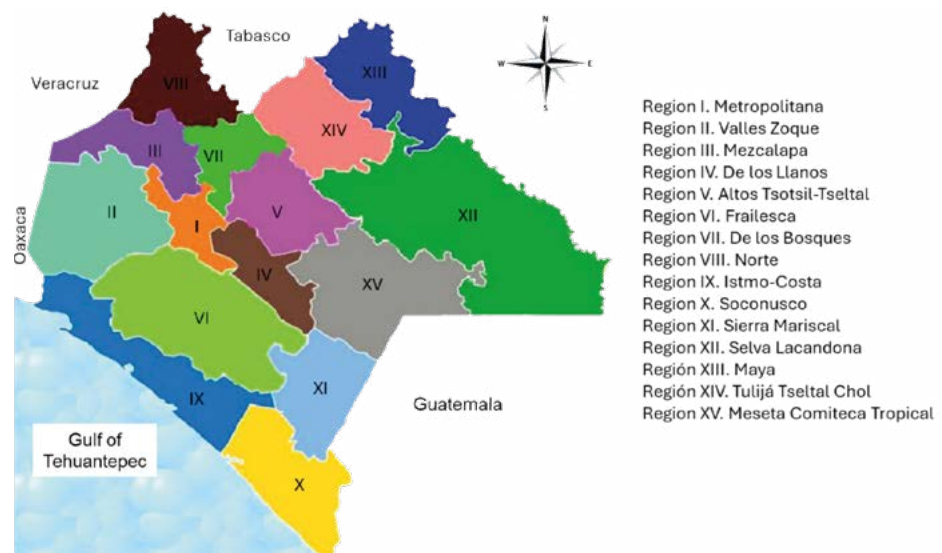
The geographic and ecological characteristics are related to the state's biodiversity (Morales & Priego, 2022) and the particular combinations determine the type of crop and confer the high agricultural diversification in the state (Flores *et al.*, 2017; CEIEG, 2023; Fernández & Méndez, 2018; Soto-Pinto *et al.*, 2021; Ubiergo-Corvalán *et al.*, 2020).

### Horticulture and Fruit growing in Chiapas

The native populations of Chiapas make use of various parts of plants, including stems, leaves, fruits, roots, and seeds. These parts have diverse purposes, including food, medicine, wood, and firewood. Therefore, plant biodiversity is part of the biocultural heritage of rural communities.

According to the FAO (2021), “fruits and vegetables are considered edible parts of plants (*e.g.*, seed-bearing structures, flowers, buds, leaves, stems, shoots, and roots), whether they are cultivated or harvested in the wild, and whether they are consumed raw or minimally processed.” A total of 174 species were identified, of which 53% are native and 47% have been introduced and acclimated to the environmental conditions of Chiapas (Table 1).

They may have been selected based on preference, family tradition, or adaptation to the environment.



**Figure 1.** Economic regions of Chiapas. Made from figure of Secretaría de Hacienda (2013).

**Table 1.** Fruits and vegetables present in Chiapas with diverse use by the population.

Common name	Species	Origin (native/exotic)	References
Chard	<i>Beta vulgaris</i> subsp. <i>vulgaris</i>	Native	Greenberg 2015; Benítez-Kanter <i>et al.</i> , 2020;
Annato	<i>Bixa orellana</i> L.	Native	Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Avocado	<i>Persea americana</i> Mill.	Native	Benítez-Kanter <i>et al.</i> , 2020; Alcazar-Sánchez <i>et al.</i> , 2025; Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024; Gerardo-Mendez 2024.
Basil	<i>Ocimum micranthum</i> Willd	Native	Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Pond apple	<i>Annona glabra</i> L.	Native	Ubierno-Corvalán <i>et al.</i> , 2020
Spleen amaranth	<i>Amaranthus dubius</i> , <i>A. hybridus</i>	Native	Greenberg 2015; Benítez-Kanter <i>et al.</i> , 2020.
Redroot pigweed	<i>Amaranthus retroflexus</i> L .	Native	Soto-Pinto <i>et al.</i> , 2022
Cacao	<i>Theobroma cacao</i> L.	Native	Ubierno-Corvalán <i>et al.</i> , 2020; Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024; Gerardo-Mendez 2024.
Mexican bayonet plant	<i>Oecopetalum mexicanum</i>	Native	Soto-Pinto <i>et al.</i> , 2022
Cocoplum	<i>Cryobalanus icaco</i> (L.) L.	Native	Gerardo-Mendez 2024.
Mexican star apple	<i>Chrysophyllum mexicanum</i> Brandegees ex Standl	Native	Almeida-Cerino <i>et al.</i> , 2024; Ubierno-Corvalán <i>et al.</i> , 2020; Gerardo-Mendez 2024.
Pumpkin	<i>Cucurbita</i> spp.	Native	Greenberg 2015; Ubierno-Corvalán <i>et al.</i> , 2020; Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Jamaica cherry	<i>Muntingia calabura</i> L.	Native	Ubierno-Corvalán <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024.
Capulín	<i>Eugenia acapulcensis</i> Steud	Native	Ubierno-Corvalán <i>et al.</i> , 2020
Capulín	<i>Prunus capuli</i> Cav.	Native	Gerardo-Mendez 2024.
Florida Trema	<i>Trema micrantha</i> (L.) Blume	Native	Almeida-Cerino <i>et al.</i> , 2024
Sacky Sac Bean	<i>Inga laurina</i> (Sw.) Willd.	Native	Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024; Gerardo-Mendez 2024.
Caspirol,tzelel	<i>Inga punctata</i> Willd.	Native	Soto-Pinto <i>et al.</i> , 2022
Chalúm	<i>Inga</i> spp.	Native	Benítez-Kanter <i>et al.</i> , 2020
Mexican forest palm	<i>Astrocaryum mexicanum</i> Liebm. ex Mart.	Native	Soto-Pinto <i>et al.</i> , 2022
Tread Softly	<i>Cnidocolus aconitifolius</i> (Mill.)	Native	Greenberg 2015; Alcazar-Sánchez <i>et al.</i> , 2025; Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Chayote	<i>Sechium edule</i> (Jacq.) Sw.	Native	Greenberg 2015; Ubierno-Corvalán <i>et al.</i> , 2020; Alcazar-Sánchez <i>et al.</i> , 2025; Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Naseberry	<i>Manilkara zapota</i> (L.) P. Royen	Native	Ubierno-Corvalán <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024; Gerardo-Mendez 2024.
Chili	<i>Capsicum</i> spp.	Native	Greenberg 2015; Ubierno-Corvalán <i>et al.</i> , 2020; Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Cayenne Pepper	<i>Capsicum annum</i> L.	Native	Soto-Pinto <i>et al.</i> , 2022
Soncoya	<i>Annona purpurea</i> Moc. & Sessé ex Dunal	Native	Ubierno-Corvalán <i>et al.</i> , 2020
Coyo Avocado	<i>Persea schiedeana</i> Nees	Native	Ubierno-Corvalán <i>et al.</i> , 2020
Longbeak rattlebox	<i>Crotalaria longirostrata</i> Hook. & Arn.	Native	Greenberg 2015

Table 1. Continues...

Common name	Species	Origin (native/exotic)	References
Hogplum	<i>Spondias mombin</i> L.	Native	Almeida-Cerino <i>et al.</i> , 2024
Coco plum	<i>Chrysobalanus icaco</i> L.	Native	Almeida-Cerino <i>et al.</i> , 2024
Spanish-plum	<i>Spondias purpurea</i> L.	Native	Almeida-Cerino <i>et al.</i> , 2024
Coral Tree	<i>Erythrina americana</i> Mill.	Native	Soto-Pinto <i>et al.</i> , 2022
Coquil 'te	<i>Inga pavoniana</i> G.Donn.	Native	Soto-Pinto <i>et al.</i> , 2022
Correlón	<i>Solanum appendiculatum</i> Dunal	Native	Soto-Pinto <i>et al.</i> , 2022
Cow-okra	<i>Parmentiera aculeata</i> (Kunth)	Native	Ubierno-Corvalán <i>et al.</i> , 2020; Almeida-Cerino <i>et al.</i> , 2024;
Ice Cream Bean	<i>Inga edulis</i> Mart	Native	Gerardo-Mendez 2024.
Churimo	<i>Inga vera</i> subsp. <i>Spuria</i> (Humb. & Bonpl. ex Willd.) J. León	Native	Soto-Pinto <i>et al.</i> , 2022
Chulli	<i>Prunus barbata</i> Koehne	Native	Cruz-Salazar <i>et al.</i> , 2020.
Mexican Tea	<i>Dysphania ambrosioides</i> (L.) Mosyakin & Clemants	Native	(Greenberg 2015; Benítez-Kanter <i>et al.</i> , 2020; Gerardo-Mendez 2024.
Yellow Passionfruit	<i>Passiflora ligularis</i> Juss.	Native	Soto-Pinto <i>et al.</i> , 2022
Guaba	<i>Inga edulis</i> Mart.	Native	Almeida-Cerino <i>et al.</i> , 2024
Bastardcedar	<i>Guazuma ulmifolia</i> Lam.	Native	Almeida-Cerino <i>et al.</i> , 2024
Guamo	<i>Inganobilis</i> Willd.	Native	Soto-Pinto <i>et al.</i> , 2022
Soursop	<i>Annona muricata</i> L.	Native	Ubierno-Corvalán <i>et al.</i> , 2020; Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024.
Stinking Toe Tree	<i>Hymenaea courbaril</i> L.	Native	Almeida-Cerino <i>et al.</i> , 2024
Guaya	<i>Talisia oliviformis</i> (Kunth) Radlk.	Native	Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024
Guava	<i>Psidium guajava</i> L.	Native	Ubierno-Corvalán <i>et al.</i> , 2020; Alcazar-Sánchez <i>et al.</i> , 2025; Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024; Benítez-Kanter <i>et al.</i> , 2020
Banana	<i>Musa acuminata</i> Colla	Native	Soto-Pinto <i>et al.</i> , 2022
Guishnay	<i>Spathiphyllum phrynifolium</i>	Native	Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022
Nightshade	<i>Solanum</i> spp.	Native	Greenberg 2015; Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Mexican pepperleaf	<i>Piper auritum</i> Kunth	Native	Greenberg 2015
Common Calabash Tree	<i>Crescentia cujete</i> L.	Native	Almeida-Cerino <i>et al.</i> , 2024
Morrito plant	<i>Crescentia alata</i> Kunth	Native	Almeida-Cerino <i>et al.</i> , 2024
Tomato	<i>Solanum lycopersicum</i> L.	Native	Soto-Pinto <i>et al.</i> , 2022
Spanish-plum	<i>Spondias purpurea</i> L.	Native	Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Pea eggplant	<i>Solanum mayanum</i> Lundell	Native	Gerardo-Mendez 2024.
Guinea arrowroot	<i>Calanthe allouia</i> (Aubl.) Lindl.	Native	Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022
Winged water-primrose	<i>Ludwigia decurrens</i> Walter	Native	Gerardo-Mendez 2024.

Table 1. Continues...

Common name	Species	Origin (native/exotic)	References
Madrone	<i>Arbutus xalapensis</i> Mart et Gal.	Native	Cruz-Salazar <i>et al.</i> , 2020.
Macuilillo	<i>Oreopanax xalapensis</i> (Kunth) Decne. & Planch.	Native	Cruz-Salazar <i>et al.</i> , 2020.
Red inkplant	<i>Phytolacca icosandra</i> L.	Native	Greenberg 2015
White sapote	<i>Casimiroa edulis</i> La Llave <i>et Lex</i>	Native	Cruz-Salazar <i>et al.</i> , 2020.
Husk-tomato	<i>Physalis philadelphica</i> Lam.	Native	Soto-Pinto <i>et al.</i> , 2022
Maricao Cimun	<i>Byrsonima crassifolia</i> (L.) Kunth	Native	Ubiergo-Corvalán <i>et al.</i> , 2020; Benítez-Kanter <i>et al.</i> , 2020; Almeida-Cerino <i>et al.</i> , 2024; Gerardo-Mendez 2024.
Naranjillo	<i>Myrsine juergensenii</i> (Mez) Lundell.	Native	Cruz-Salazar <i>et al.</i> , 2020.
Barbary fig	<i>Opuntia ficus-indica</i> , <i>Nopalea karwinskiana</i>	Native	Greenberg 2015
Pacaya	<i>Chamaedorea tepejilote</i> Liebm.	Native	Soto-Pinto <i>et al.</i> , 2022
Ilama	<i>Annona macrophyllata</i> Donn. Sm.	Native	Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Papaya	<i>Carica papaya</i> L.	Native	Ubiergo-Corvalán <i>et al.</i> , 2020; Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024; Gerardo-Mendez 2024.
Bloodberry	<i>Rivina humilis</i> L.	Native	Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022;
Macambo tree	<i>Theobroma bicolor</i> Bonpl.	Native	Soto-Pinto <i>et al.</i> , 2022
Theobroma	<i>Theobroma bicolor</i> Bonpl	Native	Ubiergo-Corvalán <i>et al.</i> , 2020
Paterna	<i>Inga inicuil</i> Schltld. & Cham. Ex G. Don	Native	Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Dragon Fruit	<i>Hylocereus undatus</i> (Haw.) Britton & Rose	Native	Ubiergo-Corvalán <i>et al.</i> , 2020
American Black Nightshade	<i>Solanum americanum</i> Mill.	Native	Soto-Pinto <i>et al.</i> , 2022
Giant potato creeper	<i>Solanum wendlandii</i> Hook. f.	Native	Soto-Pinto <i>et al.</i> , 2022
Sinzapote	<i>Licania platypus</i> (Hemsl.) Fritsch	Native	Soto-Pinto <i>et al.</i> , 2022
Tomato	<i>Solanum lycopersicum</i> L	Native	Greenberg 2015; Ubiergo-Corvalán <i>et al.</i> , 2020; Gerardo-Mendez 2024.
Husk tomato	<i>Physalis philadelphica</i> Lam.	Native	Greenberg 2015
Ground cherry	<i>Physalis pubescens</i> L.	Native	Ubiergo-Corvalán <i>et al.</i> , 2020
Sea grape	<i>Coccoloba escuintlensis</i> Lundell	Native	Almeida-Cerino <i>et al.</i> , 2024
Pod	<i>Inga inicuil</i> Schltld. & Cham. ex G. Don	Native	Ubiergo-Corvalán <i>et al.</i> , 2020; Almeida-Cerino <i>et al.</i> , 2024.
American Black	<i>Solanum americanum</i> Mill.	Native	Alcazar-Sánchez <i>et al.</i> , 2025
Mexican pepperleaf	<i>Piper auritum</i> Kunth	Native	Alcazar-Sánchez <i>et al.</i> , 2025; Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Yellow sapote	<i>Pouteria campechiana</i> Baehni	Native	Soto-Pinto <i>et al.</i> , 2022
White sapote	<i>Olmediella betschleriana</i> (Göpp.) Loes	Native	Cruz-Salazar <i>et al.</i> , 2020.
Malabar-chestnut	<i>Pachira aquatica</i> Aubl	Native	Soto-Pinto <i>et al.</i> , 2022

Table 1. Continues...

Common name	Species	Origin (native/exotic)	References
Ilama	<i>Annona macrophyllata</i> Donn. Sm.	Native	Almeida-Cerino <i>et al.</i> , 2024
Mamey	<i>Pouteria sapota</i> (Jacq.) H.E. Moore & Stearn	Native	Ubierno-Corvalán <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024; Gerardo-Mendez 2024.
Randia	<i>Randia</i> sp. Houst. ex L.	Native	Soto-Pinto <i>et al.</i> , 2022
Sansapote	<i>Licania platypus</i> (Hemsl.) Fritsch	Native	Gerardo-Mendez 2024.
Coconut	<i>Cocos nucifera</i> L.	Exotic	Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022
Aceituno	<i>Simarouba amara</i> Aubl.	Exotic	Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024.
Indian Almond	<i>Terminalia catappa</i> L.	Exotic	Ubierno-Corvalán <i>et al.</i> , 2020
Celery	<i>Apium graveolens</i> L.	Exotic	Ubierno-Corvalán <i>et al.</i> , 2020; Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024; Gerardo-Mendez 2024.
Breadfruit	<i>Artocarpus altilis</i> (Parkinson) Fosberg	Exotic	Ubierno-Corvalán <i>et al.</i> , 2020; Almeida-Cerino <i>et al.</i> , 2024; Gerardo-Mendez 2024.
Watercress	<i>Nasturtium officinale</i> A.P.Khokhr.	Exotic	Greenberg 2015; Ubierno-Corvalán <i>et al.</i> , 2020; Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Coffee Plant	<i>Coffea arabica</i> L.; <i>Coffea canephora</i> Pierre ex A. Froehner	Exotic	Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024; Gerardo-Mendez 2024.
Cinnamon	<i>Cinnamomum verum</i> J.Presl	Exotic	Soto-Pinto <i>et al.</i> , 2022
Starfruit	<i>Averrhoa carambola</i> L.	Exotic	Greenberg 2015; Ubierno-Corvalán <i>et al.</i> , 2020; Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024
Chives	<i>Allium schoenoprasum</i> L.	Exotic	Ubierno-Corvalán <i>et al.</i> , 2020)
Pea	<i>Cajanus cajan</i> (L.) Huth	Exotic	Almeida-Cerino <i>et al.</i> , 2024
Sow thistle	<i>Sonchus oleraceus</i> L.	Exotic	Cruz-Salazar <i>et al.</i> , 2020.
Fig Leaf gourd	<i>Cucurbita ficifolia</i> Bouché	Exotic	Soto-Pinto <i>et al.</i> , 2022
Manzano pepper	<i>Capsicum pubescens</i> Ruiz y Pav.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Cilantro	<i>Coriandrum sativum</i> L.	Exotic	Ubierno-Corvalán <i>et al.</i> , 2020; Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024.
Wild Cabbage	<i>Brassica oleracea</i> L.	Exotic	Greenberg 2015; Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Kohlrabi	<i>Brassica oleraceavar.gongylodes</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Peach	<i>Prunus persica</i> (L.)Batsch	Exotic	Soto-Pinto <i>et al.</i> , 2022
Green bean	<i>Vigna sesquipedalis</i> L. Fruwirth	Exotic	Soto-Pinto <i>et al.</i> , 2022
Spinach	<i>Tetragonia tetragonioides</i> (Pall.)Kuntze.	Exotic	Benítez-Kanter <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022
Indian Gooseberry	<i>Phyllanthus acidus</i> (L.) Skeels	Exotic	Greenberg 2015
Silk Banana	<i>Musa</i> spp. L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Fennel	<i>Foeniculum vulgare</i> P. Mill.	Exotic	Ubierno-Corvalán <i>et al.</i> , 2020

Table 1. Continues...

Common name	Species	Origin (native/exotic)	References
Alpine Strawberry	<i>Fragaria vesca</i> L.	Exotic	Greenberg 2015; Ubierno-Corvalán <i>et al.</i> , 2020; Gerardo-Mendez 2024.
Mint	<i>Mentha sativa</i> L.	Exotic	Ubierno-Corvalán <i>et al.</i> , 2020
Jamaica	<i>Hibiscus sabdariffa</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Kiwi	<i>Actinidia deliciosa</i> (Chev.) Liang & Ferguson	Exotic	Soto-Pinto <i>et al.</i> , 2022
Lettuce	<i>Lactuca sativa</i> L.	Exotic	Greenberg 2015; Almeida-Cerino <i>et al.</i> , 2024
Lichi	<i>Litchi chinensis</i> Sonn.	Exotic	Alcazar-Sánchez <i>et al.</i> , 2025
Lime	<i>Citrus limetta</i> Risso	Exotic	Alcazar-Sánchez <i>et al.</i> , 2025; Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Egyptian lime	<i>Citrus aurantiifolia</i> (Christm.) Swingle	Exotic	Soto-Pinto <i>et al.</i> , 2022
Mexican lime	<i>Citrus × aurantifolia</i> (Lunan) Guillaumin	Exotic	Cruz-Salazar <i>et al.</i> , 2020.
Sweet Lime	<i>Citrus × limetta</i> Risso	Exotic	Almeida-Cerino <i>et al.</i> , 2024
King orange	<i>Citrus × nobilis</i> (Lour)	Exotic	Soto-Pinto <i>et al.</i> , 2022
Mandarin lime	<i>Citrus × limonia</i> (L.) Osbeck	Exotic	Soto-Pinto <i>et al.</i> , 2022
Canton Lemon	<i>Citrus × limonia</i> (L.) Osbeck	Exotic	Ubierno-Corvalán <i>et al.</i> , 2020; Soto-Pinto <i>et al.</i> , 2022; Almeida-Cerino <i>et al.</i> , 2024; Gerardo-Mendez 2024.
Persian Lime	<i>Citrus × latifolia</i> Tanaka ex Q. Jiménez	Exotic	Soto-Pinto <i>et al.</i> , 2022
Persian Lime	<i>Citrus × latifolia</i> Tanaka ex Q. Jiménez	Exotic	Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024
Cravo Lemon	<i>Citrus × limonia</i> (L.) Osbeck	Exotic	Gerardo-Mendez 2024
Lemon	<i>Citrus × limon</i> (L.) Osbeck	Exotic	Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Mandarine	<i>Citrus reticulata</i> Blanco	Exotic	Gerardo-Mendez 2024
Tangor	<i>Citrus nobilis</i> Lour	Exotic	Soto-Pinto <i>et al.</i> , 2022; Gerardo-Mendez 2024.
Mango	<i>Mangifera indica</i> L.	Exotic	Gerardo-Mendez 2024
Mangosteen	<i>Garcinia mangostana</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Passion fruit	<i>Passiflora edulis</i> Sims	Exotic	Soto-Pinto <i>et al.</i> , 2022
Cashew	<i>Anacardium occidentale</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Blueberry	<i>Vaccinium myrtillus</i> L. m.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Moringa	<i>Moringa oleifera</i> Lam.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Dijon Mustard	<i>Brassica juncea</i> (L.) Czern.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Field mustard	<i>Brassica rapa</i> , <i>B. campestris</i>	Exotic	Soto-Pinto <i>et al.</i> , 2022
Orange	<i>Citrus × sinensis</i> (L.) Osbeck	Exotic	Soto-Pinto <i>et al.</i> , 2022
Seville orange	<i>Citrus × aurantium</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Loquat fruit	<i>Eriobotrya japonica</i> (Thunb.) Lindl.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Japanese-medlar	<i>Eriobotrya japonica</i> (Thunb.) Lindl	Exotic	Soto-Pinto <i>et al.</i> , 2022
Indian Mulberry	<i>Morinda citrifolia</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Breadfruit	<i>Artocarpus altilis</i> (Park.)	Exotic	Soto-Pinto <i>et al.</i> , 2022
Chicken Cucumber	<i>Cucumis anguria</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Cucumber	<i>Cucumis sativus</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Pear	<i>Pyrus</i> spp. L.	Exotic	Soto-Pinto <i>et al.</i> , 2022

**Table 1.** Continues...

Common name	Species	Origin (native/exotic)	References
Pineapple	<i>Ananas comosus</i> (L.) Merr	Exotic	Soto-Pinto <i>et al.</i> , 2022
Banana	<i>Musa</i> spp. L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Plantain banana	<i>Musa</i> × <i>paradisica</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Plantain	<i>Musa balbisiana</i> Colla	Exotic	Soto-Pinto <i>et al.</i> , 2022
Cavendish banana	<i>Musa acuminata</i> Colla	Exotic	Soto-Pinto <i>et al.</i> , 2022
Red Banana	<i>Musa paradisica</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Malabar plum	<i>Syzygium jambos</i> (L.) Alston	Exotic	Soto-Pinto <i>et al.</i> , 2022
Pomelo	<i>Citrus maxima</i> (Burm.) Merr	Exotic	Soto-Pinto <i>et al.</i> , 2022
Blackberry	<i>Rubus ulmifolius</i>	Exotic	Soto-Pinto <i>et al.</i> , 2022
Radish	<i>Rhapanus sativus</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Rambutan	<i>Nephelium lappaceum</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Shetland cabbage	<i>Brassica oleracea</i> var. <i>capitata</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Watermelon	<i>Citrullus lanatus</i> (Thunb.) Matsum. & Nakai	Exotic	Soto-Pinto <i>et al.</i> , 2022
Tamarind	<i>Tamarindus indica</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Tree tomato	<i>Cyphomandra betacea</i> (Cav.) Sendtn.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Melon pear	<i>Solanum muricatum</i> Aiton	Exotic	Soto-Pinto <i>et al.</i> , 2022
Grapefruit	<i>Citrus</i> × <i>paradisi</i> Macfad	Exotic	Soto-Pinto <i>et al.</i> , 2022
Common Purslane	<i>Portulaca oleracea</i> L.	Exotic	Soto-Pinto <i>et al.</i> , 2022
Jackfruit	<i>Artocarpus heterophyllus</i> Lam.	Exotic	Soto-Pinto <i>et al.</i> , 2022

Banana, cherry coffee, cacao, papaya, mango, pear, peach, apple, pineapple, avocado, and watermelon are among the top ten nationally important crops in terms of quantity produced for commercial purposes. Production records in 2023 ranged from 3,738 tons of apples to 672,021 tons of bananas (SIAP 2024; Table 2). Although the tomato is not mentioned in this list, it is also considered a commercially important crop in the state (CEIEG, 2023).

**Table 2.** Fruits grown in Chiapas considered by their national economic importance.

Product	Sown area (ha)	Harvested area (ha)	Production (t)	Production Value (thousands of mexican pesos)
Cherry coffee	243,946.87	240,301.27	391,956.99	2,116,042.81
Cacao	17,923.40	17,779.40	10,936.85	377,155.02
Banana	23,496.42	23,346.72	672,020.76	1,660,642.16
Papaya	2,077.70	2,019.20	160,251.56	825,823.43
Mango	38,781.85	36,909.85	272,174.46	1,444,154.66
Avocado	3,375.71	2,705.06	17,045.15	445,361.97
Red Tomato	1,631.66	1,631.66	82,922.79	795,676.62

Own elaboration based on data obtained from SIAP (Servicio de Información Agroalimentaria y Pesquera) (2024).

According to the CEIEG (2025), the commercial value of coffee, banana, mango, papaya, avocado, and tomato production in 2023 was \$11,044,817.17, with coffee and banana production having the highest value. These six products are cultivated in the state's XV economic region, with the greatest concentration of production in Region X (Soconusco), where all six products are grown (Figure 1).

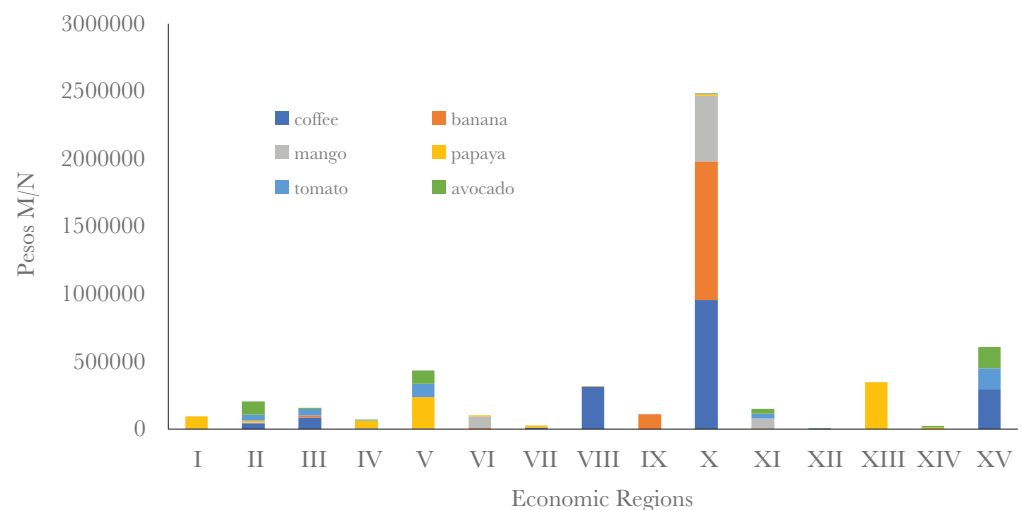
The previous crops are distributed throughout the state under different agroecosystems. Products intended for commercialization are developed in monoculture production systems. Those intended for self-consumption and local and regional trade are grown in milpa production systems alongside *Coffea arabica* L. and *Theobroma cacao* L., as well as in areas known as acahuals, which are generally associated with rural communities, family gardens, backyards, and fields. They can also be found alongside roads and in urban environments (Greenberg, 2015; Benítez-Kánter *et al.*, 2020; Soto-Pinto *et al.*, 2022; Fernández & Méndez, 2018; Ubierno-Corvalán *et al.*, 2020; Almeida-Cerino *et al.*, 2024).

Fruits and vegetables grown for self-consumption come from backyards or small plots within family production units and are part of the community's cultural, economic, and social wealth (Gerardo-Méndez *et al.*, 2024).

In addition to self-consumption, they are sold in local markets (Harvey *et al.*, 2021; Flores, 2019; Flores, 2016; Soto-Pinto *et al.*, 2022).

These vegetables are typically consumed fresh, incorporated into regional dishes, or used to make flavored water and preserves (Ubierno-Corvalán *et al.*, 2020; Gerardo-Méndez, 2024).

Family agroecological systems also preserve germplasm of edible species, primarily native ones, and contribute to the conservation of cultural traditions (Ubierno-Corvalán *et al.*, 2020). Rural communities in northern Chiapas (Ubierno-Corvalán *et al.*, 2020) and the Maya region (Martínez *et al.*, 2023) cite the extensive use of fruit trees for self-consumption.



**Figure 1.** Production value of the six main fruits by national importance. Own elaboration based on data obtained from CEIEG (Comité Estatal de Información, Estadística y Geografía de Chiapas) (2025).

## Crops in commercial productive agroecosystems

### Cacao agroecosystem

Cacao, a fruit developed in Mesoamerica, has played an important role in the region's gastronomy. It was even used as currency in Mayan and Aztec cultures and is part of their cultural heritage (Mendoza, 2011). Cacao has been an integral part of indigenous cultures, such as the Mayan and Aztec civilizations. These cultures venerated cocoa as a gift from the gods, symbolizing prosperity and fertility (Coe & Coe, 2013). Cacao cultivation in Mexico dates back to pre-Hispanic civilizations, including the Olmec, Maya, and Aztecs.

The pulp can be eaten fresh, added to water as a meal accompaniment, or enjoyed as a refreshing drink.

Currently, the main use of the fruit is for producing chocolate. Chocolate is an integral part of the regional culture and economy. It is marketed locally, nationally, and internationally.

Cultivation is usually diversified with coffee or rambutan (Mendoza *et al.*, 2024). Additionally, the cacao agroecosystem is characterized by shade-loving trees. This combination favors ecosystem services.

Among the diverse species of shade trees, several fruit trees are cultivated alongside *T. cacao* L. for personal consumption and local marketing (Salgado-Mora *et al.*, 2007).

In cocoa-producing areas of Soconusco, the following fruit trees can be identified: *Mangifera indica* L., *Persea americana* Mill., *Citrus reticulata* Blanco, *Citrus × sinensis* (L.) Osbeck, *Nephelium lappaceum* L., *Manilkara zapota* (L.) van Royen, and *Pouteria sapota* (Jacq.) H.E. Moore & Stern. And shade trees including flying guava *Terminalia amazonia* (J. F. Gmel.) Exzell., chalum, *Inga micheliana* Harms (Avenidaño-Arrazate *et al.*, 2021). In the rainforest region, the following species are found: *Coffea Musa* spp., *Citrus × sinensis* (L.) Osbeck, avocado *Persea americana* Mill., lemon *Citrus limon* (L.) Burm. f. *Manilkara zapota* (L.) van Royen, *Pouteria sapota* (Jacq.) H. E. Moore & Stern., guanabana *Annona muricata* L., tamarindo *Tamarindus indica* L., and jobo *Spondias mombin* L. (López-Baez *et al.*, 2015).

### Coffee agroecosystem

Coffee arrived in the country in the 17<sup>th</sup> century (Gómez-Pompa, 1997; Topik *et al.*, 2006) from its origin in Ethiopia (Smith, 1985) discovered by the inhabitants of the mountainous regions, when they noticed the energizing effect of its fruits.

This exotic fruit is recorded to have begun production in Soconusco in 1846, when plants were introduced from Guatemala and initially planted in Tuxtla Chico, Chiapas (Bartra, 1995).

According to the FAO (2022) classification, coffee is not considered a fruit because the seed is used to make coffee.

Nevertheless, coffee is an important agroecosystem in the Soconusco region, providing the region with significant economic benefits and ecosystem services to rural communities each year.

In Mexico, coffee represents a transient entry zone for the Mediterranean fruit fly, an important pest for global fruit and vegetable production (Flores *et al.*, 2016).

The state of Chiapas ranks first nationally in terms of production value. It cultivates 241,876 hectares distributed among 13 coffee-growing regions served by 183,761 producers. Small farms predominate.

Its cultivation is also associated with the shade of exotic and native fruit trees. This production system induces significant ecological richness. In the highlands of Chiapas, this crop is often combined with honey production.

Thirty-seven edible plant species, including fruits and vegetables, have been identified in the central region of Chiapas, which are produced for self-consumption or local trade (Gasco, 2008; Fernández *et al.*, 2013; Greenberg, 2015; Gómez-Martínez *et al.*, 2019; Alcázar-Sánchez *et al.*, 2022; Soto-Pinto *et al.*, 2022). Coffee-growing areas in the Sierra Madre also feature fruits and vegetables (Benítez-Kánter, 2020; Fernández & Méndez, 2018).

### **Export fruit farming**

Fruits destined for export must meet the high quality standards set by the importing countries and comply with pest regulation standards to prevent the dispersal of pests to those countries.

The international marketing chain includes harvesting, post-harvest treatment, packing, storage, and transportation. Thus, products must comply with quality standards, including a shelf life that guarantees consumers can consume the product as intended (Fletes *et al.*, 2016; Mendoza *et al.*, 2024).

In 2023, Chiapas exported bananas, both fresh and dried, valued at US\$95.3 million, representing 48.5% of the state's exports. Second place in exports went to *Coffea arabica* L., including roasted or decaffeinated products, coffee husks and skins, and coffee substitutes containing coffee in any proportion. These exports generated US\$70.2 million (35.7%). Noteworthy exports included pineapples, avocados, guavas, and mangoes, which generated US\$27.5 million (14%). Other fruit trees exported since 2013 include rambutan.

### ***Coffea arabica* L.**

Coffee exports from Soconusco come from the municipalities of Mapastepec, followed by Tapachula, Acapetahua, Mazatán, Huehuetán, Escuintla, Huixtla, Villa Comaltitlán, Suchiate, Pijijiapan, Tuxtla Chico, Tuzantán, Acacoyagua, Frontera Hidalgo, Cacahoatán, Tonalá, Unión Juárez, Arriaga, and Metapa. In total, 316,351 hectares are planted, with a production value of 8,051,584 thousand pesos (SIAP, 2024). The municipality of Tapachula produces the coffee with the highest commercial value. It has a planted area of 56,313 hectares, with a commercial value of 1,268,067 thousand pesos.

### ***Mangifera indica* L. var Ataulfo.**

The development of the "Ataulfo" mango variety in Soconusco has achieved widespread acceptance and economic relevance. It is valued internationally for its flavor, texture, and pulp content (Rivera-López *et al.*, 2020; Mendoza-Hernández *et al.*, 2020).

Its cash crop began in 1965 and was promoted by the National Institute for Agricultural Research at the Rosario Izapa Experimental Field (now INIFAP). From there, it expanded to other Mexican states, Central America, South America, and Spain (Infante *et al.*, 2011).

The fruit has a designation of origin for the Soconusco region as “Mango Ataulfo del Soconusco, Chiapas,” published in the Mexican Official Journal of the Federation on August 27, 2003. This designation includes the municipalities of Suchiate, Frontera Hidalgo, Metapa, Tuxtla Chico, Tapachula, Mazatán, Huehuetán, Tuzantán, Huixtla, Villa Comaltitlán, Escuintla, Acacoyagua, and Acapetahua (Secretaría de Economía, 2016).

Production in this zone is attributed to the particular agroecological characteristics that favor high production and fruit quality of *Mangifera indica* L. var. Ataulfo.

Nowadays, 32,030 hectares are dedicated to this crop in the Soconusco and Costa region, generating a production value of 1,273,402.54 thousand pesos. The municipality of Tapachula has the largest production, with 8,540.72 hectares and a production value of 407,760.39 pesos.

Production has increased in other Soconusco localities; however, Huehuetán produces the highest quality mangoes for export (SIAP, 2024).

The mangoes produced in Chiapas first reach commercial quality in February and therefore achieve a better price in the market. It is appreciated for its pulp quality, intense yellow color, thin skin, small seed, high sugar content, aroma, flavor, low fiber content, and long shelf life.

### ***Nephelium lappaceum* L.**

The rambutan is originally from Asia and was introduced to Mexico in 1965 as part of development projects carried out by the National Institute of Agricultural and Livestock Research (INIFAP). Its commercial cultivation in the region began 30 years ago.

It has been primarily cultivated alongside coffee and cocoa, and it is currently grown in backyards, small plots, and monoculture areas. When cultivated in small plots, rambutan contributes to the family economy, local trade, and national and international markets (Bello-Mendoza *et al.*, 2024).

The region has 849 hectares planted with a production value of 117,177.63 thousand pesos. The municipality of Tuxtla Chico has the largest planted area, valued at 64,691.98 thousand pesos, and it is also cultivated in the municipalities of Tapachula, Cacahoatán, Frontera Hidalgo, Metapa, Huehuetán, and Tuzantán (SIAP, 2024).

### ***Carica papaya* L.**

The Maradol papaya is another export-quality product grown on 1,401 hectares, with a production value of 491,708.6 thousand pesos. The largest area is in Mazatán, with 481 hectares and a production value of 167,063.51 thousand pesos. It is also produced in Tapachula, Acapetahua, Frontera Hidalgo, and Huixtla.

The main export markets are the United States, followed by Japan, Europe, and Canada (FAO, 2022).

### ***Musa* spp.**

Chiapas is an important banana producer, contributing 686 thousand tons (28.1%) of the total national crop. In the Soconusco region, the municipalities of Suchiate, Tapachula, and Mazatán contribute 28.4%, 13.8%, and 10.8%, respectively (SIAP, 2023).

In Soconusco, 435 banana growers have been identified within the Association of Banana Producers, who produce export-quality bananas.

These fruits can be distributed regionally, nationally, and internationally. However, the banana crop faces several challenges, including weather phenomena, pest and disease incidence, input costs, product sales prices, access to financing, and, to a lesser extent, scarce labor, producer organization, and productive technical assistance. The most important needs are the establishment of irrigation systems and the installation of warehouses. When it comes to marketing linked to export companies, they impose conditions regarding harvesting, cultivation, quantities, and prices (León-Ayala *et al.*, 2024).

In addition to export-quality production, different varieties of bananas are cultivated in backyards and small family gardens for self-consumption and local commerce.

### **Problems with Growing Fruit and Vegetables in Chiapas**

In general, world agriculture is exposed to various risk factors, the most important of which are related to climate change, poverty, inequality, and population growth. Agriculture can also be impacted by the collateral effects of pandemics, unsustainable land use and management practices, armed conflicts, and environmental degradation (FAO, 2024).

Fruit cultivation is particularly vulnerable to these factors (Álvarez *et al.*, 2021; León-Ayala *et al.*, 2024), especially in open fields. This vulnerability can be exacerbated by climate change conditions, such as prolonged droughts, which cause significant agricultural losses worldwide (FAO, 2024).

The soils are generally of medium fertility, and rugged terrain in some regions, such as northern Chiapas, makes mechanized tillage difficult. Cultivated areas face soil erosion problems and decreased fertility due to extensive use, primarily in the Soconusco region (Rodríguez *et al.*, 2017).

### **Other problems associated with crops**

The presence of pests and diseases in tropical and subtropical climates, together with monoculture production systems and environmental impact, has reduced biotic competition and impacted the biodiversity of natural pest enemies (Galindo, 2022). Fruit fly pests affect fruits and vegetables, which compromises international trade (Ramírez & Ramírez, 2020).

In Chiapas, 18 plant species have been found to be potential hosts of *Ceratitis capitata*, given the state's broad plant biodiversity (SADER, 2024).

The labor force tends to be scarce due to the abandonment of the countryside by farmers, who find it more profitable to seek employment in large cities or other countries (Alcázar-Sánchez *et al.*, 2022). Social phenomena such as armed conflicts, wars, and technological and chemical threats also impact the labor force (FAO, 2024).

Market prices can be very low in cases of oversupply, to the point that there is no return on investment or profit (Luquez, 2020; Bello Mendoza, 2024).

Regarding competitiveness, small, low-income producers face challenges that demand the modernization of farming systems to increase efficiency and competitiveness, primarily

on an international level. This requires the use of more productive crop varieties, tools, and agricultural inputs (Álvarez, 2021).

Cultivated products must meet consumption and marketing parameters, which are generally determined by consumer acceptance and include visual appearance, size, and flavor, among others.

It is also important to comply with the stipulated requirements for cultivation, harvesting, packing, and shipping, taking into account food safety and agricultural risk aspects (Osei-Kwarteng *et al.*, 2024; Mazariegos-Sanchez *et al.*, 2017; NOM-076-FITO-1999; Bello-Mendoza *et al.*, 2024).

In the case of the exporting of Ataulfo mangoes, producers mainly face attacks by fruit flies (*Anastrepha ludens* and *Anastrepha obliqua*) and the threat of infestation by the Mediterranean fruit fly. This process must comply with an established protocol during and after harvest.

Additionally, production faces the challenge of expanding crops in the face of potential market loss (Infante *et al.*, 2011; Mazariegos-Sánchez *et al.*, 2017). Marketing through intermediaries is a problem for producers in Soconusco.

Those who do not comply with export standards are lost in local and national trade. To overcome these challenges, producers should increase technological innovation to reduce costs (Mazariegos-Sánchez *et al.*, 2017), produce sticks with a defined origin to ensure crop and fruit homogeneity, reduce illegal trade chains, improve fruit quality for export, and conquer other export markets in other countries (Mendoza-Hernández *et al.*, 2020).

In the case of rambutans produced in Soconusco, there are independent producers, which causes heterogeneity in quality. One quarantine barrier to rambutan cultivation is the mealy louse for the export market. Additionally, there is a lack of knowledge regarding pest management, transportation, and quality, as well as a lack of integration among producers (Flores-Trejo *et al.*, 2016).

### **Challenges and Opportunities**

Although the recommendation for health issues is to consume raw and fresh vegetables, this limits economic development due to scarce transformation and generation of added value (Ramírez *et al.*, 2018). However, these vegetables can be offered in commercial presentations. The culinary tradition of Chiapas incorporates fruits and vegetables into dishes such as preserves, jams, and flavored water (Ubierno-Corvalán *et al.*, 2020; SIAP, 2024). Preserves, juices, jams, and cosmetics are prepared in the domestic market. Currently, the circular economy, which includes essential oils derived from fruit peels and fruit flavoring, is important. Still, the commercial importance of the industry linked to these vegetables is low.

The transformation of primary fruit and vegetable production is important because it increases income from added value. This area has not been extensively explored in the state, and its implementation requires scientific research, technological development, and agri-food innovation. To achieve this goal, interaction among producers, key stakeholders, agribusinesses, academic institutions, civil society, and government sectors

is necessary to promote innovation and more efficient production systems, improve commercial networks, and increase industrialization to generate more jobs and added value (Ramirez *et al.*, 2018).

In Chiapas, there is a wide variety of fruits and vegetables that have historically been essential components of the native population's diet. These are mainly native species, to which introduced species have been added. These vegetables are present in different agroecological environments and community sites as part of biocultural processes of cultivation and consumption. This knowledge has been passed down through generations and is an integral part of the region's culinary heritage, enriching regional gastronomy and supporting family subsistence (Ubierno-Corvalán *et al.*, 2020).

The low quantity of these commercially valuable vegetables indicates that most fruit species are underutilized. As Segura *et al.* (2018) point out, this is due to the underestimation of their potential uses and the lack of knowledge about their nutritional value. It is also due to the rapid reduction of their ecosystems. Paying attention to these species is important, mainly because they contribute to the food security of people living in extreme poverty in rural areas (Ramírez-Juárez *et al.*, 2022).

Commercially important fruits and vegetables contribute to food security at the state and national levels due to the economic benefits generated by exports and the jobs created in the production and commercialization chains. This has encouraged small producers to adopt them. However, they face difficulties complying with quality and production standards for export. They are often sold through intermediaries or local markets, which detracts from production value (Flores *et al.*, 2019).

The Soconusco zone stands out in particular for its diversity and commercial export crops, which are favored by its geographic characteristics. It is important to innovate with alternatives according to the characteristics of other areas of the state with the objective of diversifying the local food supply, including species that can potentially be commercialized.

Globalization and climate change have led to an increase in natural disasters that affect agriculture. Therefore, it is advisable to have local alternatives that guarantee food security and sovereignty. As proven by phenomena such as the pandemic, the availability of imported food can decrease, which is why regional products play a crucial role. Additionally, the current food supply in Mexico has been analyzed and found to need improvement. Fruits and vegetables are an integral part of this improvement, which can be achieved by cultivating products for which the country has arable land capacity (Ibarrola-Rivas *et al.*, 2022).

Educational and research institutions, along with governments, should promote the increased use of these vegetables in rural and urban areas. Clear examples of this can be seen with mangoes and rambutans, which, despite being exotic, have been improved to the point that they can be exported, generating foreign exchange for the country. Another important development issue is generating by-products that increase the products' added value. Another important issue in the sector is food waste.

Consuming a variety of fruits and vegetables is essential for good nutrition. The cost increases when out-of-season and imported fruits are consumed, so conserving and propagating regional fruit trees can economically complement the diet. Paying attention to

the challenges faced by commercial agriculture is also important since it has contributed to the country's development.

## CONCLUSIONS

The agroecological conditions in Chiapas have influenced the wide variety of fruits and vegetables, and conserving and cultivating them contributes to regional and even global food security through exports. The commercialization and export of these fruits and vegetables contribute to the country's economic growth. However, species richness contributes to ecosystem services and biocultural heritage, which deserves particular attention because species diversification reinforces food security. It is important to strengthen the horticultural sector in the state from technological, ecological, social, and economic points of view. This involves strengthening commercially important crops and seeking new alternatives while taking into account the existing diversity, particularly of native species. These species should be conserved due to their importance in biocultural heritage.

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# Effects of foliar-applied homobrassinolide on the *ex vitro* acclimatization of *Guarianthe skinneri* (Bateman) Dressler & W.E.Higgins

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## ABSTRACT

**Objective:** To identify the influence of foliar application of homobrassinolide at various doses and frequencies on the growth and *ex vitro* acclimatization of *Guarianthe skinneri* (Bateman) Dressler & W.E.Higgins.

**Design/methodology/approach:** *G. skinneri* seeds were germinated *in vitro* on Yasuda medium. Once the seedlings developed roots, leaves and reached a height of 2 cm, they were transferred to the nursery for *ex vitro* acclimatization. Coconut fiber was used as a substrate in 10 oz beakers. Hbr-based treatments were generated with three doses of Hbr (2, 4 and 6 mgL<sup>-1</sup>) and three application frequencies (every 7, 14 and 21 days), plus a control with 10 replicates in a completely randomized design. The following variables were evaluated at 28, 56, 70, 77, and 91 days: plant height, number of green and dry leaves, and number of dead plants.

**Results:** Foliar application frequencies and concentrations of Hbr induce differential growth and survival of *G. skinneri*. Applying Hbr more frequently at higher concentrations improves *G. skinneri* survival in the nursery.

**Limitations on study/implications:** The results may vary under different environmental conditions during the acclimatization of *G. skinneri*.

**Findings/conclusions:** Foliar application of Hbr induced greater growth in *G. skinneri*, particularly in terms of plant height. More frequent applications and higher concentrations of Hbr improved the acclimatization and survival of *G. skinneri* in nursery conditions. Different concentrations and application intervals of foliar Hbr resulted in distinct effects on the growth and survival of *G. skinneri*.

**Keywords:** Orchids, Homobrassinolide, Survival.

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## INTRODUCTION

The Orchidaceae family comprises approximately 800 genera and nearly 30,000 species, with a wide geographical distribution worldwide (Arditti & Ghani, 2000; Chase *et al.*, 2003), ranging from sea level to elevations of up to 4,000 meters (Rollke, 2007; Téllez & Flores, 2007). Orchids are highly valued and admired for the beauty of their flowers, and they are particularly popular due to their diversity of colors, sizes, shapes, and fragrances. Currently, 188 orchid species are listed under a risk category in Mexico's NOM-059 regulation (Cabrera, 2006).

Orchid populations have declined in their natural habitats due to anthropogenic factors such as habitat fragmentation (Hágsater *et al.*, 2005; Newmarch *et al.*, 2024), climate change, dependence on specific pollinators and mycorrhizal fungi (Tsiftsis & Djordjević, 2020), and their limited seed germination capacity due to the absence of endosperm (Damon *et al.*, 2004). This condition hinders natural repopulation, as germination relies on minimal carbohydrate reserves.

Currently, there are various efficient protocols for the *in vitro* regeneration of orchids (Orbovic *et al.*, 2008), and as a result, propagation protocols are now available that can support the multiplication of endangered species. During the *in vitro* stage, several plant growth regulators have been used, such as gibberellic acid in *G. skinneri* (Coello *et al.*, 2010). In *Epidendrum elongatum* Jacq., growth was also enhanced by supplementing the culture medium with activated charcoal, indole-3-acetic acid (IAA), and benzylaminopurine (BAP) (Pedroza, 2009).

In general, *in vitro* micropropagation is a useful technique for cultivating *G. skinneri* (Vázquez *et al.*, 2014; Park *et al.*, 2018); however, its effectiveness requires validation under *ex vitro* conditions (Gil Rivero *et al.*, 2017).

At this stage, it is recommended to consider nutritional, chemical, biological, and environmental factors that may influence plant growth. Growth regulators such as brassinosteroids, which are widely distributed throughout the plant kingdom, have been shown to induce pleiotropic effects in plants, among which their growth-promoting effect stands out (Yang, 2011). This is particularly significant considering that, during the early stages of acclimatization, seedlings exhibit low photosynthetic performance (Kadlecek *et al.*, 2001), as demonstrated in *in vitro* propagated *G. bowringiana*. However, once transferred to *ex vitro* conditions, a rapid increase in photosynthetic pigments (chlorophyll a, b, and carotenoids) has been observed (Buyun *et al.*, 2021). In addition, brassinosteroids have been shown to reduce the effects of abiotic stress factors such as water and heat stress (Bajguz & Tretyn, 2003), mainly by increasing cell membrane permeability (Hernández *et al.*, 2010; Bao-Fundora *et al.*, 2013).

Based on this background, the objective of this study was to identify the influence of foliar application of homobrassinolide at different doses and frequencies on the growth and *ex vitro* acclimatization of *Guarianthe skinneri* (Bateman) Dressler & W.E. Higgins.

## MATERIALS AND METHODS

The research was conducted at the Biotechnology Laboratory and the nursery of the Faculty of Agricultural Sciences, Campus IV of the Universidad Autónoma de Chiapas (15° 00' 25.02" N and 92° 23' 59.06" W, at an altitude of 32 meters above sea level). The study was carried out in two stages: the first involved seed germination and *in vitro* multiplication, and the second consisted of *ex vitro* acclimatization. The climate in which the plants were grown *ex vitro* is classified as Am(w')ig, corresponding to a tropical sub-humid climate (2,200 mm annual precipitation) with two dry periods—one occurring during the rainy season (mid-summer drought or canícula), and the other beginning in November. The average temperature is 28.5 °C, with a minimum of 15 °C and a maximum of 38 °C (García, 2004).

### Seeds and Propagation Medium

Seeds were obtained from mature plants in the Soconusco region, Chiapas, and were sown eight days after capsule collection. Capsules were first washed with a soapy solution containing Tween 80 and treated with a fungicidal solution of  $1.5 \text{ g L}^{-1}$  azoxystrobin for 20 min. Subsequently, they were disinfected with sodium hypochlorite ( $\text{NaClO}$  3%) for 15 min and then kept in an antioxidant solution until sowing. Sterile capsules were cut longitudinally to extract the seeds, which were germinated *in vitro* using Yasuda *et al.* (1985) medium, supplemented with the vitamins described by Gamborg (2002) and  $30 \text{ g L}^{-1}$  sucrose. To the medium, the following were added separately: indole-3-acetic acid (IAA) ( $1.5 \text{ mg L}^{-1}$ ), activated charcoal (AC) ( $1 \text{ g L}^{-1}$ ), and homobrassinolide (Hbr) ( $4 \text{ mg L}^{-1}$ ). One treatment was prepared using only the base Yasuda medium as a control. The medium was adjusted to pH 6.3 and solidified with Phytigel ( $5 \text{ g L}^{-1}$ ). It was sterilized in an autoclave for 15 min at  $1.0 \text{ kg cm}^2$  pressure and  $250 \text{ }^\circ\text{C}$ . Culture medium (20 mL) was dispensed into Gerber® jars, where seed sowing was carried out.

### Incubation Conditions

The experimental units for the *in vitro* growth stage were maintained in a controlled-environment growth chamber under cool white fluorescent light, with photosynthetically active radiation of  $50 \text{ ME}^2 \text{ s}^{-1}$ . The photoperiod was 16/8 h (light/dark), with an average temperature of  $26 \pm 1.0 \text{ }^\circ\text{C}$  and 50% relative humidity. Once the seeds had germinated, protocorms were selected and transferred to individual jars, which served as experimental units. The culture media used in the multiplication stage contained activated charcoal, with the Yasuda basal medium serving as the control. For the *ex vitro* stage, *Guarlanthe skinneri* (Bateman) Dressler & W.E. Higgins plantlets with developed roots and leaves were selected. These were transplanted into 10 oz Styrofoam cups containing ground coconut fiber substrate (Thomas Willey-Medal, Philadelphia, USA).

### Homobrassinolide

The homobrassinolide (Hbr) CIDEF-4 (Natura del Desierto, S.A.) contains 80% steroidal content, of which 10% is active. It is presented as a non-toxic, water-soluble formulation that is compatible with fertilizers, insecticides, and fungicides (according to label information).

### Experimental Management and Irrigation

Foliar application of Hbr was carried out at 7, 14, and 21 days after transplanting (DAT) at concentrations of 2, 4, and  $6 \text{ mg L}^{-1}$ .

### Treatments, Experimental Design, and Number of Replications

The treatments were generated by combining three doses of Hbr (2, 4, and  $6 \text{ mg L}^{-1}$ ) with three application frequencies (7, 14, and 21 days), plus a control. All treatments, with 10 replications each, were distributed in a completely randomized design on the nursery benches.

### Variables

Morphological variables and plant survival were recorded at 28, 56, 70, 77, and 91 days after transplanting (dat). Plant height was measured using a graduated ruler from the base to the apex. In addition, the number of green leaves, dry leaves, and dead plants were recorded.

### Statistical Analysis

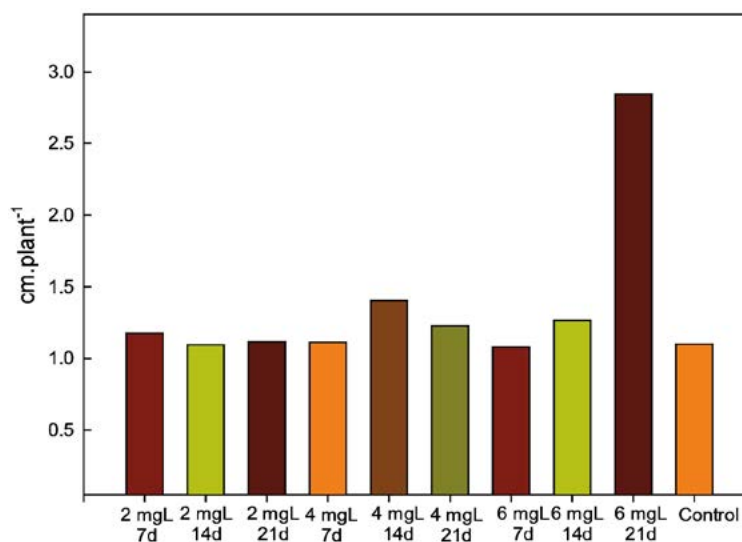
Plant height was statistically analyzed using SAS software version 9.0 for Windows (SAS Institute Inc., Cary, NC 27513, USA), and means were compared using Tukey's test ( $p \leq 0.05$ ). The other variables were graphed using Sigma Plot software (version 11.0) from Jandel Scientific.

## RESULTS AND DISCUSSION

The applications of Hbr increased the height of *G. skinneri* (Bateman) Dressler & W.E. Higgins compared to the control, but the effect depended on the concentration and the frequency of application to the plant (Figure 1).

The treatment that induced the greatest plant height was when  $6 \text{ mg L}^{-1}$  of Hbr was applied every 21 days, and this was statistically different from the other treatments ( $P \leq 0.05$ ). Plant height also increased when  $4 \text{ mg L}^{-1}$  of Hbr was applied at 7 and 14 days after transplanting (dat), but these values were not statistically different.

The increase in plant height with the application of Hbr at different frequencies has been previously reported by Joaquín-Torres *et al.* (2006) and Torres-Ruíz *et al.* (2007) in other species such as Guinea grass (*Panicum maximum* Jacq.) and maize. This effect is attributed to one of the known processes induced by Hbr application, namely cell elongation through its influence on gene expression and/or enzyme activity (Khripach *et al.*, 2000). Furthermore,



**Figure 1.** Height of *Guarianthe skinneri* (Bateman) Dressler & W.E. Higgins) with Hbr application under nursery conditions at 91 days after transplanting (dat). Means with the same letter are not significantly different (Tukey,  $p \leq 0.05$ ). CV=20.5%.

brassinosteroids play an important role in tolerance to abiotic stress such as drought and temperature fluctuations (Bajguz & Hayat, 2009; Gomes, 2011; Vriet *et al.*, 2012).

### Number of Green and Dry Leaves

The number of green and dry leaves recorded at different evaluation dates is presented in Table 1.

The initial average number of green leaves with the Hbr application was 12% higher compared to the control. At the end of the evaluation, at 91 dat, the lowest average was 12 for the treatments with 2 mg L<sup>-1</sup> applied at 7 and 14 days. In contrast, when applying 6 mg L<sup>-1</sup> at 14 and 21 dat, the highest number of green leaves was induced during the evaluation.

Regarding the interaction between application frequencies of Hbr and the concentrations of the brassinosteroid, it was found that at 21 dat, the highest number of green leaves occurred at the concentrations of 2 and 6 mg L<sup>-1</sup>, while at 14 dat, the highest number was found when the 4 mg L<sup>-1</sup> concentration was applied.

Capote *et al.* (2009) mentions that the application of Hbr MH5 stimulated the number of leaves and root formation in the Bromeliaceae *Vriesea* sp., and notes that it suggests a synergistic or additive effect with auxins in this process.

The number of dry leaves increased in all treatments with Hbr at 28 dat, but decreased in the control. The most notable effect was observed when Hbr was applied every 7 days. At the end of the evaluation, at 91 dat, the highest number of dry leaves was observed when the 2 and 4 mg L<sup>-1</sup> doses were applied every 7 days. In contrast, when applying Hbr at a concentration of 6 mg L<sup>-1</sup>, the lowest number of dry leaves was observed.

In this same context, the lowest number of dry leaves at the end of the evaluation, that is, at 91 dat, was recorded when 2, 4, and 6 mg L<sup>-1</sup> of Hbr were applied with a frequency of 14 days. In this regard, Izquierdo *et al.* (2012) report an increase in leaf area, dry biomass, and total soluble protein content in *Musa* spp.

**Table 1.** Number of green leaves and dry leaves of *G. skinneri* (Bateman) Dressler & W. E. Higgins under *ex vitro* conditions when different frequencies and concentrations of Hbr were applied in the nursery.

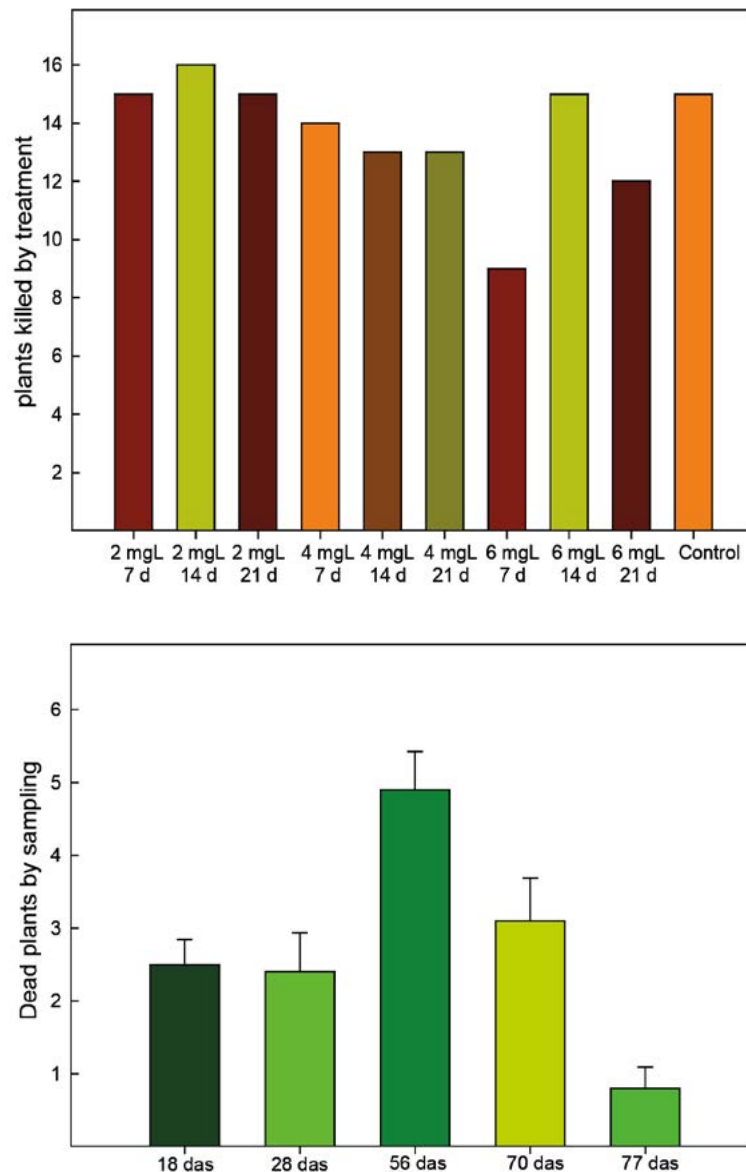
Treatment	Green leaves (Time days)						Dry leaves (Time days)					
	Initials	28*	56	70	77	91	Initials	28	56	70	77	91
2 mgL (7d)	42	31	25	31	21	12	5	18	6	1	10	5
2 mgL (14d)	35	29	32	37	23	12	3	9	12	2	11	0
2 mgL (21d)	41	22	25	32	23	18	7	16	11	8	4	2
4 mgL (7d)	35	20	26	30	31	16	0	20	4	0	2	6
4 mgL (14d)	37	22	26	28	24	17	6	9	2	1	0	1
4 mgL (21d)	40	22	29	29	25	17	10	9	2	3	3	3
6 mgL (7d)	51	30	28	29	22	14	4	14	10	4	0	0
6 mgL (14d)	38	24	31	34	29	22	10	7	4	3	4	0
6 mgL (21d)	37	32	33	33	34	27	0	9	2	0	0	3
Control	34	22	27	29	29	17	11	5	3	2	1	3

\* Days after being established in the greenhouse.

### Dead plants by treatment and sampling

The number of dead plants shows a certain relationship with the concentrations and frequencies of the applications. The highest concentration of 6 mg L<sup>-1</sup> and the most frequent application every seven days resulted in the lowest number of dead plants (Figure 2).

However, the lowest concentration of 2 mg L<sup>-1</sup>, regardless of the application frequency, and the control treatment, showed the highest number of dead plants. A similar result was reported by Francisco *et al.* (2011) at 90 days, who found that the highest value for the start of acclimatization with *Laelia eyermaniana* occurred between 60 and 90 days, primarily in



**Figure 2.** Number of dead *G. skinneri* (Bateman) Dressler & W. E. Higgins plants with different frequencies and applications of Hbr during *ex vitro* acclimatization. a) Dead plants by treatment b) Dead plants by sampling. The vertical line indicates  $\pm$  the standard error.

small (21.87) and medium-sized (31.25) seedlings. They have also reported good results when using brassinosteroids (Bajguz and Hayat, 2009; Kagale *et al.*, 2007), as they mitigate both biotic and abiotic stress.

The previous behavior suggests that the concentration of Hbr applied influences plant acclimatization, but with a lesser effect from the frequency of application. Posadas *et al.* (2016) report an 80-82% increase in survival during the acclimatization of *Carica papaya* cv. Maradol Roja with the application of Hbr Pectimorf in Cuba. In *Tuberaria major* (Willk.), a plant found near the coasts of Portugal, 97% survival was achieved six weeks after transplanting (Goncalves *et al.*, 2010).

The samples taken at 18 and 28 days after transplanting show the lowest values of plant mortality, with an average of 2.5 dead plants in each sampling. After 56 days of establishment, the number of dead plants significantly increased across treatments. The average reached 4.9, representing almost 100% in relation to the first two samplings. After this increase, plant mortality gradually decreased to 3 plants at 70 ddt, and by 77 ddt, it was less than one.

Preece and Sutter (1991) mention that *ex vitro* acclimatization presents dehydration problems due to the loss of leaf water and restricted uptake, as the roots are incapable of absorbing water in the early stages. This is one of the main causes of plant mortality when they are transferred to *ex vitro* conditions. In the first weeks after transfer to the *ex vitro* environment, plants must adapt to new growing conditions and develop a normal and functional root system physiology (Debergh *et al.*, 2000). Capote *et al.* (2009) indicate that at 28 and 49 days, significant differences were found in the survival of plants treated with MH5 compared to the control group with no application of this regulator. The lowest percentages (66%) were observed in the plants that did not receive the application of the brassinosteroid analog at the end of this phase.

## CONCLUSIONS

The foliar application of the Brassinosteroid CIDEF-4 induced greater growth of *G. skinneri* (Bateman) Dressler & W.E. Higgins, which was expressed in height. The more frequent application of Br at higher concentrations improves the acclimatization and survival of *G. skinneri* (Bateman) Dressler & W.E. Higgins in the nursery. Brassinosteroid concentrations with foliar application at different frequencies induce differences in the growth and survival of *G. skinneri* (Bateman) Dressler & W.E. Higgins.

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# Dendroclimatic potential of *Pinus hartwegii* Lindl. and its relationship with precipitation and temperature in the Sierra Juárez, Oaxaca, Mexico

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## ABSTRACT

**Objective:** To assess the dendroclimatic potential of *Pinus hartwegii* Lindl in the Sierra Juárez region of the state of Oaxaca and to describe the relationship between tree-ring width and regional precipitation and temperature patterns.

**Methodology:** A total of 66 samples were dated, in which chronologies of total ring (RWI), early wood (EWR) and late wood (LWR) were developed covering a period of 201 years (1818 to 2019).

**Results:** Significant associations were found between ring growth (RWI, EWR, and LWR) and precipitation during the December-April period ( $p < 0.01$ ), with correlation coefficients of  $r = 0.54$ ,  $r = 0.23$ , and  $r = 0.25$ , respectively. These findings indicate that precipitation is the climatic variable most strongly associated with the radial growth of *P. hartwegii*.

**Conclusions:** *P. hartwegii* demonstrates strong dendrochronological potential, supported by the results of the present study where it provides chronological information of 201 years (1818 to 2019) on the radial growth of the species, determined by climatic variables, precipitation and temperature.

**Keywords:** Growth rings; Dendroclimatology, *Pinus hartwegii*.

## INTRODUCTION

Forests around the world are under increasing pressure due to climate-induced stress factors and the growing demand for forest products (FAO, 2024). These ecosystems are home to millions of species that, in turn, influence the climate through the exchange of



water, carbon dioxide, energy, and chemical compounds with the atmosphere (Parmesan *et al.*, 2022). However, climate change—defined as significant variations in climatic parameters such as precipitation and temperature, with global warming being its most evident manifestation—has become one of the most pressing environmental issues in recent decades. The increasing frequency and intensity of droughts are disrupting ecosystem dynamics and negatively impacting forest growth (INECC, 2018; Luna *et al.*, 2022; Brichta *et al.*, 2024).

Rojas-García *et al.* (2020) point out that in natural forests, climate is the main factor regulating species growth and is responsible for the formation of tree rings. The analysis of ring growth has made it possible to improve and expand meteorological records beyond those obtained from instrumental data (Aquino *et al.*, 2019). In this regard, dendrochronological methods for historical climate reconstruction have been widely evaluated and are considered a reliable and efficient source of information (Bradley, 1999; Villanueva *et al.*, 2018; Manzanilla-Quiñones *et al.*, 2023). Specifically, tree rings serve as permanent and continuous records of the environmental conditions in which trees develop (Reyes-Basilio *et al.*, 2021). According to Manzanilla-Quiñones *et al.* (2020), the formation and thickness of tree rings are directly related to environmental, edaphic, topographic, and ecological factors of the area being evaluated. Various authors identify precipitation as the main and most limiting factor for tree growth, particularly in conifer species (Villanueva-Díaz *et al.*, 2018; Manzanilla-Quiñones *et al.*, 2020; Brichta *et al.*, 2024). Moreover, ring growth is often more evident in deep soils and areas with high light availability. Therefore, the relationship between tree growth and environmental conditions enables inferences about environmental variables based on ring width fluctuations, and even allows for the reconstruction of specific climatic variables (Gutiérrez *et al.*, 2022).

Dendrochronological studies in Mexico have primarily been documented in the north-central regions of the country, whereas studies in the southern region are limited (Aquino, 2019). The Sierra Juárez in southern Oaxaca is considered one of the most diverse mountainous systems, featuring ecosystems with high biodiversity and various vegetation types, dominated by pine-oak forests and montane cloud forests (Ponce-Reyes *et al.*, 2012). Among the most abundant species in the region is *P. hartwegii*, which has great ecological value due to its adaptability to high-altitude areas with low temperatures. Its typical altitudinal range is between 2,200 and 3,300 meters above sea level, although it may be found at elevations exceeding 3,300 meters (Pérez-Suárez *et al.*, 2022).

*Pinus hartwegii* Lindl. has been scarcely studied in Mexico. Its importance in dendrochronology lies in the fact that it is considered a subalpine species that develops in pure forests above 3,000 meters above sea level, where its growth rings are well-defined (Astudillo-Sánchez *et al.*, 2017; Manzanilla-Quiñones *et al.*, 2021). This species exhibits great longevity, surpassing 400 years (Villanueva-Díaz *et al.*, 2015), and shows a high physiological responsiveness to climatic factors. Consequently, it serves as a valuable proxy for climatic variation, from which relevant climate information can be extracted using dendrochronological techniques (Astudillo-Sánchez *et al.*, 2017). Based on the above, the objective of this study was to determine the dendroclimatic potential of *Pinus hartwegii*

Lindl. in the Sierra Juárez of the state of Oaxaca, as well as to describe the relationship between ring width and regional precipitation and temperature patterns.

## MATERIALS AND METHODS

### Study Area

The study was conducted in Santa María Jaltianguis, located in the northern part of the Sierra Juárez in the state of Oaxaca (Figure 1). The vegetation of the sites consists of coniferous forests dominated by pine and pine-oak forests. The climate is temperate subhumid with summer rainfall and warm subhumid with summer rainfall, with temperatures ranging from 26 to 32 °C and an average annual precipitation of 1,448 mm. The soil in the study area belongs to the Acrisol group (Aquino *et al.*, 2025). Sampling sites were located in specific distribution areas where *Pinus hartwegii* Lindl. is the dominant species, at an altitude between 3,000 and 3,100 meters above sea level.

### Sampling Design and Sample Collection

Using selective sampling, 66 mature *P. hartwegii* trees were selected, characterized by sparse, thin branches, free of pests and diseases, without mechanical damage, and with minimal disturbance in the stand. Two to three growth cores per tree were extracted using Pressler increment borers (Haglöf brand) with a 5 mm internal drill diameter and 48 cm length. Samples were taken at a height of 40 cm above the ground (Contreras-Mata *et al.*, 2024).

### Sample Processing and Measurement

The cores (samples) were placed in grooved molds and secured with glue and adhesive tape. They were then subjected to a sanding process, which enhances the visibility of the growth rings. Samples were processed following standard dendrochronological techniques (Stokes & Smiley, 1968).

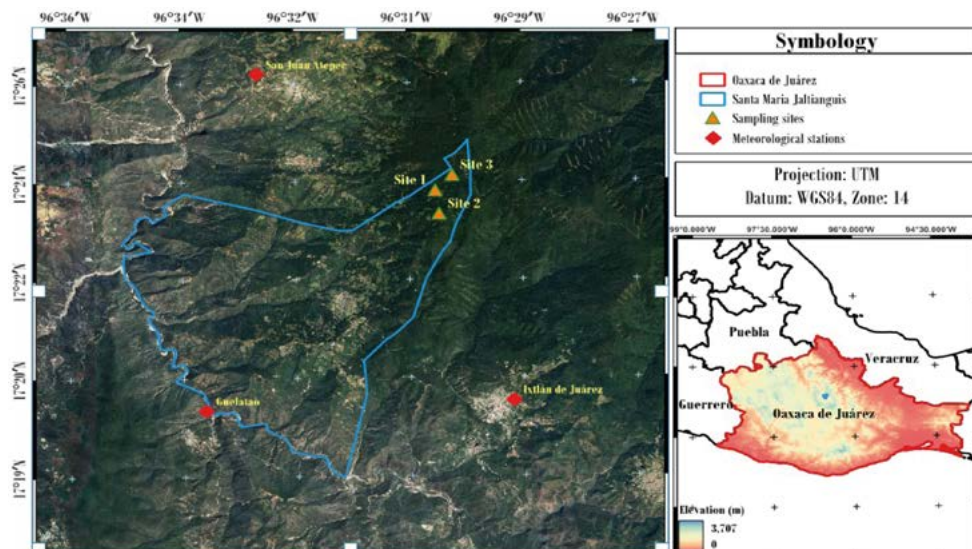


Figure 1. Geographic location of the study area.

### **Growth Ring Dating**

Using a Velmex stereomicroscope with a resolution of 0.001 mm, the growth rings of each sample were counted, and the widths of earlywood and latewood in each growth ring were measured to obtain the total ring width. These measurements were conducted in the Dendrochronology Laboratory of the Faculty of Forestry Sciences at the Autonomous University of Nuevo León. Subsequently, the quality of the dating was verified using the COFECHA program, correlating growth cores in 50-year subperiods with 25-year overlaps (Pompa-García and Camarero-Martínez, 2015).

### **Chronology Development and Dendroclimatic Potential Determination**

To remove growth trends and environmental factors unrelated to climate, a preliminary standardization of the growth ring width measurements was performed using the ARSTAN program to determine the dendroclimatic potential of *P. hartwegii*. A double standardization of the growth rings was applied to improve the fit, consisting of a cubic smoothing spline that preserved 50% of the variance contained in the series. The ring width was divided by the value of the fitted curve, transforming the increments into ring-width indices (RWI), which allowed comparison of growth series of different ages.

Using the RWI values, an annual chronology was generated based on the standard and residual chronologies provided by ARSTAN. According to Contreras-Mata *et al.* (2024), these statistics help eliminate endogenous effects caused by stand disturbance and maximize the common climatic signal contained in the growth rings.

### **Evaluation Criteria of the *P. hartwegii* Chronology**

Several parameters of the chronology were determined to assess the species' response and sensitivity to climatic variables (Buras, 2017):

- Inter-series correlation: indicates the intensity of the climatic signal that is common at the tree population level.
- Mean sensitivity: relative change in ring width from one year to the next.
- Standard deviation: variation in ring width growth.
- First-order autocorrelation: influence of the previous year's ring growth on the width of the following year's ring; low values reflect greater year-to-year growth variability.
- Signal-to-noise ratio: proportion of the climatic signal relative to other non-climatic factors.
- Expressed Population Signal (EPS): indicates the intensity of the climatic signal expressed by the population as it approaches the oldest segment of the chronology.

### **Relationship between radial growth and regional climate**

To analyze the influence of climate on the radial growth of *P. hartwegii*, the annual RWI was correlated with the series of mean annual precipitation and temperature values. This allowed for the determination of the climatic influence on ring width (Arroyo-Morales *et al.* 2023). Precipitation and temperature data were obtained from meteorological stations near the study sites with complete and reliable climate records (Table 1).

**Table 1.** Meteorological stations with complete data near the sampling sites.

Station	Latitude (N)	Longitude (W)	Altitude (m)	Period (years)
Guelatao	17° 19' 49"	96° 33' 51"	1496	1955-2002
Ixtlán de Juárez	17° 19' 59"	96° 28' 59"	2312	1955-2002
San Juan Atepec	17° 25' 59"	96° 32' 59"	1975	1955-2002

## RESULTS AND DISCUSSION

Table 2 presents the results obtained from the COFECHA program, showing that 66 cores from 45 trees were successfully dated, corresponding to 86.8% of the total analyzed sample. The remaining cores were discarded due to growth anomalies, such as sections with ring compression and release pulses that prevented the identification of the beginning and end of the growth rings (Urquijo *et al.*, 2022). According to the parameters obtained from COFECHA and ARSTAN, *Pinus hartwegii* has high dendrochronological potential for the reconstruction of climatic events (Acosta-Hernández *et al.*, 2017).

According to Díaz-Ramírez *et al.* (2016), the above results exceeded the parameters reported in various studies on conifer species. Specifically for *P. hartwegii*, the interseries correlation is notably higher compared to the results obtained by Astudio-Sánchez *et al.* (2016). The total ring-width dendrochronological series spans from 1810 to 2020 (210 years in length). The most suitable period for climate reconstruction (chronological reliability) extends from 1888 to 2020 and includes more than 10 radii, with an EPS of 0.86, surpassing the 0.85 threshold (Wigley *et al.*, 1984). Manzanilla-Quiñones *et al.* (2020) indicate that EPS is evaluated on a scale from 0 to 1; therefore, values above 0.80 are considered acceptable and recommended (Figure 2).

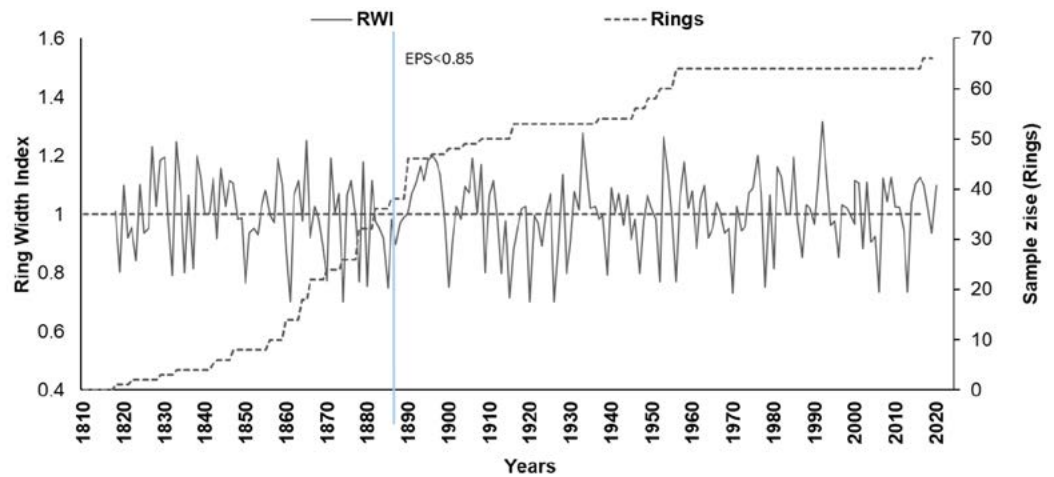
### Response Function

Climatic data from the region and the analysis of the residual chronology from 1955 to 2002 were positively correlated with three meteorological stations: Ixtlán de Juárez,

**Table 2.** Statistical results generated by the COFECHA program for the ring width of *P. hartwegii*.

Statistics	Values obtained	Range (Astudillo-Sánchez <i>et al.</i> , 2016)	Range (Manzanilla-Quiñones <i>et al.</i> , 2020)
Number of dated series	66	104	78 <sup>1</sup> , 26 <sup>2</sup> y 25 <sup>3</sup>
Intercorrelation between series	0.57	0.50	0.58 <sup>1</sup> , 0.51 <sup>2</sup> y 0.41 <sup>3</sup>
Master series	201 años (1818-2019)	307 años (1705-2012)	147 años (1869-2016) <sup>1</sup> 158 años (1858-2016) <sup>2</sup> 142 años (1874-2016) <sup>3</sup>
Average sensitivity	0.31	0.32	0.31 <sup>1</sup> , 0.24 <sup>2</sup> y 0.23 <sup>3</sup>
Average ring width	105 años	---	---
Standard deviation of ring width	0.21	---	---
First-order autocorrelation	0.39	---	0.24 <sup>1</sup> , 0.10 <sup>2</sup> y -0.15 <sup>3</sup>
Signal-to-noise ratio	12.15	---	9 <sup>1</sup> , 12 <sup>2</sup> y 18 <sup>3</sup>
Expressed population signal (ESP)	0.86	0.85	0.80

<sup>1</sup>=Nevado de Colima, <sup>2</sup>=Nevado de Toluca y <sup>3</sup>=Pico de Orizaba (Manzanilla-Quiñones *et al.*, 2020). --- Without data.



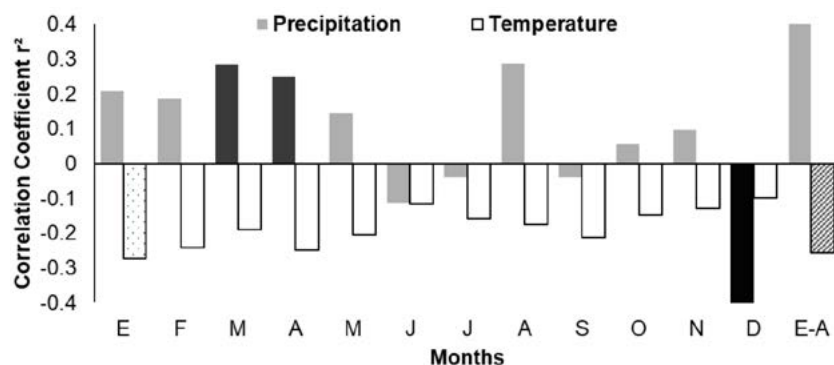
**Figure 2.** Residual chronology of total ring width (RWI) for *Pinus hartwegii* Lindl. (solid line) and sample size (dotted line). The blue line indicates the year from which the chronology exceeds the EPS value of 0.85. The dashed black line represents the threshold for a significant confidence level ( $p < 0.05$ ).

Atepec, and Guelatao. These stations are located within a radius of approximately 100 km. In general, the correlation between the residual chronology and precipitation with ring width was negative in the months of June, July, September, and December ( $-0.11$ ,  $-0.03$ ,  $-0.04$ , and  $-0.43$ ); the rest of the months showed positive correlations, with December being the most significant. Temperature correlated negatively throughout all seasons, with January showing the highest significance ( $-0.27$ ) (Figure 3).

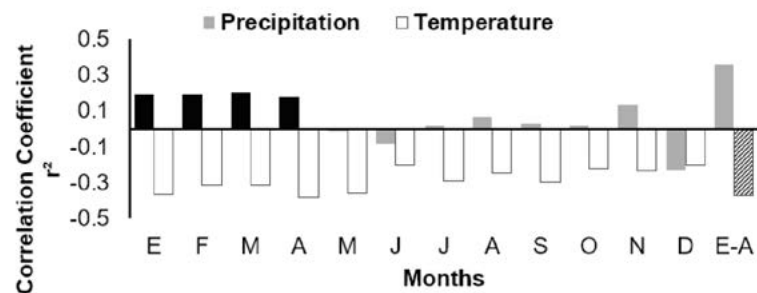
In relation to earlywood, it showed a similar behavior with precipitation, except for September; temperature was correlated with the records for the month of April ( $-0.38$ ) (Figure 4).

Finally, latewood showed a positive relationship with precipitation except for the months of May, November, and December ( $-0.02$ ,  $-0.07$ , and  $-0.24$ ). Regarding temperature, the correlation was negative throughout the year, with April showing the strongest effect ( $-0.38$ ) (Figure 5).

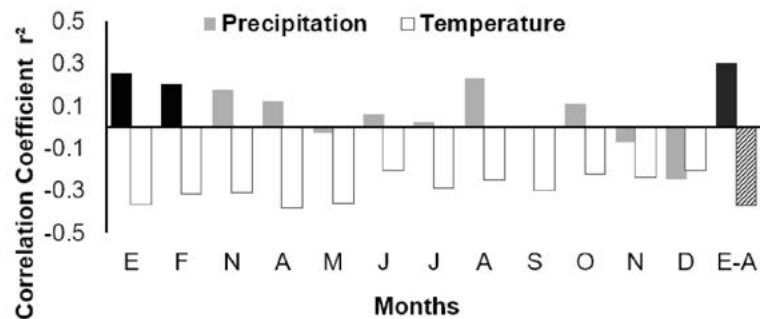
In general, the accumulated seasonal precipitation between January and April showed a significant correlation with the residual chronology of the three variables analyzed.



**Figure 3.** Response function of the residual chronology with precipitation and temperature correlated to ring width (RWI) for the period 1955-2002.



**Figure 4.** Response function of the residual chronology of precipitation and temperature with earlywood (EWR) for the period 1955-2002.

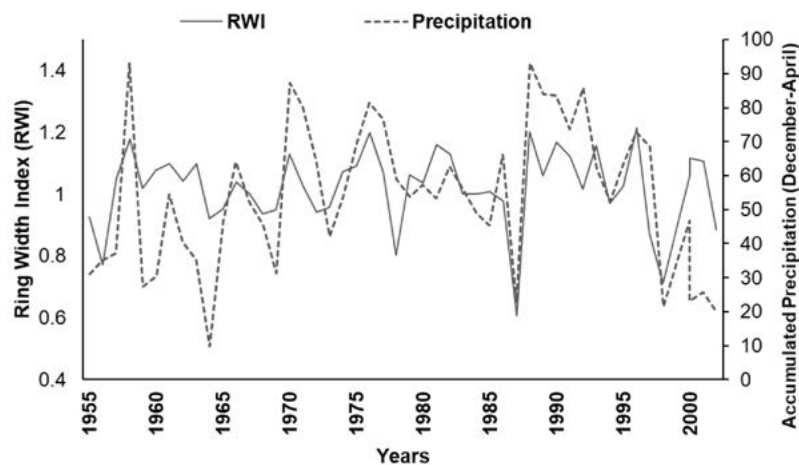


**Figure 5.** Response function of the residual chronology of precipitation and temperature with latewood (LWR) for the period 1955-2002.

Specifically, total ring width exhibited a significant negative correlation with precipitation ( $r = -0.54$ ), while earlywood ( $r = 0.36$ ) and latewood ( $r = 0.31$ ) showed significantly positive correlations. These results are consistent with Astudillo-Sánchez *et al.* (2016) for *P. hartwegii* in Nevado de Toluca and Monte Tlaloc, where they found a pattern of relationships between climate and tree growth with positive associations to precipitation and negative associations to temperature, highlighting a stronger relationship between precipitation and species growth.

On the other hand, the residual correlations of maximum temperature from January to April with total ring width ( $r = -0.26$ ), earlywood ( $r = -0.37$ ), and latewood ( $r = -0.37$ ) were significantly negative ( $p < 0.05$ ). These associations with total ring width, earlywood, and latewood growth have been reported by various authors who developed dendrochronological networks in conifers (Salem *et al.*, 2015; Villanueva *et al.*, 2015; Villanueva-Díaz *et al.*, 2018).

The standard chronology data of the total ring indicate that precipitation from December to April during the period 1955-2002 ( $r = 0.54$ ,  $p < 0.01$ ) explains up to 54% of the annual growth of *P. hartwegii* (Figure 6). These results are similar to those reported by Manzanilla-Quíñonez *et al.* (2020), who found a 53% variation in December for *P. hartwegii* at Pico de Orizaba in Veracruz. Additionally, it has been reported that the growth of *P. hartwegii* begins from March to April and ends in October-November (Biondi *et al.*, 2005). In this regard, Aquino *et al.* (2019) mention that the first months



**Figure 6.** Association between the total ring index and accumulated precipitation from December to April using meteorological station records for the period 1955-2002.

of the year show the most significant correlations with radial growth in pines, which coincides with the results of the present dendrochronological analysis. However, the increase in temperature affects radial growth (Lo *et al.*, 2010). For instance, Astudillo-Sánchez *et al.* (2019) report that in Mexico the rise in temperature was beneficial for the growth of *P. hartwegii*. The relationship of radial growth indicates an association that is regulated and mainly limited by the availability of rainfall during the winter-spring season, as it occurs with sufficient intensity to retain soil moisture. Díaz-Ramírez *et al.* (2016) state that precipitation influences up to 52% of radial growth. This has been documented in various dendrochronological analyses in North America (Gutiérrez-García and Ricker, 2019; Cerano-Paredes *et al.*, 2014; Aquino *et al.*, 2019; Manzanilla-Quñonez *et al.*, 2020).

## CONCLUSIONS

*Pinus hartwegii* shows dendrochronological potential, supported by the results of the present study which provides a 201-year chronology (1818 to 2019) on the radial growth of the species, determined by climatic variables such as precipitation and temperature.

The total ring width, earlywood, and latewood have a high potential (54%) to explain the growth of *P. hartwegii* in relation to climatic variables.

The parameters obtained from the *P. hartwegii* series reveal a reliable dating with a strong annual growth signal. The variable with the greatest influence on this growth is winter-spring precipitation; additionally, maximum temperature was negatively associated, implying that as maximum temperature increases, radial growth decreases.

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# Contribution of Home Gardens to Food Availability for Food Security

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## ABSTRACT

**Objective:** To analyze the importance and contribution of home gardens to food security and food availability, in relation to biodiversity, in communities belonging to the municipalities of Mazatán and Tapachula, Chiapas, Mexico.

**Design/methodology/approach:** A questionnaire was applied to 52 families, incorporating social and ethnobotanical aspects. The questionnaire was administered through semi-structured interviews and field visits in the communities.

**Results:** Households consisted of 4.2 to 5.6 members, and the female head of household (56.68%) was prominently involved in home garden management. The floral diversity of the 52 home gardens included up to 107 species, most notably the Fabaceae (10 species) and Solanaceae (9 species) families. These gardens also housed 15 species of wild and domestic fauna. The primary use of both plants and animals recorded was food; The main food groups found in home gardens were fruits, low-energy foods, and vegetables.

**Study Limitations/Implications:** This study was limited by the availability of rural families to complete surveys and access their home gardens.

**Findings/Conclusions:** Family home gardens harbor rich plant and animal diversity, providing both plant and animal food groups, as well as domestic and wild fauna, with women being the primary caregivers.

**Keywords:** Affordability, Biodiversity, Availability, Gardens, Poverty.

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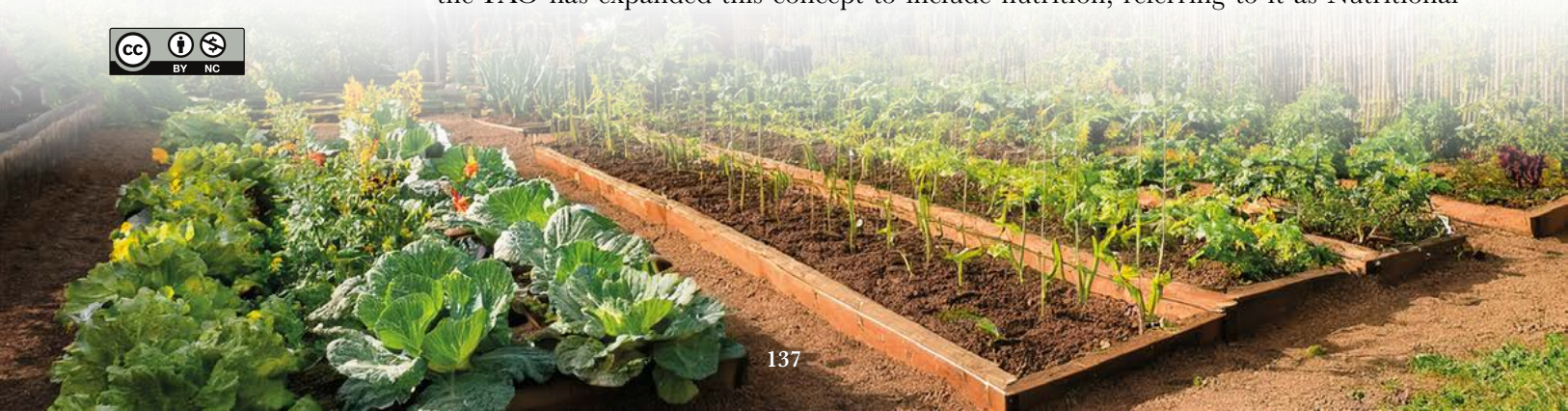
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## INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) first defined food security (FS) at the 1996 World Food Summit as “when all people, at all times, have physical, social, and economic access to sufficient, safe, and nutritious food to meet their dietary needs and food preferences for an active and healthy life.” Since 2009, the FAO has expanded this concept to include nutrition, referring to it as Nutritional



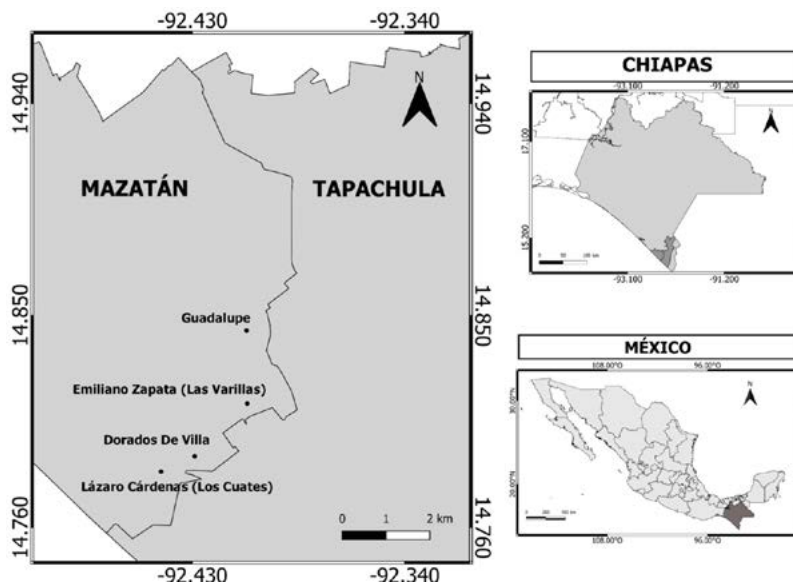
Food Security (NFS) (FAO, 2009). In relation to NFS, home gardens play an important role in food availability for rural households. These gardens are considered traditional agroforestry systems that exhibit agrobiodiversity composed of plant and animal species, as well as ecosystems used for agricultural purposes (Mariaca-Méndez, 2012; Vogl *et al.*, 2004). In academic contexts, and depending on the region, home gardens are known by various terms—such as family gardens, solares, or backyards—according to their local geographical context. In southern Chiapas, Mexico, families refer to this space as a patio (Gerardo-Méndez *et al.*, 2022). These home gardens—*patios*—are of great importance for the transmission of knowledge related to NFS, as they host both domesticated and wild agrobiodiversity that contributes to household nutrition (Cano-Contreras, 2015). In marginalized and rural areas, home gardens serve as a key resource for supplying part of the family's diet, while also promoting a healthy and varied food intake (Suárez *et al.*, 2015). Furthermore, these spaces play a crucial role in the conservation of germplasm from edible, medicinal, condiment, and ornamental species, among others (Duché-García *et al.*, 2017). Socially, they hold significant cultural value, as they contribute to the preservation of traditional and cultural roots of the communities that manage them (Lope-Alzina *et al.*, 2018; Mariaca-Méndez, 2012; Moctezuma-Pérez, 2010; Van der Wal *et al.*, 2011).

Several studies have shown that cultivated and wild plant species coexist in home gardens, forming part of the region's ethnobiological heritage. Characterizing these species allows for a better understanding of their use and significance, as well as their contribution to the food groups that make up family diets. This information can also inform development proposals at the municipal and state levels, highlight the value of traditional practices, and emphasize the ecosystem services provided by these systems. In Mexico, 25.1% of the total population experiences food access deficiencies. Specifically, in the state of Chiapas, up to 24.5% of the population faces food insecurity (CONEVAL, 2020). In rural areas of Chiapas, however, home gardens are traditionally used by families for food production aimed at self-consumption, highlighting their importance for food security in communities in southern Mexico. Therefore, the objective of this study was to analyze the importance and contribution of home gardens to food availability for food security, in relation to biodiversity, in communities located in the municipalities of Mazatán and Tapachula, Chiapas, Mexico.

## **MATERIALS AND METHODS**

### **Study Area**

This research was conducted in four communities in southern Chiapas: three located in the municipality of Mazatán: Ejido Lázaro Cárdenas (ELC), Guadalupe (GU), and Emiliano Zapata las Varillas (EZV) and one in the municipality of Tapachula, Dorados de Villa (DV). Fieldwork was carried out from January to April 2020 (Figure 1). These communities were selected due to their proximity and similar edaphoclimatic conditions. According to CONEVAL (2020), all four communities are classified as experiencing extreme poverty. They are ejido (communal land tenure) properties, located far from the municipal centers.



**Figure 1.** Geographical location of the study sites. Source: Prepared by the authors using data from (INEGI, 2020a).

The communities of the study sites share a warm subhumid climate with summer rainfall (94.22%), an average humidity of 5.78%, temperatures ranging from 26 to 30 °C, and annual precipitation between 1,200 and 2,000 mm (INEGI, 2010).

The selection of the home gardens was based on a non-probabilistic purposive sampling method (Otzen and Manterola, 2017; Zamudio-Sosa *et al.*, 2002), which included 52 gardens distributed across the communities of both municipalities. This approach was also aligned with the methodology employed by Van der Wal and Bongers (2013), which recommends selecting owners who are willing to participate. To this end, discussions were held with the women of the study communities, and the research project was presented to gain their collaboration.

After obtaining consent to participate, schedules for visits and tours of the home gardens were arranged. Additionally, a semi-structured interview was conducted with the women, focusing on social, ethnobotanical, and home garden management aspects, due to the important role women play in managing the production unit.

**Characterization of the Family:** The number of family members, educational level, time spent on home garden management activities (expressed in minutes), and the participation of family members involved in home garden activities were recorded.

**Plant Species Diversity and Use:** The methodology suggested by Colin *et al.* (2020) was applied. Taxonomic identification was verified using the database available at [www.theplantlist.org](http://www.theplantlist.org) and the VAST (Vascular Tropics) database of the Missouri Botanical Garden (Solomon and Magill, 2006). Based on the collected data, plant genera, species, and families were identified by comparison, along with the number of species and ethnobotanical data per home garden and community. Additionally, the use value of the plant species present and reported in the home gardens was recorded.

**Animal Species Diversity and Use:** This was assessed using the method proposed by Chablé-Pascual *et al.* (2015), recording the animals reported during direct interviews, as well as through home garden visits and photographic documentation for species identification. The use value of the animal species was also recorded. Subsequently, the species were compared with those reported by Mariaca-Méndez (2012).

**Diversity by Food Groups:** The total plant and animal diversity present in the home gardens was classified into food groups according to the *Mexican System of Equivalents* (Pérez-Lizaur *et al.*, 2014).

## RESULTS AND DISCUSSION

**Household Characterization:** In general, the 52 participating women reported households ranging from 4.2 to 5.6 members.

The largest households were found in the community of EZV, with 5.6/3.7 members (Table 1). The educational level of household heads in the four communities was primarily basic education (elementary school). Regarding illiteracy, a low percentage of illiterate households was found. In DV, 23% of men were illiterate, while in EZV, 31% of men and 10% of women were reported as illiterate. Conversely, in ELC, 23% of women were illiterate compared to only 7% of men. Regarding the highest level of education attained, the communities of ELC, EZV, and GU reported having completed upper secondary education (high school), while the DV community reported up to lower secondary education (middle school) only (Figure 2).

Regarding the occupation of household heads, day laborer was reported as one of the main activities across the four communities, in contrast with farmer, which was performed less frequently (Figure 3).

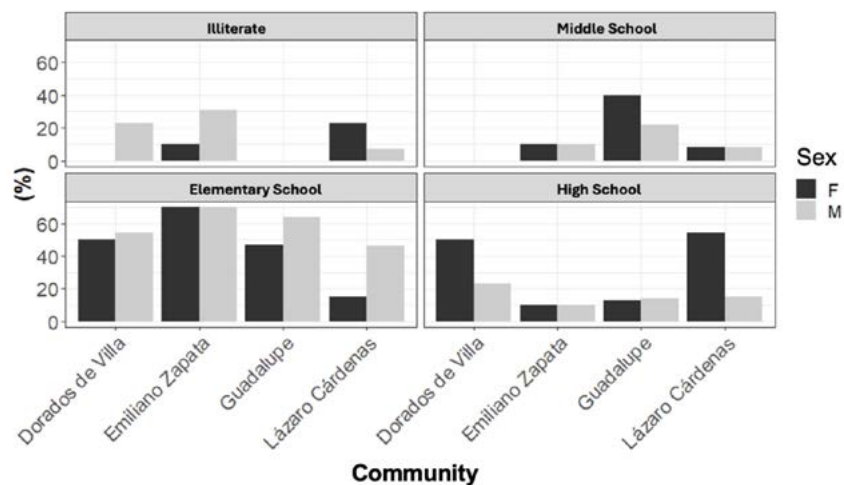
In the case of female heads of household, most are homemakers; however, in the community of DV, a small percentage (7%) of women work as day laborers.

### Yard management and activities:

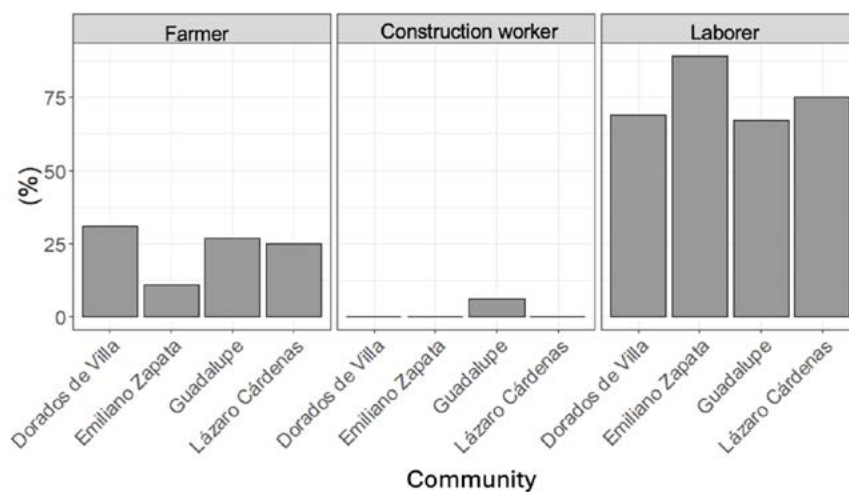
According to the results obtained, all family members participate in yard-related activities. Among the 52 studied yards, there was a marked trend of female participation, accounting for 56.68%, in contrast with the participation of male heads of household, which was only 5.49% in the communities of DV and ELC. Moreover, the participation of mothers accompanied by their children was reported in three communities: DV, ELC, and G (Table 2). Regarding the time devoted to yard tasks and management, respondents reported dedicating between 1.35 minutes and 2 hours per day.

**Table 1.** Number of family members in communities of Mazatán and Tapachula, Chiapas.

Communities	Members per family	Number of children
Ejido Lázaro Cárdenas	4.7	2.9
Guadalupe	4.2	2.2
Dorados de Villa	4.6	2.6
Emiliano Zapata las Villas	5.6	3.7



**Figure 2.** Educational level (%) of male and female heads of household in communities of Mazatán and Tapachula, Chiapas, Mexico.



**Figure 3.** Occupation of household heads in communities of Mazatán and Tapachula, Chiapas.

**Table 2.** Family members' participation and time dedicated to the yard in communities of Mazatán and Tapachula, Chiapas.

Community	Mother	Father	Mother-Father	Mother-Children	Mother-Father-Children	t (h)
Dorados de Villa	6	2	-	5	1	1.39
Guadalupe	9	-	3	3	-	2.00
Ejido Lázaro Cárdenas	7	1	1	4	-	1.73
Emiliano Zapata las Varillas	7	-	3	-	-	1.35
Total (%)	56.68	5.49	14.42	21.62	1.79	

t=time dedicated to work in the HG per day; h=hours.

**Plant Diversity and Use.** In total, across the four communities, 107 plant species were found; with the Fabaceae family standing out with 10 species, followed by Solanaceae (9 species). Meanwhile, the families that shared the same number of species (5) were: Rutaceae, Poaceae, Malvaceae, Euphorbiaceae, and Amarantaceae. On average, between 83 and 96 plant species were found per community: ELC with 90 species, GU with 85, DV with 84, and EZV with 75 species. The use of the species diversity present in the 52 patios across the four study communities was mainly for food purposes (Table 5). In the community of DV, 72.3% of the species present in the patios (68 species) are used for food; in the community of LC, 67% (67 species); GU, 63.1% (60 species); and EZV, 61.1% (56 species). The results highlight the importance of floristic diversity in the availability of food for families in the study area. Regarding the plant parts used for food, these were mainly: leaves, fruits, seeds, stems, grains, and flowers, with a notable use of fruits and leaves. Other reported uses of the diversity were: medicinal, ornamental, and shade, showing variability among the communities. Meanwhile, the contribution of wood from the patios was found in lesser proportion compared to the other uses (Table 3).

**Animal Diversity and Use:** From the fauna present in the 52 studied home gardens, six species are used for food (Table 4): chicken, turkey, pig, cow, sheep, and duck. The animal products used are: meat, eggs, and milk. This demonstrates the importance of home gardens in providing protein to the rural families' diet. The richness of the fauna in the 52 studied home gardens contains a total of 15 species distributed across the four communities: DV with 14 species, GU with 13 species, ELC with 10 species, and EZV with 8 species. Notable species include *Gallus gallus domesticus* (chicken) with 25% and *Canis lupus familiaris* (dog) with 25%, respectively. Additionally, there is the presence of wild animals such as the parrot (*Amazona auropalliata*) in three communities (DV, 11%, ELC, 11%, and GU, 6%); the chachalaca (*Ortalis vertula*) in DV and ELC with 2% in each; the mojarra (*Cichlasoma trimaculatum*), present in DV at 3%, GU at 2%, and EZV at 3%; and the casquito turtle (*Kinosternon leucostomum*) which is only found in the GU community at 2% (Table 5).

**Food group diversity:** Of the 52 yards studied, the plant and animal diversity present corresponded to eight food groups: oils and fats, animal-origin foods with low fat content and moderate fat content, fat-free cereals, fruit, legumes, energy-free foods, and vegetables. Among these, the fruit, vegetable, and energy-free food groups stand out, as shown in Table 6.

**Table 3.** Use of plant species found in home gardens in communities of Mazatán and Tapachula, Chiapas.

Use	Dorados de Villa (n)	Guadalupe (n)	Lázaro Cárdenas (n)	Emiliano Zapata las Varillas (n)
Food	68	60	67	56
Medicinal	9	9	9	9
Ornamental	9	8	10	4
Shade	5	10	7	10
Wood	1	5	3	4
Total	92	92	96	83

**Table 4.** Animal diversity and use in home gardens of communities in Mazatán and Tapachula, Chiapas.

Common name	Scientific name	Type of species	Use
Pig	<i>Sus scrofa domestica</i> L.	Domestic	Edible
Chicken	<i>Gallus gallus domesticus</i> L.	Domestic	Edible
Duck	<i>Anas platyrhynchos domesticus</i> L.	Domestic	Edible
Turkey	<i>Meleagride gallopavo</i> L.	Domestic	Edible
Sheep	<i>Ovis orientalis aries</i> L.	Domestic	Edible
Cow	<i>Bos primigenius taurus</i> L.	Domestic	Edible
Horse	<i>Equus ferus caballus</i> L.	Domestic	Working
Dog	<i>Canis lupus familiaris</i> L.	Domestic	Pet
Cat	<i>Felis silvestris catus</i> (Schreber)	Domestic	Pet
Rabbit	<i>Oryctolagus cuniculus</i> L.	Domestic	Pet
Guinea Fowl	<i>Numida meleagris</i> L.	Domestic	Pet
Parrot	<i>Amazona auropalliata</i> (Lesson)	Wild	Pet
Musk turtles	<i>Kinosternon</i> (Spix)	Wild	Pet
Mojarra	<i>Cichalsoma trimaculatum</i>	Wild	Pet
Chachalaca	<i>Ortalis vetula</i> (Wangler)	Wild	Pet

**Table 5.** Use of home garden fauna diversity in the four communities of Mazatán and Tapachula, Chiapas.

Especies	Dorados de Villa		Lázaro Cárdenas		Guadalupe		Emiliano Zapata las Varillas		Use
	F	%	F	%	F	%	F	%	
Species	14	23	13	28	13	24	8	26	Co
Perro	9	15	9	20	14	26	8	26	Ma
Chicken	8	13	4	9	3	6	4	13	Ma
Dog	7	11	5	11	3	6	-	-	Ma
Cat	6	10	3	7	1	2	2	6	Co
Parrot	5	8	6	13	3	6	2	6	Co
Turkey	3	5	3	7	3	6	4	13	Co
Duck	2	3	-	-	6	11	2	6	Co
Sheep	2	3	-	-	1	2	1	3	Ma
Pig	1	2	-	-	2	4	-	-	Co
Fish	1	2	1	2	3	6	-	-	Tr
Cow	1	2	1	2	1	2	-	-	Ma
Horse	1	2	1	2	-	-	-	-	Ma
Rabbit	-	-	-	-	1	2	-	-	Ma
Chachalaca	1	2	-	-	-	-	-	-	Ma
Total	61	100	46	100	54	100	31	100	

F: frequency; Ed: edible; Pt: pet; Wr: work.

**Table 6.** Food group diversity in home gardens of communities in Mazatán and Tapachula, Chiapas.

Food Group	Communities			
	DV	GUA	ELC	EZV
Oils and fats	2	2	2	3
Low-fat animal-based foods	3	3	3	3
Moderate-fat animal-based foods	3	3	1	2
Fat-free cereals	-	5	5	1
Fruits	23	19	25	19
Legumes	1	1	1	1
Energy-free foods	11	11	11	11
Vegetables	17	14	15	13

The number of family members in the study communities, as well as the level of education, low illiteracy rates, and occupations in families with home gardens in rural areas, align with the data reported by INEGI (2020b) for the state of Chiapas and findings by Leal *et al.* (2015). The occupation of bricklayer for the head of the household is also reported in other studies on home gardens in Mexico (Pulido-Salas *et al.* 2017). The results suggest that the differences in occupations across communities and families may be related to family characteristics and the dynamism of the family and the territory.

Some authors report that women are one of the main family members responsible for carrying out various tasks and managing the home garden, which aligns with the findings of this study (Chablé-Pascual *et al.*, 2015, Chávez-García *et al.*, 2012). Women are in charge of the operational work and creativity within the garden (Arias, 2012). This highlights the importance of women in decision-making regarding the production, management, and conservation of biodiversity in home gardens in rural communities. Regarding the time families dedicate to work in the garden, the results fall within the average time reported for gardens in Chiapas (14 hours/week) by García *et al.* (2009) and those reported by Rosales-Martínez *et al.* (2019) for rural communities in the tropical region of Mexico.

The floristic diversity of the gardens studied (107 species) is higher than that reported by Colín *et al.* (2020) for rural communities in Morelos, but lower compared to the findings of Flores-Guido (2009) for gardens in Yucatán. However, it aligns with reports on gardens in Chiapas (Corvalán *et al.* 2020, Méndez and Valenzuela, 2019). The difference in floristic diversity in gardens may be related to management practices and the edaphoclimatic characteristics of the territory, as noted by García *et al.* (2009). Regarding the use of floristic diversity, the results of this study are similar to those reported for gardens in southeastern Mexico: food, medicinal, and ornamental uses, as well as those reported for gardens in Spain by Rigat *et al.* (2009). Pérez and Uribe (2005) mention that tree and herbaceous species in the garden are used for food purposes. In terms of animal species diversity in the gardens, it is lower than that reported by Castañeda-Guerrero *et al.* (2020) in Puebla, Mexico, where 20 animal species were reported, including 11 domestic species and 9 semi-domestic species. The same author reports that the domestic home garden fauna: *G. gallus*,

*M. gallopavo*, and *B. taurus*, provides meat, eggs, and milk for the food sustenance of rural families, similar to the findings in this study. Additionally, Góngora *et al.* (2016) state that home garden birds in gardens (chickens, roosters, and turkeys) are used as both food and economic resources, species that were also found in the study area.

The number of fruit species present in the gardens is consistent with the findings reported by Montañez-Escalante *et al.* (2020). Additionally, regarding the food groups that stand out in the gardens (vegetables and fruits), these align with those reported by Sol *et al.* (2012). Furthermore, Gerardo *et al.* (2022) report the contribution of food groups such as vegetables and greens, fruits, meats and other animal-derived products, and cereals in gardens in Mazatán, Chiapas, which are also found in this study.

## CONCLUSIONS

The home gardens in the studied communities of Mazatán and Tapachula, Chiapas, Mexico, host a diversity of plant and animal species, providing physical availability of food and food groups, as demonstrated by the results. The food biodiversity present in the gardens corresponded to eight food groups of both plant and animal origin. The gardens are spaces where domestic and wild animal species converge, with women being the main caretakers of these. Therefore, the social, economic, nutritional, and biodiversity conservation role of these gardens in the region is extensive, as they provide fresh, healthy plant and animal food to families, serving as a sustainable alternative in terms of food security.

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# Control of *Hypsipyla grandella* Zeller in a commercial plantation of *Cedrela odorata* L., in Chiapas, Mexico

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## ABSTRACT

**Objective:** This study aimed to evaluate three control methods for *H. grandella* in a 2.1-year-old commercial forest plantation in Frontera Hidalgo, Chiapas, Mexico.

**Design/methodology/approach:** Three treatments were established: T1 chemical control with the application of Cypermethrin<sup>®</sup> at a dose of 10 mL L<sup>-1</sup> of water, T2 organic control with the application of the organic insecticide Vegex Kuneka<sup>®</sup> at a dose of 10 mL L<sup>-1</sup> of water, and T3 silvicultural control with sanitary pruning.

**Results:** The analysis of variance indicated that a statistical difference was found ( $p < 0.01$ ), where T1 and T3 showing the most favorable outcomes across the variables measured, although in general the three treatments presented low values in percentage of damage (22.22%), high health status (77.78%), vigor of 84.44% and a survival rate of 94.44%.

**Limitations on study/implications:** The site's environmental conditions influenced the effectiveness of the applied control methods. Additionally, the biological cycle of *H. grandella* can vary depending on ecological conditions, complicating the establishment of a management protocol.

**Findings/conclusions:** It is concluded that chemical, organic, and silvicultural control methods can reduce borer damage by up to 75%, and the species shows good growth development under these management strategies.

**Keywords:** Borer, Cedar, Silvicultural management, Control methods, Forest pest.

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## INTRODUCTION

Commercial forest plantations are of great importance in Mexico. Red cedar (*Cedrela odorata* L.) is a species from the Meliaceae family, native to tropical America (Pennington & Sarukhán, 2005), and is distributed across Mexico, Central America, the Antilles, and northern South America (Patiño, 1997). It is considered one of the precious wood species in the forest industry (Romo-Lozano *et al.*, 2017). Its high commercial value has led to a decline in natural populations due to intensive logging (Hernández *et al.*, 2018). In Mexico,

it is listed as a protected species under NOM-059-SEMARNAT-2010 (SEMARNAT, 2010) and appears on the Red List of the International Union for Conservation of Nature (Mark & Rivers, 2017).

To promote the recovery and conservation of its natural populations, in recent years *Cedrela odorata* has been included in reforestation programs and commercial forest plantations (CONAFOR, 2020), and more recently in different production systems through the **Sembrando Vida** program (DOF, 2019). To establish plantations with this species, it must be registered as a Wildlife Conservation Management Unit (UMA) subject to intensive management (SEGOB, 2000). However, its growth and development are affected by the presence of *Hypsipyla grandella* (Zeller 1848) (Lepidoptera: Pyralidae), an insect known as the Meliaceae borer, which attacks plants of species such as mahogany (*Swietenia macrophylla* King 1760) and cedar (*Cedrela odorata* L. 1756) (Cibrián *et al.*, 1995; Taveras *et al.*, 2004).

The damage caused by the insect is more severe in young plantations, which hinders the exploitation of their productive potential and creates difficulties in establishing large-scale commercial plantations (Hilje and Cornelius, 2001). The main damage consists of perforations in new shoots, especially the terminal shoot (Taveras *et al.*, 2004). This attack affects apical dominance and results in the branching of the tree (Cibrián *et al.*, 1995), which reduces the commercial value of the wood because it decreases the size of the logs that can be marketed.

Although the pest has not been eradicated, there are various alternatives to reduce its incidence. Some of these are effective (Hilje, 2020; Martínez-Vento *et al.*, 2010; Mero-Jalca *et al.*, 2021), while others are based on silvicultural practices such as mixed plantations, planting densities, and grafting with resistant species, which are more economically and ecologically profitable but have the drawback of being technically less effective. In the state of Chiapas, particularly in the Soconusco region, forest plantations with *C. odorata* have been established. Therefore, the objective of this study was to evaluate three methods for controlling *H. grandella* in a commercial plantation, aiming to explore new alternatives for its management.

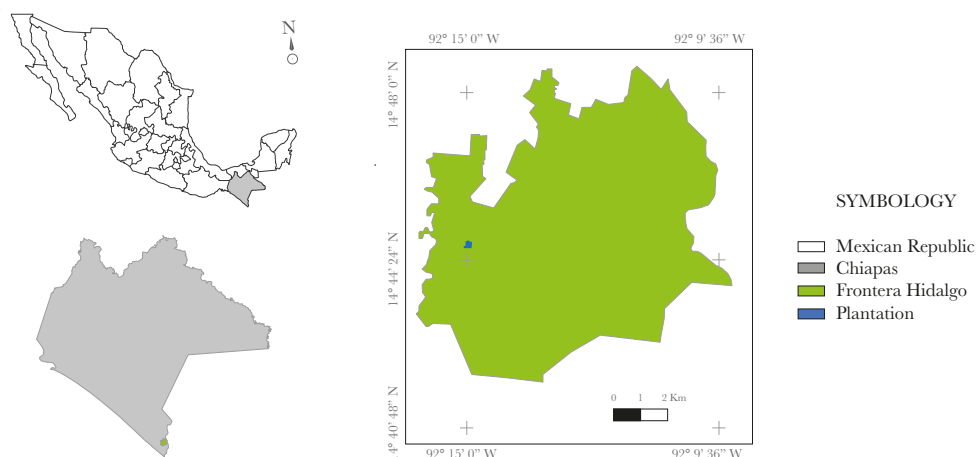
## MATERIALS AND METHODS

### Study Area

The private property El Tesoro covers a total area of 37-75-50 hectares. It is located in the municipality of Frontera Hidalgo, Chiapas, Mexico (92° 14' 52.29" W longitude and 14° 44' 48.43" N latitude), with an altitude of 25 m (Figure 1). The plantation was established in June 2017, covering an area of 7.44 hectares, with a planting density of 722 plants ha<sup>-1</sup>.

### Plantation Management

After establishing the plantation, supplemental irrigation was applied weekly during the dry season. Weed control was carried out mechanically at the beginning and end of the rainy season. A dose of 50 g per plant of the 17N-17P-17K fertilizer formula was applied, and preventive applications against the terminal shoot borer caused by *H. grandella* were



**Figure 1.** Geographic location of the private property El Tesoro of Frontera Hidalgo, Chiapas, Mexico.

made using Arrivo<sup>®</sup> (Cypermethrin) at a dose of 20 mL 20 L<sup>-1</sup> of water, applied every fifteen days during the rainy season. One month before the evaluation, the corresponding actions were carried out for each of the treatments evaluated.

### Treatments and Experimental Design

A randomized complete block design was used, consisting of three treatments (Table 1) and four replications, resulting in a total of 12 experimental units. Each unit was distributed over a 100 m<sup>2</sup> area. Each site included nine trees, covering a total surface area of 1,200 m<sup>2</sup>, with a 12-meter spacing between blocks.

### Measured Variables

Four samplings were conducted, the first in August 2019 and the last in May 2020. In each sampling, the following variables were evaluated: health status, measured as the proportion of healthy trees relative to those damaged by the borer; vigor, assessed as the proportion of vigorous individuals per unit area; and survival, measured as the proportion of living trees relative to the number of trees originally planted. Additionally, the percentage of damage was calculated based on the residual from the health status.

### Statistical Analysis

The percentage-based variables were transformed using the angular (arcsine) transformation. A Shapiro-Wilk normality test was applied to all variables to verify

**Table 1.** Description of the treatments for the control of *H. grandella*.

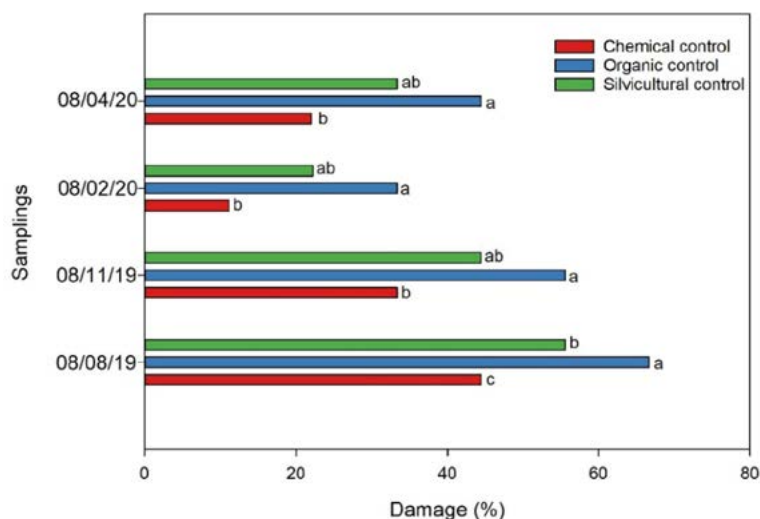
Treatments	Name	Description	Dose
1	Chemical control	Application of Cypermethrin <sup>®</sup>	10 mL·L <sup>-1</sup> of water every 15 days
2	Organic control	Organic insecticide Vegex Kuneka <sup>®</sup>	10 mL·L <sup>-1</sup> of water every 15 days
3	Silvicultural control	Sanitary pruning	Every 30 days

data distribution, and Levene's test was used to assess the homogeneity of variances. Subsequently, an ANOVA was performed, followed by Tukey's mean comparison test ( $P \leq 0.05$ ), using the R Studio statistical software, version 4.4.2 (R Core Team, 2025).

## RESULTS AND DISCUSSION

The data for each variable, according to the Shapiro-Wilk test, showed a normal distribution ( $W=0.94$ ,  $p \geq 0.67$ ). Similarly, Levene's test indicated that the variances among treatments were homogeneous ( $p \geq 0.69$ ). Regarding the percentage of damage to the trees, significant differences ( $P \leq 0.05$ ) were recorded between treatments for each sampling period. According to Tukey's test ( $\alpha=0.05$ ), T1 showed the lowest damage caused by the borer across all sampling periods, followed by T3, while T2 was classified as intermediate between T1 and T3, except during the first sampling (Figure 2). These results are similar to those reported in a study conducted in Argentina, where the incidence of the borer in *Cedrela fissilis* was lower with the application of the insecticide Fendona, compared to a silvicultural treatment (Maiocco *et al.*, 2008). However, Hilje and Cornelius (2001) mention that although pruning is not a preventive method, it is effective in mitigating the effects of *H. grandella* attacks. In the present study, the greatest damage was recorded in T2. Nevertheless, Mero-Jalca *et al.* (2021) reported that the use of a bioinsecticide based on *Azadirachta indica*, *Cinnamomum zeylanicum* Blume, and *Allium sativum* was effective in controlling the borer in a *Swietenia macrophylla* King plantation, and they also observed greater growth in the treated trees.

It is important to note that the highest percentage of damage to the trees occurred during the rainy season (August-October), reaching 44%, 55%, and 66%. During this period, the borer tends to increase in activity. However, in the absence of rain, damage is lower up to 11%, 22%, and 44% for T1, T3, and T2, respectively. These findings are consistent with Santos *et al.* (2015), who reported the greatest presence of the borer in *C. odorata* plants during the rainy season.



**Figure 2.** Percentage of damage caused by *H. grandella* on *C. odorata* trees. Different letters among treatments within each sampling period indicate statistically significant differences (Tukey,  $P \leq 0.05$ ).

The analysis of variance indicated statistically significant differences ( $p < 0.01$ ) among the treatments for each of the evaluated variables, except for vigor and survival in the last sampling (Table 2). Plant health showed an increasing trend, as the incidence of borer attacks decreased with the application of treatments, leading to a higher percentage of healthy trees. Treatments T1 and T3 recorded the highest percentages of healthy trees compared to the other treatments. Throughout the evaluation period, trees exhibited good vigor in response to the treatments, maintaining high percentages since the first sampling date. Finally, regarding survival, all treatments showed rates above 90% across the four sampling events. T1 and T3 maintained high survival percentages from the first sampling, whereas the other plots showed some variation, which was due to the replacement of dead plants in order to maintain the initial planting density of 722 trees  $ha^{-1}$ .

In general terms, the sampling sites showed a health status of 66.67%, vigor of 84.44%, and survival of 94.44%. These results are favorable when compared to those reported by Muñoz *et al.* (2011) at the El Belem property in the municipality of Múgica, Michoacán, who recorded a survival of 82.7%, vigor of 84.36%, and health status of 80.5% at 2.5 years of age. Similarly, Ramírez-García *et al.* (2008) reported a survival of 93%, vigor of 84.36%, and health status of 90.3% at 3.0 years in the Doroteo Arango ejido, municipality of González, Tamaulipas. According to Maiocco *et al.* (2008), in a *C. fissilis* plantation affected by borer incidence, survival was 74% in treated individuals, indicating the effectiveness of chemical and silvicultural treatments.

Although the borer *H. grandella* does not cause the death of the plant, it generates significant damage that leads to deformation of lateral branches, causing the tree to develop forks and lose its commercial value (Gómez *et al.*, 2017; Mero-Jalca *et al.*, 2021). Briseño (1997) states that the most susceptible phase to borer attack is between three and six years of age. If the trees are not attacked during this period, it is possible that the pest will no longer pose a problem for the plantation. Therefore, the implementation of mainly chemical and silvicultural treatments is important to mitigate borer damage (Ruíz *et al.*, 2016), as well as to guarantee the quality of the stem structure through nutritional

**Table 2.** Results of plant health status, vigor, and survival.

Variable	Treatment	Samplings			
		08/08/19	08/11/19	08/02/20	08/04/20
Health status (%)	1	55.56±1.43 a	66.67±1.57 a	88.89±0.91 a	77.78±1.90 a
	2	33.33±1.40 c	44.44±1.06 b	66.67±0.56 b	55.56±1.67 b
	3	44.44±1.57 b	55.56±0.56 ab	77.78±0.56 ab	66.67±0.64 ab
Vigor (%)	1	78.89±4.54 b	78.89±7.86 b	81.67±5.32 ab	84.44±5.56 a
	2	78.89±4.54 b	84.44±3.21 ab	81.67±2.78 ab	84.44±3.21 a
	3	87.22±2.78 a	87.22±2.78 a	87.22±2.78 a	84.44±3.21 a
Survival (%)	1	97.22±2.78 a	94.44±2.78 a	97.22±2.78 a	94.44±3.21 a
	2	88.89±4.54 b	91.67±5.32 b	91.67±5.32 b	94.44±5.56 a
	3	91.67±2.78 ab	94.44±3.21 a	94.44±3.21 ab	94.44±3.21 a

Letters that are not the same between columns indicate statistical difference (Tukey  $P \leq 0.05$ ).

management (Calixto *et al.*, 2015), maximize economic yield, and ensure the ecological sustainability of commercial plantations.

## CONCLUSIONS

Chemical and silvicultural control methods are the most effective for reducing the damage caused by the borer (*H. grandella*) in *Cedrela odorata*. Although organic control can achieve good results in infested trees by reducing damage. Despite the fact that the borer does not cause tree mortality, the application of these treatments is highly relevant because it ensures better stem straightness and, consequently, the wood attains a higher economic value.

## ACKNOWLEDGMENTS

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# Germination and morphology of *Sapindus saponaria* L. (Sapindaceae) seeds for their sustainable use

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## ABSTRACT

**Objective:** To characterize morphological traits and to evaluate different pre-germinative treatments to break the physical dormancy of *Sapindus saponaria* L. seeds.

**Design/methodology/approach:** Seeds of *S. saponaria* were collected in the municipality of Cintalapa de Figueroa, Chiapas. Seed length, seed width and seed coat width were measured. The weight of 100 seeds, germination percentage, and purity were assessed. The following pre-germinative treatments were established: (T1) Hot water (80 °C) until room temperature; (T2) Hot water (80 °C) until room temperature followed by cold water shock (14 °C) until room temperature; (T3) Mechanical scarification; (T4) Mechanical scarification plus soaking in water for 24 hours (at room temperature); and (T5) Control. A completely randomized experimental design with four replications was used.

**Results:** This study found that *S. saponaria* seeds average 10.3 mm in length, 10.2 mm in width, and have a seed coat thickness of 1.2 mm. Mechanical scarification yielded the highest germination percentage (23.3%). This finding is significant given that the untreated germination percentage in this species is moderate to low. However, this result is lower than percentages documented in studies using chemical treatments.

**Limitations on study/implications:** Despite the low germination percentage, the advantages of the treatments implemented in this study is that they are cost-effective.

**Findings/conclusions:** It is necessary to expand assessments of pre-germinative treatments, both locally and regionally, to develop effective strategies for the sustainable management of *Sapindus saponaria* while harnessing its economic and medicinal potential.

**Keywords:** Conservation, dormancy, forest germplasm, impermeable seed coat, pre-germinative treatments.

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## INTRODUCTION

*Sapindus saponaria* L., commonly known as “jaboncillo” or “soapberry,” is a tree species of great economic, ecological, and cultural importance in various regions of Latin America. It is distributed in Florida, the Greater Antilles, and from northern Mexico to southern Argentina. In Mexico, it occurs in tropical deciduous and sub-deciduous areas, in cloud forests, and occasionally in oak forests. The tannins and saponins derived from



*S. saponaria* are used as bioinsecticides, which exhibit low toxicity, degrade rapidly, and do not induce resistance (Souza *et al.*, 2023). Ecologically, this species has been used in landscaping projects and in the restoration of degraded areas (Albiero *et al.*, 2001; Sánchez and Silva, 2008). Additionally, it also has multiple traditional uses and potential industrial applications (Jozivan *et al.*, 2008; Rodríguez-Hernández *et al.*, 2016).

Studies at seed level are necessary to support the conservation of the species and its propagation, either inside or beyond its natural habitat (Lohbeck *et al.*, 2015). Seeds present several characteristics that strongly influence dispersal patterns, colonization, seedling establishment, and plant survival (Dalling, 2002). Traits such as size, shape, color, and texture can significantly influence processes like germination and seedling establishment (Luck *et al.*, 2012; Romero-Saritama and Castillo, 2021). Variation in these characteristics is commonly observed in many tropical tree species, both between and within populations; however, seed traits can also vary within a single genotype (Cohen *et al.*, 1991; Kröber *et al.*, 2012; Duncan *et al.*, 2019; Romero-Saritama and Granda, 2020; Basave-Villalobos *et al.*, 2024). Such variability, results from the combined influence of environmental and genetic factors during seed development (Upreti *et al.*, 2024). Therefore, it is essential to examine how seed traits vary in each seed source and how these variations affect germination, since the condition of the seeds themselves is one of the most critical factors governing the germination process (Shen and Cho, 2020).

Recognizing the factors that affect seed germination is particularly important for species with dormancy, such as *S. saponaria*, because the seed's morphological and anatomical characteristics regulate the development and expression of mechanisms that inhibit germination (Upreti *et al.*, 2024). *Sapindus saponaria* seeds exhibit physical dormancy, which hinders germination (Nascimento *et al.* 2009; Cabral *et al.*, 2019). In this type of dormancy, the tissues surrounding the embryo (seed coats) can interfere with water absorption or gas exchange, preventing the release of inhibitors from the embryo, or act as a mechanical barrier to radicle emergence (Bewley *et al.*, 2013). To overcome this dormancy and promote germination, scarification treatments, usually referred as “pre-germinative” treatments, are required to soften, remove, break or abrade the seed coats by applying methods of mechanical or chemical scarification (Baskin and Baskin, 2014). However, the effectiveness of each treatment depends on the specific characteristics of the seeds (above mentioned), making it essential to carefully apply and evaluate these methods, as proposed by Ramírez-Herrera *et al.* (2008).

In summary, understanding and assessing the morphological traits of seeds and germination protocols is essential for sustainable *ex situ* propagation efforts and for developing specific strategies in the management of native tropical forest species, while also preserving their genetic diversity—an important factor in conserving local populations (Jozivan *et al.*, 2008; Rodríguez-Hernández *et al.*, 2016). The aim of the present research was to describe the morphological traits and to test pre-germinative treatments used to break seed dormancy in *Sapindus saponaria*. Additionally, this study seeks to examine how knowledge of seed morphological traits can serve as a valuable tool to optimize the *ex-situ* propagation process for this tropical species and thereby contribute to its sustainable use.

## MATERIALS AND METHODS

### Collection site and plant material

Seeds were collected in the municipality of Cintalapa de Figueroa, Chiapas, Mexico (421538.60 UTM X, 1847315.60 UTM Y), at an elevation of 534 m. The seed lot evaluated was obtained directly from the tree canopy in June 2023, when seeds were observed on the ground beneath the dripline area. After collection, laboratory tests were carried out using randomly selected samples in each case. A backup herbarium specimen (ADL01) was collected, pressed, and deposited at the Herbario ECOSUR Tapachula (ECO-TA-H).

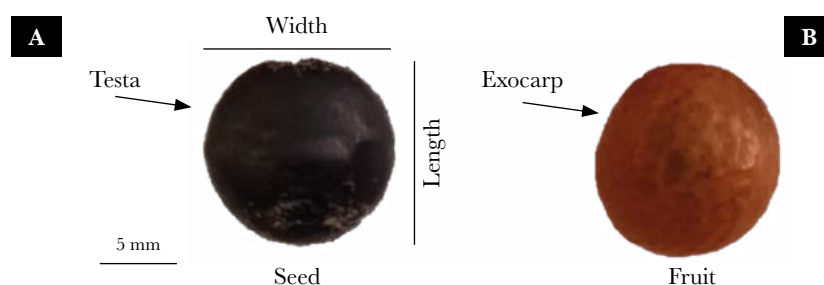
### Seed morphological characterization and yield variables

Twenty-five seeds in four replicates were used to measure seed coat thickness (mm), seed length (mm), and seed width (mm) with a digital caliper (Figure 1). Following the rules of the International Seed Testing Association (ISTA, 2010), a homogeneous sample size was used. One hundred seeds were weighed on a digital scale (0.1 g precision) in four replicates. The following weights were recorded: (a) weight of 100 uncleaned fruits, (b) weight of 100 cleaned fruits, and (c) weight of 100 clean seeds without exocarp. Inert material was then removed from the seeds with forceps to obtain pure seeds. The percentage of pure seeds was calculated with the following formula:

$$\%Purity = (Weight\ of\ pure\ seeds / Weight\ of\ the\ sample) \times 100$$

### Germination

After collection, seeds were disinfected with 2% sodium hypochlorite for five minutes and then rinsed with tap water. Germination percentage was evaluated under five different pre-germinative conditions: (T1) seeds were immersed in hot water at 80 °C until they reached room temperature; (T2) the same process was followed, but a subsequent cold-water shock at 14 °C was applied until room temperature; (T3) the seeds were cracked (mechanical scarification); (T4) seeds were also cracked and then soaked in water for 24 hours at room temperature. (T5) control, in which no treatment was applied. The germination test was carried out on paper according to Agustín-Sandoval *et al.* (2017), with four replicates of 30 seeds each. Seeds were placed on paper towels, labeled, and then kept in plastic bags at room temperature, ensuring the paper remained adequately moistened. Evaluations were made three times per week until germination.



**Figure 1.** Representation of three measured traits to determine the size of *Sapindus saponaria* L. seeds. A) Seed without protective coating: length, width, and thickness of the seeds; and B) Fruit before removing exocarp.

### Statistical analysis

Germination percentage data (p) were transformed using the arcsine square-root function to approximate a normal distribution (Sokal and Rohlf, 1981). A completely randomized design with four replicates per treatment was used. Tests of normality (Shapiro-Wilk) and homogeneity of variances (Bartlett) were applied, followed by analysis of variance (ANOVA) and Tukey's multiple comparison tests at a significance level of  $p < 0.05$ . Data were analyzed using R statistical software for Windows v3.6.3 (R Core Team, 2020).

## RESULTS AND DISCUSSION

### Study species

Trees from 3 up to 25 m tall; to 18 cm diameter to breast height, bark gray. Leaves pinnate from 10 up to 57 cm. Leaflets 5 to 10, lanceolate to oblong, unequal; petioles winged or wingless. Inflorescence a terminal panicle, from 15 to 25 cm long, the branches and pedicels densely puberulent to tomentose. Flowers white; sepals elliptic, puberulent; petals ciliolate, glabrous; disc fleshy spreading; stamens exerted, filaments filiform; ovary ovate, 3-lobed, glabrous. Indehiscent fruits of 1 to 3 cocci; cocci brown to yellow, globose, ca. 1.5 cm of diameter, shiny, often lenticellate at maturity, the surface smooth; seeds globose, 1.2 cm of diameter, set at the base of the carpel in a cottony mat pubescence. It grows from 0 to 2000 m.a.s.l., flowering and fruiting all year round (WFO, 2025).

### Morphology and yield

According to the measurements, the seeds are as long as they are wide, and the seed coat is relatively thick (Table 1). These findings are consistent with those reported by das Neves *et al.* (2018), who also found similar values for length and width. Bonilla *et al.* (2007) reported an average seed length of 10 mm and a width of 8 mm, the latter being lower than the value obtained in the present study.

The average weight of 100 seeds was  $144 \pm 0.96$  grams for uncleaned fruits;  $140 \pm 1.29$  grams for cleaned fruits; and  $76 \pm 1.26$  grams for cleaned seeds without exocarp (Table 1; Figure 1). The weight of 100 pure seeds (cleaned and without exocarp) is slightly higher in this study than that reported by das Neves *et al.* (2012) (67.2 g/100); these authors mention that variation in seed weight may be because environmental changes during seed development. It was found that *S. saponaria* seeds exhibit a purity level of 97.2%,

**Table 1.** Average weight and size of morphological and yield-related variables of *Sapindus saponaria* L. seeds.

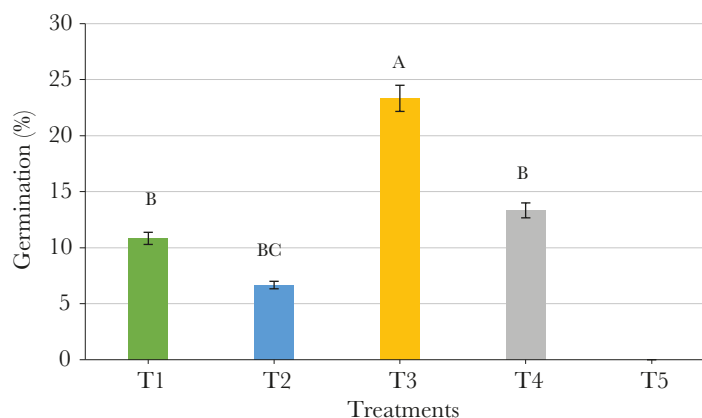
Variables	Mean $\pm$ SD
Seed coat thickness (mm)	1.2 $\pm$ 0.10
Seed length (mm)	10.3 $\pm$ 0.08
Seed width (mm)	10.2 $\pm$ 0.06
Weight of 100 uncleaned fruits (g)	144 $\pm$ 0.96
Weight of 100 cleaned fruits (g)	140 $\pm$ 1.29
Weight of 100 cleaned seeds without exocarp (g)	76 $\pm$ 1.26

SD=Standard error.

with the remaining 2.8% consisting of residual fruit material. Although *S. saponaria* seeds are relatively easy to clean, the fruit's exocarp has an oily and flexible consistency, which hinders its removal (Figure 1).

### Germination

The experiment revealed that *S. saponaria* seeds began to germinate 13 days after the start of the trial. Germination ceased after seven weeks. Hernández-Jaramillo *et al.* (2012) reported a similar germination onset of approximately 12 days, whereas Bonilla *et al.* (2007) documented a broader range, from as early as 5 days to up to 2 months. The results of this study show significant differences in seed germination among the pre-germinative treatments ( $p < 0.001$ ) (Figure 2). Manual scarification by cracking the seed coat (T3) proved to be the most effective treatment for enhancing germination (23.3%), followed by T4, which consisted of cracking followed by a 24-hour water soak (13.3%). These findings are consistent with those of Cuitláhuac (2017), who reported a 25% emergence rate after soaking seeds in water for 10 minutes. In contrast, Lima-Diniz *et al.* (2018) found a 74% germination rate using manual scarification with sandpaper (No. 100), while Oliveira *et al.* (2012) reported a 65% emergence rate using sulfuric acid as a pre-germinative treatment. The control treatment (T5) showed a 0.0% germination rate. This may be due to the presence of a narrow micropyle and micropyle canal in the seeds, which potentially hinders the imbibition of internal tissues or storage reserves (Bonilla *et al.*, 2007). Some studies have shown that sulfuric acid treatments can enhance germination rates in seeds with impermeable coats, although they may also cause damage (Santarém and Áquila, 1995; Albiero *et al.*, 2001; Mendes, 2012; Oliveira *et al.*, 2012). Nascimento *et al.* (2009) and Cabral *et al.* (2019) reported that *S. saponaria* seeds possess a very thick and sclerenchymatous seed coat, and that prolonged exposure to sulfuric acid (60 to 90 minutes) may increase germination percentage, although it may also lead to seed damage.



**Figure 2.** Germination percentage of *Sapindus saponaria* L. seeds under the established treatments: (T1) Hot water until room temperature (80 °C); (T2) Hot water until room temperature (80 °C) followed by thermal shock with cold water (14 °C) until room temperature; (T3) Mechanical cracking; (T4) Mechanical cracking followed by soaking in water for 24 h (room temperature); (T5) Control. Different uppercase letters indicate significant differences ( $P < 0.05$ ) among treatments.

## CONCLUSIONS

This study found that *S. saponaria* seeds are spherical and possess a thick seed coat. Mechanical scarification through seed cracking was observed to be effective in overcoming seed coat hardness. In spite that this treatment yielded a germination rate of less than 50%, it is an inexpensive and simple technique to accelerate germination and can be implemented by any person who wishes to reproduce the seeds, only caution is needed when performing cracks to do not damage the embryo. Understanding the dynamics of natural plant community regeneration largely depends on knowledge of seed germination processes and the application of pre-germinative treatments to break seed dormancy. Nevertheless, in the southern region of Chiapas State, as well as in many other tropical areas, little is still known about the biology of numerous native species, including *Sapindus saponaria*. This knowledge gap is closely linked to the limited availability of species proposed for ecological restoration programs in disturbed areas, particularly those with economic potential. Furthermore, collecting significant quantities of seeds should be conducted under protocols aimed at minimizing the impact on the species' natural progeny, a process that requires time and careful implementation. Therefore, it is crucial to continue investigating and deepening our understanding on the reproduction of *S. saponaria* and other native tropical species. This will aid in developing effective strategies for the conservation and sustainable management of these species both in their natural habitats and in ex situ conservation settings, while also harnessing their economic and medicinal potential.

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# Mexican Bacterial Biofertilizer Program of INIFAP

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## ABSTRACT

In 1998, the considerable reduction in agricultural profitability, as a consequence of the rise in prices of agricultural inputs —particularly chemical fertilizers and pesticides—, prompted the Mexican government to seek production alternatives to reduce the use of agrochemicals in the country. Given the importance of food self-sufficiency and the profitability of national agricultural activity, the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP) of Mexico launched the ‘Programa de Investigación sobre Biofertilizantes’. Since 1999, the initiative has had the uninterrupted support of the Mexican Secretariat of Agriculture and Rural Development (SAGARPA) to generate national technologies for the development and management of biofertilizers and to promote their use among agricultural producers. As a starting point, INIFAP sought to consolidate alliances with some of the Mexican leading research institutions, in order to promote the use of biofertilizers as an alternative to chemical fertilizers. During this first phase, microorganisms —such as *Glomus intraradices*, *Bradyrhizobium japonicum*, *Azospirillum brasilense*, and *Rhizobium etli*— were used to reduce the doses of chemical fertilizers in some of the main domestic staple crops. Given the success of this first phase, INIFAP established mycorrhizal production centers in different regions of the country and then a team of INIFAP scientists from various fields of science was integrated. Initially, this team focused on plant growth-promoting bacteria, as well as on the development and formulation of new and more efficient bacterial biofertilizers, using local strains from the main agro-ecological zones of Mexico. Later on, they focused on technology transfer and the training of Mexican producers to apply and use the products. This review describes some of the main achievements accomplished by the INIFAP Biofertilizer team, particularly in the area of bacterial bioproducts.

**Keywords:** biofertilizer, bioinoculant, *Azospirillum*, *Pseudomonas*, *Bacillus*.

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## INTRODUCTION

The use of chemical fertilizers decreased during the early 1990s. However, a growing trend for their reincorporation in agriculture —to the levels recorded during the 1980s (Ávila, 2001)— led the Mexican government to seek for alternatives that increase profitability,



while reducing the use of chemical fertilizers. For this purpose, three technologies were considered: a) The utilization of organic fertilizers—including manure, compost, compost tea, bioles (fermented liquid biofertilizers), and vermicompost— b) The determination of the optimal fertilization doses based on soil analysis, and c) The use of microorganisms capable of promoting plant growth and reducing the use of synthetic fertilizers without impacting crop productivity. After INIFAP successfully validated in 1999 the use of microorganisms such as *Glomus intraradices*, *Bradyrhizobium japonicum*, *Azospirillum brasilense*, and *Rhizobium etli* as a viable alternative to reduce the use of chemical fertilizers, various mycorrhiza production centers were established to meet the growing bioinputs demand of Mexican producers.

In 2007, Dr. Gerardo Armando Aguado Santacruz—researcher at the Bajío Experimental Field in Celaya, Guanajuato— took over the leadership of the Programa Nacional de Investigación de Biofertilizantes of INIFAP. At the beginning, the biofertilizer research team proposed the isolation of plant-growth-promoting bacteria that could complement the beneficial effects of the mycorrhiza produced by INIFAP. The potential of these bacteria, known as plant-growth-promoting rhizobacteria (PGPR), for reducing the chemical fertilization doses applied to crops has been scientifically proven during the last 20 years. Besides, In addition, the society is now aware of the importance of consuming healthier food while minimizing the environmental impact caused by the use of agrochemical products.

Biofertilizers—also known as bioinoculants, microbial inoculants, or soil inoculants—are agro-biotechnological products containing active/dormant microorganisms (bacteria or fungi, alone or in combination) that are added to crops for stimulating their growth and productivity (Aguado-Santacruz *et al.*, 2012). Although the term “biofertilizer” was initially used to facilitate the registration of strains for commercial purposes, some authors point out that the term is not completely correct, because only a few microorganisms can strictly fulfill the specific function of incorporating new nutrients into ecosystems—mainly nitrogen-fixing microorganisms (Bashan, 1998). In recent decades, several studies have focused on the role of growth-promoting bacteria in plants, proving their beneficial effects on crop productivity and health. Rhizosphere bacteria that aggressively colonize plant roots are known as rhizobacteria, while, bacteria that can also stimulate plant growth are commonly known as plant-growth-promoting rhizobacteria (PGPR). This growth-promoting activity influences various agronomic variables, including germination increase, emergence increase, seedling establishment or vigor, root system proliferation, or plant biomass or final crop yield increase.

Although this concept of PGPR emphasizes the effect of bacteria on plant growth, no less important are the activities that these microorganisms exert on the development of plants, advancing flowering times in ornamental plants or improving the quality of the fruits in terms of their size or organoleptic properties. Some researchers mention, for example, that it is possible to improve the sweetness degree (Brix degrees) of some fruits such as melon, using phosphate-solubilizing bacteria (Chien *et al.*, 2009). On the other hand, the use of biofertilizers has proven that improving maize performance is indeed possible (Fallik and Okon, 1996; Purcino *et al.*, 1996), from the reduction of germination time and

the increase of germination percentages and seedling establishment rates to high final crop yield increases. In addition, it has been stated that 13 to 20% of maize nitrogen content can be attributed to the nitrogen-fixing activities of bacteria such as *Azotobacter* (Soliman and Abdel Monem, 1994). Covarrubias-Ramírez *et al.* (2005) evaluated the kinetics and efficiency of P uptake in potato plants (*Solanum tuberosum* L.) cv. Alfa, using the  $^{32}\text{P}$  isotope technique. These researchers reported that inoculation with *Bacillus subtilis* increased potato biomass by 31.7%, and P uptake by 27.5%. They also mention that inoculation of chickpea plants with *Mesorhizobium cicerii* resulted in different beneficial effects on the growth and development of this crop, which allowed a 70% reduction of the recommended dose of nitrogen (urea) without affecting grain yield ( $2.05 \text{ ton ha}^{-1}$ ) compared to control plants fertilized with  $100 \text{ kg N ha}^{-1}$  that produced  $1.98 \text{ ton ha}^{-1}$ . According to Biswas *et al.* (2000), nitrogen-fixing microorganisms can promote plant growth by transferring fixed nitrogen to plants or by improving nutrient uptake through modulation of the hormonal activity. In this sense, Bashan (1999) pointed out that inoculation with plant-growth-promoting microorganisms (*e.g.*, *Azospirillum* sp.) results in a greater accumulation of nitrogen compounds, through the promotion of more effective nutrient uptake without apparent nitrogen fixation. In contrast, Zakry *et al.* (2012) used the  $^{15}\text{N}$  isotope technique and found that up to 89% of the N requirement of oil palm seedlings was supplied by its symbiosis with *Azospirillum*. Likewise, Khurram *et al.* (2012) evaluated the effect of ACC deaminase activity or phosphate solubilizing capacity of *Bacillus* strains, either as a single or dual growth promotion mechanism (strains possessing both capacities). Under axenic conditions, bacterial strains with dual growth-promoting traits had a higher capacity to stimulate wheat growth than strains with a single trait. Similarly, these dual-trait bacterial strains were more effective than single-trait strains under soil conditions (pot experiments) for increasing root weight (up to 3.9-fold) and elongation (up to 3.8-fold), shoot dry weight (up to 37.6%), number of shoots (up to 56%), grain yield (up to 38.5%), and grain P uptake (up to 77.4%) of wheat grown with P applied as diammonium phosphate, rock phosphate, or rock phosphate-enriched compost. An almost similar trend was recorded when the same experiment was repeated under field conditions. The inoculation with rock phosphate-enriched compost recorded growth parameter almost comparable with those obtained by diammonium phosphate. In conclusion, the simultaneous presence of two different growth-promoting bacteria capacities could have an additive effect, not only on wheat growth and yield, but also on P uptake.

Furthermore, several studies report improved nutrient uptake when chemical fertilization is paired with beneficial microorganisms. For example, Sundara *et al.* (2002) found that applying the *Bacillus megatherium* var. *phosphaticum* phosphorus-solubilizing bacterium (PSB) increased P availability in the soil, improving sugarcane growth, yield, and quality. This PSB also reduces the P dose requirement by 25%. In addition, studies reported that, in combination with phosphate rock, *B. megatherium* can save up to 50% of production costs by replacing superphosphate. Young *et al.* (2004) compared the effects on growth of a full chemical fertilization dose vs the effects of a multi-functional biofertilizer treatment (a combination of *Bacillus* sp., *B. subtilis*, *B. erythropolis*, *B. pumilus*, and *P. rubiacearum*) and 50% of the recommended chemical fertilization dose applied on

cabbage and water celery. Compared with the complete chemical fertilization treatment, the biological-chemical treatment recorded a 25% and a 34% increase in cabbage yield and water celery dry matter, respectively, which indicates, that it is possible to save at least 50% of the complete chemical fertilization by adopting a complementary approach that combines multifunctional biofertilizers and lower doses of chemical fertilizers.

Benítez-Noyola (2013) proved that maize plants fertilized with 90 and 180 kg N ha<sup>-1</sup> and inoculated with *Paenibacillus polymyxa* extracted 20 to 28% more nitrogen from the soil and produced more grain than plants that were only chemically fertilized. For their part, Das *et al.* (2004) reported a greater nitrogen (N<sub>2</sub>) accumulation in cotton, with the combination of a chemical fertilization with *Azotobacter* M4. Likewise, Naveed *et al.* (2008) proved that the grain maize yield can be maintained replacing 87 kg urea ha<sup>-1</sup>—50% of the full dose of N fertilizer (175 kg ha<sup>-1</sup>)— with 300 kg ha<sup>-1</sup> of an organic compost made from waste fruit and vegetables and enriched with 147 g N fertilizer kg<sup>-1</sup> of compost; a basal P and K dose (100 and 50 kg ha<sup>-1</sup>, respectively) was previously applied to all the plots. However, when the N-enriched compost was inoculated with different *Pseudomonas* strains and then applied to the plots along with 88 kg urea ha<sup>-1</sup>, a significant maize growth and yield increase (1.1 ton grain ha<sup>-1</sup>) was recorded over the full N fertilizer dose, being superior to the increase obtained with the the organic fertilizer (0.5 ton grain ha<sup>-1</sup>). According to these authors, *Pseudomonas fluorescens* strain N3 was particularly effective in promoting growth due to its high root colonization capacity, chitinase activity, and ACC deaminase activity. These characteristics give this strain a greater competitive advantage.

The activity of rhizobacteria with ACC deaminase enhances root growth, as a result of an ethylene synthesis reduction, through hydrolysis of ACC into NH<sub>3</sub> and  $\alpha$ -ketobutyrate in the inoculated roots (Shaharoon *et al.*, 2007). These results imply that the organic fertilizer inoculation with ACC deaminase-PGPR could help to develop improved bioproducts that combine the nutritional characteristics of compost and the beneficial activities of rhizobacteria, obtaining synergistic effects on both crop growth and productivity.

Abdullahi *et al.* (2013) studied the effect of using a biofertilizer (containing *Azospirillum* sp. and *Glomus mosseae*) combined with poultry manure on the nutrient uptake and growth of plants and microbial population associated to the sesame cultivation under field conditions. Plant height, number of leaves per plant, number of branches per plant, leaf area, and shoot and root dry biomass were higher in the plants treated with microorganisms and/or poultry manure (individually or combined) than in control plants. The combined application of the biofertilizer and poultry manure (bioorganic treatment) at a rate of 5 ton ha<sup>-1</sup> produced plants that achieved the best growth parameters, nutrient content, and N, P, and K uptake. In addition, the largest populations of *Azospirillum* sp. ( $28.6 \times 10^{-6}$  CFU g<sup>-1</sup> soil) and arbuscular fungi (69.3 spores g<sup>-1</sup> soil) were recorded in the bioorganic treatment. According to the authors, the positive growth responses of the inoculated plants could be attributed to increased nutrient supply—especially nitrogen— and growth-promoting hormones provided by *Azospirillum* sp. and to the greater absorption of phosphorus and other nutrients resulting from the mycorrhiza colonization (Zaidi *et al.*, 2004). The greater availability of nutrients reported in that study was either the result of the decomposition of organic manure or the transformation of inorganic substance into available forms produced

by microorganisms. The *Azospirillum* sp. population increase—as well as the increase of the spore density and colonization of *G. mosseae*— could be associated with the application of manure, which is a carbon source for microbes. Table 1 shows direct or indirect promotion of plant growth caused by PGPR (Glick, 1995; Persello-Cartieaux *et al.*, 2003).

Plant growth-promoting bacteria can directly impact plant growth through several mechanisms, including: atmospheric nitrogen fixation, mineral solubilization (*e.g.*, phosphorus and iron), siderophore production (solubilization and iron sequestration), and production of growth regulators (hormones) that improve plant growth during different development stages. The production of growth-promoting substances (auxins, gibberellins, and cytokinins) has been one of the main direct mechanisms used by researchers to explain growth stimulation caused by biofertilizers (García de Salamone *et al.*, 2005). The production of indoleacetic acid (IAA) is one of the most widespread growth-promoting mechanisms in bacteria, particularly in Gram-negative bacteria (Steenhoudt and Vander-Leyden, 2000). The amino acid tryptophan, precursor of this hormone, is one of the most abundant compounds in root exudates (Kamilova *et al.*, 2006). Meanwhile, the IAA hormone can be found in up to 80% of bacteria isolated from the rhizosphere of some plants (Loper and Schroth, 1986). The reduction of ethylene levels in plants is another growth-promoting mechanism linked to hormonal metabolism (Li *et al.*, 2009). Ethylene is a plant hormone that can inhibit root development, limiting the capacity of the plant to absorb nutrients and water. In higher plants, the S-adenosyl-L-methionine (SAM) synthase enzyme catalyzes the conversion of methionine to SAM (Giovanelli *et al.*, 1980). In response to damages, floods, droughts, salinity, and herbicides, the enzyme ACC synthase catalyzes the conversion of SAM into 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene. Subsequently, the ACC oxidase enzyme catalyzes the conversion of ACC to ethylene, carbon dioxide, and hydrogen cyanide (John, 1991). Increased ethylene levels in the root delays its growth. Some microorganisms, such as species of genera *Pseudomonas* and *Bacillus*, contain an enzyme called ACC deaminase that hydrolyzes ACC to form ammonia and  $\alpha$ -ketobutyrate (Glick *et al.*, 2007), preventing the formation of ethylene. Consequently, when ACC deaminase activity increases, ethylene levels in the plant decrease, and root development clearly increases (Saleem *et al.*, 2007).

Another direct mechanism for growth stimulation is the enzymatic reduction of atmospheric nitrogen ( $N_2$ ) to ammonium ( $NH_4$ ), commonly referred to as biological nitrogen fixation (BNF). In some plants, this reductive process takes place in specialized structures (such as the root nodules of legumes) and is catalyzed by the nitrogenase enzyme

**Table 1.** PGPR Plant-Growth-Promoting Mechanisms.

Direct effects	Indirect effects
Atmospheric nitrogen fixation	Antibiosis
Hormone production	Induction of systemic resistance
Increased Fe availability	Reduction in environmental Fe availability
P solubilization	Competition for ecological niche and nutrients
Reduction of ethylene levels	Parasitism and predation

complex, which consists of two distinct proteins: dinitrogenase (iron-molybdenum protein) and dinitrogenase reductase (iron protein) (Bulen and LeComte, 1966).

Vitamin synthesis is another direct mechanism that has been proposed by researchers, because the production of certain vitamins significantly contributes to the growth-promoting activity of certain microorganisms. For instance, *Pseudomonas fluorescens* strain 267 produces water-soluble B vitamins that stimulate the growth of red clover (*Trifolium pratense*) (Marek-Kozaczuk and Skorupska, 2001). Similarly, some *Azotobacter* and *Azospirillum* strains produce B vitamins that promote rooting capacity and impact microbial populations (Revillas *et al.*, 2000). On the other hand, the production of siderophores, also considered a direct mechanism, involves the synthesis of molecules with a high affinity for iron by various microorganisms and grasses (Neilands, 1995) to increase the bioavailability of this element. Some siderophores produced by different bacteria and fungi include ferrichrome (*Ustilago sphaerogena*), mycobactin (*Mycobacterium* sp.), enterobactin and bacillibactin (*Bacillus subtilis*), ferrioxamine B (*Streptomyces pilosus*), azotobactin (*Azotobacter vinelandii*), pseudobactin (*Pseudomonas* B10), and ornibactin (*Burkholderia cepacia*). *P. fluorescens* produces pyoverdine, a peptide siderophore with high affinity for iron (Madigan and Martinko, 2005).

Phosphate solubilization is another direct mechanism carried out by phosphate-solubilizing bacteria (PSB) which can increase the availability of phosphate for plants. PSB are a beneficial group of bacteria capable of hydrolyzing both organic and inorganic phosphorus from insoluble compounds (Goldstein *et al.*, 2003). These bacteria secrete organic acids and phosphatases to convert insoluble phosphates into soluble monobasic ( $\text{H}_2\text{PO}_4^-$ ) and dibasic ( $\text{HPO}_4^-$ ) ions, through a process known as rock phosphate solubilization. PSB can solubilize compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate through the production of gluconic and 2-ketogluconic acid, which are well-known phosphate solubilizers. Soil phosphate solubilization increases phosphorus availability and, consequently, phosphorous uptake by plants (Gyaneshwar *et al.*, 2002). Bacteria genera capable of solubilizing phosphates include: *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Mesorhizobium*, *Micrococcus*, *Pantoea*, *Pseudomonas*, *Rahnella*, *Rhizobium*, *Streptosporangium*, and *Yarrowia* (Paredes-Mendoza and Espinosa-Victoria, 2010).

Indirect growth promotion takes place occurs when PGPRs stimulate growth by improving growth-limiting conditions for plants (Glick *et al.*, 1999). For instance, when bacteria suppress diseases caused by pathogens (Smith *et al.*, 1999), either by producing antagonistic substances (mainly antibiotics), by inducing systemic resistance against pathogens (Glick, 1995) or by competing against pathogens for colonization of plant physical spaces (Dekkers *et al.*, 1998). Similarly, siderophore-producing bacteria use iron for themselves, reducing its availability for other competing microorganisms in the rhizosphere, which limits the growth of their competitors.

Regarding indirect mechanisms, research about the benefits of microbial inoculants extends beyond their capacity to improve plant nutrition because microbial inoculants can also induce systemic acquired resistance (SAR) against various phytopathogenic agents, such as *Blumeria graminis*, *Gaeumannomyces graminis*, *Pseudomonas syringae*, and *Fusarium*

*culmorum* (Khaosaad *et al.*, 2007; Ramos-Solano *et al.*, 2008). SAR is a globalized resistance response in plants. It takes place after a plant is exposed to a pathogen or a pathogen-derived agent. After an early and localized exposure to certain infectious organisms, SAR activates a whole-plant resistance mechanism against a wide variety of pathogens, including the pathogen responsible for the infection. This phenomenon is known as a broad-spectrum response (Bailey *et al.*, 2006). In order to exert their beneficial effects on plants, growth-promoting microorganisms must be rhizosphere-competent —*i.e.*, they must be able to compete with other microorganisms for the nutrients secreted by the root and for the physical space available in the root. Only a small portion of the root surface is covered by bacteria. The junctions between epidermal cells and the points of initiation of lateral roots are the preferred sites for bacterial growth. Once soil microorganisms colonize plant roots, they occupy spaces and consume nutrients that could otherwise be used by phytopathogen agents (O’Sullivan and O’Gara, 1992).

Siderophore production is a dual mechanism that promotes plant growth: it does not only increase iron availability for plants, but it also contributes to the biological control of plant pathogens. Growth-promoting microorganisms sequester iron from the soil, making it useful for themselves and for plant cells that can assimilate the bacterial siderophore-iron complex. Consequently, they limit the amount of iron available in the soil for other microorganisms (Callanan *et al.*, 1996).

Various plant growth-promoting microorganisms synthesize antibiotics (O’Sullivan and O’Gara, 1992); this mechanism is commonly associated with the capacity of biofertilizers to inhibit phytopathogens (Chet and Inbar, 1994; Whipps, 1997). The capacity of some bacteria to suppress pathogenic fungi depends on their capacity to produce antibiotics, such as pyoluteorin, pyrrolnitrin, phenazine-1-carboxylic acid, and 2,4-diacetylphloroglucinol (Picard, 2000). Bacteria can also inhibit the growth of phytopathogens releasing other compounds such as hydrogen cyanide (HCN) and/or lytic enzymes, which include chitinase, 1,3-glucanase, proteases, and lipases (Friedlander *et al.*, 1993; Chet and Inbar, 1994). Although pectinolytic activities are commonly associated with pathogenic bacteria, some non-pathogenic bacterial species such as *Rhizobium* sp. (Angle, 1986), *Azospirillum* sp. (Tien *et al.*, 1981), *Klebsiella pneumoniae*, *Yersinia* sp. (Chatterjee *et al.*, 1978) and *Frankia* sp. also possess this ability.

Based on the above premises, within the objectives of the National Biofertilizers Program of INIFAP, the initial objective was to isolate bacteria from the different agroecological regions of Mexico that had the capacity to stimulate plant growth. The starting point was the creation of a subproject called “Potencial de bioinoculantes microbianos como una alternativa para reducir costos de fertilización en cultivos básicos de temporal,” which was part of the SAGARPA-supported research megaproject called “Incremento en rendimiento, productividad y eficiencia en el uso de fertilizantes químicos, biológicos y abonos orgánicos de los principales cultivos básicos empleando métodos racionales de diagnóstico y recomendación” (Agreement No. 30333454).

The INI2709 biofertilizer, based on the bacteria *Bacillus subtilis*, *Herbaspirillum frisingense* and *Pseudomonas fluorescens*, was developed through this subprogram. This bioproduct was nationally evaluated in order to increase the profitability of maize grown under rainfed

systems. The results of this research proved the feasibility of reducing at least 30% of the chemical fertilization doses applied to maize in rainfed systems. As a result, the INIFAP Biofertilizer Team earned the Innovación Tecnológica 2008 award, granted by the Science and Technology Council of the State of Guanajuato (CONCyTEG) and the National Council of Science and Technology (CONACyT). Subsequently, in 2009, the INIFAP Biofertilizer Team, through the “Tecnologías de Producción y Uso de Biofertilizantes” project funded by SAGARPA (Agreement No. 143060011), developed native collections of monosporic mycorrhiza cultures to improve the bioproducts manufactured by INIFAP. In 2010, the “Impulso a nuevos productos y procesos de la bioeconomía y de investigación, transferencia de tecnología y uso de biocombustibles, biofertilizantes y abonos orgánicos” project (SAGARPA Agreement No. S19584A410111) helped to improve some of the production processes of the mycorrhizal and bacterial biofertilizers of INIFAP. In addition, this project supported the creation of the first national collection of microbial biofertilizers in Mexico. Several INIFAP researchers located in different agro-ecological zones of Mexico took part in the creation of this collection. They sent plant and soil samples to the Bacterial Biofertilizers Lab located in Celaya, Guanajuato. In this laboratory, microorganisms were isolated using selective media and their growth promotion capabilities evaluated at the laboratory and greenhouse levels. By the end of the project, approximately 5,000 growth-promoting microorganisms from different agroecological zones were part of this collection. These microorganisms were preserved using different microbiological techniques, including slant culture and cryopreservation.

The main objective of this research initiative was to provide Mexican producers with growth-promoting microorganisms isolated from different agro-ecological zones, which could adapt better to the environmental conditions of a particular local area and, in consequence, promote better the growth of the crops. Within this same project, and at the request of SAGARPA, it was proposed to monitor the quality of the main biological products marketed in Mexico, assist in the development of a proposal for an Official Mexican Standard to verify the production and quality control of biofertilizers, and perform validations of biological materials on all the agroecological regions of México using biological products from INIFAP itself, the Asociación Mexicana de Productores, Formuladores y Distribuidores de Insumos Orgánicos, Biológicos y Ecológicos (AMPFYDIOBE; currently AMPBIO), and the Benemérita Universidad Autónoma de Puebla (BUAP).

As part of the objectives of this project, the book *Introducción al Uso y Manejo de los Biofertilizantes en la Agricultura* was published in 2012. This book has been widely cited in Mexico and abroad (Aguado-Santacruz *et al.*, 2012). In 2011, the National Biofertilizer Team of the INIFAP was part of the Proyecto de Investigación en Biofertilizantes y Abonos Orgánicos (SAGARPA Agreement No. S2341HA4310111), which analyzed different basic molecular aspects of the interaction between growth-promoting microorganisms and plants. Likewise, the researchers explored the potential to:

- a) Increase biomass and sugar content in sweet sorghum through the inoculation of microorganisms.

- b) Evaluate different mycorrhizal strains for the control of nematodes in ‘Maradol’ papaya.
- c) Study the effect of inoculation with growth-promoting microorganisms in potato and husk tomato.
- d) Analyze the effect of biofertilizer inoculation on nutrient assimilation in plants.
- e) Analyze the interaction between agro-industrial by-products and mycorrhiza in vegetable cultivation.

## CONCLUSIONS

INIFAP has been an essential part of the governmental strategy to improve agricultural profitability, to enhance the quality of agricultural products, and to reduce environmental impacts by minimizing the use of agrochemicals in the field (particularly synthetic fertilizers). In 1999, the success of the first national bioproduct validation by INIFAP set a major precedent for their use in Mexico, leading to the creation of the first interdisciplinary team of INIFAP focused on research, technology transfer, and development of bioproducts (particularly biofertilizers). Since then, INIFAP has played a leading role in the implementation of technologies that lead to the establishment of a more profitable and healthier agriculture with a lower environmental impact. The various INIFAP publications about the use of bioinputs in agriculture —particularly the first handbook about the use and management of biofertilizers— and the intensive technology extension work conducted by the INIFAP scientists throughout the nearly 25 years of the biofertilizer research and training program of the INIFAP have been fundamental for the adoption of this technology in Mexico. Likewise, the monitoring carried out by this interdisciplinary group on the quality of biological products distributed in Mexico has highlighted the urgent need to develop updated regulations on the use of bioinputs in our country that ensure farmers acquire quality products and, thus, exploit the great potential of bioinputs in Mexican agriculture.

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# Background, Results, and Prospects of Microbial Biofertilizers in Mexico

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## ABSTRACT

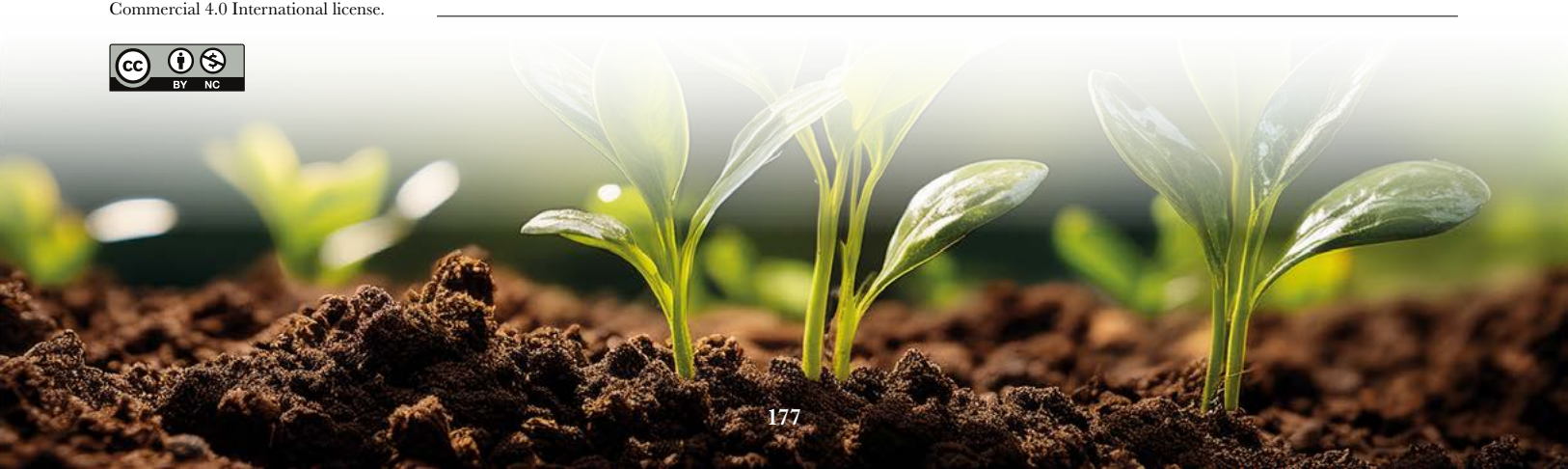
**Objective:** To convince Mexican producers and authorities about the potential of biofertilizers in different regional agricultural systems. In addition, using validation and demonstration plots with different crops, INIFAP trained producers in the appropriate use of biofertilizers.

**Design/methodology/approach:** The actions were carried out throughout the country since the 1999 SS cycle. The microorganisms used in this project were *Azospirillum brasilense*, *Rhizobium etli*, *Bradyrhizobium diazoefficiens*, and *Rhizophagus intraradices*. The training courses were attended by 9,229 producers. Biofertilization was carried out in 1,882,236 ha and 3,370,440 doses of biofertilizers were applied during three agricultural cycles. In conclusion, biofertilizers (individually or in co-inoculation treatments) can increase crop yield under various environmental and management conditions.

**Results:** In most crops, bacteria/fungi co-inoculation promoted a greater growth and yield in host crops. According to the data obtained by the biofertilization program of INIFAP, in Mexico, biofertilization can feasibly achieve more sustainable and profitable agricultural systems than conventional agricultural systems that use agrochemicals. In addition, the effective symbiosis of rhizobia promoted bean growth.

**Findings/conclusions:** Extensive and readily available research about biofertilizers proves that these microorganisms can increase yield, while providing enough nutrients to crops.

**Keywords:** rainfed agriculture, microorganisms, yield, technology transfer.



## INTRODUCTION

Around the middle of the last century, the use of synthetic fertilizers was fundamental to meet the food demand of the growing Mexican population. Consequently, the Mexican government created Guanos y Fertilizantes de México (GUANOMEX) in 1943 to commercialized synthetic fertilizers at low prices. By the 1970s, the name of this company changed to Fertilizantes Mexicanos S. A. (Fertimex) (Grageda-Cabrera *et al.*, 2012). The Green Revolution arrived in Mexico during the first years of GUANOMEX. Its purpose was to increase agricultural productivity through monocrops (Cecon, 2008), intensive agriculture (Herrera, 2006), improved seeds, agrochemicals, irrigation, technical support, and research (Pichardo, 2006). The results were encouraging (Hernández, 1988). As a consequence of the 1970s Energy Crisis, the price of energy sources used to manufacture fertilizers (such as oil, natural gas, and coal) increased, resulting in a rise in the cost of fertilizers. In addition, the privatization of state-owned companies in the late 1980s put an end to Fertimex S.A., which produced >3 million tonnes of fertilizers per year. Therefore, input purchasing and importation was difficult, particularly for small and medium producers (Sacristán-Roy, 2006). Despite the immediate increase in crop yield obtained by the application of synthetic fertilizers, their excessive use led to environmental pollution, increasing greenhouse gases and depleting the ozone layer (Bohloul *et al.*, 1992). In addition, NO<sub>3</sub> and NO<sub>2</sub> leaching polluted water sources (Martínez-Nieto *et al.*, 2011). Furthermore, the use of these products decreased organic matter decomposition and modified exchangeable cation concentration (Demiraj *et al.*, 2018; Bowden *et al.*, 2019; Mehdizadeh *et al.*, 2019). Likewise, Caballero-Mellado and Martínez-Romero (1999) reported alternations in soil microbiota. However, neither environmental pollution, nor changes in the microbial populations of the rhizosphere, helped to reduce the application of synthetic fertilizers. The reduction of the use of synthetic fertilizers in the 1990s was a consequence of price increase (Ávila Dorantes *et al.*, 2002). By 2000, Mexico was the main net importer of urea, buying 63% of fertilizers used in agriculture (Grageda-Cabrera *et al.*, 2012). This situation and the low profitability of agriculture, led the government—through the Ministry of Agriculture, Animal Production, and Rural Development (SAGAR), currently known as the Ministry of Agriculture and Rural Development (SADER)—to drive technologies developed by domestic research. These technologies were aimed to develop plant nutrition alternatives, using soil beneficial microorganisms studied by several national research and educational institutions. This agricultural practice consisted of adhering the microorganisms to the seeds in order to improve plant nutrition. Sustainable agriculture is based on the use of beneficial microorganisms that maintain soil microbial diversity and drive the association between these microorganisms and plants, promoting growth, productivity, and high yields. Microorganisms, fungi, and bacteria can improve plant growth, increasing nutrient availability (Mazid *et al.*, 2011). Therefore, the use of microbes as bioinoculants—along with chemical fertilizers—is the best strategy to increase plant growth and soil fertility (Prisa *et al.*, 2023). In 1999, SAGAR implemented and developed a biofertilization program through INIFAP. At the beginning, the Programa Nacional de Biofertilizantes was developed for marginal producers who grew maize and bean under rainfed systems, had no access to synthetic fertilizers, and only practiced

subsistence agriculture; however, producers who applied chemical fertilizers to their crops were later included in the program. During the implementation of the program, farmers included biofertilization in their agronomic management —*i.e.*, they adhered microorganisms to seeds and used conventional local crop management. The use of biofertilization in the process helped to reduce the doses of chemical fertilization, without a negative impact on yield. In many cases, it actually increases, with lower production costs. Seed inoculation favors the nutrition of host crops and strengthens the root system through several action mechanisms, consequently promoting a greater plant growth. Meanwhile, the initial objective was the expansion of the research and technology validation program for different crops of interest, in various agroecological regions of the country. In addition, promoting and spreading the use of biofertilizers through training and demonstration plots were fundamental, because, at the time, these technologies were almost unknown by farmers, technicians, and civil servants of the Mexican agricultural sector. These programs had the objective of driving the adoption of biofertilizers in the Mexican countryside and to concomitantly reduce the application of synthetic fertilizers.

### **Development of the Mexican Biofertilization Program**

Through the INIFAP, SADER invited several research and educational institutions to be part of the work group in charge of the production of biofertilizers. Both the Universidad Nacional Autónoma de México (UNAM) and the Instituto Politécnico Nacional (IPN) agreed to participate in this project. On the one hand, UNAM participated through its Centro Nacional de Fijación de Nitrógeno (currently known as Centro de Ciencias Genómicas): Mr. Jesús Caballero Mellado (ScD) —who isolated and developed the strain *Azospirillum brasilense* Tarrand, Krieg et Döbereiner— and Mr. Jaime Mora Celis (ScD) —who provided the strain *Rhizobium etli* (Segovia *et al.*, 1993)— took part in the project. On the other hand, IPN —through the participation of Mrs. María Valdés Ramírez (ScD) from the Escuela Nacional de Ciencias Biológicas— provided the strains *Bradyrhizobium japonicum* (currently *B. diazoefficiens*) (Delamuta *et al.*, 2013) for soy (*Glycine max* L. Merr) and the endomycorrhiza fungus *Glomus intraradices* (currently *Rhizophagus intraradices* (Schenck & Sm.) Walker & Schüßler (Schüßler and Walker, 2010)). The initial program was funded by the Fundación Mexicana para la Investigación Agropecuaria y Forestal A.C. (FUMIAF A.C.).

### **Actions Included in the Biofertilization Program**

The implementation of the program included validation and technology transfer activities, as well as biofertilizer training, production, and distribution. The theoretical-practical training started with researchers from the INIFAP, in three Mexican agroecological regions: central region (Puebla), northern-central region (Coahuila), and southeastern region (Tabasco). Subsequently, technicians and producers were trained on the fundamentals of microorganism reproduction. Emphasis was put on caring, transporting, and storing the microorganisms, as well as in the different ways to apply biofertilizers. Training included audiovisual presentations and in person lessons. In addition, practices regarding the adherent (5% carboxymethylcellulose) used to attach the said microorganisms

to seeds, both for hand sowing or drilled seeds were conducted in each region. Overall recommendations were issued for producers that required to inoculate large quantities of seeds. All the participants received written information about the different features of the microorganisms. A total of 231 courses were offered throughout the country: 208 courses during the 1999 spring/summer (SS) cycle and 23 courses during the 2000 autumn/winter (AW) cycle. Mass production of nitrogen fixing organisms (*Azospirillum brasilense*, *Rhizobium etli*, and *Bradyrhizobium diazoefficiens*) required specialized equipment and, consequently, a commercial service provider from Mexico City was hired. The mixture of the substrate used to soak in the bacteria, the preparation of the carboxymethylcellulose-based adhesive, and the packaging were carried out in the Centro Nacional de Investigación Disciplinaria de Microbiología-INIFAP. The final product of each bacteria had a  $100 \times 10^6$  bacteria g of peat<sup>-1</sup> concentration in 400 g bags. This quantity was enough to inoculate medium-sized seeds used to sow 1 ha of maize and/or bean. The endomycorrhizal fungi *R. intraradices* increased through a in vivo production system, using different host plants to contribute to their multiplication. The process was carried out in sterile substrate composed of a mixture of sand and soil with low phosphorous content. The strategy was implemented in experimental fields from four different representative agroecological regions of the country: Valle de México (State of Mexico), Cotaxtla (Veracruz), General Terán (Nuevo León), and Rosario Izapa (Chiapas) (Durán-Prado *et al.*, 2001; Aguirre-Medina *et al.*, 2012). The final product recorded a minimum colonization root efficiency of 95% in the host plant and 40 spores per g of soil<sup>-1</sup> plus propagule. The packaged product contained 1 kg INIFAP™ mycorrhiza. This quantity was enough to inoculate medium-sized seeds require to sow 1 ha (Figure 1). During this period, the producer demands of the product were integrated, which facilitated the planning of the distribution of the mycorrhiza throughout the different states of Mexico.

The requests and distribution of biofertilizers had just one (and sometimes two) microorganisms per crop, including *Azospirillum brasilense* and *Rhizobium etli*. The Coordination and Liaison directors of INIFAP were in charge of this stage. These directors were the links between the FUMIAF A.C., the SAGAR-Alianza para el Campo, the district chiefs of SAGAR, and the producer associations. Without their intervention, the



**Figure 1.** Packaging presentation of microbial biofertilizers distributed to producers from 1999. a) 1999 and b) 2008.

distribution of biofertilizers would have failed in Mexico. Researchers from the INIFAP, private companies, and some rural development districts (particularly in Oaxaca) were in charge of the technology validation. Researchers from the INIFAP also established the validation plots for the biofertilization technology: 244 plots for the 1999 SS cycle, 23 for the 1999-2000 AW cycle, and 240 for the 2000 SS cycle, resulting in a total of 507 plots sown with different crops. The plots were established in fields from cooperating producers (minimum area: 1 ha). The producers and researchers selected together the most appropriate biofertilizers for each site, taking into account local conditions and the characteristic of the crop. The producers were in charge of agricultural management. The aim was to drive their interaction through agricultural management and to identify the efficiency and potential of the application, both in the plots and under the specific environmental conditions of the crops of interest. The yield values recorded in each plot was used to develop a graph with the Sigma Plot v. 11.0 software. Each plot group included standard error ( $\pm$ ) for their agroecological region. Except for Sonora and Baja California, 9,229 agricultural producers and technicians from all the regions of the country took part in this training program (Figure 2).

Training activities increased during the 1999 SS and 1999-2000 AW agricultural cycles. The technicians of the Programa de Capacitación and Extensión of the Alianza para el Campo were also involved in this process. During the 1999-2000 AW cycle, biofertilizers were distributed in 411,907 ha in the states of Colima, Nayarit, Sinaloa, Sonora, and Baja California Norte and Sur. Seventy-six percent of the product was applied to sorghum crops (Table 1). Two treatments methods were applied during the technology validation process carried out in the plots of the producers: one using microorganisms (individual or co-inoculation treatment) and another using microorganisms plus the dose of chemical fertilizers used by the cooperating producer.

Table 2 includes the total number of plots established per agricultural cycle and crop. It also includes the results for maize and beans, the crops which were evaluated with greater frequency by the INIFAP researchers.



**Figure 2.** Biofertilization theoretical-practical training for agricultural producers and technicians.

**Table 1.** Microbial biofertilizers distribution and number of hectares.

Agricultural cycle	Hectares	Biofertilizer dose
SS 1999	577 657	753 191
AW1999-2000	411 907	893 764
SS 2000	893 611	723 485
Total	1 882 263	3 370 440

Source: Technical report of the Programa Nacional de Biofertilizantes. SS (Spring-Summer), AW (Autumn-Winter).

The results for maize were divided in three groups: native maize, commercial material, and commercial varieties + synthetic fertilizers. The yield of *Phaseolus vulgaris* L. increased in the 52 plots where microorganisms were used individually or in co-inoculation. The results obtained were higher than control. *R. etli* biofertilization resulted in a lower average yield than the fertilizer treatments used in the center, northeast, and south Pacific regions of Mexico; however, in the Gulf of Mexico, *R. etli* recorded higher results than fertilizers. For its part, the response to *R. intraradices* biofertilization was different between agroecological regions. *R. intraradices* increased yield in the northeastern and the south Pacific regions; however, in the latter region, fertilization surpassed microbial individual biofertilization. Meanwhile, in the Central Gulf of Mexico, *R. etli* biofertilization was 9% higher than *R. intraradices* (Figure 3).

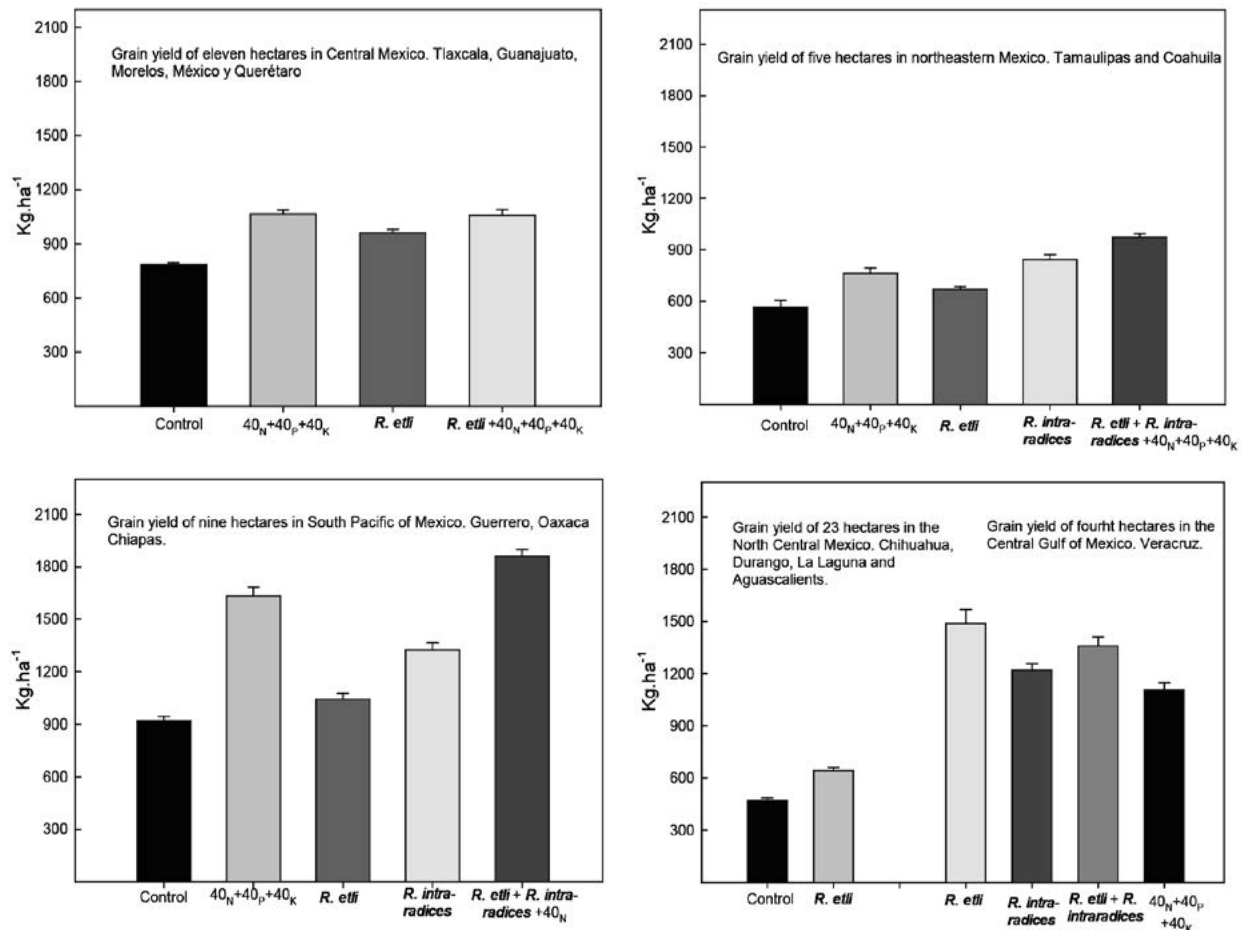
## RESULTS AND DISCUSSION

The differential yield obtained by *R. etli* suggested that regional environmental limitations restricted the yield of bean (Zahran, 1999), although bean can establish symbiotic associations with a wide diversity of rhizobia (Bernal and Graham, 2001). In addition, bean varieties strongly depend on nitrogen fertilizers to achieve a high yield in the field. This phenomenon is the result of the considerable variability of its nodulation and nitrogen fixation capacity; consequently, bean is capable of a stronger nitrogen fixation (Rahmani *et al.*, 2011) than other pulses. In the south Pacific, both environmental factors

**Table 2.** Validation plots per agricultural cycle and crop in Mexico.

Crop	Biofertilizer				Biofertilizer plus chemical fertilizer				Total in three cycles
	SS 1999	AW 1999-2000	SS 2000	Total	SS 1999	AW 1999-2000	SS 2000	Total	
Corn	62	7	32	101	69		24	93	194
Bean	28	3	6	37	26	6	6	38	75
Sorghum	16	6	15	37	14		145	159	196
Wheat	1		1	2	12		1	13	15
Barley	4			4	3			3	7
Oatmeal	3			3	2			2	5
Others	1	1	10	12	3			3	15
Total	115	17	64	196	129	6	176	311	507

Source: Technical report of the Programa Nacional de Biofertilizantes.



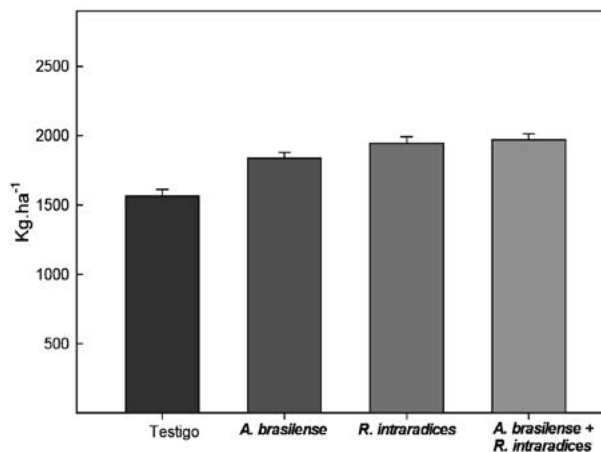
**Figure 3.** *Phaseolus vulgaris* L. yield obtained using microbial biofertilizers and/or synthetic fertilization in different regions of Mexico. The vertical line indicates the standard error ( $\pm$ ).

and local crop management can increase bean yield. Bean co-inoculated with *R. etli* and *R. intraradices* recorded an important yield increase in the south Pacific and northeastern regions of Mexico.

When endomycorrhizal fungi associate with *Rhizobium* and *Azospirillum* fixing bacteria establish a synergy with various host plants, which grown even more, increasing their capacity to explore larger soil areas and consequently gaining access to more nutrients and water.

Hidalgo-Rodríguez *et al.* (2019) reported similar results. These authors used a co-inoculation with two symbionts in Peru, achieving a higher protein production (Küçük, 2011) and plant growth increase. Compared with control, the yield of native maize biofertilized with microorganisms (individual or co-inoculation treatment) increased by 19%. *R. intraradices* and *A. brasilense* co-inoculation recorded a similar yield than the individual *R. intraradices* biofertilization (Figure 4).

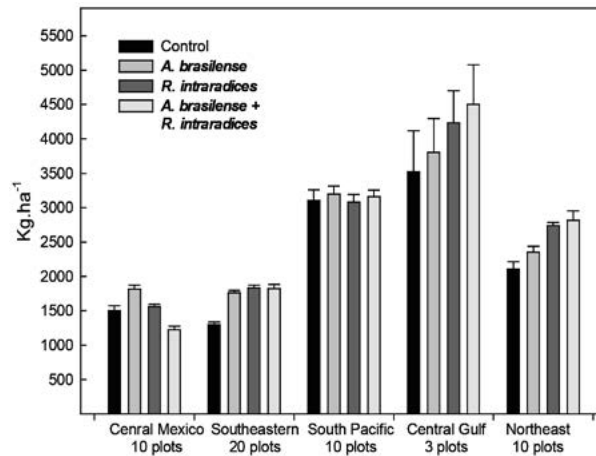
Microbial biofertilizers use several mechanisms to promote the growth of host plants, including the production of growth regulators (Naiman *et al.*, 2009) which favor root and leaf growth (Dar *et al.*, 2021) and the increase of mineralization and transport of nutrients.



**Figure 4.** Yield of native maize from different Mexican regions, using microbial biofertilizers. The vertical line indicates a standard error ( $\pm$ ) in 24 plots.

Likewise, endomycorrhizal fungi and other phosphate solubilizing microorganisms improve the availability of this element when it is found as insoluble phosphate (Djuuna *et al.*, 2022). The response of biofertilizers in the field is influenced by several abiotic factors; however, soil microbiota is also fundamental (Kumar *et al.*, 2017), because some microbes can positively or negatively impact the relationship between plants and beneficial microorganisms (Nannipieri *et al.*, 2003). Contrasting results were recorded with different maize varieties in different agroecological environments. Nevertheless, inoculated maize from the southeastern, central Gulf, and northeastern areas showed that the effect of biofertilizers surpassed control. In addition, the results reported different responses, depending on the microorganism used in the treatment. The highest yields were obtained by *R. intraradices* (individual treatment) and by *R. intraradices* and *A. brasilense* (co-inoculation treatment). In the case of the south Pacific area, no consistent differences were recorded between treatments, although the *A. brasilense* biofertilization obtained a slightly trend towards a higher yield (Figure 5).

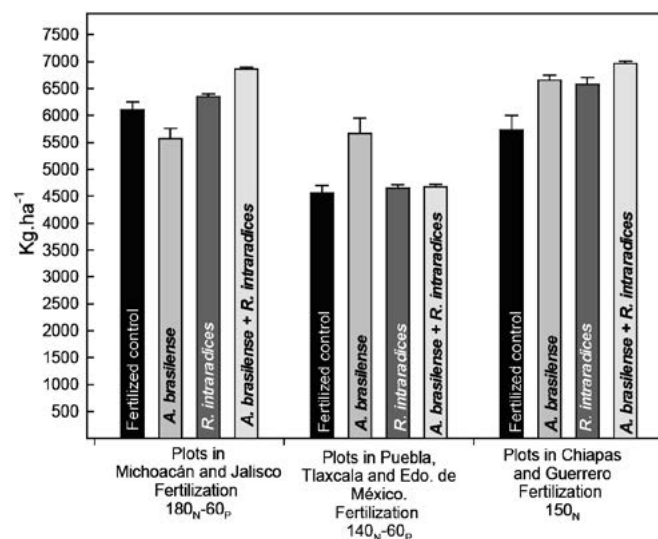
In central Mexico, *A. brasilense* and *R. intraradices* (individual treatment) recorded the highest yield increase. Xipeng Liu *et al.* (2023) developed a result meta-analysis of biofertilizers and different crops. They pointed out that individual biofertilization of microorganisms increased the growth of plants by 29%, while the biofertilization with consortia increased the said growth by 48%. The increase of yield in biofertilized maize is the result of the higher mineral absorption produced by the symbiosis. In the maize fields of Mexico, *A. brasilense* biofertilization resulted in an important yield increase (Dobbelaere *et al.*, 2001). In India, wheat yield results obtained by the co-inoculation of *Glomus fasciculatum* and several bacteria from genera *Pseudomonas*, *Bacillus*, *Azotobacter*, and *Azospirillum* were significantly higher than the results recorded by control (Parewa and Yadav, 2014). The different maize responses to *R. intraradices* can be associated with the colonization preferences of the said fungi for this plant (Bonfante and Genre, 2008). In addition, these responses can be very specific to the host plant and the fungi used in the treatments (Gonzalez-Chavez *et al.*, 2004). Endomycorrhizal fungi can increase the



**Figure 5.** Yield of native maize from different Mexican regions, using microbial biofertilizers. The vertical line indicates a standard error ( $\pm$ ).

area of effective absorption of the roots through the formation of a wide extraradical hyphae network, improving nutrient absorption efficiency (George, 2000). Currently, the use of microorganisms that promote plant growth, consequently improving the yield of different crops, has been widely spread (Radha and Rao, 2014). Figure 6 includes the response of maize to different combinations of microbial biofertilizers and high doses of fertilizers.

Using a combination of chemical fertilizers and biofertilizers can improve nutrient availability in the plant-soil system. The efficient application of several biofertilizers in the plots can greatly reduce the use of mineral fertilizers (Berg, 2009). In addition, the offer of these products is increasing, both for horticulture and for soil restoration (Gianinazzi and Vosátka, 2004).



**Figure 6.** Yield of maize varieties from different regions of Mexico, using microbial biofertilizers and different doses of chemical fertilizers. The vertical line indicates a standard error ( $\pm$ ).

The content of soil nutrients is fundamental to successfully grow sustainable and productive crops. Soil nutrients allow to obtain higher yields (Brady and Weil, 2002) and the use of rhizobacteria can reduce the doses of chemical fertilizers applied to crops (Prisa *et al.*, 2023). Ekinici *et al.* (2021) have identified several plant growth-promoting rhizobacteria (PGPR) which can significantly impact plant growth.

## CONCLUSIONS

In Mexico, microbial biofertilizers (individually or in co-inoculation treatments) can increase crop yield under different environmental and management conditions. In most of the cases, the bacteria/mycorrhizal fungi co-inoculation promoted growth and yield in host plants. Rhizobia established effective symbiosis, promoting bean growth, decreasing the application of nitrogen fertilizers, and reducing production costs. Biofertilizers are a more acceptable ecological alternative for sustainable agriculture than the agrochemical fertilizers used in other production systems. The excessive use of agrochemicals has seriously damaged the environment. Currently, research about biofertilizers is abundant. In addition, it proves that PGPRs can improve crop nutrition, achieve higher yields, and/or reduce production costs (chemical fertilization).

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# Production of Saladette-type Tomato (*Solanum lycopersicum* L.) in response to the association with aromatic species

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## ABSTRACT

**Objective:** This study analyzes tomato production in response to Volatile Organic Compounds (VOCs) emitted by the foliage and flowers of *Ocimum basilicum*, *Tagetes erecta*, and *Origanum majorana*, intercropped with the vegetable.

**Design/methodology/approach:** The development of tomato and aromatic plant species (*Ocimum basilicum*<sup>1</sup>, *Tagetes erecta*<sup>2</sup>, and *Origanum majorana*<sup>3</sup>) was evaluated during both vegetative and flowering stages. Treatments were distributed using a completely randomized block design. Indicators of tomato fruit production and quality were measured and analyzed.

**Results:** The associations with aromatic plants emitted VOCs based on alkaloids, glycosides, and terpenes, which act as stress regulators and enhancers of tomato plant growth and yield. This demonstrates the importance of aromatic species in improving the quality and size of tomato fruits.

**Limitations of the study/implications:** During the course of the research, challenges related to VOCs (aroma) arose, as they are difficult to control between experimental units and treatments. The use of 2-meter polyester nylon barriers between treatments prevented the exchange and leakage of VOCs.

**Findings/conclusions:** The association of aromatic plants with tomato affected fruit yield and quality indicators, resulting in higher production per plant and better fruit quality compared to the control.

**Keywords:** Yield, Quality, Vegetables, Volatile Organic Compounds.

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## INTRODUCTION

Under a semi-controlled exploitation model, such as greenhouses, horticultural production systems are becoming increasingly widespread and demanding in their use of agrochemicals. Tomato is the most in-demand vegetable worldwide, showing a national per capita consumption of 14.1 kilograms per person per year in 2020 (SIAP, 2024); with China positioned as the leading global producer and consumer, the United States as the main importer, and Mexico as the main exporter, placing the latter in the ninth position in the world ranking. This crop exhibited growth in Mexico of around 5.96% between 2017 and 2023, increasing from 3.47 million to 3.637 million tons, and a cumulative global increase of 125.80% is estimated by 2030 (SAGARPA, 2017; SIAP, 2025). This



demand could be met by expanding the cultivation area and improving the technification of greenhouse tomato production, an area that has not seen substantial growth in recent years (2019-2023), with a production yield of  $69 \text{ t ha}^{-1}$ . However, this is still well below the genetic potential of the crop material (SIAP, 2025). Given the need for food production and climate change resilience, horticultural systems as well as agriculture in general are becoming increasingly dependent on external agricultural inputs, specifically insecticides, fungicides, and mineral fertilizers. In tomato production, 15% of the total production cost is invested in pesticides, 25% in soluble fertilizers, 10% in nutritional foliar products, and 50% in labor expenses over the nine-month greenhouse production cycle; where product safety remains an increasingly complex challenge. In light of the need to offer healthy and safe products, biological diversity —specifically aromatic species offers highly important alternatives to address phytosanitary problems and improve the productivity of vegetables (Gallegos, 2017). The potential of aromatic species for repelling and/or allelopathic effects on various causal agents of phytosanitary issues is well documented, as is their role in human health and cuisine. However, the positive relationship of Volatile Organic Compounds from aromatic plants on the development, growth, and production of vegetables such as tomato, chili, and others is scarcely supported by scientific argumentation. Currently, humanity not only demands more food but also products free of harmful agents; in response to this immense need, the present work analyzes tomato production as a response to the Volatile Organic Compounds (VOCs) from the foliage and flowers of *Ocimum basilicum*, *Tagetes erecta*, and *Origanum majorana*, intercropped with tomato under greenhouse conditions.

## MATERIALS AND METHODS

The present study was conducted in the greenhouses of the company “Grupo Agroindustrial Chiapaneco S.C. de R.L. de C.V.” (GRACHI), located in the Sierra of Chiapas, Mexico, on the La Trinitaria - Lagos de Montebello highway, km 10, Emiliano Zapata Colony junction, km 3; at the geographical coordinates N 16.16343, W 91.97594, and an altitude of 1,525 meters above sea level. The climate in the area is warm sub-humid with summer rains, with an average monthly temperature of  $18.1 \text{ }^{\circ}\text{C}$ . The predominant soil types are limestone (77.31%), shale (9.03%), and alluvial soil (7.28%), with an organic matter content of 1.3% (Gallegos, 2017). The topological arrangement designed and implemented consisted of transplanting the aromatic plants at their development and flowering stages between the tomato plant rows, with a spacing of 20 cm between aromatic plants and 110 cm between rows. During the development of the aromatic plants, pruning of the foliage mass was performed every 15 days to promote the release of VOCs. The treatments in this study were randomized using a completely randomized block design over an area of  $1,600 \text{ m}^2$  (40 meters wide by 40 meters long), resulting in a total of 70 experimental units. Seven treatments were evaluated, represented by tomato rows intercropped with aromatic plants (*Ocimum basilicum*<sup>1</sup>, *Tagetes erecta*<sup>2</sup>, and *Origanum majorana*<sup>3</sup>) in development; associations of aromatics (1, 2, and 3) in flowering + tomato, and tomato without association, with 10 replications per treatment. The aromatic seeds were sown in 200-cell trays using peat moss as a substrate, under 70% shade mesh; the tomato seedlings were obtained in the same way. For the field phase, two areas were delineated, each 20 meters wide by 40

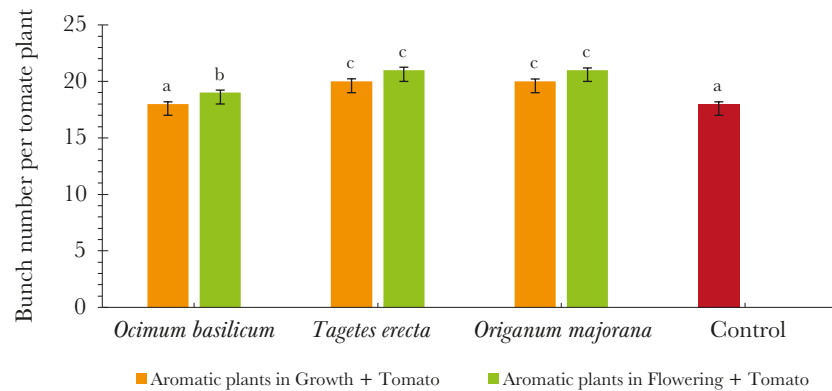
meters long, obtaining eight blocks (planting beds of four rows with 100 plants each). Between the tomato rows of each block, 80 basil (*Ocimum basilicum*) plants in development (foliage) were transplanted and 100 basil plants in flowering for the second block; likewise for the treatments with marigold (*Tagetes erecta*), marjoram (*Origanum majorana*), and the control (no association). Each block or area with the aromatic plants was enclosed with 2-meter-high polyester nylon to prevent the exchange and leakage of Volatile Organic Compounds between treatments. Tomato plant production and fruit quality indicators were measured and analyzed: number of clusters, fruits per cluster, fruit weight (g), yield (kg per plant), fruit quality, and aboveground biomass (g). Measurements began 8 days after tomato transplanting and continued until the fruit reached physiological maturity. For this purpose, a measuring tape, caliper (mm), and granataria scale (g) were used for foliar biomass, along with detailed counts for the number of flowers and days to flowering. Ten tomato plants per treatment were selected for data collection.

The data obtained during the research were analyzed using ANOVA (0.05), and based on the calculated F-value, a *post hoc* multiple range test was performed using the Tukey method (0.05). Field data analysis was carried out using the statistical software Statgraphics Centurion XVI.I.

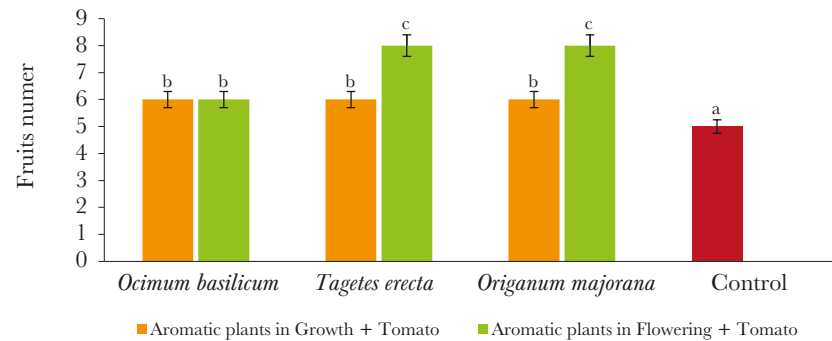
## RESULTS AND DISCUSSION

The tomato inflorescence is a cyme with different branching patterns (mono-, di-, and polychotomous), with or without axial bracts; typically having three nodes between each inflorescence (Rick, 1979). Six weeks after sowing, the plant enters its reproductive phase, producing flowers continuously depending on its developmental rate, which is influenced by the plant's physiological conditions and the agroecological environment. The inflorescences are lateral rather than apical, and this type of vegetable features highly developed axillary stems (Villegas *et al.*, 2004). The results show that associations with aromatic plants have a positive effect on the number of floral clusters in tomato plants. The effects were similar during both the vegetative growth (foliage) and flowering phases of the aromatics, with *Tagetes erecta* and *Origanum majorana* demonstrating dominance in both stages (Figure 1). The number of clusters per tomato plant increased by up to two with *Tagetes erecta* and *Origanum majorana*, whereas *Ocimum basilicum* showed results similar to the control (19 and 18, respectively). The number of tomato clusters in the associations ranged from 19 to 21, while the control showed 18 clusters per plant (Figure 1).

For the indicator “number of fruits per cluster,” greater dominance was observed with the flowering phase of *Tagetes erecta* and *Origanum majorana*, each with 8 set fruits, followed by *Ocimum basilicum* with 6 fruits, and the control with 5 fruits (Figure 2). The difference in fruit set in tomato as a response to the association with flowering *Tagetes erecta* and *Origanum majorana* was 3 fruits higher compared to the control. Similar results (2 additional fruits) were obtained for the control compared to *Ocimum basilicum*, *Tagetes erecta*, and *Origanum majorana* in their vegetative growth phase. The aromatic species improved the number of fruits set per inflorescence, with the scents emitted by each aromatic plant producing a positive effect, resulting in a 90% fruit set rate.

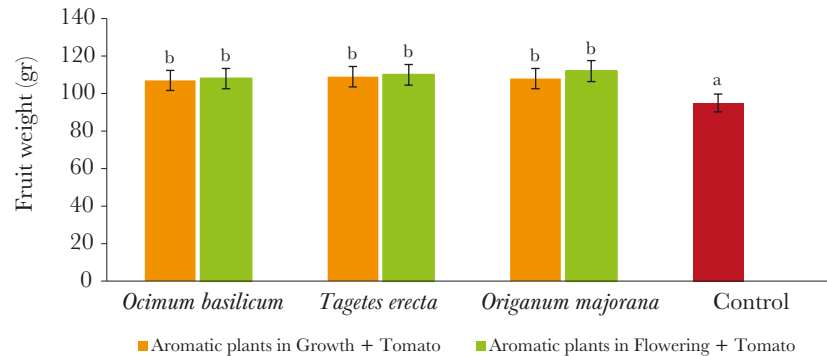


**Figure 1.** Total flower clusters per saladette tomato plant associated with aromatic plants. Values with the same letter are equal according to Tukey's multiple range test at  $P \leq 0.05$ .



**Figure 2.** Fruits per flower cluster of saladette tomato plants associated with aromatic species. Values with the same letter are equal according to Tukey's multiple range test at  $P \leq 0.05$ .

There are numerous types of tomatoes such as Saladette, Globe, Cherry, Cocktail, and Grape (SIAP, 2024), and their number continues to grow, with new varieties offering greater disease resistance, higher productivity, and improved fruit quality and shelf life (Navarro, 2011). In contrast, the effects of the phenological phases of the aromatic plants (vegetative growth and flowering) showed greater dominance in the flowering phase of *Origanum majorana*, and in the vegetative growth phase of *Tagetes erecta*, regarding the increase in tomato fruit weight. Tomatoes associated with *Tagetes erecta* yielded a fruit weight of 109 grams, followed by *Origanum majorana* with 108 grams, *Ocimum basilicum* with 107 grams, and finally the control with 97 grams per fruit. Similar results were observed in fruit weight when tomatoes were associated with aromatic plants during their flowering phase: *Origanum majorana* produced the heaviest fruits at 112 grams, followed by *Tagetes erecta* with 110 grams, *Ocimum basilicum* with 108 grams, and the control with 95 grams per fruit (Figure 3). The difference in tomato fruit weight in response to the association with flowering *Origanum majorana* was 17 grams more than the control; similar results (3 grams) were observed in comparison with *Tagetes erecta* during vegetative growth (Figure 3). These findings tangibly demonstrate the importance of aromatic species, which can be used as a form of aromatherapy for economically

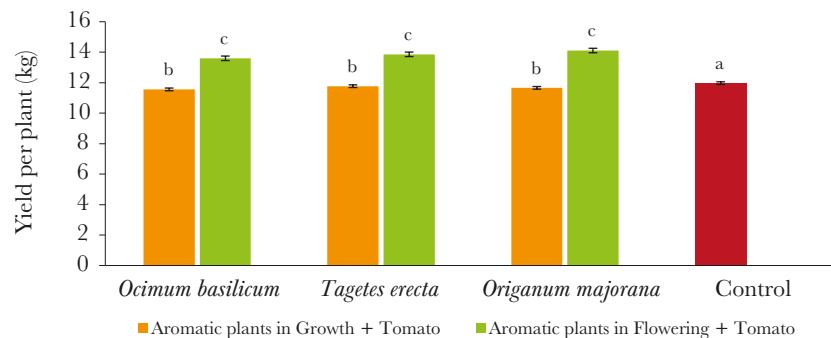


**Figure 3.** Fruit weight of saladette tomato associated with aromatic plants. Values with the same letter are equal according to Tukey's multiple range test at  $P \leq 0.05$ .

important crops such as tomato, jalapeño pepper, and bell pepper, among others, since their scents help stimulate plant metabolism, improve nutrient and water assimilation, and reduce antagonism between fertilizers present in the soil.

The differences in tomato fruit weight in response to the association with aromatic species are mainly due to the release of scents rich in alkaloids with double aromatic rings, which are found in higher concentrations during the flowering phase. This results in greater assimilation of both soil and foliar nutrients, leading to improved fruit growth.

Tomato fruit production per plant associated with *Origanum majorana* during its flowering stage reached 14.11 kg per plant, followed by *Tagetes erecta* with 13.86 kg per plant, *Ocimum basilicum* with 13.68 kg per plant, and the control with 11.97 kg per plant (Figure 4). Similarly, in the association with aromatics during their vegetative growth phase, *Tagetes erecta* recorded the highest production with 11.77 kg per plant, followed by *Origanum majorana* with 11.66 kg per plant, *Ocimum basilicum* with 11.55 kg per plant, and the control with only 10.476 kg per plant. These values are expressed per plant. From a more commercial-technical perspective, they are expressed in kilograms per square meter ( $\text{kg}/\text{m}^2$ ); in this study, the density was 2.87 plants/ $\text{m}^2$ . These results may vary depending on the planting system (plant density per greenhouse area). Similar and even higher results were reported by Villegas *et al.* (2004), stating that under greenhouse conditions and without pruning, commercial densities range from 2 to 2.5 plants/ $\text{m}^2$ .

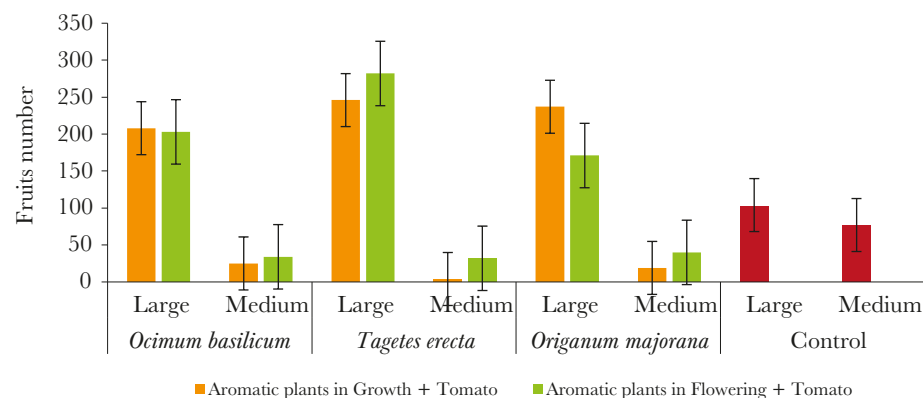


**Figure 4.** Saladette tomato production per plant associated with aromatic plants. Values with the same letter are equal according to Tukey's multiple range test at  $P \leq 0.05$ .

With an average density of 2.27 plants/m<sup>2</sup>, tomato yields a total fruit production of 20.6 kg per plant, equivalent to 46.8 kg/m<sup>2</sup>. The quality of most fruits and vegetables is significantly affected by water loss during storage, which depends on temperature and relative humidity. Temperature plays a critical role in maintaining postharvest tomato quality; the effect of storage temperature on the quality and extent of physicochemical changes in tomato fruits is highly dependent on the cultivar, exposure time, and harvest conditions (Peil and Gálvez, 2004).

Fruit quality is assessed in two stages: (1) large tomato quality (fruit weight >95 g), referring to all fruits with suitable values for national market commercialization. In this context, tomato associated with *Tagetes erecta* during vegetative growth produced 246 large fruits, followed by *Origanum majorana* with 237, *Ocimum basilicum* with 208, and the control with 57 fruits. A markedly different outcome was observed in the association with flowering aromatics: *Tagetes erecta* yielded the highest number of large fruits with 282, followed by *Ocimum basilicum* with 203, *Origanum majorana* with 171, and the control with only 104 large fruits. Additionally, medium-sized fruits (60 g-95 g) are classified as such because they do not meet the weight and size criteria for the large category, though they are still marketable at the regional level. Comparing large fruit values, the difference in response to the association with flowering *Tagetes erecta* was 178 more large fruits than the control; similar results (36 more fruits) were observed with *Tagetes erecta* in vegetative growth (Figure 5). These results demonstrate the importance of aromatic species in the assimilation of foliar fertilizers by reducing plant stress levels, which, along with temperature, relative humidity, and high plant respiration, contributes to achieving larger tomato fruit sizes.

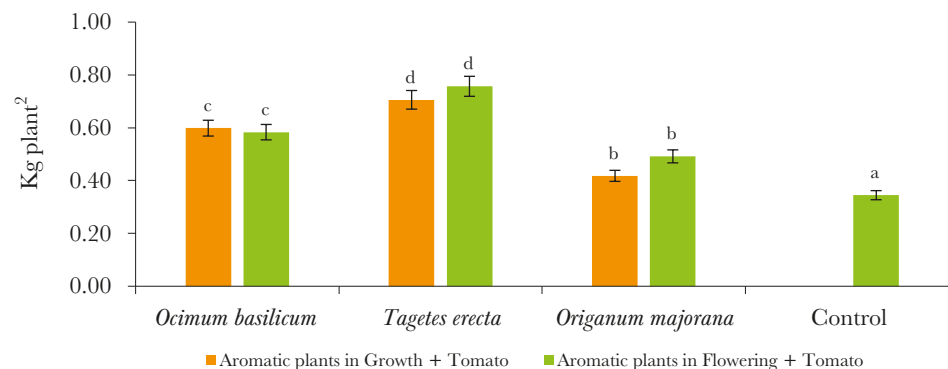
Tomato fruit quality (size) is improved by the association with aromatic species, with differences in fruit quality depending on the species and their phenological stage. The data presented above are consistent with those described by Balaguera and Álvarez (2006), who state that tomato quality is largely determined by its weight, and that fresh weight directly influences its economic value. However, variations in fruit weight have been reported depending on the time of year the crop is harvested. Modifications in tomato quality may be mechanical, physiological, or pathological in



**Figure 5.** Fruit quality per saladette tomato plant associated with aromatic plants.

nature. Mechanical damage can cause metabolic and physiological alterations, resulting in abnormal internal or external appearance and disruptions in respiratory metabolism, flavor, and firmness. Physical damage may also significantly affect the physicochemical composition of the pericarp and locular tissue in tomato fruits. Other relevant factors influencing the chemical composition of vegetables include climatic conditions, fertilization, production systems, irrigation, and the plant's developmental stage at harvest (Salomé, 2014). Regarding dry biomass production in tomato plants, the effects of the phenological stages of the aromatic plants (vegetative growth and flowering) were more dominant during the flowering of *Tagetes erecta*; the same trend was observed in its vegetative stage. Tomato associated with *Tagetes erecta* yielded 0.710 kg of biomass per plant, followed by *Ocimum basilicum* with 0.600 kg, *Origanum majorana* with 0.420 kg, and the control with 0.280 kg. In the association with flowering aromatics, *Tagetes erecta* produced the highest effect with 0.760 kg of tomato dry biomass, followed by *Ocimum basilicum* with 0.580 kg, *Origanum majorana* with 0.490 kg, and the control with 0.340 kg (Figure 6).

The dry biomass production of the tomato plant (kg) is improved by the association with aromatic species, with differences in biomass influenced by the species and their phenological stages. This difference is statistically significant in the following scenarios: basil-marigold, basil-oregano, basil-control, marigold-oregano, marigold-control, and oregano-control (Figure 6). The yield of a crop is related to the production or accumulation of biomass (fresh and dry matter) in plant organs, which may be allocated to fruit harvest and/or the synthesis of photosynthetic assimilates. As tomato fruits are sink organs for photosynthates, they compete with each other and with vegetative organs for the available assimilates (Castro *et al.*, 2014). However, in indeterminate tomato plants, other factors must be considered, as the dynamics of dry biomass accumulation are different (Ortega *et al.*, 2010). The associations with aromatic plants emit VOCs based on alkaloids, glycosides, and terpenes, which act as stress regulators and growth enhancers in tomato plants. These compounds have potential to be used as phytohormones.



**Figure 6.** Dry biomass production per saladette tomato plant associated with aromatic plants. Values with the same letter are equal according to Tukey's multiple range test at  $P \leq 0.05$ .

## CONCLUSIONS

*Tagetes erecta* is the aromatic plant with the greatest influence on the production indicators of tomato plants. The flowering of marigold releases alkaloids with a double aromatic ring structure, which the vegetable assimilates, thereby relieving stress in the tomato plant. The scents produced by aromatic plants are composed of terpenes, whose structural base is a double aromatic ring. This property acts as a growth phytohormone, stimulating and enhancing the development of tomato seedlings. Additionally, the alkaloids function as stress regulators, contributing to improved productivity. The association of aromatic plants with tomato had significant effects on yield and fruit quality indicators, as expressed through higher production per plant compared to the control.

## ACKNOWLEDGMENTS

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# Disinfection of *Guadua angustifolia* Kunth explants for *in vitro* propagation

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## ABSTRACT

**Objective:** To evaluate the effect of different disinfectants and concentrations on *Guadua angustifolia* explants collected during rainy and dry seasons for *in vitro* propagation, and to determine their impact on shoot emergence and survival.

**Design/Methodology/Approach:** Nodal segments were collected and subjected to six disinfection treatments combining 3% NaClO, the fungicide azoxystrobin (1.5 g L<sup>-1</sup>), and antibiotics (gentamicin, streptomycin, kanamycin, 5 ml) in Murashige and Skoog (MS) and Yasuda culture media supplemented with Gamborg vitamins, adjusted to pH 5.0, solidified with phytigel, and sterilized at 15 PSI for 20 minutes.

**Results:** The Yasuda medium presented lower contamination rates across treatments and improved explant survival during the dry season. Disinfection with fungicide alone resulted in 20 surviving explants, while the combination of antibiotics, fungicide, and 3% NaClO resulted in 19 surviving explants. No statistical differences were found between culture media regarding survival. Disinfection and oxidation were reduced when combining azoxystrobin, 3% NaClO, and fungicide, although survival improved in both treatments. Explants collected during the dry season exhibited lower contamination, higher survival, and greater shoot formation.

**Limitations/Implications:** Genetic variability among parent plants may influence explant response, potentially affecting propagation success and *in vitro* culture outcomes.

**Findings/Conclusions:** Explant survival improved with the combined use of azoxystrobin and 3% NaClO, as well as with azoxystrobin alone. Collection during the dry season reduced contamination and enhanced survival and shoot development, making it a more suitable period for explant procurement.

**Keywords:** *Guadua angustifolia* Kunth, *in vitro* culture, explant disinfection.

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## INTRODUCTION

Bamboo is a fast-growing forest resource (Leão *et al.*, 2020) that has become a sustainable alternative with economic, social, and cultural relevance (Aguirre-Cadena *et al.*, 2018). It has a wide range of uses, including housing construction, charcoal production, paper and cellulose manufacturing, and applications in the pharmaceutical and food sectors, among others (Leão *et al.*, 2020).

In Mexico, the highest diversity of woody bamboo species is found in Chiapas, Veracruz, and Oaxaca, which together harbor 70% of endemic species (Aguirre-Cadena *et al.*, 2018). *Guadua angustifolia* Kunth, native to Venezuela, Colombia, and Ecuador, has been introduced into Mexico and other American countries (Jiménez *et al.*, 2006). It is considered an efficient carbon sink, and more recently, tar oil has been extracted from it with reported antibiotic and antioxidant properties (Nadha *et al.*, 2012). In Mexico, its demand has increased as part of crop diversification strategies to improve farmers' income in marginalized areas, such as the northeastern region of Puebla (Aguirre-Cadena *et al.*, 2018). However, propagation by seed is difficult due to its irregular flowering patterns (Koshy & Gopakumar, 2005; Leão *et al.*, 2020). Vegetative propagation through cuttings is also practiced, but expansion is limited by the availability of propagules (Koshy & Gopakumar, 2005), requires intensive labor (Nadha *et al.*, 2012), and shows low survival rates due to poor sprouting and root emergence (Lárraga Sánchez *et al.*, 2011). In this context, *in vitro* culture methods represent an alternative for the mass propagation of *G. angustifolia*, offering advantages such as reduced propagation times and the production of disease-free plants (Luna, 2002; Sánchez *et al.*, 2002). However, fungal and bacterial contamination in *in vitro* culture of woody species is a frequent problem. *G. angustifolia* is no exception, showing a high level of bacterial contamination in culture media (Nadha *et al.*, 2012), which results in significant material loss and increased production costs (Digonzelli *et al.*, 2005; Das & Pal, 2005). Moreover, seasonal environmental changes have been shown to influence explant performance (González *et al.*, 2005). For this reason, it is essential to determine the optimal season for explant collection in *G. angustifolia* Kunth that minimizes contamination, as well as to identify effective disinfection methods for successful *in vitro* establishment.

## MATERIALS AND METHODS

Nodal segments with latent buds (explants) of *G. angustifolia* Kunth, one year old, were collected during the rainy season (May-November) and the dry season (January-April).

### Disinfection in the field and laboratory

In the field, the fungicide azoxystrobin was applied three days prior to explant collection. Once collected, explants were disinfected in the laboratory with water and soap, and subsequently immersed for 10 minutes in an antioxidant solution containing citric acid ( $100 \text{ mg L}^{-1}$ ), ascorbic acid ( $150 \text{ mg L}^{-1}$ ), sucrose (30 g), and two drops of Tween 80.

Under aseptic conditions in a laminar flow hood, explants were dissected into  $3 \text{ cm}^2$  segments and placed in Murashige and Skoog medium (Murashige & Skoog, 1962) (MS) and Yasuda medium (Yasuda *et al.*, 1985), both supplemented with Gamborg vitamins (Gamborg, 2002). Using these combinations, the disinfection treatments were established (Table 1).

### Sowing in the growing media

The MS and Yasuda culture media supplemented with Gamborg vitamins,  $30 \text{ g L}^{-1}$  sucrose,  $1.125 \text{ mg L}^{-1}$  BAP,  $8 \text{ g L}^{-1}$  Phytigel<sup>®</sup>, adjusted to pH 5.0, plus 5 ml of the

**Table 1.** Disinfection treatments applied to the nodal segments of *G. angustifolia* Kunt.

Season	Culture medium	Disinfection of explants
Rains	MS	NaClO 3%
		NaClO 3% + azoxystrobin
		Gentamicin, Streptomycin, and Kanamycin + NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin + azoxystrobin and NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin
		azoxystrobin
	Yasuda	NaClO 3%
		NaClO 3% + azoxystrobin
		Gentamicin, Streptomycin, and Kanamycin + NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin + azoxystrobin and NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin
		azoxystrobin
Dry	MS	NaClO 3%
		NaClO 3% + azoxystrobin
		Gentamicin, Streptomycin, and Kanamycin + NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin + azoxystrobin and NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin
		azoxystrobin
	Yasuda	NaClO 3%
		NaClO 3% + azoxystrobin
		Gentamicin, Streptomycin, and Kanamycin + NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin + azoxystrobin and NaClO 3%
		Gentamicin, Streptomycin, and Kanamycin
		azoxystrobin

bactericides gentamicin, streptomycin, and kanamycin, and indole-3-acetic acid (IAA) as a growth regulator at  $2 \text{ mg L}^{-1}$ , were sterilized at 15 PSI and  $120 \text{ }^{\circ}\text{C}$  for 20 minutes.

### Environmental incubation conditions

The explants were placed in test tubes ( $150 \text{ mm} \times 20 \text{ mm}$ ) containing 10 ml of sterilized culture medium. They were then transferred to an incubation chamber at  $26 \pm 1 \text{ }^{\circ}\text{C}$ , 60% relative humidity, with a light intensity of  $45 \mu\text{E m}^{-2}$ , under a photoperiod of 16 hours light and 8 hours dark.

### Evaluation for response variables

Seventy-two hours after inoculation, the presence of fungi and bacteria was evaluated as indicators of explant contamination. Oxidation percentage was also visually assessed by the degree of tissue darkening (0, 25, 50, 75, 100%). After four weeks, survival was evaluated by assigning a value of 0 (dead) or 1 (alive) to each explant. In addition, the number of

shoots emerging from new explant tissue was recorded. All variables were monitored every eight days following explant inoculation.

### Data analysis

The data were analyzed using a completely randomized design with a factorial arrangement and 20 replications.

Data were processed in Excel to obtain simple statistics such as means and percentages. Values were plotted with Sigma Plot software (version 10.0, Jandel Scientific)  $\pm$  standard error.

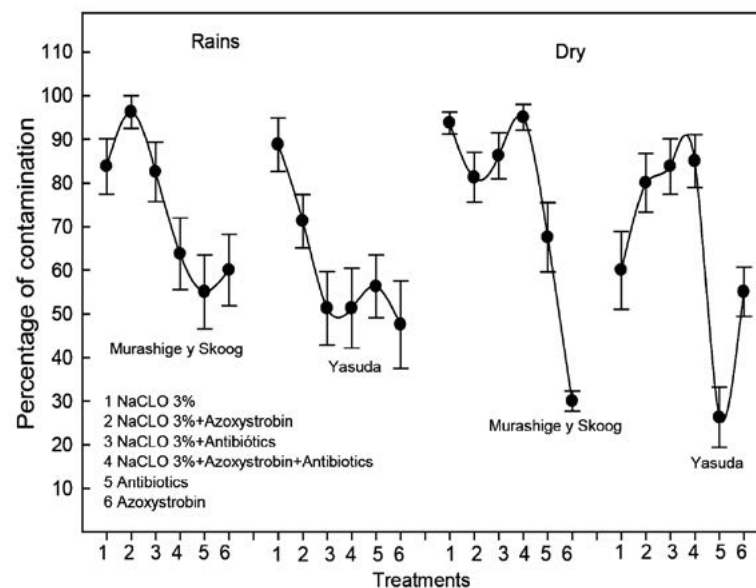
## RESULTS AND DISCUSSION

### Contamination percentage

On the third day after explant inoculation, contamination by bacteria and fungi was observed in both culture media. Contamination was identified by turbidity and a whitish appearance in the medium, as well as white and gray spores around the nodal segment.

Explants collected during the dry season showed lower contamination levels in both media when treated with azoxystrobin at  $1.5 \text{ g L}^{-1}$  (30%) in MS, and when supplemented with gentamicin, streptomycin, and kanamycin (26%) in Yasuda. However, explants placed in MS medium treated with antibiotics (gentamicin, streptomycin, and kanamycin), combined with 3% NaClO and azoxystrobin, exhibited the highest contamination level, reaching 93% (Figure 1).

In this regard, Nadha *et al.* (2012) reported that the use of kanamycin and streptomycin sulfate were the most effective antibiotics against bacteria, specifically *Pantoea agglomerans* and *Pantoea ananatis*. They also noted that kanamycin exhibited lower phytotoxic effects in the *in vitro* culture of this species.



**Figure 1.** Contamination dynamics in nodal segments of *G. angustifolia* Kunth explants under the interaction of two culture media and two collection seasons. Values represent means of 20 replications  $\pm$  standard error.

Explants obtained during the rainy season and cultured in MS medium showed the highest contamination level (95%) with the treatment of 3% NaClO plus azoxystrobin, while the lowest level (58%) was recorded under the antibiotic treatment.

In Yasuda medium, the highest contamination occurred with 3% NaClO alone (90%), whereas the lowest was observed with azoxystrobin (48%). For explants collected in the dry season and cultured in MS medium, the application of fungicide was the most efficient treatment to prevent contamination. Conversely, for explants collected during the rainy season and established in Yasuda medium supplemented with Gamborg vitamins, the addition of azoxystrobin ( $1.5 \text{ g L}^{-1}$ ) was the most effective treatment to control contamination levels.

Other authors have tested different approaches to reduce explant contamination. Jiménez *et al.* (2006) proposed using 10% NaClO for 10 minutes as an alternative for disinfecting nodal explants. Borges-García *et al.* (2004) recommended lowering NaClO concentration to 2% for 5 minutes, followed by a second treatment after 24 hours, achieving favorable results in the *in vitro* establishment of *G. angustifolia* explants. Meanwhile, Ramírez-Correa *et al.* (2014) reported that the most effective treatment for disinfection and sprouting in this species was 2% NaClO for 15 minutes. Overall, explant contamination varied according to the concentration and type of disinfectant used on *G. angustifolia* nodal segments, with harvest season exerting a decisive influence rainy season explants showing the highest contamination rates. This trend has also been observed in similar studies. Gielis & Oprins (2002) reported reduced microbial contamination during low rainfall periods in the *in vitro* establishment of bamboos. Similarly, Pérez-Alonso *et al.* (2015) found that, in the *in vitro* establishment of *Aloe vera* L. explants, dry-season collections combined with 2% NaClO yielded the highest number of contamination-free explants. In their study, microbial contamination rates ranged from 6.6% to 33.3% in the dry season, while in the rainy season contamination varied between 65% and 100%. Likewise, Ahmadpoor *et al.* (2022), working with *Melia azedarach* L., reported lower contamination levels when explants were treated with 2% NaClO (without pH adjustment) for 12 minutes, in combination with benomyl ( $2 \text{ g L}^{-1}$ ) for 2 hours and sterilization with 7%  $\text{H}_2\text{O}_2$  for 10 minutes.

### Percentage of oxidation

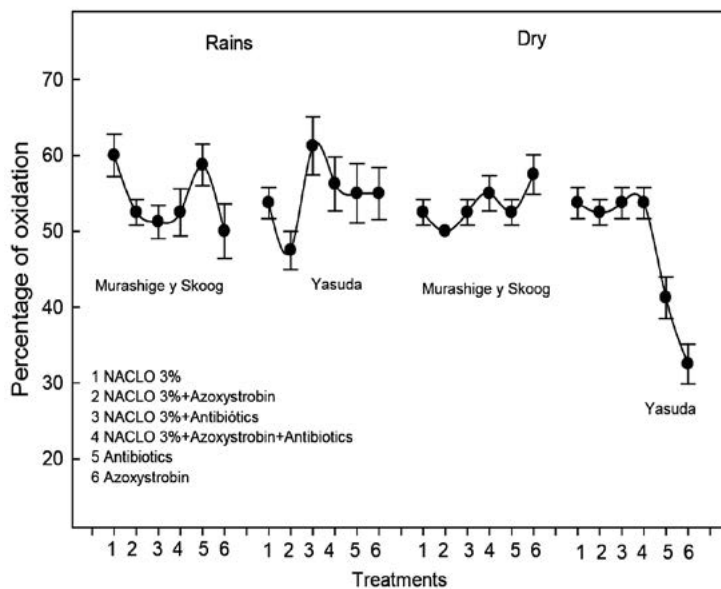
Explants collected during the dry season and cultured in MS medium exhibited oxidation levels between 51% and 58% across treatments, with the lowest value obtained using the combination of 3% NaClO plus azoxystrobin. In Yasuda medium, similar oxidation levels were observed among treatments with 3% NaClO, averaging 52%. The lowest oxidation levels of the entire study occurred when antibiotics were added to Yasuda medium, with an average of 45%, and further decreased to 33% when azoxystrobin was included. These results suggest that the combined use of antimicrobial agents and fungicides such as azoxystrobin enhances explant viability *in vitro*.

The reduction in oxidation can be explained by the lower presence of endogenous microorganisms, which typically induce the synthesis of phenolic compounds responsible for tissue browning (Ahmadpoor *et al.*, 2022; Nadha *et al.*, 2012). The accumulation of such compounds has been identified as one of the main limiting factors in the *in vitro* propagation

of woody species (Lambardi *et al.*, 2006). Furthermore, the effectiveness of Yasuda medium may be linked to its balanced mineral composition, which promotes physiological stability in plant tissues and reduces oxidative responses to disinfection-induced stress (Yasuda *et al.*, 1985). These findings are consistent with research conducted on other woody species (Espinosa-Leal *et al.*, 2018).

For explants collected during the rainy season and cultured in MS medium, the lowest oxidation level was observed with azoxystrobin alone (51%), while the highest (60%) occurred with 3% NaClO plus azoxystrobin. This difference suggests that the exclusive use of azoxystrobin may exert less phytotoxicity and act more specifically against fungal contaminants, thereby preserving tissue integrity. Under conditions of high precipitation, such as during the rainy season, explant sensitivity increases due to heightened metabolic activity, making them more susceptible to oxidation (Espinosa-Leal *et al.*, 2018). In the case of explants collected during the rainy season and cultured in Yasuda medium, the lowest oxidation was obtained with 3% NaClO plus azoxystrobin, whereas the highest occurred with 3% NaClO plus antibiotics (Figure 2). This effect may be attributed to the nutritional composition of Yasuda medium, which has proven more favorable for woody species by reducing susceptibility to oxidation in the presence of certain disinfectants (Yasuda *et al.*, 1985). Moreover, several studies have reported that antibiotics may cause secondary effects on uncontaminated tissues, inducing necrosis or oxidation through the alteration of key cellular functions (Ahmadpoor *et al.*, 2022; Nadha *et al.*, 2012).

Comparatively, studies conducted on *G. angustifolia* K. also show that combining antibiotics with NaClO can be counterproductive when no active bacterial infection is present, and instead recommend alternatives such as the use of PPM™ a mixture of isothiazolone compounds (*e.g.*, methyl-isothiazolinone) in aqueous solution designed to

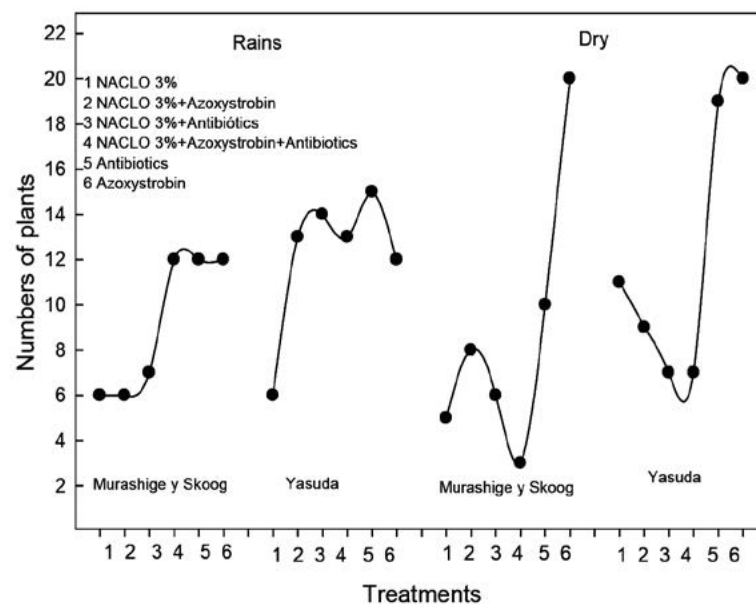


**Figure 2.** Oxidation dynamics in nodal segments of *G. angustifolia* Kunth under the interaction of two culture media and two different explant collection seasons. Values represent means  $\pm$  standard error, with 20 replications per treatment.

inhibit microbial growth without affecting plant tissues. This broad-spectrum commercial preservative has been shown to reduce contamination without increasing oxidation levels (Rodríguez & Rojas, 2020). Similarly, in species such as *Melia azedarach* and *Bambusa vulgaris*, azoxystrobin use has been associated with significant reductions in phenolic oxidation and explant necrosis (Ahmadpoor *et al.*, 2022; Singh *et al.*, 2015). These findings confirm that no single disinfection protocol is universally effective across species or environmental conditions. The choice of disinfectants must take into account the collection season, explant physiology, culture medium, and the predominant microorganisms, prioritizing treatments that minimize oxidation while ensuring effective decontamination. These results suggest that both culture medium selection and disinfectant agents are critical factors in minimizing oxidation during *in vitro* establishment of explants, particularly under high-humidity conditions. According to Azofeifa (2009), oxidation is the most frequent problem that can inhibit *in vitro* culture progression and even lead to tissue death, especially during the establishment phase of woody explants, due to metabolic reactions triggered by multiple factors. Such effects can be mitigated or prevented through the use of antioxidants or adsorbents of the released compounds.

### Survival

During the dry season, the highest explant survival was observed in MS medium with the treatment that included azoxystrobin, and in Yasuda medium when either azoxystrobin alone or antibiotics were applied. In both media, the number of surviving plants ranged between 19 and 20. The lowest survival rate was recorded when combining 3% NaClO + antibiotics + azoxystrobin (Figure 3).



**Figure 3.** Survival dynamics of seedlings from nodal segments of *G. angustifolia* Kunth under the interaction of two culture media and three explant collection periods. Values represent means of 20 replications  $\pm$  standard error.

During the rainy season in MS medium, survival increased to 12 explants with the simple treatment of 3% NaClO and when this was separately combined with azoxystrobin and/or antibiotics. However, the combined treatments showed the lowest survival values in this medium. In contrast, in Yasuda medium during the same rainy season, all treatments produced between 12 and 15 surviving explants, except for the treatment with 3% NaClO alone, which yielded the lowest value of 6 explants. No statistical differences ( $p \leq 0.05$ ) were found between MS and Yasuda media across treatments. Survival of explants was observed to depend primarily on contamination levels. According to Sierra *et al.* (1999), in their study on apical bud disinfection of *Philodendron xanadu*, explant survival reached 35% using calcium hypochlorite (2.0%) for 35 minutes. Similarly, Fajardo (2006) tested different disinfection times (5, 10, and 20 minutes) with 1.0% sodium hypochlorite in the *in vitro* establishment of *G. angustifolia* Kunth and found no statistical differences among treatments for explant contamination percentage or survival.

### Number of plant shoots

The number of shoots increased in MS medium compared to Yasuda, and among explant collection periods, the dry season showed higher values.

Within treatments, MS medium with dry-season explants produced 17 shoots under 3% NaClO treatment, which decreased to 11 when combined with 3% NaClO, antibiotics, and azoxystrobin. The number of shoots was further reduced to 9 with 3% NaClO plus antibiotics, and to 7 when antibiotics were replaced by azoxystrobin. In Yasuda medium, the highest value was six shoots in explants treated with 3% NaClO and in those treated with 3% NaClO combined with azoxystrobin and antibiotics. Similarly, in the *in vitro* culture of *M. azedarach*, improved callus induction was obtained using MS medium supplemented with  $5 \text{ mg L}^{-1}$  2,4-D and  $5 \text{ mg L}^{-1}$  kinetin (Ahmadpoor *et al.*, 2022). The presence of shoots

**Table 2.** Number of shoots in nodal explants of *Guadua angustifolia* Kunth.

Culture medium	Treatments	Explants cut during the rainy season	Explants cut in dry seasons
		Number of plant shoots	Number of plant shoots
MS	NACLO 3%	2	17
	NaCLO 3% + azoxystrobin	5	7
	NaCLO 3% + antibiotics	3	9
	NaCLO 3% + azoxystrobin	2	11
	+ antibiotics	2	2
	antibiotics	1	0
Yasuda	azoxystrobin	2	6
	NACLO 3%	0	3
	NaCLO 3% + azoxystrobin	0	0
	NaCLO 3% + antibiotics	2	1
	NaCLO 3% + azoxystrobin	3	3
	+ antibiotics	1	0

MS medium yielded the highest number of shoots from explants (Table 2).

in explants was influenced by the type and concentration of disinfectants. Contamination can be a limiting factor that inhibits shoot growth or emergence. Although shoot emission was recorded even in contaminated explants, growth was deficient and in some cases shoots died. Fajardo (2006), using different disinfection times of 5, 10, and 20 minutes with 1.0% sodium hypochlorite in the *in vitro* establishment of *G. angustifolia* Kunth, reported that five minutes of exposure to the disinfectant yielded the highest number of sprouted buds per explant, differing statistically from the other treatments.

## CONCLUSIONS

Explant collection season influences contamination, oxidation, regrowth, and survival. Contamination levels during both rainy and dry seasons decreased in both culture media when treatments included antibiotics and/or the fungicide azoxystrobin. Oxidation was reduced in explants collected during the dry season and cultured in Yasuda medium supplemented with antibiotics and/or azoxystrobin, which also improved survival.

Shoot regeneration was favored in explants collected during the dry season and cultured in MS medium with 3% NaClO plus antibiotics.

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# Taxonomic Identity of Lepidopteran Insects Associated with Mangrove Mortality in the “La Encrucijada” Biosphere Reserve, Chiapas, Mexico

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## ABSTRACT

**Objective:** To provide the responsible authority, CONANP Encrucijada, with the taxonomic identity of the folivorous larvae found on mangrove species and associated with their mortality.

**Design/Methodology/Approach:** Through field surveys in affected areas, larval specimens were collected for confinement, rearing, and observation until their metamorphosis into adults. External morphological characters were then reviewed, focusing on wing vein patterns, dorsoventral coloration, the presence of light and dark bands, and circular markings such as ocelli, to determine taxonomic identity using keys and image comparisons.

**Results:** The taxonomic identity of the collected larvae corresponded to two families within the order Lepidoptera: Nymphalidae and Hyblaeidae, and the species *Junonia evarete* (Cramer) and *Hyblaea puera* (Cramer), respectively.

**Study Limitations/Implications:** The presence of both species, each capable of consuming large amounts of foliar biomass while exploiting the same host, implies more severe damage to leaf tissue. This necessitates ongoing evaluation and monitoring to understand their impact on the optimal development and recovery of *Avicennia germinans*.

**Findings/Conclusions:** The results represent the first report of the distribution of these two Lepidoptera species in the La Encrucijada Biosphere Reserve. A comprehensive study on the impact of folivory on *Avicennia germinans* and its relationship with environmental degradation is urgently needed.

**Keywords:** folivory, mangrove mortality, taxonomic, coastal wetlands

## INTRODUCTION

Mangrove ecosystems develop in estuaries, lagoons, and river mouths along the coastlines of tropical and subtropical regions worldwide (Yáñez-Arancibia & Lara-Domínguez, 1999). These are areas of high ecological and socioeconomic value due to their rich biodiversity and productivity, driven by their ability to retain sediments and organic matter through the aerial root systems of mangrove species such as *Rhizophora mangle*. This facilitates the



development of numerous aquatic and terrestrial species, many of which are utilized by local communities for economic activities such as aquaculture, agriculture, and the extraction of tannins, wood for construction, and fuel (López *et al.*, 2020). It is estimated that mangrove ecosystems can produce from  $8.5 \text{ t ha}^{-1} \text{ yr}^{-1}$  (Félix-Pico *et al.*, 2006) to  $24 \text{ t ha}^{-1} \text{ yr}^{-1}$  (Sol-Sánchez *et al.*, 2022) of biomass, a figure considered high compared to forest (Návar-Cháidez & Jurado-Ybarra, 2009) and agricultural (Martínez-Romero & Leyva-Galán, 2014) systems. Loss of mangrove areas leads to a reduction in productivity, which is reflected in decreased fisheries output. For example, it is estimated that the loss of one hectare of mangrove may result in an annual loss of approximately 800 kg of shrimp (Sánchez-Hernández, 2002; Sol-Sánchez *et al.*, 2022). Various factors undermine the ecological stability of mangroves, including storms, hurricanes, and cyclones (Velázquez-Salazar *et al.*, 2021), which occur cyclically each year, causing significant loss of forest cover. However, mangroves also provide natural protection for the coastline and show high resilience to these meteorological events, which is positively related to the organic matter content in affected sites (Amaral *et al.*, 2023; Agraz *et al.*, 2015). At the local scale, biotic factors can also cause mortality of large mangrove areas. Reports frequently document the death of mangrove trees following severe defoliation caused by Lepidoptera larvae or stem boring by coleopterans and their symbiotic phytopathogens (Ortíz-Reyes *et al.*, 2018; Perdomo *et al.*, 2018). In the coastal regions of Brazil, atypical foliar herbivory events have been reported. Mehlig and de Menezes (2005) documented severe herbivory in *Avicennia germinans* (Cramer) caused by *Hyblaea puera* (Cramer) (Hyblaeidae) in the Ajuruteua Peninsula in 1998. In 2016, 66% of mangroves (approximately 20,300 ha) dominated by *A. schaueriana* (Stapf & Leechman) were defoliated by the same insect in Paraná (Ditzel-Faraco *et al.*, 2019). In Mexico, *Anacamptodes* sp. (McDunnough) (Geometridae) was studied in the municipality of Cárdenas, Tabasco, due to its folivory on *A. germinans* (Cramer), the dominant mangrove species (99.6%). The biomass consumed by this lepidopteran exceeded 57% of a 50-ha sample within a total affected area of 3,841 ha in 2011 (Sol-Sánchez *et al.*, 2015). More recently, in the same municipality at Laguna Mecocacán, Vázquez-Vázquez *et al.* (2024) reported intense herbivory on *R. mangle* (L.) and *Laguncularia racemosa* (L.-C.F. Gaertn) caused by the lepidopteran *Hylesia colimatifex* (Dyar) (Saturniidae). While folivory by these Lepidoptera species often occurs as part of the natural energy flow dynamics, epidemic or atypical outbreaks have been linked to progressive mangrove degradation, hydroperiod variation, hydrological flow changes, and meteorological events (Saur *et al.*, 1999; Elster *et al.*, 1999). In the mangroves of the La Encrucijada Biosphere Reserve (RBLE), Chiapas, technical personnel from the managing agency CONANP reported, following monitoring conducted between September and November 2024, a total of 187 ha of mangroves affected by Lepidoptera larval folivory. This represents 0.3% of the RBLE's total area (144,868 ha). The wetlands in this region are among the most productive, with the tallest mangrove trees on the Pacific coast of the Americas, reaching heights of up to 35 meters. Their productivity supports and shelters endemic fauna such as the bird *Campylorhynchus chiapensis* (Salvin & Godman), as well as key mammal species like the jaguar (*Panthera onca* L.), spider monkey (*Ateles geoffroyi* vellerosus Griss), and white-tailed deer (*Odocoileus virginianus* Zimm.), along with numerous commercially important

fish species such as mojarra (*Cichlasoma* sp. Swainson), mullet (*Mugil cephalus* L.), and gar (*Lepisosteus tropicus* T. N. Gill) (CONANP, 1999). Therefore, it is crucial to conduct basic scientific studies to identify the biotic factors compromising the ecological stability of the RBLE. The folivory caused by Lepidoptera larvae on mangrove trees is an atypical event, and the populations of these causal agents Lepidoptera larvae had not previously been studied in the RBLE. As such, their taxonomic identity was unknown. The aim of this work was to provide the authorities in charge of the reserve (CONANP) with the formal taxonomic identification of the Lepidoptera larvae responsible for mangrove folivory, as a first step toward guiding ecological studies, population control, and informed management strategies to mitigate their impact on mangrove ecosystem health.

## MATERIALS AND METHODS

### Sampling site

Biological material collections for the identification of the causal damage agent were conducted at the sites Embarcadero Las Garzas (15° 12' 10.5" N; -92° 48' 54.2" W) and Santa Isabel (15° 15.37' 23" N; -92° 54.48' 52.0" W) (Figure 1B and 1A. Geographical location map), where the predominant vegetation is mangrove forest composed of the species *Rhizophora mangle* (L.), *Laguncularia racemosa* (L.-C.F. Gaertn), *Conocarpus erectus* (L.), and *Avicennia germinans* (L.).

### Field collections:

Extensive surveys were conducted at two locations where mangrove tree defoliation had been reported. The first site, "Embarcadero Las Garzas" (Figure 1B. Geographical location map), included seven inspection points, and the second site, "Santa Isabel" (Figure 1B. Geographical location map), also included seven inspection points. The purpose was to record the mangrove species exhibiting defoliation and mortality. Additionally, larvae feeding on mangrove leaves were collected and transported alive to the CONANP station in La Encrucijada.

### Larval rearing:

The collected larvae were placed in one-liter jars with leaves from their host plant. The jar lids were perforated to allow air exchange. Leaves were replaced every three days until the larvae pupated. Upon successful emergence, adult Lepidoptera were transferred to waxed paper bags with their wings folded to minimize friction that could damage scale patterns and wing vein structures.

### Adult identification:

Emerging adults were removed from the waxed paper bags and mounted on a stretcher to allow clear observation of the anterior and posterior wing veins. Taxonomic identification was performed using taxonomic keys and comparison with digital images of adults, focusing on wing vein patterns, circular spots, and coloration (Comstock, 1918), as well as other external morphological traits such as antenna type (García-Barrios, 2015).

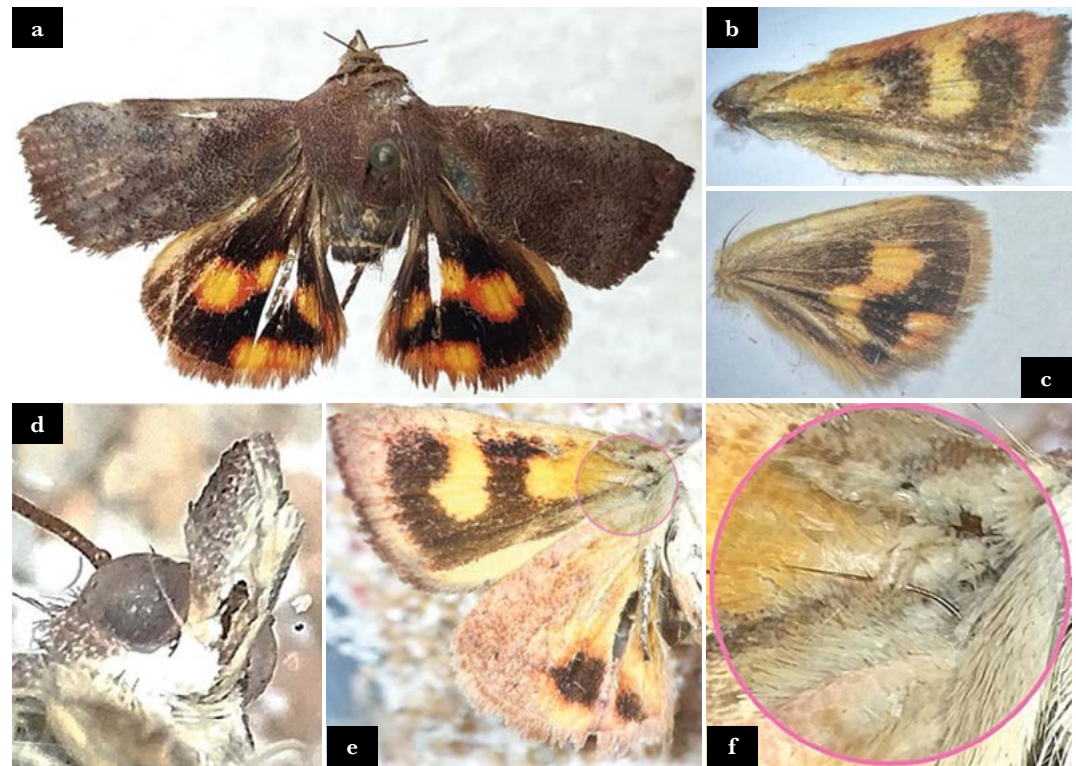


**Figure 1.** Geographic location map of the sites: A) Santa Isabel B) Las Garzas Piers.

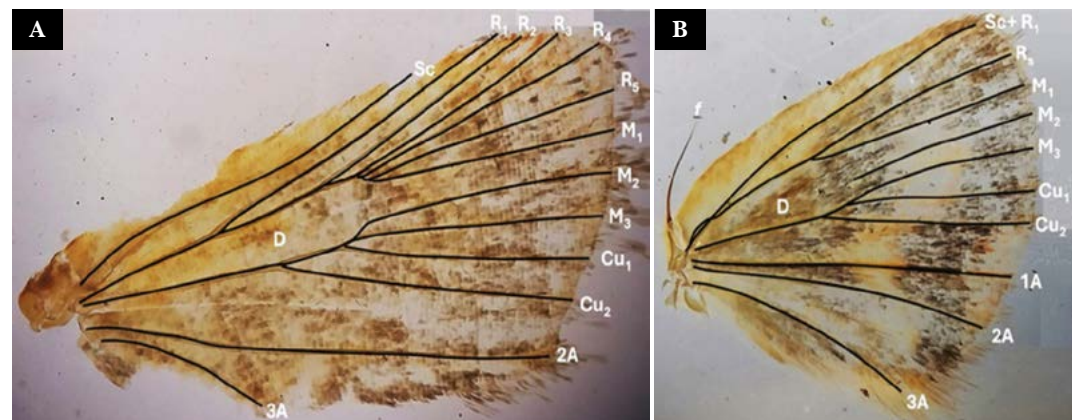
## RESULTS AND DISCUSSION

The defoliation observed during the surveys consistently affected *Avicennia germinans* trees, from which all specimens ranging from early larval stages to the final stage before pupation were collected. During the Lepidoptera larval defoliation alert, some larvae were collected at the end of August 2024 for taxonomic identification. A total of 25 larvae were collected and transported to the CONANP-Encrucijada station. After an average rearing period of ten days, 15 larvae reached the pupal stage, and of these, five successfully emerged as adults within 4 to 7 days. The successfully emerged adults were used for taxonomic identification, resulting in two Lepidoptera species: *Hyblaea puera* (Cramer) (Hyblaeidae) and *Junonia evarete* (Cramer) (Nymphalidae). Larvae of *H. puera* (Cramer) are eruciform in type, with an elongated, cylindrical body lacking urticating hairs and exhibiting a grayish-green coloration. The pupa is shiny reddish in color, smooth in appearance, and slightly curved. The adult (Figure 2. *Hyblaea puera* (Cramer)) has filiform antennae, a head with a developed proboscis, and maxillary palps. The wings span an average of 3.5 cm, with distinctive coloration between dorsal and ventral sides (Figure 2a-c), as well as between forewings and hindwings. Dorsally, the forewings are grayish brown, while the hindwings are black (Figure 2a), featuring irregular transverse orange bands extending from the inner edge of the wing to about three-quarters of the way outward. On the ventral view, a wing coupling system is observed, consisting of a jugum (a lobe on the forewing) and a frenulum (a spine on the anterior edge of the hindwing) (Figure 2e-f).

The vein configuration (Figure 3. Wing vein structure in *H. puera* Cramer) in the forewing shows the first branch of the median vein (M1) fused with the last branch of the radial vein (R5), and the median vein M2 joined with M3, resulting in an open discal cell. Additionally, a third branch of the anal vein is present (Figure 3A). In the hindwing, the median vein M2 is free, and the median vein M3 is joined with the cubital vein Cu1, also resulting in an open discal cell (Figure 3a-B-D). Three anal veins (1A, 2A, and 3A) are present (Figure 3B).



**Figure 2.** Adult *Hyblaea puera* (Cramer): a. Wing spread showing differential coloration between forewing and hindwing. b-c. Ventral view of the forewing and hindwing, respectively. d. Developed proboscis. e-f. Coupling structures: frenulum and jugum.

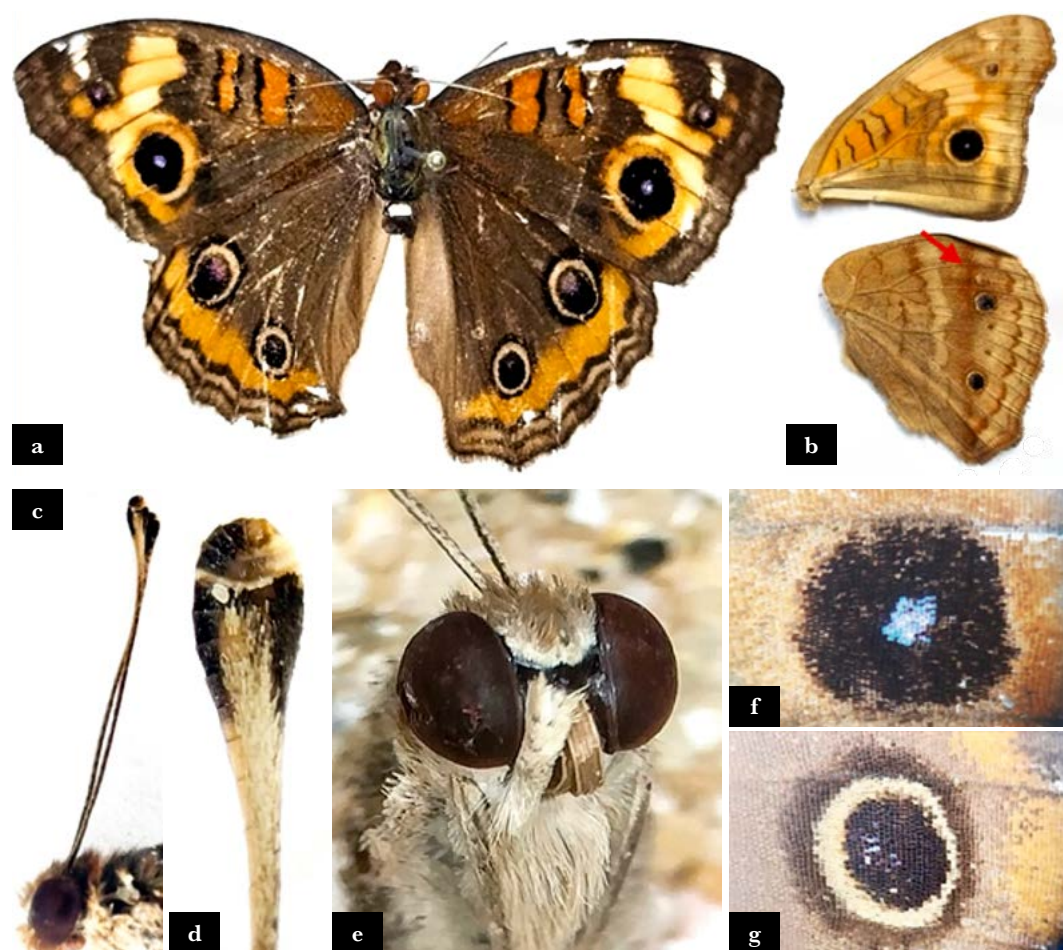


**Figure 3.** Wing vein structure of *H. puera* (Cramer). A. Forewing. B. Hindwing.

In *Junonia evarete*, the cruciform larvae are black with orange spots along the sides; the cylindrical body is adorned with spines. The pupa is grayish with spiny projections on the thorax and attaches to the substrate via a cremaster. The adult (Figure 4. *Junonia evarete* adult) has antennae on the head that are clubbed at the tip, black dorsally and light brown ventrally (Figure 4c-d), and also features well-developed palps and proboscis (Figure 4e). The wingspan measured 4.8 cm. The wings are black (Figure 4a) with distinguishable

morphological features such as two short orange bands along the costal margin of each forewing (Figure 4b, upper), while each hindwing displays two transverse bands along the inner margin one wide orange band followed by a narrow light brown band both bordered by black lines (Figure 4b, lower). Additionally, the wings show, on the dorsal side and near the inner margin, circular ocellus-like spots of various sizes (Figure 4a). On the forewings, a small ocellus is located between the median veins M1 and M2, and a larger one between the cubital veins Cu1 and Cu2. On the hindwings, two ocelli appear near the inner margin (Figure 4b), the larger one between veins M1 and M2, and the smaller between Cu1 and Cu2, similar to the forewings (Figure 4a). Each ocellus contains centrally iridescent purple scales (Figure 4e-f). All ocelli are visible in the ventral view of the wings, where a third ocellus (Figure 4c) appears on the hindwings, bordered by a faint black line and located between veins Rs and M1.

The vein configuration (Figure 5. Wing vein structure of *Junonia evarete* Cramer) in the forewing shows the median vein M2 joined with vein M1, and the median vein M3



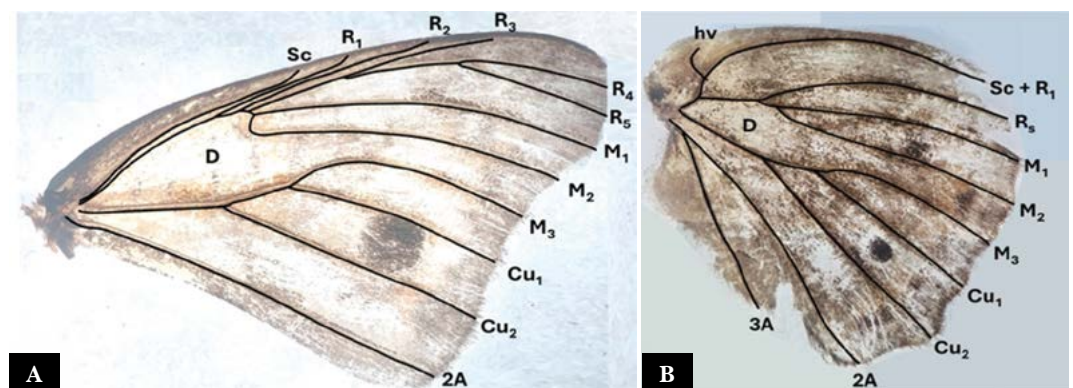
**Figure 4.** Adult *Junonia evarete* (Cramer). a. Wing spread showing the pattern and differential coloration between forewings and hindwings. b. Ventral view of the forewing (upper) and hindwing (lower). c-d. Clubbed antenna. e. Proboscis. f-g. Ocellus-like spots with light blue iridescent scales. Red arrow indicates the third distinguishable ocellus on the hindwing.

joined with the cubital vein Cu1, resulting in an open discal cell (Figure 5A-D). The same configuration occurs in the hindwing, where two anal veins, 2A and 3A, are also present (Figure 5B).

Insects, through herbivory and their various strategies to access resources, fulfill the function of reintegrating nutrients into the ecosystem by consuming biomass from leaves, branches, stems, or roots of living trees in stages of degradation, decline, or weakening. However, an increase in the intensity of herbivory particularly folivory over extended periods prevents the host plant from recovering lost vegetative tissue, thereby reducing its fitness, production of defensive secondary metabolites, and ultimately leading to death (Marquis, 1992; Návar-Cháidez, 2009).

In mangrove ecosystems, folivory occurs at all stages of host development and is mainly carried out by crustaceans of the genus *Aratus* sp., insects from the order Orthoptera (Families: Tettigoniidae and Acrididae), and Lepidoptera larvae. In this latter group, the intensity of foliar biomass consumption may be enhanced in communities with varying levels of environmental degradation (Gómez-García *et al.*, 2015). Factors such as pH alteration, salinity, and dissolved oxygen in interstitial water affect nutrient availability for mangroves, which in turn compromises their defensive capacity by limiting the production of chemical compounds that reduce leaf palatability to herbivores (Tong *et al.*, 2001; Agraz Hernández *et al.*, 2022). This creates conditions conducive to population outbreaks uncontrolled population explosions sustained as long as the susceptible resource remains available.

The Lepidoptera species *Junonia evarete* (Cramer) is distributed throughout the Neotropical zone of the Americas, ranging from Florida (USA), through the Caribbean, Mexico, Central America, to tropical and subtropical South America. This species is commonly found in secondary vegetation, disturbed environments, and riparian forests (Calhoun, 2010). Although previously unreported in the RBLE, its presence is plausible and suggests that it is part of the local ecosystem. It is worth noting that, in general, the taxonomic identification of species within this genus has not been fully resolved due to incomplete speciation, which results in a high rate of hybridization among species. Recent genomic studies have not included coastal populations from the southeastern Mexican



**Figure 5.** Structure of the wing veins of *J. evarete* (Cramer). A. anterior wing. B. hind wing.

Pacific (Coing *et al.*, 2020), highlighting the need for genetic research including these populations to confirm the identity of *J. evarete* (Cramer) in the RBLE. Regarding *Hyblaea puera* (Cramer), this species originates from Southeast Africa. According to FAO (2007), it is distributed across Southern and Eastern Africa, Northern Australia, China, and is also present in the southern U.S. states of Arizona, Texas, and Florida, as well as in the Mexican states of Campeche, Tabasco, and Veracruz, throughout Central America, and down to Paraguay. It inhabits tropical and subtropical forests and is commonly referred to as a major pest in *Tectona grandis* (L.) plantations, where its voracious feeding during the rainy season can reduce commercial wood volume by up to 44% (Cibrián, 2013). *H. puera* (Cramer) larvae exhibit similar behavior when feeding on *Avicennia germinans* (L.), having caused massive mortality of this mangrove species along the coast of Brazil (Mehlig & de Menezes, 2005).

The presence of *H. puera* (Cramer) in the RBLE, as with *J. evarete* (Cramer), has not previously been reported, and it is possible that the species was introduced via teak plantations in the area. Given its status as a potentially introduced species, it is essential to study its biology, ecology, and population dynamics within the region to determine its population status (endemic, epicentric, or epidemic) and to anticipate control strategies to mitigate its impact on *A. germinans* (L.). The presentation of wing vein structures aims to complement the morphological description of each species. Notably, the open discal cell (D cell) observed in both forewings and hindwings may represent a potential identification trait within this taxonomic group, although its diagnostic value remains tentative in the absence of a definitive reference.

## CONCLUSIONS

The identity of the larvae collected and reared for taxonomic identification as adults corresponds to the species *Junonia evarete* (Cramer) and *Hyblaea puera* (Cramer), which are reported for the first time through this study in the coastal zone of the southern Mexican Pacific. Given their potential to consume large amounts of foliar biomass, the co-occurrence of both species in the same site implies a greater impact of folivory on *Avicennia germinans* (L.). Therefore, studies are needed on their biology, ecology, and population dynamics, as well as on the estimation of herbivory impact across different developmental stages of the host. In addition to research focused on the causal agents of folivory, it is recommended to include diagnostic studies to evaluate the degree of environmental degradation that may better explain the causes of atypical (epidemic) herbivory observed in *J. evarete* (Cramer) and *H. puera* (Cramer) populations in the RBLE. This would allow for the establishment of severity criteria and the development of guidelines for their study, control, and informed management, aimed not only at reducing the impact on *A. germinans* (L.) but also at improving the environmental conditions that compromise the health of mangrove ecosystems.

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# By-Product and Residues of Tropical Crops as a Feed Source for Ruminants

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## ABSTRACT

**Objective:** To analyze the available information on the use and nutritional characteristics of tropical agro-industrial by-products with potential application in ruminant feeding.

**Design/Methodology/Approach:** A literature review was conducted on agricultural by-products of tropical origin used in ruminant diets, identifying nine residues commonly produced in the tropical region of southeastern Mexico.

**Results:** The review was performed using databases such as Google Scholar and Redalyc, with the search term “tropical agricultural residues with nutritional potential for ruminants.” The results were extensive and subsequently refined to include updated publications focused on agricultural by-products.

**Limitations/Implications:** No limitations or specific implications were identified.

**Findings/Conclusions:** Incorporating agro-industrial by-products and agricultural residues available in the region into ruminant diets offers a viable alternative for enhancing livestock production. This approach reduces dependence on conventional feed resources while minimizing animal feeding costs.

**Keywords:** By-products, livestock, agricultural waste, tropical, ruminants.

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## INTRODUCTION

According to the Servicio de Información Agroalimentaria y Pesquera (SIAP), national live cattle production in 2023 reached 4,002,013.02 t, with the main producing states being Veracruz (525,610.59 t), Jalisco (472,346.45 t), Chiapas (210,282.60 t), San Luis Potosí (231,832.14 t), and Sinaloa (197,460.16 t). In sheep farming, live sheep production was 132,328.71 t, with the highest contributions from Estado de México (18,139.97 t), Hidalgo (13,484.40 t), Veracruz (11,751.08 t), Jalisco (10,186.33 t), and Zacatecas (8,660.80 t) (SIAPa, 2023). In Mexico and other Latin American countries, livestock production is of great importance both as a source of economic income and



to meet the nutritional needs of the population (Martínez González *et al.*, 2017). For fattening purposes, the use of concentrates under confinement systems is a viable option when pasture is of poor quality or during the dry season when forage production and nutritional value decline (Desdémona Martínez, 2023). However, feeding animals with concentrates is often not economically viable for producers, especially in Latin America (Ramírez de la Ribera *et al.*, 2017). Therefore, the use of agricultural residues for ruminant feeding has proven to be an excellent alternative as feed ingredients (Guerra Italo, 2020). The use of agro-industrial and agricultural by-products from local production represents a cost-effective strategy, while also providing essential nutrients to meet the nutritional requirements of animals at different productive stages (García Ceballos, 2021). In tropical regions of Mexico, several crops are particularly relevant. According to SIAP (2023), their production included: oil palm (1,392,671.63 t), banana (2,642,338.39 t), mango (2,299,983.41 t), sugarcane (55,589,515.48 t), coffee (1,058,862.35 t), cacao (29,047.40 t), maize (27,549,917.53 t), soybean (199,162.60 t), and sorghum (4,815,930.91 t), among others (SIAPb, 2023). These crops generate agro-industrial by-products and agricultural residues such as bagasse, seeds, pulp, husks, green material residues, and stubble, which, when properly managed, can be used as alternative ingredients in ruminant diets (Vargas Corredor & Pérez Pérez, 2018).

## MATERIALS AND METHODS

The initial search was conducted using the criterion “tropical agricultural residues with nutritional potential for ruminants” in the Google Scholar and Redalyc databases, which yielded a large number of scientific articles and publications from various journals. The search was then refined to focus on the most relevant by-products produced in southeastern Mexico. Each by-product was described in terms of its nutritional composition, highlighting its production significance and use in ruminant feeding.

### Agro-industrial by-products of tropical crops

#### Banana

From banana bunch harvests, only 20-30% of the biomass is used, while 70-80% remains underutilized (Table 1), consisting mainly of the plant stem with its leaves, the floral bud, the rachis, and the peel, which represents 35-40% of the fruit (Rojas *et al.*, 2019). Proximate chemical analysis of ripe, fresh banana peel reported the following composition:

**Table 1.** Chemical composition of different parts of the banana plant.

Bromatological analysis	Leaf	Stem	Spine
Crude protein (%)	7.62	4.38	4.52
Crude energy (Mcal/kg)	4.49	4.17	4.6
Ether extract (%)	5.01	3.96	3.92
Ash (%)	11.14	16.15	17.65
Neutral detergent fiber (%)	45.34	39.04	54.46
Total dry matter (%)	13.79	3.3	7.07

Source: Vera Rodríguez *et al.*, 2021.

crude protein (CP) 5.93%, ether extract (EE) 40.28%, crude fiber (CF) 6.92%, ash 11.57%, moisture 87.22%, nitrogen-free extract (NFE) 25.30%, and gross energy (GE) 14,371.74 kJ/kg (Benítez Meza *et al.*, 2020). From an energy standpoint, its nutritional value is noteworthy, given the high dry matter content, elevated concentration of non-fibrous carbohydrates, and its potential as a source of antioxidants and antimicrobial compounds, including anthocyanins and carotenoids (Cornejo-Cornejo *et al.*, 2020). Banana peel contains a high concentration of reducing sugars, making it a valuable source of soluble carbohydrates for ruminant feeding, suitable for use either fresh or ensiled (Valverde Lucio *et al.*, 2024). In another study, banana rachis was used for silage to assess its bromatological quality. The silage mixture included 95% rachis, 5% molasses, and lactic acid bacteria (commercially known as Silobacter) to enhance its nutritional profile. The results showed: CP 5%, EE 3.7%, ash 11.39%, CF 18.07%, and NFE 23.74%. The study concluded that banana rachis silage is viable for ruminant feeding, with lactic acid bacteria improving silage quality (Macay Anchundia *et al.*, 2023). The nutritional characteristics of banana by-products represent a valuable alternative for animal feeding, particularly for ruminants due to their ability to efficiently utilize fiber, while also providing protein and energy. Banana leaves, suckers, and rachis contain adequate levels of crude protein, gross energy, ether extract, ash, and dry matter, making them suitable primarily as forage in ruminant diets (Vera Rodríguez *et al.*, 2021).

### **Sugarcane**

Agricultural residues generated from sugarcane cultivations such as tops and green leaves along with by-products from processing, including bagasse, filter cake, molasses, and vinasse, represent valuable alternatives for animal feeding, particularly during drought periods when pasture availability is reduced. These residues become essential supplements in ruminant diets (Salazar Ortiz *et al.*, 2017). Sugarcane stalks, tops, green leaves, and shoots contain on average: dry matter (DM) 19.3%, crude protein (CP) 4.3%, ash 7.4%, neutral detergent fiber (NDF) 61.2%, and acid detergent fiber (ADF) 38.3%. These characteristics highlight their potential as feed resources for animal nutrition (Hurtado *et al.*, 2021). Bagasse, obtained after juice extraction, can be used as ruminant feed because it retains about 50% of the sugars and provides a significant fiber source (Lagos Burbano & Castro Rincón, 2019). Its composition includes: DM 89.90%  $\pm$  4.6; CP 3.81%  $\pm$  0.07; crude fiber (CF) 37.89%  $\pm$  3.6; NDF 75.6%  $\pm$  3.7; ADF 58.8%  $\pm$  4.9; ether extract (EE) 0.50%  $\pm$  0.1; ash 4.87%  $\pm$  0.07; cellulose 38.4%; hemicellulose 23.2%; and lignin 25%. The fiber fractions (CF, NDF, and ADF) provide a source of energy for ruminant diets, while the protein content, though modest, can contribute to improved milk and meat production when properly supplemented (Jácome-Pilco *et al.*, 2023).

### **African or oil palm**

Three commercial products are obtained from the industrial processing of the oil palm fruit: crude palm oil, which results from the extraction of the fruit's mesocarp; palm kernel oil, which results from the extraction of the fruit's kernel; and palm kernel flour, which is a residual byproduct obtained from extracting the oil from the endocarp

(Torres Mejía, 2023). Palm kernel flour contains a high fiber content, its protein content is 14-15%, and its digestibility is between 50-60%. These characteristics make it viable for use in the production of feed for broiler chickens, ruminants, and pigs, coupled with its low economic cost and abundance in the region (Da Silva Rosa & Castillo Vargas, 2022). Among the main nutritional values provided by palm kernel meal are: DM (92.23%), CP (10.55%), ME (3000 kcal), NDF (76.56%), EE (7.27%) (Escobar-España *et al.*, 2022). In a study on lambs, the inclusion of palm kernel meal at 0, 15, 30, and 45% in the diet was evaluated. According to the results, using 20% inclusion levels in the diet did not affect production yields or meat quality (Escobar España *et al.*, 2023). Nutritional blocks were evaluated as feeding alternatives. The treatments were: T0 or control (Grazing with *Brachiaria* grass and mineral salts), T1, T2 and T3, supplemented with multinutritional blocks, which included ingredients such as molasses, urea, mineral salts, common salt, lime, cement and palm oil, varying in their content in coconut flour at 30% and palm kernel flour at 30% in treatments 2 and 3 respectively, based on the results it was concluded that the use of multinutritional blocks improves milk production, attributable to the higher consumption of energy and nitrogen, highlighting treatment 3 with 30% palm kernel flour and showing significant differences compared to treatment 1 and 2 which were similar (Villanueva Pedraza *et al.*, 2023).

### **Mango**

During mango fruit processing, waste generation ranges from approximately 32% to 50%. Additionally, the loss of fresh fruit due to postharvest handling and marketing issues constitutes a significant source of environmental contamination, particularly from the peel, seed, and pulp residues (Pacheco Jiménez *et al.*, 2022). In one study, mango waste samples (peel, pulp, and seed) were collected to perform a proximate chemical analysis. Five parameters were evaluated: Dry Matter (DM) (27.33%), Crude Protein (CP) (7.19%), Crude Fiber (CF) (12.70%), Ether Extract (EE) (3.11%), and Ash (1.99%). The study concluded that mango waste can be used as an alternative feed source for animals, provided either in the form of silage or combined with other ingredients such as vegetable or fruit residues, with additions of molasses and urea in proportions of 2% to 2.5% to enhance protein content and accelerate fermentation (Gómez Peña, 2019). In another experiment, mature discarded mangoes were used to produce silage combined with pangola grass hay in a ratio of 86% mango and 14% grass. Bromatological analysis of the silage revealed the following composition: CP (4.29%), Organic Matter (OM) (94.4%), Neutral Detergent Fiber (NDF) (64.74%), Acid Detergent Fiber (ADF) (36.14%), Hemicellulose (28.6%), and Ash (5.6%). The Metabolizable Energy (ME) was obtained through an *in vitro* fermentation process and yielded 1.86 Mcal kg<sup>-1</sup> DM. The study concluded that producing silage with 86% mature mango and 14% pangola hay could be considered a non-conventional feeding alternative for ruminants in tropical regions (Cañaveral Martínez *et al.*, 2020). Nutritional Blocks (NBs) represent an alternative feeding strategy in goat production and can incorporate regional ingredients such as mango. In one study, NBs were formulated using mango pulp (MP) and sugarcane molasses at 0%, 10%, 20%, and 30%. The NB ingredients included urea, salt, mineral mix, cement, pangola hay, coconut meal, ground corn cob, sugarcane

molasses, and MP. Bromatological analysis of the NB with 30% mango pulp showed the following composition: DM (91.89%), CP (36.16%), NDF (16.58%), ADF (13.32%), and Ash (28.79%). Additionally, animals were provided with 1 kg of pangola hay. The experiment concluded that supplementation with nutritional blocks containing varying levels of mango pulp did not affect productive performance, and thus, their use is recommended for animal supplementation (Manuel Luviano *et al.*, 2017).

### Coffee

During the processing of coffee beans and consumption of the beverage, a substantial number of by-products is generated (peel, pulp, husk, and coffee pulp residue). The coffee fruit contains two beans covered by a thin husk and surrounded by pulp. During the roasting process, a silver-colored husk (the bean's tegument) is produced. Once roasted and ground, the beans undergo heat or steam treatment to extract coffee essence, leaving behind a residue known as coffee pulp (Terán Rivera *et al.*, 2023).

Coffee pulp represents a viable alternative feed for animals, enabling producers to reduce feeding costs (Fernández Navarro *et al.*, 2024). Its nutritional value is comparable to high-quality tropical forages and can be provided to animals either dehydrated or ensiled, with ensiling being the most suitable preservation method (Table 2). In dairy-producing animals, coffee pulp can be included at levels of 20% to 40% in balanced diets or 10% to 20% of the dry base ration without affecting productive performance (Flórez Delgado & Rosales Asensio, 2017). In dairy cattle, it can replace up to 20% of commercial concentrates without compromising yields (Encalada *et al.*, 2018). In intensive beef cattle fattening systems, inclusion levels can reach 20% to 30% of the diet (Cárdenas Solano *et al.*, 2024). It has been demonstrated that dietary antioxidants benefit semen quality. Coffee pulp contains antioxidants that may reduce oxidative damage during semen cryopreservation. Therefore, a study evaluated the antioxidant properties of coffee pulp in a diet for rams, with a 5% inclusion rate. Semen was collected regularly during the experiment for analysis. The study concluded that the inclusion of coffee pulp did not significantly affect the evaluated variables; however, further research with inclusion rates above 5% is recommended (Nieto Aquino *et al.*, 2021).

**Table 2.** Nutrient results of ensiled coffee pulp at different fermentation times.

Nutrients (%)	0 (days)	90 (days)	120 (days)	240 (days)	Means
DM	87.30	95.53	86.16	88.10	89.27
Ash	9.12	12.46	22.12	23.80	16.87
OM	90.88	87.53	77.91	76.93	83.31
EE	3.86	3.27	3.24	3.02	3.34
CP	3.87	25.18	30.52	25.82	21.35
CF	22.86	22.53	35.88	36.42	29.42
NFE	60.29	26.55	8.21	10.93	26.50
Tan	0.06	0.23	0.30	0.34	0.23

0, 90, 120, 240 d, days; DM, Dry Matter; MO, Organic Matter; EE, Ether Extract; CP, Crude Protein; CF, Crude Fiber; NFE, Nitrogen-Free Extract and Tan, Tanin. Fuente: Flórez Delgado & Rosales Asensio, 2017.

In another study, the nutritional composition of coffee pulp silage was evaluated at different fermentation durations (30, 45, and 90 days), concluding that fermentation time does not significantly affect the nutritional parameters of the silage (Flórez Delgado, 2020).

### **Cocoa**

In cocoa production, only the sedes which account for approximately 20-23% of the pod are utilized, primarily for chocolate manufacturing. The remaining biomass, including the pod husk composed of endocarp, mesocarp, and exocarp (67-76% of the fruit), along with mucilage, leachate, and hull, is discarded as waste (Vega González *et al.*, 2024). A proximate chemical analysis and *in vitro* digestibility (based on dry matter) of various cocoa residues revealed the following composition for cocoa hulls: Dry Matter (DM) (91.1%), Crude Protein (CP) (23.6%), Total Digestible Nutrients (TDN) (66.35%), Net Energy (NE) (1.51 Mcal/kg), Neutral Detergent Fiber (NDF) (28.7%), Crude Fiber (CF) (32.3%), Ether Extract (EE) (10.6%), and Ash (8.9%). The study concluded that cocoa hulls exhibit considerable protein potential and represent a viable alternative for cattle feeding in tropical regions (Godoy Padilla *et al.*, 2020). The nutritional value of the “Arriba” variety cocoa husk, according to a proximate analysis on a dry matter basis, was as follows: DM (48.91%), CP (8.15%), Gross Energy (GE) (4.61 Mcal/kg), Acid Detergent Fiber (ADF) (32.22%), NDF (41.45%), Nitrogen-Free Extract (NFE) (49.94%), Fat (6.20%), and Ash (9.80%). García Villoslada *et al.* (2022) note that cocoa harvest by-products offer potential as ingredients in multi-nutritional blocks due to their protein and fiber content, as well as their high palatability for livestock, without negatively impacting weight gain or milk production. In a separate study, a balanced feed was formulated by incorporating 0%, 20%, 30%, and 40% cocoa husk flour and nacedero leaf meal, aimed at potential use in beef cattle. Bromatological analysis of the concentrate showed that the 40% inclusion met the nutritional requirements established by the NRC, and the nutritional profile of cocoa husk supports its feasibility as an ingredient in animal feed (Molina Pinza *et al.*, 2024).

### **Citrus By-products**

Citrus production in Mexico is a vital agricultural activity for both domestic consumption and export markets. The principal crops include orange, lime, mandarin, and grapefruit (Valencia Sandoval & Duana Ávila, 2019). According to SIAP (2023), the leading citrus-producing states are Veracruz, Michoacán, Tamaulipas, Puebla, San Luis Potosí, Nuevo León, Oaxaca, and Yucatán. Nationally, orange production reached 4,942,658.65 tons, lime 3,239,914.70 tons, mandarin 309,740.49 tons, and grapefruit 488,140.72 tons (SIAPc, 2023). Fermenting citrus by-products through the addition or inoculation of fungal strains specifically *Aspergillus niger* presents a promising method for biodegrading these residues and converting them into feed for large livestock, particularly cattle (Marin Machuca *et al.*, 2021). In one study, orange silage was included in dairy cattle feed, replacing 20% of commercial concentrate. The results indicated that orange silage not only enhanced milk composition but also reduced production costs (Flórez Delgado *et al.*, 2020). In another research trial, a concentrate for fattening steers was developed by incorporating citrus by-products across four treatments: T1 (control), T2 (with orange bagasse), T3 (with orange

peel), and T4 (based on orange silage). The findings demonstrated that the inclusion of citrus by-products improved weight gain, enhanced palatability, and promoted ruminal microflora activity by increasing the production of beneficial biochemical compounds, thereby enriching the substrate (Cabrera Núñez *et al.*, 2020).

### **Pineapple**

Pineapple cultivation generates a substantial volume of by-products, such as stubble, which are traditionally reincorporated into the soil as fertilizer. However, this practice may contribute to pest proliferation in agricultural zones. These residues, nonetheless, can be repurposed as forage for ruminants, either fresh or ensiled, thereby preserving their nutritional properties under optimal conditions. Additionally, the inclusion of complementary ingredients can enhance their physical and nutritional characteristics (Viveros Torres *et al.*, 2022). In Mexico, the primary pineapple-producing state is Veracruz, contributing 66% of the national total, equivalent to 840,317.47 tons. Other key producers include Oaxaca, Nayarit, Tabasco, and Quintana Roo. These five states collectively account for 93% of national production, totaling 1,193,630.43 tons out of 1,272,559.15 tons cultivated across 46,954.27 hectares (SIAPd, 2023). One study evaluated pineapple crown silage with varying inclusion levels (0%, 15%, 30%, and 45%) of musaceous fruits. Bromatological analysis was conducted on both the pineapple crown and the musaceous fruit. The pineapple crown showed the following composition: Crude Protein (CP) (12.09%), Neutral Detergent Fiber (NDF) (57.5%), Dry Matter (DM) (9.1%), Ash (8.79%), Ether Extract (EE) (2.86%), and Lignin (2.5%). The study concluded that pineapple crown is an acceptable feed resource for ruminants, whether fresh or ensiled; however, supplementing with starch-rich sources like musaceous fruits can enhance its nutritional value (Lazo Salas *et al.*, 2018). In another investigation, six treatments were formulated using different by-products: fish waste, corn stubble, molasses, and pineapple peel (PP) at inclusion levels of 15%, 30%, and 45%, with various fermentation durations. Additionally, inoculants such as *Lactobacillus* sp. or *Lactobacillus* B2 were added at 4%. It was concluded that inclusion of 15% and 30% PP resulted in better acidification at 7 days of fermentation. By day 14, all silages were stable and showed high nutrient content. Therefore, silage production using these by-products represents a simple, cost-effective, and environmentally friendly alternative for ruminant nutrition (Ramírez Ramírez *et al.*, 2020). A separate study evaluated intake, milk yield, and composition in lactating goats fed diets supplemented with fresh pineapple processing residues at inclusion levels of 0%, 10%, 20%, and 30%. It was concluded that using fresh pineapple by-products is a viable feeding strategy at 10% and 30% inclusion on a fresh matter basis (Alpízar Solís & Elizondo Salazar, 2019).

### **Crop residues**

Agricultural residues commonly referred to as stubble, straw, hay, or forage play a crucial role in agricultural and livestock systems, particularly in ruminant nutrition. These by-products are primarily derived from cereal crops such as maize and sorghum, and to a lesser extent from legumes like soybean. The volume of crop residues is directly proportional to grain production (Macías Rodríguez *et al.*, 2021). In 2023, maize

**Table 3.** Nutritional value of orange by-products.

Nutrients	OB	OP	OS
CP (%)	10	10	16
EE (%)	3.2	4.96	9.22
Ash (%)	3.3	7.92	9.22
CF (%)	14.92	30.8	33.4
NFE (%)	64.51	67.18	77.58
GE <sub>p</sub> (Mcal)	22.6	26.87	27.89
TDN (Mcal)	60.39	63.78	64.56

OB: Orange Bagasse, OP: Orange Peel, OS: Orange Silage, CP: Crude Protein, EE: ether extract, CF: Crude Fiber, NFE, Nitrogen-Free Extract, ENP: Gross Energy Production and TDN: Total Nutrient Digestible. Fuente: Cabrera Núñez *et al.*, 2020.

production in Mexico reached 27,549,917.53 tons across 6,941,031.12 hectares. The leading producing states include Sinaloa, Jalisco, Michoacán, the State of Mexico, and Guanajuato. Soybean production was 199,162.60 tons on an equivalent cultivated area, with Campeche, Yucatán, and Chiapas as the main producers. Sorghum production totaled 4,815,930.91 tons across 1,359,920.57 hectares, with Tamaulipas, Guanajuato, Michoacán, Sinaloa, and Nayarit as the top-producing states (SIAPe, 2023). Although crop residues are essential in ruminant feeding, their limited nutritional value has prompted research into strategies to enhance their composition and digestibility (Macías Rodríguez *et al.*, 2021). One study assessed the mineral content of corn stubble silage supplemented with three non-protein nitrogen sources (urea, poultry litter, and fresh pig manure) and two carbohydrate sources (molasses and bakery by-products). After 30 days of fermentation, results showed that silage enriched with poultry litter and molasses provided both macro- and microminerals suitable for ruminant diets (Domínguez Vara *et al.*, 2023). In another experiment, the productive response of dairy cows was evaluated when fed *ad libitum* corn stubble treated with 26 and 40 grams of urea in treatments one and two, respectively. Treatment three used untreated corn stubble supplemented with 760 grams of soybean meal. The results indicated that urea supplementation improved nutritional quality and reduced structural carbohydrate content. However, no statistically significant differences were observed among the treatments (García Martínez *et al.*, 2020).

## CONCLUSIONS

Formulating ruminant diets with the inclusion of agro-industrial by-products and regionally available agricultural residues represents a viable alternative to enhance livestock production, reduce dependence on conventional feed ingredients, and minimize feed costs. Additionally, the proper management of residual materials from agro-industrial processes fosters more efficient waste handling and contributes to mitigating environmental impact.

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# Remote sensing and machine learning techniques used to predict sugarcane (*Saccharum* spp.) yield

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## ABSTRACT

**Objective:** To gather information generated using remote sensing and machine learning models. These tools are already used in decision-making processes to support sugarcane yield prediction.

**Design/Methodology/Approach:** This study includes a systematic review of scientific literature. Different indicators used in studies about the prediction sugarcane yield were extracted and synthesized.

**Results:** This review retrieved 386 relevant studies from five electronic databases. Subsequently, using exclusion and selection criteria, 47 studies were selected for in-depth analysis. According to the analyses, the most frequently used variables were climatic variables (temperature, precipitation, and evapotranspiration) and crop variables (number of harvests). The most commonly used algorithms were random forests and multiple linear regression.

**Study Limitations/Implications:** The limitations are included in the in-depth analysis of the studies. These studies have great potential for further research. In this case, the analysis was limited to remote sensing and machine learning.

**Findings/Conclusions:** Satellites, such as Sentinel 2 and Landsat 8, are commonly used in remote sensing methods. The most frequently used vegetation indices include: Normalized Difference Vegetation Index (NDVI), Green Normalized Difference Vegetation Index (GNDVI), Soil-Adjusted Vegetation Index (SAVI), and Enhanced Vegetation Index (EVI). The use of spectral bands, such as near-infrared (NIR) and shortwave infrared (SWIR), was recorded.

**Keywords:** remote sensing, machine learning, vegetation indices, satellite sensors, production performance prediction.

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## INTRODUCTION

The estimation of crop yields at different scales helps to plan food systems worldwide (FAO, 2022). Sugarcane is a species with high photosynthetic efficiency. It is grown in tropical and subtropical regions. It has a long growing season and produces high yields

and high income. The importance of this crop lies in its commercial focus, fodder production, and industrial processing. Sugarcane is used in the production of sucrose, yeast, paper, chemicals, biofertilizers, fodder, and renewable energy sources (Salgado *et al.*, 2013; Wang *et al.*, 2019; Olvera-Rincón *et al.*, 2024). Sugarcane indicators show that sucrose accounts for ~70% of the global sugar production. In addition, it plays an important role in food and energy production worldwide (Salgado-García *et al.*, 2021; De los Santos *et al.*, 2022). The availability of information from satellite sensors and other types of advanced technology has improved the local and global prediction of sugarcane yield. Nevertheless, a closer link between remote sensing, meteorological data, agronomic parameters, and machine learning is required (Canata *et al.*, 2021). Remote sensing—as well as the use of orbital images—is applied in agriculture to identify spectral variations in soil and crop characteristics on a larger scale, supporting the diagnosis of crop agronomic parameters and improving agricultural management decision-making (Cao *et al.*, 2021; Geng *et al.*, 2021; Yzquierdo-Álvarez *et al.*, 2021; López-Castañeda *et al.*, 2022). Machine learning is applied in various fields, including agriculture, industry, commerce, and communications (Witten *et al.*, 2016; McQueen *et al.*, 1995). Crop yield prediction has always been—and will always be—a problem to be solved and a challenge to precision agriculture. So far, various models proposed for this purpose have been validated. Datasets should be included, considering the influence of physical and biotic factors, as well as crop variety on yield (Sharifi, 2021; Shahhosseini *et al.*, 2021). Machine learning detects data interactions, as well as patterns, generating new knowledge from this interaction. Since the operation of machine learning models uses datasets, the results are based on past experiences. The operated dataset includes 70-80% of the total dataset, while the rest is used for model testing and validation. Model performance statistics are determined during these stages (Tong and Nikoloski, 2021; Zheng *et al.*, 2021).

The literature review aims to clearly understand the application of remote sensing and machine learning techniques used to predict sugarcane crop yield. In addition, it helps new researchers to better understand this state-of-the-art technology (Slob *et al.*, 2021). According to Van Klompenburg *et al.* (2020), the main challenge of the application of machine learning to predict crop yield is the lack of high-quality datasets, which directly impacts the results of the models.

## **MATERIALS AND METHODS**

The review was conducted following the guidelines of Kitchenham *et al.* (2007). Subsequently, research questions were defined. The reference databases were used to select relevant studies about the topic. The reference databases used in this study were Science Direct, Scopus, Web of Science, Springer Link, Wiley, and Google Scholar. Afterwards, the results were filtered and evaluated using a set of exclusion and quality criteria. In addition, publication sites, initial search strings, and publication selection criteria were established (Keele, 2007). Publications were selected by reviewing all databases. Subsequently, they were mainly sorted by authors, year of publication, and type of publication. Once the required information was successfully extracted, it was synthesized to provide an overview of the relevant publications published to date. Results were documented and research

questions were addressed during the final stage, also known as review report (Snyder, 2019). Figure 1 shows the steps followed in the review protocol strategy.

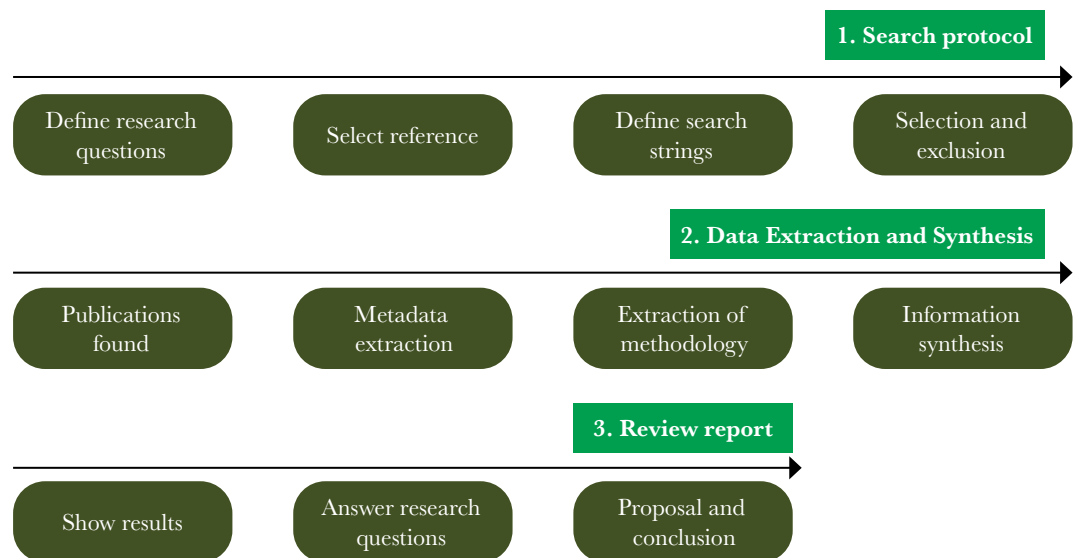
**Purpose of the research**

The following six research questions (RQs) were used in this study:

- RQ1. What type of problems do remote sensors and machine learning techniques solve?
- RQ2. What machine learning algorithms have been used for the prediction of sugarcane yield?
- RQ3. Which remote sensors are most commonly used to predict sugarcane yield?
- RQ4. What dependent and independent variables have been used for sugarcane yield prediction with remote sensing and machine learning?
- RQ5. What evaluation approaches/parameters have been used to predict sugarcane yield with remote sensing and machine learning models?
- RQ6. What challenges have been reported using remote sensing and machine learning for the prediction of sugarcane yield?

**Search strategy**

The search was narrowed down to the basic concepts relevant to the scope of this review. The initial search included “aprendizaje automático (machine learning),” “teledetección remota (remote sensing),” “predicción de rendimiento (yield prediction),” and “caña de azúcar (sugarcane).” The search resulted in 386 studies. Subsequently, the results were reported and the questions answered (Van Klompenburg *et al.*, 2020). Exclusion criteria were used to guarantee the selection of relevant studies and to reduce the possibility of biased criteria (Kitchenham *et al.*, 2007). In order to include a publication, all exclusion criteria must be false. The exclusion criteria included:



**Figure 1.** Details of the review protocol process.

1. The publication is not related to agriculture, yield prediction, and machine learning;
2. The publication is not written in English;
3. The publication is a duplicate or has already been retrieved from another database;
4. The full text of the publication is not available;
5. The publication is a review document;
6. The publication was published before 2010.

To answer the questions, data from the selected studies were extracted and synthesized. The retrieved information focused on verifying whether or not the studies met the requirements established in the exclusion criteria and on answering research questions.

The review was then conducted following the guidelines of Kitchenham *et al.* (2007). Using reference databases such as Science Direct, Scopus, Web of Science, Springer Link, Wiley, and Google Scholar, the results were subsequently filtered and evaluated based on a set of exclusion and quality criteria, in order to determine publication sites, initial search strings, and publication selection criteria (Keele, 2007). The publications were selected by reviewing all the duly-organized databases. Finally, research objectives were addressed in the results documentation (Snyder, 2019).

## RESULTS AND DISCUSSION

A total of 386 publications were reviewed in all databases. After applying the six exclusion criteria, 47 studies were selected. Table 1 shows the number of initially retrieved publications (NIRP) and the number of publications after the exclusion and selection criteria (NPAESC). In addition, it shows that 97.9% of the publications were obtained from Google Scholar, Springer Link, and Science Direct.

Figure 2 shows the distribution of the selected publications per year of publication. An increase in the use of remote sensing and machine learning techniques to predict yield in sugarcane cultivation was recorded. The trend followed a beta distribution.

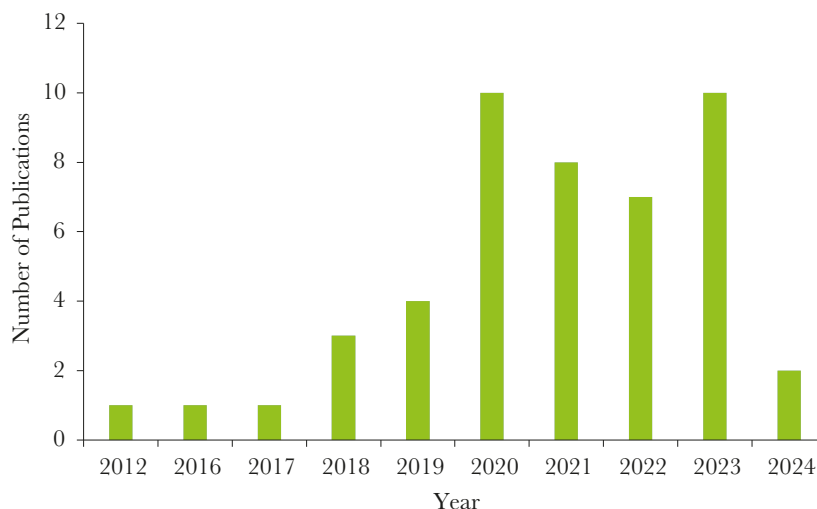
### Selected publications

The exclusion and selection criteria were used to filter the initial 386 publications that were selected to answer the research questions. These criteria incorporate elements

**Table 1.** Distribution of publications according to the databases.

Database	NPI	NPIC	PP (%)
Google Scholar	154	32	68.1
Science Direct	70	7	14.9
Springer Link	134	7	14.9
Web of science	22	0	0
Wiley	5	1	2.1
Total	386	47	100

\*NIRP (NIP)=number of initial retrieved publications; NPAESC (NPIC)=number of publications after the exclusion and selection criteria; and PP (PP)=percentage of publications according to the database.



**Figure 2.** Distribution of publications per year of publication in the databases.

included in the thesis topic. These problems are addressed using remote sensing and machine learning techniques (Miphokasap and Wannasiri, 2018; Wang *et al.*, 2019; Militante *et al.*, 2019; Wang *et al.*, 2020; Chea *et al.*, 2020; Shendryk *et al.*, 2020a). Likewise, climatic indicators, soil and crop variables, and spectral bands reported in the literature were used to predict sugarcane yield through machine learning and remote sensing techniques. The most used variables were related to climatic variables (66%). Crop yield was the dependent variable. The soil variable group included information about soil texture and moisture, slope, and the use of digital elevation models. An important finding of this sugarcane cultivation review was the use of spectral bands, such as Red, Green, Near Infrared (NIR), and Shortwave Infrared (SWIR). These spectral bands have been used as predictor variables, obtaining better adjustments than vegetation indices (Canata *et al.*, 2021). Meanwhile, in the group of crop variables, the number of cuts has been considered as a predictor variable. This situation has been fundamental to explain the high importance of machine learning models in sugarcane yield prediction (Hammer *et al.*, 2020; Pignède *et al.*, 2021; Oré *et al.*, 2022). Table 2 includes the evaluation parameters used to predict sugarcane yield with remote sensing and machine learning techniques.

**Table 2.** Evaluation parameters for sugarcane yield prediction using machine learning and remote sensing techniques.

Key	Statistical evaluation parameter	Times used
RMSE	Root mean square error	31
R <sup>2</sup>	“R-squared”	34
MAE	Mean absolute error	10
MSE	Mean square error	3
MAPE	Mean absolute percentage error	6
Cor	Pearson correlation	4

Almost all studies used Root Mean Square Error Approach (RMSE) and  $R^2$  as measures of model quality. Most machine learning models produced highly accurate results for their evaluation parameters —*i.e.*, the model made correct predictions (Canata *et al.*, 2021; Oré *et al.*, 2022). The evaluation approach was based on the K-fold method and cross-validation was the preferred choice among researchers (Chea *et al.*, 2020; Dos Santos Luciano *et al.*, 2021; Kanwal *et al.*, 2021).

Publications were reviewed to identify any issues or suggested improvements for future models in order to answer research question six (RQ6): the challenges reported in the literature for predicting sugarcane yield with remote sensing and machine learning. Several studies reported the scarce data available and the lack of real-time data to support local interventions (Canata *et al.*, 2021; Shendryk *et al.*, 2021). The studies confirmed that their prediction methods worked with the limited data available and indicated that more diverse data should be used for further testing (Xu *et al.*, 2020), including different climatic circumstances, different vegetation, and longer time series of sugarcane yield (Medar *et al.*, 2019; Rahman and Robson, 2020; Maldaner *et al.*, 2021). Adding more data sources could also improve the prediction of sugarcane yield with machine learning models (Pignède *et al.*, 2021; Galphade *et al.*, 2022).

Consequently, the authors of this research recommend the use of more machine learning algorithms, spectral bands, and vegetation indices. These indices should be calculated at different sugarcane phenological stages, in order to detect response changes in climatic variables and to correlate them with those variables, particularly in regions with clear climatic changes. Keeping an historical record of the yields obtained in sugarcane plots is fundamental to build more robust models. Due to their practicality and low cost, the use of satellite sensors is essential to capture larger areas in sugarcane mills. Therefore, the use of major and up-to-date sources, such as Sentinel 2 and Landsat 8, is fundamental. Likewise, research about the use of Unmanned Aerial Vehicles (UAVs) is essential to obtain field data that can be used to generate a larger number of observations. Currently, obtaining field data is very expensive and requires a great effort (Som-ard *et al.*, 2021). Therefore, UAVs could be integrated into the machine learning yield modeling process, because they can substitute field measurements and can cover larger areas of sugarcane plantations.

## CONCLUSIONS

This systematic literature review showed that the selected publications used a range of predictor variables, depending on the scope of the research and data availability. Each publication researched sugarcane yield prediction through machine learning and remote sensing; however, they use different predictor variables. The scale of the studies is also different. The studies indicated that models with more features did not always provide the best performance and adjustment for yield prediction. The various studies used many algorithms. No specific conclusions can be drawn about the best model used for this purpose; nevertheless, the results clearly showed that some machine learning models are used more than others. The most commonly used models were random forest and multiple linear regression. The most important satellite sources were Sentinel 2 and Landsat 8. The vegetation indices most commonly used as predictors were NDVI, GNDVI, SAVI, and

EVI. Likewise, the use of indices obtained from drone-mounted RGB cameras provided promising results for the low-cost prediction of sugarcane yield. This review would guide future research about the development of the problem for the prediction of sugarcane yield, using machine learning and remote sensing techniques.

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# Effect of biostimulation on the duration of postpartum anestrus in hair sheep: A review

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## ABSTRACT

**Objective:** To describe the effect of biostimulation, understood as the male effect and suckling management, on the reestablishment of ovarian activity during the postpartum anestrus in hair sheep.

**Design/methodology/approach:** A comprehensive review and analysis of scientific literature were conducted, focusing primarily on the biostimulatory effects of the male and the influence of suckling on reproductive activity during the postpartum anestrus in ewes.

**Results:** Suckling during the postpartum period induces a marked inhibition of reproductive activity. The sudden introduction of the male into pens containing lactating females elicits a positive stimulus for the reactivation of ovarian activity during postpartum anestrus. The abrupt exposure of ewes to the male following parturition, coupled with the limitation of mother-lamb contact during the postpartum period, significantly reduces the interval between parturition and first ovulation in hair sheep.

**Study limitations/implications:** There is limited research on the physiological effect of the male during postpartum anestrus and its association with the resumption of ovarian function in ewes, as well as its potential interactions with the inhibitory effect of suckling on reproductive activity.

**Findings/conclusions:** The positive stimulus reflected by an increase in the pulsatile secretion of luteinizing hormone (LH) exerted by the male during postpartum anestrus is more effective, can be prolonged, and may induce ovulation when suckling is managed. Specifically, restricting contact between the ewe and the lamb for a defined period helps mitigate the negative effect, preventing the suppression of LH pulsatility. According to current literature, controlled suckling is among the most effective strategies to shorten the parturition-to-first-ovulation interval in hair sheep.

**Keywords:** Male effect, suckling, postpartum anestrus, ewes.

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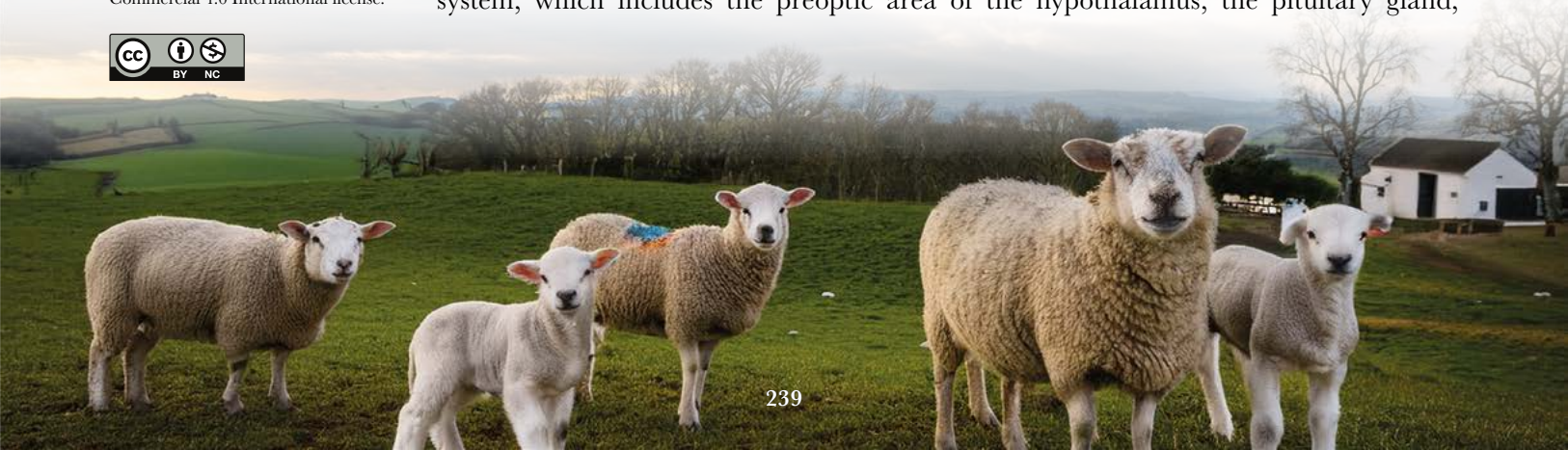
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## INTRODUCTION

Reproductive activity is a complex physiological process regulated by a series of neuroendocrine mechanisms modulated by both endogenous and exogenous factors. These factors interact dynamically to either stimulate or inhibit the pulsatile secretion of gonadotropin-releasing hormone (GnRH), the central axis of the reproductive system, which includes the preoptic area of the hypothalamus, the pituitary gland,



and the gonads (Malpaux *et al.*, 1996). Among the main factors that negatively affect reproductive efficiency in ruminants is the prolongation of the interval between parturition and the first ovulation, a condition with significant economic implications for livestock production systems. The primary cause of this postpartum inhibition is the suckling stimulus, determined by the frequency and intensity of nursing—that is, the number of events and the duration of each suckling episode exerted by the offspring on the mammary gland (Pérez-Hernández *et al.*, 2002; Camacho-Ronquillo *et al.*, 2008). Numerous scientific studies have addressed strategies to reduce the lambing interval in sheep, focusing mainly on lactation management, including practices such as early weaning, temporary weaning, restricted suckling, and controlled suckling. Additionally, interventions based on socio-sexual stimuli, photoperiod modulation, and nutritional management improvements have also been evaluated. In small ruminants inhabiting temperate and subtropical regions, reproductive seasonality constitutes a biological limitation that affects the availability and production of food and livestock derivatives throughout the year (Ramírez-Ramírez *et al.*, 2021). To mitigate the effects of this seasonality, exogenous reproductive manipulation strategies have been developed to schedule parturitions outside the natural mating season, ensuring continuous production or targeting specific periods. One such strategy is out-of-season mating, achieved by stimulating male reproductive activity to trigger, through the so-called “male effect,” ovarian reactivation in anestrus females (Martin *et al.*, 2004; Hawken & Martin, 2012). In this context, the aim of this literature review is to analyze the role of biostimulation particularly the male effect and suckling management in modulating the duration of postpartum anestrus. This strategy represents a sustainable alternative for reproductive management, as it reduces dependence on exogenous hormonal treatments and contributes to improving reproductive efficiency in sheep production systems.

### **Reproductive season**

Reproductive activity in small ruminants is finely regulated by the interaction between physiological processes and neuroendocrine mechanisms integrated within the hypothalamic-pituitary-gonadal (HPG) axis. In this context, neurotransmitters act as essential chemical messengers secreted by the central nervous system (CNS), facilitating synaptic communication between neurons (AL-Jaryan *et al.*, 2023). Within the reproductive domain, their role is crucial as they modulate the secretion and release of gonadotropic hormones such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both of which are fundamental to follicular development and ovulation. Alterations in neurotransmitter secretion patterns can significantly impact the functionality of the reproductive axis, thereby affecting the efficiency of reproductive processes (Hull *et al.*, 1999). During the reproductive season, ewes exhibit an estrous cycle averaging 17 days in length, with a range between 15 and 20 days. Estrus, or the phase of sexual receptivity, lasts for a short period averaging between 24 and 36 hours, although it may vary from 16 to 59 hours. Ovulation occurs spontaneously within this window. At both uterine and endocrine levels, functional modifications are observed, associated with fluctuations in basal concentrations of key hormones such as progesterone (P4),

estradiol (E2), and gonadotropin-releasing hormone (GnRH), the latter of which regulates the secretion of LH and FSH, promoting follicular growth and ovulation (Goodman & Karsch, 1980). Hormonally, P4 is the predominant hormone of the estrous cycle, remaining elevated for approximately 11 of the 17 days. Estradiol, in contrast, is secreted mainly over a period of three to four days and is chiefly responsible for the expression of estrous behavior (Goodman & Karsch, 1980; Karsch *et al.*, 1989). A sustained rise in E2 induces a positive feedback effect on the hypothalamic axis, generating a preovulatory GnRH surge, which in turn increases the frequency of LH secretion an essential event for ovulation to occur. Subsequently, the luteal phase is established, marked by a progressive increase in P4 levels, while the concentration and secretion frequency of GnRH and FSH rise, and LH levels gradually decline (AL-Jaryan *et al.*, 2023). However, during the annual reproductive cycle of ewes, periods of ovarian inactivity known as seasonal anestrus occur. This phenomenon is primarily influenced by environmental factors such as photoperiodicity especially extended daylight and the suckling stimulus. Both factors enhance CNS sensitivity to the inhibitory effects of estradiol through negative feedback, resulting in a diminished frequency and amplitude of GnRH and LH secretion, thereby preventing ovulation (Arroyo & Camacho-Escobar, 2009).

### **Seasonal anestrus**

In ewes, seasonal anestrus is defined by the absence of estrous cycles, estrous behavior, and ovulatory events. This period occurs during the long days of the year, when the duration of melatonin secretion a key hormone in photoperiod regulation decreases significantly (Thiéry *et al.*, 2002). In sheep located in temperate and subtropical regions of the Northern Hemisphere, the breeding season takes place during the short days, spanning from early autumn to late winter. Conversely, seasonal anestrus extends from late winter through late summer, coinciding with long-day periods. The duration of postpartum anestrus may vary depending on the timing of parturition and the length of the lactation period, both of which are critical modulators of the resumption of ovarian activity (Delgadillo *et al.*, 2020). During seasonal anestrus, sensitivity of the retrochiasmatic lateral region of the hypothalamus to the inhibitory effects of estradiol (E2) is heightened, thereby intensifying the negative feedback exerted by this hormone (Gallegos-Sánchez *et al.*, 1997). As a result, the frequency of GnRH pulses and consequently LH secretion is reduced, preventing follicular maturation and blocking ovulation (Arroyo & Camacho-Escobar, 2009). This basal state of the hypothalamic-pituitary-gonadal axis represents a physiological adaptation to environmental conditions, ensuring that reproduction is synchronized with periods of optimal resource availability.

### **Postpartum anestrus**

During the postpartum period, females undergo a series of physiological and physical changes that hinder the immediate restoration of fertility. One of the primary contributing factors to this phenomenon is the suckling effect, which activates mechanoreceptors located in the nipple. This stimulation triggers neural signals to the hypothalamus that promote the release of endogenous opioid peptides (EOPs), primarily beta-endorphin (Morales

*et al.*, 2001). These peptides exert an inhibitory effect on the secretion of gonadotropin-releasing hormone (GnRH). At the neuroendocrine level, neurons of the kisspeptin/neurokinin B/dynorphin (KNDy) complex play a central role in regulating the pulsatile secretion of GnRH in mammals (Lehman *et al.*, 2010; Maeda *et al.*, 2010; Uenoyama *et al.*, 2014, 2021). GnRH is essential for the production of the pituitary gonadotropins LH and FSH; thus, a reduction in its pulsatile release leads to decreased LH secretion, directly impairing the resumption of ovarian activity (Godfrey *et al.*, 1998). Several studies have documented the reproductive responses of ewes during the postpartum period under different management approaches. For example, Wei *et al.* (2016), in a study on anestrus Lanzhou ewes, evaluated various hormonal protocols for estrus induction. They found that treatment with fluorogestone and equine chorionic gonadotropin (eCG) resulted in a higher conception rate (85.72%) compared to the protocol based on cloprostenol and eCG (57.14%). Additionally, Ungerfeld *et al.* (2020) demonstrated that biostimulation through male exposure effectively induced estrus, ovulation, and even pregnancy, despite ongoing lactation in the ewes. These findings suggest that postpartum reproductive management can be approached either through the use of exogenous hormonal treatments or via sexual biostimulation strategies, with the latter representing a more sustainable alternative that involves reduced pharmacological intervention.

### **Management of postpartum anestrus**

In hair sheep inhabiting tropical regions, it has been observed that suckling inhibits the pulsatile secretion of GnRH during the postpartum period. However, when suckling is controlled and the presence of the male is permitted, inter-lambing intervals tend to be significantly shortened (Morales-Terán *et al.*, 2011). In this context, the presence of the lamb and the frequency and intensity of suckling constitute the most significant stimulus in suppressing the resumption of ovarian activity following parturition. Conversely, partial or total separation of the offspring promotes the return to estrus and the occurrence of ovulation in a shorter timeframe (Arroyo & Camacho-Escobar, 2009; Arroyo *et al.*, 2011; Morales-Terán *et al.*, 2011; Castillo-Maldonado *et al.*, 2013). During lactation, suckling imposes a high energetic demand on the mother, leading to a reduction in the frequency of GnRH and LH pulses. This neuroendocrine alteration contributes to ovulation inhibition and prolongation of postpartum anestrus. To counteract this effect and facilitate the early restoration of ovarian activity, various management strategies involving mother-lamb contact have been implemented, such as early weaning, temporary weaning, and controlled suckling. These practices allow modulation of mammary stimulation and, consequently, reduce the inhibitory effect on the hypothalamic-pituitary-gonadal axis, promoting a more efficient reproductive recovery in postpartum females.

### **Suckling management strategies to reduce the parturition-to-first-ovulation interval**

Suckling is one of the main factors inhibiting the reestablishment of ovarian activity during the postpartum period in ewes. Its effect on the central nervous system sensitizes the preoptic area of the hypothalamus to the negative feedback exerted by estradiol (E2),

thereby reducing the frequency of GnRH and LH pulses and consequently blocking the reactivation of the reproductive axis (Morales-Terán *et al.*, 2004; Freitas De Melo & Ungerfeld, 2016). During the first two weeks postpartum, ewes allow lambs to suckle with high frequency but low intensity. As the postpartum period progresses, this pattern reverses: frequency decreases while suckling intensity increases, maintaining the inhibitory stimulus on the hypothalamic-pituitary-gonadal axis. To counteract this effect, various management strategies have been developed to modulate mother-lamb contact with the aim of inducing earlier ovulation and shortening the duration of postpartum anestrus.

### **Early weaning**

Early weaning is a commonly used practice in hair sheep, particularly in tropical regions, to induce ovulation in lactating females. This strategy involves the abrupt separation of lambs between 20 and 30 days postpartum, triggering the reactivation of the reproductive axis and promoting ovulation within three to five days after weaning onset (Castillo-Maldonado *et al.*, 2013). Multiple studies have shown that early weaning does not negatively affect the physiological condition of ewes and, on the contrary, enables an earlier resumption of reproductive activity compared to those subjected to late weaning. This benefit is attributed to a longer period for the female to regain optimal body condition (between 2.5 and 3.0 on a 1 to 5 scale) before the next mating, significantly improving conception rates (Kinghts *et al.*, 2012). However, this strategy has been associated with severe stress in lambs, increasing the risk of disease and necessitating more intensive management by the producer. Therefore, its implementation is primarily recommended in specialized dairy production systems where lambs can be fed high-quality milk replacers.

### **Temporary weaning**

Temporary weaning consists of separating lambs from their mothers for a short period during lactation to induce the return to estrus. Castillo-Maldonado (2012) evaluated this strategy in hair sheep by implementing lamb separation on day 43 postpartum for a duration of 48 hours. The study showed that most females subjected to this practice exhibited estrus and significantly reduced the parturition-to-first-ovulation interval. However, adverse effects were reported in lambs, including behavioral stress and, in some cases, maternal rejection upon reintroduction. These issues may compromise animal welfare and productivity efficiency if appropriate complementary measures are not applied.

### **Controlled suckling**

Controlled suckling is a technique developed by the Colegio de Postgraduados with the aim of reducing the duration of postpartum anestrus in hair sheep. This strategy involves gradually reducing the lamb's dependence on the mother by limiting contact between them starting from the seventh day postpartum. The protocol includes two daily contact sessions one in the morning and one in the afternoon each lasting 30 minutes. This restricted suckling management promotes the reactivation of ovarian activity by diminishing the inhibitory stimulus that suckling exerts on the neuroendocrine axis. Simultaneously, alternative high-quality feed sources (creep feeding) are provided to ensure adequate lamb

growth. Implementing controlled suckling enables an earlier return to estrus, enhances reproductive efficiency in ewes, and supports a more uniform weight distribution among lambs within the group. Authors such as Camacho-Ronquillo *et al.* (2008), Herrera Corredor *et al.* (2008), Morales-Terán *et al.* (2011), and Castillo-Maldonado *et al.* (2013) have reported significant reductions in the parturition-to-first-insemination interval by limiting lamb access to the mother to just 30 minutes twice daily. This practice is recommended starting from the first postpartum week, once the lambs have consumed colostrum and developed sufficient immunological strength.

### **Management of socio-sexual stimuli during postpartum anestrus**

Sexual biostimulation is a phenomenon whereby males or females induce changes in the reproductive physiology and performance of other individuals through various stimuli, such as pheromones, genital stimulation, or other external signals (Fiol & Ungerfeld, 2012; Landeta-Hernández *et al.*, 2023). Sexually active males emit pheromones capable of stimulating the pulsatile secretion of GnRH and LH in anestrus females a phenomenon known as the “male effect” which induces the reinitiation of ovarian activity and potential ovulation (Scaramuzzi & Martin, 2008; Arellano-Lezama *et al.*, 2013). As a result of this stimulus, some females in the flock may enter estrus, ovulate, or even become pregnant. Sexual biostimulation is a valuable tool for inducing estrus, particularly due to its low cost, which facilitates its integration into flock reproductive management. The sudden introduction of a ram into pens with females increases the pulsatile secretion of GnRH and LH due to heightened activity of neurons in the A12 nucleus and the locus coeruleus complex (Fabre-Nys *et al.*, 2016). This increase in GnRH and LH pulse frequency persists for at least 12 hours, although pulse amplitude declines after one to two hours. Therefore, both sudden introduction and replacement of the male can induce ovulation (Ungerfeld *et al.*, 2004; Arellano-Lezama *et al.*, 2013). In some ewes, the first estrus appears with the second ovulation, occurring between 17 and 20 days after ram introduction. In others, a short luteal phase of 4 to 5 days is followed by a second ovulation without external estrus signs, after which a normal-duration luteal phase occurs. From this point, a third ovulation associated with overt estrus is typically observed (Ungerfeld *et al.*, 2004; Ungerfeld, 2007; Ungerfeld & Núñez, 2011). Ultrasound and laparoscopy observations have confirmed the occurrence of ovulations evidenced by corpora 4 and 6 days following ram introduction. Short luteal phases caused by anovulatory follicles, or normal luteal phases with luteinized follicles, have also been documented (Ungerfeld *et al.*, 2004; Pellicer-Rubio *et al.*, 2013). The rise in LH pulses coincides with an increase in the number of large follicles (>5 mm), as these pulses are essential for the development of the preovulatory follicle (Luo *et al.*, 2011; Seekallu *et al.*, 2009). Several studies have reported an increase in LH secretion frequency in response to ram introduction. The signals that trigger the resumption of reproductive activity are associated with the concentration of androgens produced by the male, which may be influenced by factors such as age and reproductive season (Arellano-Lezama *et al.*, 2013). The “female effect,” or the influence of the female on the male, induces an increase in testosterone secretion in rams, which directly affects their sexual behavior and mating success. Exposure of males to estrous females enhances their ability

to induce ovulation in anestrus females (Ungerfeld *et al.*, 2004). Meanwhile, the “female-female effect” involves stimulating ovarian activity through the introduction of a female in estrus into a group of anestrus ewes. This practice reactivates ovarian function in the other females and may potentiate the response to the male effect, increasing the number of ewes that ovulate during either seasonal or postpartum anestrus, thereby improving flock reproductive efficiency (AL-Jaryan *et al.*, 2023).

### What do the results of biostimulation during postpartum anestrus suggest?

To evaluate the efficacy of sexual biostimulation strategies (male effect) and suckling management in the reestablishment of postpartum ovarian activity in hair sheep, Arrieta *et al.* (2024; unpublished data) conducted an experimental study. The design included four treatments distributed as follows: 14 females with continuous suckling (CS), 14 females with continuous suckling plus male effect (CS+ME), 14 females with controlled suckling (Cs), and 14 females with controlled suckling plus male effect (Cs+ME).

The results showed that the interaction between the male effect and controlled suckling significantly reduced the interval between parturition and the first manifestation of estrus ( $p < 0.05$ ), compared to the other treatments (Table 1).

Moreover, as shown in Table 1, not all females exhibited estrus, with the absence of estrus occurring exclusively in the continuous suckling groups. This finding suggests that estrus inhibition may be associated with the suppression of LH pulsatile secretion induced by the suckling stimulus. On the other hand, regarding pregnancy rate and type of lambing, no significant differences were observed between treatments ( $p > 0.05$ ; Table 2), indicating that the applied strategies did not affect these reproductive parameters.

Regarding prolificacy and fertility, there is insufficient evidence to suggest that the presence or absence of the male prior to mating is a determining factor for the number of offspring born (Table 3).

The occurrence of a higher number of multiple births in some treatments does not indicate that the type of lambing is determined by sexual biostimulation, as no significant differences were found between treatments ( $p > 0.05$ ). Therefore, it is suggested that in lactating hair sheep, the male effect primarily contributes to reducing the interval between parturition and the first manifestation of estrus. These results are consistent with those reported by other authors, such as Morales-Terán *et al.* (2011), who observed

**Table 1.** Percentage of Hair Sheep Exhibiting Estrus and Average Time to Estrus Onset by Treatment.

Treatments	n	Ewes in estrus (%)	Time of onset of estrus (hrs)
CS	14	12 (85.7)	51.50±2.99 <sup>a</sup>
CS+ME	14	12 (85.7)	48.86±2.99 <sup>a</sup>
Cs	14	14 (100)	43.50±2.12 <sup>a</sup>
Cs+ME	14	14 (100)	35.50±2.04 <sup>b</sup>

Means with different superscripts are significantly different ( $p < 0.05$ ).

CS=continuous suckling; CS+ME=continuous suckling plus male effect; Cs=controlled suckling; Cs+ME=controlled suckling plus male effect.

**Table 2.** Pregnancy percentage by treatment in hair sheep.

Treatment	n	Pregnant females	Percentage
CS	12	9	75.0
CS+ME	12	10	83.3
Cs	14	12	85.7
Cs+ME	14	12	85.7

Means with different superscripts are significantly different ( $p < 0.05$ ).

CS=continuous suckling; CS+ME=continuous suckling plus male effect;

Cs=controlled suckling; Cs+ME=controlled suckling plus male effect.

**Table 3.** Prolificacy and fertility by treatment in hair sheep.

Treatment	n	Prolificacy	Fertility
CS	12	1.88	1.14
CS+ME	12	2.0	1.43
Cs	14	1.67	1.43
Cs+ME	14	1.83	1.57

Means with different superscripts are significantly different ( $p < 0.05$ ).

CS=continuous suckling; CS+ME=continuous suckling plus male effect;

Cs=controlled suckling; Cs+ME= controlled suckling plus male effect.

that the combination of continuous suckling with the male effect (CS+ME) induces the reestablishment of ovarian activity before day 60 postpartum the time at which weaning is typically performed in hair sheep.

## CONCLUSIONS

The relevance of this review lies in the description and analysis of the various strategies used for managing lactation during the postpartum period in hair sheep. It highlights that the duration of the interval between parturition and the first ovulation is a key factor in flock reproductive efficiency, and identifies critical areas of research that should be addressed in the future. Furthermore, it reveals room for improvement in sheep production systems, particularly in tropical and subtropical environments. However, a deeper understanding is still needed regarding the physiological mechanisms occurring at the hypothalamic and ovarian levels in response to sexual biostimulation, in order to optimize its application. The selection and implementation of appropriate management strategies that effectively induce the reestablishment of postpartum ovarian activity and reduce the inter-lambing interval represent a key tool for enhancing productivity and profitability in sheep production systems.

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# Implementation of nutrient kinetics concept in formulation of pig's starter feed

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## ABSTRACT

**Objective:** To compare a conventional starter feed (Milkiwean<sup>®</sup>) with a starter feed formulated under the concept of digestion and absorption kinetics of nutrients (Milkiwean Kinetio<sup>®</sup>) in piglets.

**Methodology:** A total of 440 piglets (initial body weight  $6.4 \pm 0.83$  kg and 22 days of age) were randomly assigned following a completely randomized design. The experimental treatments consisted of evaluating two starter diets: Milkiwean<sup>®</sup> and Milkiwean Kinetio<sup>®</sup>.

**Results:** Pigs fed the standard diet exhibited greater body weight gain, final body weight, and feed intake ( $P=0.08$ ); however, feed conversion ratio was significantly improved with Milkiwean Kinetio<sup>®</sup> ( $P=0.001$ ). Additionally, the cost per kilogram of pork produced was lower with Milkiwean Kinetio<sup>®</sup>.

**Implications:** Further research is needed on the synchronization of digestion and absorption of nutrients from dietary ingredients used in pig production.

**Findings/Conclusions:** Formulating pig diets based on the kinetics of nutrient digestion and absorption has the potential to enhance nutrient utilization.

**Keywords:** digestion kinetics, absorption kinetics, pigs

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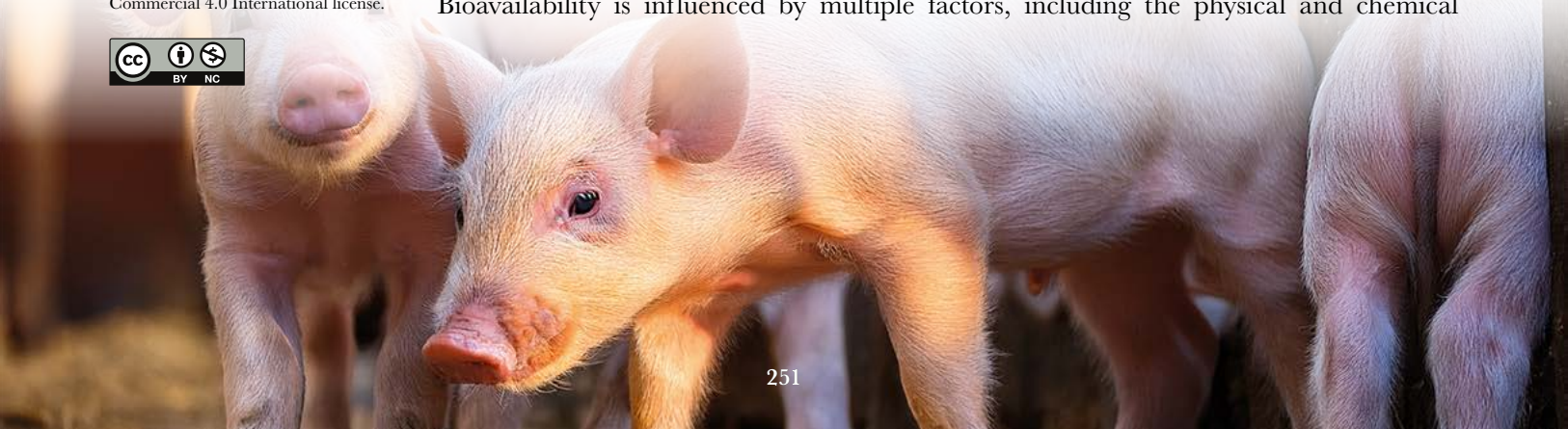
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## INTRODUCTION

Traditional systems for evaluating the nutritional value of feed ingredients have primarily relied on dietary inclusion rates, determined by nutrient concentrations and their digestibility coefficients. However, these approaches do not adequately account for differences in the rate, extent, and timing of nutrient digestion and fermentation throughout the gastrointestinal tract [1]. While nutrient composition can be considered an intrinsic characteristic of an ingredient, digestibility and bioavailability are not. Bioavailability is influenced by multiple factors, including the physical and chemical



structure of the nutrient, the source ingredient, the diet in which it is incorporated, and the animal's physiological capacity to degrade, absorb, and utilize those nutrients. Consequently, the extent to which ingested nutrients are digested and utilized depends on the interaction between diet composition and the animal's metabolism [2]. An emerging approach to assessing the nutritional value of feed ingredients involves analyzing the digestion kinetics of starch, fiber, and proteins in pigs [3]. Understanding the dynamics of nutrient digestion enables the development of more precise formulation strategies that enhance nutritional efficiency, reduce the incidence of digestive disorders such as diarrhea, and decrease nutrient excretion into the environment [2]. Currently, commercial products have been developed incorporating the concept of nutrient degradation kinetics into their formulations. One such example is Milkiwean Kinetio<sup>®</sup>, which differs from its standard counterpart (Milkiwean<sup>®</sup>) by being designed under the principle of sequential nutrient digestion potentially leading to significantly improved nutrient utilization. Within this context, the aim of the present study was to compare the productive performance of weaned pigs fed a standard starter feed versus one formulated based on the concept of nutrient digestion and absorption kinetics (Milkiwean Kinetio<sup>®</sup>).

## MATERIALS AND METHODS

The study was conducted at the commercial pig farm "Villa Rica," located in the municipality of Zapopan, Jalisco, Mexico. The region has a warm sub-humid climate, with an average annual temperature of 20.5 °C and an average annual rainfall of 943 mm. Animal management was carried out in accordance with the technical specifications for the production, care, and use of laboratory animals, complying with the provisions of the Mexican Official Standard NOM-062-ZOO-1999 (SAGARPA, 2001).

The experiment involved a total of 440 newly weaned piglets (220 castrated males and 220 females), the offspring of Large White×Hampshire crosses, with an average initial body weight (BW) of  $6.4 \pm 0.83$  kg and an average age of 22 days. The animals were housed in group pens (10 pigs per pen) equipped with nipple drinkers and hopper-type feeders, with *ad libitum* access to feed and water. Throughout the experimental period, no clinical signs of respiratory or digestive diseases were observed. The piglets were randomly assigned to two treatments, balancing initial body weight and sex across 44 pens. The treatments involved the evaluation of two starter feeds for weaned pigs, both developed by Trouw Nutrition, Mexico: T1: standard Milkiwean<sup>®</sup> and T2: Milkiwean Kinetio<sup>®</sup>. The experimental period lasted 29 days.

Milkiwean<sup>®</sup> is a pelleted starter feed (2.5 mm) structured in four phases: Phase 0: days 21-23 of age, 6-6.5 kg BW, estimated dry matter intake (DMI): 0.5 kg. Phase 1: days 24-32 of age, 6-9 kg BW, estimated DMI: 2.75 kg. Phase 2: days 33-39 of age, 9-11.2 kg BW, estimated DMI: 3.0 kg and Phase 4: days 40-49 of age, 11.2-16 kg BW, estimated DMI: 6.25 kg.

Milkiwean Kinetio<sup>®</sup>, on the other hand, is a starter feed formulated under the concept of digestion kinetics. It incorporates ingredients that provide a balanced mix of protein and starch sources with varying digestion rates (rapid, slow, and resistant), as well as fibers with different fermentation rates (rapid, slow, and resistant).

### Response variables

The following productive variables were evaluated: average daily gain (ADG), cumulative weight gain (CWG), average daily feed intake (FI), cumulative feed intake (CFI), feed conversion ratio (FCR), and final body weight (FBW). Additionally, an economic analysis was conducted based on the relationship among dry matter intake (DMI), feed conversion ratio (FCR), and average daily gain (ADG).

### Statistical analysis

The experimental design was completely randomized, with two treatments and 22 replicates per treatment; each pen was considered an experimental unit. Prior to analysis, the Shapiro-Wilk and Levene's tests were applied to verify data normality and homogeneity of variances, respectively. Subsequently, an analysis of variance (ANOVA) was performed using the GLM procedure of the Statistical Analysis System (SAS, 2010; Cary, NC, USA), with a significance level of  $P \leq 0.10$ . Mean comparisons between treatments were conducted using Tukey's test ( $P \leq 0.10$ ). Initial body weight was included as a covariate in the statistical model ( $P \leq 0.10$ ) to adjust for potential initial effects on the productive variables evaluated.

## RESULTS AND DISCUSSION

Table 1 presents the results of the productive performance of pigs during the starter phase. Final body weight (FBW), average daily gain (ADG), and cumulative weight gain (CWG) were higher in pigs fed the standard Milkiwean<sup>®</sup> diet ( $P=0.08$ ). Similarly, both daily and total dry matter intake (DMI) throughout the experimental period were significantly greater with the use of the standard Milkiwean<sup>®</sup> feed ( $P=0.001$ ). However, despite the Milkiwean Kinetio<sup>®</sup> treatment showing lower ADG and reduced feed intake, it

**Table 1.** Productive performance of starter pigs fed with two starters.

Item	T1: Milkiwean	T2: Milkiwean Kinetio	SE	P
Initial number of pigs	220	220		
Final number of pigs	217	218		
Mortality, number	3	2		
Initial body weight, kg/pig	6.42	6.41	0.83	-
Final body weight, kg/pig	15.87	15.45	0.16	0.08
ADG, kg	0.330	0.310	0.01	0.08
Cumulative ADG, kg	9.45	9.03	0.16	0.08
Daily DMI, kg	0.410	0.370	0.01	0.001
Total DMI, kg	11.78	10.70	0.20	0.001
DMI Phase 0, kg	0.51	0.50		
DMI Phase 1, kg	2.79	2.78		
DMI Phase 2, kg	3.04	3.03		
DMI Phase 3, kg	5.43	4.39		
Feed Conversion Ratio	1.25	1.18	0.01	0.001

T: Treatment; SE: Standard Error; ADG: Average Daily Gain; DMI: Dry Matter Intake.

exhibited a significantly improved feed conversion ratio (FCR) compared to the standard feed ( $P=0.001$ ), suggesting a higher nutrient utilization efficiency in this group.

The breakdown of feed cost and consumption per phase is detailed in Table 2. The price per kilogram of both feeds was similar; however, due to the higher intake observed in the group fed the standard Milkiwean<sup>®</sup> diet, the cumulative feeding cost began to diverge starting from Phase 3. The total feed cost over the entire period was higher for the standard Milkiwean<sup>®</sup> diet (\$8.271 USD) compared to Milkiwean Kinetio<sup>®</sup> (\$7.652 USD).

When the total feeding cost is related to the weight gain achieved, the cost per kilogram of pork produced was lower with Milkiwean Kinetio<sup>®</sup> (\$0.847 USD/kg) than with the standard feed (\$0.875 USD/kg), indicating greater economic efficiency of the digestion kinetics-based treatment. It is important to note that the nutritional value of feed ingredients is not strictly additive, as it is influenced by complex interactions between the diet and the animal. Nevertheless, conventional feed formulation and evaluation practices often assume nutrient additivity and rely on static nutritional values, largely due to the lack of more precise tools [4].

To advance toward more realistic models, it is essential to consider the dynamic processes of digestion, metabolism, and specific interactions between diet and animal physiology. In this context, models have been developed that integrate parameters such as digestive kinetics, variability in feed intake, enzymatic digestion, hydrolysis, and nutrient absorption providing a more robust framework for understanding the complex nutritional processes occurring in pigs [2]. The composition and origin of feed ingredients directly influence their physicochemical properties, which in turn affect nutrient degradability and solubility both for individual ingredients and for the diet as a whole. This variability impacts nutrient absorption along the gastrointestinal tract [5]. Currently, physicochemical properties of nutrients or diets such as solubility, viscosity, and pH are not routinely considered in feed formulation, despite being determined by the specific composition of the ingredients used. Although existing digestive models can provide detailed insights to support post-

**Table 2.** Feed cost of starter pigs fed with two starters.

Item	T1: Milkiwean	T2: Milkiwean Kinetio
Feed cost, \$ USD/kg*		
Milkiwean Phase 0	\$1.030	\$1.068
Milkiwean Phase 1	\$0.823	\$0.834
Milkiwean Phase 2	\$0.669	\$0.671
Milkiwean Phase 3	\$0.629	\$0.630
Cost of feed consumed, \$ USD/kg*		
Milkiwean Phase 0	0.525	0.534
Milkiwean Phase 1	2.296	2.319
Milkiwean Phase 2	2.034	2.033
Milkiwean Phase 3	3.416	2.766
Total feeding cost, \$/pig	\$8.271	\$7.652
Cost per kg of pork produced, \$ USD	\$0.875	\$0.847

\*Only includes the cost of raw materials, packaging, and manufacturing. T: Treatment.

absorptive metabolic models and predict the nutritional value of pig diets, they still have significant limitations. In particular, they are unable to simulate variation in nutrient absorption kinetics originating from diets with identical nutrient profiles but differing ingredient sources. Incorporating dietary factors such as feed viscosity, nutrient solubility, feed intake levels, and differentiation between gastric emptying of solids and liquids would allow for the development of more realistic models that simulate the transit and digestion of each ingredient within the stomach [4]. Within the scope of precision swine nutrition, significant advances can be achieved through the assessment of digestion kinetics of dietary chemical components, also considering circadian behavior, gastrointestinal microbiota, and the functional properties of dietary ingredients [2,6]. Beyond the quantitative content of nutrients or metabolites, the rates of digestion and fermentation are crucial for understanding the timing of nutrient release along the gastrointestinal tract. This information is critical for predicting the post-absorptive appearance of nutrients and their metabolic effects [7]. Information on protein digestion kinetics can be used to develop strategies that synchronize the supply of energy and protein, thereby improving protein retention and utilization efficiency in pigs [8]. This is particularly relevant because, even when amino acids are absorbed, they may not always be fully bioavailable or available at the right time for metabolic utilization [5]. The postprandial appearance of amino acids and peptides in the bloodstream is associated with the kinetics of free amino acid and di-/tripeptide release during digestion. Therefore, ingredients can be classified as sources of rapidly digestible protein such as wheat gluten or plasma protein and slowly digestible protein such as soybean meal, canola meal, or black soldier fly larvae [8]. In weaned piglets, whose gastric conditions include a relatively high pH, it is advisable to select protein sources with greater solubility at this pH, as higher solubility accelerates protein degradation. Rapidly digestible proteins reduce the risk of diarrhea by minimizing the amount of undigested protein available to pathogenic bacteria in the large intestine. Starch digestion kinetics also has significant effects on productive response. It has been observed that pigs fed higher proportions of resistant or slowly digestible starch exhibit longer intervals between meals and more prolonged feeding patterns compared to those fed rapidly digestible starch [9]. This kinetic behavior is influenced by starch characteristics such as the amylose-to-amylopectin ratio, particle size, processing method, and interactions with other dietary components. Based on the rate and extent of enzymatic digestion, starch can be classified as rapidly digestible, slowly digestible, or resistant [10]. It is worth noting that gastric starch digestion is often underestimated, which can lead to overestimation of the starch fraction reaching the small intestine, thereby affecting the accuracy of *in vitro* models [10]. Given that starch is the primary quantitative macronutrient in pig diets, it may also alter the digestion kinetics of other nutrients, such as proteins and minerals. In swine diet formulation, the concept of fiber degradation and fermentation kinetics is applied to improve gastrointestinal health, digestive system development, and digesta transit. Proper characterization of dietary fiber fractions is essential, as they can influence the production of short-chain fatty acids, lactate, gases, and the composition of the intestinal microbiota [11]. Poorly fermentable fibers tend to support intestinal epithelial regeneration and improve fecal quality, while highly fermentable fibers serve as substrates for intestinal

bacteria, promoting digestive health benefits. However, excessive fermentable fiber may induce osmotic imbalances and intestinal disorders [12].

## CONCLUSION

Based on the results obtained in this study, it can be concluded that formulating starter diets for pigs based on the concept of nutrient digestion kinetics has the potential to optimize dietary nutrient utilization. This strategy enhances feed efficiency and reduces production costs, while promoting a more precise and sustainable approach to swine nutrition.

## ACKNOWLEDGMENTS

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# Characterization of forage weeds associated with grasslands under Voisin grazing system

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## ABSTRACT

**Objective:** To characterize forb and browse species associated with cattle pastures under the Voisin grazing system.

**Design/Methodology/Approach:** Floristic inventories were conducted at four distinct sites: a weed-dominated pasture (WeedDom), a secondary vegetation pasture (SecVeg), a pasture invaded by weeds (WeedInv), and a fallow field with woody vegetation (WoodVeg). The phenological transition phase was recorded and the growth stage identified for the most abundant species in each paddock. Cattle foraging behavior was assessed using scan sampling of 10 cows during active grazing. Chemical analyses were performed on 18 forage species from all sites to determine crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, total phenolics, and tannins.

**Results:** A total of 65 species were identified in WeedDom, dominated by *Sida spinosa* L.; 51 species in WoodVeg, dominated by *Solanum adscendens* Sendtn.; 51 species in SecVeg, dominated by *Cleome viscosa* L.; and 57 species in WeedInv, with *Andropogon gayanus* (Kunth) as the dominant species. Most plants were in vegetative or early blooming stages during the transition phase. Cattle diets were diverse; *S. spinosa*, *Trianthema portulacastrum* L., *Gomphrena globosa* L., *Lagascea mollis* Cav., and *A. gayanus* were among the most preferred species. Crude protein content ranged from 5.8% to 20.3%, NDF from 31.5% to 72.9%, and ADF from 21.0% to 40.0%, with lignin levels reaching up to 29.4%. Forage species also contained phenolic compounds (1.28-14.48%) and tannins (1.15-13.78%).

**Limitations/Implications:** This was an exploratory study.

**Findings/Conclusions:** Most forbs and browse species associated with grazing areas across varying growth stages and exhibiting diverse nutrient and phenolic profiles were effectively utilized as forage by cattle under the Voisin grazing system.

**Keywords:** forbs, grasses, chemical composition, phenology.

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## INTRODUCTION

Inadequate grazing management represents one of the main causes of pasture and grassland degradation. Nevertheless, overgrazing and the associated plant successional process often maintain levels of plant diversity that are generally not considered functional for a productive pastoral system and are indiscriminately eliminated through the use of herbicides, intensive mowing, or burning (Espinosa-Palomeque *et al.*, 2020; Bautista-García *et al.*, 2022). However, this vegetation, often classified as undesirable, constitutes

a potential source of biomass with relevant nutritional value that, under proper management, can be utilized as part of cattle diets (Rodríguez *et al.*, 2018). Rational management involves establishing appropriate grazing and rest periods for pastures, as well as adjusting the stocking rate based on forage availability. This strategy promotes better forage utilization by cattle, including associated weedy species that might otherwise invade and displace dominant grasses. This research supports the use of the Voisin grazing system as a management tool to allow cattle to incorporate a greater diversity of associated weedy species present in grazing sites into their diets, particularly in areas with high plant heterogeneity and reduced grass dominance. Our study objective was to characterize the weedy species associated with pastures that form part of the cattle diet in sites with different plant composition and diversity, identifying their phenological stages and quantifying the chemical-nutritional composition of those species effectively consumed under a Voisin grazing system.

## **MATERIALS AND METHODS**

### **Location and study area description**

The research was conducted in a tropical dry deciduous forest ecosystem. The predominant climate corresponds to type AW1(w)(i)g (warm, dry, with regular rainfall), according to the Köppen classification modified by García (2004). The average annual precipitation is 1,500 mm.

### **Study site floristic diversity**

Four grazing environments with varying degrees of vegetation intervention were evaluated, all managed under the Voisin grazing system with cattle, during the period from July to October 2022. The environments included: (1) a pasture dominated by forb species (WeedDom, 9 paddocks), (2) a fallow field with woody vegetation (WoodVeg, 5 paddocks), (3) a site with established secondary vegetation (SecVeg, 5 paddocks), and (4) a pasture invaded by forbs and browses but still dominated by grasses (WeedInv, 8 paddocks). In each paddock, five sample points were randomly selected. At each point, a 1 m<sup>2</sup> frame was placed to record the plant species present, followed by an extrapolation to characterize the general floristic composition of each site.

### **Optimal rest period and plant phenological stage**

The optimal rest period (ORP) is defined as the moment when the pasture completes its vegetative stage and transitions toward floral bud formation (Klapp, 1977). In each paddock, the ORP was determined based on the most abundant species present. At that time, the phenological stage of each species was recorded using the methodology proposed by Van Soest (1994), with necessary adaptations for our study objectives.

### **Forb species in cattle diets**

Scan sampling was conducted on 10 randomly selected cattle during active grazing, the frequency of consumption of each plant species was recorded to identify the preferred forbs in each evaluated environment.

### **Chemical analysis of selected forbs**

Foliage of the forb species consumed by cattle was collected at each study site for chemical analysis. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin content were determined using the filter bag technique (Van Soest *et al.*, 1991). Crude protein was quantified using the Kjeldahl method (AOAC, 1990). Additionally, total phenolics were measured using the Folin–Ciocalteu method, with absorbance reading at 725 nm, and total tannins were quantified using the PVPP (polyvinylpyrrolidone) method following FAO (2000).

## **RESULTS AND DISCUSSION**

### **Floristic diversity of evaluated sites**

In the WeedDom site, 20 botanical families, 40 genera, and 65 species were identified. In WoodVeg, 19 families, 37 genera, and 51 species were recorded, while WeedInv contained 20 families, 34 genera, and 57 species. Finally, the SecVeg site recorded 15 families, 35 genera, and 51 species.

Overall, the species richness observed in this study follows that reported by other authors in similar grazing environments within the same region (Soto-Calderón *et al.*, 2018; Espinosa-Palomeque *et al.*, 2020). This difference may be attributed to the fact that, in the present study, species richness is reported specifically per site, rather than as an aggregate from all sites. Although floristic richness was dominated by forb species, the values recorded suggest that cattle may be selecting diets with diverse composition, taking advantage of the plant heterogeneity available across the evaluated environments.

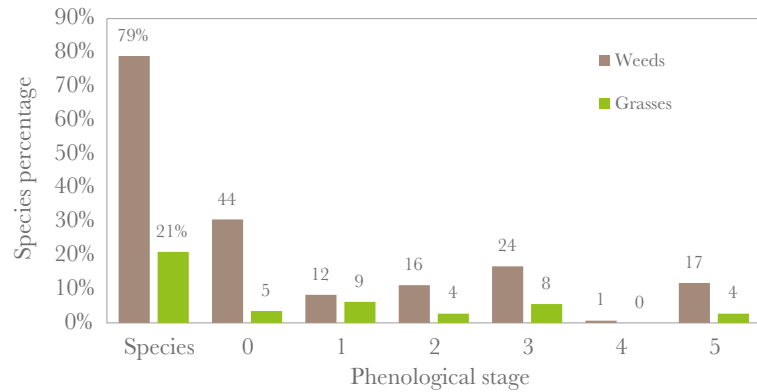
### **Phenological stages of plants in the paddocks**

At the time of identifying the optimal rest period (ORP), plant species were distributed across various stages of development. In general, most plants fell within phenological categories 0 to 5, according to the classification proposed by Van Soest (1994). Only a few species were observed at more advanced stages of physiological maturity. In the WeedDom site, both forb and grass species exhibited a higher proportion of individuals in the vegetative (categories 0 to 2) and early budding stage (stage 3). Only a small fraction of species was in more advanced reproductive phases (stages 4 and 5) (Figure 1).

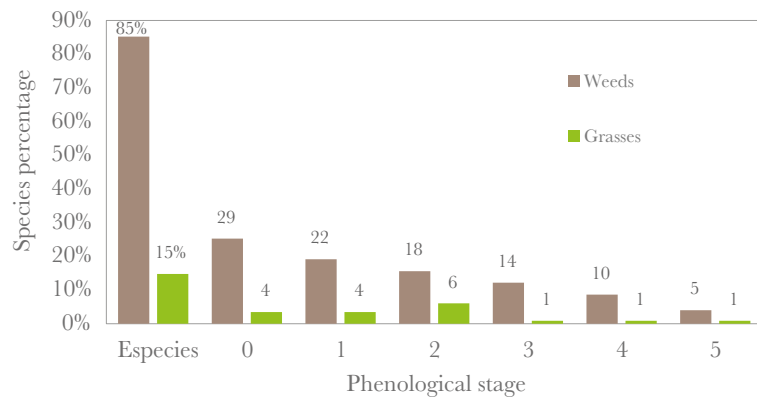
At the WoodVeg site, more species were observed in early developmental stages, with progressive reduction as they advanced to later phenological phases. In particular, native grasses were predominantly found in initial vegetative stages 0, 1, and 2 (Figure 2).

At the SecVeg site, most forb species were in the early vegetative stage (stage 0), with only a small number distributed across subsequent phenological stages (1 to 5). In contrast, grasses present at this site were mainly in stages 3 and 4, corresponding to the onset of floral budding (Figure 3).

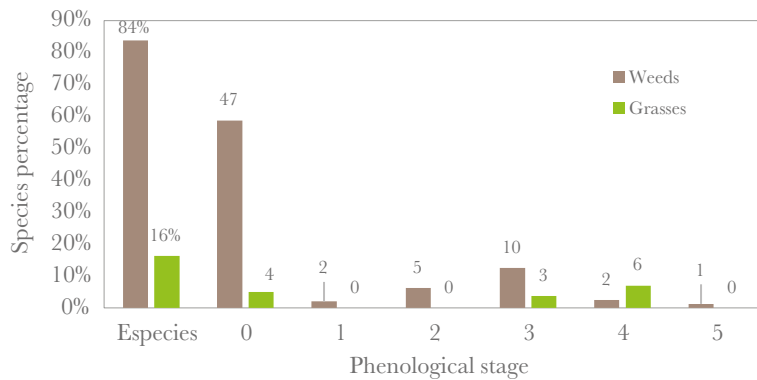
Finally, at the WeedInv site, fewer forb species were observed, as the grass *Andropogon gayanus* consistently dominated these paddocks. The forbs present were mostly in the early vegetative stage (stage 0), with a progressive decline in frequency toward more advanced phenological stages. Most grasses were found in stage 2, corresponding to the intermediate vegetative state (Figure 4).



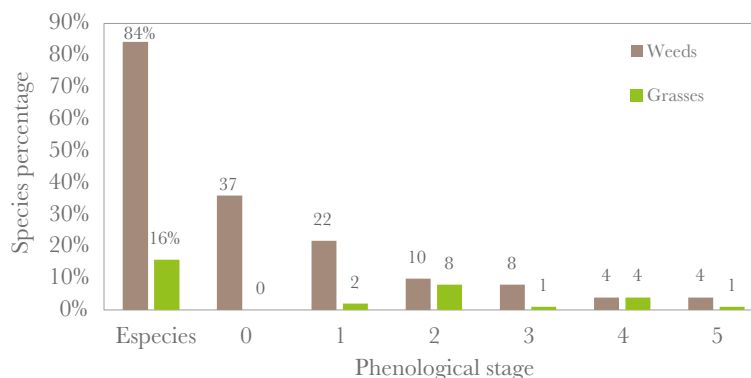
**Figure 1.** Frequency of phenological stages of grasses and associated species in a weed-dominated pasture (WeedDom) at the optimal pasture rest period. Numbers above the bars indicate the number of species corresponding to each percentage.



**Figure 2.** Frequency of phenological stages of grasses and associated species in a fallow field with secondary vegetation (WoodVeg) at the optimal pasture rest period. Numbers above the bars indicate the number of species corresponding to each percentage.



**Figure 3.** Frequency of phenological stages of grasses and associated species in a site with secondary vegetation (SecVeg) at the optimal rest period. Numbers above the bars indicate the number of species corresponding to each percentage.



**Figure 4.** Frequency of phenological stages of grasses and associated species in a pasture invaded by forbs (WeedInv) at the optimal rest period. Numbers above the bars indicate the number of species corresponding to each percentage.

At the time of determining the optimal rest period (ORP) in all evaluated paddocks, most plant species were in the vegetative state, although distributed across different stages of development. This phenological variability is attributed to intrinsic differences among species regarding their growth and developmental rates (Rua, 2015). Additionally, paddock recovery may vary even within the same site due to the influence of abiotic factors such as soil moisture, solar radiation, microrelief, and soil fertility (Brizuela *et al.*, 2015).

#### Forb species selected by cattle

Cattle selected different plant species depending on the floristic composition of each grazing site. Within each environment, certain species were consumed more frequently than others, although the observed diets were diverse overall. At the WeedDom site, the most frequently selected species were *Sida spinosa*, *Trianthema portulacastrum* L., and *Ruellia nudiflora* (Engelm. & A. Gray) Urb. In the WoodVeg site, *Gomphrena globosa* L., *S. spinosa*, *Lagascea mollis* Cav., and *Zornia diphylla* (L.) Pers. were prominent. In the SecVeg site, the most consumed species were *L. mollis*, *Ipomoea purpurea* (L.) Roth, and *Cleome viscosa* L., while in the WeedInv site, *Andropogon gayanus*, *L. mollis*, and *Borreria laevis* (Lam.) Griseb. constituted a significant portion of the diet (Table 1).

A notable diversity of forb species included in the cows' diet was observed across all study sites. Variations in dietary composition were mainly influenced by the differential presence and availability of species in the various paddocks (Table 1). According to Soto-Calderón *et al.* (2018), even when floristic diversity is high, dominant species in the cattle diet are commonly identified, with herbaceous plants being the most representative across different grazing environments.

#### Chemical composition of selected forbs

The chemical composition of the species consumed by cattle was diverse. Crude protein (CP) content showed wide variation, ranging from 5.7% to 20.3%. *Indigofera thibaudiana* DC. stood out with the highest CP concentration and the lowest neutral detergent fiber (NDF) content, positioning it as a species of high nutritional quality (Table 2).

**Table 1.** Bite frequency at which plant species were selected by cattle in each grazing environment.

Species	Freq	Species	Freq
<b>WeedDom</b>		<b>WoodVeg</b>	
<i>Sida spinosa</i>	178	<i>Gomphrena globosa</i>	74
<i>Trianthema portulacastrum</i>	109	<i>Sida spinosa</i>	41
<i>Ruellia nudiflora</i>	37	<i>Lagascea mollis</i>	39
<i>Tridax procumbens</i>	32	<i>Zornia diphylla</i>	34
<i>Cleome viscosa</i>	23	<i>Desmodium procumbens</i>	30
<i>Croton argenteus</i>	18	<i>Dalea cliffortiana</i>	29
<i>Lagascea mollis</i>	15	<i>Cleome viscosa</i>	23
<i>Mimosa pudica</i>	13	<i>Solanum adscendens</i>	23
<b>SecVeg</b>		<b>WeedInv</b>	
<i>Lagascea mollis</i>	63	<i>Lagascea mollis</i>	24
<i>Ipomoea purpurea</i>	43	<i>Borreria laevis</i>	15
<i>Cleome viscosa</i>	32	<i>Diphysa minutifolia</i>	15
<i>Gomphrena globosa</i>	19	<i>Sida spinosa</i>	12
<i>Solanum adscendens</i>	17	<i>Desmodium procumbens</i>	8
<i>Cracca greenmanii</i>	11	<i>Cleome viscosa</i>	8
<i>Sida acuta</i>	11	<i>Andropogon gayanus</i>	7
<i>Chamaecrista nictitans</i>	10	<i>Sida acuta</i>	5
<i>Randia punctata</i>	10	<i>Tridax procumbens</i>	3

Only the most frequently selected species were included.

In contrast, *Andropogon gayanus* showed the highest values of NDF and acid detergent fiber (ADF), typical characteristics of grasses that have not yet reached full physiological maturity (Velázquez-Martínez *et al.*, 2011).

Crude protein values recorded in this study fall within the range reported by Soto-Calderón *et al.* (2018), indicating that the species consumed by cattle possess adequate nutritional quality. In general, forb species exhibited higher CP content than grasses, suggesting that these plants complement the cattle diet by providing essential nutrients that improve overall nutritional balance. Regarding secondary compounds, total phenolic concentrations up to 14.48% and total tannins up to 13.78% were observed in the evaluated species. *Randia punctata* showed the highest concentrations of both compounds, while grasses exhibited the lowest levels of phenolics and tannins (Table 3).

Broadleaf species tended to exhibit higher concentrations of phenolic compounds compared to grasses. These results highlight that all species selected by cattle contained phenolic compounds, although with notable variability among them. Nevertheless, the presence of these secondary metabolites did not prevent ingestion by cattle, which aligns with the findings of Villalba and Provenza (2007), who indicate that herbivores possess physiological and behavioral mechanisms that allow them to self-regulate the intake of such compounds. When present in moderate concentrations, phenolic compounds may offer metabolic benefits and contribute to the overall well-being of livestock (Avella *et al.*, 2008). In this study, no adverse effects were observed associated with the consumption of

**Table 2.** Chemical composition (%) of the collected species.

Species	CP	NDF	ADF	Lig	CC	Hemicel	Cel
<i>Sida spinosa</i>	14.33	41.35	20.52	5.58	58.65	20.83	14.94
<i>Indigofera thibaudiana</i>	20.3	31.35	21.03	8.8	68.65	10.32	12.23
<i>Chamaecrista nictitans</i>	17.04	47.04	31.5	13.55	52.96	15.54	17.95
<i>Ruellia nudiflora</i>	13.2	40.99	26.73	11.03	59.01	14.26	15.7
<i>Tamonea curassavica</i>	10.44	49.87	30.87	11.27	50.13	19.0	19.6
<i>Zornia diphylla</i>	10.77	47.7	27.21	8.77	52.3	20.49	18.44
<i>Desmodium procumbens</i>	17.28	49.43	30.92	8.13	50.57	18.51	22.79
<i>Gliricidia sepium</i>	19.14	48.74	41.34	29.4	51.26	7.4	11.94
<i>Gomphrena globosa</i>	11.66	44.75	26.32	4.16	55.25	18.43	22.16
<i>Guazuma ulmifolia</i>	12.53	54.04	32.8	18.01	45.96	21.24	14.79
<i>Ipomea purpurea</i>	8.69	38.99	26.89	6.17	61.01	12.1	20.72
<i>Randia punctata</i>	9.13	57.66	38.09	24.31	42.34	19.57	13.78
<i>Solanum adscendens</i>	11.84	36.74	27.2	8.34	63.26	9.54	18.86
Annual grass sample	8.0	68.52	31.88	4.24	31.48	36.64	27.64
<i>Aeschynomene americana</i>	13.12	45.5	30.79	8.69	54.5	14.71	22.1
<i>Andropogon gayanus</i>	5.65	72.97	40.06	4.05	27.03	32.91	36.01
<i>Borreria laevis</i>	7.55	47.1	39.71	18.35	52.9	7.39	21.36
<i>Lagascea mollis</i>	13.3	51.13	38.28	21.78	48.87	12.85	16.5

CP=Crude Protein, NDF=Neutral Detergent Fiber, ADF=Acid Detergent Fiber, Lig=Lignin, CC=Cell Content, Hemicel=Hemicellulose, Cel=Cellulose.

**Table 3.** Total phenolic and tannin concentrations in forb species selected by grazing cattle.

Scientific Name	Total Phenolics (%)	Total Tannins (%)
<i>Sida spinosa</i>	3.11	2.44
<i>Indigofera thibaudiana</i>	4.76	3.38
<i>Chamaecrista nictitans</i>	13.76	13.19
<i>Ruellia nudiflora</i>	1.44	1.35
<i>Tamonea curassavica</i>	7.15	6.05
<i>Zornia diphylla</i>	1.5	1.15
<i>Desmodium procumbens</i>	2.45	1.8
<i>Gliricidia sepium</i>	2.16	1.53
<i>Gomphrena globosa</i>	1.58	1.24
<i>Guazuma ulmifolia</i>	8.59	8.03
<i>Ipomea purpurea</i>	9.18	8.22
<i>Randia punctata</i>	14.48	13.78
<i>Solanum adscendens</i>	2.69	2.00
Annual grass sample	1.28	1.18
<i>Aeschynomene americana</i>	3.13	2.74
<i>Andropogon gayanus</i>	4.27	3.92
<i>Borreria laevis</i>	4.23	2.94
<i>Lagascea mollis</i>	1.39	1.24

species with higher phenolic content, which may be attributed to the animals' varied and selective feeding behavior under grazing.

## CONCLUSIONS

The Voisin grazing system promotes the inclusion of a diversity of forb and browse species in cattle diets, which are present in various phenological stages, with equally variable chemical-nutritional composition. These species constitute part of the available forage in the paddocks and provide nutrients that complement cattle diets, enhancing their nutritional balance under extensive grazing conditions. Beyond their nutritional value, this dietary selection may represent an ecological mechanism for weed control particularly in invaded sites by reducing their relative dominance through targeted consumption. In the long term, this strategy could contribute to the restoration of the botanical composition of pastures, promoting greater grass predominance and thereby improving the structure and functionality of the forage system.

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# Environmental niche of *Pennisetum setaceum* (Forssk.) Chiov., 1923, invasive species in Mexico, and climate change scenarios

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## ABSTRACT

**Objective:** The goal of this work was to know the potential distribution of *Pennisetum setaceum* (Forssk.) Chiov., 1923 in Mexico, at present and in the future, after its introduction as an ornamental species, which carries a risk of invasion. However, its presence has not been exhaustively monitored, and there are favorable environmental conditions for its distribution in México.

**Design/methodology/approach:** we used MaxEnt algorithm as the methodology of ecological niche modeling. For climate change models, the four SSP scenarios of the IPCC Sixth Report were used.

**Results:** The model generated for *P. setaceum* presents a potential environmental niche with high probability for the entire Pacific coast and in central Mexico; in general, the future scenarios maintain the central area of the country as the most likely distribution area. These results represent the first models for the potential environmental niche distribution of *P. setaceum* in Mexico.

**Limitations on study/implications:** However, it is necessary to increase the data from historical records.

**Findings/conclusions:** Monitoring the species could generate more fresh data to improve accuracy in futures models and start reasoning about containment measures, such as restricting its use or pursuing eradication.

**Keywords:** alien species, climate change, desertification, ecological niche.

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## INTRODUCTION

*Pennisetum setaceum* (Forssk.) Chiov. (1923) is a herbaceous plant belonging to the Poaceae family. It is cultivated as an exotic ornamental or forage plant in various parts of the world and has proven to be an aggressive coloniser (GIBD, 2021). It is native to North Africa, East Africa, and the Arabian Peninsula (EPP0, 2009) and grows in arid coastal and pre-desert areas of the Sahara (Williams *et al.*, 1995), reaching altitudes of up to 2,800

metres. Today, this plant is considered cosmopolitan, as it is found on all continents. It has a wide climatic range, provided that annual rainfall does not exceed 1,270 mm and temperatures do not fall below 0 °C (Pasta *et al.*, 2010). This triploid species has a lifespan of up to 20 years. It becomes productive from the second year, producing up to 100 seeds per inflorescence (Pasta *et al.*, 2010).

*P. setaceum* is highly resilient to the impact of fire due to its strong root system and rapid germination following a burning event (Alcamisi, 2019). It can adapt to degraded and infertile soils, as well as drought conditions, and has become invasive in several countries, reducing biodiversity, impoverishing the soil, and displacing native grass species (PIER, 2021).

This weed is an adaptable grass that is highly competitive and efficient in its use of water. These characteristics have endangered the biodiversity of tropical dry forests in Hawaii (Tunison, 1992; Wagner *et al.*, 1990) since its introduction as an ornamental plant. In this environment, *P. setaceum* competes with the native grass *Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult., utilising water more effectively during the rainy season (Williams & Black, 1994). Cordell and Sandquist (2008) reported that changes in land use, grazing and forest clearance enabled this species to become established, resulting in the conversion of Hawaii's tropical dry forests into monospecific grasslands. Richardson *et al.* (2000) concluded that *P. setaceum*'s peculiarities make it a species capable of invading and modifying the ecological features of the territory it colonises. This species benefits from roads and motorways, as these facilitate the physical movement of seeds through air currents created by traffic. These tiny seeds germinate easily in poor and ruderal soils (Pasta *et al.*, 2010). Conversely, Georgen & Daehler (2001) state that the plant produces large quantities of seeds that can germinate within three to five days under optimal moisture conditions. Under adverse conditions, the seeds can maintain their germination potential for up to six years (Tunison, 1992).

Of the 1,100 recorded alien species in Mexico, 348 are considered invasive (CONABIO, 2021). *P. setaceum* is on the Ministry of Environment and Natural Resources' list of high-risk invasive alien species in Mexico. According to this agency, it was only found in the states of Baja California, Sonora and Nuevo León until 2016 (SEMARNAT, 2017).

A fundamental approach to understanding and managing species involves determining their current and potential distribution using tools such as ecological niche modelling (Guisan & Thuiller, 2005; Peterson *et al.*, 2012). ENMs contribute to predicting areas in which environmental conditions are suitable for species survival, describing potential distributions or fundamental environmental niches (Anderson *et al.*, 2003; Guisan & Thuiller, 2005; Simoes *et al.*, 2020). ENMs have been used to determine the ecological and spatial delimitations of native species (Martinez-Sifuentes *et al.*, 2021), alien species (Marcer *et al.*, 2012; Xue *et al.*, 2022), and, in one case, *P. setaceum* (Da Re *et al.*, 2020). The latter study used presence data related to invasion to determine the potential for adaptation to non-native habitats.

The aim of this study was to compile all available information on *P. setaceum* in Mexico, in order to create and analyse an ecological niche model for this species under current and future climate scenarios. This information is therefore crucial for assessing the potential

invasion of this species in Mexico. These results will inform the development of invasion mitigation strategies in Mexico.

## MATERIALS Y METHODS

### Creation of the database

The database was generated using data from the National Commission for the Knowledge and Use of Biodiversity (CONABIO, 2021), the Global Biodiversity Information Facility (GBIF, 2023) and the correspondance author's personal collection data (VB). This data was collected in the Guadalajara metropolitan area using a random transect method, with georeferenced self-sown individuals identified in urban and peri-urban areas in 2021. A total of 313 points of interest georeferenced to *P. setaceum* were included in the database. Duplicate occurrences were removed to reduce the effects of spatial autocorrelation, with records within 5 km of individual occurrence points being filtered using the spThin package in R (Ailello-Lammens *et al.*, 2015).

The final database comprises 29 unique occurrence points, each at least 5 km from the next. These points are located in the Mexican states of Baja California Sur, Baja California Norte, Sonora, Chihuahua, Sinaloa, Nayarit, Jalisco, Coahuila, Nuevo León, Michoacán, Guanajuato, Guerrero, Puebla and Chiapas.

### Accessible region M

Accessible region M (Barve *et al.*, 2011) was generated using a 100 km radius zone of influence around each occurrence point and subsequently overlaid on the World Wildlife Fund ecoregion shapefile (Olson *et al.*, 2001), according to the methodology proposed by Moo-Llanes *et al.* (2019) to avoid possible modelling bias related to model calibration. It was decided to use invasion area data rather than natural distribution data because it was thought that the prediction would be underestimated due to the significant difference in climate between Mexico and the Arabian Peninsula, as well as the species' high level of adaptability to new environments. In fact, the same position has been taken in several studies, with the intention of being able to more accurately approximate the model regarding biological invasion at the local level, when the invasive species has demonstrated a high capacity to adapt to climatic situations very different from its natural context (Marcer *et al.*, 2012; Xue Y *et al.*, 2022; Da Re *et al.*, 2020). The occurrence records were randomly divided into two subsets using the 'random k-fold' method: 70% for model calibration and 30% for assessments (Moo-Llanes *et al.*, 2020; Moo-Llanes *et al.*, 2021). This method randomly divides occurrence locations into a user-specified number of bins, as detailed in the Muscarella *et al.* (2014) protocol.

### Bioclimatic variables

Fifteen of the 19 WorldClim bioclimatic variables (Fick & Hijmans, 2017) were used to construct the ecological niche models (ENMs). Variables combining temperature (Bio08 and Bio09) and precipitation (Bio18 and Bio19) were excluded (Escobar *et al.*, 2014; Moo-Llanes *et al.*, 2021). All variables had a spatial resolution of 1 km<sup>2</sup>. Four sets of bioclimatic variables, each comprising 15 WorldClim variables, were used: a)

Set1: the fifteen bioclimatic variables from WorldClim (Moo-Llanes *et al.*, 2021). b) Set2: the Jackknife method in MaxEnt was used to select different bioclimatic variables that contributed most to the model (>90%) (Moo-Llanes *et al.*, 2013; Moo-Llanes *et al.*, 2021), removing one variable per pair with Pearson's correlation ( $r < 0.8$ ). To select the variable to be eliminated, it was verified that the variable presenting the lowest spatial autocorrelation with the pair of variables to be evaluated and with other combinations of bioclimatic variables would be selected. c) Set 3: nine variables were established for constructing ecological niche models for different species (Moo-Llanes *et al.*, 2013). Finally, Set4 (d): the variance inflation factor (VIF) was considered (Table 1).

This is a measure of the levels of multicollinearity between pairs of variables in the USDM package in R. VIF values greater than 10 indicate potentially problematic correlations with covariates and suggest that these should be carefully evaluated during model development (Estrada-Peña *et al.*, 2013; Moo-Llanes *et al.*, 2023). ENMs were projected for the current period (1970-2000) and for the year 2041-2060 using the Socio-Economic Shared Pathways (SSPs). The ENMs were projected using the four SSPs of the IPCC Sixth Assessment Report: SSP1-2.6 (445.6 ppm CO<sub>2</sub>), SSP2-4.5 (602.8 ppm CO<sub>2</sub>), SSP3-7.0 (867.2 ppm CO<sub>2</sub>) and SSP5-8.5 (1135.2 ppm CO<sub>2</sub>) (IPCC, 2021; Moo-Llanes *et al.*, 2021; Moo-Llanes *et al.*, 2023).

### Ecological Niche Models

These models were constructed using the MaxEnt algorithm based on the kuenm package in R (Cobos *et al.*, 2019). The generated models were projected onto region G, comprising the entire Mexican national territory, given that the species is cultivated as an ornamental plant in almost all of Mexico (personal observation).

**Table 1.** Sets of bioclimatic variables used for the construction of the ecological niche model of *Pennisetum setaceum* in Mexico.

Bioclimatic variables	Code	Set1	Set2	Set3	Set4
Average annual temperature	Bio01	X		X	X
Average diurnal range	Bio02	X			X
Isothermality	Bio03	X			X
Seasonal temperature	Bio04	X	X	X	
Maximum temperature of the hottest month	Bio05	X			
Minimum temperature of the coldest month	Bio06	X			
Annual temperature range	Bio07	X	X	X	
Average temperature of the hottest quarter	Bio10	X	X	X	
Average temperature of coldest quarter	Bio11	X	X	X	
Annual precipitation	Bio12	X		X	
Precipitation of the wettest month	Bio13	X		X	
Precipitation of the driest month	Bio14	X	X	X	X
Seasonal precipitation	Bio15	X		X	X
Precipitation in the wettest quarter	Bio16	X			X
Precipitation of the driest quarter	Bio17	X			

A total of 1,736 candidate models were obtained by combining four sets of environmental variables and 14 regularisation multiplier values (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 3.0, 4.0 and 5.0). These were then combined with the 29 possible combinations of the five entity classes (linear=l, quadratic=q, product=p, threshold=t and hinge=h) (Cobos *et al.*, 2019).

First, the best candidate models were selected for statistical significance: partial Receiver Operating Characteristic (ROC) and omission rate at 5% predictive ability. Secondly, they were selected for their performance based on Akaike's information-corrected criterion (AICc) for small sample sizes (Núñez-Penichet *et al.*, 2021). The number of parameters was determined by identifying all parameters with a non-zero weight in the lambda file produced by MaxEnt. This is a small text file containing details of the model produced by MaxEnt as part of the modelling process (Warren & Seifert, 2011). Finally, models with  $\Delta AICc \leq 2$  were selected from those that were statistically significant and had omission rates below 5% (Cobos *et al.*, 2019).

After the calibration process, the final models were created using all occurrences with the selected parameter values and 10 bootstrap replications with logistic outputs for Mexico. In order to identify high-risk geographical areas for model transfer, the environmental distance between sites in general, and to the nearest portion of the calibration region M, was calculated using Mobility-Oriented Parity (MOP) analysis. The environmental amplitude of the predictors within region G (10% of the sampled benchmarks) was then compared for the total projection area. It should be noted that the extrapolation risk analysis defines areas with strict extrapolation (*i.e.* they represent the degree of similarity between conditions in M and G, where zero values correspond to areas of strict extrapolation) to avoid the risk of overprediction in non-analogue settings (Owens *et al.*, 2013).

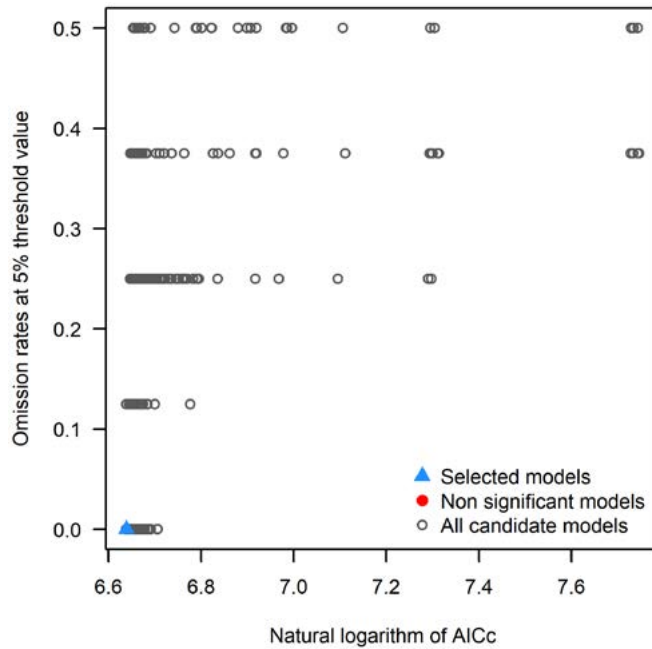
## RESULTS AND DISCUSSION

A total of 1,736 models were analysed, 1,254 of which were statistically significant. Five models that met the omission rate and AICc criteria were selected (Figure 1).

The model with environmental predictors (Set2), class (Threshold), multiple regularisation (N=0.9), partial ROC (1.39), omission rate at 5% (0.00), AICc (764.25),  $\Delta AICc$  (0.00), WAICc (0.16) and numerical parameters (N=9) was chosen as the best candidate (Table 2).

The model indicates that *P. setaceum* is likely to find favourable environmental conditions for establishment along the Pacific coast and in the central regions of Mexico (Chiapas, Oaxaca, Guerrero, Michoacán, Colima, Jalisco, Nayarit, Puebla, Mexico City, Guanajuato, Zacatecas, Sinaloa, Baja California Sur and Baja California Norte), as well as in the states of Veracruz, Hidalgo, San Luis Potosí and Coahuila (Figure 2a). However, care must be taken when interpreting the results for the areas corresponding to the Yucatán Peninsula, the Gulf of Mexico, San Luis Potosí, Durango, Zacatecas, Coahuila, Sinaloa, Sonora, Baja California Sur and Baja California Norte, as there is high standard deviation (Figure 2b).

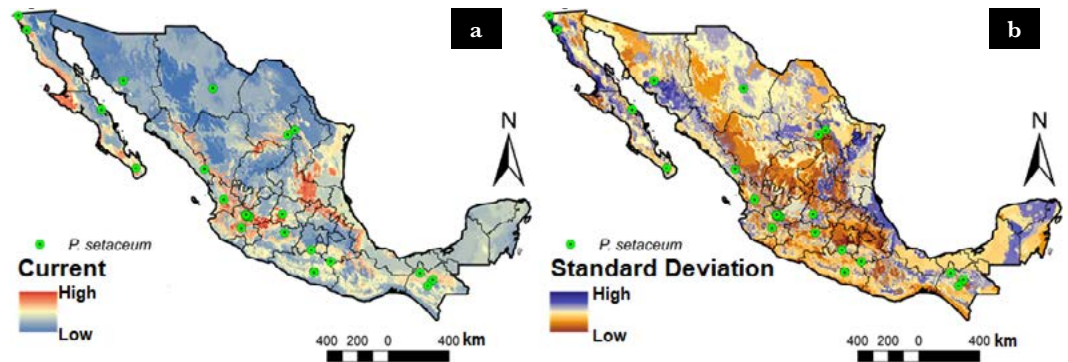
In all four climate change scenarios (SSP1-2.6; SSP2-4.5; SSP3-7.0; and SSP5-8.5), the areas of potential *P. setaceum* invasion and standard deviation are minimal. As can be seen in Figure 3, the increase in the areas of encroachment is smaller in each of the different scenarios.



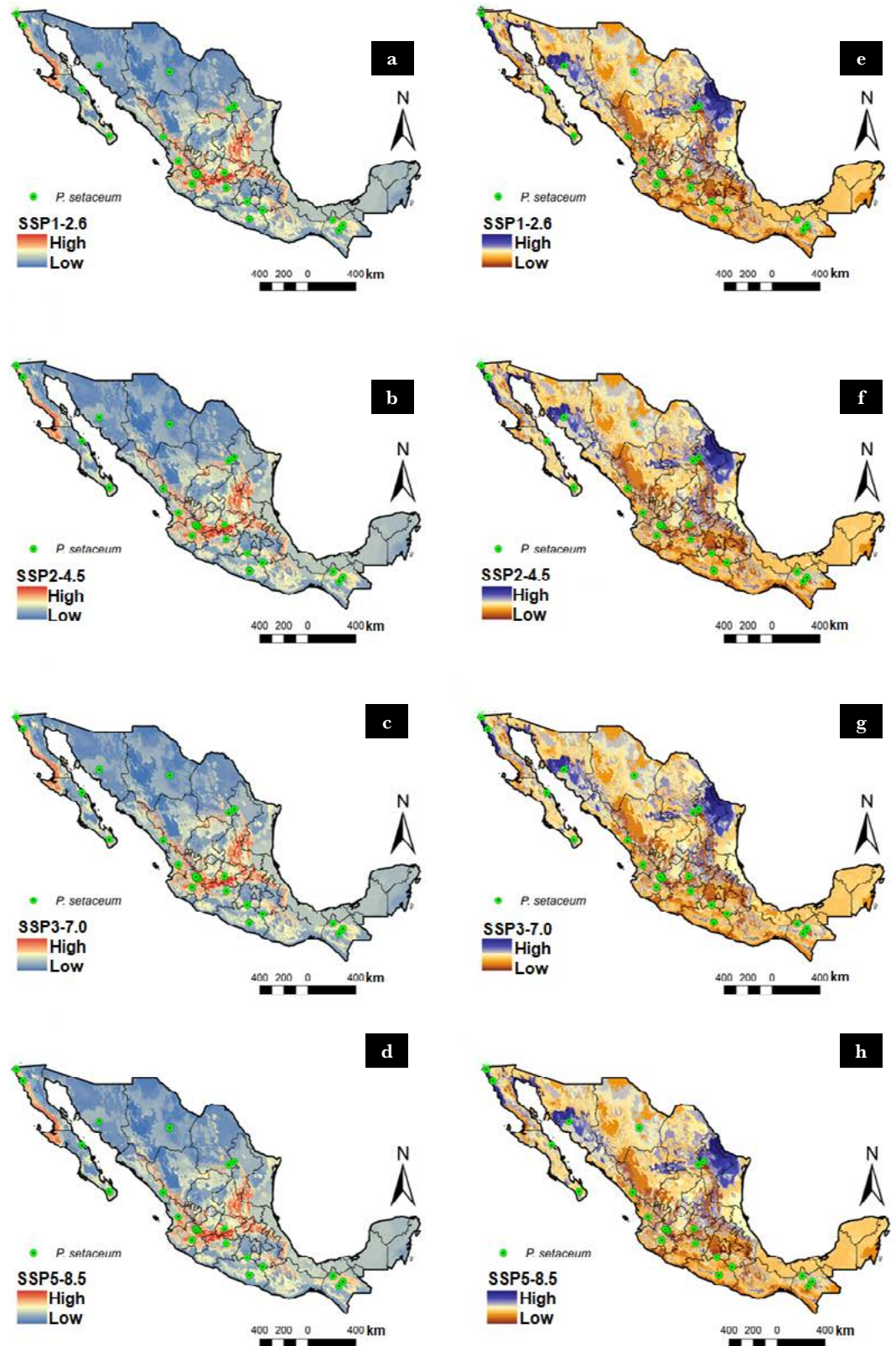
**Figure 1.** Distribution of selected models, those statistically non-significant and the total set, according to predefined criteria for *P. setaceum* in Mexico.

**Table 2.** Performance statistics for the selected models according to the criteria established for the construction of the ecological niche of *P. setaceum* in Mexico. \*Features: T (Threshold), P (Product), L (Linear) y Q (Quadratic).

Classes*	Multiple regularisation	Set	ROC partial	Omission rate 5%	AICc	Delta AICc	W AICCc	# Parameters
T	0.9	Set2	1.39	0.00	764.25	0.00	0.16	9
PT	0.9	Set2	1.38	0.00	764.25	0.00	0.16	9
LPT	0.9	Set2	1.38	0.00	764.25	0.00	0.16	9
QPT	0.9	Set2	1.38	0.00	764.25	0.00	0.16	9
LQPT	0.9	Set2	1.38	0.00	764.25	0.00	0.16	9



**Figure 2.** Ecological niche model for *P. setaceum* in Mexico. a) Shows the ENM of *P. setaceum* in Mexico, with the highest probability of distribution shown in red. b) Shows areas of uncertainty, with the highest uncertainty shown in blue.



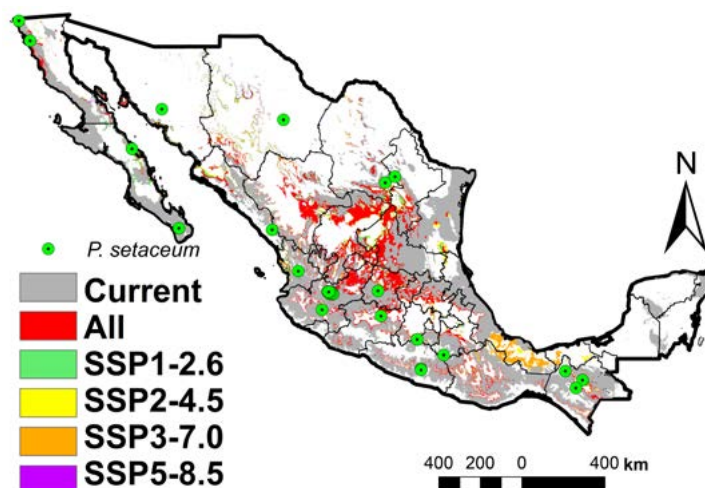
**Figure 3.** Climate change scenarios for the ENM of *P. setaceum* in Mexico. The left panel shows each of the four SSP scenarios (SSP1-2.6, SSP2-4.5, SSP3-7.0 and SSP4-8.5), while the right panel shows the uncertainty of the ENMs.

Overlapping the current scenario with the different climate change scenarios reveals that the zones where they overlap remain largely unchanged across Mexico, particularly in the centre of the country (Figure 4).

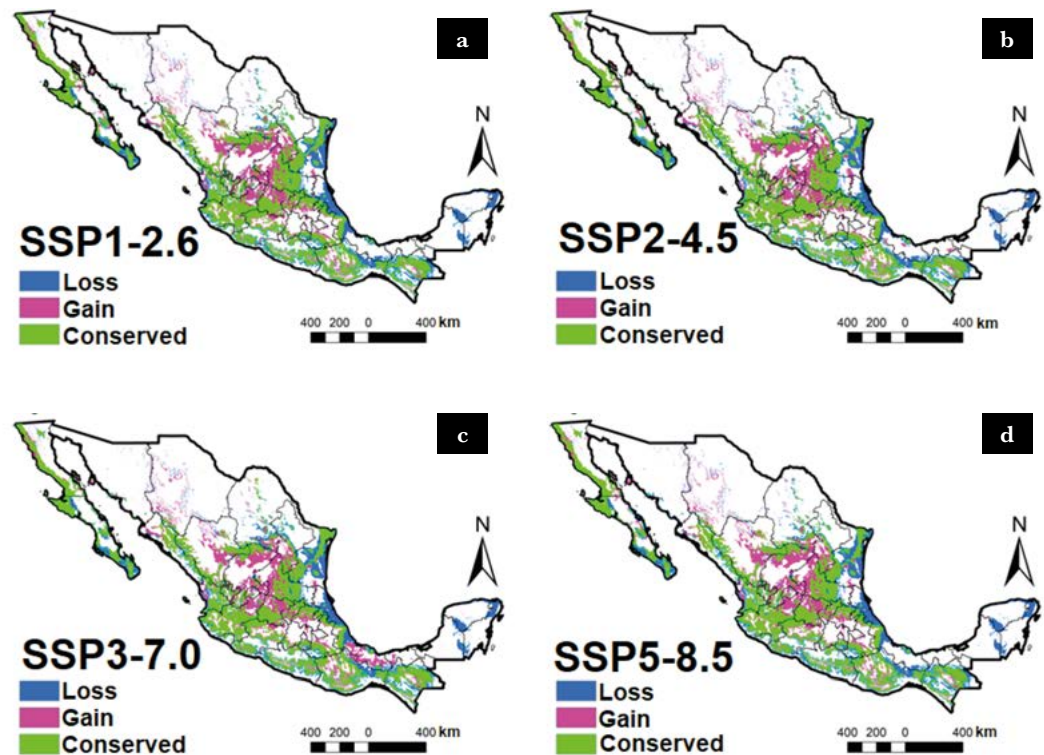
Depending on the scenario, there are exclusive areas of encroachment, as is the case with SSP370 in the Veracruz area. The areas that remain constant between the current period and the climate change scenarios are consistent across the four SSPs (SSP1-2.6, SSP2-4.5, SSP3-7.0 and SSP4-8.5). The areas lost correspond mostly to parts of the Yucatán Peninsula, the Gulf of Mexico (Veracruz) and Baja California Sur, while the areas gained (areas of new invasion) correspond mostly to central Mexico (Figure 5).

The territorial scale of the data presented here is much larger than that of a similar study conducted by Da Re *et al.* (2020) on the island of Tenerife. In that study, it was shown that the entire coastal area was suitable for the spread of *Pennisetum*. In contrast, Mexico offers an extremely large territorial space with a wide range of climates, including tropical conditions that could be suitable for the species, which naturally occurs in arid zones, as seen in its natural distribution. The models analysed could be improved to better describe reality if new distribution records for this species in Mexico could be accessed. This work may therefore stimulate interest in generating new knowledge about this species in Mexico, as the field study demonstrates the severity of the invasion in the studied sites, particularly in the Guadalajara metropolitan area (Jalisco), resulting in biodiversity loss and desertification (Pasta *et al.*, 2010; PIER, 2021; Tunison, 1992). Having observed that the plant is being used on a national scale as an ornamental grass, we are keen to see the results of this work. The collection points used are those of self-propagated individuals, *i.e.* individuals that originated in urban and peri-urban areas due to the species' invasive potential after seeds were spread from ornamental plantings in city flower boxes.

Raising awareness of this specific problem in Mexico could encourage the implementation of effective measures to prevent situations like the one in Italy, where eradication is now economically impossible (Alcamisi, 2019). The species was first introduced to the botanical garden in Palermo, Italy, in 1939 for study purposes, as a forage and ornamental plant. It is



**Figure 4.** Areas of overlap of the four SSP scenarios based on the ENM construction of *P. setaceum* in Mexico.



**Figure 5.** Areas of gain, loss and maintenance (conserved) of ENM of *P. setaceum*, according to each of the scenarios and the current period in Mexico.

now fully naturalised, having almost completely displaced local grasses such as *Hypparrhenia hirta* (L.) Stapf. (Pasta *et al.*, 2010; Giovanni Provenzano, personal communication; manager of the Monte Pellegrino Oriented Nature Reserve, Palermo). A similar situation could occur in some areas of Mexico if intervention is not carried out promptly and effectively.

Studies on possible increases in invasions depending on climate change scenarios on Tenerife warn of the potential expansion of this exotic species on the island, including the possible conquest of the high-altitude ecological niche of local pines. This could have serious consequences, including increased desertification and forest fires, as well as a loss of biodiversity (Da Re *et al.*, 2020). A similar situation could easily occur in the La Primavera Forest in Guadalajara, an area subject to frequent forest fires (Jardel-Peláez, 2021). The extensive root system of *P. setaceum* reduces the availability of water for neighbouring plants, stunting the growth of tree species (Cordell & Sandquist, 2008). It therefore limits the number of young trees in areas where it becomes established (Cabin *et al.*, 2002). Given the species' characteristics, we should not expect less aggressive results in Mexico than in previous studies conducted in other parts of the world. Currently, several cities in Mexico use this species for public landscaping, planting it in tree surrounds and roundabouts. There is strong support from citizens, who consider the species aesthetically pleasing. However, the risk of invasion is considered to be very high, and it has been observed that the species is reproducing spontaneously and colonising cracks in pavements, planters, and vacant lots in several areas of these cities (personal observation, Figure 6).



**Figure 6.** On the left, entrance to the city of *San Cristóbal de las Casas*, Chiapas, on the right, *Avenida de las Américas* avenue in the city of Guadalajara, Jalisco.

This situation is evident to a critical observer and deserves to be studied and reported, hence the need for this research. The models generated in this study demonstrate that there is indeed territory within the country susceptible to colonisation. Fieldwork data also show that the species is capable of colonising the Mexican environment independently. The large quantity of viable seeds produced by the species, its ecological niche and the complacency of the population, which propagates and sows the species unknowingly, could therefore generate a serious future problem.

## CONCLUSIONS

This study is the first to model the environmental niche of *P. setaceum* in the Americas. It is expected to lay the groundwork for further research on this invasive species in Mexico. Specific censuses must be carried out in several metropolitan areas of the country to update databases and produce increasingly accurate models, providing an up-to-date view of the level of invasion and enabling the development of control and eradication plans.

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