ISSN: 2594-0252

AGRO PRODUCTIVIDAD

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Año 18, Volumen 18, Número 6, junio 2025, Agro productividad es una publicación mensual editada por el Colegio de Postgraduados. Carretera México-Texcoco Km. 36.5, Montecillo, Texcoco, Estado de México. CP 56264. Tel. 5959284427. www.colpos.mx. Editor responsable: Dr. Jorge Cadena Iñiguez. Reservas de Derechos al Uso Exclusivo No. 04-2017-031313492200-203. ISSN: 2594-0252, ambos otorgados por el Instituto Nacional del Derecho de Autor. Responsable de la última actualización de este número, M.C. Valeria Abigail Martínez Sias. Fecha de última modificación, 5 de agosto de 2025.

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Sustainable production of melon seedlings (*Cucumis melo* L.) Zacapa with different substrates

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Citation: Miranda Ramírez, J.M., Perales-Segovia, C., González-Gaona, E., & Perales-Aguilar, L. (2025). Sustainable production of melon seedlings (*Cucumis melo* L.) Zacapa with different substrates. *Agro Productividad*. https://doi.org/10.32854/y8m7gk39

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

Received: November 21, 2024.
Accepted: May 15, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 3-17.

ABSTRACT

Objective: Different substrates were evaluated in polystyrene containers for the production of Harper-type melon (*Cucumis melo*) seedlings, focusing on growth performance and preliminary management prior to transplantation.

Design/Methodology/Approach: A completely randomized design was implemented, consisting of 15 treatments with 30 replicates each, using one plant per experimental unit. The treatments included four pure substrates: T1 peat moss (PM), T2 rice husk (RH), T3 vermicompost (V), T4 red clay (RC), and eleven combinations of these substrates in varying proportions. Plant height, leaf length and width, stem diameter, root length and diameter, among other variables, were recorded.

Results: Significant differences were observed across all treatments for all evaluated variables. The highest statistically significant plant heights were achieved with T10 (PM 10% + RH 70% + V 10% + RC 10%) and T11 (PM 33.33% + RH 33.33% + V 33.33%), reaching 4.74 cm and 4.78 cm, respectively. The greatest leaf length and width were recorded in T14 (PM 30% + V 70%), with values of 3.25 cm and 3.29 cm, respectively, corresponding to a leaf area of 10.69 cm^2. Study

Limitations/Implications: This study focused exclusively on the technological evaluation of melon seedling production. Future research should include an assessment of the economic profitability of the most promising treatments.

Findings/Conclusions: The PM-based substrate demonstrated a germination rate of 97.85%, indicating strong commercial potential for the production of melon seedlings.

Keywords: Cucurbitaceae, germination, growth, seedling management, containers.

INTRODUCTION

Melon (*Cucumis melo*) is an annual, herbaceous, creeping or climbing plant belonging to the Cucurbitaceae family. It is a phanerogamous species that reproduces through seeds (Mármol, 2008). In Mexico, approximately 19,759 hectares are cultivated annually,

with a production value of USD $219.35 million. The leading melon-producing states, in order of importance, are Guerrero, Sonora, Michoacán, Coahuila, and Chihuahua, with 3,583, 3,573, 3,532, 3,375, and 1,522 hectares planted, respectively (SIAP, 2024). Melon cultivation can be established either directly in the field or through transplantation (Robles Trinidad *et al.*, 2005). Direct sowing involves placing seeds directly into the prepared soil, whereas transplantation depends on obtaining seedlings in optimal sanitary conditions and with strong vigor before being transferred to the field (Jasso Chaverría *et al.*, 2012). In this context, the substrate becomes an essential input for the production of high-quality seedlings (Cabrera, 1999). According to Barbaro and Karlanián (2020), the substrate must meet specific physical properties that ensure an appropriate balance between air and water content properties that are closely linked to plant development and root distribution within the container. Additionally, Crawford and Abarca (2017) emphasize the importance of optimal phytosanitary control and the use of containers with the ideal number of cavities for production, under temperatures ranging from 28 to 30 °C. However, several challenges arise during the seedling production process in containers, with one of the most common being the improper use of substrates (Robles-Trinidad *et al.*, 2005; Domínguez-Liévano and Espinosa-Zaragoza, 2021). Such misuse often results in low germination rates, compact root balls that are difficult to extract without damaging the roots, or fragile root balls that crumble easily. Despite the practical relevance of substrate management, this area has received relatively little attention from researchers (Barret *et al.*, 2016). Moreover, due to the high cost of imported substrates, there is a growing need to identify locally produced materials that are physically stable and of proven quality and safety (Quesada-Roldán and Méndez-Soto, 2005). This scenario led to the formulation of the following research question: Which type of substrate can be used in the production of melon seedlings in containers to achieve optimal growth, facilitate easy handling, ¿and utilize local resources at the time of transplantation? Currently, a variety of substrates are used for horticultural seedling production in containers. These substrates can be classified as organic of natural origin such as peat moss, coconut fiber, sugarcane bagasse, rice husk, cereal straw, and vermicompost (Cruz-Crespo *et al.*, 2013). Among these, compost and vermicompost are notable because they are produced through biological processes that transform organic waste into relatively stable materials (Claassen and Carey, 2004). There are also inorganic substrates of natural origin, including perlite, vermiculite, sand, volcanic soils, and expanded clay, among others (Baixauli Soria and Aguilar Olivert, 2002; Cruz-Crespo *et al.*, 2013). Importantly, the physical properties of a substrate are considered more critical than its chemical properties, as the latter can be adjusted through nutrient solution management, while the former are much harder to modify (Baixauli-Soria and Aguilar-Olivert, 2002). These physical properties are determined by the internal structure of the particles, their granulometric distribution, and structural stability (Pastor-Sáez, 1999). In this context, several studies have highlighted the impact of substrates on the production of horticultural seedlings across various crops. For instance, García López *et al.* (2022) reported that tomato seedlings exhibited the highest germination rate (96%) when grown in a mixture of 50% commercial peat substrate and 50% vermicompost, outperforming other mixtures such as 70% vermicompost with 30% vermiculite, 100% peat, and 100%

vermicompost, which achieved germination rates of 93%, 92%, and 87%, respectively. Similarly, García-Velázquez *et al.* (2024) found that tomato plants (*Solanum lycopersicum* L.) grown in containers with a substrate mix of soil, humus, and rice husk (3:2:1) had superior stem diameter growth (3.87 mm) and total fresh biomass (5.302 g) compared to those grown with soil, peat, and rice husk (3:2:1), which exhibited 3.59 mm stem diameter and 4.995 g fresh biomass. In another study, Meneses Fernández and Quesada-Roldán (2018) reported that Dutch cucumber plants reached heights of 110 cm and 105 cm, 25 days after transplantation, when grown in mixtures of 40% coconut fiber + 40% palm fiber + 20% commercial organic fertilizer and 70% coconut fiber + 30% commercial organic fertilizer, respectively. These results were superior to other treatments, including mixtures of 50% coconut fiber + 50% palm fiber, 70% palm fiber + 30% melina sawdust, and a control using 100% commercial coconut fiber tablets, which only achieved heights between 45 and 78 cm. Additionally, Fernández-Zárate *et al.* (2022) found that *Cinchona pubescens* Vahl. (Rubiaceae) seedlings achieved an 88.3% germination rate using 100% forest soil, surpassing mixtures of 50% forest soil + 50% sand and 75% forest soil + 25% sand, which exceeded 50%, whereas the 25% forest soil + 75% sand mixture and 100% sand substrate resulted in germination rates below 50%. Furthermore, Cardoza-Viera *et al.* (2024) reported that maize seedlings exhibited the highest plant height values at all sampling times when grown in a 50% rice husk + 50% vermicompost mixture, reaching 32.47 cm at 18 days after emergence (DAE). This performance was significantly superior to substrates based on 100% sand, 75% sand + 25% rice husk, and 50% sand + 25% rice husk + 25% vermicompost, which achieved plant heights of 19.5 cm, 23.23 cm, and 29.37 cm, respectively. Therefore, the aim of this study was to evaluate the effect of different substrates in polystyrene containers on the growth and early management of Harper-type melon seedlings for transplantation purposes.

MATERIALS AND METHODS

Location and characterization of the experiment

The experiment was conducted in the municipality of Buenavista, Michoacán, Mexico, located at kilometer 2 on the highway from Crucero de la Ruana, Buenavista, Michoacán, to Tazumbos, Jilotlán de los Dolores, Jalisco, at coordinates 19° 13' 52" N, 102° 40' 13" W, and an elevation of 418 meters above sea level (Google Earth, 2024). The site features a BS1 (h') W (W) dry climate, classified within the group of dry climates BS1 (García, 2004), with an average annual temperature of 33.5 °C and an average annual precipitation of 599.3 mm (SMN-CONAGUA, 2024).

Plant material

The study used Harper-type melon (*Cucumis melo*) 'Zacapa' seeds, provided by the seed company US Agriseeds® (US Agriseeds, 2024).

Seedling management

For seedling production, 45 polystyrene containers with 248 cavities each were used, with a volume capacity of 35 cc per cone. Direct sowing was performed at a depth of 1 to 2 cm under the roof of a metal structure, where the trays remained for 48 hours.

Subsequently, the containers were transferred to a black shade house with 20% shade netting, measuring 10×20 m, to complete the germination cycle. Once germinated, the seedlings were placed on an open-air metal structure for 10 days. To manage sucking insect pests, the commercial repellent Oleotech Garlic® (20% *Allium sativum* oil) from Biotech/México and the adherent Inex A® (20.2% ethoxylated fatty alcohol) from Cosmocel/México were applied at a dose of 1.0 mL L^{-1} each, twice throughout the cycle. Applications were carried out using a manual backpack sprayer with a 15-liter capacity, brand SWISSMEX, model COSMOS®, directing the spray toward the foliage. For fungal disease prevention, *Trichoderma harzianum* (6.1×10^7 CFU mL^{-1}) was applied at a dose of 5.0 mL L^{-1}, four times throughout the cycle, using a 5-liter capacity metal watering can during irrigation. Nutritional management involved the application of 16-16-16 (N-P_2O_5-K_2O) fertilizer, GRO GREEN by Campbell, at a dose of 1.0 g L^{-1}, diluted in irrigation water and applied seven days after germination. Irrigation was performed manually, daily, in the early morning hours, using 1.0 L of water per container.

Experimental design

A completely randomized experimental design was employed, with 15 treatments, 30 replicates, and one melon plant per experimental unit.

Treatments

Among the 15 treatments, four were pure substrates (controls), and eleven were mixtures of these substrates in varying volumetric proportions (Table 1). A total of 3,720 melon seedlings were distributed across 15 rows, with each row representing one treatment and containing 744 seedlings arranged in three containers. For data collection, 10 seedlings

Table 1. Treatments used for the evaluation of melon seedlings.

Treatment	Substratum	Percentage
T1	PM	100%
T2	RH	100%
T3	WC	100%
T4	RC	100%
T5	PM+RH	50% + 50%
T6	RH+WC	50% + 50%
T7	RH+RC	50% + 50%
T8	PM+RH+WC	20% + 30% +50%
T9	RH+WC+RC	50% + 25% + 25%
T10	PM+RH+WC+RC	10% + 70% + 10% + 10%
T11	PM+RH+WC	33.33% + 33.33% +33.33%
T12	PM+RH+WC+RC	25% + 25% + 25% + 25%
T13	RH+WC	80% + 20%
T14	PM+L	80% + 20%
T15	PM+CA	10% + 90%

PM: peat moss; RH: rice husk; WC: wormcompost; RC: red clay were randomly selected from each container, resulting in 30 seedlings per treatment. The remaining 714 seedlings were used as buffer plants to ensure reliable data collection.

Study variables

The recorded variables were: (1) Plant height [cm], measured from the root collar to the point where the stem ends and the cotyledon leaves begin; (2) Leaf length [cm], measured from the base to the apex of the leaf along an imaginary vertical axis; (3) Main leaf width [cm], measured from the left lateral edge to the right edge of the leaf along an imaginary transverse axis; (4) Stem diameter [mm], measured at the basal starting point of the stem; (5) Root length [cm], measured from the root collar to the apex where the root terminates; (6) Root diameter [mm], measured at the thickest point of the main root collar. For measurements expressed in millimeters, a digital caliper (Hardened brand) with a graduated scale from 0.01 to 150 mm and an accuracy of 0.01 to 0.3 mm was used. For measurements expressed in centimeters, a 30 cm metal ruler was employed. Data collection was carried out 15 days after sowing. Additionally, (7) Germination percentage [%] was recorded five days after sowing, determined per container.

$$\% = \frac{NGP}{NC} \times 100\%$$

Where: NGP=Number of Germinated Plants, NC=Number of Cavities (248).

Root ball extraction, plant handling, and substrate consistency

To establish the parameters for root ball extraction, plant handling, and substrate consistency, and to mechanically characterize the substrate materials, representative numerical values were assigned as follows: 0=difficult and 1=easy. This coding system generated a binary scale. After the fieldwork, a coding matrix was developed for data interpretation. The quantitative data coding process involved assigning numerical values to represent each category or item, where each category was assigned a specific numerical value or symbol with defined meaning (Hernández-Sampierí, Fernández-Collado, and Baptista-Lucio, 2014). To confirm the internal validity of the instrument, the global value of Cronbach's alpha internal consistency index was used.

$$\alpha = \frac{K}{K-1}\left[1 - \frac{\sum Vi}{Vt}\right]$$

Where: α=Alpha, K=number of variables, Vi=variance of each variable, Vt=total variance. This index is conceptualized as the average of the correlations among the variables that comprise an instrument (Streiner, 2003). It measures reliability in terms of internal consistency (Celina and Ocampo, 2005; Cervantes, 2005), with the final value being considered an ordinal variable (Supo, 2013).

Statistical analysis

Normality and homoscedasticity tests of the variance data were performed [Post-Hoc], followed by an analysis of variance (ANOVA) to evaluate the effects of each treatment, along with Tukey's mean separation test (P≤0.05). All analyses were conducted using the STATISTICA software package, Version 13.3 (TIBCO Inc., 2017).

RESULTS

The results of the normality and homoscedasticity tests of the variance data (Post-Hoc) indicated a satisfactory linear alignment of the data along the straight line. All variables exhibited a normal distribution, confirming the reliability of the experiment. The analysis of variance revealed significant differences among all treatments for all evaluated variables (Table 2).

Plant Height

Tukey's mean comparison test (P ≤ 0.05) for this variable showed that treatments T7, T10, T11, and T13 were statistically similar, recording the highest values. Treatments T9 and T15 also demonstrated a significant growth effect, with 3.89 cm and 3.85 cm, respectively, both surpassing T1, which recorded 3.47 cm. However, the highest numerical value was observed in T9, reaching 5.00 cm (Table 3).

Leaf length

The best results with significant effects (P≤0.05) were observed in treatments T11 and T12, with values of 3.15 cm and 3.04 cm, respectively. These treatments exhibited greater longitudinal leaf growth compared to T8 and T9, which also showed significant effects with 2.79 cm and 2.43 cm, respectively, though belonging to a different statistical group (Table 3). However, T2 did not show statistical significance and recorded the lowest numerical value among all treatments, with 0.70 cm.

Leaf width

Treatments T12 and T13 were statistically similar (P≤0.05), with values of 3.21 cm and 2.96 cm, respectively, showing the greatest increase in leaf width, differentiating them

Table 2. Analysis of variance for the variables evaluated.

Variable	Degrees of Freedom	Mean Square Effect	Mean Square Error	Calculated F	P-value	CV
PH	14.35	0.43738	0.18631	2.3476	0.0039*	29.44
LL	14.35	0.16271	0.07066	2.3028	0.0047*	44.73
LW	14.35	0.25727	0.07992	3.2191	0.0001*	49.62
SD	14.35	60.66080	23.14329	2.6211	0.0012*	28.75
RL	14.35	0.96921	0.42632	2.2734	0.0053*	15.76
RD	14.35	21.75461	4.80519	4.5273	0.0000*	31.05
GP	14.35	3.519766	1.077517	3.2665	0.0031*	9.17

PH: plant height; LL: leaf length; LW: leaf width; SD: stem diameter; RL: root length; RD: root diameter; GP: germination percentage; CV: coefficient of variation (%); Significant (P≤0.05).

Table 3. Mean values of the treatments for all variables evaluated in melon seedlings.

Treatment	PH	LL	LW	SD	RL	RD	GP
T1	3.47 cd	1.63 d	1.55 c	1.94 ab	6.81 bc	1.74 ab	97.85 g
T2	2.87 bc	0.70 a	0.60 a	2.16 a	6.63 abc	1.51 a	79.97 bcd
T3	2.16 a	0.78 ab	0.70 ab	2.00 ab	5.48 a	1.55 ab	80.11 bcd
T4	2.84 bc	1.18 d	1.03 c	2.17 bc	6.23 ab	1.79 ab	74.46 ab
T5	2.73 ab	1.13 bc	0.98 ab	2.02 ab	7.11 c	1.68 ab	82.26 cd
T6	3.29 bcd	1.72 d	1.67 c	2.60 de	6.62 abc	2.15 cd	70.16 a
T7	4.62 fg	2.36 e	2.36 d	2.79 ef	6.91 bc	2.27 de	90.32 ef
T8	5.00 g	2.79 fg	2.89 ef	3.09 g	7.02 bc	2.70 fg	90.05 ef
T9	3.89 de	2.43 fg	2.47 d	3.15 g	7.3 cd	2.92 h	94.09 efg
T10	4.74 fg	2.42 e	2.54 de	3.03 fg	6.99 bc	2.58 ef	95.30 fg
T11	4.78 fg	3.15 gh	3.32 g	3.17 g	6.51 abc	2.81 fg	90.05 ef
T12	4.37 efg	3.04 gh	3.21 fg	3.15 g	7.3 cd	2.81 fg	87.10 de
T13	4.66 fg	2.47 ef	2.96 fg	2.41 cd	8.13 d	1.85 bc	77.15 abc
T14	4.23 ef	3.25 h	3.29 g	3.59 h	6.98 bc	3.58 h	90.05 ef
T15	3.85 de	1.75 d	1.82 c	2.47 d	6.98 bc	1.75 ab	94.09 efg
HSD=	0.04	0.03	0.03	0.52	0.07	0.31	0.40

PH: plant height (cm); LL: leaf length (cm); LW: leaf width (cm); SD: stem diameter (mm); RL: root length (cm); RD: root diameter (mm); GP: germination percentage (%); T1: peat moss (PM); T2: rice husk (RH); T3: wormcompost (WC); T4: red clay (RC); T5: PM 50% + RH 50%; T6: RH 50% + WC 50%; T7: RH 50% + RC 50%; T8: PM 20% + RH 30% + WC 50%; T9: RH 50% + WC 25% + Topure 25%; T10: PM 10% + RH 70% + WC 10% + RC 10%; T11: PM 33.33% + RH 33.33% + WC 33.33%; T12: PM 25% + RH 25% + WC 25% + RC 25%; T13: RH 80% + WC 20%; T14: PM 30% + WC 70%; T15: PM 10% + RH 90%; HSD: honestly significant difference; different letters within the same column indicate significant differences according to Tukey's test ($P \leq 0.05$).

from the other treatments. Treatment T8 also showed a statistically significant difference ($P \leq 0.05$), with a value of 2.89 cm, which was 9.96% and 2.36% lower than T12 and T13, respectively. As in the case of leaf length, T2 recorded the lowest numerical value for leaf width, with 0.60 cm (Table 3).

Stem diameter

Treatments T1, T3, T4, T5, T6, T7, T10, and T13 showed statistically significant differences ($P \leq 0.05$) and were distinct from one another (Table 3). The highest value was observed in T10, with 30.30 mm, while the lowest was recorded in T1, with 19.40 mm, which is considered the commercial control. However, treatments T11, T12, and T14 showed only numerical values of 31.77 mm, 30.50 mm, and 35.97 mm, respectively, without statistical significance.

Root length

Twelve treatments presented statistically significant differences ($P \leq 0.05$), ranging from 6.23 cm to 7.02 cm. Among these, T1, T7, T8, T10, T4, and T15 stood out for showing statistical similarity and forming the largest group, with intermediate average values of 6.81 cm, 6.91 cm, 7.02 cm, 6.99 cm, 6.98 cm, and 6.98 cm, respectively. However, T13 exhibited the highest numerical value of 8.13 cm without statistical significance.

Root diameter

This variable was characterized by six statistical groups (P≤0.05). The group formed by T8, T11, and T12 reached the highest average values of 27.03 mm, 28.17 mm, and 28.13 mm, respectively, showing statistical similarity. In contrast, the group consisting of T1, T3, T4, T5, and T15 exhibited the lowest average values, with 17.40 mm, 15.50 mm, 17.93 mm, 16.87 mm, and 17.53 mm, respectively, also showing statistical equality.

Germination percentage

Treatments T9 and T15 recorded the best statistically significant results (P≤0.05), both achieving an average value of 94.09%. Treatments T7, T8, T11, and T14 also showed statistical similarity, with average values of 90.32% and 90.05% for the last three. Meanwhile, T4 recorded a statistically significant value of 74.46%, although this was lower compared to the aforementioned treatments. T1 achieved a numerical value of 97.85%, which, while not statistically different, represented the highest value among all evaluated treatments (Table 3).

Root ball extraction, plant handling, and substrate consistency

The calculated value of α=0.71 indicated that the measurement instrument demonstrated high internal consistency and stability (Table 4). According to Celina and Ocampo (2005), the minimum acceptable value for Cronbach's alpha coefficient is 0.70;

Table 4. Cronbach's alpha calculation matrix.

Treatment	Substratum	Percentage	Item or variable			∑ Vt
			EP (VNR)	MP (VNR)	CS (VNR)	
T1	PM	100%	1	1	1	3
T2	RH	100%	1	0	1	2
T3	WC	100%	0	0	0	0
T4	RC	100%	0	0	0	0
T5	PM+RH	50% + 50%	1	0	1	2
T6	RH+WC	50% + 50%	0	0	1	1
T7	RH+RC	50% + 50%	1	0	1	2
T8	PM+RH+WC	20% + 30% + 50%	1	1	1	3
T9	RH+WC+RC	50% + 25% + 25%	0	0	1	1
T10	PM+RH+WC+RC	10% + 70% + 10% + 10%	1	0	1	2
T11	PM+RH+WC	33.33% + 33.33% +33.33%	1	1	1	3
T12	PM+RH+WC+RC	25% +25% +25% + 25%	0	0	0	0
T13	RH+WC	80% + 20%	1	0	1	2
T14	PM+WC	30% + 70%	0	0	0	0
T15	PM+RH	10% + 90%	1	0	1	2
		∑ Vi	0.24	0.16	0.22	1.26

EC: root ball extraction; PH: plant handling; SC: substrate consistency; NRV: numerical representative values [EC and PH: 0=difficult, 1=easy; SC: 0=crumbles, 1=does not crumble]; α: 0.71 (Cronbach's alpha); ∑ Vi: variance of each variable; ∑ Vt: total variance; PM: peat moss; RH: rice husk; WC: wormcompost; RC: red clay.

values below this threshold reflect low internal consistency, while values above 0.90 may suggest redundancy or duplication.

DISCUSSION

Plant height

The best growth effects in melon seedlings for plant height were observed with substrate mixtures T7 (RH+RC [50% + 50%]), T10 (PM+RH+WC+RC [10% + 70% + 10% + 10%]), T11 (PM+RH+WC [33.33% + 33.33% + 33.33%]), and T13 (RH+WC [80% + 20%]), with values of 4.62 cm, 4.74 cm, 4.78 cm, and 4.66 cm, respectively. These results were superior when compared to the pure substrates T1 (PM [100%]), T2 (RH [100%]), T3 (WC [100%]), and T4 (RC [100%]), which recorded 3.47 cm, 2.87 cm, 2.16 cm, and 2.84 cm, respectively, and were considered the controls. The evaluated mixtures were also characterized by the presence of wormcompost and whole, raw rice husk. These findings align with those reported by David-Santoya *et al.* (2018) for habanero pepper seedlings, where greater plant height was achieved with substrate mixtures of bocashi + *Gliricidia sepium* (cacahuananche) + plant residues [50% + 25% + 25%], cocoa husk + *G. sepium* + plant residues [50% + 25% + 25%], and cocoa husk + *G. sepium* + sheep manure [50% + 25% + 25%], with values of 7.1 cm, 7.3 cm, and 8.1 cm, respectively. All these mixtures were combined with vermicompost and soil at a 1:1 ratio and produced through a primary wormcomposting process using plant residues, outperforming the control soil treatment (100%) which recorded 5.0 cm.

Leaf length and width

Treatment T14 (PM 30% + WC 70%) achieved the highest growth rates in both leaf length and width among all evaluated substrates, with values of 3.25 cm and 3.29 cm, respectively, corresponding to a leaf area of 10.69 cm^2. In contrast, the control treatments T1 (PM 100%) and T2 (RH 100%) showed the lowest leaf area growth rates, recording 2.52 cm^2 and 0.42 cm^2, respectively. Similarly, Chirinos *et al.* (1997) used leaf length and width as reliable indicators for estimating leaf area growth in melon under field conditions, with measurements that are easily obtained. According to Bidwell (1993), such growth whether measured by length, thickness, or area results in an increase in volume or mass, occurring in different directions and at varying rates. The observed differences in leaf area growth rates of 8.17 cm^2 and 10.27 cm^2, respectively, are attributed to the mineral concentrations present in these three substrates. In this regard, Durán-Umaña and Henríquez-Henríquez (2010) reported nutrient contents in peat moss of 18, 130, 128, 76, and 252 mg kg^{-1} for N, P, K, Ca, and Mg, respectively. For wormcompost, they reported contents of 23.5, 18.8, 33.4, 7.8, 3, and 4.6 g kg^{-1} for N, P, Ca, Mg, K, and S, respectively, as well as levels of 5, 149, 181, 813, 633, and 25 mg kg^{-1} for Fe, Cu, Zn, Mn, B, and other trace elements. Additionally, Sanchez-Hernández and Domínguez (2019) emphasized that wormcompost contains a well-established microbial community that enhances substrate fertility through nutrient supply. In comparison, Cruz-Crespo *et al.* (2023) reported that rice husk-based substrates contain lower levels of N, P, K, Ca, and Mg, with respective concentrations of 13, 782, 2,195, 86, and 939 mg kg^{-1}.

Stem diameter

Melon seedlings grown in the T14 mixture (PM 30% + WC 70%) again exhibited the greatest stem diameter growth, with an average value of 3.60 mm. In contrast, the lowest significant growth was observed in the control substrate T1 (PM 100%), with 1.94 mm. This difference of 1.66 mm between both treatments represents a 46.11% higher growth in T14. Such a sudden increase is likely attributed to the effect of the microorganism Trichoderma harzianum, applied during substrate preparation, which promotes rhizosphere development enriched with organic matter from peat moss and wormcompost. Alfiky and Weisskopf (2021) noted that *T. harzianum* enhances plant growth as a plant growth promoter. Additionally, it can decompose organic matter, a process closely linked to its high enzymatic capacity for substrate degradation (Infante *et al.*, 2009).

Root length

The best significant results for root length growth were observed in the substrates of treatments T1 (PM 100%), T7 (RH 50% + RC 50%), T8 (PM 20% + RH 30% + WC 50%), T10 (PM 10% + RH 70% + WC 10% + RC 10%), T14 (PM 70% + WC 30%), and T15 (PM 10% + RH 90%), with values of 6.81 cm, 6.91 cm, 7.02 cm, 6.99 cm, 6.98 cm, and 6.98 cm, respectively. This growth effect is primarily due to the arrangement of substrate particles within the container cavities, optimizing pore space for oxygenation and water availability to the melon seedling roots. This interpretation aligns with the observations of Valenzuela *et al.* (2012), who highlighted the critical importance of oxygen availability in the root zone for water absorption. However, the highest root length growth was recorded in treatment T13 (RH 80% + WC 20%) with a value of 8.13 cm. Similarly, Medina Saavedra *et al.* (2023) reported root length values of 6.95 cm in cucumber seedlings grown in T5 (PM 100%), a result comparable to that of T1 in the present study. According to Valenzuela *et al.* (2012), peat moss features a porosity of 85.86%, water retention capacity of 59.91%, and air-filled pore space of 25.95%. These physical properties make peat moss a highly versatile substrate for vegetable seedling production.

Root diameter

The substrate mixtures of T8 (PM 20% + RH 30% + WC 50%), T11 (PM 33.33% + RH 33.33% + WC 33.33%), and T12 (PM 25% + RH 25% + WC 25% + RC 25%) achieved the highest growth rates in root diameter when compared to the commercial control T1 (PM 100%), with increases of 28.14%, 38.29%, and 38.07%, respectively, in root volume. From a physical perspective, these results can be attributed to two key characteristics: high water retention capacity at low tension (0 to 100 cm water column) and high aeration capacity, as described by Burés (1997). These conditions facilitated efficient water absorption by the seedling roots, leading to rapid root diameter growth. These same mixtures also exhibited superior growth in stem diameter compared to the commercial control, confirming the physical continuity of growth between the root system and the stem (Table 3). The T14 mixture (PM 30% + WC 70%) recorded the highest numerical value for root diameter among all evaluated substrates, with an average of 3.58 mm. In contrast, the lowest value was observed in T2 (RH 100%), with 1.59 mm. This represents an increase of 1.99 mm, equivalent to a 57.82% higher root volume growth. According to Barret (2016), the container environment provides a very shallow layer of growing medium, which quickly becomes saturated during irrigation and has limited water storage capacity. In this context, rice husk demonstrated a physical structure unable to retain water effectively when compared to the peat moss + wormcompost mixture, thereby limiting root diameter growth. On the other hand, the combination of textures in the mixture confirmed greater water retention capacity. Additionally, Bidwell (1993) noted that the organic matter present in a substrate significantly influences water retention, nutrient holding capacity, and provides substrates for microbial metabolism. Water and nutrient absorption occur primarily in the younger regions of the root, which are structurally heterogeneous and continuously changing in their anatomical and physiological characteristics. As Esau (1985) pointed out, water absorption rates along the root vary according to its length, age, and the internal conditions of the seedlings. Consequently, this leads to longitudinal growth as well as an increase in the vascular cylinder diameter of the root.

Germination percentage

According to Mármol (2008), melon seeds should achieve a germination rate above 90%. However, seven of the evaluated substrates in this study did not meet this threshold. In contrast, the substrate T1 (PM 100%) and the mixtures T7 (RH 50% + RC 50%), T8 (PM 20% + RH 30% + WC 50%), T9 (RH 50% + WC 25% + RC 25%), T10 (PM 10% + RH 70% + WC 10% + RC 10%), T11 (PM 33.33% + RH 33.33% + WC 33.33%), T14 (PM 30% + WC 70%), and T15 (PM 10% + RH 90%) recorded values exceeding the established parameter, with 97.85%, 90.32%, 90.05%, 94.09%, 95.30%, 90.05%, 90.05%, and 94.09%, respectively. These results are mainly attributed to particle size, pore capacity for water retention, and overall water availability, which directly influenced melon seed germination. These germination percentages are comparable to those reported by Vera-Velázquez *et al.* (2020) in papaya, where substrates based on T1 (100% black soil, control), T2 (50% black soil + 25% worm humus + 25% sand), T3 (50% black soil + 25% compost + 25% sand), T4 (50% black soil + 30% worm humus + 20% sand), and T5 (50% black soil + 25% compost + 25% sand) achieved germination rates of 79%, 92%, 94%, 90%, and 92%, respectively.

Root ball extraction, plant handling, and substrate consistency

Regarding seedling management in containers, the substrates based on T1 (PM 100%), T8 (PM 20% + RH 30% + WC 50%), and T11 (PM 33.33% + RH 33.33% + WC 33.33%) exhibited easy root ball extraction and plant handling, with substrate consistency classified as non-crumbly (root balls remained intact, porous, and soft without root breakage during extraction). These substrates demonstrated stable physical properties for melon seedling production, and the fibrous nature of peat moss contributed to strong root adherence and homogeneous root distribution. One of the main criteria for substrate selection is ease of handling or compatibility, particularly when mixing different materials (Cruz-Crespo *et al.*, 2013). In contrast, the pure substrate T2 (RH 100%) exhibited easy root ball extraction but difficult plant handling, with a crumbly substrate consistency characterized by very loose root balls lacking mechanical root anchorage (Figure 1). These conditions confirmed the absence of fibrous structure in the substrate. Additionally, this mixture presented an overly open pore space, typical of lightweight materials with large, loose particles, which resulted in poor root adherence. Martínez and Roca (2011) emphasized that substrate materials must maintain structural stability to support the plant throughout its growth cycle. Once roots occupy the substrate within the containers, modifying its physical properties is no longer feasible.

Substrates based on T3 (WC 100%) and T4 (RC 100%), as well as mixtures T12 (PM+RH+WC+RC [25% + 25% + 25% + 25%]) and T14 (PM+WC [30% + 70%]), exhibited difficult root ball extraction and plant handling, with a substrate consistency classified as easily crumbling. These treatments presented sandy, loose root balls with root breakage during extraction and handling (Figure 1). Therefore, it is concluded that these three physical characteristics are inadequate for melon seedling management in containers. These findings are supported by Barret *et al.* (2016), who state that a substrate must meet the practical requirements of the production system in which it is used and must promote healthy root growth in the challenging environment of a container. The T5 mixture (PM+RH [50% + 50%]) was characterized by easy root ball extraction but

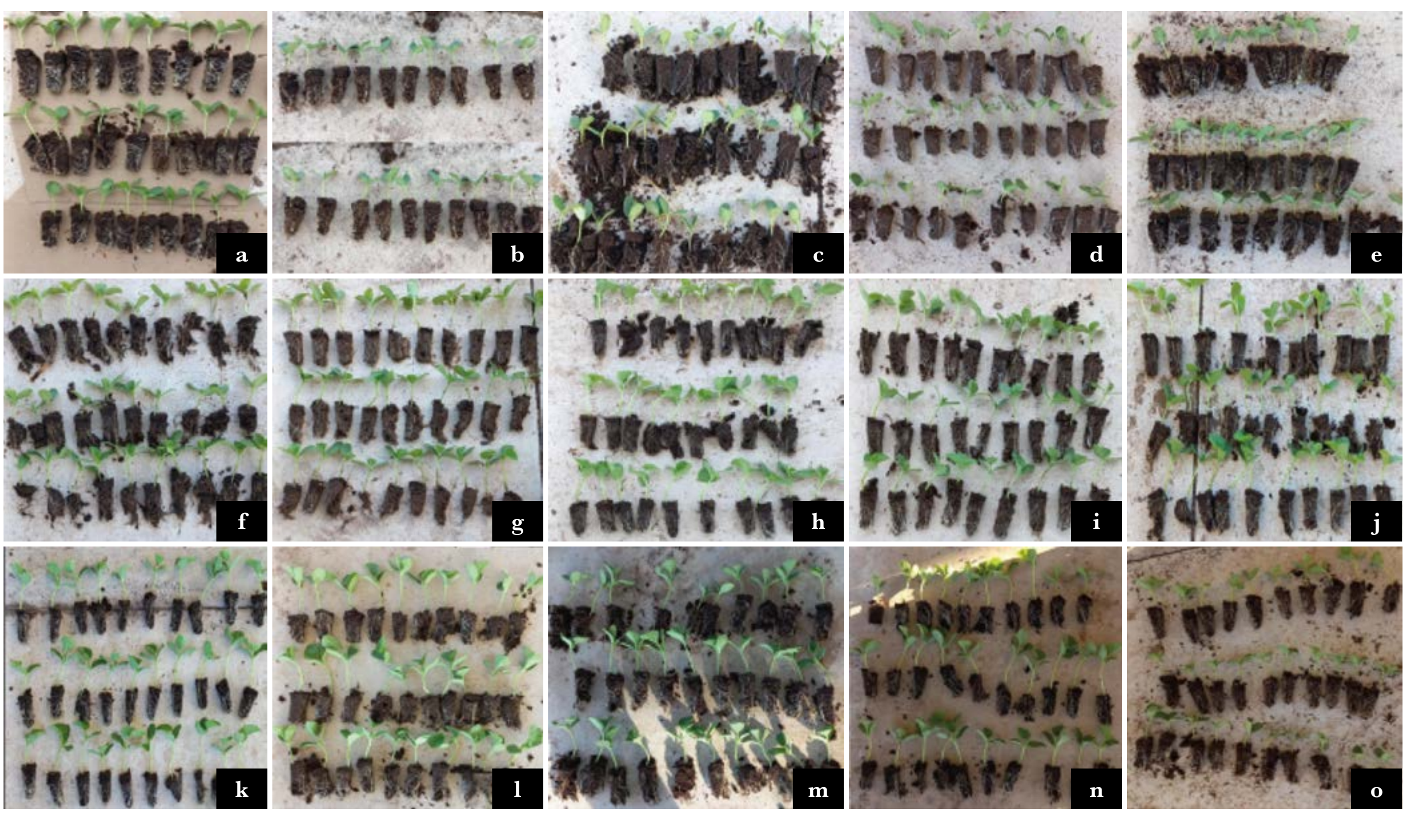

Figure 1. Harper-type melon (*Cucumis melo*) seedlings of the Zacapa hybrid after extraction from polystyrene containers with 248 cavities using the evaluated substrates: peat moss (PM), rice husk (RH), wormcompost (WC), red clay (RC), PM 50% + RH 50%, RH 50% + WC 50%, RH 50% + RC 50%, PM 20% + RH 30% + WC 50%, RH 50% + WC 25% + Topure 25%, PM 10% + RH 70% + WC 10% + RC 10%, PM 33.33% + RH 33.33% + WC 33.33%, PM 25% + RH 25% + WC 25% + RC 25%, RH 80% + WC 20%, PM 30% + WC 70%, PM 10% + RH 90%. El Crucero de La Ruana, municipality of Buenavista, Michoacán, Mexico.

difficult plant handling, with a soft, non-crumbly substrate consistency (intact root balls without root breakage). However, this mixture demonstrated instability in the second physical characteristic required for successful transplantation, as handling led to root ball disintegration. A noted issue in substrate characterization is the lack of standardized methodologies, which complicates result comparison (Cruz-Crespo *et al.*, 2013). The substrate mixtures T7 (RH+RC [50% + 50%]), T10 (PM+RH+WC+RC [10% + 70% + 10% + 10%]), T13 (RH+WC [80% + 20%]), and T15 (PM+RH [10% + 90%]) showed easy root ball extraction (very soft) but difficult plant handling due to the easily crumbling substrate consistency when removing plants from the containers (Figure 1). These findings confirmed that these evaluated mixtures are unstable regarding the second physical characteristic assessed. Zanin *et al.* (2011) also noted that when RH is used in substrates, its proportion should not exceed 50% by volume, due to its slow decomposition rate compared to the higher bio-stability of other organic materials. Therefore, further research is needed on other plant species to better characterize these mixtures physically. The physical mixtures of T6 (RH+WC [50% + 50%]) and T9 (RH+WC+RC [50% + 25% + 25%]) were characterized by combining a lightweight, porous material (RH) with denser, compact materials (WC and RC), resulting in difficult root ball extraction and plant handling, with a soft, easily crumbling substrate consistency. Regarding plant handling, Luna *et al.* (2012) emphasized that containers must often be moved, making transportation to the planting site a key issue in terms of logistics and safety. Consequently, these mixtures proved to be unviable for melon seedling production in containers due to the high porosity of the raw, whole RH, which negatively affected the last two evaluated conditions.

CONCLUSIONS

Among all evaluated treatments, the greatest growth effects in leaf length and width, stem diameter, root length, and root diameter were obtained with the mixture based on peat moss (PM) 30% + wormcompost (WC) 70%. In contrast, the lowest growth effects in leaf length and width, as well as root diameter, were observed in the substrate based on rice husk (RH) 100%.

The substrate consisting of peat moss (PM) 100% achieved a germination rate of 97.85%, thus maintaining its commercial potential for melon seedling production. Additionally, substrates based on PM 100%, PM 20% + RH 30% + WC 50%, and PM 33% + RH 33% + WC 33% demonstrated the best results in terms of root ball extraction, plant handling, and substrate consistency, thereby confirming their usability potential. However, mixtures based on RH 50% + WC 50% and RH 50% + WC 25% + red clay (RC) 25% proved to be inefficient for seedling production due to difficult root ball extraction and plant handling, combined with a substrate consistency that was prone to crumbling.

ACKNOWLEDGMENTS

The author expresses gratitude to Mr. José Manuel Ochoa Morales, General Director of the agro-company Papayas y Melones El Jefe 8A, for providing all the necessary support and access to the production unit, which made this research possible.

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Colegio de Postgraduados

Influence of *Rhizophagus intraradices* (Schenck & Sm.), Chemical and Organic Fertilization on the Growth of *Swietenia humilis* Zuccarini in Nursery Conditions

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ABSTRACT
Objective: To evaluate the growth of *Swietenia humilis* Zuccarini (caobilla) seedlings when grown in different substrates and biofertilized with and without *Rhizophagus intraradices* in combination with chemical fertilization.
Design/Methodology/Approach: Caobilla seeds were collected in Huehuetán, Chiapas, and once germinated, were transplanted into plastic bags filled with a soil-based substrate supplemented with 30% cattle manure. The following treatments were established: (1) control; (2) cattle manure; (3) cattle manure + *R. intraradices*; (4) 100N-50P; (5) 100N-50P + *R. intraradices*; (6) 50N-25P + *R. intraradices*. Each treatment had five replicates and was arranged in a completely randomized design. Morphological variables were recorded every 28 days, and a final destructive sampling was performed at 121 days after transplanting (DAT) to determine dry biomass. Data were analyzed using analysis of variance (ANOVA).
Results: The morphological and physiological variables indicated an increase in the growth of caobilla when cattle manure was added to the substrate and plants were biofertilized with *R. intraradices*.
Study Limitations/Implications: The plant response may vary if caobilla is biofertilized with a different species of arbuscular mycorrhizal fungus.
Findings/Conclusions: The greatest growth in the biomass of morphological and physiological components of *S. humilis* Zucc. was observed in the treatment with cattle manure combined with *R. intraradices*. The highest chemical fertilization dose also promoted biomass accumulation, particularly at the end of the evaluation period.

Keywords: caobilla, arbuscular mycorrhizal fungi, alternative substrates, biomass.

Citation: Aguirre-Medina, J. F., Pérez-Flores, S. C., Agulrre-Cadena, J. F., Reyes-Reyes, J., Espinosa-Zaragoza, S., & Cadena-Zamudio D. A. (2025). Influence of *Rhizophagus intraradices* (Schenck & Sm.), Chemical and Organic Fertilization on the Growth of *Swietenia humilis* Zuccarini in Nursery Conditions. *Agro Productividad*. https://doi.org/10.32854/zjm4me79

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

Received: March 14, 2025.
Accepted: May 19, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 19-28.

INTRODUCTION

Swietenia humilis Zuccarini, commonly known as caobilla, is a species with high commercial demand due to the quality of its wood. However, its natural populations have significantly declined. Therefore, for the successful establishment of this species in areas with low population density, the production of high-quality nursery-grown plants is essential prior to field transplantation. A crucial first step in this process involves the

use of high-quality seeds and appropriate substrates for germination. Without these considerations, reforestation efforts are likely to be unsuccessful. Traditionally, the substrate used for this purpose is imported peat moss (*Sphagnum magellanicum*), which is costly. This situation highlights the need to explore alternative local options, including organic and chemical amendments, capable of promoting rapid and vigorous plant growth in nursery conditions. One such alternative involves the incorporation of cattle manure, which is abundantly available in the coastal region of Chiapas, Mexico. Additionally, the growth of forest seedlings has been improved through the inclusion of arbuscular mycorrhizal fungi (AMF) during planting. These fungi establish symbiotic relationships with host plants across nearly all ecosystems (Strack *et al.*, 2003), enhancing plant growth through nutrient and water transport (Redecker, 2000; Aguirre-Medina *et al.*, 2016; Aroca *et al.*, 2011). Although AMF are not host-specific, there is evidence of preferential associations between certain fungal species and specific plant hosts or fungal isolates (Anzueto-Herón *et al.*, 2023; Aguirre-Medina *et al.*, 2023). The beneficial effects of AMF symbiosis in promoting plant growth have been reported in various perennial crops, where it helps reduce the time needed for plants to reach transplant readiness. This has been demonstrated, for example, in robusta coffee (*Coffea canephora* Pierre ex Froehner) (Ibarra-Puón *et al.*, 2014). Furthermore, mycorrhizal symbiosis has been shown to increase the accumulation of key nutrients such as phosphorus and nitrogen in plant tissues (Aguirre-Medina *et al.*, 2012). Based on this background, it is expected that the application of arbuscular mycorrhizal fungi to caobilla seeds in the nursery stage will promote early-stage growth and reduce the time required for seedlings to achieve the stem thickness necessary for successful field establishment. Therefore, the aim of this research was to evaluate the growth of *Swietenia humilis* when amended with cattle manure and biofertilized with and without *Rhizophagus intraradices*, in combination with chemical fertilization.

MATERIALS AND METHODS

Study site

The research was conducted at the nursery of the Faculty of Agricultural Sciences, Universidad Autónoma de Chiapas (UNACH), located in Huehuetán, Chiapas, Mexico, at an altitude of 44 m. The site is geographically positioned between 15° 00' and 15° 30' N latitude and 94° 30' and 95° 00' W longitude. The climate is classified as Am (w') i g, corresponding to a warm humid climate with summer rains, an average annual precipitation of 2500 mm, and maximum and minimum temperatures of 38 °C and 15 °C, respectively, with an average temperature of 25.4 °C (García, 2004). The substrate consisted of fluvisol soil (INEGI, 2004) collected from Ejido Huehuetán, Chiapas, mixed with 30% cattle manure ground using a hammer mill (Swrssrnex model 610120) and solarized for 72 hours. The substrate's physical-chemical properties were as follows: sandy loam texture with 80.76% sand, 13.36% silt, and 5.88% clay; 2.63% organic matter; pH 5.78; 0.13% nitrogen; 14.1 ppm phosphorus; 64.2 ppm potassium; 474.00 ppm calcium; 58.00 ppm magnesium; 102.50 ppm sodium; and electrical conductivity (EC) of 0.05.

Rhizophagus intraradices (Schenck & Sm.) was obtained from the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP) at the Rosario Izapa

Experimental Field. The inoculum had a concentration of 40 spores per gram of soil and 95% root colonization in the host plant *Brachiaria brizantha* (Hochst. ex A. Rich.) according to the product data (Micorria INIFAP®). The experimental unit was a container with a single plant. Seedlings were irrigated with deep well water four times per week using a manual watering can, with subsequent irrigation depending on environmental conditions.

Sowing and application of microorganisms and chemical fertilizer

Caobilla seeds were collected from a selected tree on the premises of the Faculty of Agricultural Sciences, Huehuetán Campus IV, chosen for its dense canopy and absence of diseases and pests. For germination, seeds were coated with carboxymethylcellulose and inoculated with *R. intraradices* biofertilizer at 4% of the seed weight. Biofertilized seeds were sown in trays with 24 cones of 150 cm^3 each, measuring 19.5 cm in height and 4.0 cm in upper diameter, at a depth of 3.0 cm. After 10 days, seedlings were transplanted into black plastic bags (25×35 cm) with a 1 kg soil capacity and placed on metal benches. The bags were perforated at the bottom to ensure proper drainage.

Treatments, replications, and experimental design

The following treatments were evaluated: (1) control, (2) cattle manure, (3) 100N+50P, (4) cattle manure + *R. intraradices*, (5) 100N+50P + *R. intraradices*, and (6) 50N+25P + *R. intraradices*. All treatments were arranged in a completely randomized block design with five replicates. Destructive sampling was performed at 121 days after transplanting (DAT).

Evaluated variables

Morphological variables (plant height, stem diameter, and number of leaves) were recorded at 14, 28, 42, 56, 70, 84, 98, and 112 DAT. Physiological variables (dry weight of aerial and root components) were measured at 112 DAT. Root and shoot biomass were weighed using a semi-analytical balance (Ohaus Adventurer Pro, USA) after drying in a forced-air oven at 75-80 °C until reaching constant weight. The root-to-shoot ratio was determined following Boonstra (1931).

Statistical analysis

The results were analyzed using SAS software version 8.1 (SAS Institute, 1999-2000) with a completely randomized design. Treatment means were compared using Tukey's test ($P \leq 0.05$), and graphs were generated using Sigma Plot (version 11.0).

RESULTS AND DISCUSSION

Plant height exhibited consistent changes starting at 56 days after transplanting (DAT), with the treatments that induced the greatest height being cattle manure alone, cattle manure combined with seed biofertilization using *R. intraradices*, and the chemical fertilization treatment with 100N+50P (Table 1).

Plants grown in the soil substrate supplemented with cattle manure and biofertilized with *R. intraradices* showed a 5% increase in height at the final sampling and were

Table 1. Mean comparisons of morphological components in *Swietenia humilis* (caobilla) biofertilized with *Rhizophagus intraradices* in interaction with cattle manure and chemical fertilization under nursery conditions.

	Time (days)	**Witness**	**Bovine manure**	**Bovine manure + *R. intraradices***	**100_N-50_P**	**100_N-50_P + *R. intraradices***	**50_N-25_P + *R. intraradices***	****CV (%)**
Height (cm.floor^{-1})	14	12.7 b*	13.7 ab	15.1 a	12.7 b	11.8 b	12.0 b	8.12
	28	15.0	14.8	16.9	15.7	14.9	15.6	8.5(NS)
	42	17.3	18.1	18.0	17.9	16.4	16.6	5.3(NS)
	56	19.3 b	20.9 ab	22.3 a	21.0ab	19.2 b	20.1 b	5.2
	70	19.7 c	21.5 bc	23.5 a	22.2ab	20.7 bc	21.0 c	4.5
	84	20.8 c	25.1 ab	27.2 a	24.4 b	22.5 bc	21.0 c	5.7
	98	24.7 c	28.3 ab	30.3 a	28.6 a	24.3 c	24.8 bc	6.8
	112	28.3 b	31.4 ab	32.9 a	31.4ab	29.3 ab	28.6 b	6.4
Stem diameter (mm.plant^{-1})	14	2.4 ab	2.5 a	2.6 a	2.5 a	2.4 ab	2.1 b	5.8
	28	3.1 ab	3.2 a	3.2 a	2.7 b	3.0 ab	2.9 ab	7.3
	42	3.5	3.6	3.5	3.1	3.4	3.3	6.9(NS)
	56	4.5 b	5.5 a	5.6 a	5.3 a	5.4 a	5.4 a	7.4
	70	7.3 bc	8.0abc	8.6 a	8.2 ab	7.1 cd	6.2 d	6.9
	84	8.3 ab	8.9 a	9.3 a	8.9 a	7.8 bc	7.2 c	6.2
	98	9.4bcd	10.8abc	11.3 a	10.9ab	9.4 cd	8.7 d	7.4
	112	9.9 b	11.5 ab	12.4 a	11.5ab	10.0 b	9.9 b	8.3
Number of leaves.plant^{-1}	14	2.8 ab	3.6 a	3.2 a	3.8 a	2.0 b	3.2 a	16.6
	28	4.0 b	7.4 a	3.0 bc	3.0bc	2.4 c	2.4 c	17
	42	5.2	5.6	6.0	6.2	5.2	5.2	11.8(NS)
	56	5.6 b	7.2 a	4.8 bc	5.4 b	4.8 bc	3.8 c	13.2
	70	7.6 ab	8.4 a	8.4 a	8.6 a	6.8 b	6.8 b	7.6
	84	7.6 c	8.8abc	9.4 a	9.2ab	7.8 bc	7.4 c	9.1
	98	8.8 ab	8.6 ab	9.4 a	9.6 a	8.0 b	8.8 ab	7.7
	112	9.8 ab	9.6 ab	10.6 a	10.0ab	9.0 b	9.8 ab	7.2

* Values with the same letter within each factor and row are statistically similar according to Tukey's test ($P \leq 0.05$). ** CV=Coefficient of variation.

statistically different ($P \leq 0.05$). The initial lack of response to biofertilization during the first 42 days coincides with the root colonization period of the fungus, during which the benefits of nutrient and water transport to the host plant are still limited. In the other treatments, the early root system was small, with reduced capacity for nutrient uptake and transport. The increase in plant height due to mycorrhizal symbiosis with *R. intraradices* has been documented in several perennial crops, including cacao (Aguirre-Medina *et al.*, 2007), arabica coffee (Anzueto-Herón *et al.*, 2023), and robusta coffee (Ibarra-Puón *et al.*, 2014). Once established, the mycorrhizal fungus uses its hyphal network to explore a larger soil volume, supplying essential nutrients to the host plant, particularly phosphorus (Garza-Cano *et al.*, 2005), nitrogen (Anzueto-Herón *et al.*, 2023), and water, as observed in *Casuarina equisetifolia* (Valdéz *et al.*, 2004).

For stem diameter, biofertilization demonstrated a consistent effect, showing differences from the first evaluation. From 56 DAT until the final measurement, treatments with cattle manure alone, cattle manure combined with *R. intraradices*, and the 100N+50P fertilization formed the leading statistical group, significantly outperforming the other treatments ($P \leq 0.05$). Among these, stem diameter was 8% greater when cattle manure was included in the substrate and seeds were biofertilized with *R. intraradices*. Similar findings were reported in cedar, where increased aerial biomass was observed with biofertilization using inoculum from two tropical ecosystems (Méndez-Cortés *et al.*, 2013). Other forest species, such as teak (*Tectona grandis*) and ronrón (*Astronium graveolens*), also showed increases in stem diameter by 11.8% with mycorrhizal application compared to controls (Hernández and Salas, 2009).

The number of leaves showed differences from the first sampling, with consistent results in treatments using cattle manure alone and cattle manure plus *R. intraradices*. This variable also increased under high chemical fertilization. At 112 DAT, the treatment combining *R. intraradices* and cattle manure resulted in a 9% higher leaf count compared to cattle manure alone, and a 7% increase when combined with 50N+25P fertilization and biofertilization with *R. intraradices*. These findings emphasize the effectiveness of combining mycorrhizal fungi with cattle manure in the substrate. In *Robusta coffee*, an average increase of 15 leaves was reported when grown with cattle manure and *R. intraradices* (Ibarra-Puón *et al.*, 2014). Similar results were observed in cacao (Aguirre-Medina *et al.*, 2007) and oro azteca coffee (Aguirre-Medina *et al.*, 2011). In other crops, mycorrhizal fungi have also been associated with similar leaf numbers in biofertilized plants, as seen in *Acacia farnesiana* and *Prosopis glandulosa* grown in substrates such as tepetate, forest soil, and agricultural soil (Hernández-Martínez *et al.*, 2006).

Physiological components

Root biomass accumulation in *Swietenia humilis* (caobilla) exhibited significant variations among treatments (Figure 1).

Root dry matter allocation in *Swietenia humilis* (caobilla) increased across all treatments compared to the control, with statistically significant differences ($P \leq 0.05$). The highest increase was observed in the treatment with 100_N+50_P fertilization and in the combination of cattle manure with seed biofertilization using *R. intraradices*. This response may be associated with nutrient availability provided by the cattle manure and high chemical fertilization, as other studies, such as those in cacao, have shown reduced root biomass allocation when plants are biofertilized with *R. intraradices* (Aguirre-Medina *et al.*, 2007). Additionally, the greater root development observed with the *R. intraradices* treatment could be linked to an increase in growth regulators induced by the symbiotic interaction. Aguirre-Medina *et al.* (2011) reported enhanced root system dry weight in *Coffea arabica* after inoculation with *R. intraradices*. Rovira *et al.* (1983) also emphasized the importance of root growth stimulation by microorganisms as a major contribution of symbionts to plant development. Once root colonization is established, plants show an improved capacity for nutrient uptake and distribution, enhancing overall nutrition (Aguirre-Medina and Kohashi, 2002). Similar responses have been observed in other

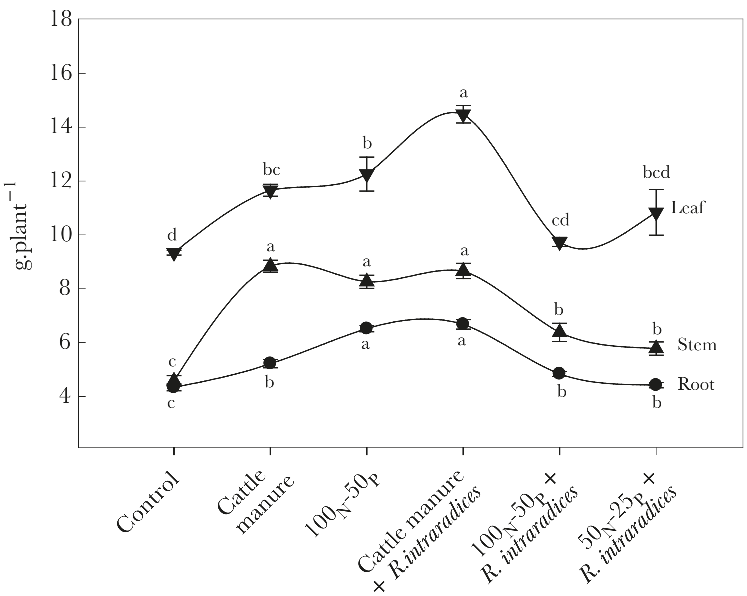

Figure 1. Dry weight of physiological components of *Swietenia humilis* Zucc. biofertilized with *Rhizophagus intraradices* in interaction with cattle manure and chemical fertilization. Values represent means of five replicates ± standard error, and different letters indicate statistical differences (Tukey $P \leq 0.05$). CV=Root 55%, Stem 6.1%, Leaf 89.1%.

forest species, such as *Swietenia mahagoni* L. Jacq., where endomycorrhizal association led to greater root and shoot biomass accumulation (Falcón *et al.*, 2021). In terms of stem dry weight, all treatments showed increases compared to the control, with the highest values observed in treatments that included cattle manure, with or without *R. intraradices*, and in the 100_N+50_P fertilization treatment. These treatments formed the top statistical group, with significant differences from the remaining treatments ($P \leq 0.05$). The increased biomass allocation to the stem under these conditions suggests a differential partitioning of photosynthates, possibly influenced by changes in the rhizosphere microbial community due to root exudates (Linderman, 1988). The mycorrhizal association allows plants to allocate fewer carbohydrates to root system maintenance, favoring above-ground growth (Pereira *et al.*, 2001). The positive effect on stem thickness has also been documented in various crops. Aguirre-Medina *et al.* (2011) reported increased stem thickness in the coffee variety Oro Azteca following biofertilization with *R. intraradices* in the nursery stage. Similar results were observed in Marsellesa, Geisha, Sarchimor, and Costa Rica 95 coffee varieties (Anzueto-Herón *et al.*, 2023) and in species such as *Acacia farnesiana* and *Prosopis glandulosa* inoculated with mycorrhizal fungi (Hernández-Martínez *et al.*, 2006). Manjarrez-Martínez *et al.* (2000) noted that morphological and physiological responses of the host plant depend on the availability of carbon and phosphorus in the soil. Leaf dry weight (including lamina and petiole) also increased significantly in the treatment with cattle manure combined with *R. intraradices*, showing the highest biomass accumulation at 140 DAT and statistically outperforming the other treatments. In *S. mahagoni* L. Jacq., biofertilization with *Glomus cubense* and *Rhizophagus irregularis* similarly improved seedling growth and nutrition (Falcón *et al.*, 2021). These results support the variable functionality of *R. intraradices* in plant interactions, which can induce physiological changes in the host plant (Jäderlund *et al.*, 2008; Barea *et al.*, 2002), as observed in *Leucaena leucocephala* (Aguirre-Medina *et al.*, 2018). The differential biomass allocation among caobilla growth components may also be influenced by root exudates, which facilitate colonization by other microorganisms affecting root morphogenesis and nutrient uptake (Berrabah *et al.*, 2018). In this study, the significant biomass accumulation observed with *R. intraradices* and cattle manure suggests a functional affinity between the fungus and the host plant, potentially supported by sufficient carbon allocation from the plant to the mycorrhizal partner. This finding confirms the differential growth induction in caobilla seedlings in response to the various treatments applied at sowing.

Root-to-shoot ratio

The root-to-shoot ratio exhibited contrasting changes among treatments, whether applied individually or in combination. The increase in the root-to-shoot ratio appears to be associated with the modular growth pattern of the plants. A notable increase in root biomass was observed with the 100_N+50_P fertilization treatment, while the highest shoot biomass was recorded when seeds were biofertilized with *R. intraradices* and cattle manure was incorporated into the substrate. In contrast, treatments that combined fertilization with biofertilization using *R. intraradices* showed a reduction in both root and shoot biomass (Figure 2).

The differences observed in biomass allocation between shoot and root components indicate an interaction between the plant and microorganisms (Olanrewaju *et al.*, 2019). In the case of *R. intraradices*, the benefits to both aerial and root organs are linked to improved

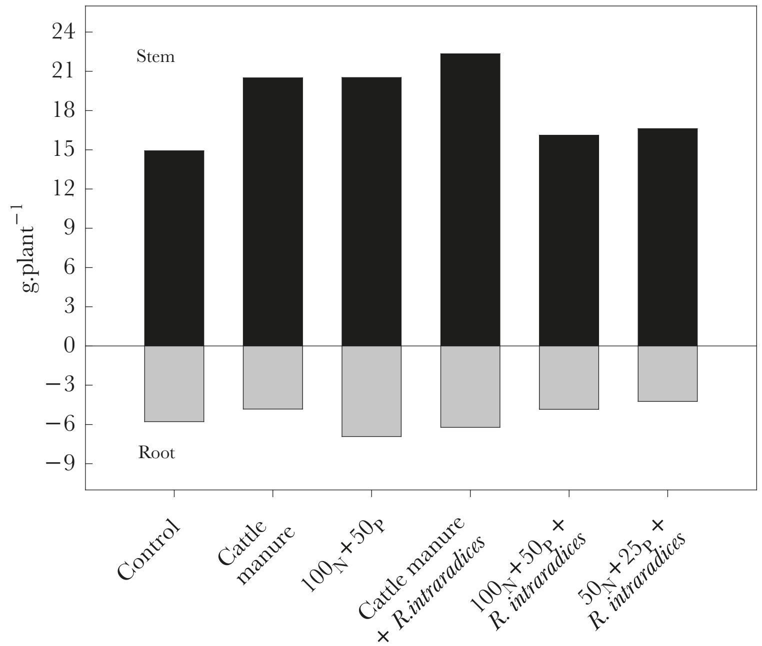

Figure 2. Root-to-shoot ratio of *Swietenia humilis* Zucc. biofertilized with *Rhizophagus intraradices* in interaction with cattle manure and chemical fertilization.

nutrient and water transport (Pereira *et al*., 2001). This enhancement is facilitated by the hyphae of the fungus, which are thinner than plant roots and root hairs, allowing them to access soil zones that roots cannot penetrate, thus expanding the effective soil volume explored for nutrient acquisition (Ness and Vlek, 2000; He *et al*., 2020). According to Perreta and Vegetti (2005), plant growth is regulated by genetic traits that vary within a specific range of phenotypic plasticity. The expression of modular growth across the plant's strata physiologically integrates the modules into a cohesive whole (Collado-Vides, 1997). In general, biofertilization of *Coffea arabica* L. with *R. intraradices* and *Azospirillum brasilense* significantly increases dry matter allocation across various yield components compared to control treatments (Vosatka and Albrechtova, 2009; Cogo *et al*., 2017). In some cases, specific growth components may be more prominently expressed depending on the fungal isolate or when plants are biofertilized with microbial consortia (Vallejos-Torres *et al*., 2019). Reduced growth induction in certain plants may be attributed to environmental and soil conditions that influence biomass accumulation responses. Endomycorrhizal fungi from one soil type may behave differently compared to those from other soils (Hart and Reader, 2002). This variability is also highly influenced by several environmental factors, including climatic conditions, as well as the age and variety of the host plant (Jefwa *et al*., 2004).

CONCLUSIONS

Biofertilization with *Rhizophagus intraradices*, combined with the addition of cattle manure to the substrate and the application of 100_N+50_P chemical fertilization, promoted an increase in the biomass of morphological and physiological yield components of *Swietenia humilis* Zucc. compared to the control. The most notable differences in the morphological components of caobilla were observed from 56 days after transplanting (DAT). Both shoot and root biomass in caobilla exhibited differential responses depending on the interaction between cattle manure, *R. intraradices*, and chemical fertilization.

ACKNOWLEDGMENTS

The authors would like to thank the Faculty of Agricultural Sciences-Campus IV, Autonomous University of Chiapas, Huehuetán, Mexico, for providing the necessary facilities in the nursery and laboratory.

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Determination of the technical and economic optimum in piglet production under a semi-technified system

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ABSTRACT

Objective: To estimate the optimal technical and economic levels for semi-technified piglet production based on the number of farrowings.

Design/Methodology/Approach: A linear additive statistical model was applied to the parameters, while a nonlinear cubic model was employed for the variables, using field data collected in 2023 from a semi-technified farm located in Temascaltepec, State of Mexico. The dependent variable was the number of piglets, and the independent variable was the number of farrowings.

Results: At the technical optimum, the number of farrowings was 2.54, corresponding to a maximum piglet production of 10.4749 and a net cash gain of $2,974.9. At the economic optimum, the number of farrowings was 2.43, equivalent to a production of 10.4731 piglets and a net cash gain of $3,298.1.

Limitations/Implications: In this type of analysis, the technical optimum is closely aligned with the economic optimum.

Findings/Conclusions: The point of maximum piglet production at weaning did not necessarily correspond to the point of maximum net cash gain at sale.

Keywords: Piglets, births, technical optimum, economic optimum, money profit.

Citation: Rebollar-Rebollar, S., Gómez-Tenorio, G., & Velázquez-Villalva, H. H. (2025). Determination of the technical and economic optimum in piglet production under a semi-technified system. *Agro Productividad*. https://doi.org/10.32854/n633mc67

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

Received: November 21, 2023.
Accepted: May 14, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 29-XXX.

INTRODUCTION

Mexico is characterized by a wide diversity of pig production systems, which, based on their primary characteristics, are classified into three categories: technified, semi-technified, and backyard or self-sufficiency (extensive) systems (INTAGRI, 2019; SENASICA, 2021). The technified system represents approximately 46% of the national swine inventory, the semi-technified system 20%, and the backyard system 34%. In terms of pork production, the technified system contributes 55%, the semi-technified 20%, while the remaining production, which typically does not enter formal marketing channels, originates from the backyard sector (Hernández *et al.*, 2020a).

In the southern region of the State of Mexico particularly in the municipalities of Luvianos, Tejupilco, Temascaltepec, Zacazonapan, Texcaltitlán, among others backyard

or extensive pig farming predominates, accounting for 63.6% of production, with herds ranging from five to 50 pigs per production unit (PU). Within these systems, piglet production per farrowing remains the most critical productive variable of concern for pig farmers, who continuously seek to improve outcomes provided that the input-output ratio specifically, the efficiency of variable input utilization during piglet production achieves greater technical and economic efficiency. Conversely, the semi-intensive or semi-technified system represents the remaining 36.4% of production in this region, whereas the technified system remains scarce due to the absence of conditions necessary for its proper development (Hernández *et al.*, 2020b).

Undoubtedly, optimizing both the number and weight of piglets per farrowing, along with achieving the optimal number of farrowings per sow, requires strategic management beginning well before farrowing. This involves effective herd management and high-quality nutrition from mating through gestation. However, it is widely recognized that one of the key factors leading to productive imbalance within the herd is the weaning-to-estrus interval (WEI), which significantly influences the percentage of repeated services (PRS), increasing it up to 15%. Prolongation of the WEI and an elevated PRS contribute to a rise in non-productive days (NPD), ultimately decreasing the number of farrowings per sow per year (FSY) and reducing the number of live-born piglets (LBP) per litter. This combination of reproductive inefficiencies including extended WEI, higher PRS, and increased NPD along with the premature culling of young females, adversely affects production costs within pig production systems (PPS) (Ordaz *et al.*, 2014). In this context, and considering the farm's production conditions, it becomes relevant to determine the number of farrowings that maximizes piglet production, and to identify the production point at which achieving the highest number of farrowings does not necessarily translate into the greatest monetary profit. Therefore, the aim of this research was to determine the technical and economic optimum levels of piglet production based on the number of farrowings in a semi-technified system. The underlying hypothesis posits that maximum piglet production does not inherently guarantee the highest sales profitability.

MATERIALS AND METHODS

Study Site: The field data were obtained from a full-cycle farm classified as semi-technified, dedicated to live pig production between April and June 2023. The farm is located in a locality within the municipality of Temascaltepec, State of Mexico.

Biological Material: At the time of data collection, the farm maintained a population of 123 hybrid sows, consisting of 50% Yorkshire and 50% Landrace genetics, along with boars carrying 50% Pietrain and 50% Duroc genetics.

Data Analysis: The dataset encompassed records from seven farrowings per sow over an approximate five-year period. The data collected for each sow were analyzed through correlation using the Ordinary Least Squares (OLS) statistical model, as described by Gujarati and Porter (2010):

$$L = \beta_0 + \beta_1 P + \beta_2 P^2 + \beta_3 P^3 + e$$

Where L=piglets weaned per female per year; β_i, for i=1, 2, 3, were the parameters of the model; P=Number of farrowings, and e=Statistical, stochastic, or random error. The statistical significance of the estimated model was obtained through the calculated F, the calculated t-values (tc) (Student's t-test), the adjusted R-squared, and the predicted R-squared (Wooldridge, 2010). For the economic significance of the model, the sign of the coefficients β_2 and β_3 was considered, based on the necessary economic logic to define the concavity of the production function curve (Doll and Orazem, 1984; Rebollar *et al.*, 2022).

The statistical model established that the optimal number of piglets is a function of the sow's optimal average number of farrowings. This model enabled the estimation of the optimal average number of weaned piglets associated with the maximum number of farrowings on the referenced farm. Two optimization levels were derived from this model.

The Technical Optimum Level (TOL) was determined by applying the first derivative of the estimated production function and equating it to zero (marginal product, MP) (Rebollar *et al.*, 2014), expressed as $dL/dP=0$, where the variable to be solved was the number of farrowings.

The Economic Optimum Level (EOL) (Rebollar *et al.*, 2014) was calculated by equating the MP to the price ratio between the input (farrowing cost) and the output (weaned piglet price), represented as (farrowing price/piglet price), according to the equation: $dL/dP=FP/PP$. Upon determining the value of P (number of farrowings) corresponding to both the TOL and the EOL, the total cost (TC), total revenue (TR), and profit (G) were subsequently calculated for each optimization level. According to Rebollar *et al.* (2014), the total cost (TC) was calculated using the equation $TC=PpP$, where Pp represents the average farrowing cost and P denotes the optimal number of farrowings. The Pp value corresponded to the estimated cost per sow at farrowing, as reported by the farm owner, and was established at $7,500. Total revenue (TR) was determined using the equation $TR=PlL$, where TR is the total revenue, Pl represents the average price per weaned piglet valued at $1,000 as of October 2023 and L refers to the optimal number of weaned piglets per farrowing. Profit (G) was calculated using the formula $G=TR-TC$, where G represents the monetary profit. The results from the statistical model were generated using the PROC REG procedure of SAS (Statistical Analysis System) in its online version (SAS, 2021). Additionally, the predicted R-squared value was obtained through the PRESS (Prediction Sum of Squares) statement within the PROC REG procedure of SAS.

RESULTS AND DISCUSSION

The estimated production function, which explained the number of piglets (dependent variable L) as a function of the number of farrowings (independent variable P) of the sows, is presented in Table 1.

Based on the results of the Fc statistic, the model was statistically significant, as the Fc value was 32.42 (ANOVA, $P<0.05$) under the default 95% confidence level provided by the model output. With this level of confidence, the null hypothesis stating that any β coefficient is equal to zero is rejected. Additionally, empirical evidence suggests that an Fc value of 2.0 or higher, depending on the degrees of freedom, is already sufficient to assign

Table 1. Results of the statistical model of weaned piglets on a semi-technified farm, October 2023.

Variable	Estimated parameter	SE	t-c	Significance
Intercept	9.31286	0.26770	34.79	P<0.05
P	1.03472	0.27009	3.83	P<0.05
P^2	−0.27452	0.07596	−3.61	P<0.05
P^3	0.01861	0.00627	2.97	P<0.05

Source: own elaboration with results from the SAS output. SE: Standard error. Tc: calculated t-value.

statistical significance to a model (Wooldridge, 2010; Rebollar *et al.*, 2008). The adjusted coefficient of determination (ACD) was 0.9402, or 94.02%, indicating that 94% of the total variation in the number of piglets per farrowing (dependent variable) was explained by the number of farrowings (independent variable P). In this context, each β parameter estimate exceeds twice its standard error (SE) (Table 1), supporting the statistical significance of these estimates. Moreover, the predicted coefficient of determination (PCD) was 0.46, reflecting an acceptable predictive power of the regression model (Wooldridge, 2010; Gujarati and Porter, 2010). According to microeconomic production theory (Doll and Orazem, 1984; Rebollar *et al.*, 2022), the signs of the P^2 and P^3 coefficients were as expected, confirming the presence of a concave production function with diminishing marginal returns in the short term (MRST), which allows for the determination of optimization levels. The coefficient of the β_0 estimator, valued at 9.3, lacks economic interpretation (Doll and Orazem, 1984), as it is not feasible to conceptualize piglet production when the number of farrowings (P) is zero. The value of 1.03 suggests that for each additional farrowing achieved by the sows, piglet production is expected to increase by 1.03 piglets. As outlined by Rebollar *et al.* (2011), the technical optimum level (TOL) does not involve product or input prices, as its primary objective is to identify the level of maximum physical production the peak of the production function curve. At this point, as supported by production theory (Doll and Orazem, 1984), production reaches its maximum, and no additional amount of the input variable will yield higher output than that achieved at the TOL. Consequently, the values corresponding to both the TOL and the economic optimum level (EOL), derived from the estimated production function, are presented in Table 2.

Table 2. Cost, revenue, and profit at the TOL and EOL in piglet production on a semi-technified farm, 2023.

Concept	TOL	EOL
X, given in number of births per female	2.54	2.43
P, number of piglets per sow	10.4749	10.4731
TR, $	7,500.0	7,175.0
IT, $	10,474.9	10,473.1
P, $	2,974.9	3,298.1

Economic Optimum Level (EOL). TOL: Toluca. Source: Prepared by the authors based on results output, October 2023.

Thus, the technical optimum level (TOL) of the estimated cubic production function corresponds to a value of the dependent variable L (piglets) of 10.4749 average piglets per farrowing, which is similar to the 10.65 reported by García *et al.* (2011), 10.4 average piglets per sow by Ordaz *et al.* (2013), 9.6 confirmed by Gruhot *et al.* (2017), and 9.8 piglets by Ordaz *et al.* (2019). The production optimum (technical efficiency), or the highest point on the production function curve, is thus associated with a P value (number of farrowings) of 2.54, consistent with the findings of Ordaz *et al.* (2013) and García *et al.* (2014), who reported an average of 3.4 farrowings per sow.

At the economic optimum level (EOL), according to microeconomic production theory (Rebollar *et al.*, 2022), the prices of both the variable input and the product are relevant, as the goal is always to achieve economic efficiency. In the EOL, the producer is not interested in reaching maximum production, but rather in determining the monetary profit, which does not necessarily coincide with the point of maximum total output. Here, a P value of 2.43 corresponds to the EOL of the estimated cubic production function for piglets. Notably, this P value is lower than the P value at the TOL (P=2.54). This finding demonstrates that the TOL occurs before the EOL in relation to L, which aligns with microeconomic production theory (Rebollar *et al.*, 2022). Additionally, the L value at the EOL (10.4731) is lower than that at the TOL (10.4749), which is also consistent with microeconomic theory (Doll and Orazem, 1984; Portillo *et al.*, 2015). At the TOL, the producer's focus is on achieving the maximum level of physical production. Under microeconomic production theory (Rebollar *et al.*, 2022), prices of the product and input are not relevant at this optimum; however, it remains important to estimate the total cost (TC), total revenue (TR), and profit (P) in monetary terms corresponding to the TOL. According to microeconomic production theory (Portillo *et al.*, 2015), in a classic production function graph, the EOL is always located below the TOL. At the EOL, the prices of both the variable input and the product are considered, as this optimum reflects the fact that maximum production does not necessarily imply maximum monetary profit. This insight is especially important for any producer aiming to maximize monetary profit in the production process. In this study, the monetary profit at the EOL ($3,298.1) was higher than at the point of maximum piglet production ($2,974.9). This demonstrates that maximum production does not necessarily lead to maximum monetary gain. In fact, in this research, the highest piglet production did not correspond to the highest profit, thereby confirming both the general objective and the hypothesis of the study.

CONCLUSIONS

The number of farrowings corresponding to the technical optimum level (TOL) was higher than that of the economic optimum level (EOL). The number of piglets associated with the TOL exceeded that of the EOL; however, the monetary profit achieved at the EOL was greater than at the TOL. This demonstrates that maximum production does not necessarily result in maximum monetary profit. Any pig producer operating under similar production conditions as those described in this study may benefit from these findings to improve the economic profitability of piglet production.

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Colegio de Postgraduados

Analysis of the number of bibliographic publications on the presence of microplastics in sediments in the Americas

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Citation: Castañeda-García, F., Castañeda-Chávez, M.R., Navarrete-Rodríguez, G., & Zamudio-Alemán, R.E. (2025). Analysis of the number of bibliographic publications on the presence of microplastics in sediments in the Americas. *Agro Productividad*. https://doi.org/10.32854/x60vz574

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

Received: June 20, 2024.
Accepted: May 28, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 35-44.

ABSTRACT

Objective: To examine the scientific activity of the American continent in detail during a period of ten years (from 2014 to 2024).

Design/Methodology/Approach: A quantitative methodology was employed, analyzing articles related to the presence of microplastics (MPs) in sediments, sourced from the Scopus database.

Results: A total of 2,050 articles were retrieved, of which 123 (6%) focused specifically on MPs in sediments. The leading contributors were Brazil, the United States, Canada, and Mexico. The most frequently cited authors included Alexander Turra, Patricia L. Corcoran, and A.D. Vethaak. A variety of methodologies were used, including density separation of MPs, oil extraction protocols, and FTIR spectroscopy, among others.

Study limitations/Implications: No standardized methodology exists for the main steps involved in the determination of microplastics (MPs), even as their detection continues to increase across diverse environmental sediments.

Findings/Conclusions: The production of scientific articles on microplastics (MPs) in sediments has increased over the past ten years, driven by the growing detection of these contaminants in sediment samples.

Keywords: microplastics; sediments; marine pollution; ocean, American continent.

INTRODUCTION

The increasing use of single-use plastic products and various types of packaging is a growing trend in today's society (Ronda *et al.*, 2023). As a result, an estimated 11 million additional tons of plastic waste are expected by 2025 (Naidu *et al.*, 2022). Although many plastics can be recycled or reused, the majority are still disposed of through incineration, landfilling, or simply released into the terrestrial environment (Bi *et al.*, 2023). Once released into the environment, plastics undergo physicochemical and biological processes —such as mechanical abrasion, photodegradation, hydrolysis, thermal oxidation, chemical oxidation, and biodegradation (Iannacone *et al.*, 2019; Wang *et al.*, 2022)— which lead to

the aging and fragmentation of plastic materials into tiny particles smaller than 5 mm, known as microplastics (MPs). Due to their abundance, persistence, and unregulated components, MPs are considered a high-impact threat to marine ecosystems, particularly in sediments, where economically important benthic species inhabit (Gil *et al.*, 2012; Olivatto *et al.*, 2019; Jorquera *et al.*, 2022).

Currently, MPs are predominantly found in seawater, marine organisms, and coastal sediments (Olivatto *et al.*, 2019; Rusinque-Quintero *et al.*, 2022). It has been identified that the terrestrial environment accounts for approximately 80% of marine plastic pollution, making it a major source of contamination (Wang *et al.*, 2022). A gradual increase in MP abundance has been reported across different land uses, with urban areas contributing more significantly to MP presence and transport than fishing-related activities (Naidu *et al.*, 2022). In densely populated areas, MP pollution levels have been found to closely correlate with population density (Bi *et al.*, 2023). For instance, Guanabara Bay in Brazil exhibits some of the highest levels of microplastic pollution worldwide, which is attributed to inadequate urban solid waste management. In general, developing countries often lack appropriate solid waste management technologies, contributing to MP accumulation in marine sediments (Olivatto *et al.*, 2019; Nchimbi *et al.*, 2022).

The objective of this study was to examine the scientific output of the American continent over the period 2014-2024, using a quantitative approach based on articles referring to the presence of MPs in sediments.

MATERIALS AND METHODS

This analysis focused on scientific activity based solely on articles published in the Scopus database, selected for its recognized rigor in the indexing process, which adheres to criteria of editorial quality, peer review, and scientific relevance. Scopus provides broad multidisciplinary coverage, granting access to specialized literature across a wide range of fields. Additionally, it offers standardized bibliometric indicators and robust analytical tools that are essential for conducting quantitative studies on scientific output, collaborative networks, and thematic trends. Its technical features further facilitate data collection, organization, and analysis. The platform's widespread acceptance within the scientific community reinforces the methodological validity of this study (Baas *et al.*, 2020).

The selected documents were meticulously analyzed using a quantitative approach, year by year, over a ten-year period (2014 to 2024) across the American continent. Articles in English and Spanish were filtered using the keywords "*microplastics*", "*microplastic*", and "*sediments*" in the title and abstract fields, with data collected up to April 15, 2024. The search criteria within the Scopus database were restricted to research articles and review papers within the subject areas of Environmental Science, Agricultural and Biological Sciences, Chemistry, and Chemical Engineering. Finally, the search was refined by selecting countries in the American continent, including: the United States, Brazil, Canada, Mexico, Chile, Peru, Colombia, Ecuador, Uruguay, Costa Rica, Venezuela, Panama, Dominican Republic, Bahamas, Puerto Rico, Paraguay, Guatemala, Trinidad and Tobago, Jamaica, Cuba, Bolivia, Belize, Barbados, and Honduras. This process resulted in a total of 2,050 articles.

From the 2,050 articles obtained, a detailed review was conducted to extract information specifically related to the presence of MPs in sediments across the American continent. During this process, studies focusing solely on organisms inhabiting marine sediments were excluded. An Excel database was developed to record key information from each publication, including the year of publication, study location, and types of MPs identified (Supplementary Material 1). A total of 123 articles met the inclusion criteria.

To support the analysis, VOSviewer and Research Rabbit software were used to construct bibliometric networks and trace connections between studies. These tools helped identify patterns of collaboration, key authors, and emerging research trends in the field. A Pearson correlation analysis was also performed to assess whether article production was strongly correlated among countries in the Americas. Additionally, journals with the highest number of publications on MPs in sediments were identified.

The image generated in VOSviewer is composed of colors, edges, and nodes. The proximity between two nodes indicates the strength of their relationship: the closer they are, the stronger the connection. Edges (lines between nodes) represent relationships such as co-citation, co-authorship, or co-occurrence of terms, with thicker lines denoting stronger associations. The size of the labels or nodes reflects the frequency or relevance of the term —larger labels correspond to terms that appear more frequently in the analyzed documents.

In the visualizations, the size of the circles and the distance between nodes indicate the number of publications and the strength of the relationships between items. Node color represents thematic groupings or clusters —nodes sharing the same color are more closely related to each other, suggesting subtopics within the broader field of study.

Additionally, a Pearson correlation analysis was conducted to determine whether article production was strongly correlated among countries in the Americas. The journals with the highest number of publications on MPs in sediments were also identified.

RESULTS AND DISCUSSION

The bibliographic analysis identified the presence of MPs in sediments based on 123 studies conducted in the American continent over the past 10 years, as indexed in the Scopus database. The lowest publication outputs were recorded in 2014 and 2017. However, a notable increase occurred over a four-year period, peaking in 2021 with 30 published articles, followed by 25 articles in 2023. Although the publication trend is not linear, there is clear evidence of growing research interest and scientific production in this field (Table 1). It is important to note that different sampling and analytical strategies used across studies contribute to significant variations in reported MP concentrations (Wang *et al.*, 2022).

The analysis of the relationship between the number of documents published each year and time, using Pearson correlation analysis, yielded a correlation coefficient of P=4.3512 with 95% confidence. This suggests a significant relationship between the number of articles published and the year of publication over the last decade. The predominant subject area was Environmental Science, with 314 documents, followed by Agricultural and Biological Sciences, with 129 documents. Additionally, among the 75 selected articles, the majority

Table 1. Overview of recent studies reporting MP concentrations across the Americas.

Country	Study area	Sample type	MPs concentration	MPs type of form or color	Source
USA	San Andres Bay	Sediment	3,16 ± 1,59 MP kg^{-1} at 34,03 ± 11,69	11,600 particles per kg dry sediment	Ridall *et al.* (2022)
Argentina	Central Andes	Trout intestines, and catfish streams		85% fiber, 15% blue fragments, 50% brown	Ríos *et al.* (2022)
Ontario, Canada	Muskoka -Haliburton Ontario	Terrestrial and aquatic environment	1.78 pieces from May to June	In the lake: fibers (70%) Sediment (fibers and fragments 51-67%)	Welsh *et al.* (2022)
Ecuador	San Cristobal Island Beaches and Tortuga Island	Sediment	2213 MPs, where 93% were of 1mm	Rubber presence	Jones *et al.* (2022)
USA	Narragan-sett Bay, Rhode Island	Sediment	40 and 4.6 million pieces per 100 g	Cellulose acetate fibers	Cashman *et al.* (2022)
Mexico	Tampico Beach	Sediment	256 to 283 particles in 20 g	Transparent (32%), White (8%), Yellow (6%), Pink (4%), Red (1%) Multicolored (1%) fibers.	Flores-Ocampo *et al.* (2023)
Mexico	Southern Gulf of Mexico	Sediment	1.3 to 95.2 particles per kg	Fragments (82.44%), Fibers (16.19%), Films (1.35%)	Rendón-von Osten *et al.* (2023)
Argentina	Bahía Blanca Estuary	Sediment	1693 ± 2315 MPs kg^{-1}	Pellets	Arias *et al.* (2023)

were authored by researchers from the Americas, with 27 publications highlighting the presence of MPs in marine sediments (Figure 1). Although research on MPs is ongoing, it remains limited: out of 2,050 studies published in the last ten years on this topic, only 6% focused specifically on MPs in sediments. This indicates a significant gap in assessment and monitoring efforts. It has been reported that at least 66.7% of plastics in the ocean are difficult to monitor under current observation frameworks (Ugwu *et al.*, 2022).

Significant knowledge gaps remain regarding the occurrence, distribution, aging, fragmentation, transport, flux, fate, and sinks of microplastics (MPs) in urban runoff, particularly from a continental perspective and its impact on the oceans (Wang *et al.*, 2022)

The indiscriminate use of plastic products, coupled with a deficient recycling culture, has led to the widespread disposal of plastic waste into the environment, causing significant harm to biota. The reviewed studies report a high presence of microplastics (MPs), predominantly fibers and fragments (Olivatto *et al.*, 2019). Authors with greater seniority tend to receive a higher number of citations. The data obtained from the network of cited authors across the 123 articles on microplastics in sediments in the Americas highlight key research works and their impact. These references are concentrated in six major scientific journals from the region. The journal with the highest impact factor was *Science of the Total Environment*, with an impact factor of 10.753 and 29 published articles. It was followed by *Environmental Pollution*, which has an impact factor of 8.9 and 12 articles published. The third journal in terms of impact factor was *Marine Pollution Bulletin* with 5.8; this journal, however, published the greatest number of articles, totaling 34. The lowest impact factors were found in *Marine Environmental Research* and *Journal of Great Lakes Research*, with 3.3 and 2.2 respectively, each having

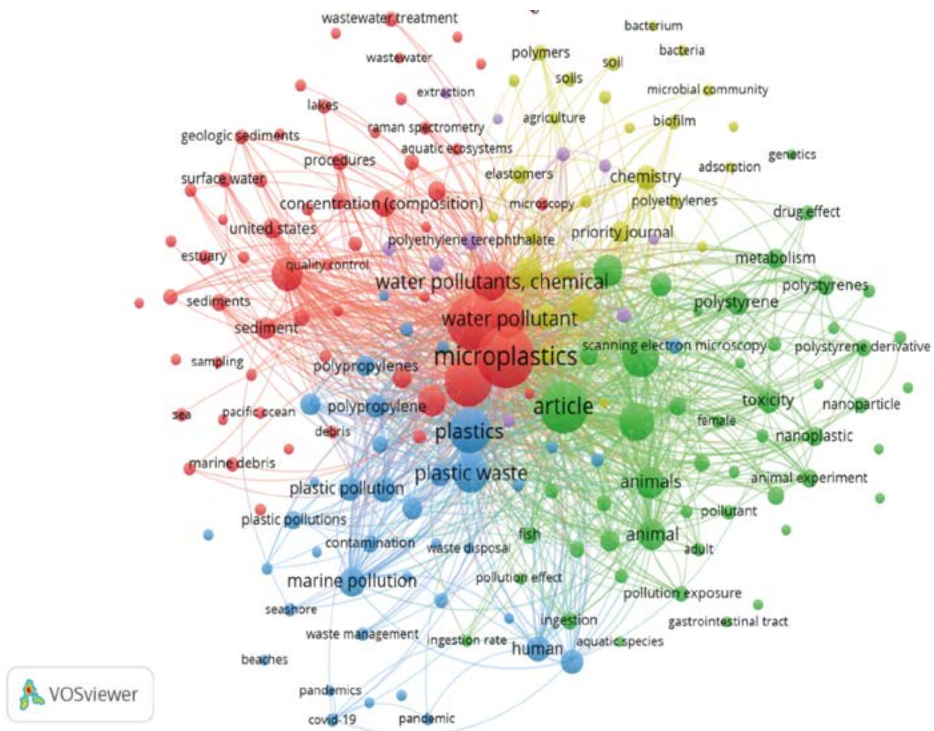

Figure 1. Keyword network of publications on microplastics.

only 3 published articles. Meanwhile, *Bulletin of Marine and Coastal Research* had the lowest number of articles published, with only 2. The keywords commonly used across these publications include water pollution, plastics, animals, chemicals, and microscopy, indicating shared thematic concerns. The analysis of microplastic (MP) levels found in urban runoff indicates higher concentrations compared to those released in wastewater effluents. The small size of MPs accelerates their transport in urban runoff waters. Most MPs (up to 97-98%) entering wastewater treatment plants (WWTPs) become trapped in the sludge (Wang *et al*., 2022). Fibers are the most abundant type of MPs attributed to wastewater discharges (Jorquera *et al*., 2022).

In a 2014 study conducted in Canada, 52 microbeads per square meter were reported in freshwater sediments (Castañeda-Rowshyra *et al*., 2014). That same year, bibliographic records already documented the ingestion of MPs by plankton, mostly juvenile vertebrates and invertebrates, as well as interactions between MPs and chemical contaminants present in sediments worldwide (Ivar do Sul & Costa, 2014). By 2015, microbeads were identified in facial cleansing products, which can enter the marine environment, leading to bioaccumulation and the transmission of organic toxins to predators (Chang, 2015).

The elutriation method was implemented to separate sediment using a PVC device, followed by filtration with coffee filters and characterization under a 40x magnification loupe (Zhu, 2015). On the southeastern coasts of Brazil in 2016, researchers investigated MPs using a density separation methodology, applying a gradient approach in dune regions with transects and depth profiling (Moreira *et al*., 2016). Santana *et al*. (2016) identified and quantified MPs using a polarized light microscope.

In 2017, Canadian researchers proposed a novel method for collecting MPs on sandy beaches, suggesting the replacement of conventional density separation techniques with a protocol inspired by petroleum extraction processes used in the oil industry (Crichton *et al*., 2017). In Brazil, additional efforts focused on determining the optimal diameter of a manual auger, aiming to estimate sediment volume and enable heterogeneous sampling based on beach profiles (Fisner *et al*., 2017).

In 2018, studies conducted in Canada reported higher concentrations of MPs at the mouths of the Detroit and Grand Rivers, areas characterized by significant urban influence, as well as in tributaries with high population densities and maritime traffic (Dean *et al*., 2018).

Researchers in Mexico monitored 21 beaches over the course of a year, detecting MPs through filtration, visual observation, and verification using FTIR spectroscopy (Piñon-Colin *et al*., 2018). In Campeche, Mexico, monitoring was conducted at seven sampling sites to detect MPs and phthalate esters using a Varian 3800 gas chromatograph (Borges-Ramírez *et al*., 2019). In Brazil, the presence of MPs in sediments was observed in 2019 using flotation techniques involving the addition of NaCl to the samples, followed by filtration through commonly used coffee filters (Baptista-Neto *et al*., 2019).

Concerns regarding the quality of sample collection and analysis across various matrices —such as sediment, tissue, water, and air— have prompted a strong response from the scientific community. In the United States, scientists have proposed the development of standardized guidelines to address the challenges associated with sampling in diverse environments. These guidelines aim to streamline collection methods, optimize sample processing while minimizing secondary contamination, and establish a comprehensive database of identified MPs (Brander *et al*., 2020). In Canada, a prospective model developed in 2020 assessed the potential accumulation of MPs in crustaceans in the Northeastern Pacific, enabling the evaluation of risks associated with inadequate plastic waste management (Alava, 2020).

In 2021, there was a notable increase in scientific publications related to MPs in sediments, including the first report from Puerto Rico. This study employed a methodology similar to that of Baptista-Neto (2019), in which six sandy beaches were sampled and the results were compared with beaches from other parts of the world (Pérez-Alvelo *et al*., 2021).

In Mexico, samples of water, sediment, and commercially available fish were analyzed through visual inspection. For sediment samples, the methodology involved drying, followed by wet oxidation, and the subsequent addition of $ZnCl_2$ɑ—an approach capable of extracting up to 90% of the polymers. This represents a novel advancement in MP identification. Additionally, chemical characterization was performed using Fourier-transform infrared spectroscopy (Sánchez-Hernández, 2021).

In 2022, Brazil introduced an innovative approach to MP analysis by using a hot needle as an alternative to chemical analysis for confirming whether particles are composed of plastic. Meanwhile, in the United States, the presence of MPs in coastal sediments had already been documented using X-ray fluorescence (XRF) for physical identification, along with the application of potassium metaphosphate for organic matter degradation (Culligan *et al*., 2021).

It has been demonstrated that the sedimentation time of MPs positively correlates with the frequency of wind speed >1 m, especially for coarse particles, the dry sedimentation rate of coarse particles increases with the increase in wind speed (Jia *et al*., 2022). This implies that their concentration will be higher in areas with favorable wind conditions. In addition to the high concentration of sediments and sand particles, the transport of MP particles is favored (Zhao *et al*., 2023).

High-density MPs, such as PVC and PET, tend to accumulate in sediments rather than being transported over long distances (Wang *et al*., 2022). In contrast, films and granules exhibit a greater tendency to settle on surfaces with larger specific surface areas compared to fiber-type MPs. Fibers are more likely to adhere to moisture and particulate matter in water during rainfall events, resulting in their broader dispersion into the atmosphere and soil (Jia *et al*., 2022). Although data on MP pollution in soils with varying land uses remain limited, the occurrence, fate, and ecological impacts of MPs in terrestrial environments warrant further investigation (Bi *et al*., 2023).

The abundance of MPs varies according to land use, nutrient content in surrounding soils, and atmospheric deposition (Table 1), which can act as a medium for transferring MPs from the atmosphere to terrestrial and aquatic ecosystems —an aspect that remains underexplored (Jia *et al*., 2022). Factors such as unregulated municipal waste disposal, insufficient waste reduction strategies (Naidu *et al*., 2022), and marine aquaculture practices (Jorquera *et al*., 2022) contribute to the continuous accumulation of MPs, exerting harmful effects on aquatic ecosystems, organisms, and adjacent coastal communities (Naidu *et al*., 2022). Moreover, MPs accumulated in soils during dry seasons may undergo seasonal mobilization, becoming incorporated into the hydrological cycle during rainy periods. Due to their small size and high mobility, MPs can serve as vectors for the transport of pathogenic microorganisms, heavy metals, invasive species, and other pollutants. During this process, they may also adsorb lipophilic contaminants, amplifying their ecological risks (Jorquera *et al*., 2022; Rusinque-Quintero *et al*., 2022).

MPs are transported by surface runoff to coastal areas, where they can enter the ocean and accumulate locally on beaches. Their distribution along the coastline is influenced by tidal cycles and sediment grain size, as soil characteristics determine the extent to which MPs can accumulate. These patterns also reflect the dynamic interactions of marine environments influenced by the sediment deposition capacities of rivers that discharge into the sea (Rusinque-Quintero *et al*., 2022). Coastal hydrodynamic factors —including current direction and magnitude, wind, and tidal regimes— are key drivers of oceanic circulation and vertical mixing, which significantly affect MP distribution (Jorquera *et al*., 2022). Additionally, marine dynamics and wave-induced abrasion promote the fragmentation of MPs, increasing their ecological risk. Studies have shown that deeper marine environments tend to harbor higher concentrations of MPs than coastal waters, due to reduced water movement and circulation (Rusinque-Quintero *et al*., 2022; Jorquera *et al*., 2022; Wang *et al*., 2022). MPs with densities greater than seawater, or those subjected to biofouling by microorganisms, may settle and accumulate in marine sediments, the water column, and deep-sea environments.

Marine organisms provide valuable insights into the current state of marine biota, MPs can obstruct the digestive tract (Olivatto *et al.*, 2019) adhere to gills, potentially causing asphyxiation, and may be ingested accidentally or through contaminated food, negatively affecting the reproduction of oysters, the metabolism of copepods. In *Arenicola marina* polychaetes the ingestion of polystyrene MPs leads to weight loss (Olivatto *et al.*, 2019, Jorquera *et al.*, 2022). Future studies should consider wind as a transport factor for fibers since it is a factor that cleans areas where the wind favors, potentially transporting MPs to different locations and causing further impact. Furthermore, a strong correlation has been observed between the density of permanent residents and local MP contamination. In contrast, temporary residents primarily contribute to coastal water pollution through urban and terrestrial runoff, including riverine inputs, rather than fishing-related activities.

CONCLUSIONS

The production of scientific articles on MPs in sediments has increased significantly over the past decade; however, a standardized methodology for their separation, quantification, and classification is still lacking. Despite this, countries such as Brazil, the United States, Canada, and Mexico have continued to detect MPs in sedimentary environments by applying a variety of methodological approaches. Scientific journals have shown growing interest in publishing research related to MPs, particularly studies that include keywords such as plastics, water pollutants, chemical contaminants, toxic substances, microscopy, toxicology, polystyrene, scanning electron microscopy, animals, and fish. Additionally, relevant topics include MP concentration, agricultural contexts, lakes, geological sediments, and estuarine systems.

ACKNOWLEDGMENTS

This research was carried out with support to the Instituto Tecnológico Nacional de México/ Instituto Tecnológico de Boca del Río (TecNM/ITBOCA) and to the Consejo Nacional de Ciencia y Tecnología (CONAHCYT) for funding.

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Colegio de Postgraduados

Technical and financial feasibility of using plastic poles for chayote production [*Sechium edule* (Jacq.) Swartz]

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ABSTRACT

Objective: To evaluate the technical and financial feasibility of using recycled plastic poles for chayote production.

Design/methodology/approach: A structure (pergola) was designed to produce chayote [*Sechium edule* (Jacq.) Swartz], size 8 m×8 m, using recycled plastic poles of 2.5 m length, a square section of 95.25 mm and 16.26 kg, using eight balances to record the biomass weight during production. A financial run of 10 years was conducted to evaluate the plastic pole and its projection for one hectare of tapanco, comparing it with wood and concrete poles.

Results: The plastic poles did not show fractures or deformations, even when an average load of 200 kg was found in the production cycle. For the financial analysis, the following were used: current interest rate, investment, and operation costs. The results indicated that the benefit/cost ratio and the internal rate of return were favorable when using plastic poles, since for each peso invested there is a recovery of $1.04, compared to $0.81 and $0.80 of wood and concrete poles, respectively; the internal rate of return was nearly 9 times the interest rate.

Limitations on study/implications: The plastic poles are sold as ornamental elements and for the installation of fences. The company does not have a certificate of resistance tests.

Findings/conclusions: Recycled plastic poles can substitute wood poles in the productive structure of chayote.

Keywords: sustainable production, plastic recycling, pergola, standing poles, biomass.

Citation: Herrera-Gómez, Á. E., Ortiz-Laurel, H., Rosas-Calleja, D., & Leyva-Ovalle, O. R. (2025). Technical and financial feasibility of using plastic poles for chayote production [*Sechium edule* (Jacq.) Swartz]. *Agro Productividad*. https://doi.org/10.32854/97tqwj06

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

Received: June 26, 2024.
Accepted: May 18, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 45-53.

INTRODUCTION

Mexico is the primary global producer of chayote [*Sechium edule* (Jacq.) Swartz], with a surface of 3,334 ha, averaging a yield of 64 t ha^{-1} for an annual production of 213,269 t (Axayacatl, 2023; SIAP, 2021).

To achieve the full development and higher productivity of the crop, a wire structure (tapanco, pergola) must be built, similar to a net, since the plant is of climbing habit; it is the scaffolding raised from the floor at 2.0 to 2.2 m, held by wood posts/poles, which are the ones most frequently used.

Although cultivating chayote is profitable, it faces difficulties; on the one hand, the high investment in the purchase of poles, followed by the uncertainty about their timely supply and the safety in maintenance of the tapanco (Gamboa, 2005). In the chayote production cycle, the weight of the biomass (leaves, guides and fruits) rests on the mesh (net) of the scaffolding formed by thin wires with diameter of 1.291 mm (AWG #16), which covers the tapanco. They are supported by thick wires with diameter of 3.264 mm (AWG #8), and the load is transferred to them. At the same time, each end of the wires is secured on the superior ends of the opposite poles located on the periphery of the tapanco.

These last poles are the ones that resist the greatest effects of the biomass load during production, which is why the ones that show greater robustness and vigor are selected. Therefore, the importance of ensuring a constant and reliable supply of these materials (Figure 1).

Some producers are making concrete poles to replace the wood poles. Although they could have more durability, standardizing their manufacture could increase the costs of using finite raw materials.

Presently, recycled plastic poles are sold, which could be considered for their inclusion in the tapancos and to replace the wood poles, while they represent an alternative that is friendlier with the environment. The objective was to explore the technical and financial viability of using commercial poles made with recycled plastic, which are satisfied in the dimensions required for the correct installation of tapancos for chayote production.

MATERIALS AND METHODS

This study was conducted in the municipality of Coscomatepec in the state of Veracruz, Mexico (19° 04' 32.42" N and 97° 01' 33.8" W) at an altitude of 1515 masl. It is important to point out that all the poles used in the tapanco are subject to the effects of climate, such as rain, hail, temperature, wind, and solar radiation. All these events contribute to decreasing their useful life, which is why wood poles deteriorate (rot) easily, mainly in the low part of the pole, from the effect of moisture (Guevara-Hernández *et al.*, 2014). This reality cannot be avoided for any of the poles considered as viable.

Figure 1. Production infrastructure (tapanco) for chayote.

Although there are different densities of chayote plantations, from 6 m×6 m, 8 m×8 m, to 10 m×10 m (IICA, 2016), in the study zone there are no accurate records of the preference of producers for any of these. However, the tapancos surrounding the study site use the 8 m×8 m model, so they were taken as reference for the study. Likewise, from the records of producers in the zone of Coscomatepec, Veracruz, 866 poles ha^{-1} were counted, which include those on the inside and the periphery, called crown, in addition to 40 storage units to supply the possible contingencies during the (one year) cycle. The decision was made to build a single experimental module of 8 m×8 m with faithful likeness to a conventional production tapanco, although using only nine recycled plastic poles, with separation of 4 m between the poles and forming a square of 8 m length and 8 m width, with a chayote plant in its geometric center (Figure 2).

Thin (galvanized) wires with a diameter of 1,291 mm (AWG #16) were placed to build the roof (grate), with a separation of 25 cm between them; and thick wire with diameter of 3.264 mm (AWG #8) was placed in the entire perimeter and the interior central cross that is formed (Ortiz-Laurel *et al.*, 2019).

The pole elaborated from the recovered plastic has a length of 2.5 m with a square transversal section of 95.52 mm per side and with an average weight of 16.26 kg. Each pole is buried at 0.5 m depth in the soil. The management of the crop in this module is similar to the one carried out by the producer in the commercial plantation. Therefore, during the production cycle, biomass pruning was carried out every 15 days, and the pruning was weighed in an electronic balance Rhino brand, model BAPCA-100.

The weight recorded exhibited the vegetative development of the chayote crop, in addition to determining the load capacity of this experimental module on its surface of 64 m^2. For this purpose, eight balances were installed (Roman Pretul with spring model BAS-100P) with load capacity of 100 kg (Figure 3). Each was placed in pertinent sites and between the ends of different sections of the thick load wires (Figure 4). Thus, the balances recorded the different weight variations; increase (new buds of biomass-leaves, guides, flowers, small chayotes, and heavier chayotes), and decrease (biomass pruning and fruit harvesting).

Figure 2. View of the experimental module with separation between poles of 4 m.

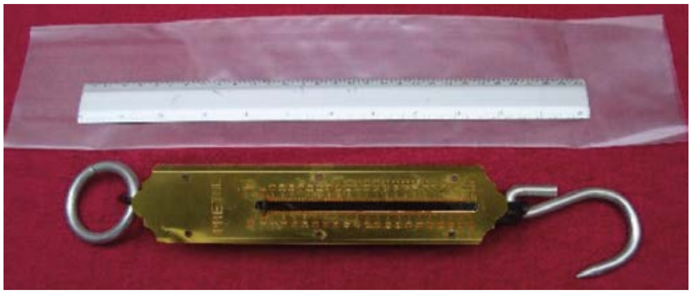

Figure 3. Roman spring balance used to record the biomass weight.

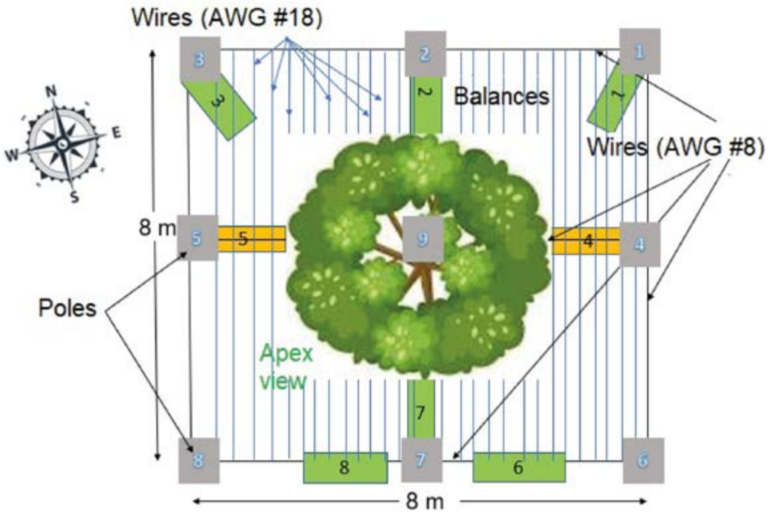

Figure 4. Distribution of the 8 balances inside the experimental tapanco.

Financial evaluation of three pole alternatives in the construction of tapancos

For the financial analysis, the production costs of the chayote crop were obtained from among a group of producers in the region of Coscomatepec, Veracruz. The main parameters were the initial investment cost ($500,000.00 MX pesos) and the operation costs of all the agricultural activities during the production cycle. Likewise, the average cost of the kg of chayote was obtained with the data obtained, which fluctuates between $5.00 and $12.00 over the different months of the year.

The production costs of chayote were integrated into their main sectors, with the purpose of differentiating their magnitude and their variation regarding time. These were grouped into: costs for the establishment of the plantation (design of planting, soil preparation tasks, hollowing, seed conditioning, etc.); the costs of the tapanco structure (poles, wires, staples, tools, etc.); the costs of inputs (seed, fertilizers, phytosanitary products, etc.); the maintenance costs of the plantation (pruning, irrigation, pole replacement, etc.); the costs of machinery and equipment (tractor, farming implements, backpack pumps, machetes, scissors, irrigation pumps, etc.); the harvesting costs (bars, sacks and bags for packaging, yarn, raffia, etc.); and other expenditures and unforeseen costs, such as electricity, fuels, repairs, replacement parts, etc.

It is important to highlight that the workforce is and was used as workdays in every sector, although with different intensity. Likewise, it is emphasized that these concepts, their breakdown and costs were obtained directly with chayote producers in the region of Coscomatepec, Veracruz. Therefore, Table 1, Table 2 and Table 3 represent a concentrate of all the previous ones, differentiating between fixed costs and variable costs. It can be seen in these that they are the fixed costs, variable against time (year), referring to costs of the different types of poles and the costs associated to their maintenance and replacement in time.

The financial run was projected for a period of 10 years, where three scenarios were analyzed; the first is chayote production with tapanco built with wooden poles; the second with tapanco built with concrete poles (with the producer's manufacturing costs); and the third, built with recycled plastic poles (supplied directly by the manufacturer). These scenarios maintain a surface of one hectare, the same number of chayote plants (156), the same number of interior, plant and crown poles (866 in total), the same arrangement of the plants (8 m×8 m), equal agronomic management, a yield per hectare of 64 t ha^{-1}, and an average sale price of $8.7 kg. For each scenario, the following indicators of profitability were estimated: net value added (NVA), benefit/cost ratio (B/C), and internal rate of return (IRR).

The economic information used for the financial evaluation was obtained from official documents (INEGI, 2023), with an interest rate of 1.17% during January to July of 2023. Data from the Banxico portal (2023) for the equilibrium interbank interest rate (TIIE, for its initials in Spanish) was used, with a value at 182 days of 11.5% reported in August 2023, as well as the percentage of fixed-term investment, of 6.8% for June 2023.

RESULTS AND DISCUSSION

The nine recycled plastic poles from the experimental module did not exhibit adverse effects in bending, fissures or deformations from the effects of the weight of

Table 1. Production costs for one ha of chayote by using wooden poles for the pergola in the region of Coscomatepec, Ver. (2022).

Concept	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8	Year 9	Year 10
Fixed costs	$218,453.0	$30,000.0	$151,620.0	$30,000.0	$151,620.0	$30,000.0	$151,620.0	$30,000.0	$151,620.0	$30,000.0
Variable costs	$227,049.0	$146,050.0	$227,049.0	$146,050.0	$227,049.0	$146,050.0	$227,049.0	$146,050.0	$227,049.0	$146,050.0

Table 2. Production costs for one ha of chayote by using concrete poles for the pergola in the region of Coscomatepec, Ver. (2022).

Concept	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8	Year 9	Year 10
Fixed costs	$413,303.0	$30,000.0	$38,400.0	$30,000.0	$38,400.0	$379,570.0	$38,400.0	$30,000.0	$38,400.0	$30,000.0
Variable costs	$227,049.0	$146,050.0	$227,049.0	$146,050.0	$227,049.0	$146,050.0	$227,049.0	$146,050.0	$227,049.0	$146,050.0

Table 3. Production costs for one ha of chayote by using plastic poles for the pergola in the region of Coscomatepec, Ver. (2022).

Concept	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8	Year 9	Year 10
Fixed costs	$451,407.0	$30,000.0	$38,400.0	$30,000.0	$38,400.0	$30,000.0	$38,400.0	$30,000.0	$38,400.0	$30,000.0
Variable costs	$227,049.0	$146,050.0	$227,049.0	$146,050.0	$227,049.0	$146,050.0	$227,049.0	$146,050.0	$227,049.0	$146,050.0

the biomass and climatological events during the production cycle. The installation remained stable.

Figure 5 illustrates the weight recorded for each of the balances during the production cycle of six months. The curves show a continuous increase and decrease; the decrease shows the fruit harvest and the pruning biomass. This confirms the dynamic development of the plant, because of the accumulation of dry matter (Valverde and Sáenz, 1985).

When the average of these weights was calculated, there would be a distribution of more than 30 kg in each perimeter point, where the balances of the modular structure of the tapanco were placed. This weight could be considered permanent, which represents a constant load on the recycled plastic poles. It is also quite likely that a similar weight is found in the central points of the modules.

The maximum biomass weight found was 252 kg in the month of October. This weight has a relatively short presence in time and space, due to: a) fruit harvesting, and b) pruning of guides and leaves; this is perhaps the most notable effect on the temporary decrease of records.

Figure 6 shows that 400 kg is the amount of plant material that is taken from the module in this six-month production period. From this, practically half (50%) corresponds to the harvested fruit, even when in percentage the chayotes harvested represent 4% of all the plant biomass.

Regarding financial feasibility, the production costs and income were processed to determine the Net Present Value (NPV) (Table 4). It is obtained from the sum of the actualized income from year one to year 10, with the same process for the actualized costs. Therefore, the total costs are subtracted from the total income, defined as NPV (Table 4).

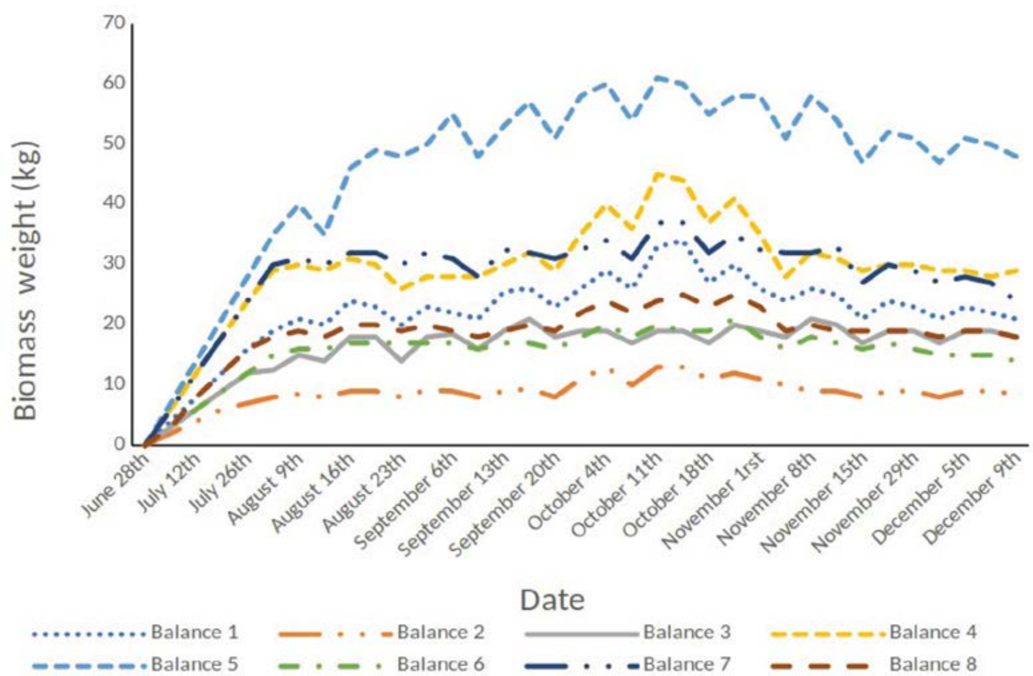

Figure 5. Accumulated weight of the biomass during the chayote cultivation cycle in the experimental tapanco.

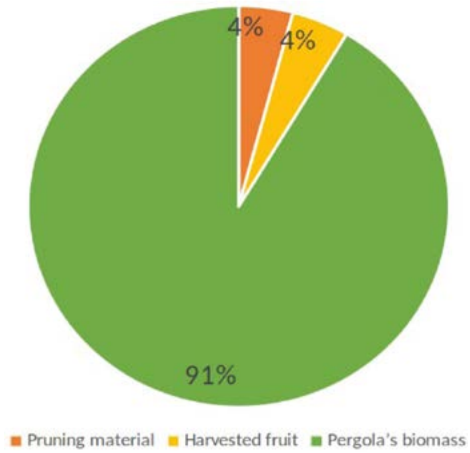

Figure 6. Percentage distribution in the plant biomass weight on the modular structure of 64 m^2 during the chayote production cycle.

Table 4. Net present value (NPV) for three materials for the poles.

Poles	Total income ($)	Total cost ($)	NPV ($)	Result	Decision
Wood	8,054,069.46	4,452,879.10	3,601,190.36	NPV>0	Accepted
Concrete	8,054,069.46	4,462,235.39	3,591,834.07	NPV>0	Accepted
Plastic	8,054,069.46	3,931,945.05	4,122,124.41	NPV>0	Accepted

The NPV variable determines the economic viability of the project. In this study, the profitability is accepted in the three scenarios, since they all have a value higher than zero and the highest value of NPV corresponds to the tapanco with wooden poles, which is the one of greatest use, equally expensive than using concrete poles; meanwhile, the use of recycles plastic poles is up to 12% less expensive according to the evaluation period of 10 years.

The benefit/cost ratio helps with decision making regarding the investment, for which both the total income and the benefits of the project are considered, and they are divided by the total costs (Table 5). It can be observed that the income is higher than the costs in the ten-year period of the project, and it is defined as profitable.

According to the results from the financial run (10 years), it is established that for the plastic poles, for each peso invested in the production costs, there is a profit of $1.04; meanwhile, for the concrete and the wooden poles, it is only $0.80 and $0.81, respectively.

The profitability of an investment implies considering the interest rate of the business, and the Internal Rate of Return should be calculated; therefore, with the data processed from the actualized income and costs, the benefits are estimated, from which the initial

Table 5. Benefit-Cost ratio (B/C) for three materials for the poles.

Poles	Total income ($)	Total cost ($)	B/C	Results	Decision
Wood	8,054,069.46	4,452,879.10	1.81	B/C>1	Accepted
Concrete	8,054,069.46	4,462,235.39	1.80	B/C>1	Accepted
Plastic	8,054,069.46	3,931,945.05	2.04	B/C>1	Accepted

investment is discounted, and thus the net cash flows are obtained from year one to year 10 for each of the scenarios in this study.

Once the cash flows are determined, the IRR value is calculated (Table 6). The fixed investment rate provided by Banxico (2023) was 6.8% at the time of determining the results presented. It is evident that chayote production is profitable with the traditional exploitation system, using wood materials for the construction of tapancos. However, it is a reality that this resource needs more attention in its management and use.

Table 6. Value of Internal Rate of Return (IRR) for each pergola assembled.

Type of pergola	Value of IRR (%)
Wood poles	86
Concrete poles	58
Recycled plastic poles	60

Likewise, the proposal of this study to use recycled plastic poles is viable because the value of this financial indicator is as competitive as the concrete poles. The existing difference from the Internal Rate of Return (IRR) regarding the traditional system with wooden poles does not mean that it should not be considered. The project of using the recycled plastic poles is profitable since it provides an interest rate that is nearly 9 times the value established by Banxico as fixed interest rate. Rather than a value or decision from the economic point of view, the importance of the contribution to a friendlier management with the environment for the preservation of natural resources that are exploited to be used in the construction of traditional tapancos with wood materials should be considered.

CONCLUSIONS

The recycled plastic poles are a technically and economically viable option for the construction of tapancos. These poles kept their stability during the six months of evaluation in the experimental module, where the biomass weight reached a continuous average of 200 kg. The poles did not exhibit fractures or deformities that would affect their functionality. This alternative must be disseminated for its acceptance and adoption by producers of the different chayote-producing zones. Regarding the financial feasibility (analysis at 10 years), the financial indicators of the benefit/cost ratio (B/C), as well as the internal rate of return (IRR) illustrate the viability of the proposal, since for each Mexican peso invested, $1.04 are generated in comparison to the $0.81 for the case of the wooden trunk poles. Concerning the internal rate of return, the value is almost 9 times the fixed interest rate established, which means that it is a totally profitable project considering the investment and that it is risk-free.

AKCNKOWLEDGEMENTS

I wish to thank the Consejo Nacional de Ciencia y Tecnología (CONACYT) for granting me a scholarship to carry out graduate studies; and Colegio de Posgraduados Campus Córdoba, for providing me with the opportunity of academic growth.

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Evaluation of the hydrological response of a forest small catchment of Lake Patzcuaro, Michoacán

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ABSTRACT

Objective: To analyze the hydrological response of the Malacate forest small catchment, based on rainfall and runoff event variables recorded between 2013 and 2016.
Design/Methodology/Approach: Rainfall was recorded with digital rain gauges (0.2 mm accuracy), at a gauging station in the outlet of the small catchment. It was equipped with a long-throat gauger and an ultrasonic sensor. Runoff was recorded every 3 minutes. Linear and multiple regression analysis were used to study 11 characteristic rainfall and runoff variables. The aim was to identify those characteristics that influence the hydrological response of the small catchment.
Results: The highest correlation with surface runoff (R^2=0.7657) was recorded by the following variables: total rainfall of the event (TRE), previous 2-day rainfall (P2DR), rainfall intensity in 30 minutes (RI30), and runoff time (ROT). Meanwhile, the correlation between the measured and estimated runoffs recorded R^2=0.8558 (1:1 ratio). TRE is the most influential variable in the behavior of surface runoff (R^2=0.6706).
Study Limitations/Implications: The role of rainfall variables and their influence in the runoff and hydrological response of the forest small catchment must be subjected to further studies.
Findings/Conclusions: The results allow a first approximation to the hydrological response of a forest small catchment. In conclusion, the hydrological response depends on the moisture level of the small catchment and the characteristics of the rainfall.

Keywords: runoff, rainfall, hydrological response, forest small catchment, regression analysis.

Citation: Rivera-Ruiz, P., Rubiños-Panta, J. E., Fernández-Reynoso, D. S., Mendoza-Pérez, C., Prado-Hernández, J. V., Khalil-Gardezi, A., & Dehesa-Carrasco, U. (2025). Evaluation of the hydrological response of a forest small catchment of Lake Patzcuaro, Michoacán. *Agro Productividad*. https://doi.org/10.32854/ar11yc97

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

Received: August 13, 2024.
Accepted: May 11, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 55-63.

INTRODUCTION

Currently, experimental basins are of interest for hydrological researches. These studies evaluate the effects and changes of soil use, as well as management actions implemented in the catchment. They can also relate and arrange the influence of the different factors

that take part in the hydrological cycle. Hydrological studies are frequently based on experimental basins, where inflow (rainfall) and outflow (water flow) can be quantified. In addition, the effect of different types of plant covers and the relationship between inflows and outflows can be evaluated in experimental basins (García and Gallart, 1997).

Brown *et al.* (2005) and Van Dijk *et al.* (2012) studied the hydrological response to forest change in <100 km^2 basins and provided a deep insight about the impact of forest change of annual runoffs in small catchments. For their part, Beck *et al.* (2013) and Zhang *et al.* (2015) concluded in their researches about small catchments that deforestation, forest exploitation, urbanization, soil cover change, forest fires, and pests can increase annual runoff, while afforestation or the maintenance or improvement of forest covers has a positive impact on the flow of streams.

Other studies about small catchments reported that great variations in the hydrological response to forest change and in forest cover throughout time are a consequence of factors such as forest type, typology, climate, hydrological regimes, soil, geology, and landscape morphology (Zhang and Wei, 2014). In most cases, the inconsistent hydrological responses are just the consequences of the complex processes of basins and the heterogeneity of the landscape, weather, and geology (Stednick, 1996; Vose *et al.*, 2011).

Therefore, the objective of this study was to evaluate the hydrological behavior of the Malacate forest small catchment, to establish the hydrological response to the intensity of rainfall events and to determine factors that provide a better explanation for the runoff response. This study has a double purpose: (i) to provide data about the hydrological functioning of a mountain area where plant cover has not been removed; and (ii) to provide data about a potential future trend of many basins of the Trans-Mexican Volcanic Belt, in order to strengthen the importance of maintaining forest covers in the basins of the mountains in Mexico and all over the world.

MATERIALS AND METHODS

Study area

The Malacate small catchment is located in the basin of Lake Patzcuaro and has an area of 149.24 ha. The soil use is divided as follows: 74.13% forest (pine-oak), 16.64% scrubland, 5.53% areas without plants, 2.51% eroded pasture-forest, and 1.19% rainfed agricultural lands (Figure 1). Small catchment tributaries directly discharge in the lake. It has a 576-m elevation gradient between the watershed (2,651 m) and the gauging station (2,075 m).

Instruments and equipment

The hydrological response of the outflow of a small catchment should be studied through the continuous recording of the runoffs. Hydrographs are developed using water expenditure. They show the constant behavior of water level rises and can be used to report the instant changes of water flow throughout time. These phenomena are the result of the different hydrological processes in the small catchment.

In order to carry out a comparative analysis of the hydrological response of the Malacate small catchment, a temporal scale (per event or water level rise) was used to

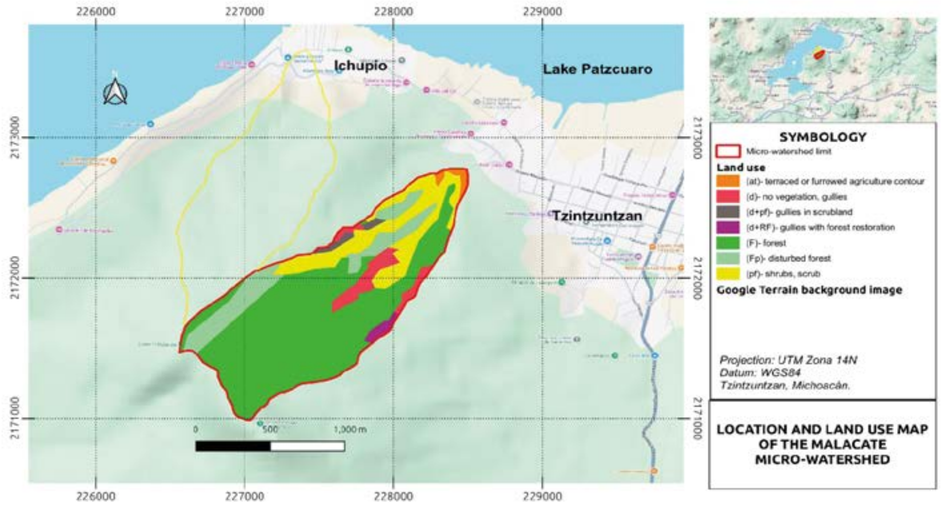

Figure 1. Location and soil use of the Malacate small catchment, Tzintzuntzan, Michoacán.

obtain the most reliable data. Water level rise is usually defined as a sudden increase in the water flow of a riverbed —*i.e.*, it is a response to a high volume of rainfall in a catchment basin (Nadal-Romero *et al.*, 2010). The bed of the Malacate small catchment is intermittent and it only has runoffs during the raining season. Consequently, no base-runoff was recorded.

A gauging station was built in the lower part of the Malacate small catchment to monitor its activity during four years (2013-2016). The following measurement equipment and instruments installed in the small catchment were used to gather data from both the inflow (rainfall) and the outflow (water flow):

1) A HoBo rain gauge with a rocker connected to a data logger to measure rainfall (0.2 mm accuracy).
2) A long-throat gauger (Figure 2a), equipped with an ultrasonic sensor (Figure 2b). This technology was developed by the Instituto Mexicano de Tecnología del Agua (IMTA) and records water level every 3 minutes and measures it as water expenditure.

Figure 2. long-throat gauge (a) and ultrasonic sensor equipment (b) used to record the runoffs.

Variable recording and data analysis

Thirty major superficial runoff events with a maximum water flow were selected to analyze the hydrological response of the small catchment from 2013 to 2016. Events 1-4, 5-9, 10-21, and 22-30 took place in 2013, 2014, 2015, and 2016, respectively. Serrano *et al.* (2005) maintain that a hydrograph by itself is not enough to provide information about the runoffs and the processes that take part in their creation. Consequently, once the 30 events were established, the following variables were analyzed: total rainfall of the event (TRE; mm); previous 2-day rainfall (P2DR; mm); rainfall time (RT; min); rainfall intensity in 30 minutes (RI30; mm/h); runoff (RO; mm); runoff time (ROT; min); maximum runoff (MAXRO; l/s); average runoff (ARO; l/s); runoff response time (ROREST, min), from the beginning of the rainfall to the beginning of the runoff; discharge time (DIST; min), from the end of the rainfall to the end of the runoff; and time to peak runoff (TPRO; min), from the beginning of the rainfall to the peak of the runoff.

A database was developed to create a correlation matrix, in order to determine the relation between the variables. Meanwhile, a multiple regression analysis was conducted to identify and explain the relationship between the different variables and their influence in the hydrological response, as well as to appropriately evaluate the impact of the different parameters. A graphical analysis and the coefficient of determination (R^2) were used for this purpose. These procedures were used to identify which variables have the best correlation to explain their influence on the superficial runoff of the Malacate small catchment. In addition, rainfall events were grouped in three ranges based on the total rainfall of the event, to analyze the average behavior of the variables with higher correlation with the rainfall.

RESULTS AND DISCUSSION

The runoff events recorded during the four-year evaluation were very diverse, ranging from 3.8 to 44.4 mm. Overall, the hydrological response was depended on the rainfall conditions, including the amount of rainfall in previous days. Data from 2 to 5 days before the runoff response were analyzed. The results indicated that the rainfalls of 2 previous days had a better response. This situation is a consequence of the shallow luvisols with clay present in the small catchment.

Figures 3 and 4 show the normalization of the values of the analyzed variables. Each of their values was divided between the maximum value for the 30-event data set, with the following results: 44.4 mm (TRE), 32.2 mm (P2DR), 292.2 min (RT), 86.0 mm/h (RI30), 11.3 mm (RO), 567.0 min (ROT), 2,432.3 l/s (MAXRO), 486.4 l/s (ARO), 141.5 min (ROREST), 464.1 min (DIST), and 159.5 min (TPRO).

The first rains of the rainy season do not usually have a hydrological response, likely because the soil of the small catchment is dry, after several months without rain (November-April). However, relatively intense rains will still cause runoffs.

The year-long relationship between rainfall and runoff is conditioned by constant rainfall events on previous days; therefore, prior moisture in the soil likely plays a major role in the hydrological response of the small catchment. Serrano *et al.* (2005),

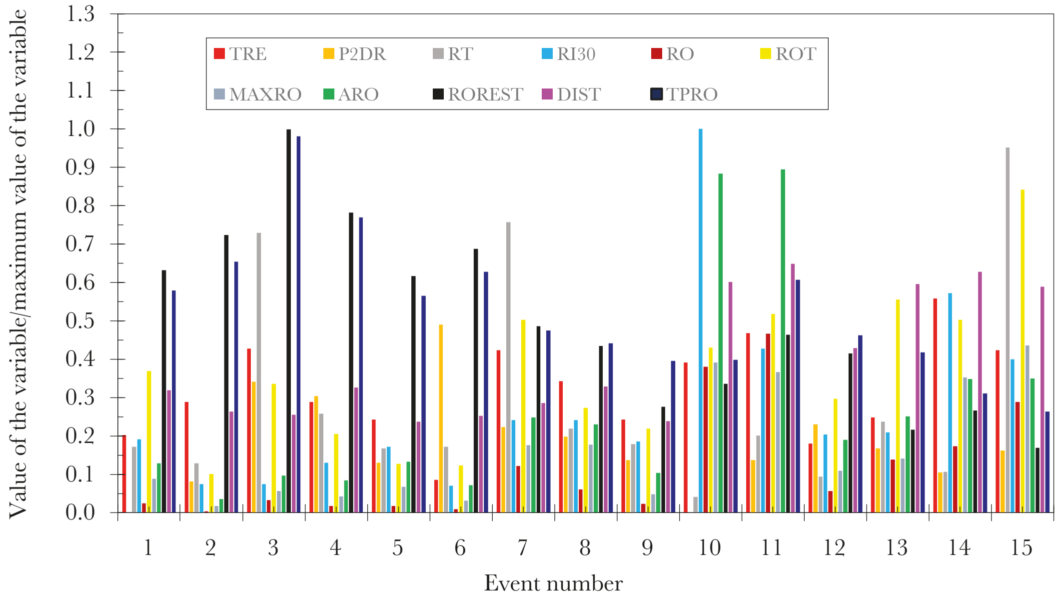

Figure 3. Normalization of the values of the rainfall and runoff characteristics for events 1 to 15.

TRE: total rainfall of the event; P2DR: precipitation for 2-day rainfall; RT: rainfall time; RI30: rainfall intensity in 30 minutes; RO: runoff; ROT: runoff time; MAXRO: maximum runoff; ARO: average runoff; ROREST: runoff response time; DIST: discharge time; TPRO: time to peak runoff.

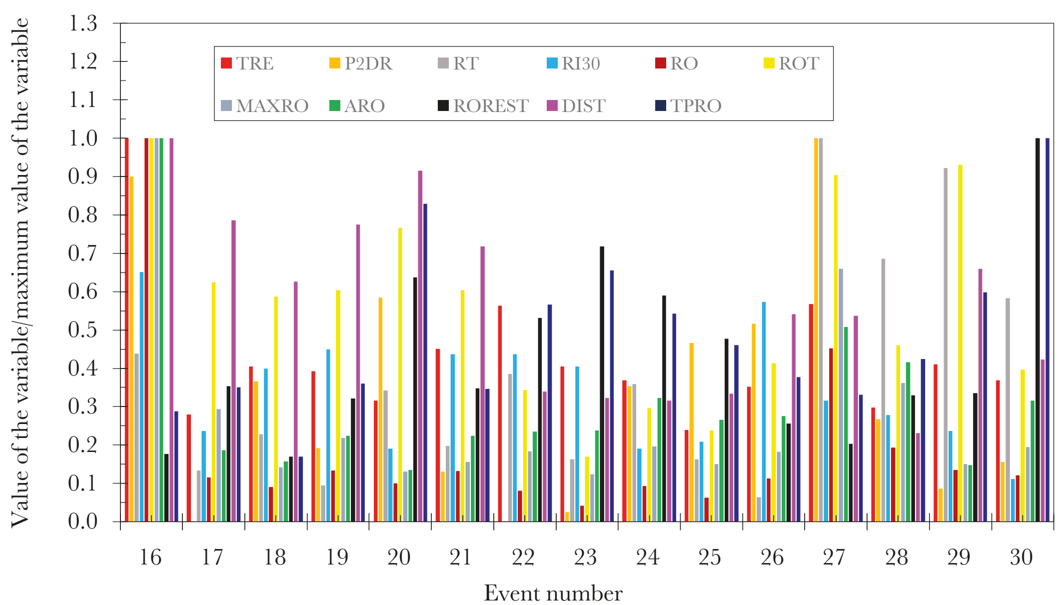

Figure 4. Normalization of the values of the rainfall and runoff characteristics for events 16 to 30.

TRE: total rainfall of the event; P2DR: precipitation for 2-day rainfall; RT: rainfall time; RI30: rainfall intensity in 30 minutes; RO: runoff; ROT: runoff time; MAXRO: maximum runoff; ARO: average runoff; ROREST: runoff response time; DIST: discharge time; TPRO: time to peak runoff.

Nadal-Romero *et al.* (2008), Latron and Gallart (2008), Bart and Hope (2010), and Penna *et al.* (2011) reported similar trends. Additionally, the role of rainfall intensity is fundamental for the hydrological response in the Malacate small catchment, with different intensities and similar rainfall volume, but with different prior moisture conditions, resulting from previous rainfalls (Ares, 2014; Nadal-Romero *et al.*, 2010; Nunes *et al.*, 2011).

Zabaleta *et al.* (2007), Latron and Gallart (2008), Nunes *et al.* (2011), and Yao *et al.* (2021) have reported that even soils with a dry hydrological condition can record greater runoff, as a result of the more intense rainfall.

The results provide an overview of the complex operation of the hydrological response of the small catchment: as rainfall increases, the runoff does not increase, because similar rainfall values can have very different responses. Therefore, in face of a given volume of rainfall in the small catchment, the hydrological response is conditioned by other factors beyond rainfall itself (Boulet *et al.*, 2021; Ares, 2014).

Runoff usually increases as the rainfall volume increases, although the same total rainfall can also quantify different runoffs. Therefore, the response to this variable is conditioned by other factors, including rainfall intensity and rainfall time —which are in turn dependent on the rainfall volume accumulated in previous days. This phenomenon is reflected in the response time before the runoff starts and is recorded at the outflow of the small catchment, as well as in the discharge time and the time to peak runoff.

In the case of the Malacate small catchment, a higher maximum runoff is expected with a more intense rainfall in a 30-minute period (Nu Fang *et al.*, 2011; Yao *et al.*, 2021). Meanwhile, a greater rainfall intensity decreases the response time —*i.e.*, a shorter time elapses between the beginning of the rainfall and the first runoff at the outflow of the small catchment.

Discharge time is defined as the time that elapses from the moment it stops raining to the moment when the runoff ends. Therefore, with a higher maximum runoff, the time it takes for the runoff to exit the small catchment increases. Meanwhile, a greater maximum runoff rate results in a shorter response time before the runoff exits the area.

The multiple regression analysis identified the variables that have the best correlation and weighed up their influence on runoff: total rainfall of the event (TRE), the previous 2-day rainfall (P2DR), rainfall intensity in 30 minutes (RI30), and runoff time (ROT). The resulting equation has a R^2 of 0.7657 and is set forth as:

$$RO = -2.8725 + 0.1376TRE + 0.0727P2DR + 0.0345RI30 + 0.0032ROT$$

where RO, TRE and $P2DR$ are measured in mm, while $RI30$ and ROT are measured in mm/h and min, respectively.

Figure 5 shows the graphical representation of the results from the multiple regression model, including the measurements for the runoff and their estimations (R^2=0.8558; ratio 1:1). Overall, the multiple regression model underestimates the runoff by 14.42%.

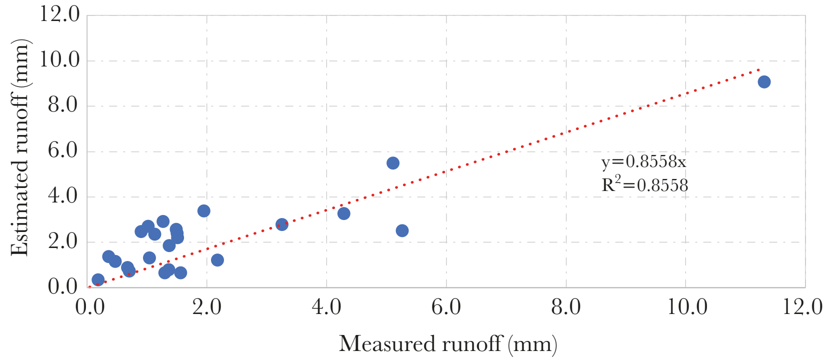

Figure 5. Ratio between the runoff measured and estimated using the multiple regression model on four variables from the Malacate small catchment.

The total rainfall of the event (TRE) by itself is the analyzed variable with the best correlation with the runoff (RO), with an R^2 of 0.6706. When the runoff values are estimated based on a multiple regression analysis, R^2 reaches 0.8608 (Figure 6), proving that the four identified variables are correlated and provide a better explanation of the runoff behavior in the Malacate small catchment. Rodríguez-Blanco *et al.* (2010) and Nu Fang *et al.* (2011) reached similar conclusions. However, just like Nadal-Romero *et al.* (2008), this study concluded that the model is more accurate when it is used to estimate the runoff for >10 mm rainfall events; however, it is inaccurate with lower rainfalls.

Meanwhile, the results were analyzed and the average values for seven variables were correlated with the total rainfall of the event (TRE). Table 3 shows how the events were grouped into three rainfall ranges: 10 to 15 mm, 15 to 20 mm, and 20+ mm.

As the total rainfall of the event (TRE) increases, the following characteristics likewise increase: rainfall intensity in 30 minutes; runoff; maximum and average runoffs; runoff length; and discharge time. Evidently, the runoff response time decreases as rainfall increases.

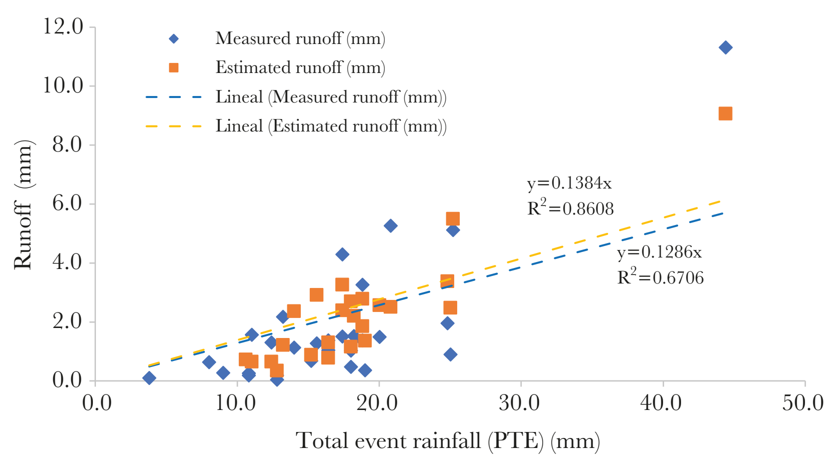

Figure 6. Relation between the measured and estimated runoffs, based on the multiple regression model used to determine the total rainfall of the event in the Malacate small catchment.

Table 3. Average characteristics of the rain and runoff per range of the total rainfall of the events (TRE) recorded from 2013 to 2016 in the Malacate small catchment.

Range of total event rainfall (TRE) (mm)	RI30 (mm/h)	RO (mm)	MAXRO (l/s)	ARO (l/s)	ROT (min)	ROREST (min)	DIST (min)
10 a 15	16.13	0.84	338.47	86.97	207.67	69.33	202.52
15 a 20	31.00	1.52	495.31	141.45	273.08	68.53	221.26
>20	40.73	4.34	1101.51	260.23	366.00	46.92	299.48

RI30: Rainfall intensity in 30 minutes; RO: Runoff; MAXRO: Maximum runoff; ARO: Average runoff; ROT: Runoff time; ROREST: Response time; DIST: Discharge time.

CONCLUSIONS

The data recorded in the Malacate small catchment during four water years have enabled a first approach to its hydrological response and the following conclusions have been reached:

(i) Some rainfall variables play a major role in hydrological response, including the total rainfall of the event, the rainfall of the 2 previous days, and the rainfall intensity in the first 30 minutes.

(ii) In most cases, the catchment can absorb even the most intense rainfall; nevertheless, the higher soil moisture resulting from rains in the previous days will cause surface runoffs —*i.e.*, the hydrological response of the small catchment depends on the previous rainfall, as well as the characteristics of the rainfall.

ACKNOWLEDGMENTS

The authors would like to thank the Instituto Mexicano de Tecnología del Agua for the facilities and opportunities provided for this research and the recording and compilation of data and information about the Malacate forest small catchment, established through internal research projects.

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Colegio de Postgraduados

Biochemical and Functional Characterization of *Guazuma ulmifolia* Lam Biomass: A Novel Source of Fructose and Fiber for Sustainable Applications

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Citation: Martínez-Espinosa, J. C., Macías-Alonso, M., Ramírez-Rizo, M.A., Pablo-Hernández, A., Carrera-Cerritos, R., & Gutiérrez-Chávez, A.J. (2025). Biochemical and Functional Characterization of *Guazuma ulmifolia* Lam Biomass: A Novel Source of Fructose and Fiber for Sustainable Applications. *Agro Productividad*. https://doi.org/10.32854/gqg7wb87

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

Received: August 03, 2024.
Accepted: May 20, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 65-76.

ABSTRACT

Objective: Quantification of phytochemical content of *Guazuma ulmifolia* Lam biomass from Veracruz, Mexico, with the aim of carrying out future biotechnological applications, such as animal feed formulations.

Design/methodology/approach: This work characterized the peel and seed of *G. ulmifolia* L. Fructose and glucose were identified as the primary components in the peel extract using high-performance liquid chromatography. HPLC also revealed traces of unknown components. In addition, combined glucose and fructose were quantified at different extraction times (up to 90 min) and in triplicate using refractometry as a rapid and complementary technique. The seed extract was analyzed using Fourier transform infrared spectroscopy, and the dry peel underwent bromatological evaluation to determine the analytical constituents.

Results: High-performance liquid chromatography results corroborated the presence of fructose and glucose after 90 min of extraction, with retention times of 2.1 and 2.4 minutes, respectively. HPLC analysis revealed a larger area under the chromatogram at a retention time of 2.1 minutes, indicating that fructose (76.2%) was the predominant sugar in the peel extract. The concentration of combined fructose and glucose in the extract obtained was 12.2 %wt at the beginning of the extraction and 36.727 %wt at the end of the extraction (90 minutes). The total soluble saccharide content (fructose and glucose) in the peel was 15.42 %wt. Bromatological analysis of the remaining biomass showed a crude fiber content of 35.4% and a crude protein content of 7.12%

Limitations on study/implications: The seasonal nature of the fruit *G. ulmifolia* L. posed a limitation for its conservation and storage.

Findings/conclusions: These findings suggest that *G. ulmifolia* L. biomass, particularly from dried fruit, is rich in carbohydrates and crude fiber, presenting a potential alternative for livestock fodder and its sugares may serve as an alternative feed for honey bees. Finally, and applying the adjusted mathematical model, the refractometry technique is proposed as a rapid alternative for the measurement of sugars in extracts of *G. ulmifolia* L., avoiding the use of robust techniques.

Keywords: *Guazuma ulmifolia* L., biomass, fructose, analytical constituents.

INTRODUCTION

Recently, the veterinary industry has been looking for food alternatives through biomasses that the environment generates naturally in a cycle of circular economy and

reproduction [1-5], as well as obtaining natural products for human consumption that are free of preservatives and synthetic additives that harm animal and human health in the medium term [6-8]. There are different biomasses that have been used for different applications such as microalgae biomass, which are used to extract biofertilizers and biofuels [9-11]. Also, due to the problem of large accumulations of *Sargassum* spp. biomass in different parts of the world [12-13], its use has been studied to generate ecological filters for the treatment of contaminated water [14]. Additionally, it has been shown to be a natural source of alginates, cosmetics, biopolymers and bioplastics [15-18]. In another case, *Agave sisalana* biomass is being used to generate green plasticizers, fructans, and even biofuels [19-21]. Globally, we can find many more sources of biomass for the generation of products of daily use in humans and the veterinary industry that have recently become of commercial interest [22].

In Mexico there are several sources of organic biomass, including some that have been mentioned previously, such as agave, coconut, and the large quantities of sargassum that reach the coasts of the Yucatan peninsula. Another important source is the *G. ulmifolia* L. tree, which has been used as a source of fodder for cattle and goats mainly [24-25]. In addition, this tree has beneficial health properties, for example, it has been reported to reduce oxidative stress, contains anti-diabetes properties, and its fruit is rich in dietary fiber and phenolic compounds [26-30]. The dried fruit of the *G. ulmifolia* L. tree has been used in traditional medicine, treating some diseases such as diarrhea, elephantitis, control and treatment of hemorrhage and uterine pain [31]. Despite the above-mentioned properties, no attention has been given to expanding its use in animal food formulations at present. Although there is information of phytocomponents of *G. ulmifolia* L. from other countries [32], there is limited information on the phytochemical composition of the native species of Veracruz, Mexico. For this reason, a phytochemical characterization of the shell and the seed is shown in the present work.

In addition, the glucose and fructose content of fruits is a determinant key to the sweetness of these fruits. Accurate monitoring of these sugars allows a predictive evaluation of the quality of vegetable products. High-performance liquid chromatography (HPLC) coupled with a high-performance mass spectrometer is commonly employed to measure glucose and fructose in different fruit extracts of *G. ulmifolia* L. [33-34]. However, the availability of HPLC-MS equipment in Veracruz is not very high for performing daily monitoring. For this reason, refractometry measurement of sugars in aqueous extracts of the seed shell is included, as a rapid and cheap methodology for the determination of glucose and fructose.

MATERIALS AND METHODS

Collection, treatment and seed separation of *G. ulmifolia* L. fruit

G. ulmifolia L. dried fruit was collected from two different regions of Mexico, from the coast of the State of Chiapas and the Huasteca region of the State of Veracruz. Disinfection of the dried fruit was carried out by washing using neutral soap and a 10% sodium hypochlorite solution for 10 minutes. Drying was carried out for 36 hours at a temperature of 60 °C in a conventional oven (Memmert UN110). Subsequently, the seed was extracted from each fruit and stored in a Falcon tube at room temperature, as well as the fruit peel in sterile plastic containers for further processing.

Obtaining the peel and seed extracts

To obtain the peel extract, 100 g of the disinfected dried fruit peel was placed in a 1000 ml beaker. 500 ml of distilled water was added, and the heating process was started until it reached 60 °C, maintaining the temperature for 60 minutes. The resulting extract was brought to boiling point by maintaining the temperature for 90 minutes. During this boiling process, seven samples were extracted at different times (0, 20, 30, 40, 50, 70 and 90 minutes) and were stored in Eppendorf tubes at 4 °C. The extraction process was repeated three times for the same fruit peel to ensure complete extraction of soluble solids. The peel of the fruit was removed from the liquid solution and taken to a drying process for 36 hours at 60 °C for the determination of the total soluble solids of the sample by weight difference.

The seed extract was carried out in a 1:10 ratio (weight: volume) of seed and distilled water, whereby 10 g of seed was placed in 100 mL of distilled water in a beaker and heated for one hour at 60 °C until a viscous consistency was reached. The extracted mucilage was separated from the seed and stored in 250 mL glass containers and stored refrigerated at 4 °C.

Lyophilization of seed extract and FTIR characterization

The mucilage was freeze-dried using a 4.5 L benchtop freeze-drying system (LABCONCO FreeZone 4.5 Liter Benchtop Freeze Dry System). Prior to lyophilization, the mucilage was flash frozen (−81 °C) for 24 hours. Subsequently, the sample was subjected to the freeze-drying system at a temperature of −47 °C and a pressure of 0.25 ± 0.1 mbar for 72 hours. Finally, the lyophilized sample was placed in Eppendorf cups. 1 mg of freeze-dried sample was placed on the sample holder for analysis by FTIR. The spectroscopy system (Thermo Scientific Nicolet iS10 SMART iTR) was operated at a resolution of 8 cm^{-1} and acquisition range of 400-4000 cm^{-1}.

Characterization of the peel extract by HPLC (LC/MS spectra of sugars)

Both extracts from *G. ulmifolia* L. fruits and reactive grade standards of glucose and fructose were injected into the UHPLC (Dionex Ultimate 3000) equipped with a ACQUITY UPLC BEH Amide 1,7 micrometer column (2.1×100 mm). The system was operated with acetonitrile and ammonium hydroxide solution (0.1% NH_4OH) with a volume ratio of 75/25. The samples were diluted in an acetonitrile/water solution (1/1) before being injected.

Obtaining the calibration curve and characterization of the peel extract by refractometer

Six dilutions (w/w) were prepared with distilled water of pharmaceutical secondary standard (fructose, Sigma Aldrich, CAS 57-48-7) to obtain the calibration curve. Table 1 shows the dilution ratios for the different concentrations used. Refractometry measurements were carried out in triplicate using a Brix Digital 0-90% digital refractometer (Brixometer, Aosihui brand) using 80 μL of the different dilutions of fructose as shown in Table 1. Dispersion analysis of the raw data was performed using OriginLab v 8.0 software. Final concentration of soluble carbohydrates was calculated by using the Brix measurements and the total mass of the peel extract.

The extracts obtained from the fruit of *G. ulmifolia* L. at 0, 20, 30, 40, 50, 70 and 90 minutes of the process were evaluated by the refractometer and the carbohydrates concentration was calculated by applying the mathematical model adjusted from the calibration curve and the total volume of the peel extract (42 g).

Bromatological analysis of *G. ulmifolia* L. peel flour

For the bromatological analysis, the *G. ulmifolia* L. fruit peel was ground (Insely 1000A mill) to a flour consistency. 500 g of the flour were placed in a completely sealed plastic container and sent to the certified external laboratory (EuroNutec Laboratory). The parameters analyzed were: ash (AOAC method 942.05), nitrogen free extract (FAO manual difference), crude fiber (method 06AI-75-025), fat ethereal extract (method 06AI-75-048, official AOAC 2003.05), moisture (method 06AI-75-049, NMX-F-083-1986), crude protein by Dumas (method ISO_16634-1_2008).

RESULTS AND DISCUSSION

Characteristics of the fruit of *G. ulmifolia* L.

G. ulmifolia L. fruit has five cavities, where the small seeds are located. The fruit has an average length of 2.5 cm and an approximately 1 cm width. A fruit contains an average of 50 seeds with light brown shades and whose average dimensions are 0.5 cm long and 0.3 cm wide (Figure 1).

The peel presents a unique and very characteristic aroma of some type of floral honey or even sugar with some characteristic flavoring. The biochemical properties of the dried fruit (shell and seed) are of great importance for future research that will provide relevant information for the development of alternative biotechnological applications.

Characterization of the seed extract by FTIR spectroscopy

The FTIR spectrum presented in Figure 2 corresponds to the mucilage extracted from the seed of *G. ulmifolia* L. It shows a large and broad peak centered at 3292 cm^{-1} that is due

Table 1. Relationship of concentrations used for calibration curve generation. Abbreviations: F, Fructose; DW, Distilled Water; C, Concentration.

F (g)	DW (g)	C (%wt)
0.250	0.250	50
0.250	0.500	33.3
0.250	0.875	22.3
0.250	1.460	14.7
0.250	2.460	9.2
0.250	4.710	5

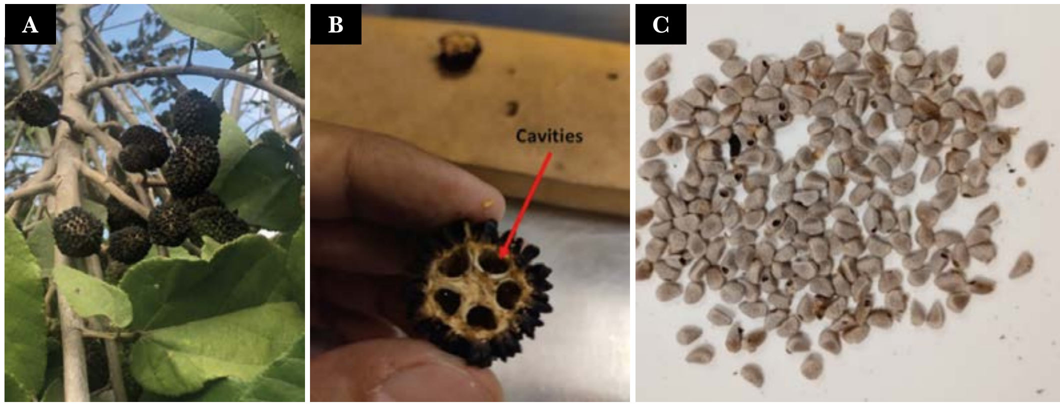

Figure 1. *Guazuma ulmifolia* L. fruit A) Representative image of *G. ulmifolia* L. tree with dried fruit. B) Cross section of the fruit where the five cavities of the fruit are observed. C) Seeds of *G. ulmifolia* L.

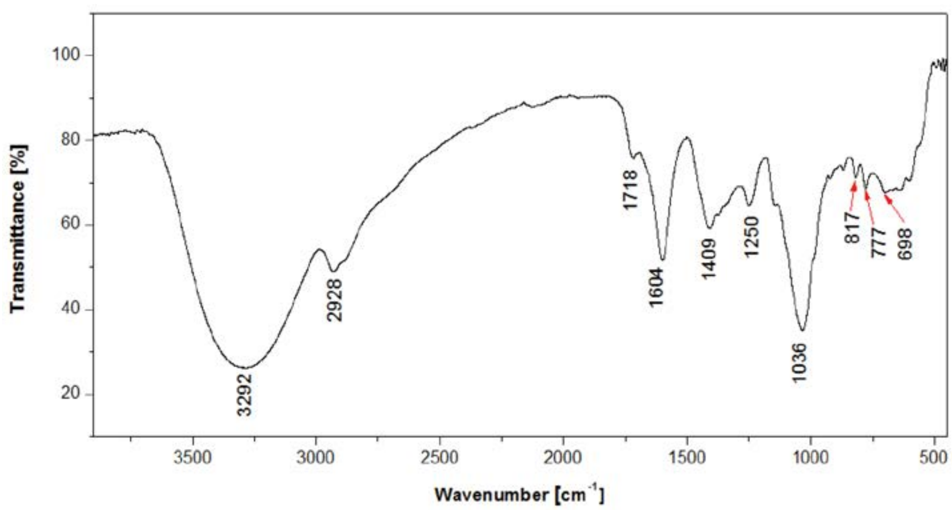

Figure 2. FTIR spectra of freeze-dried mucilage extracted from *G. ulmifolia* L. seed.

to stretching of the O-H bond. The peaks centered at 2928 and 1409 cm^{-1} were assigned to elongation and bending motions of the C-H bond, respectively. The signal located at 1250 cm^{-1} was associated with C-O-C bond elongation from an ester group, while the neighboring peak located at 1036 cm^{-1} was associated with elongation of C-O bonds of alcohols. The vibrations described so far strongly suggest the presence of polysaccharides in high concentration. Additionally, the vibrations located at 1718 and 1604 cm^{-1} were associated with bond stretching. C=O and C=C, respectively. Ramírez and collaborators reported a high concentration of polysaccharides and fiber in the seed extract of *G. ulmifolia* L. [35], considering these results, we could associate the vibrations to aromatic rings and ketone groups present in the lignin.

Characterization of peel extract by HPLC (LC/MS spectra of sugars)

Glucose and fructose standards exhibited molecular ions at m/z 179, indicating the presence of the ions [glucose−H]− and [fructose−H]− (Figure 3). [36]

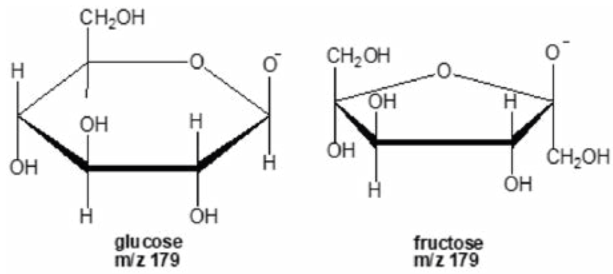

Figure 3. Anions [glucose−H] − and [fructose−H]− ions.

Figure 4A shows chromatogram of the fructose standard and chromatogram of the glucose standard (Figure 4B), with retention times of 2.1 min and 2.4 min, respectively. Chromatogram C, corresponding to the *G. ulmifolia* L. fruit extract, displays two clear peaks with retention times of 2.1 min and 2.4 min, with m/z values of 179, corresponding to fructose and glucose, respectively (see supplementary information). Instinctively, the area under the curve of the chromatogram indicates that the fructose content in the extract of *G. ulmifolia* L. is the highest. Interestingly, the chromatogram of *G. umifolia* L. fruit extract presented two additional peaks centered at 0.8 and 4 min. The small area of these peaks indicates that their concentration was very small: therefore, almost all the soluble carbohydrates consisted of fructose and glucose (76.2 and 23.8 %wt, respectively) in the present study.

Characterization of the extract (glucose plus fructose) of the peel by refractometry

The Brix degrees allowed to measure the total number of soluble solids present in the *G. ulmifolia* L. fruit extract. Considering that HPLC analysis showed that almost all the weight of soluble solids in the extract consisted of fructose and glucose (76.2 and 23.8%), in

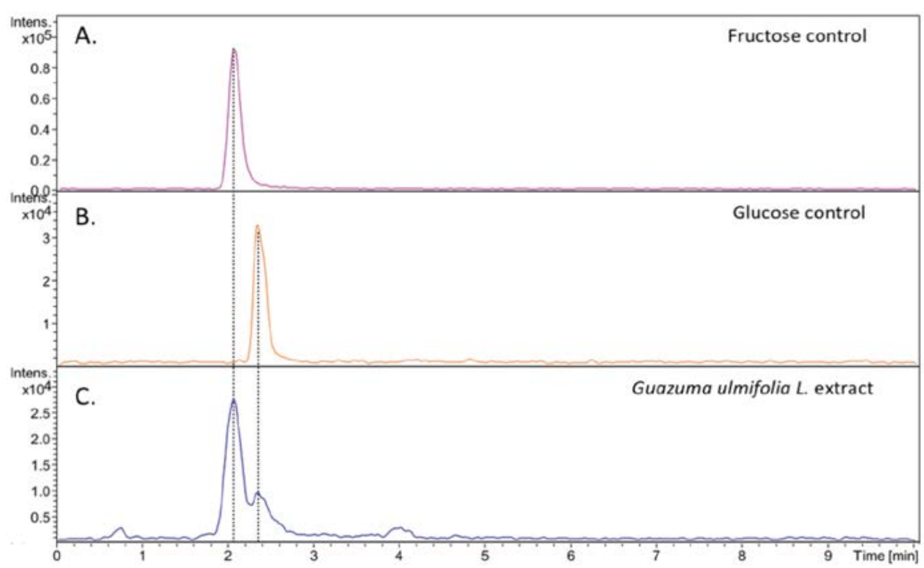

Figure 4. Qualitative analysis of chromatograms. A. Chromatogram of fructose control, B. Chromatogram of glucose control, C. *G. ulmifolia* L. extract chromatogram.

the present section it is evaluated the refractometry as a quick technique to determine the total carbohydrates (glucose and fructose) by using fructose as calibration sugar. This sugar content must be quickly determined for a potential large scale application, for instance, in the area of beekeeping, and food industry. The calibration curve was obtained by using a digital refractometer with the different standard fructose concentrations as defined in Table 1. For each concentration Brix degrees were determined, obtaining a calibration curve with a linear behavior with an R^2=0.967 (Figure 5A).

The following equation (equation 1) determines the concentration (C) of fructose, with BG being the Brix degrees for each concentration evaluated.

$$C = 1.550(BG) - 2.023 \quad (1)$$

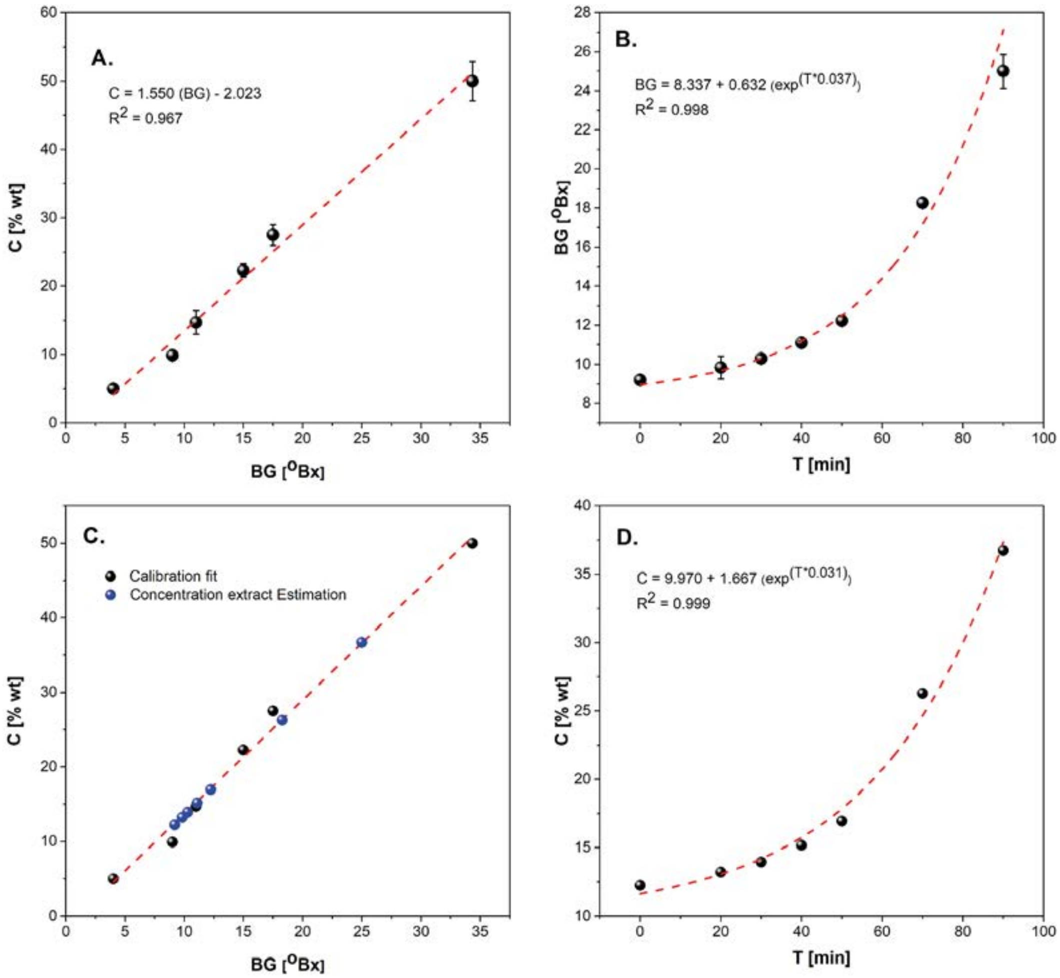

Figure 5. Brix measurement and determination of fructose concentration. A. Calibration curve with an exponential regression obtained from the standard fructose standard, B. Exponential behavior curve during the extraction process. Exposure of *G. ulmifolia* L. extract at different times after its boiling point, C. Concentration obtained from each sample of *G. ulmifolia* L. extract, compared with the calibration curve obtained from the standard, D. Estimation of fructose and glucose concentration during the extraction process as a function of time.

On the other hand, the *G. ulmifolia* L. fruit extract was heated until it reached boiling point. Afterwards, samples were obtained at 0, 20, 30, 40, 50, 70, and 90 minutes to evaluate the degrees °Bx. The behavior of the BG during the extraction process as a function of time (T) reflected an exponential increase with an $R^2=0.998$ (equation 2, Figure 5B)

$$BG = 8.337 + 0.632\left(\exp^{T\times 0.037}\right) \tag{2}$$

Figure 5C shows the comparison between the concentrations used for the calibration curve and the results of fructose concentration (C) obtained at different times of the extraction process. These results are summarized in Table 2. Using equations (1) and (2) it was possible to estimate the behavior of fructose and glucose concentration as a function of time during the extraction process (Figure 5D) with an $R^2=0.999$. The concentration increases exponentially due to the amount of water that evaporates during the extraction process. Applying the calibration function obtained in Figure 5A (equation 2), a determination of the fructose plus fructose concentration was made from the Brix degrees obtained in each sample obtained from the extract at different times. According to the time range (T) exposed the extract to boiling point, the glucose plus fructose concentrations for the *G. ulmifolia* L. extract ranged from 12.23 %wt (T=0 min, BG=9.200) to reach a viscous solution of 36.727 %wt (T=90 min, BG=25).

The total concentration of soluble saccharides in the *G. ulmifolia* L. peer calculated from the total volume of extract and the glucose plus fructose concentration is 15.42% wt. If the results from HPLC are considered, a 11.75 %wt of fructose and 3.7%wt of glucose are the total mass concentration in the peel of the fruit. Pereira *et al.,* reported a total concentration of carbohydrates of mono and disaccharides of 25.94 % wt for *G. ulmifolia* L. pulp and seed extract [30]. The source of the extract could explain the difference between these results, as the peel seed was used in the present study.

Although the calibration curve was obtained with fructose, the present results showed that refractometry is not selective for this sugar and can be used for a quick and cheap determination of combined glucose and fructose concentration in *G. ulmifolia* L. aqueous

Table 2. Fructose concentrations used to obtain the calibration curve and determination of fructose plus fructose concentration during different times in the extraction process. Abbreviations: C, Concentration; BG, Degrees Brix; T, Time.

Calibration curve			***G. ulmifolia* L. extract**			
C (%wt)	**BG (°Brix)**	σ	**T**	**C (%wt)**	**BG (°Brix)**	σ
50	35.333	0.012	0	12.23	9.200	0.264
33.3	17.000	0.014	20	13.22	9.833	0.577
22.3	15.000	0.035	30	13.94	10.300	0.173
14.7	11.000	0.020	40	15.182	11.100	0.100
9.2	9.000	0.030	50	16.94	12.233	0.230
5	4.000	0.057	70	26.29	18.267	0.251
			90	36.727	25.000	0.866

extracts. However, validation measurements with other techniques must be performed in future work.

Bromatology analysis of *G. ulmifolia* L. peel flour

The use of the total biomass of the fruit can be an alternative natural source of complementary fiber for cattle, pigs, horses, among others. For this, the bromatological analysis of *G. ulmifolia* L. meal is essential for the determination of analytical constituents (ash, nitrogen free extract, crude fiber, fat, ethereal extract, moisture and crude protein) that will allow to generate a balance in the formulations that integrate the *G. ulmifolia* L. meal. The samples were analyzed by an external laboratory authorized by SENASICA (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria), which is a regulatory body that promotes the application and certification of food contamination risk reduction systems and their agri-food quality, in order to facilitate application and commercialization. Table 3 shows the bromatology results and the method applied.

In the resulting analytical constituents shown in Table 3, it can be observed that it has a high content of nitrogen-free extract (47.81%), which are soluble carbohydrates (starches, sugars, organic acids, pectins and mucilages) and can be easily digested. Another parameter with high content was crude fiber (35.4%), which is non-nitrogenous organic substances. Crude protein or crude protein (7.12%) is normally one of the constituents of special importance in the veterinary industry, since it has a significant impact mainly in the growth and production stages. Finally, in a small fraction of the biomass is found the ethereal extract fat (2.72%).

Table 3. Analytical constituents determined from the peel of *G. ulmifolia* L. in the form of flour through a bromatological analysis.

Constituent	Method	Results (%)
Ash	AOAC 942.05	2.76
Nitrogen free extract	FAO manual difference	47.81
Crude fiber	06AI-75-025	35.40
Fat ethereal extract	06AI-75-048, AOAC 2003.05	2.72
Moisture	06AI-75-049, NMX-F-083-1986	4.19
Crude protein	ISO_16634-1_2008	7.12

CONCLUSIONS

The extract from the peel of *G. ulmifolia* L. reveals the presence of both fructose and glucose, with fructose being more abundant (almost 3 times than glucose). This was confirmed through High-Performance Liquid Chromatography (HPLC) coupled with high-resolution mass spectrometry, which provided accurate monitoring and identification of the sugars based on their retention times and molecular ions. The concentration of total soluble saccharides (fructose and glucose) in the peel extract was determined using a digital refractometer. The saccharides concentration is a function of extraction time. The maximum saccharide concentration (36.727) was achieved at longer extraction time

(90 min). The total content of soluble glucose and fructose in the peel was 15.42 %wt. The freeze-dried seed extract was analyzed by FTIR spectroscopy, where the presence of vibrations corresponding to polysaccharide and lignin functional groups were verified. Finally, the biomass of *G. ulmifolia* L. has a high content of nitrogen free extract (47.8%), crude fiber (35.40%) and crude protein (7.12%), which makes it a source of biomass that can be balanced to integrated rations for animals.

ACKNOWLEDGMENTS

The authors thank the Secretary of Research and Postgraduate Studies of the National Polytechnic Institute for the financial support with the SIP 20231512 project. We also thank the CONAHCYT for the financial support for a sabbatical stay scholarship applied for this project.

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AGRO PRODUCTIVIDAD

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Family farming in Mexico: a review of its evolution and exclusion over time

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ABSTRACT

Objective. This paper aims to analyze the evolution and various forms of exclusion experienced by Mexican family farming over time.

Results. Family farming originated with the agrarian reform that followed the Porfiriato era. However, the 2007-2008 global food crisis catalyzed renewed attention to this production system as a means of providing food, employment, and income to the poorest populations, particularly in rural areas. Its development and marginalization have been shaped by the so-called agro-export phase, which was integrated into the neoliberal export-oriented model that entered Latin America between the 1980s and 1990s. Despite this, over time and as family farming has come to be recognized as a cornerstone of food security for rural households it has gained relevance and has increasingly attracted the attention of stakeholders in Mexico. The trajectory of family farming in the country is closely linked to both national and international reforms in public and trade policy, as well as to Mexico's bilateral relationship with the United States.

Conclusion. Current rural development policies fail to explicitly and adequately incorporate family farming. Nevertheless, this sector represents a key opportunity for reducing poverty and hunger in marginalized regions of Mexico.

Keywords: Subsistence agriculture, rural development, agrarian reform, public policies.

Citation: Vázquez-Alfaro, M., Gómez-Carreto, T., & Saavedra-Jiménez, L. A. (2025). Family farming in Mexico: a review of its evolution and exclusion over time. *Agro Productividad*. https://doi.org/10.32854/jtcdw442

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

Received: August 05, 2024.
Accepted: May 11, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 77-83.

INTRODUCTION

Family Farming (FF) emerges as a vital mechanism to support the livelihoods of rural and Indigenous households, contributing to the eradication of hunger and poverty, the achievement of food sovereignty, and the sustainable management of natural resources (Fondo Internacional de Desarrollo Agrícola [FIDA], 2014). In Mexico, FF comprises 4.33 million Rural Economic Units (REUs), with agriculture as the predominant activity. Key crops include maize, beans, wheat, sorghum, coffee, sugarcane, oranges, and alfalfa (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación [SAGARPA]; Organización de las Naciones Unidas para la Alimentación y la Agricultura [FAO], 2012). Despite its significance, family farming operations face socioeconomic

constraints and limited access to productive resources (Comisión Económica para América Latina y el Caribe; Organización de las Naciones Unidas para la Alimentación y la Agricultura; Instituto Interamericano de Cooperación para la Agricultura, 2017). In this regard, it is essential to conduct research aimed at promoting the sustainability of FF and moving toward ensuring food security in Mexico. It is therefore necessary to provide further information that enhances understanding of the context in which FF has developed.

Within this framework, the objective of this paper is to analyze the evolution and forms of exclusion experienced by family farming in Mexico over time. To that end, the study first addresses the conceptualization of FF, followed by its historical background and development up to the present. The third section examines public policies implemented in support of FF in Mexico, and the final section discusses the current advantages, opportunities, and limitations faced by FF, with the aim of contributing to the ongoing theoretical debate surrounding this activity.

Conceptualization of Family Farming

The interpretation of the concept of FF carries significant implications for both social frameworks and public policy, offering a deeper understanding of the development of this activity from its origins to the present. The concept of FF has been recognized since the mid-20th century, although no single, universally accepted definition exists (Samper, 2016), as each author emphasizes their own contextual and theoretical framework. According to Maletta (2011), FF is a family-based economic unit carried out on a farm of sufficient size to support a household. Piza *et al.* (2016) add that it may sometimes involve the use of improved seeds, fertilizers, agrochemicals, and even agricultural machinery. FF integrates economic activities, product exchange, land tenure arrangements, social interaction networks, power dynamics, governance structures, and collective identity (Samper, 2016). In essence, a family unit engages in agricultural and livestock activities to produce goods and services (Schneider, 2014).

In Mexico, SAGARPA as a national agency and FAO as an international organization define FF for the purpose of designing effective public policies as agricultural, livestock, forestry, fisheries, and aquaculture production systems characterized by limited access to land and capital, predominant use of family labor, and the direct participation of the head of household in the production process (SAGARPA and FAO, 2012). Across the definitions presented, the family unit emerges as the foundational element in the development of this activity.

Background and current situation of family farming in Mexico

With a clearer definition of FF, it is pertinent to examine its historical development and present-day context. The progression of FF in Mexico has been fraught with challenges, marked by exclusion and disadvantages compared to commercial agriculture. Nevertheless, over time and as FF has come to be recognized as a pillar of food provision for rural households it has gained prominence and drawn the attention of stakeholders in Mexico. Understanding its evolution is thus essential to ensuring its sustainability. The trajectory of FF in the country is closely tied to both national and international reforms in public and trade policy, as well as to Mexico's relationship with its neighboring country, the United States. According to Quintero (2017), FF in Mexico originated as a result of the agrarian reform that followed the Porfiriato era (1876-1910). This reform began with the Mexican Revolution of 1910, during which peasants fought for access to farmland (Warman, n.d.), ultimately leading to the establishment of small-scale holdings and the creation of a subsistence peasant sector (Quintero, 2017). In the 1930s, under the administration of President Lázaro Cárdenas, a perceived need arose to modernize agriculture (Otero, 2004). This "modernization" involved the adoption of innovations and strategies that widened the gap between small-scale and large-scale producers. Traditional agriculture lost value in comparison to industrialization and the adoption of new technologies and techniques, leading to a devaluation of peasant labor (Jarquín *et al.*, 2017). Industrialization was fueled in part by the overproduction of staple grains in the United States following the Green Revolution of the 1940s, which introduced industrial-scale agricultural production and enabled transnational agribusinesses to dominate the global agri-food market. As a result, the U.S. sought external markets for its agricultural surpluses and pursued strategies aimed at increasing production and reducing costs (Rubio, 2014). In response, Mexico implemented strategies to remain competitive, promoting agricultural development as a central component of an import-substitution model aimed at producing food and raw materials for domestic consumption and export. This facilitated resource transfers toward national industrialization (Reyes *et al.*, 1974). However, this phase became unsustainable by the late 1960s (Ramírez-Juárez, 2022). In the agricultural sector, this decline manifested in a reduced capacity to provide rural families with adequate income and employment, leading to the unequal distribution of natural resources, capital, education, and infrastructure. Consequently, rural households adopted a range of subsistence strategies according to their income levels (Escalante *et al.*, 2007), often resulting in livelihood diversification and migration to urban centers in search of better opportunities. This trend has led to a gradual abandonment of agriculture, particularly FF. The neoliberal agro-export phase significantly deepened the exclusion of rural producers, especially small-scale family farmers. According to Rubio (2002), this phase aligned with the secondary export model and was introduced in Latin America between 1980 and 1990. It was characterized by the dominance of transnational agribusinesses. This exclusion arose from three key dynamics: (1) the state withdrew from direct involvement in productive activities, ceding control to agribusiness; (2) trade liberalization and open borders facilitated an influx of imported products; and (3) U.S. agricultural policy promoted food expansion into developing countries. In Mexico, domestic food-producing agribusinesses gained control over raw materials and internal prices, generating adverse effects such as intensified internal and external competition for raw materials, where only the most competitive farmers prevailed. Another factor that disadvantaged Mexican farmers was the rise of the neoliberal model in the 1990s, particularly through the Uruguay Round of the World Trade Organization (WTO), which established rules for so-called "free" trade (Rubio, 2014). One key outcome was the 1994 North American Free Trade Agreement (NAFTA), which gradually eliminated tariffs. NAFTA represented a major trade agreement, especially for Mexico, as it largely benefited export-oriented sectors, including agricultural products such as tomatoes and avocados. However, one major drawback was the exclusion of farmers unable to compete under this trade regime due to unequal conditions particularly family farmers, who lacked the necessary productive resources and were thus placed at a disadvantage compared to large-scale producers. The condition and dynamics of FF, which became more widespread in the 2000s (Garner and de la O, 2014), are linked to Mexico's development trajectory over the past fifty years and to the role of the state in agricultural and rural development (Reyes *et al.*, 1974). In recent decades, FF has not been perceived as economically or socially viable. However, the 2007-2008 global food crisis revived interest in this production system. In response, the debate intensified around a new phase of FF development and the formulation of public policies to enhance food production and alleviate rural poverty (Schneider, 2014). Today, FF in Mexico is primarily characterized by holdings of no more than 15.5 hectares, a reliance on family labor up to 15 individuals per production unit and a transitional status in which production is intended both for self-consumption and for market sale. This segment comprises 56.8% of the country's REUs (SAGARPA and FAO, 2012). Looking ahead, population growth and changing dietary patterns are anticipated, positioning family farmers as key actors in this transition. Most of them already incorporate ecological and sustainable practices that contribute to environmental conservation.

Current public policies on family farming in Mexico

The adjustments in agriculture prompted by neoliberal globalization during the 1980s and 1990s led Mexico to implement various public policy reforms. These reforms aimed primarily at mitigating the negative impact on small-scale farmers while enabling export-oriented producers to remain competitive in the new globalized environment. This analysis, however, focuses specifically on the public policies that have been implemented to support FF. In Mexico, rural development policies prioritize regions with significant social and economic marginalization, where production units with potential for FF are located (SAGARPA and FAO, 2012). Programs such as PROCAMPO, Alianza por el Campo, and the Programa Especial Concurrente para el Desarrollo Rural Sostenible have been implemented over time, often overlapping and evolving under the influence of political shifts and the NAFTA, to provide support to the rural sector (Maletta, 2011). PROCAMPO offered direct support to farmers cultivating staple crops such as barley, beans, maize, cotton, rice, sorghum, soybeans, sunflower, and wheat. Alianza por el Campo aimed to engage low-income producers in rural agribusiness initiatives. Meanwhile, the Programa Especial Concurrente para el Desarrollo Rural Sostenible sought to integrate public policies geared toward rural development (Maletta, 2011). Despite these efforts, FF in Mexico continues to face challenges due to the absence of a constitutional framework that explicitly supports it. In response, the Mexican Network for Family and Peasant Farming was established in 2013 with the goal of strengthening differentiated public policies and promoting a new constitutional framework grounded in gender equity and youth participation (Red Mexicana por la Agricultura Familiar y Campesina, 2015).

Current opportunities and limitations for family farming in Mexico

Family Farming has historically undergone periods during which it was not granted economic or social relevance, and thus was not considered a driver of structural transformation. Nevertheless, numerous scholars and international organizations have demonstrated that FF is a strategic sector for achieving food security and alleviating rural poverty (Yúnez *et al.*, 2013). Today, FF occupies a strategic position in addressing food supply challenges, fostering competitive and sustainable development, and offering viable solutions to the problems facing the Mexican countryside. Despite its potential, FF faces several limiting factors that are nonetheless critical to its success. From a natural resource standpoint, FF is challenged by changing agroecological conditions, insufficient or degraded productive resources, and the loss of genetic diversity. These factors compel rural families to improve their tools, technologies, and organizational systems (Hernández & Salazar, 2018). Socioeconomically, FF is affected by rural outmigration and the abandonment of farmland, as remittances have become a primary source of income for peasant households (Hernández & Salazar, 2018). On the market front, FF grapples with price volatility for both agricultural products and inputs. Limited access to technology, credit, services, capital, and specialized technical assistance further widens the gap between FF and commercial agriculture (Hernández & Salazar, 2018). Compounding these issues is FF's constrained access to and control over productive means particularly land and wáter which must also be shared with other competing productive sectors. Moreover, while the Secretaría de Agricultura y Desarrollo Rural (2016) acknowledges that agricultural output in Mexico has improved through modernization, innovation, and mechanization especially among small producers FF continues to face challenges stemming from limited public investment (Yúnez *et al.*, 2013). Nonetheless, FF presents distinct advantages and opportunities that position it as a cornerstone of national development. It has demonstrated resilience in marginal environments by using locally adapted resources and requiring minimal wage labor. Consequently, FF exhibits higher survival potential than commercial agriculture (Hernández & Salazar, 2018). It can also contribute to economic development by leveraging locally available rural resources (Ramírez-Juárez, 2022). FF units typically maintain greater biodiversity, adopt practices that promote soil and water conservation (Hernández & Salazar, 2018), and offer a pathway toward sustainable production (Ramírez-Juárez, 2022), while enhancing food security. Additionally, FF plays a vital role in supplementing rural household incomes (Yúnez *et al.*, 2013). Beyond its socio-economic, environmental, and cultural contributions, FF also holds significant relevance in shaping public policy and promoting rural development (Hernández & Salazar, 2018). Today, FF is fundamental to the eradication of hunger and poverty, ensuring food security, safeguarding natural resources and the environment, and promoting the holistic development of rural areas (Ramírez-Juárez, 2022).

CONCLUSIONS

Family Farming has evolved in response to the structural reforms that have taken place in Mexico and globally. This production system has faced numerous challenges that have hindered its economic and social advancement. Nevertheless, FF holds significant productive potential to combat poverty and hunger in marginalized areas, thereby capturing the attention of various stakeholders. Studies such as this one shed light on the historical processes that have shaped the role of families in agriculture in Mexico. Moreover, they open the door to further lines of inquiry, such as the specific roles played by different family members. This, in turn, could lead to a more in-depth analysis of the respective contributions of men and women throughout the history of family farming in Mexico.

ACKNOWLEDGMENTS

The authors wish to thank the National Council for the Humanities, Sciences, and Technologies (CONAHCYT) for the scholarship awarded to the first author under the 2023 Call for Postdoctoral Fellowships in Mexico.

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Colegio de Postgraduados

Coffee prices: Mexican, Colombian and future quotes, a statistical cointegration analysis

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ABSTRACT

Objective: to estimate an econometric model for exports price dynamics of Mexican Arabica coffee with the joint influence of Colombian Arabica spot prices, and Arabica coffee futures from the New York Stock Exchange.

Design/Methodology/Approach: we applied unit root tests, cointegration analysis, estimation of a vector model with error correction, Granger's sense causality tests, impulse-response function analysis, and variance decomposition analysis.

Results: results made evident the dynamic relationship between the long-term prices of Colombian Arabica and Mild Arabica under the temporary control of futures quotes.

Limitations/Implications of the study: the estimated model can be expanded by incorporating financial, climate, and policy variables. However, the lack of information available with the same periodicity makes it difficult to develop more robust models.

Findings/Conclusions: the volatility inherent in coffee prices was corroborated, which suggests the importance of properly understanding and modeling these fluctuations in the market. This study provides a deeper insight into the interconnectedness among the various determinants of the coffee market, highlighting the need for effective risk management strategies for participants in this market.

Keywords: vector error correction model (VECM), unit root, cointegration analysis.

Citation: Jorge-Vázquez, O., González-Estrada, A., Martínez-Damián, M. A., & Reyna-Izaguirre, D. A. (2025). Coffee prices: Mexican, Colombian and future quotes, a statistical cointegration analysis. *Agro Productividad*. https://doi.org/10.32854/1vpyxx59

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

Received: May 14, 2024.
Accepted: May 18, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 85-92.

INTRODUCTION

According to the report of the International Trade Centre (ITC, 2021), the International Coffee Organization (ICO, 2023) and Tuyển *et al.* (2020), coffee is one of the most traded tropical crops in the world. Coffee production involves more than 52 producing countries to supply global demand.

Within the coffee production chain, one of the variables of greater interest corresponds to the prices of this aromatic product. Two major varieties of coffee are distinguished in the world: Arabica and Robusta (Otero and Milas, 2001; Tuyển *et al.*, 2020). Derived from this differentiation, the International Coffee Organization registers four types of prices: Colombian Arabica, Mild Arabica, Brazilian beans and Robusta (Figueroa-Hernández *et al.*, 2019).

To study the dynamism of coffee prices, various studies have used bivariate and multivariate models. Some examples of those independent studies are, Otero and Milas (2001); Frey and Manera (2007); Hussien (2015); Jaramillo-Villanueva and Benitez-García (2016); Tuyển *et al.* (2020); Babu and Muniyappa (2021); among others. Within the econometric models used, the following are more relevant, Vector Autoregressive models (VAR), vector error correction models (VECM), cointegration analysis, impulse-response functions, variance decomposition analysis, Granger's sense causality, analysis of cycles and structural changes. Each of them is used to investigate dynamics of price transmission; determinants of exports and demand; asymmetry and dynamics of production costs; as well as their relationships with financial and economic variables affecting coffee prices.

In Mexico, there is not much research on coffee prices where multivariate time series models such as VAR, VECM or cointegration analysis are used. One research under this approach was developed in Mexico by Jaramillo-Villanueva and Benitez-García (2016), who investigated the transmission of international prices to the domestic prices paid to the producer.

On that background, this study aimed to develop a cointegration analysis and the definition of a VEC model to capture the joint price dynamics of Arabica coffee, to deduce the dynamic relationships present among the main prices of this aromatic bean, which in the end rule over Mexican coffee exports. So, the objective was to estimate an econometric model fed by three time-series which correspond to spot and futures prices for Arabica coffee types, with monthly records from 1990 to 2019.

MATERIALS AND METHODS

To form the data vectors, time series were compiled from two different sources, International Coffee Organization (ICO) for spot prices and the investing.com website for futures quotes on the New York Stock Exchange. Time series from the ICO (2023) correspond to the monthly prices of Mild Arabica beans (PM_t), the group to which Mexican Arabica coffee belongs, and the monthly prices of Colombian Arabica beans (PU_t). Meanwhile, the time series from the *investing.com* site correspond to the average monthly quotes of futures contracts listed on the New York Stock Exchange (PW_t). For performing the statistical analyses of this research, the analytical tools integrated in the software Econometric Views v. 12 were used (EViews 12).

A statistical analysis was developed to identify the presence of unit root in the three time-series. Augmented Dickey-Fuller (ADF) and Phillips-Perron (PP) were applied to test null hypothesis (H_0) that there is a unit root in each of the time series, against the alternative hypothesis that there is no such root.

Taking into account the nature of the time series, spot and futures prices, a vector autoregressive model was proposed with endogenous variables, corresponding to the spot prices of Colombian Arabica and Mild Arabica beans, because dynamics of those develop at spot markets. On the other hand, futures were considered as an exogenous variable, since they are defined in advance by the participants of the exchange, in which speculation plays an important role.

To define the system of dynamical equations we followed the notation of VAR-X models presented in other research (Espinosa and Vaca, 2014; Nicholson *et al.*, 2017):

$$\begin{bmatrix}\Delta PM_t\\ \Delta PU_t\end{bmatrix}=\begin{bmatrix}\theta_1\\ \theta_2\end{bmatrix}=\begin{bmatrix}\varnothing_{11}^{1} & \varnothing_{12}^{1}\\ \varnothing_{21}^{1} & \varnothing_{22}^{1}\end{bmatrix}\begin{bmatrix}\Delta PM\\ \Delta PU\end{bmatrix}_{t-1}+\ldots+\begin{bmatrix}\varnothing_{11}^{p} & \varnothing_{12}^{p}\\ \varnothing_{21}^{p} & \varnothing_{22}^{p}\end{bmatrix}\begin{bmatrix}\Delta PM\\ \Delta PU\end{bmatrix}_{t-p}+pw_t\begin{bmatrix}\Phi_1\\ \Phi_2\end{bmatrix}+\begin{bmatrix}\varepsilon_{1t}\\ \varepsilon_{2t}\end{bmatrix} \quad (1)$$

The lag order considered in the VAR-X model was a function of the lowest value of five criteria, namely: likelihood ratio (LR), final prediction error (FPE), Akaike information criterion (AIC), Schwarz information criterion (SC) and Hannan-Quinn information criterion (HQ), as indicated by Gebru and Ramakrishna (2018). These criteria for selecting the optimal lag order for a dynamic multivariate system are those usually used in econometric studies involving time series (Gebru and Ramakrishna, 2018; Hamzah *et al.*, 2020).

In order to study the long-term dynamics of the time series, a cointegration vector was estimated. To estimate the cointegration equation, the methodology proposed by Johansen (1988) was followed. In this analysis, the null hypothesis (H_0) that there are maximum r vectors of cointegration was tested against the alternative hypothesis (H_a) that there are more than r vectors of cointegration (Johansen, 1988; Babu and Muniyappa, 2021), where r must be less than the number of endogenous variables of the dynamic system (Engle and Granger, 1987; Johansen, 1994).

With the estimated cointegration equation, a vector with error correction model (VEC) was proposed, in order to generate an econometric model that describes the dynamics of the time series in the short and long term. To identify the causality among the prices of Colombian Arabica and Mild Arabica spot prices and futures, the Granger's sense causality test was applied. Time series were evaluated in pairs with null hypothesis (H_0) that x_t does not cause y_t in the Granger sense, against the alternative hypothesis (H_a) that x_t causes y_t in the Granger's sense.

To synthesize the dynamic structure of the time series, the effects caused by some random shocks of the endogenous variables, on each dependent variable in the VAR-X model were investigated. In the analysis of the impulse-response functions, no random innovations of the exogenous variable of the model were incorporated, according to Brugger and Ortiz (2012). The Cholesky variance decomposition analysis was performed, which allowed isolating the percentage variability of each endogenous variable in the event of a shock in any of the endogenous variables.

RESULTS AND DISCUSSION

According to the results of the unit root test against (ADF) Augmented Dickey-Fuller test and (PP) Phillips-Perron test (Table 1), it was determined that the time series are integrated in the same order since stationarity is achieved in first differences (Table 2). This attribute of the time series is common in the economic and financial series, which are usually (I(1)), this is, they are integrated of order 1 (Granger, 1988; Brugger and Ortiz, 2012).This condition is essential to propose multivariate models which aim to capture the dynamics of stochastic variables (Stock and Watson, 1993; Jordán, 2014).

Time series were adjusted to an autoregressive vector model co-integrated with exogenous variable (VAR-X). This specification differs from some research on coffee, such as Jaramillo-Villanueva and Benitez-García (2016) and Tuyển *et al.* (2020). These discrepancies could be attributed to the difference in the period of analysis and the number of series considered, which are usually bivariate models.

According to the lowest value of these five criteria, Likelihood Ratio (LR), Final prediction error (FPE), Akaike information criterion (AIC), Schwarz information criterion (SC) and Hannan-Quinn information criterion (HQ); it was concluded that the time series under study fit a model with two lags (Table 3).

The cointegration analysis with Johansen's methodology made it possible to observe that both the trace statistic (Table 4) and the maximum Eigenvalue statistic show that there is at least one cointegration vector at a significant level of 5% (Table 5); which agrees with the results of Jaramillo-Villanueva *et al.* (2016). These results confirm, from a statistical point of view, that the model under consideration has the capacity to capture the short- and long-term dynamics of spot prices of Arabica coffees, taking into account the dynamics of futures.

Table 1. Unit root test results with levelled data.

Variable	Augmented Dickey-Fuller			Phillips-Perron			Conclusion
	t-Statistic	tc 5%	Prob.*	t-Statistic	tc 5%	Prob.*	
PM	−2.9553	−3.4229	0.1466	−2.1152	−3.4228	0.535	Unit root
PU	−2.2935	−3.423	0.4359	−2.5854	−3.4228	0.2873	Unit root
PW	−2.9074	−3.4231	0.1614	−2.655	−3.4228	0.2563	Unit root

**p-value* for Augmented Dickey-Fuller and *p-value* for Phillips-Perron. PM: Mild Arabica; PU: Colombian Arabica; PW: future quotes.

Table 2. Unit root test results with data in first differences.

Variable	Augmented Dickey-Fuller			Phillips-Perron			Conclusion
	t-Statistic	tc 5%	Prob.*	t-Statistic	tc 5%	Prob.*	
PM	−10.553	−1.9418	0.0000	−15.6477	−1.9418	0.0000	Stationary
PU	−9.3416	−1.9418	0.0000	−18.9764	−1.9418	0.0000	Stationary
PW	−7.3654	−1.9418	0.0000	−15.6773	−1.9418	0.0000	Stationary

**p-value* for Augmented Dickey-Fuller and *p-value* for Phillips-Perron. PM: Mild Arabica; PU: Colombian Arabica; PW: future quotes.

Table 3. Results in lag length for the Vector Autoregressive model (VAR).

Lag	LR	FPE	AIC	SC	HQ
0	NA	8525.000	14.727	14.749	14.735
1	671.090	1191.500	12.759	12.826	12.786
2	43.4624*	1071.408*	12.653*	12.765*	12.697*
3	7.1675	1073.600	12.654	12.812	12.717
4	2.4471	1091.000	12.671	12.873	12.751

* Minimum value. LR: likelihood ratio; FPE: final prediction error; AIC: Akaike information criterion; SC: Schwarz information criterion; HQ: Hannan-Quinn information criterion.

Table 4. Results of cointegration analysis with trace statistic.

H_0, number of CI vectors	Eigenvalue	Trace statistic	0.05 Critical value	Prob.*
No vectors**	0.7304	464.7	25.872	0.0000
At most 1 vector	0.0355	12.481	12.518	0.0507

* *p-value* of MacKinnon-Haug-Michelis (1999); ** Null hypothesis rejection.

Table 5. Results of cointegration analysis with the maximum Eigenvalue statistic.

H_0, number of CI vectors	Eigenvalue	Max Eigenvalue statistic	0.05 Critical value	Prob.*
No vectors **	0.7304	452.22	19.387	0.0001
At most 1 CI vector	0.0355	12.481	12.518	0.0507

* *p-value* of MacKinnon-Haug-Michelis (1999); ** Null hypothesis rejection.

This latter variable was not considered by Jaramillo-Villanueva and Benitez-García (2016) or by Tuyến *et al.* (2020); however, the inclusion of the dynamics of coffee futures quotes leads to a better modelling between these two linked markets, spot and futures, as it was stated by Babu and Muniyappa (2021).

The linear combination that maintains a long-term equilibrium relationship between the spot price of Colombian Arabica coffees and the spot prices of mild Arabica coffees, such as coffees from Mexico, fits a model with a linear trend and a constant term, according to Equation (2):

$$PM_{t-1} = 107.6943 + 0.076325 PU_{t-1} + 0.066254 T \tag{2}$$

As we found a cointegration equation between the endogenous variables of the model VAR-X(2) means that the specification and estimation of a error correction model (VEC) is justified (Jordán, 2014), with which it is possible to study the price dynamics in the spot markets of Arabica coffees. Based on the above, the following two equations were estimated:

$$\begin{aligned}\Delta PM_t = & -0.798 EC_{(2)} + 0.3015 \Delta PM_{t-1} + 0.2021 \Delta PM_{t-2} - 0.2351 \Delta PU_{t-1} \\ & -0.1226 \Delta PU_{t-2} + 0.751 PW_t - 90.549\end{aligned} \tag{3}$$

$$\Delta PU_t = -0.928EC_{(2)} + 0.5978\Delta PM_{t-1} + 0.2398\Delta PM_{t-2} - 0.5749\Delta PU_{t-1} - 0.1923\Delta PU_{t-2} + 0.8755PW_t - 105.5853 \quad (4)$$

The notation $EC_{(2)}$ represents equation (2).

Colombian Arabica coffees were found to have a higher adjustment speed compared to Mild Arabica in the long term. This is due to Colombia's level of participation in international Arabica coffee markets, since that is the country with the highest production and marketing of Arabica variety (ICO, 2023). On the contrary, countries producing Mild Arabica coffees have a lower percentage share in international trade. This is the case of Mexico, which reflects in a lower adjustment speed; however, this value is higher than that found by Jaramillo-Villanueva and Benitez-García (2016). Such discrepancies can be attributed to the difference in the period of analysis and the specifications of the model, since those authors developed the analysis only with spot prices. One of the series was adjusted with the producer price index (domestic price), which probably introduced biases, as this was the result of a series that was constructed with interpolations.

The Granger's sense causality test showed that the time series referring to the spot prices of Mild Arabica, Colombian Arabica and Arabica futures coffee prices present a bidirectional causality in the Granger sense. Similar results were reported by Otero and Milas (2001) and Babu and Muniyappa (2021), who stated that precisely in the Arabica coffee market there is this particular situation of bilateral causality between spot and futures prices, unlike what occurs in the Robusta coffee market.

Finally, with the impulse-response function analysis, using the Cholesky decomposition with one-standard-deviation and the variance decomposition analysis over a length of 12 monthly periods (one year), shocks of the endogenous time series in the short term in the face of shocks in each of the variables were identified. From these tests, temporal responses were identified in only three periods (a quarter). In the face of a positive shock in the prices of Colombian Arabica, the price of Mild Arabica presented a negative temporal response for three periods. Whereas in the face of a shock in the spot prices of Mild Arabica, the prices of Colombian Arabica presented a very notable positive temporary response. After the three periods of impact from any random shock in each of the variables, the responses tend to an asymptotic equilibrium (Figure 1).

The variance decomposition of the time series corresponding to the spot prices of Mild Arabica coffees and Colombian Arabica coffees shows that in the face of an innovation in the spot prices of Mild Arabica, the dynamics of the same variable is more than 97% explained throughout the period, with very little influence of the temporary behavior of the spot prices of Colombian Arabica. On the other hand, a random shock in the prices of Colombian Arabica showed a significant variability in the prices of Mild Arabica, which reveals the market power of Colombian Arabica coffees. Despite the great variability of Mild Arabica facing Colombian Arabica shocks, after three quarters, price variations in the dynamic system were observed to be equally distributed (close to 50%).

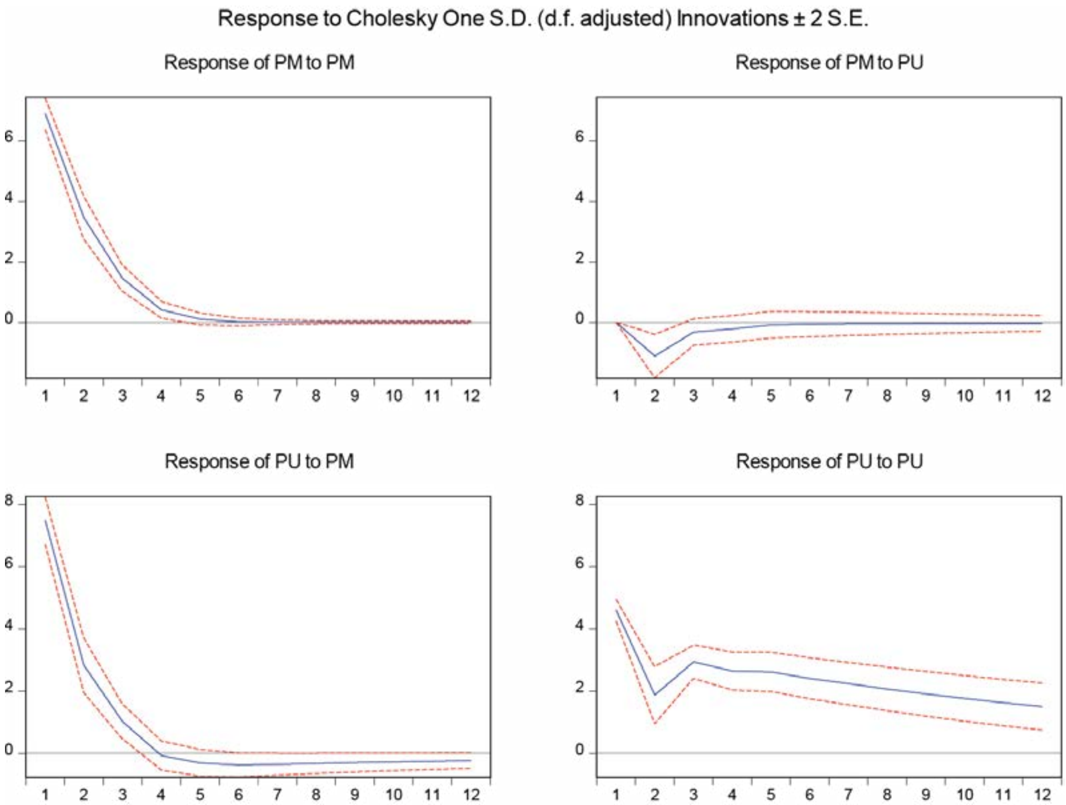

Figure 1. Impulse-Response Functions.

CONCLUSIONS

The spot prices of Colombian Arabica coffee and the spot prices used by Mexico for green coffee exports (Mild Arabica) are closely related in the long term. Sudden changes in any of these prices have only temporary effects, which dissipate over time. This research offers quantitative tools for coffee producers in Mexico to manage risks and thus minimize the losses they frequently face.

A strategic alternative for risk management by domestic producers is the consideration of financial instruments, such as coffee future quotes. In order to reduce the gap between spot prices and futures prices, since it is currently coffee futures that direct the dynamics of spot prices. However, this approach presents significant challenges for the national coffee production sector, especially due to the predominance of smallholdings in that sector.

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Colegio de Postgraduados

Proximate and Mineral Evaluation of Six Edible Wild Mushroom Species from the Nahuatl Region of the State of Mexico

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ABSTRACT

Objective: To evaluate the nutritional and mineral content of six species of edible wild mushrooms, from a Nahuatl region in the State of Mexico.

Design/methodology/approach: Standard analytical techniques were used to determine moisture content, partial dry matter, ash, crude protein, crude fat, and mineral composition of six edible wild mushroom species.

Results: The edible wild mushrooms exhibited high moisture content, ranging from 87.0% to 91.6%. The highest dry matter content was found in *Lyophyllum decastes*. *Hypomyces macrosporus* showed the highest ash content, with a value 2.22 times greater than that of *Lactarius deliciosus*. Regarding protein content, *Infundibulicybe gibba* had the highest percentage of crude protein among the species analyzed.

Limitations on study/implications: The production of sporomes of edible wild mushrooms is subject to environmental conditions, mainly rainfall.

Findings/conclusions: Edible wild mushrooms are a valuable source of nutrients and easily accessible to the Indigenous communities living in the forests of the State of Mexico. Additionally, there was a conspicuous nutritional variation among the different species studied.

Keywords: food, environment, forest, beneficial, ectomycorrhiza, fungi.

Citation: Barragán-Soriano, J. L., Pérez-Moreno, J. Crosby-Galván, M. M., &Almaraz-Suárez, J. J. (2025). Proximate and Mineral Evaluation of Six Edible Wild Mushroom Species from the Nahuatl Region of the State of Mexico. *Agro Productividad*. https://doi.org/10.32854/zx4p6785

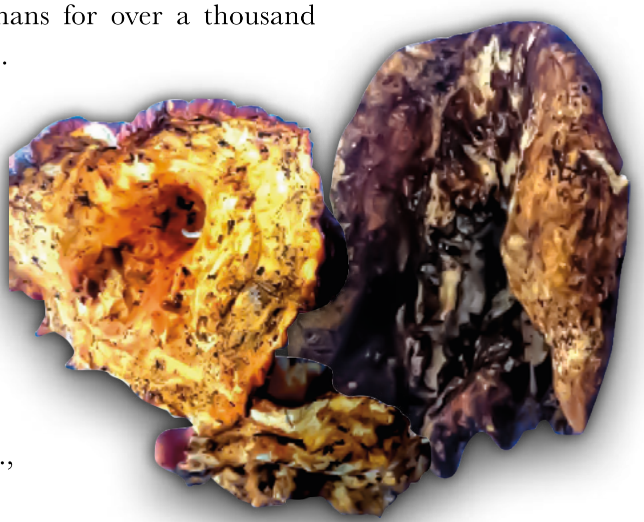
Academic Editor: Jorge Cadena Iñiguez
Associate Editor: Dra. Lucero del Mar Ruiz Posadas
Guest Editor: Daniel Alejandro Cadena Zamudio

Received: June 22, 2024.
Accepted: May 19, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 93-99.

INTRODUCTION

Wild mushrooms are an important forest resource and play several essential roles in forest ecosystems, such as decomposers, mycorrhiza formers, and pathogens. In addition, they serve as a food source (Mleczek *et al.*, 2021). Edible mushrooms have served as a food source for humans for over a thousand years (Jacinto-Acevedo *et al.*, 2021). It is estimated that there are 2,189 species of edible wild mushrooms worldwide (Li *et al.*, 2021). Their commercial and culinary importance lies primarily in their organoleptic properties, such as aroma and flavor. In addition, they are rich in protein and fiber, contain essential amino acids, and have a low lipid content (Rugolo *et al.*,



2022). Many edible wild mushrooms have also been reported to contain significant amounts of minerals such as magnesium, calcium, sodium, iron, zinc, selenium, among others (Khumlianlal *et al.*, 2022). Mexico harbors a high diversity of wild mushroom species. The presence of temperate forests dominated by gymnosperms and angiosperms across the national territory supports the development of approximately 200,000 fungal species (Jiménez *et al.*, 2013). It is estimated that around 450 species are consumed and 350 are used in traditional medicine by the various Indigenous groups inhabiting the country (Perete & Velázquez, 2023). It is particularly evident that many Indigenous communities in Mexico possess extensive knowledge to distinguish edible mushrooms from toxic ones (Torres-Gómez *et al.*, 2023). Mexico ranks as the second country in the world with the greatest diversity of edible wild mushrooms, following China (Pérez-Moreno *et al.*, 2021). In addition to their consumption, gathering communities generate income by selling them in markets and local fairs (Molina-Castillo *et al.*, 2023). Several studies have shown that nutritional content and bioactive compounds may vary even among mushrooms of the same species (Jacinto-Acevedo *et al.*, 2021). The consumption of edible wild mushrooms has contributed to the well-being of various cultures since ancient times due to their high nutritional value (López-Hernández *et al.*, 2022). There are reports of wild mushroom commercialization in traditional markets such as the one located in Ozumba, State of Mexico, where at least 90 species are traded and designated with more than 100 local names (Pérez-Moreno *et al.*, 2018). The objective of this study was to evaluate the nutritional content through a proximal analysis of moisture, dry matter, protein, total fat, and mineral content in six species of edible wild mushrooms from a Nahuatl region in the State of Mexico.

MATERIALS AND METHODS

Study Site

The mushrooms were obtained from the local market in the municipality of Ozumba de Alzate, State of Mexico. The municipality of Ozumba is inhabited by an Indigenous ethnic group of Nahuatl origin. The mushrooms were identified based on key ethnomycological knowledge, and taxonomic identification techniques were also applied (Bautista-Bautista *et al.*, 2024).

Nutritional Content

Moisture and Dry Matter: Sixty grams of fresh mushrooms from each species were weighed in triplicate and placed in 120-milliliter vials. Subsequently, they were freeze-dried using a FreeZone 6 lyophilizer (Labconco®). Moisture loss was recorded. For dry matter determination, one gram of the lyophilized sample was weighed and placed in an oven (Feliza®) at 180 °C for 8 hours.

Total Ash Determination: This technique was performed using the incineration method (Gómez-Flores *et al.*, 2019).

Protein Determination: Crude protein was determined using the micro-Kjeldahl method (AOAC, 2005). The methodology followed was that described by Gómez-Flores *et al.* (2019).

Crude Fat Determination: Two grams of lyophilized sample were weighed and placed in a cellulose cartridge, which was then mounted in the Goldfish extraction device. Fat extraction was performed using 100 ml extraction flasks (Labconco®) containing 30 ml of petroleum ether (Meyer Cat. 1290-41®).

Mineral Analysis: Nitrogen (N) was determined by the semi-micro Kjeldahl method (Bremner, 1965). Total phosphorus (P) and potassium (K) were determined following the method described by Allen *et al.* (1997). Micronutrients were evaluated using the ammonium acetate extraction method combined with flame photometry.

Statistical Analysis

Values were calculated as the mean of triplicate samples for each mushroom species. The data for the evaluated variables were subjected to analysis of variance and the Shapiro-Wilk normality test. When the data sets did not meet the normality criteria, square root or logarithmic transformations were applied to allow subsequent mean comparison using Tukey's test ($p \leq 0.05$) with the Statistical Analysis System software, version 9.00 (SAS, 2004).

RESULTS AND DISCUSSION

Six edible wild mushrooms with high biocultural importance in the studied area were identified: *Lactarius* aff. *deliciosus* (L.) Sf. Gray, *Infundibulicybe gibba* (Pers.) Harmaja, *Lyophyllum decastes* (Fr.) Singer, *Hypomyces lactifluorum* (Schwein.) Tul. & C. Tul., *Hypomyces macrosporus* Seaver, and *Cantharellus cibarius* s.l. Singer (Figure 1, d, e). Common names were provided by the collectors and vendors at the market (Figure 1, a, b, c). The identified species were previously reported by Pérez-Moreno *et al.* (2009) in the study area, who analyzed 150 mushroom species commercialized in the markets of the Izta-Popo National Park region.

The dry matter content varied among the species, with *C. cibarius* presenting the highest total dry matter (Figure 2). In general, mushrooms contain approximately 90% moisture and 10% dry matter (Assemie & Abaya, 2022). Neh *et al.* (2021) reported a dry matter increase of 17.77% in *Polyporus dictyopus* Mont. and 12.69% in *Auricularia polytricha* (Mont.) Sacc., compared to average values. These differences were attributed by the authors to fluctuations in environmental factors during growth and storage, which affect mushroom metabolism. Srikram and Supavanich (2016) stated that differences in dry matter are mainly due to the mushroom species, climatic conditions, growth substrates, and geographical distribution.

The ash content varied among the different species, with *H. macrosporus* showing the highest percentage, 2.22 times greater than that of *L. deliciosus* (Table 1). Srikram and Supavanich (2016) reported low ash percentages in both wild and cultivated edible mushrooms, with contents ranging from 0.27% to 2.56% in *Russula cyanoxantha* (Schaeff.) Fr.

Protein percentages varied, with *I. gibba* showing a significant difference ($P=0.05$), containing up to 2.6 times more protein than *C. cibarius* (Table 1). Previous studies have reported varied protein percentages; Agrahar-Murugkar and Subbulakshmi (2005) reported that *Lactarius quieticolor* (Romagnesi) had the highest percentage at 27.5%. Gunasekara *et al.* (2021) mentioned that differences in protein content and percentage may be due to various

Figure 1. Wild mushrooms: mushroom vendor (a); commercialization of edible mushrooms at the Ozumba market, State of Mexico (b, c); *Hypomyces lactifluorum* (d); *Hypomyces macrosporus* (e).

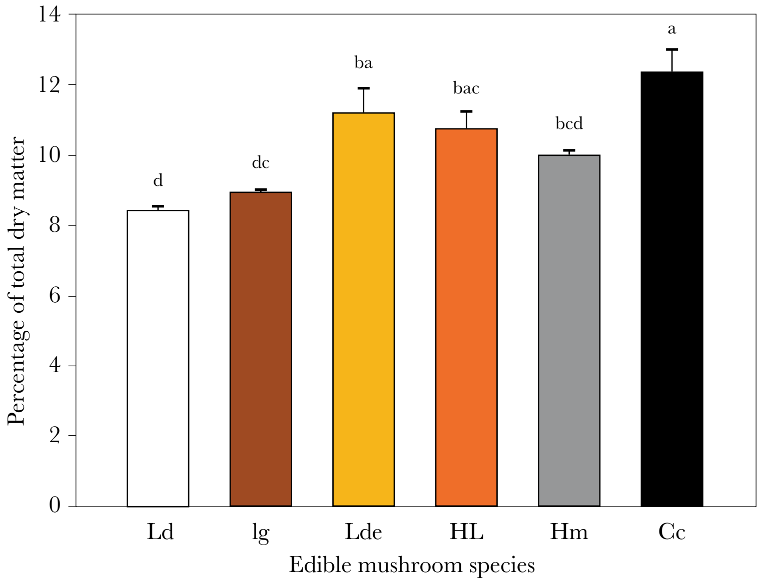


Figure 2. Total dry matter percentage of edible wild mushrooms. L=*Lactarius* aff. *deliciosus*, Ig=*Infundibulicybe gibba*, Ld=*Lyophyllum decastes*, Hi=*Hypomyces lactifluorum*, Hm=*Hypomyces macrosporus*, and C=*Cantharellus cibarius* s.l. Error bars represent the standard error of the mean (n=3). Means with different letters (a-d) above the bars are significantly different according to Tukey's test (P=0.05).

Table 1. Proximate composition of wild edible mushrooms.

	Ash (%)	**Protein** (%)	**Total fat** (%)
L. deliciosus (L)	4.77±0.03e	13.27±0.13b	1.49±0.13c
I. gibba (Ig)	6.45±0.01d	25.67±0.53a	3.57±0.07b
L. decastes (Ld)	9.64±0.01b	24.94±0.24a	6.19±0.27a
H. lactifluorum (Hl)	8.20±0.04c	13.08±0.23b	3.40±0.18b
H. macrosporus (Hm)	10.62±0.10a	14.46±0.44b	3.51±0.19b
C. cibarius (C)	9.59±0.16b	9.90±0.23c	2.75±0.05b

Values with the same letter in the same column are not significantly different according to Tukey's test (P=0.05). ±Standard error of the mean (n=3).

factors, such as intraspecific genetic variation and developmental stages. The protein fraction of wild edible mushrooms is characterized by significant concentrations of essential and non-essential amino acids. (Altaf *et al.*, 2020). The percentage of crude fat varied among the different species, with *L. decastes* presenting the highest fat content, showing significant differences (P=0.05), with up to 4.15 times more than *L. deliciosus* (Table 1). Gunasekara *et al.* (2021) reported wide ranges of crude fat percentages, with *Termitomyces* spp. recording a maximum value of 12.35%. The species *L. decastes* showed significant differences (P=0.05) in nitrogen (N) content, with 6.24%. The highest phosphorus (P) content was also recorded in *L. decastes*, with 8.88 g. Potassium (K) content ranged between 21.23 g and 9.34 g, with *C. cibarius* showing the highest K content (Table 2).

Table 2. Macronutrients in wild edible mushrooms.

	N (%)	**P** (g)	**K** (g)
L. deliciosus (L)	2.95±0.15b	2.72±0.67b	9.34±2.27b
I. gibba (Ig)	6.06±0.29a	7.97±0.09a	12.33±0.06b
L. decastes (Ld)	6.24±0.53a	8.88±0.24a	16.71±0.74ba
H. lactifluorum (Hl)	3.33±0.18b	3.58±0.04b	16.98±2.21ba
H. macrosporus (Hm)	3.05±0.03b	3.50±0.07b	17.46±2.53ba
C. cibarius (C)	2.35±0.11b	3.18±0.27b	21.23±1.99a

Values with the same letter in the same column are not significantly different according to Tukey's test (P=0.05). ± Standard error of the mean (n=3).

Table 3. Micronutrients in wild edible mushrooms.

	Ca (g)	**Mg** (g)	**Cu** (g)	**Fe** (g)
L. deliciosus	0.57±0.16b	0.74±0.18b	0.01±0.003d	0.04±0.011b
I. gibba	0.79±0.15ba	1.05±0.02ba	0.06±0.001ba	0.11±0.003a
L. decastes	0.65±0.02b	1.42±0.04a	0.05±0.002b	0.08±0.001ba
H. lactifluorum	1.17±0.11a	1.11±0.02ba	0.07±0.002a	0.09±0.021ba
H. macrosporus	0.55±0.06b	1.11±0.02ba	0.07±0.002a	0.10±0.008ba
C. cibarius	0.88±0.02ba	0.87±0.07b	0.04±0.004c	0.09±0.012ba

Values with the same letter in the same column are not significantly different according to Tukey's test (P=0.05). ± Standard error of the mean (n=3).

Fogarasi *et al.* (2020) reported that potassium is the element with the highest concentration in mushrooms. Neh *et al.* (2020) observed that wild mushrooms have high levels of phosphorus and potassium. Therefore, mushrooms are rich in mineral content and are an excellent source of minerals (Sifat *et al.*, 2020).

In this study, we report a high calcium (Ca) content in the fungus *H. lactifluorum* with 1.17 g. The highest magnesium (Mg) content was found in the species *L. decastes*. *H. lactifluorum* showed the greatest copper (Cu) content. We observed that the fungus *I. gibba* presented the highest concentration of iron (Fe). Krupodorova and Sevindik (2020) pointed out that mineral content can vary depending on the habitats where the fungi were collected. This study is one of the first to demonstrate the nutritional contribution of Mexican wild edible mushrooms to the diet of the people who consume them. The research shows that these mushrooms have a high mineral content, which contributes to the improvement of the diet and dietary diversification. Previously, it has been shown that wild mushrooms contain higher amounts of nutrients and minerals compared to cultivated mushrooms (Krupodorova and Sevindik, 2020).

CONCLUSIONS

The nutritional profile of the studied wild edible mushrooms was characterized by high protein concentrations, low fat levels, and interspecific variation in micronutrient composition. Dietary intake of these fungi provides significant nutrient supplementation to consumers, with heightened dietary relevance for ethnic populations utilizing them as traditional food sources. Consequently, they represent a nutritionally valuable non-timber forest product.

ACKNOWLEDGMENTS

We thank the Consejo Mexiquense de Ciencia y Tecnología for their support in carrying out this research, which is part of the COMECyT Chair project. Support provided by Colegio de Postgraduados for the corresponding author's research visit to the Kunming Institute of Botany, Chinese Academy of Sciences in Kunming, China is also gratefully acknowledged.

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Colegio de Postgraduados

Bibliometric analysis of studies about *Phaseolus vulgaris* L., 2013-2023 period

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ABSTRACT

Objective: To identify the scientific production about the *Phaseolus vulgaris* L. species through a bibliometric analysis.

Design/Methodology/Approach: Bibliographic data were downloaded from the Scopus platform to determine their bibliometric indicators: published documents, growth rate, type of document published, areas with the highest number of publications, countries, journals, authors, and institutions and foundations with the highest number of publications.

Results: A total of 7,447 publications were identified (2.16% annual growth rate) and 44,144 citations were recorded. The area with more publications was agricultural and biological sciences. The most productive countries were Brazil, the United States, and Mexico. Twenty-three thousand six-hundred sixty-seven authors were identified, resulting in an average of 5.34 authors per publication. The most researched topics focused on the performance and development of the plant.

Study Limitations/Implications: Publications outside the Scopus platform were not considered.

Findings/Conclusions: Determining the scientific production about *P. vulgaris* will result in more relevant research works.

Keywords: beans, scientific production, agro-food systems.

Citation: Ruiz-Hernández, R., & Pérez-Vázquez, A. (2025). Bibliometric analysis of studies about *Phaseolus vulgaris* L., 2013-2023 period. *Agro Productividad*. https://doi.org/10.32854/6m53y267

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

Received: June 03, 2024.
Accepted: May 15, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 101-108.

INTRODUCTION

Phaseolus vulgaris L. is native to Mesoamerica and the Andean region. This species is currently distributed throughout Mesoamerica and South America (Bitocchi *et al.*, 2017). The essential nutrients contained in their seeds contribute to food security (Uebersax *et al.*, 2023). Their agronomic and nutrimental characteristics have led to a significant increase in researches about *P. vulgaris* throughout the years. Several bibliometric studies have covered the global scientific production from 1903 to 2019 (Capanema *et al.*, 2022). Meanwhile, other bibliometric studies have focused on very specific subjects, such as the research trends regarding the improvement of the resistance of bean to the common bacterial blight (Aparecida *et al.*, 2025) and the agronomical practices that increase the yield and quality

of beans (Karavidas *et al.*, 2022). No up-to-date and comprehensive bibliometric analysis about *P. vulgaris* is currently available. The lack of information limits the identification of the trends for recent years, the type of documents that influence the global production about this species, and the areas where more research has been carried out. Likewise, it hinders the development of relevant research about *P. vulgaris.* Therefore, bibliometric indicators are required to determine the degree of scientific knowledge about *P. vulgaris* and to facilitate the development of increasingly relevant projects. Therefore, the objective of this analysis was to identify the scientific production about *Phaseolus vulgaris* L. from 2013 to 2023.

MATERIALS AND METHODS

A bibliographic search of the documents published from 2013 to 2023 was carried out in Elsevier's Scopus platform. Since some metrics are influenced by the period in which they were measured, the aim of this study was to determine the trends of the last years, in order to identify future trends. Scopus is the most complete multidisciplinary database and it was chosen as a result of its comprehensive research metrics, which cover 44,070 scientific journals. *Phaseolus vulgaris* was used as search term, in order to collect all bibliographical information available about that species. The bibliographical data was downloaded in a .csv file, which included all the information for each document published as of March 4, 2024. Every kind of published document was taken into account to achieve a comprehensive understanding of the researches about *P. vulgaris*. Consulting a single database prevented duplicate results. The data were analyzed with the bibliometrix package of the R software (version 4.3.1). The following bibliometric indicators were determined: number of published documents, annual growth rate, total publication sources, type of published documents (scientific articles, reviews, books, book chapters, conference papers, scientific notes, short surveys, and letters to the editor), areas with the greater number of published documents, countries, institutions, foundations, journals, and authors with the greatest number of published documents, total number of citations, average number of citations per document, most quoted articles, total number of authors, documents with a single author, average number of co-authors per document, most quoted authors, total keywords and keywords plus, and trending themes. The world scientific production graph was developed with Excel 2016. The keyword network was based on the word frequency recorded by Scopus and the corresponding graph was developed with VOSviewer version 1.6.20. The thematic map was developed with the biblioshiny function of the bibliometrix package (R software, version 4.3.1).

RESULTS AND DISCUSSION

A total of 7,447 publications were identified from 2013 to 2023. The annual growth rate reached 2.16%. The 7,447 documents included a total of 313,344 bibliographical references. As a whole, the publications included 44,144 citations. The highest number of publications (820) and citations (3,761) were recorded in 2021 (Figure 1).

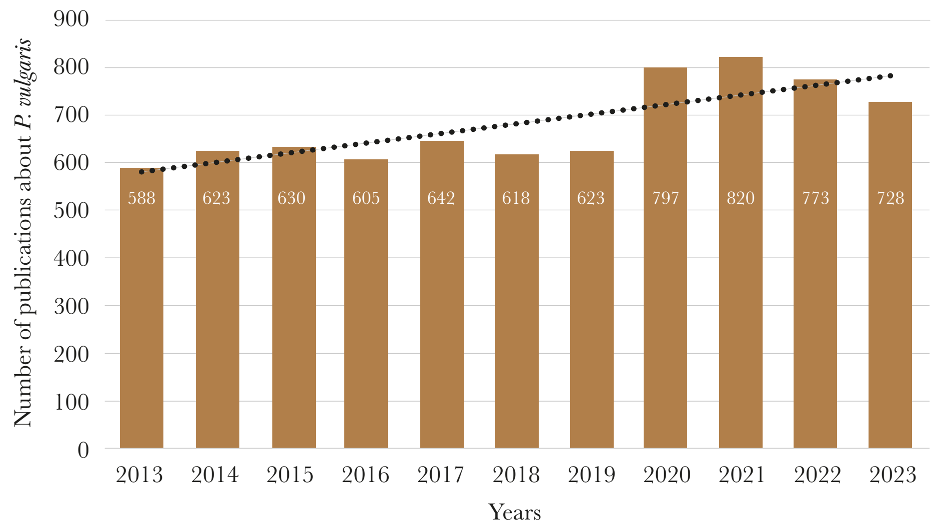

Figure 1. Global scientific production and citations recorded about the *Phaseolus vulgaris* theme from 2013 to 2023.

Published documents and subjects per percentage

One-thousand six-hundred sixty-one publications sources were identified. The published documents were divided as follows: scientific articles (91.95%), reviews (3.47%), book chapters (1.86%), conference papers (1.36%), scientific notes (0.53%), and books (0.03%). In addition, other documents (such as short surveys and letters to the editor) accounted for the remaining 0.81%. The Scopus database includes several types of documents, 97.81% of which were peer-reviewed. Steer and Ernst (2021) mentioned that peer-reviewing is important, because works published in journals must be truthful, valid, and accurate. Only a minimum percentage of publications (2.19%) were not subjected to a peer-review process. This study included all types of documents, in order to achieve a comprehensive understanding of the research about *P. vulgaris*. Hoang (2023) mentions that there are no standards for the selection of certain type of documents. However, a poor selection of documents impacts the overall calculation of the metrics. *P. vulgaris* has been the subject of study in several areas of knowledge. Scopus considers several categories, including: agricultural and biological sciences (42.82%), biochemistry, genetics, and molecular biology (16.94%), environmental science (7.64%), medicine (4.75%), and chemistry (4.60%). The remaining articles were included in the microbiology, pharmacology, and multidisciplinary sciences categories. Capanema *et al.* (2022) had likewise reported that, since the object of study is a species, agricultural and biological sciences and biochemistry, genetics, and molecular biology accounted for the highest frequencies. Biological, agronomic, and genetic characterization are frequent subjects for the existing varieties.

Countries with most publications and citations

A total of 125 countries have worked on the subject of *P. vulgaris*. The countries with the highest number of publications were Brazil, the United States, Mexico, and China (Table 1). Capanema *et al.* (2022) evaluated the scientific production about *P. vulgaris* from 1903

Table 1. Countries with more publications and citations about *Phaseolus vulgaris* (2013-2023).

Country	Total number of publications	PSP*	PCP*	total number of citations	Average citation for publication
Brazil	1222	1065	157	12802	10.48
EUA	653	446	207	15505	23.74
Mexico	475	378	97	5660	11.92
China	459	352	107	8188	17.84

Total number of publications PSP* PCP* total number of citations Average citations per publication.
PSP*: single-country publications; PCP*: publications in collaboration with other countries.

to 2019, identifying that the United States and Brazil had contributed 24 and 17% of the publications, respectively. This study concluded that Brazil published most research works (16.40%) and its most frequent collaborations were with the United States, Mexico, and Spain. Mexico held the third place in the *P. vulgaris* subject, with 475 publications (6.37%) and its most frequent collaborations were the United States, Brazil, Spain, and China. The United States held the first place, with 15,505 citations (an average of 23.74 citations per publication); meanwhile, Mexico reached the fifth place with 5,660 citations (11.92 citations per document). Determining the countries with the most significant scientific production and the greatest number of citations regarding a specific subject is highly important, because this result indicates if the research about a given species is relevant.

Institutions and foundations with greater scientific production

Most research works have been carried out by the following institutions: Embrapa Arroz e Feijão (259), USDA Agricultural Research Service (247), and Universidade Federal de Vicosa (206). The main foundations that have funded the research about *P. vulgaris* include: Conselho Nacional de Desenvolvimento Científico e Tecnológico (609), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (516), National Natural Science Foundation of China (217), and Consejo Nacional de Ciencia y Tecnología (207). This data is related to the production and citations per country, since the institutions and research centers that hold the first places in this ranking are based on Brazil, the United States, China, and Mexico.

Journals with more publications and most quoted articles

Frontiers in Plant Science published the greatest number of documents (161). This journal has a 6.62 impact factor and is classified in quartile Q1. Food Chemistry and Plos One were the second and third most prolific journals, with 115 and 103 publications, respectively. Forty-four thousand one-hundred fifty-four citations were obtained from 7,447 publications, resulting in an average of 15.33 citations per document. The three most quoted articles were written by J. Schmutz (2014) in Nature Genetics (904 citations), D. Bhardwaj (2014) in Microbial Cell Factories (681 citations), and D.J. Bertioli (2016) in Nature Genetics (622 citations). Schmutz *et al.* (2014) described the genome and identified the genes associated with bigger leaves and seeds. They also discussed the effect of domestication on the composition of the genome. For their part, Bhardwaj *et al.* (2014)

discussed the importance of biofertilizers for phenology and as defense mechanism for crops. During the last ten years, both works have been benchmarks in the field and have laid the foundations for the development of sustainable agro-food systems, through the efficient use of genetic resources and agroecological practices.

Number of authors and citations per author

Twenty-three thousand six-hundred sixty-seven authors participated in the scientific production about *P. vulgaris*, which included 158 documents written by a single author. In average, 5.34 co-authors collaborated in these documents, 25.42% of which were international co-authorships. The authors with more publications were: L.C. Melo (71 publications), J.D. Kelly (68 publitacions), H.S. Pereira (68 publications), A.F. Chiorato (63 publications), and S.A.M. Carbonell (57 publications). The authors with more citations were: P. Gepts (3,662 citations), M.W. Blair (3,132 citations), J.D. Kelly (2,885 citations), S. Beebe (2,006 citations), and S.P. Singh (1,989 citations). J.D. Kelly recorded the highest H index (24). P. Gepts is the ninth most productive author and holds the first place in number of citations. The bibliometric analysis identified the impact of the scientific production of each author and connected the number of publications with the number of citations. This study determined that the number of publications is not related to the number of citations: researches with theoretical and methodological contributions influence the number of citations per documents, but not the volume of publications.

Keywords and keywords plus

In total, 15,127 keywords per author and 24,232 keywords plus were identified. The keywords per author are located after the abstract. The authors established the following keywords: *P. vulgaris* (2,284), common bean (861), yield (145), germination (99), and abiotic stress (88). Keywords plus are allocated by the Scopus system through an algorithm and their wider descriptive range helps in the delimitation of a scientific area (Zhang *et al.*, 2016). The keywords plus were: *P. vulgaris* (3403), faseolo (bean) (2,469), plant metabolism (864), genetics (840), pulse (792), and chemical compounds of the plant (627). For example, the chemical compounds of the black jamapa variety underscore its potential as a medicine for people with high glucose levels (Diéguez *et al.*, 2023). For their part, Nagessa *et al.* (2023) found out that soaking and pressure cooking diminish the amount of phytates and tannins in the seeds. The frequency of keywords is closely related to the most researched areas of knowledge. The co-occurrence network of the 15,058 keywords generated 9 clusters (Figure 2). Cluster 1 covered themes related to nutritional content and the functional compounds of the seeds. Researches about the application of UV-B radiation to increase the nutrient content of pods and seeds were particularly relevant (Ha *et al.*, 2023). Cluster 2 included themes related to production and its related factors. Determining the various types of stress that impact production will enable the development of agronomic practices that mitigate their effect. Cluster 3 grouped the molecular biology themes, including phylogenesis and gene expression. Cluster 4 was characterized by themes such as plant health and breeding and the determination of gene diversity through molecular markers. Studies about the gene diversity and structure of *P. vulgaris* populations help to determine

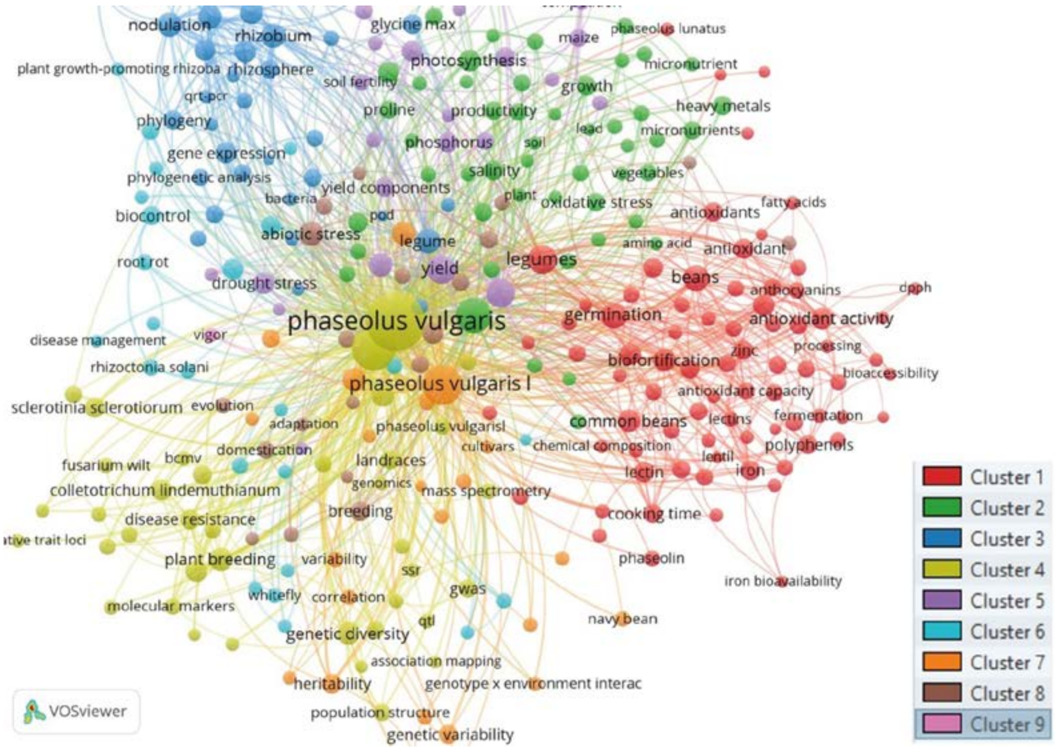

Figure 2. Co-occurrence network of keywords related with the *P. vulgaris* theme (2013-2023). Each node stands for a keyword and its size illustrates its frequency. Lines connect keywords and their width shows the frequency with which two keywords are connected.

the gene divergence and flow between the materials from a given region. Molecular markers provide relevant information about the gene base of the species and facilitate the preservation and development of resistant varieties (Nawaz *et al.*, 2023). Cluster 5 incorporated agronomic management themes. This is a major cluster and these keywords make reference to the agronomic practices that impact the yield of *P. vulgaris* (Karavidas *et al.* 2022). Cluster 6 included the biological control in the root, while cluster 7 grouped the studies about the interaction between genotype and environment. The descriptor-based morphological evaluation of bean varieties enables the identification of materials with interesting characteristics, as the production of new cultivars leads to greater yields (Elkoca *et al.*, 2022). Cluster 8 incorporated themes related to evolution and domestication. Wild materials are currently an alternative for production and consumption, given their adaptability and disease resistance. Finally, cluster 9 grouped themes related to the initial stages of the crop, such as germination and survival evaluation. Keywords enabled the identification of groups within an overall theme. Likewise, they show the relation between groups and the size of the cluster.

Trending themes and thematic map

In the last years, the trending themes have been the following: evaluation of genotypes (2015-2020), plant breeding (2017-2022), metabolomics (2019-2022), abiotic stress (2018-2022), genome-wide association studies (2019-2022), biotic stress (2016-2022), DNA sequencing (2022-2023), and gene expression (2022-2023). The abiotic stress theme was predominant from 2016 to 2022. These themes included research works focused on the evaluation of the factors that cause stress on *P. vulgaris* (Koleva *et al.*, 2022) and some of the mechanisms used to fight it (Alghamdi *et al.*, 2023). The thematic map classified the published documents in declining, primary, niche, and motor themes (Figure 3).

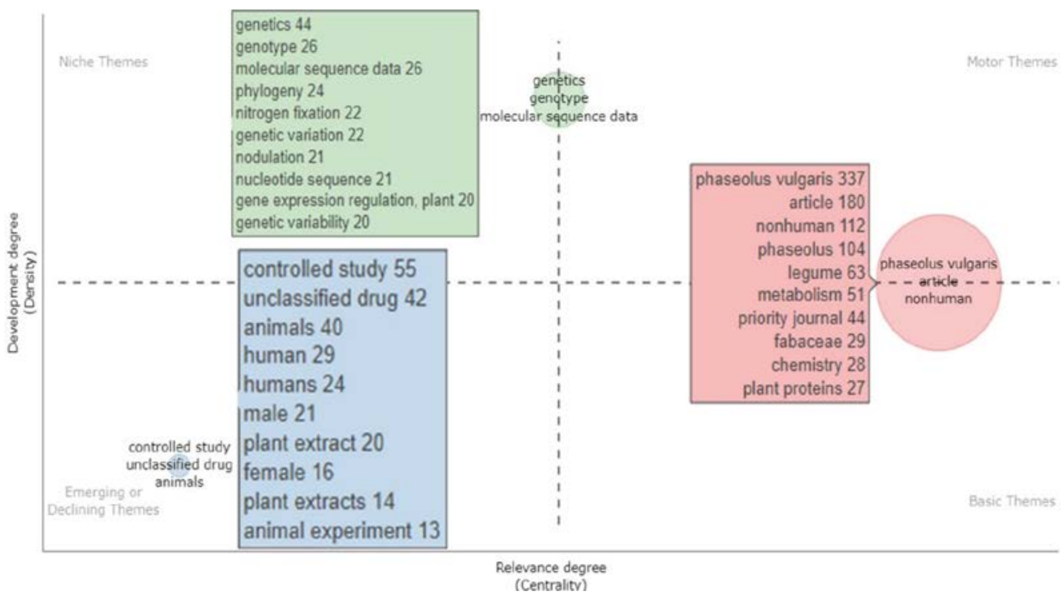

Figure 3. Thematic map of the *P. vulgaris* theme (2013-2023).

The declining themes included studies about plant extracts. The primary themes covered taxonomic and chemical studies of the plants. This type of research describes the morphology of local materials, through an analysis of the chemical composition of the seeds. Meanwhile, motor themes include nucleotide sequencing, regulation of gene expression, phylogenetics, and genotyping. Omics sciences are currently in vogue, because they provide a great amount of information for the understanding of a phenomenon. Transcriptomics identifies gene expression under several types of abiotic stress (Du *et al*. 2022). These themes are found in the frontiers of knowledge and enable a better understanding of the physiology of the species. Globally, the themes are distributed in four groups. However, the period or type of documents under evaluation impact the said distribution. A similar situation happens when the research is limited to a single country, because the research about *P. vulgaris* is a recent endeavor in some of them.

CONCLUSIONS

Determining the bibliometric indicators of *P. vulgaris* allows researchers to develop more relevant work, in line with current trends. These indicators also facilitate the identification of the most popular areas of knowledge, the most prestigious authors on the field, and which works are global academic benchmarks.

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Colegio de Postgraduados

Evaluation of the Nutritional Value of Glycerol in Broiler Chickens

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ABSTRACT

Objective: To evaluate the apparent metabolizable energy corrected for zero nitrogen balance (AMEn) of glycerol (GLY), as well as the productive performance, carcass yield, and meat stability and antioxidant status in broilers fed increasing levels of GLY.

Design/methodology/approach: Energy balance trials were conducted on broilers aged 10-20 and 28-38 days to determine AMEn using diets containing 0, 10, 20, and 30% GLY. A separate performance trial was carried out using diets with 0, 2.5, and 5% GLY. Data were analyzed using analysis of variance and simple linear regression.

Results: AMEn values of 3,123 and 3,242 kcal/kg of GLY were obtained in broilers aged 10-20 and 28-38 days, respectively. Final body weight and weight gain increased linearly ($p \leq 0.05$) with higher inclusion levels of GLY in the diets of floor-raised chickens. Carcass yield and its components, water loss, lipid oxidation, and antioxidant status were similar among treatments.

Limitations of the study/implications: Only one GLY sample was evaluated, and information on its free fatty acid content and composition was not available.

Findings/conclusions: The AMEn values of GLY were 3,123 and 3,242 kcal/kg for starter and grower broilers, respectively. GLY can be considered an alternative energy ingredient and may be included at levels up to 5% in broiler chicken diets.

Keywords: Broiler chickens, glycerol, energy value, productivity, antioxidant status.

Citation: Ángeles, M. de L., Gómez-Rosales, S., & Jiménez- Ocampo, R. (2025). Evaluation of the Nutritional Value of Glycerol in Broiler Chickens. *Agro Productividad*. https://doi.org/10.32854/pqzp5z69

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

Received: July 04, 2024.
Accepted: May 23, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 109-117.

INTRODUCTION

Glycerol (GLY) is generated as a by-product of the biodiesel industry from various oil sources; one ton of biodiesel produces approximately 100 kg of GLY (Badia-Fabregat *et al.*, 2019; Zhou *et al.*, 2018). It is obtained through the transesterification of triglycerides, catalyzed by KOH or NaOH, in the presence of fat or oil and an alcohol (Arif *et al.*, 2019). In Mexico, biodiesel production has been promoted using different oils such as African palm, castor bean, and jatropha, generating a residue that contains approximately 80%

GLY. Glycerol is considered a suitable ingredient in the production of soaps, cosmetics, pharmaceuticals, and food products. However, due to the limited quantities produced during biodiesel manufacturing and the high cost of purification, the most viable option is to use GLY as an energy source in animal feed (Monteiro *et al.*, 2018).

GLY is an essential structural component of phospholipids and triglycerides in both plant and animal tissues. During digestion, pancreatic lipase hydrolyzes triglycerides to form GLY and free fatty acids. The resulting GLY is water-soluble and freely enters the portal circulation, where it can be rapidly converted into glucose through gluconeogenesis, subsequently generating energy via glycolysis and the citric acid cycle (Rotondo *et al.*, 2017; Shah *et al.*, 2022). When the body utilizes stored fat as an energy source, fatty acids and GLY are released into the bloodstream. GLY produces energy through the tricarboxylic acid cycle and glycolytic pathways (Shah *et al.*, 2022), and upon oxidation, yields 22 moles of ATP per mole (Guyton and Hall, 2020).

In several previous studies on broiler chickens, the energy value of GLY derived from biodiesel production has been estimated, and various performance trials have been conducted. In one experiment evaluating different sources of biodiesel-derived GLY, the apparent metabolizable energy corrected for zero nitrogen balance (AMEn) ranged from 3,085 to 4,100 kcal/kg. AMEn values of 3,621, 3,331, 3,349, and 3,434 kcal/kg for the starter, grower, finisher, and overall phases, respectively, have been previously reported (Dozier *et al.*, 2008). More recently, AMEn values of 2,651 and 3,013 kcal/kg were found in broilers during the starter and grower phases, respectively, when fed GLY derived from soybean oil (Tavernari *et al.*, 2022). Other studies have estimated metabolizable energy (ME) values of 3,527 and 3,585 kcal/kg based on the gross energy (GE) content of the sample (Cerrate *et al.*, 2007; Merval *et al.*, 2019). In a performance trial that included 0 to 12% GLY, it was found that productive variables in broilers from 1 to 42 days of age were optimized at a level of 6.60% GLY, while carcass yield was improved at a level of 4.66% GLY (Tavernari *et al.*, 2022). It has also been reported that adding 2.0 to 8.3% GLY to feed throughout the entire broiler growth period promoted better performance efficiency during the 1-7 and 8-21 day stages (Merval *et al.*, 2019). Another study found that up to 6% GLY can be included in the diet of broilers from 22 to 42 days of age without adverse effects on productivity (da Silva *et al.*, 2017). Previously, it had been reported that including 5% GLY had no negative effects on broiler performance (Cerrate *et al.*, 2007; Jung and Batal, 2011a).

The reviewed results indicate that GLY is a good energy source for broiler chickens; however, its energy value depends on the content of other compounds derived from biodiesel production, such as free fatty acids, methanol, and water (Dozier *et al.*, 2011), as well as salt and total ash (Suchý *et al.*, 2011). GLY may also show wide variations in nutrient concentrations; therefore, it is recommended to analyze its energy value before including it in poultry diets (Jung and Batal, 2011b). The hypothesis of this study is that the energy value of GLY derived from recycled oils is lower than that of GLY from non-recycled oils; therefore, lower inclusion levels than those previously recommended may be required to maintain adequate productivity and prevent detrimental effects on the antioxidant status of broiler meat. Accordingly, the objective of this study was to evaluate the AMEn content of GLY and the productive performance, carcass yield, and stability and antioxidant status of meat from broiler chickens fed increasing levels of GLY.

MATERIALS AND METHODS

Experiment 1: Two energy balance trials were conducted in broilers of two different ages housed in metabolism cages equipped with trays for excreta collection (Angeles and Gómez-Rosales, 2021; Angeles *et al.*, 2024). Each trial lasted 10 days, divided into two days of adaptation to the cages, four days of adaptation to the experimental diets, and four days of excreta collection. On the first day of adaptation to the experimental diets, the birds were weighed and randomly assigned to four treatments. Increasing levels of GLY were included in a control diet using the substitution method (Dozier *et al.*, 2008). During the collection period, feed was offered at 95% of the amount consumed on previous days. Total excreta were collected over four consecutive days at 24-hour intervals. After each daily collection, excreta were weighed and frozen. After the last collection, the birds were weighed again (Angeles and Gómez-Rosales, 2021; Angeles *et al.*, 2024).

In the first balance trial, broilers aged 10 to 20 days were used, with two birds per cage, and in the second trial, broilers aged 28 to 38 days were used, with one bird per cage. Both trials had eight replicates per treatment. The treatments were: 1) Control diet formulated based on corn and soybean meal, meeting the nutritional requirements of the broilers; diets 2-4) the control diet was partially substituted with 10%, 20%, and 30% levels of GLY. The GLY was obtained from a company dedicated to biodiesel production by transesterification of recycled oils. In the nutrition laboratory, excreta samples were thawed, lyophilized, and ground. Dry matter (DM) and GE were determined in feed and excreta samples. Based on the results and feed intake data, GE intake on a DM basis was estimated; using the weight of the excreta, GE excretion was estimated; and GE retention was calculated by difference. Subsequently, the AMEn of each diet was calculated using the following formula:

$$AMEn, \text{ kcal / kg} = \frac{Consumed\ energy - \left(Excreted\ energy + \left(8.22 \times \text{g}\ of\ nitrogen\ retained\right)\right)}{\text{g}\ of\ \ feed\ consumed}$$

Based on the obtained results, the proportional AMEn of the control diet was estimated, and by difference, the AMEn of GLY was calculated according to each level of substitution.

Experiment 2. A total of 360 one-day-old broiler chickens were randomly distributed into groups of 20 birds per pen in 18 floor pens. Diets were formulated using corn and soybean meal and divided into two production phases: starter (1-21 days) and grower (21-42 days) (Table 1). The estimated contents of ME digestible lysine, calcium, and available phosphorus were 3060 kcal/kg, 1.22%, 0.98%, and 0.49% for the starter phase, and 3200 kcal/kg, 1.13%, 0.90%, and 0.45% for the grower phase, respectively. The AMEn values for GLY estimated in Experiment 1 were used to formulate the diets supplemented with GLY. Treatments were as follows: 1) Control without GLY; 2) Control + 2.5% GLY; and 3) Control + 5% GLY in both production phases. Diets were offered on free access, and

Table 1. Composition of the experimental diets.

Addition of glycerol, %	Starter			Grower		
	0	2.5	5.0	0	2.5	5.0
Ground corn	55.00	52.93	50.04	57.40	55.22	52.00
Soybean meal	36.10	36.40	36.80	32.20	32.60	33.18
Glycerol	0.00	2.50	5.00	0.00	2.50	5.00
Soybean oil	4.10	3.90	3.90	5.70	5.50	5.60
Ca orthophosphate	1.73	1.73	1.74	1.56	1.56	1.61
Ca carbonate	1.55	1.55	1.54	1.43	1.43	1.43
Salt	0.33	0.00	0.00	0.30	0.00	0.00
Methionine	0.28	0.28	0.28	0.27	0.27	0.27
Lysine	0.23	0.23	0.22	0.24	0.23	0.22
Threonine	0.08	0.08	0.08	0.08	0.07	0.07
Sodium bicarbonate	0.20	0.00	0.00	0.20	0.00	0.00
Minerals	0.10	0.10	0.10	0.10	0.10	0.10
Vitamins	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.10	0.10	0.10	0.07	0.07	0.07
Antibiotic	0.05	0.05	0.05	0.05	0.05	0.05
Nicarbacin	0.05	0.05	0.05	0.00	0.00	0.00
Salinomycin	0.00	0.00	0.00	0.05	0.05	0.05
Pigment	0.00	0.00	0.00	0.25	0.25	0.25

feed intake and feed conversion ratio were recorded. Birds were weighed on days 1 and 42 to calculate weight gain. On day 42, three birds per pen were slaughtered to evaluate carcass weight and yield, as well as its components. Breast meat samples were collected to measure color, pH, and water losses by filtration, centrifugation, and drip loss. Lipid oxidation was determined by the thiobarbituric acid reactive substances (TBARS) assay, and antioxidant status was analyzed by ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity following standardized procedures (Domínguez-Negrete *et al.*, 2021).

Data Analysis

The energy balance results and AMEn were subjected to analysis of variance and simple linear regression to determine the energy value of the diets and the GLY during each collection period. In the productive performance trial, carcass and its parts, and response variables in breast meat, the results were also analyzed using analysis of variance and simple linear regression.

RESULTS AND DISCUSSION

The energy balance results in broilers aged 10-20 days are presented in Table 2. The consumption of diets with increasing levels of GLY had a quadratic effect on DM intake ($y=33.596-0.6247x+0.0373x^2$; $R^2=0.98$; $p\leq0.01$) and GE intake ($y=138.68-1.9116x+0.1445x^2$; $R^2=0.99$; $p\leq0.05$). In both cases, intake was higher in

Table 2. Dry matter and energy balance in broilers aged 10-20 and 28-38 days (Experiment 1).

	Glycerol, %				SEM[a]
	0	10	20	30	
10-20 days of age					
DM intake, g/day	33.66[b]	31.50[c]	31.18[c]	31.40[c]	0.174
DM excretion, g/day	14.42	13.75	13.29	13.25	0.568
DM retention, %	57.19	56.35	57.36	57.81	1.742
Energy					
Intake, kcal/day	138.78[d]	133.03[e]	133.94[e]	135.44[e]	0.740
Excretion, kcal/day	54.16	53.71	53.13	55.01	2.491
Retention, %	61.00	59.63	59.02	60.70	1.805
AMEn, kcal/kg	2400	2395	2440	2480	78.061
AMEn of glycerol, kcal/kg	.	3024	3162	3182	92.477
28-38 days of age					
DM intake, g/day	65.78	63.04	66.32	62.54	1.695
DM excretion, g/day	13.29[f]	14.34[fg]	15.36[fg]	16.45[g]	0.863
DM retention, %	79.79[f]	77.15[fg]	76.83[fg]	73.50[g]	1.348
Energy					
Intake, kcal/day	262.26	271.61	289.61	273.01	7.347
Excretion, kcal/day	49.54[f]	54.20[fg]	58.16[fg]	64.51[g]	3.254
Retention, %	81.10[f]	79.95[fg]	79.92[fg]	76.21[g]	1.231
AMEn, kcal/kg	3049	3237	3303	3098	49.843
AMEn of glycerol, kcal/kg	.	3574	3604	2547	46.224

[a] SEM=Standard error of the mean. [b-c] Quadratic effect of GLY, $p \leq 0.01$.
[d-e] Quadratic effect of GLY. [f-g] Linear effect of GLY, $p \leq 0.01$.

broilers fed the control diet without GLY (0%) compared to broilers fed diets with 10, 20, and 30% GLY. The excretion and retention of DM and the excretion and retention of GE, as well as AMEn, were similar among the different GLY levels. The estimated AMEn for GLY was similar across treatments, averaging 3123 kcal/kg. The AMEn value obtained in broilers aged 10-20 days was higher than the 2651 kcal/kg reported in broilers aged 10-18 days (Tavernari *et al.*, 2022) and lower than the 3621 kcal/kg found in broilers aged 4-11 days (Dozier *et al.*, 2008). This difference was likely due to variations in the composition of the GLY used in each experiment, which affects the amount of GE and its efficiency in being converted to AMEn. For example, the GE content in 10 GLY samples ranged from 3337 to 6742 kcal/kg (Jung and Batal, 2011b), whereas in the present study, the GE of GLY was 5160 kcal/kg. The conversion rate from GE to AMEn was 60.4% (3123 AMEn/5160 GE), indicating that 40% of the energy in GLY was not utilized, probably because the evaluated GLY sample was derived from recycled oils.

The energy balance results in broilers aged 28-38 days are also shown in Table 2. DM and GE intake were similar among treatments. DM excretion increased ($y=13.287+0.1049x$; $R^2=0.99$), whereas DM retention decreased ($y=79.695-0.1919x$; $R^2=0.92$) as the GLY level in the feed increased (linear effect, $p \leq 0.01$). Similarly, GE excretion increased

(y=49.269+0.4887x; R^2=0.99) while GE retention decreased (y=81.502−0.1472x; R^2=0.79) due to the increasing level of GLY in the feed (linear effect, p≤0.01).

Gross energy intake, the AMEn of the diets, and the estimated AMEn of GLY were similar among treatments. On average, the AMEn of GLY was 3,242 kcal/kg. This observed value was higher than the 3,013 kcal/kg AMEn reported in broilers aged 25-33 days (Tavernari *et al.*, 2022), but lower than the 3,331 and 3,349 kcal/kg values reported in broilers aged 17-24 and 38-45 days, respectively (Dozier *et al.*, 2008). In other studies, ME values of 3,527 and 3,585 kcal/kg have been estimated based on the GE content of the sample (Cerrate *et al.*, 2007; Merval *et al.*, 2019). These studies assume a GLY digestibility of 95-98%, whereas in the present study, the estimated utilization rate of GLY was 60.4% and 62.8% during the starter and grower phases, respectively. These results are only close to the 65.5% value found in a GLY sample with a high content of free fatty acids that escape the transesterification process (Dozier *et al.*, 2011). In a study evaluating different GLY samples, it was suggested that their energy value depends on the content of other residual compounds during biodiesel production, such as fatty acids, methanol, and water (Dozier *et al.*, 2011), as well as salt and total ash (Suchý *et al.*, 2011).

The results of productive performance and carcass yield and its parts are presented in Table 3. For the performance trial, the inclusion of GLY was limited to 2.5% and 5.0% because it had a higher GE compared to carbohydrates, but its utilization efficiency was only 60.4-62.8%. This suggests the presence of free fatty acids remaining from the transesterification process, which is considered a factor that may negatively affect broiler growth (Lindblom *et al.*, 2019). Additionally, considering that the evaluated GLY was derived from recycled oils, it has been documented that oil recycling increases oxidation

Table 3. Productive performance and carcass weight and yield and its parts (Experiment 2).

	Glycerol, %			
	0	**2.5**	**5.0**	**SEM[a]**
Body weight at day 1, g	41.56	41.30	42.00	0.429
Body weight at day 42, g	2906.8[b]	3090.7[c]	3118.0[d]	61.407
Feed intale, g/day	104.36	107.18	108.83	1.533
Weight gain, g/day	69.88[b]	74.38[c]	75.02[d]	1.501
Feed conversion	1.50	1.45	1.45	0.037
Weight and yield of carcass				
Breast, g	788.20	821.10	811.61	22.479
Breast, %	26.49	26.23	26.15	0.431
Legs, g	248.35	256.93	253.74	7.940
Legs, %	8.38	8.23	8.21	0.143
Thighs, g	273.33	285.47	278.65	7.950
Thighs, %	9.20	9.13	9.01	0.166
Carcass, g	1309.88	1363.51	1344.00	46.802
Carcass, %	44.08	43.59	43.37	0.434

[a] SEM=Standard error of the mean. [b-d] Linear effect of GLY, p≤0.01.

and rancidity of feed, generating the production of free radicals and, in some cases, the formation of trans fatty acids, which may pose a health risk (Brühl, 2014).

At the end of the floor pen trial, the addition of increasing levels of GLY in the diet had a linear effect ($p \leq 0.05$) on body weight at 42 days ($y=2827.4+105.58x$; $R^2=0.85$) and weight gain ($y=67.955+2.5698x$; $R^2=0.84$). Feed intake and feed conversion ratio were similar among treatments. No differences were found in carcass weight and yield or its parts between broilers fed the control diet and those fed diets with increasing levels of GLY during the starter and grower phases. In a performance trial where 2.0 to 8.3% GLY was included in the feed throughout the entire broiler growing period, it was reported that GLY promoted improved performance efficiency during the 1-7 and 8-21 day stages (Merval *et al.*, 2019). Previously, it had been reported that including 5% GLY had no negative effects on broiler productivity (Cerrate *et al.*, 2007; Jung and Batal, 2011a). Other studies found that up to 6% GLY can be included in broiler diets from 1 to 42 days of age without observing negative effects on productivity or on carcass yield and its parts (da Silva *et al.*, 2017; Araujo *et al.*, 2018; Broch *et al.*, 2018).

The color of the breast meat, pH, water loss, lipid oxidation, and antioxidant status are presented in Table 4. No differences were found among the GLY levels in the feed for any of the evaluated response variables. The potential presence of unsaturated and/or peroxidized free fatty acids in the GLY is considered a factor that may trigger a peroxidation process in the cell membranes of the organism, leading to the production of free radicals and oxidative stress (Brühl, 2014; Lindblom *et al.*, 2019). The results of the present study agree with a research that determined the abundance of genes associated with counteracting reactive oxygen species in the liver and muscle of chickens fed diets supplemented with

Table 4. Color, pH, water losses, lipid oxidation, and antioxidant status of breast meat (Experiment 2).

	Glycerol, %			
	0	**2.5**	**5.0**	**SEM[a]**
Meat color				
L	77.06	76.34	76.71	0.389
a	3.70	3.69	3.69	0.170
b	9.98	9.71	9.54	0.466
pH	6.09	6.07	6.13	0.033
Water loss				
Filtration, %	21.60	19.77	21.02	1.219
Centrifugation, %	15.76	16.50	15.12	2.122
Dripping, %	1.79	1.12	1.47	0.246
Antioxidant status[b]				
TBARS, mg MDA/kg meat	0.92	0.80	0.84	0.136
FRAP, μmol trolox/kg meat	1176.14	1197.90	1174.72	44.637
DPPH, μM trolox/kg meat	177.11	148.92	180.97	15.186

[a] SEM=Standard error of the mean. [b] TBARS, thiobarbituric acid reactive substances; FRAP, ferric reducing antioxidant capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity.

GLY, finding that the inclusion of 6% GLY did not affect the expression of any of these genes (Araujo *et al.*, 2018).

CONCLUSIONS

The AMEn value of the GLY was 3123 and 3242 kcal/kg, showing an energy efficiency of 60.4 and 62.8% in starter and growing broilers, respectively. In floor-reared broilers, the inclusion of 2.5 and 5% of GLY in the diet improved final weight and weight gain, and carcass yields similar to those observed with the control diet. Variables in breast meat, such as color, pH, stability, and those associated with antioxidant status, were similar among treatments. Therefore, it is concluded that up to 5% of GLY derived from recycled oils can be used as an alternative energy source in broilers throughout the entire production cycle.

ACKNOWLEDGMENTS

The funding for this work is gratefully acknowledged from Universidad del Noroeste and the SEMARNAT-SENER Sectoral Fund.

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Colegio de Postgraduados

Non-Centrifugal Sugar Production in Three Mexican States: A Theoretical Review and *in situ* Observations

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ABSTRACT

Objective: To determine the status of non-centrifugal sugar production in the three main producing states of Mexico through a literature review and direct field observations.

Design/methodology/approach: Statistical databases and literary sources were consulted to gather information on non-centrifugal sugar production. Articles were selected based on five criteria to ensure precise information, which was analyzed and complemented with field observations. Additionally, in Veracruz, the locations of the sugar mills were georeferenced.

Results: It was found that sugarcane is produced in all three states, primarily for non-centrifugal sugar production. The production process is similar, with some variations in the type of equipment and materials used, as well as in the presentation and packaging of non-centrifugal sugar for marketing. The state of Veracruz is the most technologically advanced in its production units and stands out for its greater diversification of non-centrifugal sugar products, while San Luis Potosí and Hidalgo continue to rely on traditional methods, infrastructure, and marketing practices.

Limitations on study/implications: Scarse information is available on the topic.

Findings/conclusions: The information presented in this study provides new and relevant data on the current production of non-centrifugal sugar in the three states, particularly from *in situ* observations. Notable findings include the location of sugar mills in Veracruz, variations in sugarcane harvesting methods (burned *vs.* green), different types of sugar mills, mold options, and the various forms of non-centrifugal sugar.

Keywords: sugar mills, production, traditional, burning.

Citation: Santiago-Hernández, E., Soto-Estrada, A., Pérez-Hernández, P., & Pérez-Bautista, J. J. (2025). Non-Centrifugal Sugar Production in Three Mexican States: A Theoretical Review and *in situ* Observations. *Agro Productividad*. https://doi.org/10.32854/nj2sv641

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

Received: July 05, 2024.
Accepted: May 12, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 119-128.

INTRODUCTION

In Mexico, non-centrifugal sugar (NCS) production is a longstanding tradition with significant economic and social importance. This activity has been carried out since the introduction of sugarcane cultivation in 1493, along with the arrival of mills (trapiches, in Spanish), sugarcane grinding, and its by-products (SIAP, 2018). Since then, non-centrifugal sugar has been considered the primary sweetener for farmers and rural inhabitants.

As a natural sweetener, non-centrifugal sugar maintains the nutritional properties of sugarcane juice, mainly sucrose and other minor sugars such as glucose and fructose (Muñoz, 2018). Additionally, unlike refined sugar, it contains minerals, vitamins, and proteins. However, the establishment of sugar refineries has placed non-centrifugal sugar at a disadvantage in terms of consumption (Jaffé, 2015). Nevertheless, some producers continue the tradition of making non-centrifugal sugar, as it represents a source of livelihood for their families and remains an integral part of Mexico's cultural identity since it is commonly used to sweeten beverages, bread, and traditional sweets such as candied pumpkin and cassava, particularly during Día de Muertos celebrations.

Non-centrifugal sugar is produced in eight states in Mexico, with notable production in San Luis Potosí, Hidalgo, and Veracruz (SIACON, 2024). The production process involves extracting juice from sugarcane using mills (trapiches), removing impurities, evaporating the juice through high temperatures, crystallizing the sucrose, and molding it into various shapes and sizes before it cools completely, with these shapes varying by region (Cabrera & Aguilar, 2018).

Despite the importance of NCS, there is limited current information on the process from sugarcane production to its commercialization. Therefore, the objective of this study was to determine the status of non-centrifugal sugar production in the three main producing states in Mexico through a literature review and direct field observations.

MATERIALS AND METHODS

To obtain statistical data related to non-centrifugal sugar production in the states of San Luis Potosí, Hidalgo, and Veracruz, the database of the Sistema de Información Agroalimentaria de Consulta (SIACON) was consulted.

A literature search was conducted in the databases Google Scholar, Scielo, and Redalyc, as they promote the visibility of scientific production in Latin America, a region where non-centrifugal sugar production, including in Mexico, is prominent. Scientific and review articles, book chapters, scientific bulletins, and theses were consulted. The search terms used were non-centrifugal sugar production (10 results), panela (6 results), non-centrifugal sugar (4 results), jaggery (4 results), and rapadura (2 results). Additionally, the "snowball" methodology was employed, where one reference led to others that contributed to a better understanding of the topic.

As an exclusion criterion, studies published more than 10 years ago were discarded, except for those considered relevant for the development of the sections outlined in this document. The document selection criteria were based on the following factors: raw material (sugarcane), type of mill (trapiche), material of the containers used for processing the sugarcane juice, presentation (shape), and quantity of non-centrifugal sugar production. This information was complemented with 95 incidental observations made directly at the mills during field visits across the three states, from September 2023 to June 2024. Specifically, for the state of Veracruz, the locations of the mills were georeferenced. Based on the obtained data, the topics described below were developed.

National Production of Non-Centrifugal Sugar

The most recent information on national NCS production and the main producing states corresponds to the year 2019 (SIACON, 2024). The eight producing states and their respective production are presented in Table 1; San Luis Potosí, Hidalgo, and Veracruz stand out, representing 61.78%, 12.90%, and 11.09% of the total national production, respectively.

In San Luis Potosí, the production activity is concentrated in the Huasteca Potosina region, particularly in the municipalities of San Antonio, Aquismón, Huehuetlán, Tancanhuitz, Tanlajás, Tampamolón, Tamazunchale, and Coxcatlán, where 4,834 producers are reported, of which 98% belong to the Teenek ethnic group and 2% to the Nahuatl (Baca *et al*., 2018). Hidalgo has a non-centrifugal sugar-producing region known as the Huasteca Hidalguense, which includes the municipalities of Huejutla, Atlapexco, San Felipe Orizatlán, Huazalingo, Yahualica, Jaltocán, Xochiatipan, and Huautla (Rivero, 2017). The NCS production in Veracruz is primarily concentrated in the Huatusco non-centrifugal sugar-producing region, consisting of the municipalities of Huatusco, Zentla, Comapa, Totutla, and Sochiapa (Garcia-Barojas *et al*., 2021).

Sugarcane Production for Non-Centrifugal Sugar

Table 1 presents the current data on the planted area and sugarcane production for non-centrifugal sugar production. These data have fluctuated over time. For example, during the 2010-2019 period, the total planted area decreased from 14,059 ha in 2010 to 10,821.93 ha in 2019 (SIACON, 2024).

According to the observations made in the three states, non-centrifugal sugar producers grow their own sugarcane to supply their mills; when the supply is insufficient, they purchase sugarcane from other producers. To produce one ton of sweetener, 10 tons of sugarcane are required, which aligns with the data in Table 1, where, for example, 177,849.44 t of sugarcane correspond to 17,784.94 t of NCS. Prior to harvesting, in Hidalgo and San Luis Potosí, the sugarcane is not burned before cutting, which is an environmentally friendly practice; in Veracruz, it can be either burned, unburned, or both. In all three states,

Table 1. Non-Centrifugal sugar producing states and their respective sugarcane production in 2019.

State	Non-centrifugal sugar production (t)	Sugarcane surface (ha)	Sugarcane production (t)
San Luis Potosí	17,784.94	7,711.68	177,849.44
Hidalgo	3,714.08	1,196.75	37,140.77
Veracruz	3,193.08	998.00	31,930.78
Nayarit	2,163.80	276.00	21,638.00
Oaxaca	1,728.12	546.50	17,281.29
Chiapas	99.16	21.00	991.62
Puebla	80.30	58.00	803.00
Michoacán	19.80	14.00	198.00
Total	28,783.28	10,821.93	287,832.90

SIACON, 2024.

harvesting is done manually. Harvesting occurs during a single period when the sugarcane is mature. This contrasts with the method used in India, where harvesting is performed using the thinning method, which consists of cutting the mature sugarcane and leaving the immature sugarcane for later harvest (Ordoñez-Díaz and Rue-da-Quiñónez, 2017).

Sugarcane Transportation

In the Huasteca Potosina region, sugarcane is transported from the fields to the mills using pack animals (horses and donkeys) where the terrain is difficult to access (Muñiz-Márquez *et al.*, 2017). In Hidalgo, some producers were observed carrying rolls of sugarcane on their shoulders, because their mills were constructed next to their plots of land. In Veracruz, trucks are used due to the larger volumes of harvested sugarcane.

Number and Type of Mills

The number of mills in each state is not fully documented, except in Veracruz. Cabrera and Aguilar (2019) report 650 mills in the state, of which 450 are located in the central region. These data are consistent with field observations, where 88 mills were located, 85 in the Huatusco region and three in the municipality of Atzalan (Zapotitlán locality). Although this number is low compared to the reported figures, the contribution lies in providing their locations (Table 2), which would facilitate their identification.

In San Luis Potosí, it was found that there are two types of mills; the most common is the diesel engine-powered mill, and the electric one is rare. In Hidalgo, most mills are made of wood with animal traction, followed by iron mills with animal traction, and rarely diesel engine-powered mills. Diesel engine and electric mills are more frequent in Veracruz. Morales-Ramos *et al.* (2017) state that in the Huasteca Potosina, the diesel engine mill is common, while in Veracruz, the electric one is more common, which coincides with our findings. Likewise, our data are consistent with those of Rodríguez-Borray *et al.* (2022), who report that in Colombia, mills are powered by animal traction or engines.

A relevant observation in Veracruz was the presence of two electric motor-powered mills in series. Their operation involves crushing the sugarcane in the first mill and then using the resulting bagasse to be crushed in the second mill, with the aim of extracting all the juice. This is an innovative technology similar to the double mill system used in Colombia, which consists of two mills mounted on a single base with two levels, so that the first mill, which receives the cane, is positioned higher than the second one; the second mill then processes the cane again (Hernández-Cely & Torres Zamudio, 2021).

Non-Centrifugal Sugar Production Process

This process begins with the crushing of the sugarcane in the mills. An extraordinary practice in San Luis Potosí is washing the sugarcane prior to crushing (Muñiz-Márquez *et al.*, 2017), which, according to our observations, does not occur in Hidalgo and Veracruz; in the latter state, the sugarcane is even milled with either burned or green leaves. The juice extracted from the cane falls into plastic tubs, mainly in San Luis Potosí and Hidalgo, while in Veracruz, these are made of galvanized sheet metal or stainless steel. The juice is clarified by separating the impurities (Morales-Ramos *et al.*, 2017); these authors report the

Table 2. Location of sugar mills in the non-centrifugal sugar region of Veracruz.

Municipality	Locality	Latitude	Longitude	Altitude	Exactitude
Comapa	Boca del Monte	19.15196106°	−96.84443769°	910.0 m	3.7900925 m
Comapa	Boca del Monte	19.15348371°	−96.80960776°	908.0 m	8.434771 m
Comapa	Boca del Monte	19.1398491°	−96.81765721°	988.0 m	3.7900925 m
Comapa	Boca del Monte	19.13873563°	−96.80207401°	778.0 m	5.7422833 m
Comapa	Boca del Monte	19.13299311°	−96.81318269°	816.0 m	5.006217 m
Comapa	El Platanar	19.1248426°	−96.8069486°	768.0 m	4.56 m
Comapa	El Platanar	19.12254654°	−96.80352276°	768.0 m	5.7722464 m
Comapa	El platanar	19.1235202°	−96.79518136°	768.0 m	10.719584 m
Comapa	Pochote Nuevo	19.10408388°	−96.77425093°	660.0 m	9.935046 m
Comapa	Pochote Nuevo	19.10368561°	−96.76707819°	660.0 m	4.8223195 m
Comapa	Comalcuavitl	19.11010754°	−96.76350391°	660.0 m	5.747801 m
Comapa	El Paraíso	19.14491484°	−96.74970803°	662.0 m	3.7900925 m
Comapa	Palo Verde	19.13991975°	−96.7386071°	662.0 m	9.935046 m
Comapa	Paso Pimiento	19.12609221°	−96.69701838°	643.0 m	3.7900925 m
Comapa	Paso Pimiento	19.12545068°	−96.69550698°	643.0 m	3.7900925 m
Comapa	San Felipe	19.1287413°	−96.69991776°	643.0 m	3.7900925 m
Comapa	San Felipe	19.14734847°	−96.69259445°	546.0 m	3.7900925 m
Comapa	San Felipe	19.14425009°	−96.69460251°	546.0 m	3.7900925 m
Comapa	San Felipe	19.12869347°	−96.69975731°	819.0 m	3.7900925 m
Comapa	San Felipe	19.13484162°	−96.69993337°	819.0 m	3.7900925 m
Comapa	San Felipe	19.13472925°	−96.70040562°	819.0 m	3.7900925 m
Comapa	San Felipe	19.13454258°	−96.70073847°	819.0 m	3.7900925 m
Comapa	San Felipe	19.13322192°	−96.70216677°	819.0 m	3.7900925 m
Comapa	San Felipe	19.12696607°	−96.70305324°	819.0 m	3.7900925 m
Comapa	San Felipe	19.12706526°	−96.70282402°	819.0 m	3.7900925 m
Comapa	San Felipe	19.12586272 °	−96.69704892°	819.0 m	3.7900925 m
Comapa	San Felipe	19.12531352°	−96.69554746°	819.0 m	3.7900925 m
Comapa	La Divina Providencia	19.17104387°	−96.86345559°	988.0 m	3.7900925 m
Comapa	El Coyol	19.17598039°	−96.69341114°	546.0 m	3.7900925 m
Zentla	La Flor	19.05480826°	−96.70715954°	501.0 m	5.9501853 m
Zentla	Pueblito de Matlaluca	19.06148242°	−96.78588498°	689.0 m	3.7900925 m
Zentla	Pueblito de Matlaluca	19.06327292°	−96.78934917°	705.0 m	3.7900925 m
Zentla	Pueblito de Matlaluca	19.06881671°	−96.78408779°	686.0 m	3.7900925 m
Zentla	Pueblito de Matlaluca	19.07124945°	−96.79669257°	723.0 m	3.7900925 m
Zentla	Pueblito de Matlaluca	19.09911351°	−96.83905346°	721.0 m	3.7900925 m
Zentla	Tenanzintla	19.0747807°	−96.79681944°	717.0 m	3.7900925 m
Zentla	Tenanzintla	19.07392972°	−96.792479473°	711.0 m	3.7900925 m
Zentla	Col. Manuel González	19.10416048°	−96.86784044°	947.0 m	3.7900925 m
Zentla	Col. Manuel González	19.10419303°	−96.86808488 °	931.0 m	3.7900925 m

Table 2. Continues...

Municipality	Locality	Latitude	Longitude	Altitude	Exactitude
Zentla	Col. Manuel González	19.11285977°	−96.85573589°	929.0 m	4.552489 m
Zentla	Col. Manuel González	19.11548793°	−96.86648455°	975.0 m	4.077302 m
Zentla	Maromilla	19.0414432°	−96.78957062°	654.0 m	5.059679 m
Zentla	Maromilla	19.03926496°	−96.78663428°	646.0 m	5.5583487 m
Zentla	Ejido la Piña	19.02844984°	−96.78256556°	637.0 m	3.7900925 m
Zentla	Ejido la Piña	19.02905767°	−96.78170286°	637.0 m	3.9529984 m
Zentla	Ejido la Piña	19.02798115°	−96.77930254°	631.0 m	3.7900925 m
Zentla	Ejido la Piña	19.02556802°	−96.77845316°	631.0 m	3.7900925 m
Zentla	Ejido la Piña	19.03079953°	−96.78583853°	631.0 m	9.935046 m
Zentla	Ejido la Piña	19.02839495°	−96.79299528°	631.0 m	3.7900925 m
Zentla	Ejido la Piña	19.03331286°	−96.80526003°	631.0 m	3.7900925 m
Huatusco	El Ocote	19.128115183°	−96.90397489°	1064.0 m	3.7900925 m
Huatusco	Sabanas	19.13802801°	−96.93106863°	1149.0 m	3.7900925 m
Huatusco	Capulapa	19.09906139°	−96.88403782°	949.0 m	3.7900925 m
Huatusco	Capulapa	19.09965357°	−96.88143887°	941.0 m	4.1745257 m
Huatusco	Cotecontla	19.0964532°	−96.89247157°	979.0 m	9.935046 m
Huatusco	Carrizal	19.1021776°	−96.9038511°	1020.0 m	3.7900925 m
Huatusco	Cotecontla	19.1184295°	−96.90823108°	1063.0 m	3.7900925 m
Huatusco	Cotecontla	19.12353567°	−96.91500292°	1098.0 m	3.7900925 m
Totutla	Tlapala	19.26329697°	−96.91887461°	31.0 m	4.3579392 m
Totutla	Tlapala	19.27022282°	−96.91213209°	1060.0 m	3.9121404 m
Totutla	Tlapala	19.27076912°	−96.91167141°	1060.0 m	5.527287 m
Totutla	Tlapala	19.27150751°	−96.910445°	1060.0 m	7.307498 m
Totutla	Tlapala	19.27220657°	−96.90969235°	1060.0 m	7.31859 m
Totutla	Tlapala	19.27303559°	−96.90749074°	1043.0 m	3.7900925 m
Totutla	Tlapala	19.275535°	−96.894554912°	976.0 m	3.7900925 m
Totutla	Santuario	19.27660548°	−96.89483617°	976.0 m	4.1985655 m
Totutla	Santuario	19.275535°	−96.894554912°	976.0 m	3.7900925 m
Totutla	Santuario	19.27462936°	−96.89823463°	976.0 m	4.4797688 m
Totutla	Santuario	19.2721348°	−96.90102286°	976.0 m	9.935046 m
Totutla	Santuario	19.270569648°	−96.90280111°	976.0 m	9.935046 m
Totutla	Rincón de Cacahualco	19.23968929°	−96.91641857°	1190.0 m	3.7900925 m
Totutla	Rincón de Cacahualco	19.26143569°	−96.893715°	1007.0 m	8.133801 m
Totutla	Rincón de Cacahualco	19.26145294°	−96.89369947°	1007.0 m	6.274433 m
Totutla	Rincón de Cacahualco	19.2642383°	−96.88977546°	979.0 m	4.542377 m
Totutla	Rincón de Cacahualco	19.2639108°	−96.88862514°	982.0 m	4.3063827 m
Totutla	Rincón de Cacahualco	19.26372804°	−96.8876152°	982.0 m	3.411135 m
Totutla	Rincón de Cacahualco	19.26480562°	−96.88456159°	981.0 m	9.935046 m
Totutla	Rincón de Cacahualco	19.26492345°	−96.88292768°	974.0 m	3.7900925 m

Table 2. Continues...

Municipality	Locality	Latitude	Longitude	Altitude	Exactitude
Totutla	El Capricho	19.20790827°	−96.94552391°	1190.0 m	3.7900925 m
Sochiapa	Tomatlancillo	19.17328623°	−96.92812966°	1226.0 m	3.7900925 m
Sochiapa	Tomatlancillo	19.17329772°	−96.92814135°	1226.0 m	3.7900925 m
Sochiapa	Rancho Nuevo	19.17035785°	−96.92153386°	1190.0 m	3.7900925 m
Sochiapa	2 de Abril	19.04784802°	−96.81106836°	725.0 m	4.084675 m
Sochiapa	2 de Abril	19.04551479°	−96.81396418°	755.0 m	3.7900925 m
Tomatlán	Tomatlán	19.02110701°	−97.01239769°	1327.0 m	3.7900925 m
Atzalan	Zapotitlán	19.81608526°	−97.15944212°	1014.0 m	3.7900925 m
Atzalan	Zapotitlán	19.82216884°	−97.15612775°	866.0 m	3.7900925 m
Atzalan	Zapotitlán	19.83274418°	−97.15910054°	814.0 m	3.7900925 m

Data taken with the Note Cam application.

use of lime slurry (Ca (OH)2) to control the pH and induce the precipitation of impurities, a practice observed in all three states. For this process, the use of clarifying agents or plants with agglutinating properties is also reported (Quezada-Moreno & Gallardo-Aguilar, 2014; Hernández-Cely & Torres-Zamudio, 2021). Other alternatives to Ca (OH)2 could include sodium carbonate, sodium bicarbonate, superphosphate, alum, or sodium hydrosulfate (Kumbhar, 2016).

After clarifying the juice, evaporation is performed to increase the sucrose content (Morales-Ramos *et al.*, 2017). For this, open pands (punteras or pailas, in Spanish), are used, generally made of stainless steel (Muñiz-Márquez *et al.*, 2017); however, it was recorded that these pands are mostly made of sheet metal and stainless steel in Hidalgo and Veracruz, respectively. The pands are placed over a furnace, whose combustion source is the bagasse obtained from the crushing process (Muñiz-Márquez *et al.*, 2017) once it is dry; some producers in Hidalgo also use firewood in smaller quantities.

After evaporation, the process concentration (punteo, in Spanish) is carried out, which involves stirring the syrup to check its viscosity and identify the right moment for cooling and molding (Muñiz-Márquez *et al.*, 2017). This process varies in each state; thus, in SLP and Hidalgo, the same pand used for evaporation is also used for concentration. In contrast, in Veracruz, producers use three to five pands. When three are used, the first one is used to remove remaining impurities, the second undergoes complete evaporation, and the third is for concentration. In the case of five pands, the first two are for impurity removal, the third and fourth are for evaporation (known as consumer 1 and consumer 2), and the last one is for concentration.

Molding varies in each state. Muñiz-Márquez *et al.* (2017) report the use of wooden molds in SLP for NCS in the shape of a cone. However, communication from some producers in this state indicates the use of clay molds; in Hidalgo, only clay molds are used. The use of mango wood molds was also confirmed in Veracruz, which are rectangular prisms approximately 1 meter long with 12, 28, and 30 cavities for NCS in the shape of a cone, pirinola, and stopper, respectively. Other molds measuring 1.30 meters long by 40 cm wide with 78 cavities are used for the truncated cone shape. The molds are filled prior to a stirring process, which may be manual (SLP and Hidalgo) or mechanical (Veracruz). After filling, the molds are left to cool for the crystallization of the non-centrifugal sugar, followed by the unmolding.

Presentations or Shapes of Non-Centrifugal Sugar

There are various presentations or shapes of non-centrifugal sugar, as well as different colors and sizes (Cabrera and Aguilar, 2019). In SLP, the most common presentations are granulated and cone (Romero *et al.*, 2011; Baca *et al.*, 2018); the latter presentation weighs 250 g (Muñiz-Márquez *et al.*, 2017). In addition to these reports, it was observed in this state that there are also cones that weigh 1 kg. In Hidalgo, the main presentation found is the "mancuerna" (two cones joined at their base and wrapped with sugarcane leaves) weighing 2 kg, and occasionally one weighing 85 g (Figure 1). In Veracruz, a greater variability of presentations was detected: cone, truncated cone, stopper, pirinola, circular (cheese), and bar (soap) with average weights of 250, 180, 45, 39, 466, and 510 g, respectively (Figure 1). The cone is the most common form in the non-centrifugal sugar-producing region of Huatusco, followed by the circular and bar forms. The truncated cone proved to be typical of the municipality of Atzalan. The wooden molds facilitate the unmolding process. Another presentation is granulated non-centrifugal sugar, found in SLP (Muñiz-Márquez *et al.*, 2017; Baca *et al.*, 2018) and in the Huatusco region (Cabrera and Aguilar, 2018). This presentation has not been adopted in the production areas as much as the other forms; it was recorded that only the companies Tzejkom Tzimaxtalab and Endulzantes La Esmeralda S.P.R. de R.L. from SLP and Veracruz, respectively, produce this type of NCS.

Among these presentations, the circular (cheese), bar (soap), and granulated shapes are also produced in Colombia, Brazil, and India (Barbosa *et al.*, 2016; Kumbhar, 2016;

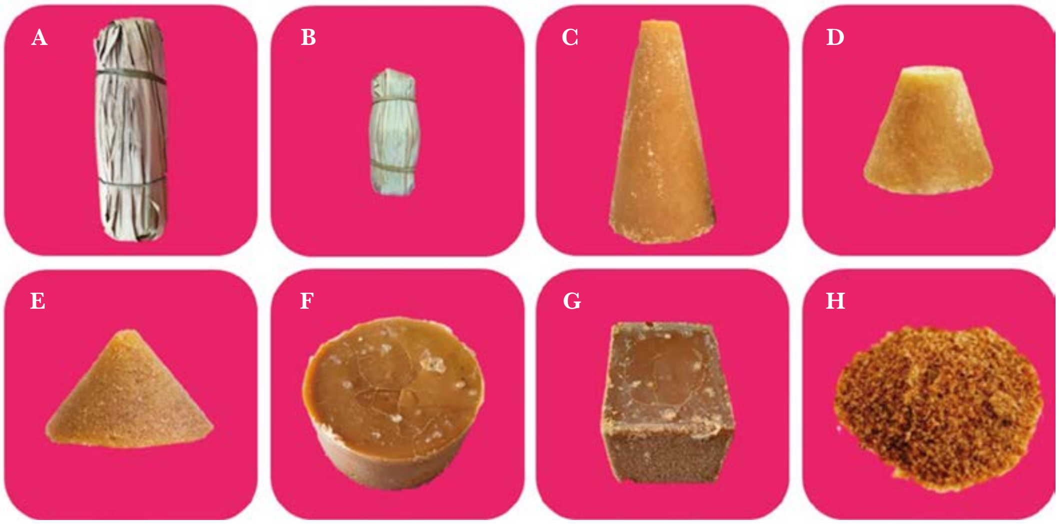

Figure 1. Presentations and weights of non-centrifugal sugar in three states of Mexico. A) "mancuerna" (2 kg), B) "mancuerna" (85 g), C) cone (250 g), D) stopper (45 g), E) pirinola (39 g), F) cheese (466 g), G) bar (510 g), and H) granulated (500 and 1000 g).

Gutiérrez-Mosquera *et al*., 2018); however, in India, the individual weights are larger (1, 5, 10, 19, 20, and 30 kg). Additionally, Ingale *et al.* (2024) report the production of liquid non-centrifugal sugar. This last presentation and the granulated form represent an opportunity area for enhancing the competitiveness of the product in Mexico.

Marketing

In San Luis Potosí, marketing is done through regional collectors or intermediaries (Baca *et al*., 2018); however, according to our findings, the representative from the company Tzejkom Tzimaxtalab states that they currently have direct customers, which results in higher profits.

In Hidalgo, marketing is done by selling “mancuernas” in retail markets, through community rounds, or to tourists. Wholesale sales are made to intermediaries and cane liquor ("aguardiente") producers, and the product is sold in sacks containing approximately 80 “mancuernas”. The production is aimed at the local or municipal market.

In the Huatusco region, during months with excess supply (April to October), production is stored in large distribution centers and marketed from November to March (Cabrera and Aguilar, 2018). Additionally, our findings show that marketing is done wholesale to collectors of the wholesale markets of Veracruz, Mexico City, Monterrey, and Guadalajara, primarily. The product is packed in cardboard boxes. Occasionally, the company La Esmeralda sells retail directly from the factory, via its website, or on Mercado Libre.

Marketing in San Luis Potosí and Veracruz is broader, mainly for the Huatusco region. This is due to the geographic location and existing communication routes, which make it possible for NCS marketing to reach important markets (García-Barojas *et al*., 2021).

CONCLUSIONS

The information provided in this work contributes new and relevant insights into the current production of non-centrifugal sugar in the three main producing states: San Luis Potosí, Hidalgo, and Veracruz. A significant contribution is the results from the *in situ* observations, whose data had been either scarcely reported or not reported at all. Among these, the location of the sugar mills in Veracruz, variations in the sugarcane harvesting methods (burning and green cutting), optional types of sugar mills, mold types, and the different shapes of non-centrifugal sugar stand out.

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Colegio de Postgraduados

Microencapsulation of anthocyanins from *Hibiscus sabdariffa* and the association of stability and phenolic compounds and antioxidant activities

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Citation: Vargas-Álvarez, D., Matus-Reyes, L., Moreno-Dimas, V., Cruz-Cruz., E., Flaviano-Godínez, & Damián-Nava, A. (2025). Microencapsulation of anthocyanins from *Hibiscus sabdariffa* and the association of stability and phenolic compounds and antioxidant activities. *Agro Productividad*. https://doi.org/10.32854/f9fy5n89

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

Received: October 30, 2024.
Accepted: May 26, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 129-140.

ABSTRACT

Objective: To design an extraction, characterization, and microencapsulation process for two *Hibiscus* (Sudan and Tecoanapa) cultivars.

Design/Methodology/Approach: The plant material was collected. The samples were first crushed and then optimized with water and alcohol. Afterwards, the extract obtained in this process was microencapsulated. Anthocyanin content and antioxidant activity were evaluated, before and after the treatment. Finally, the efficiency of the preserved extract was determined.

Results: The Sudan cultivar recorded a higher anthocyanin content (1,319.8 mg 100 g^{-1}) than Tecoanapa (557.2 mg 100 g^{-1}). Maltodextrin was the best microencapsulation process for the extracted and encapsulated matrix of Sudan. The matrix had phenols and anthocyanins. The size of the particles recorded a 11.50±0.5 μm diameter. In addition, microencapsulation efficiency reached 85%±5%.

Study Limitations/Implications: The preservation of extracted matrices can be profitable, because it keeps the color and health benefits of the flower; however, the equipment required for the microencapsulation process is very expensive.

Findings/Conclusions: Maltodextrin was the best resin for this procedure, recording a higher percentage of matrix preservation than phenols and anthocyanins.

Keywords: resin, preservation, anthocyanins, maltodextrin, phenols.

INTRODUCTION

Jamaica belongs to the family Malvaceae (Jung and Joo, 2013). This plant has different names in different countries: roselle (UK, USA), karkadé (Arabia, Sudan), byssap (Senegal), l'oiselle (France), alazán (Caribbean), krachiap daeng (Thailand), and *jamaica* (Mexico and Spain) (Ramírez-Rodrigues *et al.*, 2012). The calyces of *Hibiscus* are used to prepare cold and hot beverages, as well as marmalade and jelly (Mohamed *et al.*,

2012). *Jamaica* is widely consumed by Mexicans, including children and elders (Mercado-Mercado, 2015). In addition, *Hibiscus* is used all over the world to treat different diseases (Picking D., *et al.*, 2011).

Sudan, Thailand, China, Mexico, Egypt, Senegal, and Tanzania are the major producers of *Hibiscus* (Villanueva-Carvajal *et al.*, 2013). In Mexico, *jamaica* is grown in Oaxaca, Michoacan, Nayarit, Puebla, and Guerrero (Pérez-Torres, 2009). According to the Servicio de Información Agroalimentaria y Pesquera (SIAP) of SAGARPA, Guerrero is the major jamaica producer of the country. The municipality of Ayutla de los Libres is the main producer of Guerrero, followed by Tecoanapa, San Luis Acatlán, and Acapulco de Juárez (SIAP, 2023).

During the last few years, researchers worldwide have been focused on discovering the full potential of medicinal plants grown under traditional systems. *H. sabdariffa* is one of several medicinal plants that have been studied and that could be used as a powerful phytochemical agent to treat different diseases (Montaño *et al.*, 2024). The therapeutic effects of *H. sabdariffa* have been associated with bioactive and functional components, such as phenolic acids, flavonoids (Vargas *et al.*, 2018), anthocyanins, organic acids, and dietary fiber (Izquierdo-Vega *et al.*, 2020). Given their biological activities, the bioactive compounds found in the calyces are used as anti-inflammatory, hepatoprotective, antihypertensive, antimicrobial, and antioxidant agents (Montalvo-González *et al.*, 2022; Ghazala-Riaz *et al.*, 2018).

Anthocyanin content is responsible for the characteristic red color of the calyx of *jamaica* flowers (Sumaya *et al.*, 2014). Anthocyanins attract pollinators, which spread the seeds of the plants and protect them from the effect of UV radiation and viral and microbial infections (Garzón, 2008). However, factors such as pH, temperature, light, antioxidants, and metal ions impact anthocyanin stability (Xue *et al.*, 2024). Microencapsulation is a great option for the food industry to preserve bioactive compounds that could be added to the matrices as food supplements in order to develop functional food (Aguilera-Chávez *et al.*, 2022). Therefore, the objective of this research was to design an extraction, characterization, and microencapsulation process for two jamaica cultivars. The hypothesis was that protective resins can be used to preserve the anthocyanins and phenols responsible for biological activities.

MATERIALS AND METHODS

Samples from the Sudan and Tecoanapa varieties were collected in the community of Tecoanapa, Guerrero. The samples of the two *jamaica* (*Hibiscus sabdariffa* L.) varieties were transported in bags. The *jamaica* calyces were dried in the shade for four days. During the evenings, the samples were left in the open, covered with a waterproof coat. Afterwards, a hammer mill was used to crush the samples until they reached the desired particle size. Finally, the samples were placed in amber glass jars. A thin-layer chromatography (TLC) was used for the preliminary analysis. In order to determine the presence of flavonoids, a mobile phase was conducted with 100 ethyl-acetate: 26 formic acid: 26 acetic acid: 1 water. For this purpose, 0.5g of dry and crushed plant material and seeds of the Sudan and Tecoanapa varieties were weighted. The sample was placed in a test tube with 15 mL of distilled water. Subsequently, it was subjected to a bain-marie for 20 minutes at 60 °C. Afterwards, the content was filtered, in order to obtain a first extract of the calyces and the seeds of *H. sabdariffa*. Three-mL of this extract were poured into three test tubes. Three drops of olive oil were added to the first test tube; the test tube was then stirred to observe the foam and to detect saponin content. Three drops of hydrochloric acid were added to the second test tube to establish the presence of flavonoids. Finally, three drops of ferric chloride (3%) were added to the third test tube to detect tannin content. Based on the preliminary analysis, the following extracts were then obtained from the calyces: cold water, methanol, and ethanol.

Subsequently, 4 g of dry and crushed plant material were added to the three extraction solvents (30 mL of methanol, ethanol, and cold water). The 6-hour extraction process was carried out at room temperature. Four g of dry and crushed plant material were used to prepare boiling aqueous extracts, with different extraction times (3, 5, and 10 min). Once the boiling time of each extraction was over, they were filtered and stored at 4 °C.

Quantification of phenolic content

The Folin-Ciocalteu method described by Waterman and Mole (1994) was used to determine the total phenolic content. One g of calyx sample of each of the two varieties and 5 mL of 80% methanol were weighted. The sample was centrifuged for 20 minutes. Subsequently, 250 μL of the sample and 750 μL of distilled water were poured into test tubes. Afterwards, 250 μL of this solution were placed in test tubes, where 15.75 mL of deionized water and 1 mL of Folin reagent were added. After one minute (but not longer than 8 minutes), 3 mL of 20% Na_2CO_3 were added to the mixture. The mixture was rested for two hours in the dark. The readings were taken at 760 nm, with a medium sensitivity and visible light. Deionized water was used as blank. Based on the calibration curve, the phenolic concentration of the sample was calculated and expressed in mg L^{-1} of gallic acid.

Determination of anthocyanin content

The method described by Nakata (2014) was used to determine anthocyanin content. Ten mL of HCl 0.1 N were added to a test tube in which 0.1 g of dry and crushed *jamaica* calyx had been placed. The mixture was stirred for 10 minutes. Afterwards, it was centrifuged at 3,500 rpm, for 30 minutes, to clarify the solution. Absorbance was taken at 516 nm. The molar extinction coefficient was used for the calculations.

Determination of antioxidant activity (DPPH)

The DPPH method described by Brand-Williams (1995) was used to determine antioxidant activity. In order to determine antioxidant activity, 30 μL of methanol extract were added to each sample, along with 2 mL of DPPH. The mixture was rested for two hours in the dark. Subsequently, an Unico® 2800 UV/VIS spectrophotometer was used to measure absorbance at 517 nm. Antioxidant activity was expressed as an inhibition percentage. The results were expressed in IC50.

Microencapsulation

Aqueous extracts

Based on the total phenolic and monomeric anthocyanin content and the antioxidant activity of 10 g of Sudan and 40 g of Tecoanapa, the extract was analyzed in a boiling aqueous system for 25 minutes.

Control extract

Five mL of aqueous extract were placed in an amber glass jar and kept in the dark, at room temperature, for a month.

Emulsion preparation

Core Gum E0 gum arabic (Gomas Naturales, S.A. de C.V., México) and maltodextrin (with a 10 dextrose) were used. Sixty g of gum arabic and 40 g of maltodextrin were weighted in a beaker for the C1, S1, C2, and S2 extracts. All the extracts were gauged at 1,000 ml in the appropriate aqueous extract. The final arrangement for Tecoanapa (C) and Sudan (S) was as follows: two concentrations for C1 and S1 (10 g L^{-1}) and C2 and S2 (40 g L^{-1}) and two resins (R1 and R2) for the microencapsulates (maltodextrin:gum arabic). The R1 and R2 ratios were 60M:40GA and 40M:60GA, respectively. In addition, three types of extracts were used: aqueous extract (E), microcapsules (M), and control extract (T).

Microencapsulation

The spray drying process was carried out with a Niro Mobile Minor® R&D Spray Dryer (Niro Atomizer, Denmark). The feed volumetric flux reached 2.77 mL min^{-1}, with an air pressure of 2.8 bars, inlet air of 160 °C, and outlet air of 60 °C.

Statistical analysis

The data of each measurement was used to develop a database in the R 4.0.3 statistical package, which was also used to conduct a statistical analysis. Parameters such as means, standard deviation, variances, and ranges were determined based on the distribution of each variable. ANOVA and Tukey's Test were used to compare groups. A $P<0.01$ value was statistically significant in the design for the two varieties of *jamaica*, the two resins, and the type of extract.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis showed a higher tannin content in the Sudan variety than in the Tecoanapa variety. The anthocyanin, saponin, flavonoid, and potential antioxidant contents were similar in both varieties. The content of the seeds did not record variations; however, they did have saponins (Table 1).

The phytochemical composition of the calyces of the Sudan and Tecoanapa varieties recorded an anthocyanin content difference in preliminary analysis. Table 1 shows that the Sudan variety recorded a higher anthocyanin content (1,319.8±265.8 mg 100 g) than the Tecoanapa variety (557.2±5.5 mg L^{-1}). Nevertheless, both cultivars recorded a 63% antioxidant activity (minimum difference). Meanwhile, consumers prefer the Sudan variety

Table 1. Comparison between the organs of two *jamaica* varieties (Sudan and Tecoanapa).

Plant material	Saponins	Tannins	Flavonoids	Anthocyanins (mg L^{-1})	Antioxidant potential (%)
Cáliz Sudan	(+)	(+++)	(+)	1319.8±265.8	63.7±3.5
Cáliz Tecoanapa	(+)	(++)	(+)	557.2±5.5	63.2±3.4
Semilla Sudan	(+)	(−)	(−)	ND	ND
Semilla Tecoanapa	(+)	(−)	(−)	ND	ND

ND=Not determined. Mean ± Standard deviation (n=5).

due to its intense color and flavor, which are a result of its high anthocyanin concentration. Ramírez-Cortés *et al.* (2011) reported concentrations ranging from 205.93 to 1,110.74 mg 100 g, while Salinas-Moreno *et al.* (2012) registered a concentration of 1,488 mg 100 g in dry calyces of *H. sabdariffa*. Meanwhile, Cid-Ortega *et al.* (2012) pointed out that the percentage or number of nutritional compounds are different in each *jamaica* variety, depending on the part of the plant where they are found. Consequently, the percentage is different in calyces, dry leaves, and even in seeds.

Figure 2 shows that the Sudan (1.39±0.22 mg gallic acid 100 g) and Tecoanapa (31±0.17 mg gallic acid 100 g) varieties did not record differences in the total phenolic content. These results can be the consequence of the quantification of the total phenolic fraction using the Folin-Ciocalteu method. This method includes anthocyanins and flavonoids (flavone, isoflavonoids, flavonols, and tannins), which also have antioxidant activities. Mercado-Mercado *et al.* (2015) associated the values of the antioxidant capacity with the molecular structure of the phenolic compound found in *jamaica* calyces.

Most of the studies agree that the beneficial effects of *jamaica* are mainly a consequence of its anthocyanin, phenolic acid, and flavonoid content (Izquierdo-Vega *et al.*, 2020). However, the antioxidant activity recorded in this study is not directly associated with anthocyanin content, but rather with other metabolites or a mixture of metabolites. Cid-Ortega and Guerrero-Beltran (2012) pointed out that the content of bioactive compounds (such as phenols and anthocyanins) depends on the *jamaica* variety and the extraction method. The solvents used to obtain metabolites were cold and hot water, along with

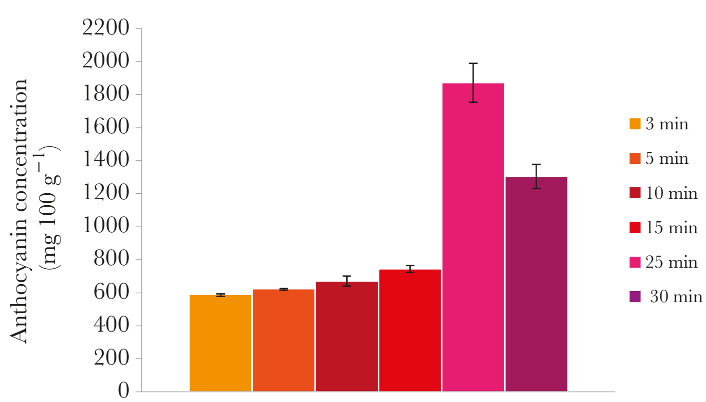

Figure 1. Anthocyanin concentrations of the Sudan variety, based on different extraction times and using boiling water. Mean values ± standard deviation (n=3).

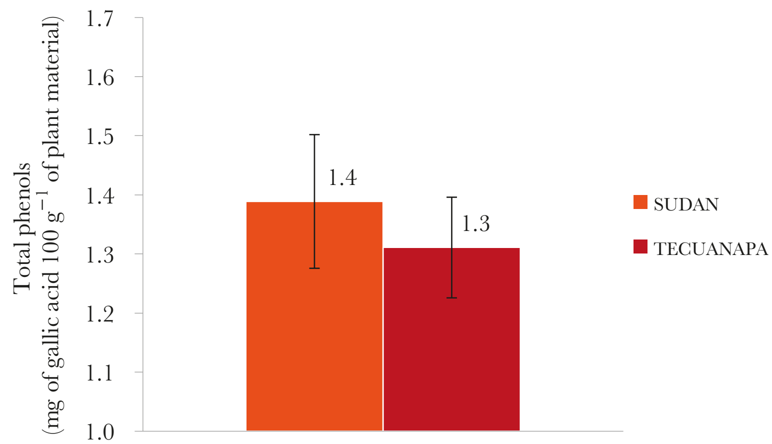

Figure 2. Comparison of the total phenolic content between the Sudan and Tecoanapa cultivars. Means ± standard deviation (n=3).

methanol and ethanol. Hot water was the best solvent for the extraction. Hot water is the most polar of the solvents used in this study, because its temperature produces a higher membrane permeability and increases the exit of secondary metabolites to the extraction medium. In addition, increasing the exposure time of the plant material to boiling water increases the concentration of the anthocyanins extracted. Although anthocyanins are thermolabile, no reduction in their concentration was recorded. This phenomenon suggests that the exposure time could be extended until a significant reduction of the anthocyanin content takes place. This method will establish the appropriate exposure time for the extraction (Table 2). Meanwhile, ethanol was less efficient than methanol (Table 3). However, methanol is toxic and its use is not recommended in food (Salinas-Moreno *et al.*, 2005). Nevertheless, Mercado-Mercado *et al.* (2015) used methanol-acetone extracts to obtain antioxidant activity from *H. sabdariffa*. Izquierdo-Vega *et al.* (2020) explained that, since *Jamaica* calyces are mostly used to prepare cold, warm (tea), and fermented beverages, most researchers work with these aqueous extracts for their analyses. In addition, Salinas-

Table 2. Comparison of the extraction time of anthocyanins using water.

Plant material	Anthocyanin content (mg L^{-1})		
	3 min	5 min	10 min
Sudán	590.1±7.7	624.7±4.9	768.9±4.3
Tecoanapa	137.3±3.1	146.8±2.4	153.1±1.4

Mean ± standard deviation (n=5).

Table 3. Comparison of the solvents used to extract anthocyanins.

Cultivar	Extraction solvent and concentration of anthocyanins (mg L^{-1})			
	H_2O (boiling 10 min)	H_2O (cold 1 h)	Methanol	Ethanol
Sudán	768.9±4.3	560.2±4.3	164.7±0.5	92.7±0.6
Tecoanapa	153.1±1.4	95.2±2	86.9±0.6	59.9±0.5

Mean ± standard deviation (n=5).

Moreno *et al.* (2012) mentioned that increasing the exposure time of the plant material to boiling water increases the concentration of anthocyanins.

According to Escobar-Ortiz *et al.* (2020), the main effect on the extraction yield is a consequence of the solid-liquid ratio. Cissé *et al.* (2012) showed that increasing the solid-liquid ratio improves yield extraction of the anthocyanins found in *jamaica*. These results match the principles of mass transfer. The evaluation of the extraction time recorded an increase of 60.2% in anthocyanin concentration from 15 to 25 minutes. This increase suggests that the total permeability or rupture of the cell membrane occurred at 25 minutes. Afterwards, a falling in the anthocyanin concentration took place (Figure 3). This phenomenon suggested that degradation (diminished concentration) started when anthocyanins were directly released and exposed to a hot aqueous medium.

In descending order of importance, the four types of anthocyanins found in *jamaica* calyces are: delphinidin-3-sambubioside (D3S), cyanidin-3-sambubioside (C3S), delphinidin-3-glucoside (D3G), and cyanidin-3-glucoside (C3G) (Riaz *et al.*, 2018). D3S recorded a total anthocyanin content of 85%. It is the main source of the antioxidant capacity of the extracts of *H. sabdariffa* L. (Da-Costa-Rocha *et al.*, 2014).

According to Hopkins *et al.* (2013), anthocyanins —particularly D3S and C3S— are responsible for some biological effects and they can be found in great quantities in aqueous extracts. Meanwhile, Lo CW *et al.* (2007) associated them with anti-cancer activities, as a result of their capacity to stimulate the apoptosis.

Abubakar *et al.* (2019) pointed out that, 4 hours after consuming polyphenol-rich *H. sabdariffa* beverages, the antioxidant activity of 25 apparently healthy individuals was higher than the control group, who only drank water. These results were possibly the consequence of the antioxidant properties of gallic acids (4-O- and 3-O-methyl gallic acids). In addition, Vargas *et al.* (2018) mentioned that the extract of both Tecoanapa and Sudan varieties have flavonols and flavones.

According to the analysis of variance, the average size of the spheres of Teconapa was 11.50±1.62 μm for C1M (R1) and 11.38±1.38 μm for C2M (R2); meanwhile, Sudan

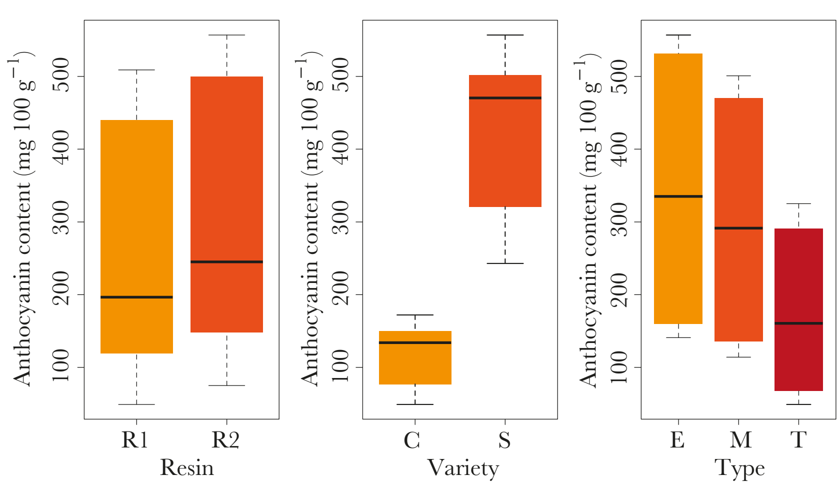

Figure 3. Anthocyanin concentration of the Sudan variety, at different boiling water extraction times. Mean values ± standard deviation (n=3).

obtained 11.59±1.38 μm for S1M (R1) and 11.28±1.33 μm for S2M (R2) (Figure 4).The effectiveness of the spray drying encapsulation method for *jamaica* aqueous extracts reached 81.15, 87.37, 85.95, and 89.95% for C1M (R1), S1M (R1), C2M (R2), and S2M (R2), respectively (Figure 5).

For its part, the total phenolic encapsulation method recorded a 71.02, 73.83, 76.0, and 81.25% efficiency for C1M (R1), S1M (R1), C2M (R2), and S2M (R2), respectively (Figure 6). After a month of microencapsulation, the encapsulation on the antioxidant activity recorded an 87.86, 88.13, 92.32, and 92.58% efficiency for C1M (R1), S1M (R1), C2M (R2), and S2M (R2), respectively. A control kept in a refrigerator was compared with a recently extracted product (Figure 7). Carneiro *et al.* (2013) researched the efficiency of encapsulation and the oxidative stability of flaxseed oil and recorded an encapsulation efficiency of 63-95.7%. These authors used maltodextrin mixed with gum arabic and a buttermilk protein concentrate. Chatterjee *et al.* (2014) reported lower encapsulation efficiency percentages (60-65%) for the dry extract encapsulation of *Phormidium valderianum* with maltodextrin and gum arabic. This variation in the results

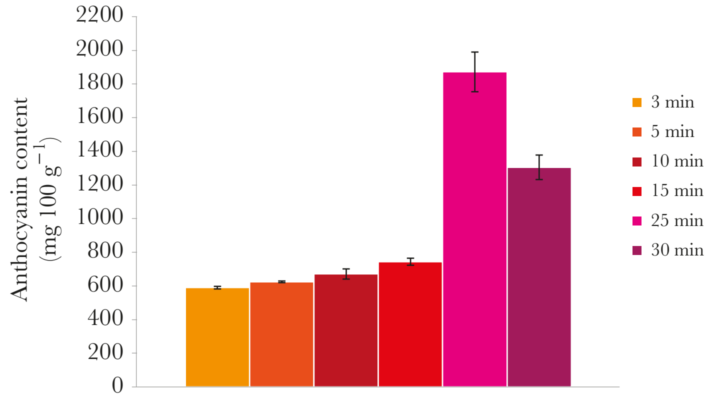

Figure 4. Distribution of the anthocyanin content means, depending on resin, variety, and type variables.

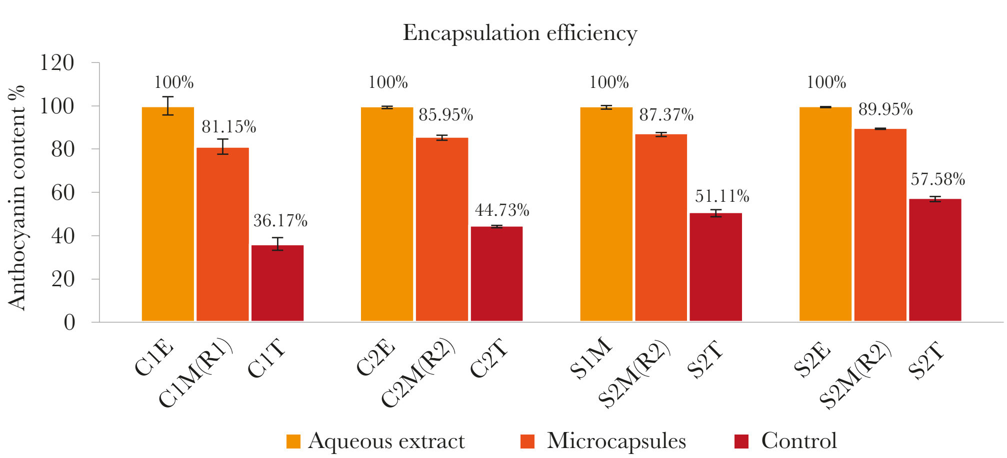

Figure 5. Preservation of anthocyanins in a fresh and microencapsulated extract and control, a month after the spray drying process.

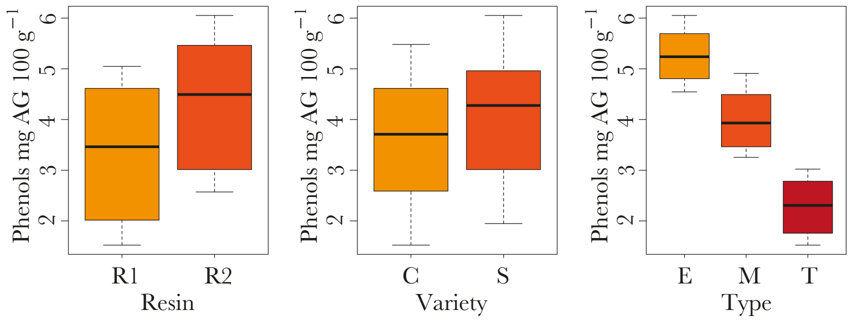

Figure 6. Distribution of the phenol means depending on resin, variety, and type variables. E: recently extracted extract; M: microencapsulated; T: control (extracted extract, kept in the refrigerator for the same period).

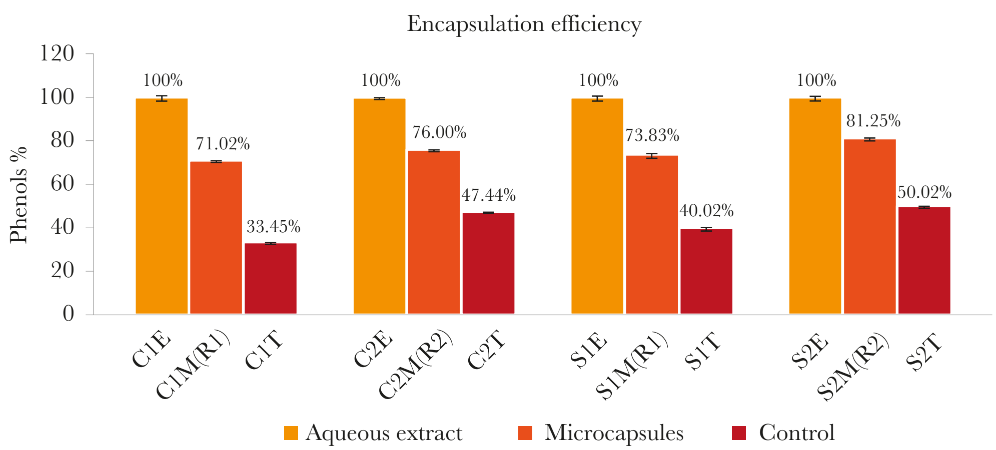

Figure 7. Percentage of phenol preservation in a microencapsulation with two resins, a month after the spray drying process.

can be the consequence of the type of metabolites responsible for the antioxidant activity and the encapsulation process used in the experiments.

Based on the means and variances of the microencapsulation process (anthocyanins, phenols, and antioxidant activity (DPPH)), R2 (60M:40GA) was the best resin for encapsulation. The Sudan variety recorded the best anthocyanin preservation (429.0 mg 100 g^{-1}), followed by Tecoapana (119.03 mg 100 g^{-1}). Based on the type of treatment, S2M (R2) recorded the best anthocyanin preservation (499.6 mg 100 g^{-1}), followed by S1M (R1) (438.8 mg 100 g^{-1}), C2M (R2) (147.8 mg 100 g^{-1}), and C1M (R2) (119.4mg 100 g^{-1}). These results showed a better preservation than the results recorded by control (S1T, C2T, C1T, S2T).

Regarding microencapsulation, Tecoanapa had a better response to the spray drying process (4.08 mg AG 100 g^{-1}) than Sudan (3.60 mg AG 100 g^{-1}). Meanwhile, the best response to the spray drying process was recorded by S2M (R2) with 4.86 mg of gallic acid 100 g^{-1}, followed by C2M (R2), S1M (R1), and C1M (R1) with 4.15, 3.67, and 3.27 mg of gallic acid 100 g^{-1}, respectively. Sudan recorded the highest antioxidant activity (49.88%). For its part, Tecoanapa reached 49.13%. S2M (R2) obtained the highest antioxidant activity (60.00%), followed by C2M (R2), S1M (R1), and C1M (R1) with 59.20, 43.83, and 43.20%, respectively. These percentages account for their antioxidant potential.

Descriptive analysis of the phenol variables

A significant effect ($p \leq 0.01$) was recorded for *Hibiscus sabdariffa* L. regarding the resin, variety, and treatment variables and their response to anthocyanins, phenols, and DPPH preservation and the distribution of particle size. S2E obtained more anthocyanins (555.4 mg 100 g^{-1}) and phenols (5.983 mg of gallic acid 100 g^{-1}), as well as a better antioxidant activity (64.77%). The similarity regarding antioxidant activity for both cultivars is determined and established by the total phenolic fraction and not just by the anthocyanin concentration. The best solvent to extract anthocyanins was boiling water (H_2O) for 25 minutes.

CONCLUSIONS

The Sudan and Tecoapana varieties of *Hibiscus sabdariffa* L. share chemical-taxonomic characteristics; nevertheless, they are quantitatively different. Sudan has a higher anthocyanin content (1,319.8 mg 100 g^{-1}) than Tecoanapa (557.2 mg 100 g^{-1}). Maltodextrin was the best microencapsulation process for the extracted and encapsulated extract of Sudan. It recorded the highest percentage of preservation of phenols and anthocyanins in the microencapsulation (85% ± 5%).

ACKNOWLEDGEMENTS

The authors would like to thank Mr. Efraín Cruz Cruz (ScD) (INIFAP) and Ms. Ruth Pedraza Islas (ScD) (Universidad Metropolitana) for their collaboration. The authors would also like to acknowledge the internal funding granted by the Universidad Autónoma de Guerrero.

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Early production of dry matter and its relationship to grain yield in maize (*Zea mays* L.) populations

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Citation: Ramírez-Ceh, G. A., Ruiz-Torres, N.A., Rincón-Sánchez, F., & & Benavides-Mendoza, A. (2025). Early production of dry matter and its relationship to grain yield in maize (*Zea mays* L.) populations. *Agro Productividad*. https://doi.org/10.32854/0v269y25

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

Received: December 27, 2024.
Accepted: May 19, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 141-150.

ABSTRACT

Objective: To determine whether early production of dry matter can be considered as a selection criterion, and to analyze the association of the traits in dissimilar environments with grain yield. The genetic material consisted of nine maize populations adapted to the environmental conditions of the southeast of the Coahuila state in Mexico, and the direct crosses between them.

Design/methodology/approach: The genotypes were evaluated for the root dry weight (RDW) and stem dry weight (SDW) under laboratory and greenhouse trials, with and without stress due to salinity, and in the field, for grain yield.

Results: There was no evidence of a significant relationship between dry weight (RDW and SDW) in laboratory trials with the grain yield whereas, in greenhouse trials, a positive RDW and SDW relationship with stress and non-stress was found with grain yield, where the RDW (stress) showed a correlation of r=0.308*. There is a genetic variation among populations for the evaluated traits, which is manifested in their expression, and in the potential of genetic combination of the crosses.

Findings/conclusions: Populations 6R and 7R were identified, and a significant group of crosses showing the combinations of them with positive values of grain yield and root and stem dry weight under salinity stress. Root dry weight (RDW) determined under salinity stress, jointly with RDW and SDW under non-stress conditions in the greenhouse can be considered as potential indicator for early selection of maize genotypes.

Keywords: *Zea mays* L., salinity stress, environmental effects, native populations.

INTRODUCTION

Genetic improvement is a long process and success depends, in addition to the selection of the germplasm, on the enhancement of the selection technique. In this sense, the identification of correlated traits in early stages can be an alternative for the selection

of genotypes with outstanding agronomic behavior, as a basis for the development of new varieties that provide genes associated with resistance to biotic and abiotic factors (Lamz and González, 2013). Exposure of genetic materials to salinity stress conditions may be an alternative for identifying alleles present in genotypes. Salinity affects the growth and development of plants through their impact on photosynthesis, respiration, starch metabolism, water ratios and nutrient absorption; at high levels causes toxicity, reduces plant growth and crop productivity (Torabi *et al.*, 2013; Farooq *et al.*, 2015). In a study under salinity stress conditions, Giaveno *et al.* (2007) reported significant presence of genetic variation, and proposed that the use of vigor traits, such as the weight and growth rate of seedlings under stress conditions may be used as a selection criterion for salt-tolerant in maize breeding programs. In seed quality tests, the dry weight of seedling and seed are considered as vigor tests, as well as the expression of the response of genotypes to different stresses conditions, to determine differences in the physiological potential of seeds (Marcos-Filho, 2015). Considering the needs to identify alleles that can contribute to mitigate fluctuations in the environment, the present research work was developed, with the following objectives: to determine whether dry root and stem weight under salinity stress conditions can be considered as selection criteria; and to analyze the association of the traits in dissimilar environments with grain yield performance. The research work is based on the premise that there is important genetic variation in the genotypes under study, and that early-stage dry matter production traits can be considered as a selection criterion in genetic improvement of maize.

MATERIAL AND METHODS

The genetic material included in the research work consisted of nine native maize populations, representative of five racial groups, adapted to the environmental conditions of the southeast of the Coahuila state in Mexico, which were identified based on a sample of genetic variation and performance potential in the region of study (Espinosa *et al.*, 2019). In the PV-2016 agricultural cycle, direct crosses were made between the nine populations under study. Genotypes (populations and 36 crosses) were subjected to evaluation trials in three stages: 1) Laboratory trials to determine the physiological quality of seeds under conditions with and without salinity stress, 2) Greenhouse trials to analyze early-stage development with two environments, with and without salinity stress, and 3) Field evaluation trial in a target study location in the southeast of the Coahuila state. In laboratory and greenhouse trials, an electrical conductivity of 22.8 mS cm^{-1} was used for salinity stress conditions, obtained with a NaCl concentration of 16.29 g L^{-1}. Electrical conductivity was determined based on an *ad hoc* study to obtain the optimal concentration of salts (unpublished data).

Laboratory trials

Laboratory trials were carried out in the Seed Physiology and Biochemistry Laboratory of the Centro de Capacitación y Desarrollo en Tecnología de Semillas, Universidad Autónoma Agraria Antonio Narro (UAAAN). The physiological quality of the seeds through the standard germination test was determined, according to the ISTA rules (ISTA, 2009), with modification in the sample size and number of replications, since, in some of the crosses not enough seed was obtained.

To know the response of the genotypes to different environmental conditions, two trials were established, one under salinity stress (SL) conditions and one under non-salinity stress (NL) conditions. In both cases, three replicates of 25 seeds were sown on anchor paper, which was moistened with saline solution for the stress conditions, and only distilled water for the non-stress conditions, the latter, used as a check in this study. The paper with the seeds was rolled in the form of a "taco" and placed in polyethylene bags according to the corresponding identification plot number and placed inside a basket, which were subsequently placed in a growth chamber at 25±1 °C for seven days. From the germination test, only the seedlings that had normal development, using as a criterion, those that had a length equal to or greater than three centimeters, both in the stem and the root, were considered. Remnant of the seed was removed from each seedling, separating the stem from the root system. Later, they were dried in a stove at a temperature of 70 °C for 24 h, resulting in the stem dry weight (SDW) and the root dry weight (RDW), expressed in milligrams (mg plant^{-1}).

Greenhouse trials

The evaluation trial was conducted in the greenhouse number two of the Department of Plant Breeding, located on the Saltillo campus of UAAAN. The greenhouse has passive temperature control, which was recorded every four hours during the experiment, with a daily average of 31.0 °C during the day and 18.3 °C at night. Planting was done in polyethylene bags, using a mixture of peat moss, perlite, and vermiculite as a substrate in a 2:1:1 ratio, respectively. Four replications were set in the evaluation test, and for more efficient handling, each repetition was established on a different planting date. Two seeds were sown at a depth of 3 cm; in the emergency, one of them was removed to leave a plant per bag as an experimental unit, for both non-stress (NG) and salinity stress (SG) conditions.

To subject the seed of the genotypes to different evaluation environments, for the stress condition, the irrigation was performed by applying 100 ml of saline solution, beginning at the planting day, and only distilled water, for testing under non-stress conditions; these settings were applied in subsequent irrigations. In both evaluation environments, 35 days after planting, each plant, through a carefully washing process, the seed residue was removed, and the aerial part or stem was separated from the root system. Subsequently, both parts were subjected to drying in a dehydrating stove at a temperature of 70 °C for 48 h. This resulted in the stem dry weight (SDW) and root dry weight (RDW) traits, expressed in mg plant^{-1} in both environments.

Field evaluation trial

The genotypes were established for agronomic evaluation in the Spring-Summer (2017) agricultural cycle, in the location of El Mezquite, Galeana, N. L. (1890 m; 25° 05' N; 100° 42' W), located 10 km from the limits of Saltillo, Coah., under irrigation conditions. The experiment was established using an incomplete block design with an alpha lattice arrangement and three replications, and a fertilization dose of 120-60-60 units of N, P and K, respectively. The experimental unit consisted of a 4 m row, with a distance between plants of 0.20 m and a distance between rows of 0.80 m. In this study, only the grain yield was expressed in t ha^{-1}, considering a 15% seed humidity content.

Data analysis

In each evaluation environment, analysis of variance was performed according to the experimental design with the GLM procedure of SAS (SAS Institute, 2022). To identify outstanding genotypes, in each of the traits under study, a decision value was calculated determined by the mean plus one and twice the standard error of the mean ($\mu + Se$; $\mu + 2Se$). A simple Pearson correlation analysis (r) was performed for the studied traits by the trials × the evaluation environments. The genotype means of each combination of traits by trials × the evaluation was used to perform a graphical display analysis based on the Principal Component Analysis (PCA) with the GGEbiplot model (Yan and Kang, 2003; Yan, 2014).

RESULTS AND DISCUSSION

An overview of the analysis of variance results for both, root dry weight (RDW) and stem dry weight (SDW) is stated in the trials performed under stress and non-stress conditions in Laboratory and Greenhouse, and the grain yield performance on the field evaluation (Data not shown). In the laboratory trials, significant differences for RDW and SDW ($P \leq 0.01$) were found between the environments, corresponding to the stress and non-stress (check) salinity trails, respectively; while, in the greenhouse trials, no differences were found between environments in the two traits under study. In Genotypes (populations and crosses), statistical differences were only found in the RDW in laboratory, greenhouse and grain yield trials; basically, these differences were observed in the crosses between the populations under study, so in no case there was any statistical evidence of differences between populations. In general, no differences were found in the genotype × environment interaction, except in the SDW ($P \leq 0.05$) in laboratory trials, which refers to the populations × environments interaction. In the laboratory trials, there was a reduction of 49.0 and 60.0 % for root dry weight (RDW) and stem dry weight (SDW), respectively, as a response to the salinity stress with respect to the non-stress (check). In greenhouse trials, although there is a numerical difference between stress conditions with respect to the check, those differences are not statistically thought, with a reduction of 5.0 and 13.0 % for RDW and SDW, respectively. Means of grain yield and dry weight of root and stem for populations, and the best 25 crosses from the evaluation environments are presented in Table 1.

Although, populations are different base on their genetic constitution and the area of adaptation source (Espinosa *et al.*, 2019), no differences were found among them, due in part to the relative comparison and confounded effects on the evaluation environments (stress and non-stress) in laboratory and greenhouse; while in the field, possibly to the response to the environment effects, since, with the exception of populations 1CN and 2CN, which are specifically adapted to the evaluation location (transition-highland adaptation area), the

Table 1. Means of grain yield and dry weight of maize populations, and the top 25 crosses evaluated in 2017.

Genotypes	Yield (t ha^{-1})	Greenhouse trials		Laboratory trials	
		RDW (mg)	SDW (mg)	RDW (mg)	SDW (mg)
7R †	9.25 *	375.7	2,343.6	23.9	43.1 *
8T	9.10	432.6	2,264.2	26.4	37.4
6R	8.13	333.3	2,436.6	25.8	42.0 *
5TN	8.09	380.8	1,960.6	21.3	38.4
3C	7.61	371.2	2,303.1	27.2	40.4
2CN	7.18	317.7	2,146.5	28.9 *	36.5
9T	6.99	350.8	2,295.6	17.6	37.6
1CN	6.97	377.6	2,269.8	24.3	40.1
4R	6.75	434.6	2,341.8	25.2	38.1
Populations means	7.79	374.9	2262.4	24.5	39.3
8×9	10.22 **	454.7	2,635.6	27.6	39.8
5×7	10.21 **	423.9	2,225.6	19.7	37.0
4×7	9.89 *	440.2	2,599.9	28.3	42.1 *
6×9	9.78 *	349.7	2,241.2	22.8	45.0 **
2×7	9.69 *	355.3	2,260.8	25.9	38.8
4×8	9.49 *	322.0	2,111.6	28.4 *	37.7
7×9	9.46 *	573.2 **	2,823.1 *	34.5 **	41.4
6×7	9.38 *	463.5	2,440.8	27.1	43.3 *
1×7	9.34 *	330.4	2,453.5	24.3	38.0
5×6	9.33 *	373.0	2,458.7	25.7	39.3
3×9	9.32 *	410.0	2,539.8	31.4 **	42.6 *
2×6	9.25 *	346.1	2,429.4	26.0	36.8
3×8	9.13	405.5	2,623.1	27.4	38.8
7×8	9.11	375.9	2,462.7	21.8	37.2
4×9	9.07	385.6	2,087.2	18.7	34.2
2×9	8.91	320.0	2,361.8	16.1	35.3
5×9	8.83	476.4 *	2,573.2	26.3	46.5 **
3×7	8.79	446.4	2,219.7	22.7	35.0
4×6	8.75	373.8	2,614.8	26.0	35.4
6×8	8.73	472.1 *	2,669.7 *	25.7	39.8
1×9	8.70	411.9	2,652.6 *	22.1	37.6
2×8	8.65	446.8	2,330.8	24.7	37.3
3×6	8.59	445.7	2,345.9	31.0 *	39.8
2×5	8.27	369.5	2,050.6	24.3	39.0
3×4	8.16	504.1 *	2,693.1 *	28.9 *	39.9
Crosses means	8.60	397.2	2,393.5	25.5	38.8
Genotypes means	8.43	392.7	2,367.3	25.3	38.9
Se	0.81	75.2	281.9	3.1	2.7

*, **, Selection base on $\mu+Se$ and $\mu+2Se$, respectively; † The first digit indicates the population number and the letter, the race id; C=Celaya, CN=Cónico Norteño, R=Ratón, T=Tuxpeño and TN=Tuxpeño Norteño; RDW, SDW=Root and Stem dry weight, respectively; Se=Standard error of the mean.

rest of the populations come from intermediate adaptation area, within the study region. However, by comparing the average performance of the crosses against populations, except for SDW in the laboratory trial, where a reduction of 1.3% was obtained, in the rest of the cases, a positive response of the crosses was found with 11.4% increase in grain yield, with 4.1 and 6.0% of RDW in laboratory and greenhouse tests, respectively. Of the 12 outstanding crosses in grain yield, six involve the population 7R, and four the population 9T, corresponding to the Ratón and Tuxpeño races, respectively, suggesting a positive response of the genetic combination of these two populations. In particular, the 7R×9T (Ratón×Tuxpeño) cross with outstanding grain yield, also achieved significant values in RDW in laboratory and greenhouse trials. The rest of the upper crosses in grain yield, generally, also showed higher values in the average crosses in RDW, both, in laboratory and greenhouse trials. It can be verified from Table 1 that populations with higher values in grain yield, in general, also showed positive relationship in RDW values in the evaluation environments.

The differences found between populations and crosses of 4.1 and 6.0% in root dry weight in laboratory and greenhouse trials, and 11.4% in grain yield, suggests that the potential of the genetic combination between populations, as an indicator of genetic divergence and the average heterotic effects between them (Reif *et al*., 2005). The positive genetic combinations identified on the traits in this study may be useful to transfer favorable alleles to adapted population, and through recurrent selection schemes, to develop new genetic materials. Although, the performance of 7R and 9T populations in genetic combinations is also emphasized, which, according to their contribution on the best crosses in grain yield, reveals their overall combined ability, but also, the cross of both (7R×9T), with outstanding values in grain yield, obtained significant values in root dry weight in laboratory and greenhouse tests (Table 1). In much of the upper crosses, a positive association of root dry weight values in the evaluation environments is shown, which represent a source of alleles that may be useful to incorporate them into the selection process, complementing grain yield (Mercer and Perales, 2010). Results in Table 1 suggest analyzing the association of the traits under study to the evaluation environments, including grain yield. The results of Pearson's correlation analysis are presented in Table 2.

It is apparent from the results of Table 2 that, except for the laboratory stress trial, the relationship between RDW and SDW is significant ($P \leq 0.01$) within trials, and within stress and non-stress conditions. In the case of the dry weight, the relationship between laboratory and greenhouse trials, only under non-stress laboratory conditions, a positive and significant correlation between RDW and SDW was found under greenhouse stress conditions. No evidence of a significant relationship between dry weight (RDW and SDW) was found in laboratory trials with grain yield. In the greenhouse trials, a positive relationship, although a low value, for dry weight with grain yield was found, where RDW under stress conditions (RDW_SG) obtained a correlation of $r=0.308$*. This is partially consistent with Giaveno *et al.* (2007), who suggest that seedling weight can be used as a selection criterion, however, in this study, RDW and SDW under non-stress conditions in laboratory trials showed positive correlation with RDW and SDW in the greenhouse trials. On the other hand, under greenhouse stress conditions the RDW showed a positive

Table 2. Grain yield and dry matter correlation coefficients across environments based on the genotypes evaluated in 2017.

	Laboratory trials			Greenhouse trials				Yield
	SDW_SL†	RDW_NL	SDW_NL	RDW_SG	SDW_SG	RDW_NG	SDW_NG	
RDW_SL	0.028	0.304 *	0.223	0.044	0.208	−0.132	−0.061	−0.192
SDW_SL		−0.288	−0.099	0.010	−0.078	0.117	0.237	0.126
RDW_NL			0.465 **	0.486 **	0.366 *	0.224	0.090	0.102
SDW_NL				0.401 **	0.335 *	0.168	0.276	0.079
RDW_SG					0.549 **	0.281	0.386 **	0.308 *
SDW_SG							0.182	0.234
RDW_NG							0.408 **	0.157
SDW_NG								0.148

*, **, Significant at 0.05 and 0.01 probability levels, respectively; RDW, SDW=Root and Stem dry weight, respectively; † In the name of the variables, S and N, refers to the salinity stress and non-stress (Check) trials, respectively; L and G, Laboratory and Greenhouse trials, respectively.

correlation with SDW (r=0.549**), and positive correlations of r=0.281 and r=0.386** for the RDW and SDW, respectively, in the greenhouse trial under non-stress conditions.

The genetic variation of populations and their crosses regarding the study traits (Table 1) and the relationship to the evaluation environments (Table 2) can be graphically analyzed to understand the interrelationships between genotypes and combinations of traits with environments and grain yield performance. Figure 1 presents the graphical display of the interaction of genotypes with the traits of this study, based on the principal component analysis, using the GGEbiplot model for graphical analysis (Yan 2014).

In Figure 1, vectors representing the combination of traits with environments originate from the origin, and the magnitude of these, is an indicator of the proportion of variation associated with the traits and environments; the cosine of the angle between vectors approximates the correlation coefficient and indicates the level of association between them (Yan and Kang, 2003). Similarly, each genotype projects a vector from the origin, and therefore the relationship to the traits and environments will depend on the place in the quadrant. In this sense, the interrelationships between the genotypes and the traits×environments can be used as a process of indirect selection of genotypes according to the traits of interest. In this analysis, the two first principal components (PC1 and PC2) were considered, which are those that most explain the cumulative variation presented in the original traits (48.8%).

According to the projection and angles of the vectors, grain yield is highly and positively correlated with the dry root and stem weight under non-stress greenhouse conditions (RDW_NG and SDW_NG); however, these values being r=0.157 and r=0.148, respectively (Table 2). There is also a positive correlation between grain yield and RDW under stress conditions in the greenhouse (RDW_SG), being r=0.308* in Table 2.

The discrepancy between the projection of the vectors in Figure 1 and the correlation results (Table 2) in these traits, is due to the fact that, in Table 2, 100.0% of the cumulative variation was used, while in Figure 1, only 48.8% of that variation was exploited; but data on Table 2 explain the phenotype values ($P = G + E + GE$), while Figure 1 means

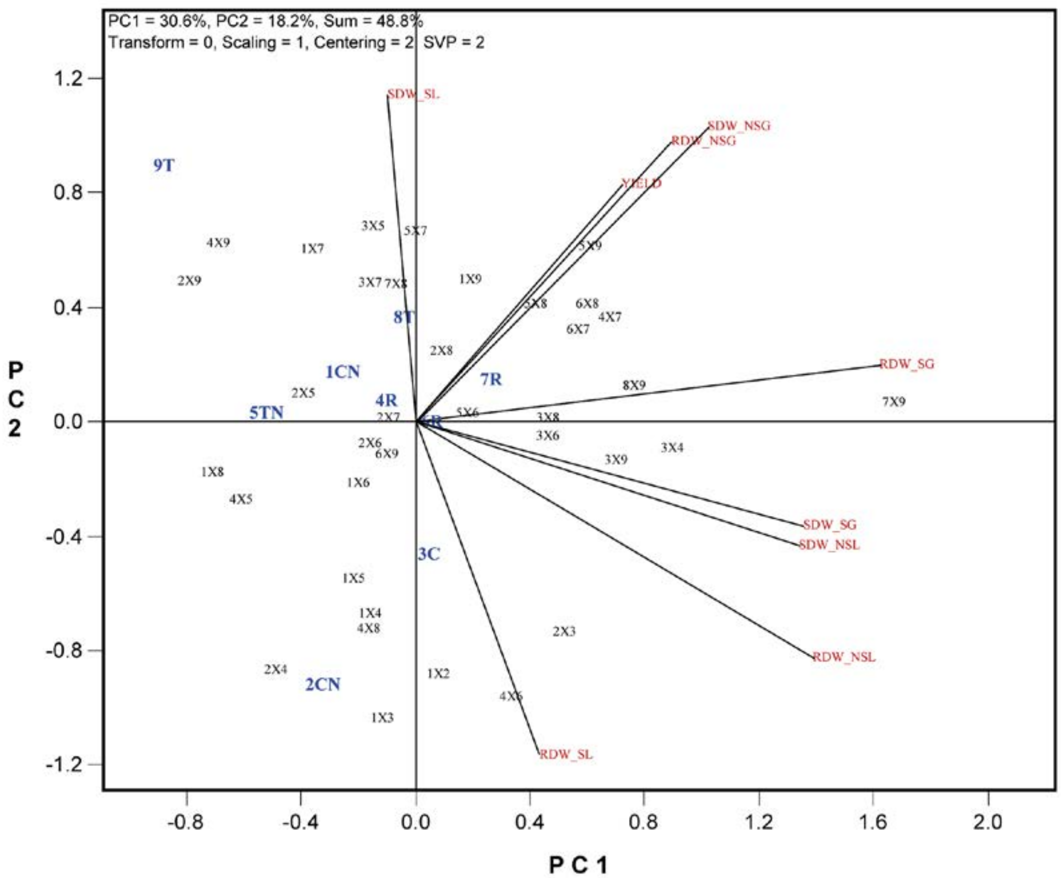

Figure 1. Graphical dispersion of the interaction of maize populations and their crosses with dry weight and grain yield traits; RDW, SDW=root and stem dry weight, respectively; SL and SG, stress conditions in laboratory and greenhouse, respectively; NL and NG, non-stress conditions test in laboratory and greenhouse, respectively; in populations, the first digit indicates the population number and the letters, the identification of race: C=Celaya, CN=Cónico Norteño, R=Ratón, T=Tuxpeño y TN=Tuxpeño Norteño.

only to the $G+GE$, referred as the GGE model (Yan and Kang, 2003; Yan, 2014). The association of RDW and SDW in the laboratory trials under non-stress conditions with RDW and SDW under greenhouse stress conditions, as shown in Table 2, is also revealed in Figure 1. The 48.8% of the cumulative variation through the GGEbiplot model (Figure 1) regarding the phenotype relationship of the traits under study (Table 2), can be analyzed to understand the combination of traits×evaluation environments with the genotypes under study.

The dispersion analysis of the GGE model (Figure 1) can validate the variation that exists between the populations under study, which are sorted according to the interactions between the traits × evaluation environments. The genotypes×traits in the environments shows that 7R, 6R and 8T populations create a group, which positively correlates with grain yield and RDW and SDW under greenhouse non-stress conditions; the rest of the populations, except for 9T, are negatively associated forming another group. Similarly, the crosses 5×9, 6×8, 4×7, 6×7, 5×8, 1×9, set up a group closely related to this group of traits.

In these results, RDW and SDW stands out under stress conditions in the greenhouse trial, which correlate positively with grain yield (Table 2) and based on the interaction with genotypes (Figure 1), the 6R, and 7R populations can be identified, as well as a significant group of crosses showing the combinations of these populations, additionally, five of outstanding crosses, involve the 9T population. On the other hand, populations 4T, 5TN, 1CN and 2CN, and four of the crosses involving the 2CN population, correlate negatively with the RDW under stress conditions in the greenhouse. Based on the above results, the RDW under greenhouse stress conditions, jointly with RDW and SDW under non-stress conditions in the greenhouse, can be considered as potential indicator for early selection of maize genotypes, which strengthens improvement strategies, as pointed out by Lamz and González (2013).

In genetic improvement, it is important to identify attributes that determine quality levels in early stages of development, and that can be considered as a selection criterion. However, physiological quality indices are commonly not associated with good agronomic behavior. In a trial of lines and their crosses in maize, Antuna *et al.* (2003) found no direct association between seed physiological quality and agronomic traits, including grain yield. In a study to analyze the ratio of seedling dry weight as an indicator of vigor with maize growth and yield, TeKrony *et al.* (1989) conclude that the vigor of the seed had no effect on grain yield. Also, in a review of seed vigor, basically referring to the dry weight of seed in maize, TeKrony and Egli (1991) conclude that, in general, there is no relationship of seed vigor with grain yield, because in crops with complete reproductive maturity, yield is generally not closely associated with vegetative growth. From a trial under salinity stress conditions, Giaveno *et al.* (2007) suggested that vigor traits such as seedling weight and growth rate can be used as a selection criterion in maize with salinity tolerance.

CONCLUSIONS

Base on the conditions under which the study was developed, it is verified that there is genetic variation between populations for the traits included in the study, which manifests itself in the expression of the potential of the genetic combination in crosses. Populations 6R and 7R were identified, and a significant group of crosses showing the combinations of them with positive values of grain yield and root and stem dry weight under salinity stress. Root dry weight (RDW) under salinity stress, jointly with RDW and stem dry weight under non-stress conditions in the greenhouse can be considered as a potential indicator for early selection of maize genotypes.

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Effect of the conversion factor *212 on the estimation of log volume in the forest industry of Durango, Mexico

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Citation: Soto-Cervantes, J. A., Mora-Silva, B. A., Montiel-Antuna, E., Prieto-Ruíz, J. Á., Compeán-Guzmán, F. J., Pérez-Luna, A., & García-Montiel, E. (2025). Effect of the conversion factor *212 on the estimation of log volume in the forest industry of Durango, Mexico. *Agro Productividad*. https://doi.org/10.32854/2advnv78

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

Received: January 10, 2025.
Accepted: May 19, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 151-161.

ABSTRACT

Objective: to analyze Doyle and Smalian cubing methods, comparing their behavior in the application of conversion factors in the forest industry in Durango, Mexico.

Design/Methodology/Approach: a total of 38 731 logs were measured in different log yards and cubed using the Doyle (board ft) and Smalian (m^3) methods. The volume calculated using the Smalian method was multiplied by the operational standard *212, a conversion factor which is used to obtain volume in tables (board feet) from roundwood. The volume estimate by diameter category and log length was analyzed using the Kruskall-Wallis test ($p \leq 0.05$) to test differences between the Smalian×212 and Doyle cubing methods.

Results: eighty percent of the surveyed forestry companies use the Smalian method (m^3) to calculate the volume of roundwood in their log yards, then, use the factor *212 to convert cubic meters to board feet. The volume was found to be significantly greater with the Smalian equation; however, for diameters of 65 cm and larger, the estimated volume is greater with the Doyle equation. For logs with diameters 37-55 cm, or lengths 2.44-6.71 m, estimation with the conversion factor is closer to the actual metric volume.

Limitations/Implications of the study: due to internal policies, 20% of the companies interviewed did not respond. However, because of the significant nature of the differences, this study confirms underestimation or overestimation of log volume when using standardized estimation methods based on economic utility. Furthermore, the accuracy of log volume estimates is relevant in terms of sustainability.

Findings/Conclusions: to use empirical factors is now obsolete and should not be mixed with different measurement systems, as their theoretical framework is different. Cubing methods should not be mixed, since the conversion factor *212 currently used with Smalian equation renders estimations not equivalent to actual cubic measures under the official International Standard of Units in terms of roundwood cubing.

Keywords: conversion factors, Doyle method, Smalian method, log yard, International System of Units, Imperial System.

INTRODUCTION

Mexico's forest area covers 137.8 million hectares (CONAFOR, 2019), dominated by arid ecosystems, cold temperate forests, and tropical forests. There is an annual potential for harvesting 15 to 18 million hectares of forests and tropical forests. However, in 2019, only five million hectares were under authorized commercial forest use, of which 4.4 million belong to social ownership (3.7 million to *ejidos* and 0.7 million to community property) and the rest is private property (CCMSS, 2020).

The Secretariat of Environment and Natural Resources (SEMARNAT, 2018) recorded in 2018 that the states of Durango and Chihuahua contributed 46% of the nationally authorized volume. The state of Durango leads the list of forest production with around two million cubic meters of wood per year. In addition, this state has a forest area of 10.5 million hectares, which includes forest sites with 1.9 m^3 ha^{-1} $year^{-1}$ as annual current increment– ACI. (Madrid and Barrera, 2008; Soto *et al.*, 2021). These notable forest sites are managed by *ejidos*, communities, and small private owners (Suárez, 2018; Blackman and Villalobos, 2021). The main species harvested belong to the genera *Pinus* spp. (70.9% of the authorized volume) and *Quercus* spp. (9.8%); the rest (19.3%) of wood harvest belongs to other genera (CONAFOR, 2019).

The purpose of roundwood cubing is to determine the volume of harvested logs (Ordoñez *et al.*, 2012; Carvajal *et al.*, 2023; De Miguel-Díez *et al.*, 2023). According to Juárez (2014) and Hernández-Ramos *et al.* (2018), the commercial volume of a log is the quantity of solid wood with acceptable dimensions in the market. Different measurement methods are used to measure roundwood volumes. One of those is the English System which includes units that are very different to those in the Metric System. The English System is currently used in the United States; based on it, for a quick estimation of the commercial volume, it considers the smaller diameter of a log without bark in inches, and the length in feet.

The Smalian formula considers the average of the major and minor diameters (cm) of the ends of the log without bark width, as well as the length (m) to determine log volume (Vásquez and Ramírez, 2005). Whereas, the Doyle method considers the minor diameter without bark in inches and the length in feet (Romahn and Ramírez, 2010).

In Durango's forest industry, volume conversions from cubic meter to board foot are based on conversion factors such as *212, which causes confusion in the production chain. This occurs because the differences between the two methods, when using mixed with conversion factors are not clearly documented (Hernández *et al.*, 2024a). The cubing method *212 is traditionally used in Durango's forests. Although it is not recommended, because it is a common-use rule for estimating sawn wood production in board feet that also underestimates the volume of logs.

For all the above, with the hypothesis that the conversion factor *212 used to convert cubic meters to board feet is not equivalent to an actual dimensional estimation based on the official metric standard; the objective of this research was to analyze Doyle and Smalian cubing methods for roundwood, and their behavior when applying the conversion factor *212 in the forest industry of Durango.

MATERIALS AND METHODS

Data collection and log classification

Managers, whose logging yards are established in the city of Durango, (Durango), Mexico were randomly visited. Semi-structured interviews were conducted to learn about the cubic measurement methods and forest product conversion factors used in the marketing of roundwood. Volume estimation data were collected by direct measurement of logs in five log yards; additional data records were obtained with information on roundwood cubing.

A total of 38 731 logs were measured to estimate and compare the volume of roundwood from both cubing methods. These measurements consisted of taking the length (m), and the smallest and largest diameters (cm) without bark width at the ends of the log, using a Truper® length-meter and a tape measure, respectively. Logs were classified according to their diameter (cm) and length (m), separating them into diameter categories every five centimeters, and into length categories of one meter apiece, according to the commercial sizes used by the company (Table 1).

Cubing of logs using the Smalian formula

The logs were cubed using the Smalian formula after classification by diameter category (Equation 1, Vásquez and Ramírez, 2005).

$$Vol = \frac{\pi}{4} \times \frac{\left(d_M + d_m\right)}{2} \times L \qquad (1)$$

where: Vol=wood volume, m^3; d_M: largest diameter of the log without bark width, m, d_m: smallest diameter of the log without bark width, m; L: length of the log, m.

Volume result was multiplied by the conversion factor *212 to obtain the volume in board feet, then to compare volume estimates with the Doyle system. This factor is the most widely used in the Durango forest industry, according to the log yard managers interviewed.

Considering theoretical framework, this factor is based on the equivalence that the volume of a board one foot long (0.3048 m)×one foot wide (0.3048 m)×one inch thick (0.0254 m) is by definition, one board foot, which is a volumetric measure. Based on these dimensions, the metric volume of one board foot is 0.00236 m^3, therefore, 1 m^3 is equivalent to 423.84 board feet (Vásquez and Ramírez, 2005; INAB, 2019). However, under the general assumption that the transformation coefficient from roundwood (logs) to sawnwood (boards) is 50%, it is empirically estimated that one cubic meter of roundwood is "equivalent" to 212 board feet.

In this regard, Hernández *et al.* (2024a) indicated that this factor is still used as the primary reference for estimating the equivalence between the volume of logs (roundwood) entering the sawmill and the resulting volume of timber (boards). Accordingly, it is assumed that one board foot is obtained from each Doyle foot.

Table 1. Logs by diameter categories and length categories, used for cubing and comparing volume estimates between methods.

Diameter class (cm)	N	DAvg± StdDev (cm)	Length class (m)	n	LAvg± StdDev (m)
1=10-15	15	14.60±0.65	1=1-2	12	1.80±0.09
2=15,1-20	4711	14.86±1.05	2=2-3	3474	2.45±0.05
3=20,1-25	13439	22.73±1.39	3=3-4	4225	3.37±0.30
4=25,1-30	9256	27.66±1.42	4=4-5	4709	4.67±0.29
5=30,1-35	6082	32.48±1.43	5=5-6	1295	5.48±0.04
6=35,1-40	3015	37.54±1.42	6=6-7	3263	6.30±0.29
7=40,1-45	1344	42.26±1.43	7=7-8	3149	7.52±0.29
8=45,1-50	553	47.44±1.42	8=8-9	1302	8.53±0.02
9=50,1-55	183	52.14±1.32	9=9-10	15360	9.69±0.18
10=55,1-60	64	57.55±1.43	10=10-11	1802	10.43±0.19
11=60,1-65	35	62.43±1.45	11=11-12	60	11.58±0.00
12=65,1-70	22	66.00±2.16	12=12-13	80	12.20±0.07
13=70,1-75	12	71.75±1.77	--	-	--

N: number of logs per diameter class, n: number of logs per length class; DAvg: average diameter; Lvg: average length; StDev: standard deviation.

Cubing of the logs with the Doyle formula

Logs were also cubed using the Doyle formula (Equation 2), considering that the diameter to be measured is the smallest diameter of the log without bark width, in English units (Romahn and Ramírez, 2010).

$$V = (D'' - 4)^2 \times \frac{L'}{16} \quad (2)$$

Where, V: volume, board ft; D'' minor diameter without bark, in; L': length, ft.

Statistical analysis

Data obtained using the Smalian equation multiplied by the factor 212 and Doyle equation were compared with the Kruskal-Wallis test, after proving data met the assumptions of normality (Shapiro-Wilkinson) and homogeneity of variances (Levene) tests. All statistical analyses were performed using the statistical software R® version 4.3.1 (R Core Team, 2023).

RESULTS AND DISCUSSION

In 80% of the log yards in the forestry companies where we surveyed the owners, the Smalian method (m^3) is used to size roundwood, using the *212 factor to convert roundwood volume to board feet. The remaining 20% of the companies did not respond. It is important to mention that the Mexico's Federal Metrology and Standardization Law (Ley Federal sobre Metrología y Normalización, 2015) does not permit the use of English system measurements. However, this conversion is used anyway as customary, because in extraction operations based on the purchase agreement, loggers are paid per thousands of board feet.

Significant differences ($p \leq 0.01$) were found in volume estimation between the Smalian×212 and the Doyle method. When done by diameter category, there were differences in volume in most of the categories evaluated, except category 13 (70.1-75 cm). Volume estimation by length category also showed statistically significant differences ($p \leq 0.001$) in all the categories evaluated (Table 2).

Volume estimate is statistically higher with the equation Smalian×212, except for in diameter classes 65.1-70 and 70.1-75. That is, from diameters of 65 cm onwards, although it was not significant, the volume estimate is higher with the Doyle equation (Figure 1). These results coincide with Romahn and Ramírez (2010), who argued that the Doyle method generates underestimation errors in small diameter logs and overestimation errors in large diameter logs.

Likewise, when considering all length categories evaluated, volume estimates obtained with the equation Smalian×212 were significantly greater, compared to Doyle estimates (Figure 2).

Distinct equations are used to calculate log volume. The most commonly used are those of Newton, Huber, Doyle and Smalian (Zavala and Hernández, 2000; Spelter, 2004). The Smalian and Doyle methods involve diameter measurements at the ends of the logs; whereas Newton and Huber methods are impractical equations, as those require recording the diameter in the middle of the log (Chinchilla and Gómez, 2005; Ghorani *et al.*, 2014).

Cruz and Uranga-Valencia (2013) stated that the Smalian method is less precise than the Huber method, but it is used more frequently, particularly when logs are arranged

Table 2. Statistical comparison per diameter category and length category between the Smalian×212 and Doyle methods, according to the Kruskal-Wallis test.

Diameter classes (cm)	p-value	Length classes (m)	p-value
1=10.0-15	0.0090	1=1-2	0.0001
2=15.1-20	0.0001	2=2-3	0.0001
3=20.1-25	0.0001	3=3-4	0.0001
4=25.1-30	0.0001	4=4-5	0.0001
5=30.1-35	0.0001	5=5-6	0.0001
6=35.1-40	0.0001	6=6-7	0.0001
7=40.1-45	0.0001	7=7-8	0.0001
8=45.1-50	0.0001	8=8-9	0.0001
9=50.1-55	0.0001	9=9-10	0.0001
10=55.1-60	0.0001	10=10-11	0.0001
11=60.1-65	0.0001	11=11-12	0.0001
12=65.1-70	0.0066	12=12-13	0.0001
13=70.1-75	0.1213 ns	--	--

p-value ≤ 0.01 and p-value ≤ 0.001 are significant; ns: non-significant.

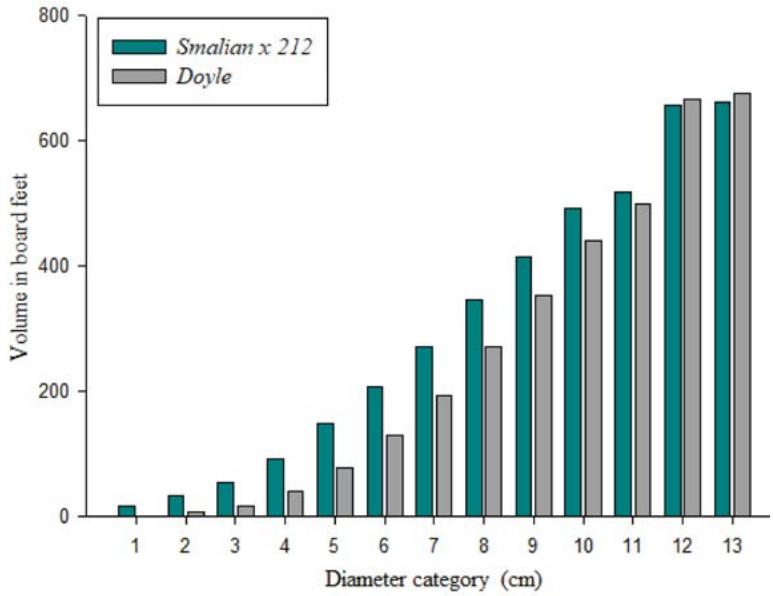

Figure 1. Volume averages in board feet by diameter class (cm).

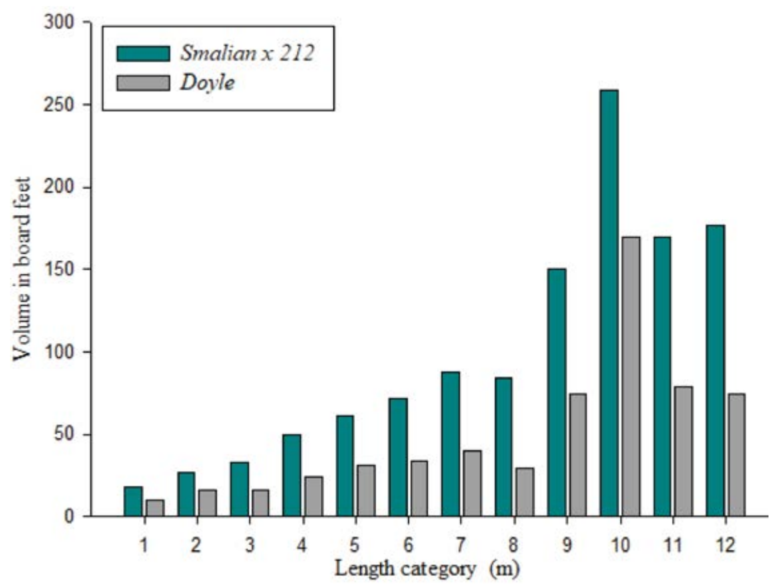

Figure 2. Volume averages in board feet by length class (m).

in piles where the midpoint diameter cannot be measured. In this research, many of the logs were stacked, so the Smalian method was used. When accurate log sizing is required, sections of equal or different lengths must be made and cubic measured separately, so that the cubing accuracy is greater as shorter is the length of the sections into which the trunk is divided (Alpízar, 2020).

Nájera-Luna *et al.* (2011) observed that roundwood sizing methods using the International Units System (SI), which is the updated name of the Metric System, are considered better than methods using board feet, since decimal rules give more accurate estimates of log volumes using only diameters and lengths. Likewise, Romahn and Ramírez (2010) emphasized that the Doyle method is based on a mathematical function, but this does not mean that it is accurate, since it is based on false assumptions and as a result the generated volumes are inaccurate. Therefore, the Smalian method is one of the most widely used for commercial log sizing in Mexico (Hernández *et al.*, 2024a).

In this study, in the larger diameter categories, the volume estimates using Doyle were greater compared to those obtainded with Smalian×212. However, the best option for forest owners in the state of Durango is the Smalian method, since the harvested trees come from native forests; those trees have diameters less than 65 cm in most cases.

Additionally, Alpízar (2020) indicated that the Doyle method has the disadvantage that the producer can only sell usable wood, since this method is based on estimating the final volume in the square dimensions that are usual in the sawmills. This means not to take into account the edges and coarse debris of the log from which other products of commercial interest are obtained; such as cape, broom handle stick, box-boards, among others (Chinchilla and Gómez, 2005).

According to Spelter (2004) and Romahn and Ramírez (2010), the use of the Doyle rule presents several drawbacks, such as mathematical oversimplicity. This formula does not consider important factors such as conicity (decrease in the diameter of the trunk from the base to the tip) and this can result in less precision in the estimation of the wood volume. Because the Doyle rule is based on an ideal cylindrical projection of the trunk, which results in precision inconsistencies. Also, this method does not take into account trunk defects such as knots, rot or curvature, which can lead to overestimation of the usable wood volume.

Log volume results from cubing when the conversion factor approximates the metric volume estimates are those with diameters ranging from 37 to 55 cm; they are also consistent with logs with lengths ranging from 2.44 to 6.71 (Table 3).

Spelter (2004) noted that the factors used to convert volumes from one cubing method to another have become obsolete. Thus, a transition into more accurate standards would improve transparency in the national log market by reducing inequalities from scales based on board feet. This would facilitate regional comparisons, as well as supporting fair play in the international market operations.

Hernández *et al.* (2024b) reported that forest managers do not supervise the spot use of the International System of Units, as opposed to the English system or any other, to measure yield in logging operations. As a result, loggers often use board feet, which are generally obtained using imprecise conversion factors. This makes it difficult to monitor the distribution and conversion of harvested volumes, which were originally projected and planned in the metric system when the forest management program and the yields in the cutting plan were formulated for each year. What means that management plan is not consistent with logging operations, in regard to commercial yields.

The use of allometric equations to estimate commercial timber volume allows for planning forest management (Revilla-Chávez *et al.*, 2021). Therefore, it is important to estimate with the greatest possible certainty the volume of logs extracted in sustainable forest management, since this estimate is equivalent to the amount of marketable volume

Table 3. Diameters and lengths in which the conversion factor *212 approximates the SI metric estimated volume.

Diam 1 (cm)	Diam 2 (cm)	Average diameter (cm)	Length (m)	Vol Smalian (m^3)	Vol Doyle (board ft)	Vol Smalian×212 (board ft)	Diff. (board ft)
37	37	37	2.44	0.26	55.83	55.58	0.25
37	37	37	4.27	0.46	97.70	97.27	0.43
39	38	38.5	2.44	0.28	60.07	60.18	0.11
39	38	38.5	2.44	0.28	60.07	60.18	0.11
39	38	38.5	3.05	0.35	75.08	75.23	0.14
39	38	38.5	2.59	0.30	63.82	63.94	0.12
39	38	38.5	6.10	0.71	150.17	150.45	0.28
39	38	38.5	5.49	0.64	135.15	135.41	0.25
44	41	42.5	2.59	0.37	78.32	77.92	0.40
46	42	44	2.59	0.39	83.48	83.52	0.04
46	42	44	4.27	0.65	137.50	137.55	0.06
46	42	44	6.71	1.02	216.06	216.16	0.09
48	43	45.5	2.59	0.42	88.81	89.31	0.50
55	47	51	2.44	0.50	105.18	105.60	0.42
55	47	51	2.59	0.53	111.76	112.20	0.45
60	50	55	2.44	0.58	123.01	122.82	0.19

Diam 1: Largest diameter; Diam 2: shortest diameter; Vol: volume; Diff: difference between volume estimates from both methods.

(Syed *et al.*, 2020). When calculating the volume of roundwood, it is important to have equivalents that allow for conversion from one system of units to another during processing, transportation, or marketing. Based on data from the National Forest Institute of Guatemala (INAB, 2019), 1 m^3 is equivalent to 424 board feet. Therefore, it is important that forestry companies in the state of Durango consider these equivalencies when assuming a sawing coefficient of 50% to convert from m^3 to board feet.

Knowledge and records of timber (standing trees) in metric units will allow monitoring the original volume and understanding how it is distributed among the various products, by-products, and waste throughout the entire sawnwood production chain (Hernández *et al.*, 2024a). Those authors suggested that logs should be cubed directly in metric units, without applying the English system or other usual conversion factors. The most convenient approach would use the Smalian or Huber formulas, as well as promoting the use of the International Standard Units (SI), thus avoiding underestimation or overestimation in customary commercial conversion.

Most of the studies on roundwood cubing agree on the use of a single method as standard, since differences between systems have strong economic repercussions. Moreover, differences probably impact on the sustaninability of native forests, from which the raw material (timber) is obtained (Ek *et al.*, 2003; Romero, 2024).

This study contibuted with significant evidence of different results when using the Doyle and Smalian cubing methods. Therefore, when using two different methods, it is advisable to use equivalent conversion factors that adapt to the reality of the forests. For this reason, it is suggested not to use the Doyle rule and to emphasize the use of the International Standard of Units, which is metric, as it is established in forest regulations (Alpízar, 2020; Hernández *et al.*, 2024b). This will be in compliance with the Regulations of the Mexican General Law on Sustainable Forest Development (Reglamento de la Ley General de Desarrollo Forestal Sustentable– LGDFS, Article 32) which establishes that raw materials and wood forest products must be expressed in cubic meters (m^3), the volumetric unit of the standard International System of units (SI).

CONCLUSIONS

In the volume estimation of logs there was significant difference between the Smalian×212 and Doyle cubing methods. Volume estimates were significantly greater ($p \leq 0.01$) with the Smalian equation. Only for logs with diameters 37 cm to 55 cm, and lengths 2.44 m to 6.71 m, the conversion factor used approximates the actual estimated volume. It is important to emphasize, according to national forest regulations, that two measurement systems, expressed with very different units, should not be mixed.

In the state of Durango, volumetric measurement methods still are used with a mix of units of measurement and conversion factors, thus affecting the marketing of roundwood. The use of the metric international standard should be prioritized over the Doyle rule, in accordance with Mexico's forest regulations (LGDFS, article 32). As a recommendation, stakeholders in the production chain should be aware of the volumetric differences of the methods analyzed in this study, to get an informed consensus on those possible economic repercussions of an imprecise cubing at the spot of roundwood marketing.

ACKNOWLEDGEMENTS

To the forestry companies in the city of Durango (Durango, Mexico) that allowed us gathering data in their log yards.

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Biocontrol of Chickpea Wilt Caused by *Fusarium oxysporum* f. sp. *ciceris* Race 0 with Plant Growth-Promoting Bacteria

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Citation: Edeza-Urías, J. A., López-Orona, C. A., Angulo-Castro, A., López-Urquídez, G. A., Castro-Diego, J. A., Estrada-Vázquez, R., Gomez, G., & Rubio-Aragón, W. A. (2025). Biocontrol of Chickpea Wilt Caused by *Fusarium oxysporum* f. sp. *ciceris* Race 0 with Plant Growth-Promoting Bacteria. *Agro Productividad*. https://doi.org/10.32854/chbkcr36

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

Received: January 15, 2025.
Accepted: May 21, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 163-170.

ABSTRACT

Objective: To evaluate the antagonisms of the plant growth-promoting bacteria, A46, P61 (*Pseudomonas tolaasii*), R44 (*Bacillus pumilus*), BSPS1.1 (*Paenibacillus polymyxa*), and CPPC55 (*Serratia plymuthica*) strains to *Fusarium oxysporum* f. sp. *ciceris* race 0 under *in vitro* and *in vivo* conditions.

Design/methodology/approach: A growing chamber and greenhouse assays were carried out to evaluate the antagonistic effect of the plant growth-promoting bacteria strains against *Fusarium oxysporum* f. sp. *ciceris* race 0 in Petri dishes and chickpea plants, respectively.

Results: The five bacteria strains significantly reduce the pathogen radial growth, and the strains A46, P61, R44 and CPPC55 reduced the chickpea plant disease symptoms and the affectation of different plant morphological traits such as height, stem width, number of leaves and root volume, plus nitrogen and potassium content.

Limitations on study/implications: *Fusarium* Wilt is one of the main causes of chickpea production losses worldwide. The identification and evaluation of promising biocontrol agents are crucial for their inclusion in integrated management programs to the disease.

Findings/conclusions: Chickpea seeds inoculation with plant growth-promoting bacteria strains A46, P61, R44 and CPPC55 are recommended to *Fusarium oxysporum* f. sp. *ciceris* race 0.

Keywords: Control, Fusariosis, *Cicer arietinum*, wilt, PGPB.

INTRODUCTION

Mexico is in the top ten of main producers of chickpea (*Cicer arietinum* L.) worldwide and in the second place in the Americas with around 111,143 ha cultivated in 2022 (FAOSTAT, 2023). Northwestern region, including Sinaloa, Sonora and Baja California states is the main producer area of chickpea in Mexico, where production reached in 2022 about 74.13% of the total national area cultivated to chickpea (SIAP, 2023).

Different factors affect chickpea yield in Northwestern Mexico such as wilting diseases caused by soil born fungi complex, including *Fusarium oxysporum* (Cota-Barreras *et al.* 2024). For chickpea, eight *F. oxysporum* f. sp. *ciceris* races have been described so far which are grouped according to their symptomatology into yellowing (0 and 1B/C) and wilting pathotypes (1A, 2, 3, 4, 5 and 6) (Jendoubi *et al.* 2017). Five out of the eight races have been reported in Mexico (0, 1B/C, 4, 5 and 6) and two in the Northwestern states (0 and 5) (Velarde-Félix *et al.* 2015; Guerrero-Aguilar, 2015). In this region, wilting by *F. oxysporum* f. sp. *ciceris* causes losses up to 60% (Arvayo-Ortiz *et al.* 2012), and commercial cultivars and breeding lines are susceptible, including race 0 provoking farmer's efforts to manage the disease to not work, needing control alternatives.

The use of plant growth-promoting bacteria is an effective, eco-friendly, and sustainable control strategy to complement or substitute the use of chemical fungicides (Shaikh and Sayyed, 2014). Plant growth-promoting bacteria are known to promote the plant development by producing siderophores, auxins, phytohormones and enzymes, nitrogen fixation, solubilizing phosphate, releasing ammonia or by supplying essential vitamins to plants (Saini *et al.* 2023). Nevertheless, these microbes also exhibit protection against phytopathogenic fungi mediated by volatile metabolites including hydrogen cyanide and ammonia, iron acquisition by siderophores production of antibiotic, exopolysaccharides, production of cell wall degrading enzymes and antioxidant enzymes like superoxide dismutase, catalase, peroxidase and phenols (Chowdhury *et al.* 2020; Khalil *et al.* 2022). In Mexico, different screening studies have been conducted to detect, identify, characterize and test different plant growth-promoting bacteria, strains A46, P61 (*Pseudomonas tolaasii*), R44 (*Bacillus pumilus*), BSPS1.1 (*Paenibacillus polymyxa*), and CPPC55 (*Serratia plymuthica*) have proved to promote different plant growth parameters and nutrients uptake on several crops such as peppers (*Capsicum* spp.), maize (*Zea mays*) and cucumber (*Cucumis sativus*) (Perez-Rosales, 2012; Benítez-Noyola, 2013; Angulo-Castro *et al.* 2018; Castillo-Aguilar *et al.* 2017; Quiroz-Sarmiento *et al.* 2019), however, evaluations against phytopathogenic fungi are limited (Pérez-Rosales 2012; Pineda-Mendoza *et al.* 2018; Florencio-Anastasio *et al.* 2022) and for *Fusarium oxysporum* f. sp. *ciceris* race 0 no study has conducted. Therefore, the goals of the present study were to evaluate the antagonisms of these five bacteria strains to *Fusarium oxysporum* f. sp. *ciceris* race 0 under *in vitro* and *in vivo* conditions.

MATERIAL AND METHODS

The *Fusarium oxysporum* f. sp. *ciceris* race 0 (FOC 0) isolate used was provided by Dr. Sixto Velarde-Félix (INIFAP, Culiacán, Sinaloa) with isolate number 130CS and accession number KJ000583. The PGPB strains *Pseudomonas tolaasii* "R46" (KY933652) and "P61" KY933651, *Bacillus pumilus* "R44", *Paenibacillus polymyxa* "BSPS1.1", and *Serratia plymuthica* "CPPC55" (KX259564) were provided by the Colegio de Postgraduados, Montecillo, México.

The fungal isolate was prepared in Petri dishes (90×15 mm) using the portion inclusion method (Leslie and Summerell 2006). Mycelium disks of 1-cm in diameter cut with a perforating punch were placed in the center of Petri dishes supplemented with PDA-Difco® culture medium (39 g L^{-1}), incubated at 25 °C for 7 days in complete darkness inside a Friocel® growth chamber (Landa *et al.*, 2006), the final conidia suspension of the isolate was adjusted to 1×10^6 spores/mL. On the other hand, the bacterial strains were prepared in Petri dishes without division (90×15 mm) with Difco® nutritional culture agar (23 g L^{-1}) under aseptic conditions in a laminar flow hood. A sample of each strain was taken following the streak plate method using a sterile bacteriological loop. Dishes were incubated at 28 °C for 24 h for their development under darkness in Friocel®, the final concentration for each strain was adjusted to 1×10^8 CFU/mL.

A growing chamber assay (25 °C) was carried out to evaluate the antagonistic effect of the five PGPB strains against FOC 0 in Petri dishes with the dual culture technique (Ezziyyani *et al.*, 2004). For this, a 1-cm diameter disk of the PDA-grown phytopathogen was placed at one end of the plate and, after 72 h, the bacterial strain was sown in the center of the plate using a thick loop forming a vertical barrier (1.0 cm thick); a control dish with only the fungal inoculum was also included. The bacterial inhibitory capacity was determined after seven days. A digital Vernier was used to measure the pathogen radial growth (cm), subsequently, the inhibitory percentage of radial growth (IPRG) was calculated with the formula from Ezziyyani *et al.* (2004).

$$IPRG=(R1-R2)/R1\times100$$

where: $R1$=larger *Fusarium* growth radii and $R2$=smaller *Fusarium* growth radii.

A completely randomized design was used with four replications per treatment where each challenging disk was considered as replication. The experiment was performed twice.

An *in vivo*-greenhouse experiment (25-30 °C and 50-60% HR) was carried out to evaluate the effect of the five PGPB strains to control FOC 0 in chickpea plants (Blanco Sinaloa-92). Disinfected seeds (2.5% sodium hypochlorite) were submerged in a 1×10^8 CFU/mL bacterial inoculum for 5 min. Inoculated seeds were germinated and grown individually in polystyrene trays of 128 cavities (autoclaved peat and vermiculite [2:1]). Each plant (3 true leaves) received 15 mL of fungal inoculum (1×10^6 spores mL^{-1}). To fertilize the plants, a Steiner solution (0.5 ds/m and pH 6.5) was used with a daily dose of 50 mL per plant. Plants symptoms were recorded every 15 days after the inoculation (DAI) until 75 DAI using the Fusariosis wilt visual scale (0-4) proposed by Marlatt *et al.* (1996), where: 0=asymptomatic plant; 1=mild wilting symptoms, leaf chlorosis, growth delay; 2=moderate chlorosis symptoms, wilting, growth delay; 3=severe chlorosis symptoms, wilting or growth delay; 4=dead plant, completely wilted. The disease severity index (Chiang and Bock, 2022).

$$(DSI)(\%)=\left[\sum\frac{(class\ frecuency\times score\ of\ rating\ class)}{(total\ number\ of\ observations)}\times(maximal\ disease\ index)\right]\times100$$

On the other hand, the mean area under disease progress curve (AUDPC) was calculated using the formula of Pandy *et al.* (1989),

$$AUDPC = D\left[1/2(Y1+Yk)+(Y2+Y3+...+Yk-1)\right]$$

where D=time interval; $Y1$=first DSI; Yk=last DSI; and $Y2$, $Y3$, $Yk-1$=intermediate DSI.

Besides the visual damage, plant growth parameters such as plant height (cm), stem thickness (mm), number of leaves, and root volume (cm^3) were assessed in every evaluation. Additionally, in the last evaluation, five plants were randomly selected from each treatment and the content of the nitrogen, phosphorus, and potassium (mg kg^{-1}) was determined as follows: nitrogen was determined through the semi-micro Kjeldahl procedure (Etchevers, 1987). Phosphorus was determined through colorimetry of molybdophosphoric acid complexes reduced with ascorbic acid (AOAC, 1980). Potassium was determined through flame photometry according to Rodríguez and Rodríguez (2015). The handling of the sample included dehydrating at 70 °C, 24 h, grinding and weighing 0.5 g of dry matter from all the organs of the plant (root, stem and leaves).

All data were analyzed with the XLSTAT statistics software (Addinsoft, 2019). Data generated from both experiments was combinate since no statistical difference was exhibited ($P \leq 0.05$). The data was subjected to Shapiro-Wilk and Barlett tests to verify the statistical assumptions of normality and homogeneity of variances, respectively. The radial growth from the *in vitro* assay did not meet these statistical assumptions, therefore, a nonparametric variance analysis was used. In the *in vivo* assay, the damage scale and plant phenotype indicators (plant height, stem thickness, number of leaves and root volume) met the statistical assumptions, therefore, a parametric variance analysis was used. On the other hand, the content of the nitrogen, phosphorus, and potassium did not meet these assumptions. For the multiple comparison among treatments, an ANOVA and the Tukey mean test were used in the parametric variables to determine the significance among treatments ($P \leq 0.05$). For the non-parametric factors, the Kruskal-Wallis and the Dunn median tests were used ($P \leq 0.05$).

RESULTS AND DISCUSSION

The efficacy of the five PGPB *P. tolaasii* "A46" and "P61", *B. pumilus* "R44", *P. polymyxa* "BSP1.1", and *S. plymuthica* "CPPC55" was observed to reduce *Fusarium oxysporum* f. sp. *ciceris* race 0 (FOC 0) radial growth *in vitro*. The five bacteria exhibited a statistical difference with the control but not between them ($H=14.000$; df=5; $P=0.016$), indicating a strong inhibition action against the pathogen with a range from 40.92 to 58.51% (Table 1) (Figure 1).

These results indicate an antibiotic ability by the strains; A46 and P61 have been reported to produce siderophores while BSP1.1 and R44 do not, and CPPC55 has not been tested (Angulo-Castro *et al.*, 2018; Pineda-Mendoza *et al.*, 2019), suggesting the involvement of a different compound. Further studies need to be carried out to in order to identify it. These results partially agree with those reported by other authors such as Pérez-

Table 1. Inhibition of *in vitro* mycelial growth of *Fusarium oxysporum* f. sp. *ciceris* races 0 by the five plant growth-promoting bacteria strains on PDA media.

Strain	Pathogen growth (cm)	IPRG (%)*
A46 (*Pseudomonas tolaasii*)	3.87±0.64 b**	48.45
P61 (*Pseudomonas tolaasii*)	3.96±0.40 b	47.28
R44 (*Bacillus pumilus*)	3.76±0.32 b	49.88
BSP1.1 (*Paenibacillus polymyxa*)	4.43±0.09 b	40.92
CPPC55 (*Serratia plymuthica*)	3.11±0.08 b	58.51
Control	7.50±0.08 a	/

* $IPGR = inhibition\ percentage\ of\ radial\ growth = \left(\left[control - treatment\right] / control\right) \times 100.$

**=Means with different letter in columns indicate significant difference according to Dunn's median test (P≤0.05).

Rosales (2012) who determined strain A46 inhibiting *F. oxysporum* and *Alternaria* sp. growth by 36.52 and 53.83% while strain P61 did 20.05 and 55.14%, respectively, and R44 did not inhibit anything at all on both pathogens. On the other hand, Pineda-Mendoza *et al.* (2018) reported 36 and 44% inhibition to *Rhizoctonia solani* by strains A46 and P61, respectively, and Florencio-Anastasio *et al.* (2022) 13.0, 20.5 and 44.7% to *F. oxysporum* f. sp. *cubense* by strains P61, R44 and BSP1.1, respectively. This variation among the results of these studies indicates that the strains have a different respond according to the plant pathogen, needing an individual pathogen by pathogen evaluation. To the best of our knowledge, this is the first time evaluating this bacteria species and strains to FOC 0.

In the *in vivo* assay, the significant differences among strains and the control started after 45 days post FOC 0 inoculation (DAI), nevertheless, in this evaluation no strain was statistically different from the control but between them, R44 exhibited the lowest disease severity index (DSI), and CPPC55 and P61 the highest. In the 60 DAI evaluation, strains A46, P61 and R44 exhibited the lowest disease, and in the 75 DAI strains P61, R44 and CPPC55 did. On the mean area under disease progress curve (AUDPC) calculated with the five evaluations, A46, P61, R44 and CPPC55 significantly differed from the control and only BSP1.1 did not (Table 2).

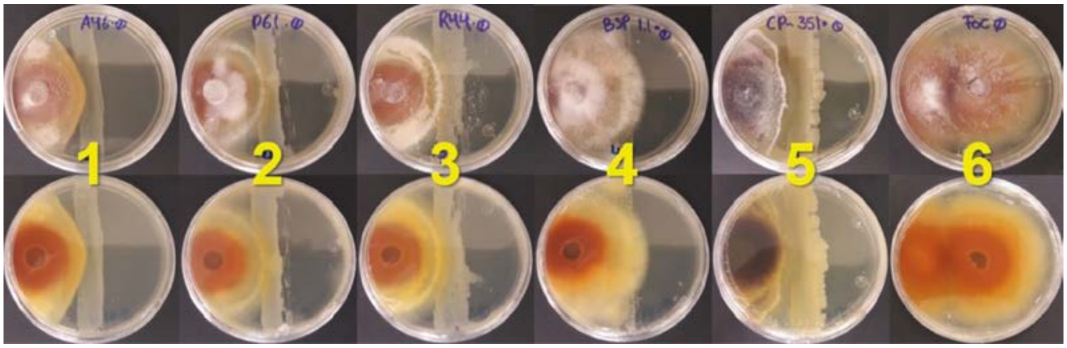

Figure 1. Inhibition of *in vitro* mycelial growth of dual culture against FOC-0 in Petri dishes with PDA-Difco medium® at 25 °C for 7 days in total darkness in growth chamber (Friocel®). Where: 1=FOC 0 *vs.* A46; 2=FOC 0 *vs.* P61; 3=FOC 0 *vs.* R44; 4=FOC 0 *vs.* BSP1.1; 5=FOC 0 *vs.* CPPC55; 6=FOC 0.

These results indicate that FOC 0 has a destructive effect on chickpea plants and the inoculation of chickpea seeds with PGPB can reduce the disease severity caused by the pathogen, however, there is variation among bacteria. Diseased plants may show affections through the reduction of morphological traits and nutrients content, and on the other hand, PGPB role is to improve morphological plant traits and nutrients content, in the current experiment morphological parameters such as plant height, stem width, number of leaves and root volume plus nitrogen (N), phosphorus (P) and potassium (K) content was compared 75 DAI in order to evaluate the PGBP strains on inoculated FOC 0 chickpea plants. Strain A46 had higher N and K content in comparison with the control; strain P61 showed a higher plant height, N and K content; strain R44 exhibited a higher plant height, stem width and root volume; strain CPPC55 had a higher plant height, number of leaves, N and K content in comparison with the control (Table 3).

These results indicate that chickpea plants inoculated with these four PGPB can besides reducing FOC 0 symptoms, can reduce the plants morphological traits affections in major agronomic parameters linked to yield such as plant height, stem width, number of leaves

Table 2. Effect of the plant growth-promoting bacteria strains inoculation on chickpea seeds on the *Fusarium oxysporum* f. sp. *ciceris* race 0 disease severity index (DSI %) and the area under disease progress curve (AUDPC).

Strain	Disease severity index (%)			AUDPC
	45 dai	60 dai	75 dai	
A46 (*Pseudomonas tolaasii*)	16.7 ab*	46.7 b	57.5 ab	3313 b
P61 (*Pseudomonas tolaasii*)	21.7 a	48.3 b	51.7 bc	3187 b
R44 (*Bacillus pumilus*)	13.3 b	47.5 b	53.3 bc	3168 b
BSP1.1 (*Paenibacillus polymyxa*)	16.7 ab	49.2 ab	57.5 ab	3388 ab
CPPC55 (*Serratia plymuthica*)	25.0 a	50.0 ab	50.0 c	3190 b
Control	16.7 ab	55.0 a	64.2 a	3769 a
P	0.042	0.003	0.001	0.001

*=Means with different letter in columns indicate significant difference according to Tukey's mean test ($P \leq 0.05$); dai=days after inoculation.

Table 3. Response of chickpea plants morphological traits and nutrient content of five previous seed inoculated growth-promoting bacteria strains 75 days after *Fusarium oxysporum* f. sp. *ciceris* race 0 inoculation.

Strain	Plant morphological traits				Nutrient content		
	PH (cm)	SW (mm)	NL	RV (cm^3)	N	P	K
A46 (*Pseudomonas tolaasii*)	31.2±4.5 b	2.6±0.4 ab	20.5±2.9 b	2.5±1.0 ab	6.6±1.0 ab	2.0±0.3 a	15.6±0.9 a
P61 (*Pseudomonas tolaasii*)	33.6±3.2 a	2.5±0.3 ab	20.4±1.9 b	2.4±0.8 ab	6.2±1.9 b	2.1±0.2 a	12.3±0.5 a
R44 (*Bacillus pumilus*)	33.3±2.3 a	2.7±0.4 a	21.0±2.8 ab	2.7±0.8 a	5.0±1.0 bc	1.9±0.3 a	10.6±2.3 ab
CPPC55 (*Serratia plymuthica*)	35.7±2.8 a	2.7±0.5 ab	22.5±2.7 a	2.5±0.7 ab	8.4±0.9 a	1.8±0.9 a	13.7±0.5 a
Control	30.1±3.9 b	2.5±0.3 b	20.7±2.0 b	2.2±0.8 b	4.1±0.8 c	1.8±0.2 a	9.8±0.6 b
P	0.001	0.038	0.008	0.010	0.001	0.384	0.001

PH=plant height; SW=stem width; NL=number of leaves; RV=root volume; N=nitrogen; P=phosphorus; K=Potassium. Means with different letter in plant morphological traits columns indicate significant difference according to Tukey's mean test ($P \leq 0.05$) and in nutrient content indicate significant difference according to Dunn's median test ($P \leq 0.05$).

and root volume, plus nutrient content including N and K. To the best of our knowledge, this is the first *in vivo* assay designed to evaluate these strains as biocontrol agents.

CONCLUSIONS

PGPB strains A46, P61 (*Pseudomonas tolaasii*), R44 (*Bacillus pumilus*), BSPS1.1 (*Paenibacillus polymyxa*), and CPPC55 (*Serratia plymuthica*) significantly reduces mycelial growth of *Fusarium oxysporum* f. sp. *ciceris* race 0. *Fusarium oxysporum* f. sp. *ciceris* race 0 has a destructive effect on chickpea plants and the inoculation of chickpea seeds with PGPB can reduce the disease severity caused by the pathogen, however, there is variation among PGPB. Strains A46, P61, R44 and CPPC55 reduce the chickpea plant disease symptoms and the affectation of different plant morphological traits such as height, stem width, number of leaves and root volume, plus nitrogen and potassium content.

ACKNOWLEDGEMENTS

Authors thanks the Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT) for the scholarship granted Jorge Alberto Edeza-Urías to carry out his Postgraduate Studies on Ciencias Agropecuarias, Colegio de Ciencias Agropecuarias at the Universidad Autónoma de Sinaloa – Facultad de Agronomía.

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Colegio de Postgraduados

Isolation and identification of fungi with bio-herbicidal activity in *Cyperus rotundus*

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ABSTRACT

Objective: to isolate and to identify selected fungi with bio-herbicidal potential in *Cyperus rotundus*.

Design/Methodology/Approach: for this research we collected *Cyperus* spp. plants in four sites of the municipalities Cárdenas and Huimanguillo (Tabasco) Mexico. From various tissues of healthy and diseased plants of *Cyperus*, different strains of fungi were isolated for morphological and molecular characterization. They were then identified through the ITS (Internal Transcribed Spacer) region. Some fungi that were isolated from diseased plants were selected to perform some pathogenicity and severity tests on seedlings of *C. rotundus*

Results: nine genera of fungi were isolated and associated with diseased and healthy plants of *Cyperus* spp. Only *Fusarium*, *Curvularia* and *Pestalotiopsis* were pathogenic in seedlings of *Cyperus rotundus*. These three genera of fungi caused different degrees of symptomatology and severity of damage on *C. rotundus*.

Limitations/Implications of the study: infrastructure and climate variables in the greenhouse could affect the variables evaluated. One clear limitation is found in the high costs of performing the complete molecular identification of selected fungi up to the taxonomic level of species.

Findings/Conclusions: this study provided evidence on the bio-herbicidal potential of fungi from genera *Fusarium*, *Curvularia* and *Pestalotiopsis* in seedlings of *C. rotundus*, However, more detailed studies are required on determining the range of hosts; on the combinations of these fungi, both of spores and extracts; and on the effect on sexual or asexual propagation of different species of *Cyperus*. As well as to evaluate the effect on wild plants considered weeds, and cultivated plants in order to test their full potential as bio-herbicide.

Keywords: weeds, endophyte fungi, phytopathogens, bio-herbicides.

Citation: Ortiz-Gálvez, G. C., García-De la Cruz, R., Martínez-Hernández, A., Acosta-Pech, R. G., & Ortiz-García, C. F. (2025). Isolation and identification of fungi with bio-herbicidal activity in *Cyperus rotundus*. *Agro Productividad*. https://doi.org/10.32854/7newwt06

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

Received: January 15, 2025.
Accepted: May 23, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 171-182.

INTRODUCTION

Around the world, one of the biotic-related problems that negatively impact the agriculture sector is the presence of large numbers of weeds. The species *Cyperus rotundus* L. and *Cyperus esculentus* L. are among the most well-known weeds of global importance. These plants have a great capacity to adapt to different soil conditions, moreover, they disperse rapidly through bulbs or rhizomes. Due to this, these wild plants can establish in various agri-climatic regions, where they can cause losses between 20 and 90% in agricultural crops worldwide (Moncada, 2017; Peerzada, 2017). In general, weed populations are controlled through commercial chemical herbicides, however, these products result in negative impacts on the environment and health when they are used in the long term (Franco *et al.*, 2015; Mohd Ghazi

et al., 2023). Thus, the use of alternatives that are responsible with the environment is suggested, such as integrated weed management (Pavlović *et al.*, 2022).

Recent research focused on the microbial endobiome, in particular of those plants considered as weeds, in search of potential agents for biological control (Saitheja *et al.*, 2024). Thus, for example, endophyte fungi (EF) are found in the plant endobiome, which colonize internal plant tissues without causing apparent symptoms of disease in their host. Nonetheless, under certain conditions (environmental, from stress, or senescence of the plant) they can become pathogenic (Dos Reis *et al.*, 2022).

This study reports the presence of endophyte fungi and fungi with potential as phytopathogens in *Cyperus* spp. Some strains that could have potential as biological control agents were identified; those were evidenced by high level of pathogenicity and virulence to *C. rotundus*. This study set the foundations for future studies with these fungi to expand the characterization of bio-herbicide potential, by determining a range of hosts and by assessing combination with other weed management methods on land evaluations.

MATERIALS AND METHODS

Isolation and characterization of fungi

Sampling in four sites consisted of collecting six plants with a similar size per site, either with or without symptoms of *Cyperus* spp. infection. Sites were the "Benito Juárez" sugar mill (18° 00' 02.7" N, 93° 34' 46.5" W), the C-34 village (17° 57' 56.3" N, 93° 35' 54.5" W), and two plots of "Ejido La Esperanza" (17° 54' 23.4" N, 93° 36' 26.2" W; 17° 54' 22.8" N, 93° 37' 31.1" W).

Collected plants were cut into fragments of roots, stem, leaves and inflorescence which were disinfected with 70% ethanol inside a laminar flow hood for 2 min. They were then immersed in 0.5% sodium hypochlorite for 2 min. Finally, they were rinsed twice with sterile distilled water. Fragments were inoculated in Petri dishes with Potato Dextrose Agar (PDA) medium and left in incubation at 27±2 °C for 12 h light and 12 h dark until hyphae growth was observed. These isolates were classified into morphotypes according to Arnold *et al.* (2001). In addition, monosporic cultures were done following the method of Molina -Cárdenas *et al.* (2021).

Morphological identification

The morphological characterization of the fungi was done by observation through an optical compound microscope (Leyca Microsystem DM750). The structures were analyzed following Dugan's (2006) taxonomic identification keys.

Molecular identification

DNA extraction

For DNA extraction, the protocol of Raeder and Broda (1985) was used, with the modifications established at the Pest Microbial-control Lab under Colegio de Postgraduados Campus Campeche. Integrity was observed through 0.7% agarose gels added with ethidium bromide (BrEt). The concentrations of the gDNA (genomic DNA) were quantified with a

NanoDrop (Thermo Fisher). All gDNA concentrations were adjusted to 100 ng μL^{-1} prior to PCR amplification (Castle *et al.*, 2018).

Amplificación por PCR

To perform the PCR amplification of the fungal gDNA, a reaction of 40 μL was performed for each sample with the following concentrations: 100 ng μL^{-1} gDNA, PCR Buffer- $MgCl_2$, 4mM $MgCl_2$, 0.6mM dNTP mix, 0.4 pmol μL^{-1} of each primer ITS1-TCCGTAGGTGAACCTGCGG, or ITS4-TCCTCCGCTTATTGATATGC, 0.1 U μL^{-1} of Taq Polymerase (Invitrogen Cat. No. 10342-020) and sterile ultrapure H_2O.

The C1000TM Thermal Cycler (BIO-RAD) was programmed based on the temperatures mentioned by White *et al.* (1990) plus specific modifications in timing. An initial denaturation at 95 °C for 7 min, 40 cycles with denaturation of 95 °C for 1 min, alignment at 55 °C for 1 min and extension at 72 °C for 1 min, ending with a final extension of 72 °C for 5 min. At the end of the reactions, the PCR products were visualized in 1% agarose gel plus BrEt. Subsequently, the PCR purifications were done with the commercial QIAquick PCR Purification Kit (Quiagen Cat. Num. 28104) and the integrity of the product was verified with Nanodrop.

Preliminary pathogenicity tests in *Cyperus rotundus*

Plants of *Cyperus rotundus* that had the same height and age characteristics were collected and selected. Twelve *C. rotundus* plants were placed per plastic germination tray in rows of three plants for each of the selected fungi; which belong to the genera *Curvularia*, *Pestalotiopsis*, *Fusarium*, *Helminsthosporium*, *Trichoderma*, *Pseudopestalotiopsis* and *Penicillium*. Subsequently, in the greenhouse, inoculations were done following the methodology of Jyothi *et al.* (2010). Plants were under observation until they developed symptoms, affected by the inoculated fungi. Finally, Koch's postulates were tested.

Evaluation of the severity of damage caused by *Pestalotiopsis*, *Curvularia* and *Fusarium* on *Cyperus rotundus*

From the preliminary tests, three fungi (*Fusarium* sp., *Curvularia* sp. and *Pestalotiopsis* sp.) were selected that were able to produce symptoms on *C. rotundus* plants. The conidia solutions were prepared following the methodology of Wang *et al.* (2021) and were adjusted to two concentrations of conidia (1×10^7 and 1×10^8 conidia mL^{-1}). In the greenhouse, two trays were used for each genus of fungus (each inoculated with its respective concentrations of fungi) and one as a control. Subsequently, they were inoculated with 1 mL of the conidia suspension during the evening, when the environment was at a 90% relative humidity. For 20 days we evaluated the height, number of tillers emerged (regrowth) and severity of damage as result of the disease in the plants (Figure 1).

Statistical analysis

A completely randomized design was used with two treatments (conidia concentrations) and six replicates. The response variables of interest were height and number of tillers. Normality and homogeneity tests were performed on the data obtained to run the analysis

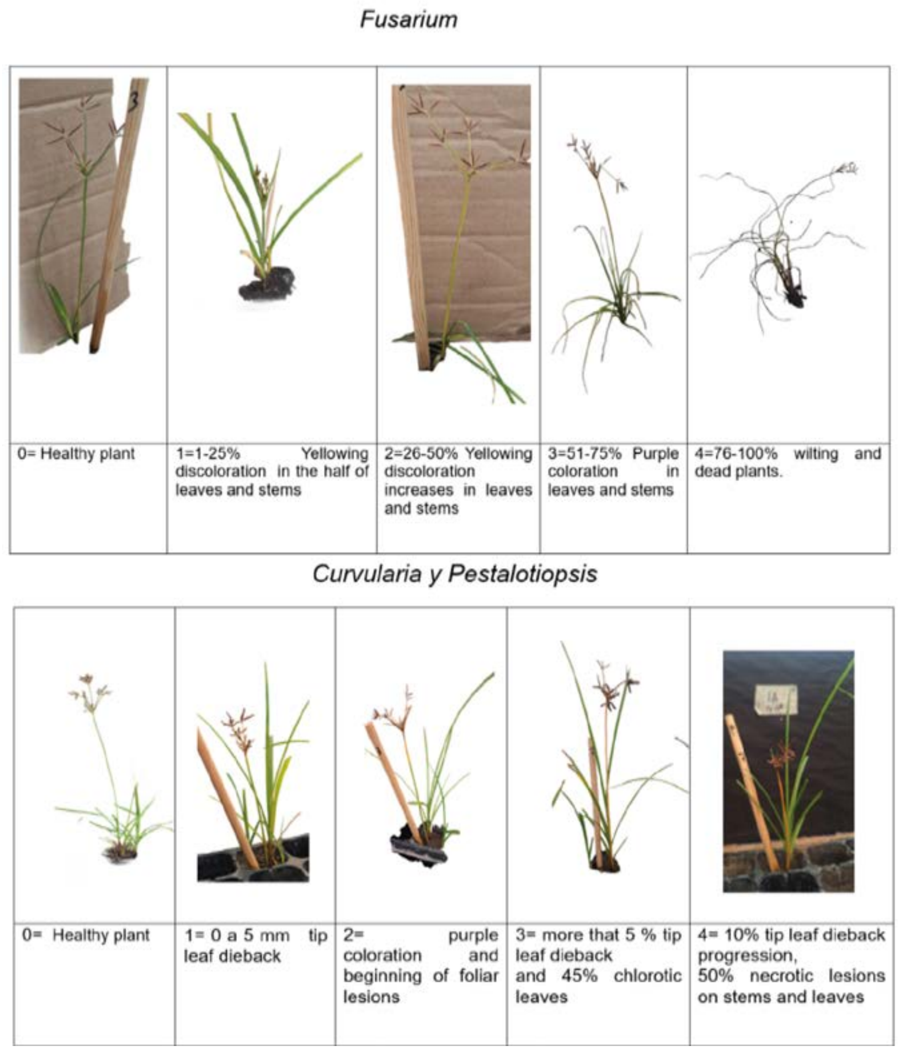

Figure 1. Severity score used in the greenhouse bioassays at Colegio de Postgraduados Campus Tabasco, in 2024.

of variance with the RStudio software version 4.4.1; When this was significant, multiple comparisons of means was done with the Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Isolation and morphological identification of fungi

A total of 146 isolates of endophytic and epiphyte fungi were obtained from different parts of *Cyperus* spp. plants with and without symptoms. These isolates were grouped into 20 morphotypes. Seven genera of fungi were isolated from healthy plants, corresponding to the genera *Nigrospora*, *Aspergillus*, *Penicillium*, *Fusarium*, *Pseudopestalotiopsis*, *Curvularia* and *Trichoderma*. On the other hand, in plants with symptoms, the same fungi that were identified in plants without symptoms were found, plus two more genera of fungi, *Pestalotiopsis* and *Helminthosporium* that only were isolated with low frequency. It is well documented that there are many microorganisms that can be found in almost all parts of plants. However, the identification of weed-colonizing fungi is a topic that is little researched and, therefore, little known. In this study, we identified fungi whose colonies were found in different vegetative parts, in both healthy plants and symptomatic plants of *Cyperus rotundus* and *C. esculentus*.

Triolet *et al.* (2022) mentioned that weeds, like cultivated plants, possess and host a large amount of endophytic microflora, micro-epiphytes, and rhizosphere microorganisms. Within the community of microorganisms there are phytopathogenic fungi and some of them with potential for use as bio-herbicides. The starting point for developing a bio-herbicide is the isolation of microorganisms associated with weeds with disease symptoms. In addition, isolation from asymptomatic plants is also necessary to contrast and identify possible fungi with biological control potential in unexpected genera. In this study, the aforementioned principles were taken as a reference and from that it was possible to identify nine genera of fungi associated with *Cyperus* plants. Some authors such as Maharachchikumbura *et al.* (2011) and Kubiak *et al.* (2022) among others, in studies on the diversity of fungi and their possible use as biological weed controls, reported founding genera of fungi similar to those we isolated in this research.

Molecular analyses

Results of molecular identification are consistent with the results of macro and microscopic morphological analyses. Therefore, in all cases it was possible to confirm through the ITS region the genus to which the strains belonged. However, when analyzing the sequences in the NCBI and UNITE databases, the data we obtained only coincided with two strains of *Trichoderma* (*Trichoderma koningiopsis* and *T. harzianum*) which could be identified up to the taxonomic level of species (Table 1). Raja *et al.* (2017) indicated that although the ITS region is considered as the barcode of fungi, it is not widely well accepted. One of the reasons is because it does not work properly to distinguish species from highly specific genera. This is because some fungi have very narrow or no spaces in their ITS regions. Then, to achieve identification, it would be necessary to resort to the use of complementary primers, thus obtaining an accurate identification.

Identification, characterization and pathogenicity of *Fusarium* sp., *Curvularia* sp. and *Pestalotiopsis* sp. in *Cyperus rotundus*

Strains from the genera *Fusarium*, *Curvularia*, *Trichoderma*, *Penicillium*, *Pestalotiopsis*, *Pseudopestalotiopsis*, and *Helminthosporium* were taken from symptomatic plants. Days after inoculation, only *Fusarium*, *Curvularia* and *Pestalotiopsis* managed to cause damage to *C. rotundus* plants (Table 2).

Figure 2 shows the macro and microscopic characteristics of the strain which belongs to the genus *Fusarium*. This strain was isolated from the stem of a *Cyperus* spp. plant which presented symptoms of yellowing and stunting. This colony has a flat, smooth surface with a floccose appearance and creamy-orange color (Figure 2A). We observed the presence of conidia; thin, elongated, smooth, and crescent-shaped macroconidia can be seen, hialine and multi-septated (Figure 2B). These characteristics coincide with those reported by Lestari *et al.* (2021).

Table 1. Identification of fungi isolated from *Cyperus* spp., based on reference data from the NCBI and UNITE databases.

Strain	Identification	Base pairs	Accession	Percentage of identity (%)
HP	*Pestaloptiosis*	592	NCBI: KT459349.1 UNITE: MW404604	100 99.81
H2	*Pseudopestaloptiosis*	548	NCBI: OR588134.1 UNITE: OR588134	99.82 99.82
R1	*Penicillium*	408	NCBI: PP665290 UNITE: PP665290	100 100
RP	*Aspergillus*	555	NCBI: OR354739.1 UNITE: PQ106409	99.46 99.46
R8	*Trichoderma*	578	NCBI: KP965729.1 UNITE: OR2669177	99.83 99.83
R5	*Trichoderma koningiopsis*	580	NCBI: PP647347.1 UNITE: PP647347	100 100
R6	*Trichoderma harzianum*	595	NCBI: KU530201.1 UNITE: MT378439	100 100
T8	*Fusarium*	521	NCBI: MW391509.1 UNITE: PQ039530	100 100
TE	*Curvularia*	272	NCBI: LR994025.1 UNITE: PQ047117	99.26 99.26
TP	*Nigrospora*	413	NCBI: LC704396.1 UNITE: PP939049	99.52 99.52

Table 2. Symptomatology observed after fungal inoculation during the experiment with *Cyperus rotundus*.

Fungus	Symptoms
Pestalotiopsis sp.	Yellowing, spotting, and death on the tips of the leaves.
Curvularia sp.	Brown spots on leaves, yellowing, and death of leaf tips
Fusarium sp.	Yellowing, laxity and necrosis of stems and leaves, from the base of the plant.

On the other hand, Figure 3 shows the observed characteristics that correspond to the descriptions of the genus *Pestalotiopsis*. This strain was isolated from leaves with symptoms of yellowish leaf spots. Colony presents the following macro and microscopic characteristics, semi-elevated surface with a cottony white appearance and curved growth along its entire periphery (Figure 3A), small and circular black acervuli formed by smooth and septate sub-cylindrical fusiform conidia (Figure 3B). The apical and median appendices contained a coloration ranging from brown to dark brown. These characteristics coincide with those reported by Yin *et al.* (2024).

Finally, Figure 4 shows the isolation of the strain belonging to the genus *Curvularia*. This strain was isolated from the stem of a plant that had brown spots and yellow-brown colorations on the stem. It has an elevated cottony, dark brown surface with circular growth (Figure 4A). At the microscopic level, their conidia are straight and septated, it was observed that the conidia had a more marked and pigmented septum in the middle compared to the rest of the septa (Figure 4B). These characteristics coincide with those reported by Santos *et al.* (2018).

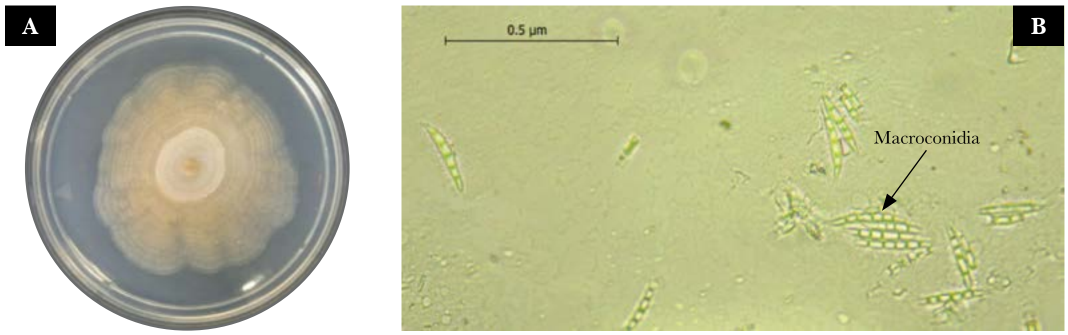

Figure 2. *Fusarium*. A: colony on PDA medium; B: microscopic structures observed using a compound optical microscope at Colegio de Postgraduados Campus Tabasco, Laboratorio de Control biológico.

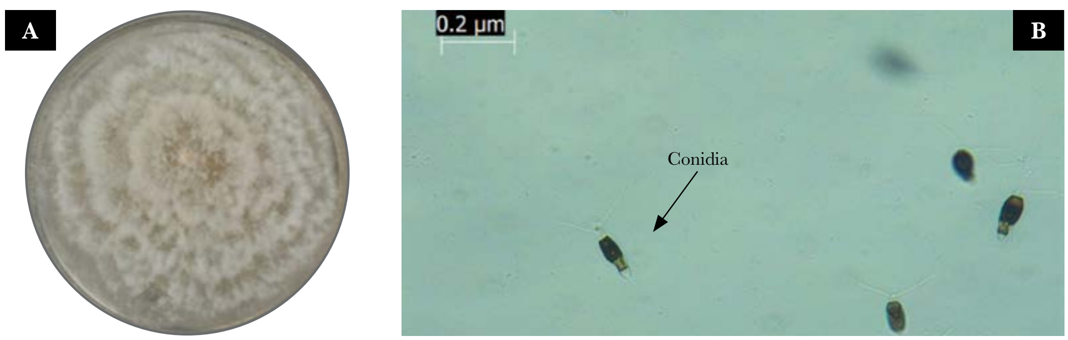

Figure 3. *Pestalotiopsis*. A: colony on PDA medium; B: microscopic structures; Compound optical microscopy at Colegio de Postgraduados Campus Tabasco, Laboratorio de Control biológico.

Figure 4. *Curvularia*. A: colony on PDA medium. B: microscopic structures; Compound optical microscopy at Colegio de Postgraduados Campus Tabasco, Laboratorio de Control biológico.

Severity of damage caused by fungi of *Curvularia*, *Fusarium* and *Pestalotiopsis* genera

Only three fungi were selected since those were pathogenic to *Cyperus rotundus* plants in greenhouse. These strains were selected to test for severity of damage analyses and to evaluate the effects of inoculum under semi-controlled conditions. After 20 days from inoculation, the damage shown in Figure 5 was observed. Damages were observed in most of the plants, in addition to stem necrosis and damage to the leaves (necrotic spots or dead leaves).

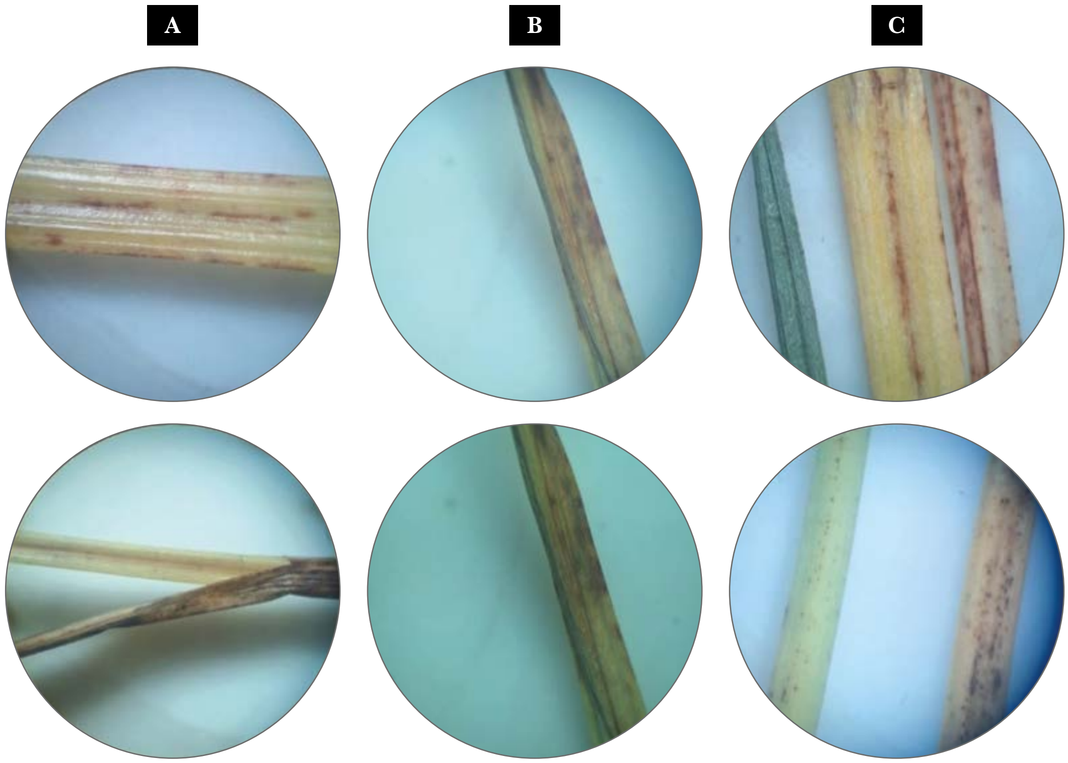

Figure 5. Damage on *Cyperus rotundus* 20 days after inoculation with A: *Fusarium* sp., B: *Curvularia* sp. and C: *Pestalotiopsis* sp.

Height of *C. rotundus* plants was affected after 20 days since inoculation (Figure 6). The inoculated control (with only distilled water) showed greater growth than the plants infected with fungi. Although non-significant statistical differences were found between both concentration treatments, there were statistical differences among the control and the treatments with fungal inoculation at two different concentrations. In addition, treatments C1, F2, P1, and P2 displayed greater dispersion of data compared to the treatments

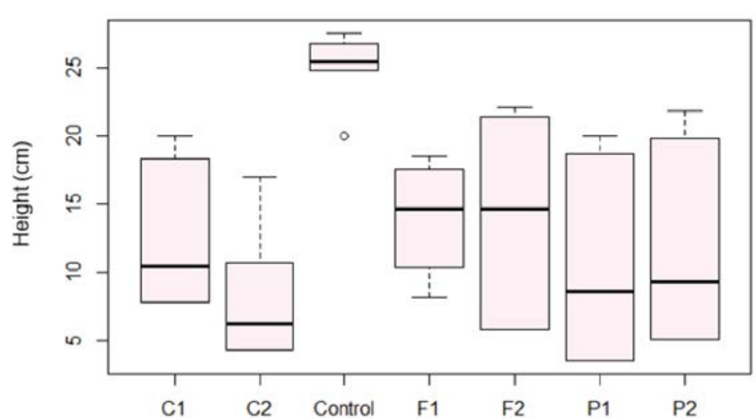

Figure 6. Effect of fungal concentrations (conidia mL^{-1}) on the height of *Cyperus rotundus* 20 days after inoculation. F1: *Fusarium* sp. 1×10^8; F2: *Fusarium* sp. 1×10^7; C1: *Curvularia* sp. 1×10^8; C2: *Curvularia* sp. 1×10^7; P1: *Pestalotiopsis* sp. 1×10^8; P2: *Pestalotiopsis* sp. 1×10^7.

with *Curvularia* 1×10^7 conidia mL^{-1} and *Fusarium* 1×10^8 conidia mL^{-1}, which had less dispersion and homogeneous damage.

For the variable of number of tillers, Figure 7 shows the trend in the emergence of new tillers on six different dates over a 20-day period. The control showed a higher number of tillers sprouted per tray cavity (4.5), while plants with fungal treatments had fewer tillers (2.5 and 3.5).

Non-significant statistical differences were found between the treatments with the fungi evaluated (Table 3). The results obtained from the analysis of variance indicated that there were significant differences ($p\leq0.05$) between the control and the concentrations of conidia used.

Regarding the severity of damage caused by the three fungi and the two concentrations, the reference values used were those in the severity score presented in the materials and methods section (Figure 1). Results obtained showed that there was a negative effect of fungi on plants of *C. rotundus*. The *Pestalotiopsis* strain at a concentration of 1×10^8 conidia mL^{-1} proved to be more aggressive to the plants inoculated with it, causing the damage described above in a shorter period of time (Figure 8). In the case of Curvularia, both inoculated concentrations managed to cause damage to the plants in a period of time

Table 3. Effect of fungi of *Fusarium*, *Curvularia* and *Pestalotiopsis* genera on the height (cm) of *Cyperus rotundus* plants.

Treatment (conidia mL^{-1})		Mean	SEM
Control		24.954 a	2.371
Curvularia	1×10^8	12.447 b	2.371
Curvularia	1×10^7	8.093 b	2.371
Fusarium	1×10^8	13.935 b	2.371
Fusarium	1×10^7	14.035 b	2.371
Pestalotiopsis	1×10^8	10.443 b	2.371
Pestalotiopsis	1×10^7	11.708 b	2.371

SEM: standard error of the mean; Tukey ($p\leq0.05$), n=8 plants.

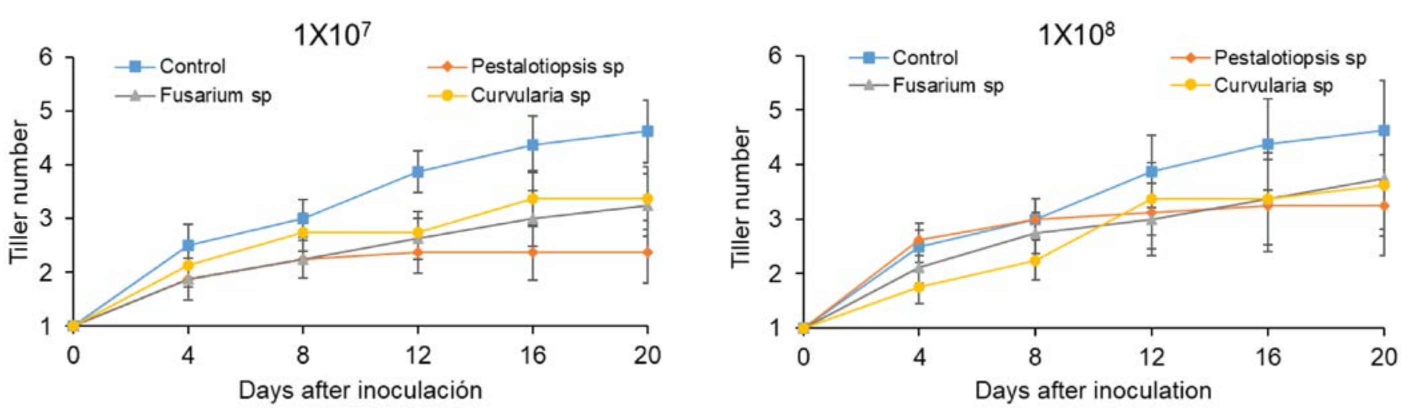

Figure 7. Effect of inoculation and concentration (conidia mL^{-1}) of the solutions with fungi *Fusarium* sp., *Curvularia* sp., and *Pestalotiopsis* sp. on tiller emergence in *Cyperus rotundus* plants.

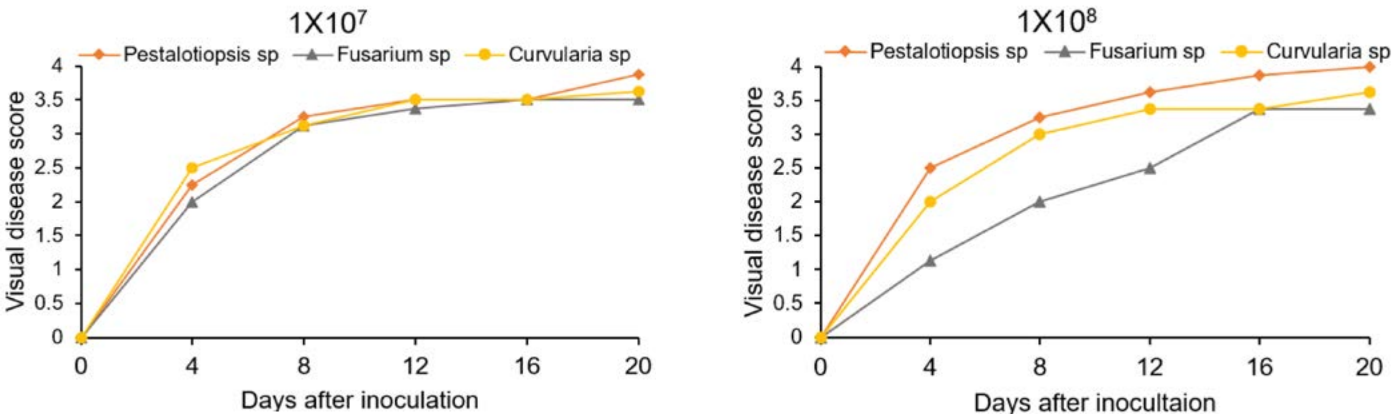

Figure 8. Severity of damage by *Pestalotiopsis* sp., *Curvularia* sp. and *Fusarium* sp. (1×10^8 and 1×10^7 conidia mL^{-1}) in *Cyperus rotundus*.

similar to that of Pestalotiopsis. On the other hand, concentrations of 1×10^8 conidia mL^{-1} of *Fusarium* caused less severe damage to *C. rotundus*. Therefore, despite the effects caused on *C. rotundus* plants, *Fusarium* did not demonstrate that it has the potential to cause significant damage under the experimental conditions reported here.

In the results of these tests, it was observed that the fungi evaluated have the ability to decrease the development of *C. rotundus* plants; however, they were not able to inhibit the growth of new tillers. Although non-significant statistical differences were found between the fungal inoculums, the *Fusarium* sp. strain at a concentration of 1×10^8 conidia mL^{-1} had a lower rank on the severity score compared to the rest of the treatments.

In a study by Raghavendra *et al*. (2013) on *Centaurea stoebe*, they mentioned that the infection of seeds caused by the *Fusarium* fungus subsequently produced a decrease in the rate of reemergence of this weed. This agrees with our results since a lower rate of emergence of regrowth was observed, compared to control plants. On the other hand, Tan *et al*. (2002) evaluated the effect of *Fusarium* sp. on *Alternanthera philoxeroides* they reported that the fungus is capable of producing an effect on that weed, but the efficiency of the bio-herbicide varies depending on the concentration of the inoculum; better results were obtained when the concentration reached high densities of spores.

Regarding the genus *Pestalotiopsis* sp., there are few studies referring to it as a potential agent for biological control of weeds. Santos *et al*. (2008) conducted studies on the bio-herbicide potential of *Pestalotiopsis guepinii*. Their results showed that its chemical compounds had inhibitory potential in the germination of *Senna obtusifolia* seeds. In another study, it was reported that *Pestalotiopsis vismiae* showed high suppression both in the germination and in the reduction of rhizome length of different weeds (Radi and Hamedi, 2017). This may be consistent with our results, since we did not observe abundant presence of tillers emerged from the rhizome of *C. rotundus* due to the effect of the evaluated fungi.

In another study on weeds, in which De Luna *et al*. (2002) tested the bio-herbicide effect using *Curvularia*, those authors reported two isolates (*Curvularia tuberculata* and *Curvularia oryzae*) obtained from diseased plants of *Cyperus difformis* and *Cyperus iria* in Philippines in

1993. When these fungi were inoculated at a concentration of 1×10^{8} conidia mL^{-1} with a dew period of 24 h at 28 °C, rapid and severe blighting of the leaves was observed, which caused the death of the seedlings within two weeks after inoculation with fungi. In the case of our study, although inoculation in *Cyperus rotundus* is reported, symptoms and virulence were different. Since indeed there was death of *C. rotundus* plants, but not all the plants in the treatments died.

Our results in this research are consistent with what was reported by those authors, since necrosis and dieback were observed starting at the tips of the leaves of *C. rotundus* plants, but no sporulation was observed. This may be partly due to the experimental conditions, since we applied the inoculations during the dry season when the temperatures inside the greenhouse were around or above 30-35 °C. In contrast, it could be interpreted that our results on the height of the plants were favorable; however, there was a negative impact on the emergence of tillers or a regrowth delay which may be a sign that there was an effect on rhizomes. More experiments are required to evaluate, quantify and thoroughly report these significant effects of fungi with bio-herbicidal potential in the reduction of the wild populations of *C. rotundus*.

CONCLUSIONS

In this research, we identified fungi associated with *Cyperus* plants. Morphological and molecular identification techniques in the ITS region allowed us to identify and accurately verify nine genera *Fusarium*, *Curvularia*, *Trichoderma*, *Aspergillus*, *Penicillium*, *Pestalotiopsis*, *Pseudopestalotiopsis*, *Helminthosporium* and *Nigrospora*. Moreover, two of them (*Curvularia* and *Pestalotiopsis*) showed to be related with potential phytopathogenic activity to *Cyperus rotundus*. And three isolates, *Fusarium* sp., *Curvularia* sp., and *Pestalotiopsis* sp. proved to be pathogenic to *C. rotundus*.

Findings in this study contribute to the knowledge of the diversity of fungi from wild plants in Mexico and set the foundations for future research aimed at the biological control of invasive weeds. Also, this study adds to the research aiming to explore ecological interactions among groups of microorganisms that inhabit the endobiome and rhizosphere of weeds.

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Colegio de Postgraduados

Nutrient composition and chromatic parameter of Bolita pigmented maize (*Zea mays* L.)

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Citation: Juárez-Segovia, K. G., Varapizuela-Sánchez, C. F., Matías-Pérez, D., Díaz-Barrita, A. J., Pérez-Santiago, A. D., García-Montalvo, I. A., & Sánchez-Medina, M. A. (2025). Nutrient composition and chromatic parameter of Bolita pigmented maize (*Zea mays* L.). *Agro Productividad*. https://doi.org/10.32854/9rdxp887

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

Received: January 24, 2025.
Accepted: May 15, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 183-191.

ABSTRACT

Objective: To determine the nutrient composition and chromatic parameters of two pigmented (blue and red) Bolita maize varieties from the communities of the Valles Centrales region, Oaxaca, Mexico.

Design/Methodology/Approach: The grains of two maize varieties were evaluated to determine their moisture, ash, crude fiber, ether extract, protein, and carbohydrates content, as well as their chromatic parameters. In addition, their macro- and micro-elements were established. Data were analyzed with the Student's-t test for independent samples with a >95% confidence level ($p \leq 0.05$).

Results: A significant difference ($p \leq 0.05$) was recorded in the nutrient composition of each parameter: 7.46-9.70% (moisture), 1.33-1.61% (ash), 5.43-6.6% (fat), 8.32-9.3% (proteins), 3.25-4.38% (fiber), and 70.83-71.78% (carbohydrates). Macro- (Mg, Ca, K, Na, and P) and micro-elements (Cu, Fe, Zn, and Mn) were identified. In addition, the L*, a*, b*, C*, and h chromatic parameters were determined.

Study Limitations/Implications: This study had no limiting factors.

Findings/Conclusions: The blue and red Bolita maize varieties recorded a nutrient composition that matched the findings of other authors. The differences may be mainly the result of genetic factors and the development conditions of the plants.

Keywords: *Zea mays* L., nutrient composition, color.

INTRODUCTION

Maize (*Zea mays* L.) is an extensively grown cereal worldwide. It is used to feed both humans and animals. Maize is native to Mexico, where it was domesticated and diversified (Mendoza-Mendoza *et al.*, 2020), leading to different morphological characteristics (plants) and chemical composition (grains) (Mex-Álvarez *et al.*, 2016). In addition, it is the most popular cereal in the country, due to its taste and year-long availability (Peña *et al.*, 2017) and it is used in a wide variety of dishes (Salinas-Moreno *et al.*, 2024).

Maize is a staple crop worldwide, because it provides macro- and micro- nutrients that meet the requirements of the human metabolism (Jaradat and Goldstein, 2018). It is one of the most important food sources of the population and it provides nutrients that complement human diet, such as minerals, dietary fiber, and unsaturated fats (Chan-Chan *et al.*, 2021).

As a result of its topography and different climates, Mexico houses 64 different maize varieties (Salinas-Moreno *et al.*, 2024), out of which, 59 are native to the country (CONABIO, 2020). These native species include accessions with different grain color and pigment modifications (Serna-Saldívar *et al.*, 2013), including shades of yellow, blue, black, orange, and red (Salinas *et al.*, 2013). Thirty-five native varieties have been identified in Oaxaca. They have a high genetic variation (Aragón *et al.*, 2006) that improves the basic diet of rural and indigenous communities (González-Cortés *et al.*, 2016). In addition, these varieties have been traditionally commercialized, providing an important source of income (Peña *et al.*, 2017). Native maize is a source of a great variety of secondary metabolites, including flavonoids, anthocyanins, stilbene, phenolic acids, coumarins, and tannins (Francavilla and Joye, 2020), which are fundamental for the functioning of the plant and are part of its defense mechanism against pathogens. In addition, maize has nutraceutical and antioxidant properties (Cabrera-Soto *et al.*, 2009). However, based on the stress conditions undergone by the plant during its development, these compounds may vary (Peña, 2013). Nevertheless, biotic and abiotic factors, as well as variety, influence the presence and amount of the phenolic compounds in the plant. Consequently, their chemical composition and properties, as well as their final use are different, as a result of the great diversity of maize varieties (Méndez *et al.*, 2005).

The secondary metabolites of maize have been the subject of several researches, given their biological activities. However, the characteristics of native maize from Oaxaca require further studies: its remarkable genetic variability and preservation favor food security and guarantee an income for thousands of families from indigenous communities. Therefore, the objective of this study was to determine the nutrient composition and the chromatic parameters of two pigmented maize varieties (blue and red Bolita maize) grown in the communities of the Valles Centrales region, Oaxaca, Mexico. The hypothesis was that the pigmented grains of native maize are an improved source of macro- and micro-nutrients that can complement daily diets.

MATERIALS AND METHODS

Collection and variety identification

Red maize and blue maize samples were collected from San Francisco Telixtlahuaca (17.3176416 N, −96.9070607 E) and Trinidad Zaachila (16.9372.1 N, −96.7967.1 E), respectively. Both towns are located in the Valles Centrales region, Oaxaca, Mexico. The germplasm samples of the two varieties were harvested in October-November, 2023. The germplasm was classified within the Bolita variety group, based on the criteria described by Aragón *et al.* (2006) and CONABIO (2020), which include morphology, cob size, and number and grains per row, as well as grain size and shape.

Nutrient composition

In order to analyze the nutrient content, 100 g of grains were ground in a homogenizer to obtain flour. The official AOAC methods (2005) were used to determine the nutrient content of the grains. The Kjeldahl method (method 984.13) was used to establish the total protein content, while methods 962.09, 942.05, 920.039 (Soxhlet method), and 930.15 were used to determine crude fiber, ash, ether extract, and moisture content, respectively. Meanwhile, the method established by Nielsen (2017) was used to obtain the total carbohydrate content, subtracting the sum of protein, crude fiber, ash, ether extract, and moisture percentage from 100%.

Mineral content

Ten g of ashes from each maize sample were analyzed to determine their mineral content in the Departamento de Química Analítica, Servicio Geológico Mexicano, Centro Experimental Oaxaca, using the dissolution method with four acids: hydrochloric acid (HCl), nitric acid (HNO_3), perchloric acid ($HClO_4$), and hydrofluoric acid (HF).

Analysis of the color of maize grains

Reflectance was used to analyze the color of maize grains. A HunterLab UltraScan® Vis spectrophotometer and the EasyMachQC software in the #2-RSIN Reflectance Specular Included-9.525 mm mode were used for this purpose. The chromatic parameters of the CIE-LAB system (L*, a*, and b*) were obtained. L* (luminosity) indicates if the color is light ($51 \leq L \leq 100$) or dark ($0 \leq L \leq 50$) (Mendoza-Mendoza *et al.*, 2017). Meanwhile, a* and b* stand for the following chromaticity coordinates: a* (+) red and a*(−) green and b*(+) yellow and b*(−) blue (Rodríguez-Salinas *et al.*, 2020). The b*(+) and b*(−) parameters were used to calculate h° (hue), which is the hue angle that indicates the sample color quadrant: 0°=red-purple, 90°=yellow, 180°=greenish blue, and 270°=blue (García-Campos *et al.*, 2020). In addition, this parameter is deeply associated with the visual assessment of color (Salinas-Moreno *et al.*, 2021). Chromaticity (C*) refers to the color saturation index, which indicates that high values are pure colors, while low values stand for matte or less pure colors. The h°=arctg(b*/a*) and C*=(a*2+b*2)1/2 expressions were used (Ballesteros *et al.*, 2019). The values of each evaluated sample were input in the ColorHexa website to obtain the color image.

Statistical analysis

The mean result of three replicates —including the nutrient composition, color analysis— was recorded per sample. The PSPP v. 3 Software for Windows was used to calculate the mean and standard deviation. Data were analyzed with the Student's-t test for independent samples with a >95% confidence level ($p \leq 0.05$). Based on the methodology established by the Servicio Geológico Mexicano, the geochemical analysis was performed in duplicate.

RESULTS AND DISCUSSION

Nutrient composition

Significant differences were recorded between the two Bolita varieties regarding moisture, ash, ether extract, protein, and fiber content (Table 1). Overall, the two varieties recorded normal concentrations for each of the evaluated parameters.

The nutrient composition analysis establishes the main components and percentages of macro-nutrients of a given species to describe its characteristics (Cadena *et al.*, 2024). The moisture content of blue Bolita was higher than in red Bolita; however, the percentage found in both varieties fell below the critical value. A higher moisture content (>14%) is a major hazard for all cereal species (Cadena *et al.*, 2024). A low moisture value is fundamental, because low moisture content helps cereals to develop a higher resistance to the deterioration caused by microorganisms and insects during their storage (García-Campos *et al.*, 2020). These results match the findings of Mex-Álvarez *et al.* (2016), who reported values between 7.45 and 10.43% for maize varieties from Campeche. For their part, Rodríguez-Salinas *et al.* (2020) recorded 7.98-9.67% values for pigmented genotype maize. Red Bolita recorded a higher ash and fat content than blue Bolita. The first parameter is related to mineral content, most of which can be found in the germ, just like fat (Bello-Pérez *et al.*, 2016). Rodríguez-Salinas *et al.* (2020) reported a pigmented maize ash and fat content of 1.00-1.46% and 3.38-6.15%, respectively. Meanwhile, Chan-Chan *et al.* (2021) recorded an ash and fat content of 1.36-1.50% and 5.01-5.05%, respectively, for varieties from Yucatan. The differences in ash content can be the result of the use of fertilizers and environmental factors. In addition, a good quality maize must not record a >2% ash content. Higher percentages are associated with calcium and salt pollution and the lack of cleanness during harvest and sample collection (Rodríguez *et al.*, 2023).

The protein and carbohydrate content were higher in red Bolita. A significant amount of the protein content is found in the endosperm (8.2-9.4% in pigmented maize). Some segregating forms of the Bolita variety have a positive ratio between the hardness of the grain and the protein content, which has been attributed to a higher presence of the storage proteins (prolamins) that surround the starch granules (Bello-Pérez *et al.*, 2016). Mex-Álvarez *et al.* (2016) reported a lower protein percentage in purple (6.76%) and red (7.45%) varieties. However, Rodríguez-Salinas *et al.* (2020) reported a higher protein content (9.72-12.75%), which contrasts with the results of this study. Carbohydrates are the main component of maize grains. Starch is the main carbohydrate found in maize and it is formed by amylose (linear constituent) and amylopectin (branched polysaccharide) (Bello-Pérez *et al.*, 2016). Mex-Álvarez *et al.* (2016) reported a higher carbohydrate content for the red (72.31%) and purple (74.30%) varieties.

Table 1. Nutrient content of two pigmented maize varieties from the Valles Centrales region, Oaxaca, Mexico.

Variety	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrates (%)	Fiber (%)
Bolita azul	9.70 ± 0.097^a	1.33 ± 0.012^b	5.43 ± 0.282^b	8.32 ± 0.095^b	70.83 ± 0.340^b	4.38 ± 0.297^a
Bolita rojo	7.46 ± 0.127^b	1.61 ± 0.049^a	6.60 ± 0.310^a	9.30 ± 0.295^a	71.78 ± 0.372^a	3.25 ± 0.402^b

^{a,b} Means with different superscripts in the same column are significantly different ($p \leq 0.05$).

Fiber content is related to the plant material that is resistant to digestive enzymes (Dhingra *et al.*, 2012). In the case of maize, this material is located in the pericarp and is mainly made up of arabinoxylans, heteroxylans, cellulose, and phenolic acids (Bello-Pérez *et al.*, 2016). The phenolic compounds of dietary fiber are chemically bond with various insoluble macromolecules; therefore, grains with a higher fiber content can be linked to the presence of bound phenolic compounds (Gálvez *et al.*, 2021). The content of this parameter was higher in blue Bolita. It was even higher than the range (1.2-1.76%) reported by Rodríguez-Salinas *et al.* (2020). However, Mex-Álvarez *et al.* (2016) reported a higher percentage of fiber in red maize (6.39%) and in purple maize (3.32%). Each parameter has a wide nutritional range, because the ratio of each macronutrient in maize depends on genetic and environmental factors, as well as on the conditions of the crop.

Mineral content

The following macro-elements (minerals) —which are necessary for the development of the plant— were found in the grains of red and blue Bolita maize: magnesium (Mg), phosphorous (P), calcium (Ca), potassium (K), and sodium (Na) (Table 2).

Table 3 shows the essential micro-elements whose physiological function is also fundamental for the organism and which significantly contribute to the mineral content of the diet (Saulnier, 2012; Caballero *et al.*, 2014). The said minerals include iron (Fe), zinc (Zn), cupper (Cu), and manganese (Mn).

The macro- and micro-elements identified in the grains of red and blue Bolita are important for human and animal diet and health (Jordan-Meille *et al.*, 2021). Additionally, they play a major role in seed quality (Jaradat and Goldstein, 2018). The predominant compounds in both varieties were P, K, and Mg. The same results were reported by Rodríguez-Salinas *et al.* (2020), who analyzed 15 genotypes of pigmented native maize and recorded the following results: P (334.40-719.99 mg 100 g^{-1}), K (314.20-397.18 mg 100 g^{-1}), and Mg (116.24-146.75 mg 100 g^{-1}). Chí-Sánchez *et al.* (2021) reported lower Ca concentrations in native maize grown in several regions of Yucatan, Mexico: 3.8 and 5.61 mg 100 g^{-1} for purple and red maize, respectively.

Table 2. Macro-element content in the grain of two pigmented maize varieties in the Valles Centrales region, Oaxaca, Mexico.

Variety	Macroelements (mg 100 g^{-1})				
	Mg	**Ca**	**K**	**Na**	**P**
Bolita azul	108.66	5.32	271.72	1.53	309.89
Bolita rojo	135.72	7.41	258.73	1.10	365.63

Table 3. Micro-element content in the grain of two pigmented maize varieties in the Valles Centrales region, Oaxaca, Mexico.

Variety	Microelements (mg 100 g^{-1})		
	Cu	**Fe**	**Zn**
Bolita azul	0.22	2.00	1.98
Bolita rojo	0.24	2.58	2.87

In contrast, Rodríguez-Salinas *et al.* (2020) detected higher concentrations than those reported in this study: 24.58 to 32.63 mg 100 g^{-1}. Likewise, they reported 24.08 to 70.30 mg 100 g^{-1} Na concentrations.

Meanwhile, red Bolita had a higher concentration of micro-elements (Table 3). The Cu content of the two varieties under study fell within the range (0.20-0.53 mg 100 g^{-1}) reported by Rodríguez-Salinas *et al.* (2020). However, it exceeded the concentrations (0.0073-0.1169 mg 100 g^{-1}) reported by Chí-Sánchez *et al.* (2021). For its part, the Fe content ranged from 0.454 to 1.8 mg 100 g^{-1} concentrations. However, Qamar *et al.* (2017) reported higher concentrations in white (8.20 mg 100 g^{-1}) and yellow (11.5 mg 100 g^{-1}) maize. The Zn and Mn concentrations in blue Bolita were slightly lower than those reported by Rodríguez-Salinas *et al.* (2020), who recorded 2.80 to 4.50 mg 100 g^{-1} (Zn) and 0.3 to 0.73 mg 100 g^{-1} (Mn) concentrations. In this case, the varieties under analysis fell within this range.

Several factors determine the nutrient concentration of the grain. Therefore, although both blue and red Bolita belong to the same variety, they showed differences in mineral content. Dourado *et al.* (2015) reported that the concentration of nutrients is impacted by their availability in the soil, which in turn is determined by such factors as texture, organic matter, and pH. In this regard, soil texture is particularly important. For example, compared with sandy soils, clay soils are more frequently associated with grains with a higher mineral content (Jordan-Meille *et al.*, 2021). Likewise, pH differences in the soil influence the solubility of minerals and consequently their absorption by the plant and the composition of the grain (Gálvez *et al.*, 2021). For example, an increase in soil pH reduces the availability of Cu, Mn, and Zn. These elements are particularly important, because Cu and Mn are part of the defense mechanisms of plants and can provide them with various levels of resistance (Dourado *et al.*, 2015). Meanwhile, Zn and Fe are essential micro-elements for human metabolism. Therefore, the biogeochemistry of the soil has a massive impact and influences mineral solubility, their potential absorption by crops, and the mineral content of its grains and tissues. Nevertheless, other factors also impact this availability, including agricultural practices (*e.g.*, fertilization and lime application), consequently neutralizing soil acidity (Jordan-Meille *et al.*, 2021). Additional fertilization interacts with other nutrients and soil pH, diminishing certain micronutrients or increasing the solubility of the nutrient provided (Chí-Sánchez *et al.*, 2021). The presence of P in maize grains is influenced by the application of phosphorous fertilizers, while the Ca content can be influenced by its concentration in the soil where the crop was sown (Martínez *et al.*, 2017).

Analysis of the color of maize grains

Table 4 shows the values of the color parameters of the grains of both maize varieties. All the parameters recorded significant results and, overall, showed that both varieties have a dark color. However, red Bolita had a lower L* value (luminosity or brightness of the sample), while its hue (h°) and chroma (C*) fell within the red scale. For its part, blue Bolita recorded a lower chroma, with a hue angle that places this variety in the greenish yellow area, with some grey hues.

Table 4. Chromatic properties of two varieties of pigmented maize in the Valles Centrales region, Oaxaca, Mexico.

Variety	**Color parameter**					
	L*	**a***	**b***	**C***	**h°**	**Color image**
Bolita azul	37.36 ± 0.52^{a}	0.44 ± 0.30^{b}	2.58 ± 0.59^{b}	2.62 ± 0.64^{b}	81.05 ± 4.51^{a}	
Bolita rojo	32.43 ± 0.43^{b}	26.85 ± 1.39^{a}	14.60 ± 0.90^{a}	30.57 ± 1.65^{a}	28.76 ± 0.59^{b}	

^{a,b} Means with different superscripts in the same column are significantly different ($p \leq 0.05$).

The L* values indicated that the germplasm under study has dark grains and that red Bolita was more luminous than blue Bolita. Rodríguez-Salinas *et al.* (2020) reported L* values ranging from 25.13 to 42.75 for purple grain genotypes. Blue Bolita falls within this range. In contrast, Mendoza-Mendoza *et al.* (2017) recorded L* values of 5.7-24.5 in dark purple and reddish-purple grains (darker grains). Red Bolita recorded an L* value of 32.43, closer to the mean reported by Salinas-Moreno *et al.* (2021) for cherry red grains (34.41) and lower than the value recorded by Rodríguez-Salinas *et al.* (2020) for a red genotype (38.22). Meanwhile, Salinas *et al.* (2012) reported higher values for magenta red (38.5) and light red (51.4) varieties. The color of red Bolita was more saturated, since high values of C* (degree of saturation) indicate pure colors, while lower values point out matte or less pure colors (Ballesteros *et al.*, 2019). Therefore, the color of the blue Bolita grain was less pure. For their part, Rodríguez-Salinas *et al.* (2020) established that the C* of purple genotypes fluctuated between 2.42 and 22.17. Blue Bolita fell withing this range. In addition, the value of two genotypes was similar to the value reported in this study (2.41 and 3.63). Likewise, Mendoza-Mendoza *et al.* (2017) reported dark purple grains with a low saturation (1.8 and 2.5). Red Bolita was brighter than the colors reported by Mendoza-Mendoza *et al.* (2017): reddish purple, red, and dark blue grains (6.2 and 11.8). Finally, Rodríguez-Salinas *et al.* (2020) recorded a 16.54 color for a red genotype.

Meanwhile, the value of the h° (hue or hue angle) parameter in purple blue maize can be similar to the value associated with a yellow or greenish yellow hue (Salinas-Moreno *et al.*, 2021), due to the uneven color of the grain surface. Areas such as the pedicel and the germinal area usually lack pigments. Additionally, since the pericarp is translucent, the values of the surface of the grain do not match the characteristics of the purple-blue hue (Salinas-Moreno *et al.*, 2024). The red Bolita grain had a more even color and fell within the red hue area. However, the h° value of the blue Bolita placed it in the greenish yellow hue area. The color of the grain and the content of anthocyanin compounds are correlated: as the L*, C*, and h° values decrease, the anthocyanin content increases (Rodríguez *et al.*, 2020). Therefore, darker grains have a higher content of these compounds.

CONCLUSIONS

Significant variations were reported in the concentration of minerals between both maize varieties and the data reported by other authors, particularly in macro-elements (*e.g.*, Ca and Na) and micro-elements (*e.g.*, Cu, Fe, and Zn). The grains recorded remarkable differences in protein content, as well as low luminosity (L*); therefore, they were classified as dark, due to the pigments in the pericarp or the aleurone layer. The location where they were sown or the agricultural practices employed could explain these differences between samples from the same variety. Therefore, these pigmented maize varieties —which are grown in several communities of the state of Oaxaca— should be the subject of further studies, because they are an abundant source of macro- and micro-nutrients.

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Colegio de Postgraduados

In vitro effectiveness of plant extracts on the mortality of root-knot nematodes

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ABSTRACT

Objective: To identify plants with nematicidal potential against *Meloidogyne enterolobii* and *Nacobbus aberrans* for potential use in sustainable agricultural practices.

Design/methodology/approach: Six aqueous extracts (AEs) from the leaves of *Origanum vulgare*, *Foeniculum vulgare*, *Dracocephalum moldavica*, *Thymus vulgaris*, *Dysphania ambrosioides*, and *Melissa officinalis* were evaluated for the control of second instar (J2) juveniles of *Meloidogyne enterolobii* and *Nacobbus aberrans*. The solution obtained was designated as standard (100%) and was diluted with distilled water to concentrations of 25%, 50% and 100%. Sterile distilled water was included as a test sample. Each concentration was repeated four times, using 400 J2/concentration. Mortality observations were performed at 6, 24 and 48 h. A nonparametric factorial analysis was performed using aligned ranks in R.

Results: The factorial analysis of variance for the main effects —P (Plant) and T (Time)— on the mortality of *M. enterolobii* (Me) and *N. aberrans* (Na) J2 showed highly significant differences ($p \leq 0.05$), as well as a significant P*T interaction effect. All aqueous extracts at 100% concentration caused the highest J2 mortality for *M. enterolobii* at 24 hours and for *N. aberrans* at 12 hours using the 25% concentration.

Limitations on study/implications: This study serves as a foundational step for future research and represents the first attempt to propose the use of these plant species for the control of root-knot nematodes through nematicidal activity.

Findings/conclusions: Our results demonstrate, for the first time, the in vitro nematicidal activity of plant extracts of *Dysphania ambrosioides*, *Melissa officinalis*, *Foeniculum vulgare* and *Thymus vulgaris* against the nematodes *M. enterolobii* and *N. aberrans*. These findings support the need for further research under soil conditions and metabolomic studies to identify the active compounds responsible for this effect.

Keywords: Aqueous extracts, *Meloidogyne enterolobii*, *Nacobbus aberrans*.

Citation: Leon-Tello, M. I., & Gómez-Rodríguez, O. (2025). *In vitro* effectiveness of plant extracts on the mortality of root-knot nematodes. *Agro Productividad*. https://doi.org/10.32854/1k8wxf07

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

Received: January 24, 2025.
Accepted: May 13, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 193-201.

INTRODUCTION

Root-knot nematodes are considered among the most important soil-borne phytoparasites. They are responsible for economic losses in agricultural crop productivity exceeding 170 billion USD annually worldwide (Bernard *et al.*, 2017). Their polyphagous capacity and considerable phenotypic plasticity have allowed them to establish in various geographical areas, where they can achieve higher rates of infection, development, and reproduction (Mbow *et al.*, 2019). This, in turn, poses significant challenges for agricultural systems due to their impact on both crop yield and quality.

In the search for more sustainable and environmentally friendly alternatives to chemical pesticides for the control of various plant pathogens, several strategies are being developed that involve the use of phytochemicals. In recent years, there has been a significant increase in scientific and industrial interest in the development of new biopesticides, with plant extracts gaining prominence over the last decade as a sustainable and ecological alternative (Khursheed *et al.*, 2022). Interest in extracts obtained from various plants lies in their content of bioactive compounds —such as alkaloids, terpenoids, phenols, and saponins— that have demonstrated nematicidal properties by interfering with the physiological and metabolic processes of nematodes. These include motility, glycolysis, giant cell formation, neurotransmission, the urea cycle, polyamine synthesis, antioxidant activity, and nematode reproduction (Subramanian *et al.*, 2017; Naboulsi *et al.*, 2018). Metabolites such as kaempferol and its derivatives have been shown to affect root-knot nematode invasion, acting as defense compounds against these microorganisms by reducing gall formation in tomato plants (*Solanum lycopersicum* L.) and inhibiting egg hatching (Chin *et al.*, 2018; Zhao *et al.*, 2023). Other compounds —such as quercetin, myricetin, rutin, patuletin, and glyceollin— produced by various antagonistic plants have also been studied for their nematicidal properties (Faizi *et al.*, 2011; Hamaguchi *et al.*, 2019). Despite advances in this field of research, the number of plant species analyzed for such properties remains limited. Therefore, the objective of the present study was to evaluate *Origanum vulgare*, *Foeniculum vulgare*, *Dracocephalum moldavica*, *Thymus vulgaris*, *Dysphania ambrosioides*, and *Melissa officinalis* as potential plant species with nematicidal activity against *Meloidogyne enterolobii* and *Nacobbus aberrans*.

MATERIALS AND METHODS

Six aqueous extracts (AE) from the leaves of *Origanum vulgare*, *Foeniculum vulgare*, *Dracocephalum moldavica*, *Thymus vulgaris*, *Dysphania ambrosioides*, and *Melissa officinalis* were evaluated for the control of second-stage juveniles (J2) of *Meloidogyne enterolobii* and *Nacobbus aberrans*. A total of 50 g of plant leaves were macerated in 200 mL of distilled water for 48 hours. The resulting mixture was blended and filtered through Whatman No. 1 filter paper. The solution obtained was designated as the standard (100%) and subsequently diluted with distilled water to obtain concentrations of 25%, 50%, and 100%. Sterile distilled water was included as a control treatment. Each concentration was replicated four times, using 400 J2 per concentration. Mortality observations were recorded at 6, 24, and 48 hours.

For the efficacy tests, 140 μL of each concentration were placed into ELISA plates (Thermo Scientific, Nunclon™ Delta Surface). In each well, 100 J2 nematodes —obtained from tomato plants (*Solanum lycopersicum*) cultivated monoxenically under greenhouse conditions at the Colegio de Postgraduados, Montecillo Campus— were added. Each concentration was replicated four times, for a total of 400 J2 per concentration evaluated. The plates were incubated in a controlled growth chamber (APT.line™ KBWF model, E5.1) at 28±1°C (Pagamas and Nawata, 2007).

Since the assumption of normality in the distribution of residuals was not met in the factorial analysis of variance of the original data, a non-parametric factorial analysis based on the use of aligned ranks was employed. This methodology involves centering or aligning the data by subtracting the group mean from each observation. The aligned data, corresponding to the desired main and interaction effects, are then ranked, and parametric tests are applied to these aligned ranks (Wobbrock *et al.*, 2011). Following this analysis, pairwise mean comparisons were performed using Tukey's test on the adjusted group means. Three separate analyses were conducted according to the extract concentration used: 1) Factor 1 (six different plant extracts and a control consisting of distilled water), Factor 2 (three exposure times to the extract), at a 25% extract concentration; 2) the same two factors as the previous set, at a 50% extract concentration; 3) the same two factors as the first set, at a 100% extract concentration. All analyses were performed using the RStudio statistical software package. The experiments were conducted under a completely randomized design. To determine nematode mortality, individuals showing immobility were stimulated with a fine bristle near the cephalic region and then maintained in sterile water for 24 hours. Nematodes that did not move after this procedure were considered dead.

RESULTS AND DISCUSSION

The factorial analysis of variance of the main effects, P (Plants) and T (Time), for the variable mortality of J2 of *Meloidogyne enterolobii* (Me) and *Nacobbus aberrans* (Na) indicated highly significant differences ($p \leq 0.05$), as well as a significant interaction effect between P and T. All aqueous extracts evaluated at a 100% concentration achieved higher mortality in J2 of *M. enterolobii* after 24 hours of exposure (Table 1). Furthermore, it was observed that as both the concentration and exposure time increased, the mortality of J2 *M. enterolobii* with all evaluated extracts progressively increased.

During the first 6 hours of exposure, significant differences ($p \leq 0.05$) were observed among the plant species evaluated at a 25% concentration. The *Melissa officinalis* leaf extract achieved the highest mortality percentage (39%) compared to the control (4%). This was followed by extracts from *Foeniculum vulgare* and *Thymus vulgaris*, which reached mortality rates above 30% (Figure 1).

The remaining extracts ranged between 25% (*Dysphania ambrosioides*) and 30% (*Origanum vulgare*). Only the *Dracocephalum moldavica* leaf extract showed a lower mortality percentage (20%) compared to the other extracts evaluated ($p \leq 0.05$); however, its performance was still superior to the mortality observed in the control group. From 12 hours of exposure onward,

Table 1. Maximum mortality of *Meloidogyne enterolobii* J2 juveniles in relation to concentration and exposure time for each evaluated extract.

Extract	Concentration (%)	Exposure time (h)	Mortality (%)
Origanum vulgare	100	24	100
Foeniculum vulgare	100	24	100
Dracocephalum moldavica	100	24	100
Thymus vulgaris	100	24	100
Dysphania ambrosioides	100	24	100
Melissa officinalis	100	24	100

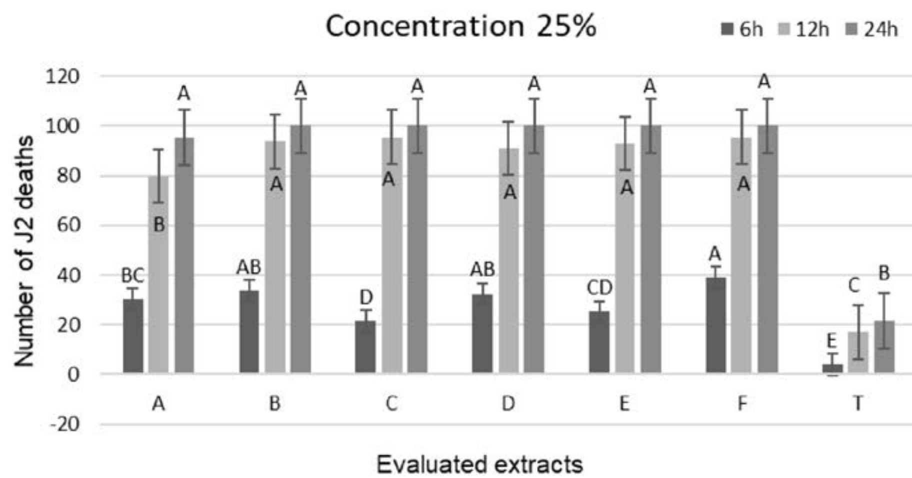

Figure 1. Effect of 25% aqueous plant extracts on the mortality of *M. enterolobii*. A: *Origanum vulgare*; B: *Foeniculum vulgare*; C: *Dracocephalum moldavica*; D: *Thymus vulgaris*; E: *Dysphania ambrosioides*; F: *Melissa officinalis*; T: Control. Test: Tukey. Alpha=0.05. Degrees of freedom (df)=6. Means sharing the same letter are not significantly different ($p \leq 0.05$).

mortality of *M. enterolobii* J2 juveniles was homogeneous, as all aqueous extracts evaluated achieved mortality rates higher than 80%. Specifically, the plant species *Foeniculum vulgare*, *Dracocephalum moldavica*, *Thymus vulgaris*, *Dysphania ambrosioides*, and *Melissa officinalis* reached 100% mortality at 24 hours (Figure 1).

This behavior positively increased mortality as the concentration of the aqueous extract increased from 50% to 100% (Figures 2 and 3). Starting at 6 hours of exposure at a 50% concentration, leaf extracts of *Foeniculum vulgare*, *Dracocephalum moldavica*, *Thymus vulgaris*, and *Dysphania ambrosioides* achieved mortality rates of Me J2 exceeding 50% compared to the control (Figure 2). An exponential increase in Me J2 mortality was observed at 100% concentration from 6 hours of exposure in all extracts evaluated, reaching maximum

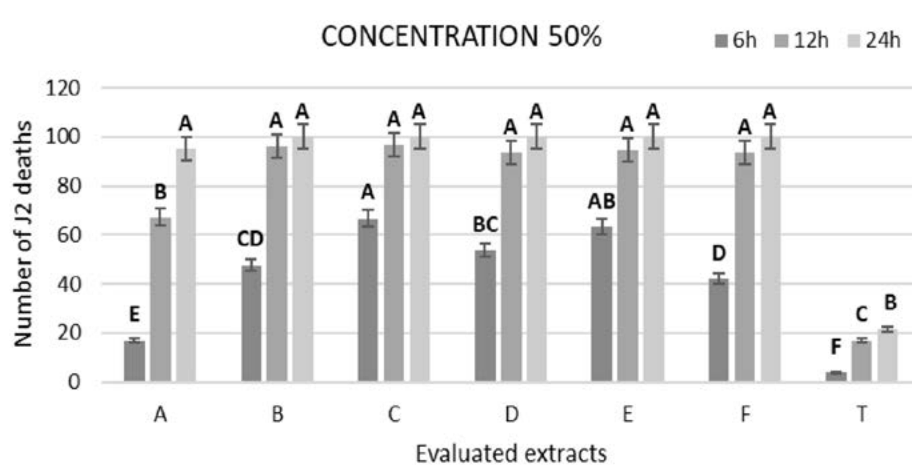

Figure 2. Effect of 50% aqueous plant extracts on *M. enterolobii* mortality. A: *Origanum vulgare*; B: *Foeniculum vulgare*; C: *Dracocephalum moldavica*; D: *Thymus vulgaris*; E: *Dysphania ambrosioides*; F: *Melissa officinalis*; T: Control. Test: Tukey. Alpha=0.05. df=6. Means sharing the same letter are not significantly different ($p \leq 0.05$).

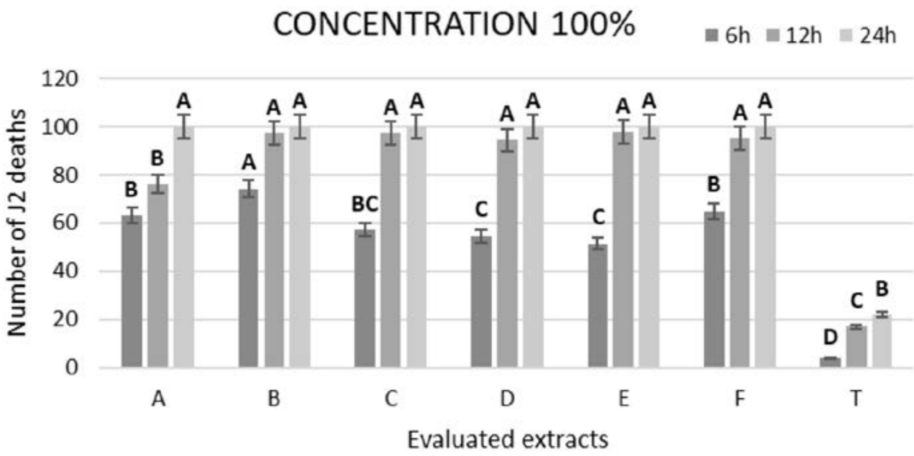

Figure 3. Effect of 100% aqueous plant extracts on *M. enterolobii* mortality. A: *Origanum vulgare*; B: *Foeniculum vulgare*; C: *Dracocephalum moldavica*; D: *Thymus vulgaris*; E: *Dysphania ambrosioides*; F: *Melissa officinalis*; T: Control. Test: Tukey. Alpha=0.05. df=6. Means sharing the same letter are not significantly different ($p \leq 0.05$).

mortality (100%) of Me J2 after 24 hours of exposure, with significant differences ($p \leq 0.05$) compared to the control (Figure 3).

In turn, the mortality observed in J2 of *Nacobbus aberrans* (Na) with all the aqueous extracts evaluated at a 25% concentration showed greater effectiveness after 12 hours of exposure (Table 2).

Similarly, it was observed that as the concentration and exposure time increased (12 and 24 h), the mortality of Na in all evaluated extracts exceeded 95% compared to the control, with highly significant differences ($p \leq 0.05$). However, unlike Me, *Nacobbus aberrans* reached 100% mortality after 12 hours of exposure at the lowest concentration of most evaluated extracts compared to the control (Figure 4). Similar to Me, the leaf extracts of *Melissa officinalis* and *Thymus vulgaris* at the lowest concentration (25%) showed the highest mortality rates (27% and 22%, respectively) within the first 6 hours of exposure, compared to the control and with significant differences from the other evaluated extracts (Figure 4). These were followed by *Foeniculum vulgare* (17%) and *Origanum vulgare* (15%). Although the extracts of *Dracocephalum moldavica* and *Dysphania ambrosioides* recorded the lowest J2 mortality rates of *N. aberrans* among the evaluated extracts ($p \leq 0.05$), their effectiveness remained higher than that of the control.

Table 2. Maximum mortality of J2 juveniles of *Nacobbus aberrans* in relation to the concentration and exposure time for each evaluated extract.

Extracts	Concentration (%)	Exposure time (h)	Mortality (%)
Origanum vulgare	25	24	100
Foeniculum vulgare	25	12	100
Dracocephalum moldavica	25	12	100
Thymus vulgaris	25	12	100
Dysphania ambrosioides	25	12	100
Melissa officinalis	25	12	100

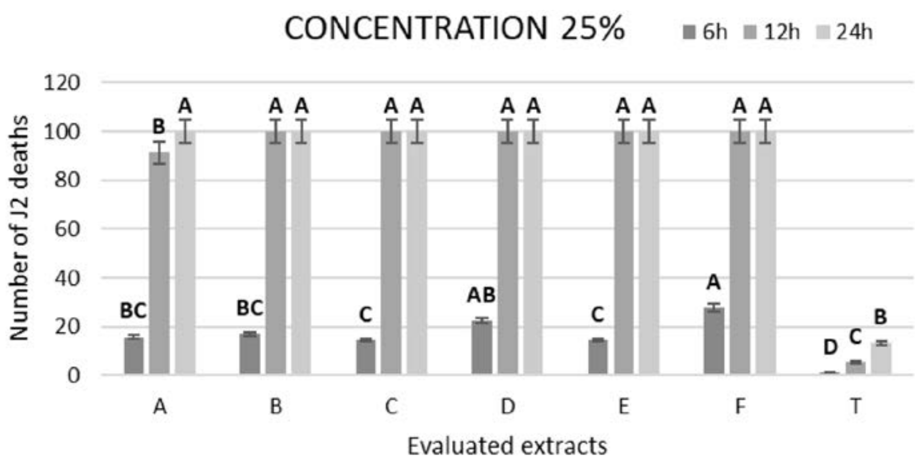

Figure 4. Effect of aqueous plant extracts at 25% concentration on the mortality of *N. aberrans*. A: *Origanum vulgare*; B: *Foeniculum vulgare*; C: *Dracocephalum moldavica*; D: *Thymus vulgaris*; E: *Dysphania ambrosioides*; F: *Melissa officinalis*; T: Control. Test: Tukey. Alpha=0.05. df=6. Means sharing a letter are not significantly different ($p \leq 0.05$).

The mortality observed with the *Melissa officinalis* extract remained the highest (43%) at the same exposure time (6 hours) and at a 50% concentration compared to the other evaluated extracts (Figure 5), followed by *Foeniculum vulgare* (41%). Extracts based on *Origanum vulgare*, *Dracocephalum moldavica*, *Thymus vulgaris*, and *Dysphania ambrosioides* showed *N. aberrans* J2 mortality ranging between 12% and 22%. Although the mortality recorded with these extracts was lower than that of the two aforementioned plant species, they still had a significant effect ($p \leq 0.05$) on Na mortality compared to the control. As the concentration of the evaluated extracts increased to 100% (Figure 6), J2 control was similar to that observed in *M. enterolobii*. However, Na proved to be more susceptible to these

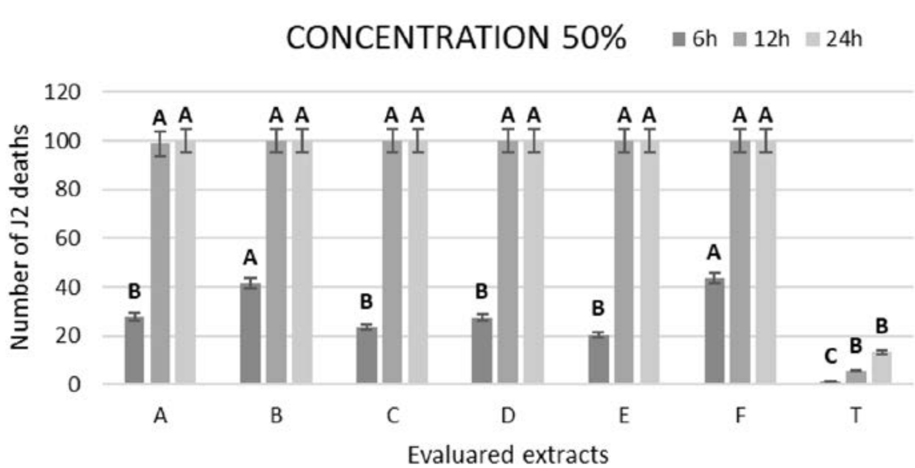

Figure 5. Effect of aqueous plant extracts at 50% concentration on the mortality of *N. aberrans*. A: *Origanum vulgare*; B: *Foeniculum vulgare*; C: *Dracocephalum moldavica*; D: *Thymus vulgaris*; E: *Dysphania ambrosioides*; F: *Melissa officinalis*; T: Control. Test: Tukey. Alpha=0.05. df=6. Means with the same letter are not significantly different ($p \leq 0.05$).

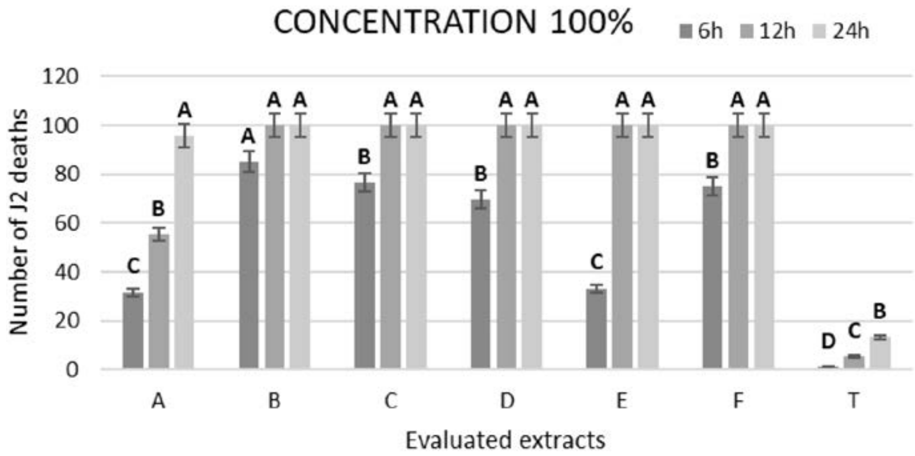

Figure 6. Effect of aqueous plant extracts at 100% concentration on the mortality of *N. aberrans*. A: *Origanum vulgare*; B: *Foeniculum vulgare*; C: *Dracocephalum moldavica*; D: *Thymus vulgaris*; E: *Dysphania ambrosioides*; F: *Melissa officinalis*; T: Control. Test: Tukey. Alpha=0.05. df=6. Means with the same letter are not significantly different ($p \leq 0.05$).

extracts, as control rates greater than 80% were observed from the lowest concentration starting at 12 hours of exposure, with all evaluated species showing effectiveness.

The observed behavior can be explained by the specific biochemical characteristics of the evaluated plant species, as well as by the effect of the undiluted pure extract concentration. In aqueous extracts, it is more likely to obtain secondary metabolites with hydrophilic or moderately polar properties, such as certain phenols, tannins, and triterpenes, which could influence the results obtained in terms of the observed mortality. Within these groups, compounds such as kaempferol can be found. This metabolite and its derivatives have shown the ability to restrict the motility of Meloidogyne incognita and exhibit repellent effects on this species by inhibiting its penetration rate in tomato (*Solanum lycopersicum* L.) roots, resulting in a lower gall index due to reduced penetration (Zhao *et al*., 2023). A similar effect has been observed in the extract of false arnica (*Heterotheca inuloides*), which inhibits egg hatching and J2 motility of *N. aberrans* (Rodríguez-Chávez *et al*., 2019). This plant species has been reported to possess pesticidal properties associated with its high content of quercetin- and kaempferol-type flavonoids (Rodríguez-Chávez *et al*., 2017). These metabolites, like the flavonol myricetin, have been shown not to require large doses to exert a nematicidal effect on *Meloidogyne* species, as reported by Wuyts *et al.* (2006). They observed that concentrations of 46 μg ml^{-1} of kaempferol, quercetin, and myricetin repel and limit the mobility of *M. incognita* juveniles. Similarly, in this study, despite using a small volume (140 μl) of the aqueous extracts evaluated, all showed a positive effect on the mortality of the root-knot nematodes studied.

The aqueous leaf extracts of *Dysphania ambrosioides*, *Melissa officinalis*, *Foeniculum vulgare*, and *Thymus vulgaris*, and their effect on the mortality of *Nacobbus aberrans* and *Meloidogyne enterolobii*, may be related to the metabolites present in these plants. All of these species have been reported to contain compounds such as quercetin and kaempferol (Barros *et al*., 2013; Petrison *et al*., 2022; Shamkant *et al*., 2014; Komaki *et al*., 2015). However, the heterogeneous susceptibility observed between the two nematodes to these extracts may likely be explained by differences in chemosensory receptors, the binding affinities of flavonoid receptors, cellular signaling, and the permeability of metabolites through the cuticle in the different nematode species, or by gene expression prior to nematode infection —although this has not yet been studied. For example, the concentration of lauric acid has been shown to influence the expression of the Mi-flp-18 gene (Dong *et al.*, 2016). It was demonstrated that low concentrations (0.5-2.0 mM) of lauric acid from the root exudates of crown daisy (*Chrysanthemum coronarium*) attract *Meloidogyne incognita*, whereas a higher concentration (4.0 mM) repels the nematode. However, there is a lack of studies on the factors that regulate this gene expression. While some investigations show that root exudates regulate the expression of genes such as flp, which encode FMRFamide-like peptides —a diverse group of neuropeptides involved in nematode feeding, reproduction, and mobility, playing a key role in nematode chemotaxis (Peymen *et al.*, 2014)— it remains unknown which specific components of the exudates are involved in genetic regulation and what factors trigger that signaling.

Although research is increasingly focused on understanding the dynamics between metabolomics and interactions with pathogens, our knowledge of the molecular responses triggered by attractants, repellents, and toxins within the nematode's body —and their impact on host crops under greenhouse or field conditions, including their effectiveness in controlling root-knot nematodes, potential phytotoxicity on the host plant, or their effect on the microbial loop— remains limited.

CONCLUSIONS

To the best of our knowledge, our results demonstrate for the first time the *in vitro* nematicidal activity of plant extracts from *Dysphania ambrosioides*, *Melissa officinalis*, *Foeniculum vulgare*, and *Thymus vulgaris* against the root-knot nematodes *Nacobbus aberrans* and *Meloidogyne enterolobii*. These plant species are proposed as subjects for future research aimed at identifying additional compounds responsible for their nematicidal activity and at providing non-toxic alternative chemical products for crop protection.

ACKNOWLEDGMENTS

We thank the Consejo Mexiquense de Ciencia y Tecnología for the financial support provided through project PI EESP2024-009, which made this research possible, and the Colegio de Postgraduados, Campus Montecillo, for their collaboration in carrying out this work.

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Germination, survival, and growth of *Cordia dodecandra* A.DC. under Field Conditions

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ABSTRACT
Objective: *Cordia dodecandra* A.DC., an endemic species of the Yucatán Peninsula, Mexico, is valued for its fruit and timber production in the market. This study evaluated seedling emergence and growth from seeds directly sown in the field using pregerminative treatments.
Design/methodology/approach: The research was conducted in the town of Castamay, Campeche. The land was prepared using agricultural machinery. Scarification treatments consisted of T1: 10%, T2: 20%, and T3: 50% hydrogen peroxide in purified water, as well as an untreated control, using seeds harvested in the same year.
Results: The highest number of seedlings emerged in the control treatment. Height and diameter growth varied across measurement periods, and both temperature and ambient humidity significantly affected these variables.
Findings/conclusions: The use of *C. dodecandra* seeds for reforestation in deforested areas is viable, with up to 80% success. Scarification is not required when seeds are sown in the same year they are harvested. Seedlings of *C. dodecandra* grown from directly sown seeds in the field exhibit steady growth, which is influenced by temperature and humidity conditions.

Citation: González-Lazo, E., Novelo-Moha, B. del P., Arriola-Escalante, M. T. Vanoye-Eligio, M., Pérez Vera, A. I., Rodríguez-Uc, G. R., & Dzib-Castillo, B. B., (2025). Germination, survival, and growth of *Cordia dodecandra* A. DC. under Field Conditions. *Agro Productividad*. https://doi.org/10.32854/96fj0y79

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

Received: January 27, 2025.
Accepted: May 08, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 203-211.

INTRODUCTION

Cordia dodecandra A.DC. (commonly known as Ciricote) (Boraginaceae) is native to the forests of Mesoamerica and is adapted to a variety of environments. It is a multipurpose species that provides several products, including timber, its rough leaves that can be used as sandpaper, edible flowers and fruits (Yam *et al.*, 2014; Morales & Herrera, 2009), and is also used in traditional medicine according to local knowledge, in addition to its ornamental value due to its beauty (Yam *et al.*, 2014). However, there are no studies on the geographic distribution of this tree species in Mexico (Cámara *et al.*, 2020).

It is a heliophilous species of great ecological importance, making it a key component of tropical forests due to its fruit production during dry seasons, which is essential for wildlife. It serves as a year-round nectar source for bees, and its abundant foliage protects the soil surface against erosion through the production of organic matter (Yam-Chin *et al.*, 2014). On the international market, *C. dodecandra* wood is in high demand, which has been the main cause of the decline in its populations since the 1990s. Today, it has been reported that populations in tropical forests are either nonexistent or reduced to a single individual per hectare (Vester & Navarro, 2007). *C. dodecandra* has been classified by the National Forestry Commission (CONAFOR) as a priority species for conservation due to overexploitation and the destruction of its ecosystem. One factor contributing to this destruction is vegetation fragmentation, which reduces and isolates its populations (Morales & Herrera, 2009; Yam *et al*., 2014).

This situation has prompted various studies on different aspects related to the growth of this species in forest plantations (Cámara-Romero, 2021), ethnobotany (Yam *et al*., 2014), domestication (Ferrer *et al*., 2020), and agroforestry systems (Campos-Bobadilla *et al*., 2016). However, studies on its population dynamics under natural and managed conditions are still lacking, which are necessary to design effective recovery strategies for this species. Changing climatic conditions have led to prolonged droughts or, conversely, excessive rainfall in the forest, both of which reduce the number of seedlings. Most seedlings are found beneath or near the mother plant, leading to competition for resources such as light and water (Urrego & Valle, 2001). In addition, herbivory by wild animals can increase mortality during early developmental stages (Ferrufino *et al*., 2016). Therefore, the aim of this study was to evaluate the emergence and growth performance of seedlings obtained from seeds directly sown in the field under different pregerminative treatments.

MATERIALS AND METHODS

The research was conducted in the town of Castamay, Campeche, Mexico (Longitude −90.430556, Latitude 19.838611), located 11 kilometers east of the state capital, at an elevation of 19 meters above sea level. The predominant climate is warm sub-humid with summer rainfall, with an average annual precipitation of 1,124.67 mm and a mean temperature of 27.02 °C (Figure 1).

Land preparation

The soil in the experimental area was classified as Ferric Luvisol. Land preparation was carried out in May 2022 using agricultural machinery for brush removal and soil harrowing. This process loosens the soil, thus improving the rooting zone conditions for the plants by enhancing the soil's capacity to retain and store water and oxygen, all with the aim of facilitating seed germination, seedling emergence, and subsequent growth (Martínez, 2015).

Seed collection and scarification

Ripe fruits were collected in June from healthy and vigorous trees located in the town of Castamay and the city of Campeche. To extract the seeds, the pulp was removed through soaking and maceration. Once the pulp was eliminated, the seeds were left to dry in the sun for two days.

Treatments

The experiment consisted of three treatments and one control, using 30 seeds per treatment. The treatments were as follows: 10%, 20%, and 50% hydrogen peroxide solution

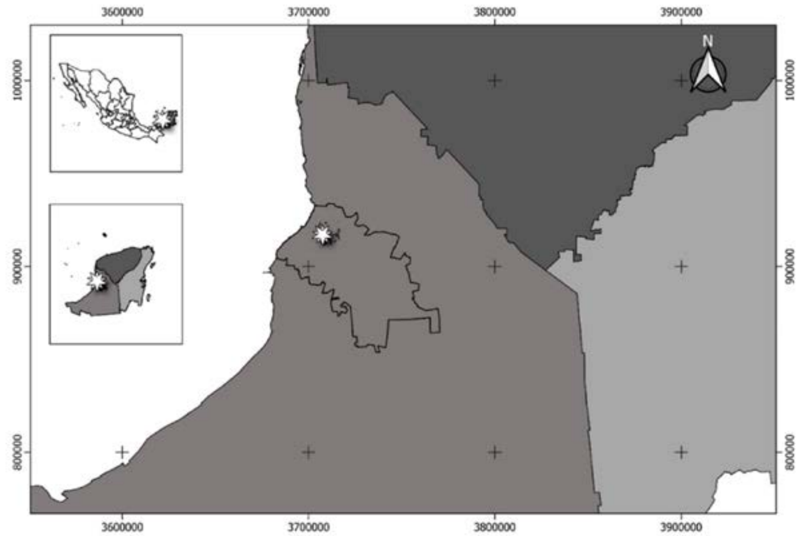

Figure 1. Location of the research site in the town of Castamay, Campeche, Mexico.

in purified water. Seeds were soaked for 10 minutes in each solution, then rinsed with water before being sown in the field. The control group did not receive any treatment. Seeds were sown directly in the field in July using a randomized block design, with the treatments distributed across the entire area.

Sowing was done in four blocks with a spacing of 1.5×1.5 m between planting points, with 30 seeds per block. After sowing, seedling emergence was recorded weekly until emergence ceased.

Seed viability

To determine the viability of *C. dodecandra* seeds, 100 seeds were selected and sown in trays filled with substrate (50% Luvisol soil and 50% coconut fiber). Observations were recorded as seedlings emerged from the substrate. In the field, data on seedling emergence, height, and stem diameter were collected once all seedlings had emerged from the soil. The data were analyzed using INFOSTAT software (Di Rienzo, 2020).

The experiment was conducted at the agroforestry research area of the Instituto Tecnológico de Chiná, located in the town of Chiná, Campeche, Mexico, at coordinates 19.77° N latitude and −90.50° W longitude. The predominant climate is warm sub-humid with an average annual temperature of 27 °C (García, 2004).

Evaluated variables

The basal stem diameter at substrate level (mm) was measured using a Cadena® digital caliper model A020. Total height (cm) was measured with a graduated ruler from the substrate level to the plant apex. These measurements were taken every seven days, recording 12 data points over a period of 114 days. Using the height and diameter data, growth increments for each seven-day period, averages, and total increments were calculated. The increment for each period was obtained by subtracting the previous measurement from the subsequent one, continuing this process for all data points.

The survival percentage of plants established in each treatment was recorded by counting living and dead plants.

For statistical analysis, the Shapiro-Wilks normality test was applied to determine data distribution ($P<0.0001$), which indicated the need for a non-parametric analysis. Variance analysis was performed using the Kruskal-Wallis test to compare means and identify differences between treatments, using the INFOSTAT statistical software (Di Rienzo *et al.*, 2020).

RESULTS AND DISCUSSION

Germination

Seed germination showed a viability of 75%, a value obtained from seeds sown in trays. This was achieved over a period of 24 days, with the highest germination occurring between days 12 and 18 of the evaluation period, which started on August 18 and ended on September 10 (Figure 2).

Seedling emergence from direct seeding in the field

Seedling emergence in the field under different scarification treatments showed no statistically significant differences ($P=0.05$). Despite this, it can be observed that the highest emergence occurred in the control (T1=30%, T2=37%, T3=13%, and Control 80%) (Figure 3).

Seedling Growth

Height increment analysis using the non-parametric Kruskal-Wallis test showed statistically significant differences ($P=0.0001$) in height growth between measurement periods. The greatest height increases occurred during the initial stages of *C. dodecandra* seedling growth. The first data collection was on August 25 and the last measurement on

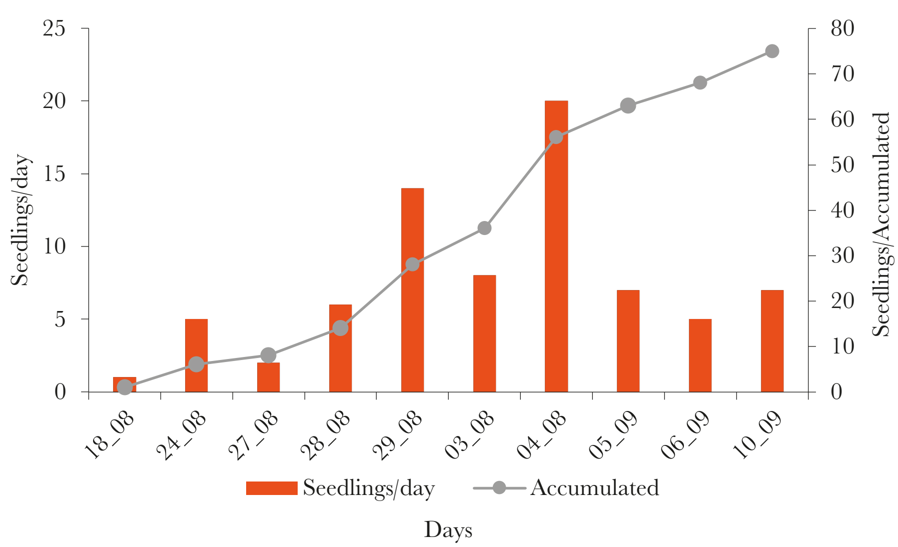

Figure 2. Germination behavior of *Cordia dodecandra* A.DC. seeds in trays under shade conditions and temperatures between 30 and 38 °C.

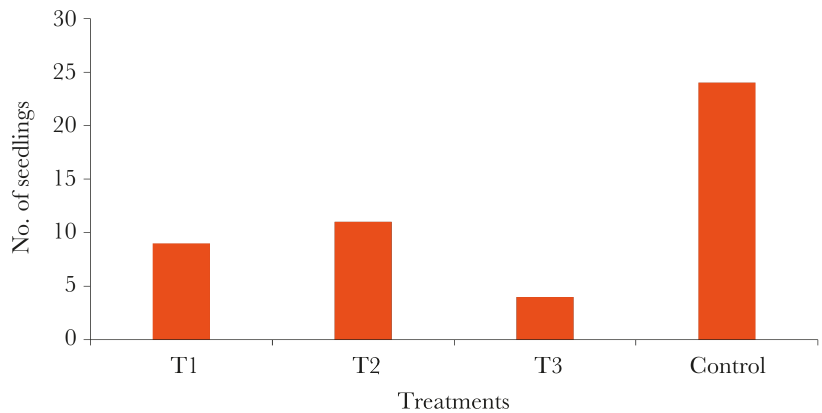

Figure 3. Seedling emergence in the field on a Ferric Luvisol soil in Campeche, Mexico. Using seeds treated with hydrogen peroxide at 10%, 20%, and 50%, soaked for 10 minutes, and a control without treatment.

December 17, covering a total of 114 days. Measurement periods were spaced seven days apart. The highest growth increment was observed during the weeks from September 24 to October 2, while the lowest increase occurred between October 8 and 15 (Figure 4). On average, seedling growth per period was 2.27±1.27 cm, with a total average height increase of 35.3 ± 7.8 cm over the 3.5-month evaluation period.

The Pearson correlation analysis showed a negative correlation ($r=-0.52$, $P=0.04$) between humidity and height increment. This can be observed in Figure 5, where an increase or decrease in humidity corresponds to an inverse change in height growth. Meanwhile, temperature showed a positive correlation ($r=0.45$, $P=0.08$), with height increasing during a period as temperature decreased (Figure 6).

Diameter increments

The average total increment in stem diameter during the evaluated period was 10.31±1.85 mm, while the average increment per period was 1.31±0.44 mm. Analysis of variance showed statistically significant differences between the stem diameter increments of *C. dodecandra* seedlings across the increment periods (Figure 7). The greatest increases were observed from August 25 to September

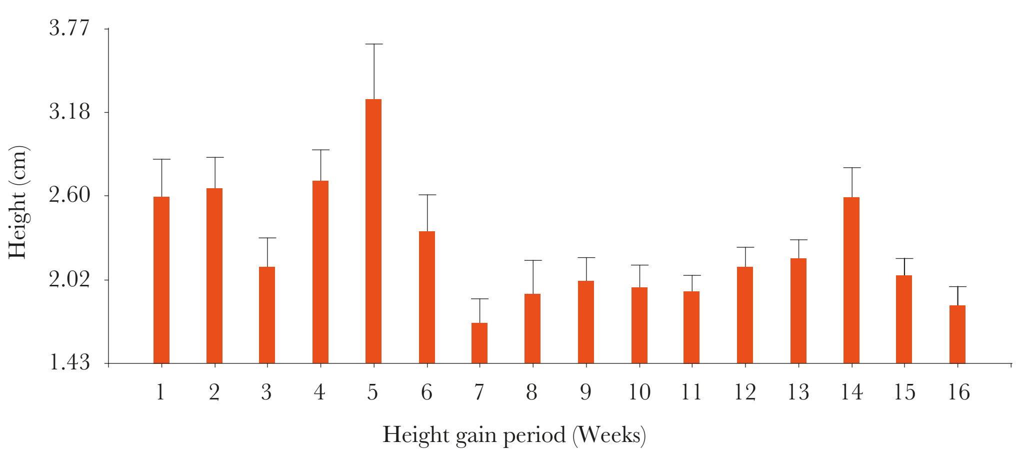

Figure 4. Height increments of seedlings obtained from direct seeding of *Cordia dodecandra* A.DC. in the field.

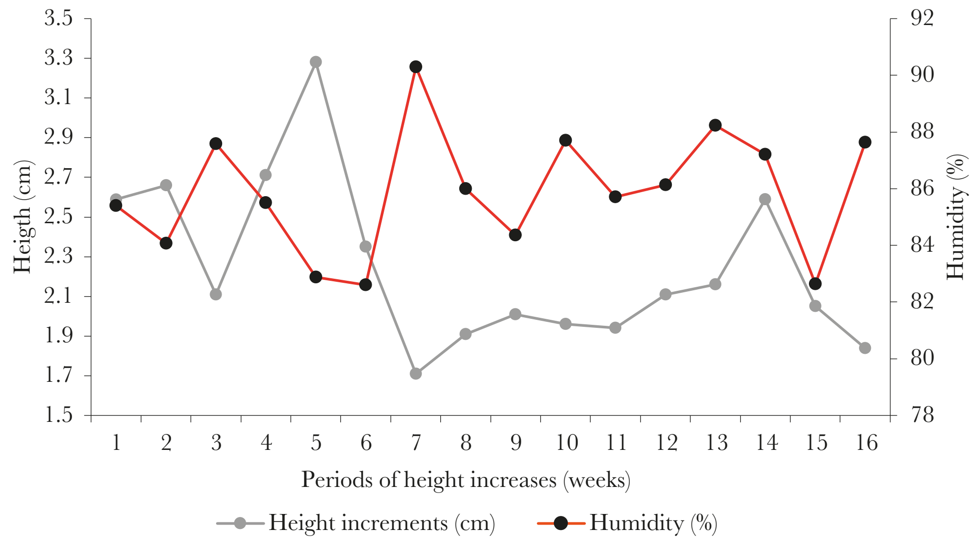

Figure 5. Behavior of height increment in *Cordia dodecandra* A.DC. seedlings in relation to humidity.

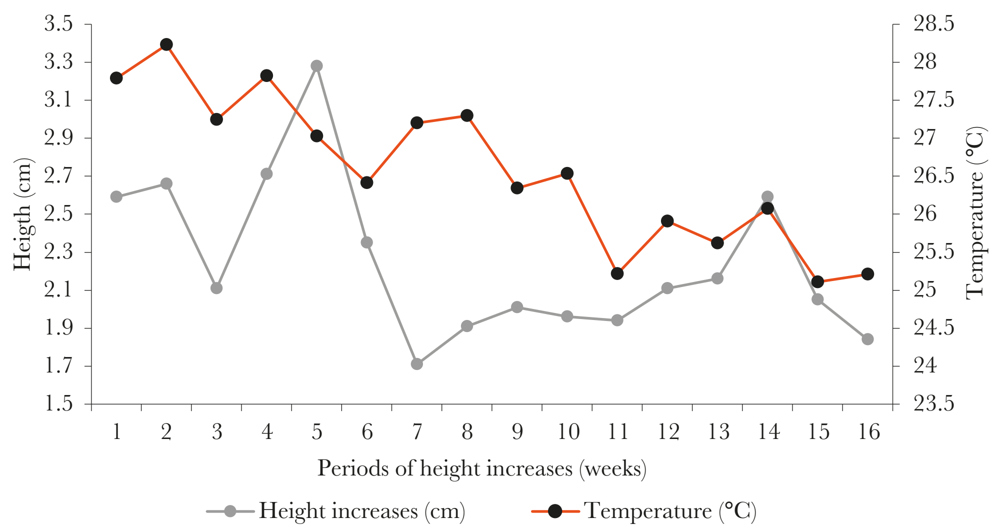

Figure 6. Behavior of height increment in *Cordia dodecandra* A.DC. seedlings in relation to temperature.

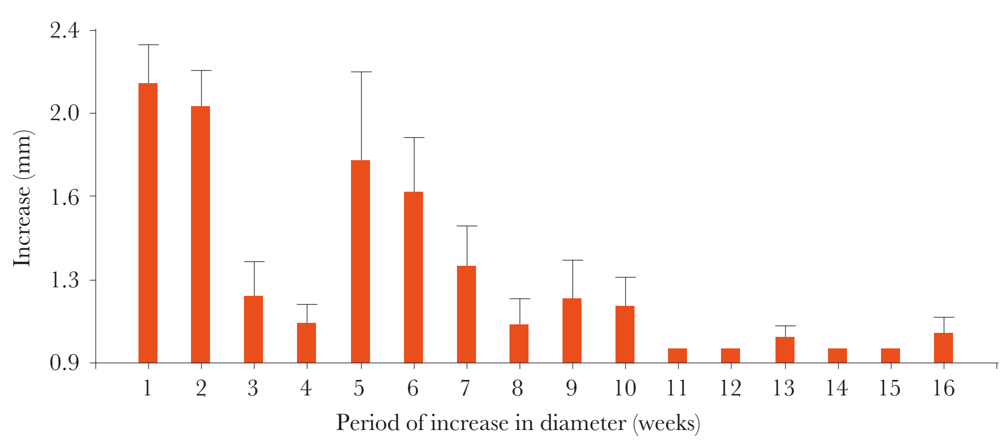

Figure 7. Stem diameter increments in *Cordia dodecandra* A.DC. seedlings obtained from direct seeding in the field.

2 and from September 3 to 10, while the smallest increments occurred starting November 5 (Figure 8). It was observed that when temperatures remained high, the diameter increment was low, and as temperature decreased, the increment increased (Figure 9).

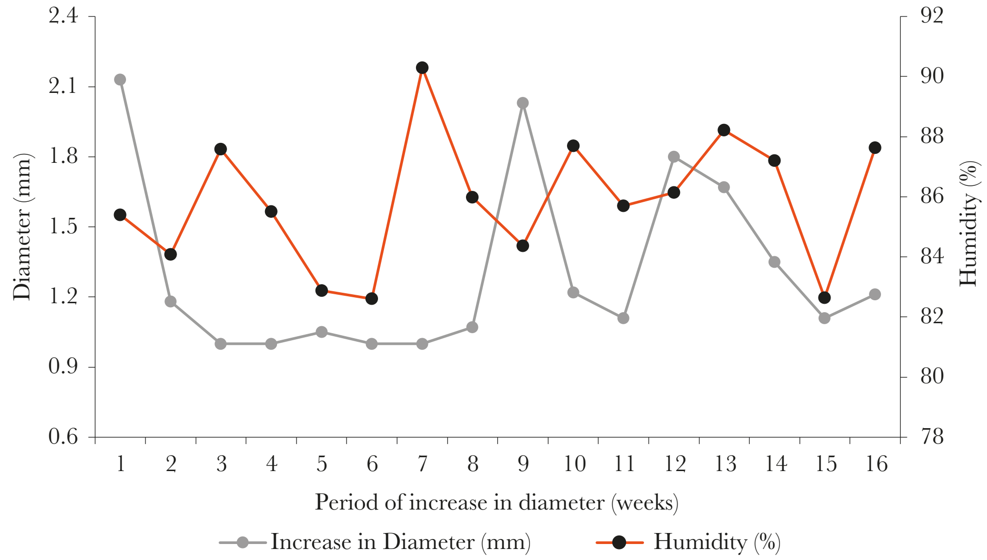

Figure 8. Stem diameter increment behavior of *Cordia dodecandra* A.DC. seedlings in relation to humidity.

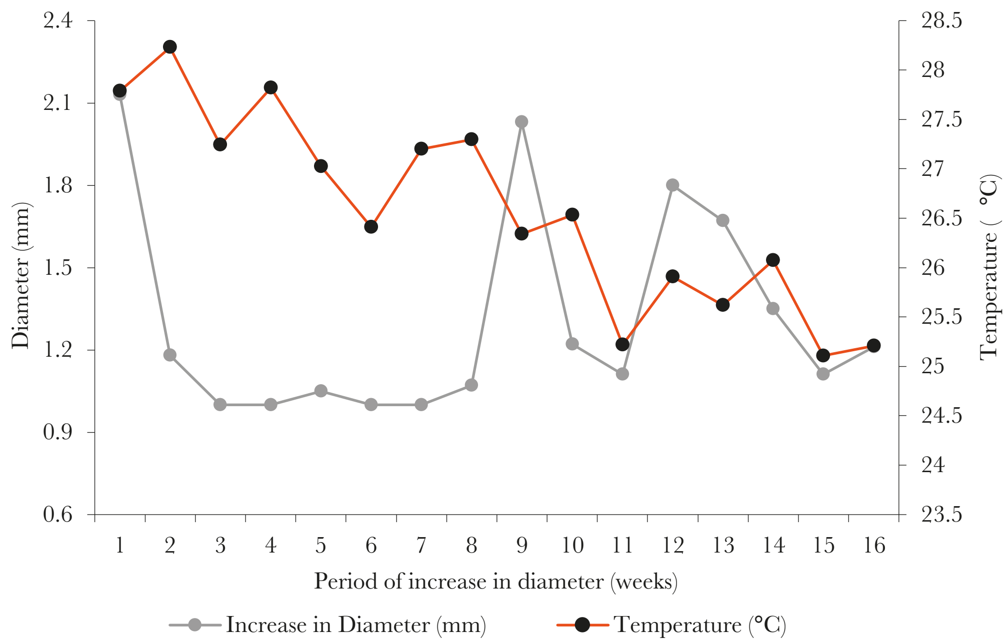

Figure 9. Behavior of diameter increment of *Cordia dodecandra* A.DC. seedlings in relation to temperature.

CONCLUSIONS

The use of *C. dodecandra* seeds for reforestation in deforested areas is viable up to 80%. Scarification of seeds is not required when seeds are from the same year of harvest and sowing. The growth of *C. dodecandra* seedlings obtained from seeds sown directly in the field shows a steady increase, which is influenced by temperature and humidity conditions.

ACKNOWLEDGMENTS

We thank the project with code 18370.23-PD, funded by the Tecnológico Nacional de México, and all the individuals who contributed to the development of this research.

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Effect of seasons on days open of breeding cows

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ABSTRACT

Objective: to evaluate the effect of seasons on days open and calving date of breeding cows of the Cristo Rey cattle ranch, San Andres Tuxtla, Veracruz based on 15-years records.

Design/Methodology/Approach: using 15 years of production records from the Cristo Rey cattle ranch, the relationship between date of calving and next breeding was analyzed to obtain the days open (number of days defined from calving to next conception). Data used were the most recent calving date, and next breeding date. Based on calving date cows were classified into four seasonal calving groups, then the days open (calving interval) for each cow were quantified. Descriptive statistics of calving intervals were used to obtain a frequency distribution, and an analysis of variance with InfoStat© was used to determine differences among calving intervals, also to determine if days open were associated with calving per season.

Results: a total of 985 calving events were recorded, with an overall average of 240 days open. Only 15.8% of the breeding cows had less than 103 days open. The highest percentages were counted between 104 and 358 days open. It was also recorded that 17 cows took more than a year and a half (≥ 528 days open) to breed again. With a significant difference among seasons ($p=0.0003$), winter was identified as the season in which the cows that calved had the fewest days open (219).

Limitations/Implications of the study: a correlation analysis between calving and breeding was not performed in two cycles. However, the identified trend is an approximation to the usefulness of performing such correlations as a further step.

Findings/Conclusions: breeding and calving do not occur synchronously in the same herd. This is due not only to the calving intervals in each season, but also to other nutritional, health, and genetic factors that influence the date of breeding and calving in the same year or the following year.

Keywords: seasons, days open, breeding.

Citation: Acar-Martínez, N. B., Tadeo-Rosado, A., Cartas-Domínguez, V. M., Ramírez Valencia, J. O.& Lechuga-Hidalgo, J A. (2025). Effect of seasons on days open of breeding cows. *Agro Productividad*. https://doi.org/10.32854/knk1aa96

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

Received: January 28, 2025.
Accepted: May 14, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 213-219.

INTRODUCTION

Bovine reproduction is a multifactorial process influenced by diverse environmental conditions that can affect both the physiology and productivity of animals (Góngora and Hernández, 2010). Mexico is a country with significant climate diversity. Environmental effects on days open of cows are relevant, especially in tropical and subtropical regions. High temperature and relative humidity, plus the seasonal variations can induce thermal stress on animals that affects reproductive capacity. All of which may result in very long calving intervals, this is, greater numbers of days open between calving and the next breed (Cordova, 2017).

In tropical regions, such as southern Veracruz (Mexico), climate conditions, especially temperature and humidity, significantly affect the reproductive physiology of cows inducing heat stress (Arias and Escobar, 2008). Previous studies have shown that an increase in ambient temperature alters ovarian function, estrus, embryonic development, birth, and affects semen quality; (Brito *et al*., 2002; Vergara, 2018). This type of stress not only affects reproductive activity, but also affects behavior, decreased food intake, and the overall health of the animals. All of those are factors that have effects on days open (Bustillo Parrado and Melo Colina, 2020).

Despite growing concern about this phenomenon, research on the specific effects of the environment on days open and calving of cows in southern Veracruz remains limited. This study aimed to evaluate the effect of seasons on days open and calving date of breeding cows of the Cristo Rey cattle ranch (San Andres Tuxtla, Veracruz) based on 15-years records.

MATERIALS AND METHODS

This study was conducted at the "Cristo Rey" cattle ranch, located in the community of 'El Popotal', municipality of San Andres Tuxtla (Veracruz) Mexico, at coordinates 18.236058 N and −95.289757 W (Figure 1). The local climate is predominantly warm sub-humid according to the modified Köppen classification (García, 2004), with approximately 1282 mm of annual average rainfall and 25.7 °C average temperature. Soils are clayey in texture, classified as Vertisols. Topographically, terrain relief are plains, although there are low areas saturated with residual moisture (CONAGUA, 2024).

An Ombrothermal diagram of the area was graphed with 30-years data of monthly average temperature and monthly average rainfall in the study area. January to May are the drier months in the year, with the greatest amount of rainfall concentrated between June and October (Figure 2).

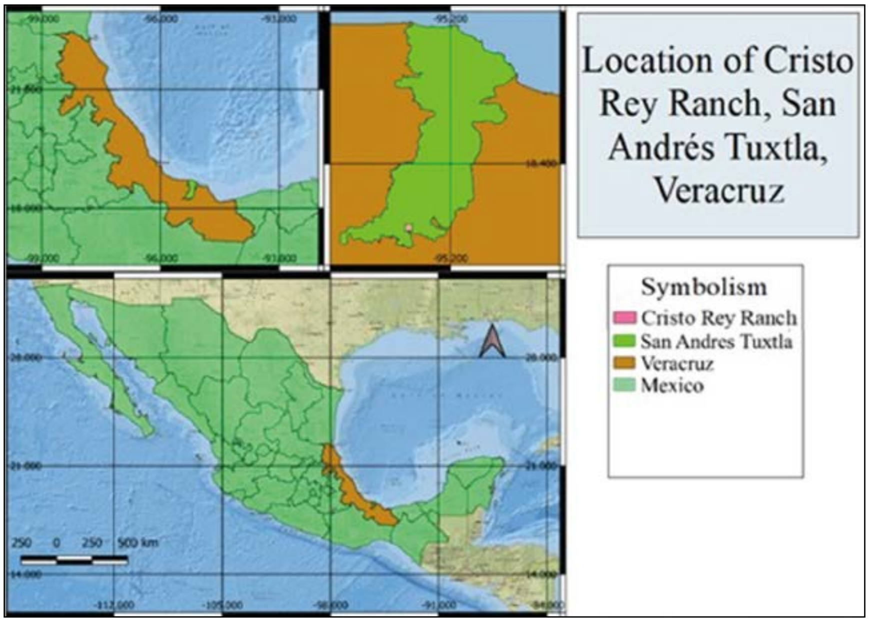

Figure 1. Location of the study area; San Andres Tuxtla (Veracruz), Mexico.

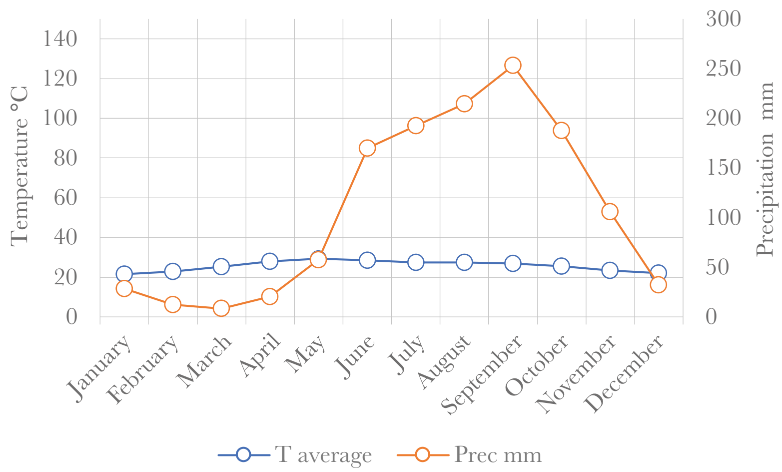

Figure 2. Ombrothermal diagram, based on normalized (1991-2020) climate data for 'El Popotal', San Andres Tuxtla (Veracruz) Mexico. Source: CONAGUA (2024) database.

The production system of the Cristo Rey cattle ranch can be classified as a livestock farm dedicated to the production of breeding stock, with natural mating. Breeds managed are American Swiss (*Bos taurus*), Gyr (*Bos indicus*) and their cross breeds. The grazing system is rotational; in December, January and February, a feed supplement of sugarcane silage (*Saccharum officinarum*) is offered to animals. Staff follows a vaccination schedule; they administer vitamins, and deworm the animals; also, they provide free access to mineral salts. Female calves born are destined for breeding, and male calves are sold as livestock.

Variables evaluated

The study consisted of two stages; first stage was the collection of records at the livestock farm, 15-year data (2009-2024). Second stage was the design and performing of analytics (Figure 3).

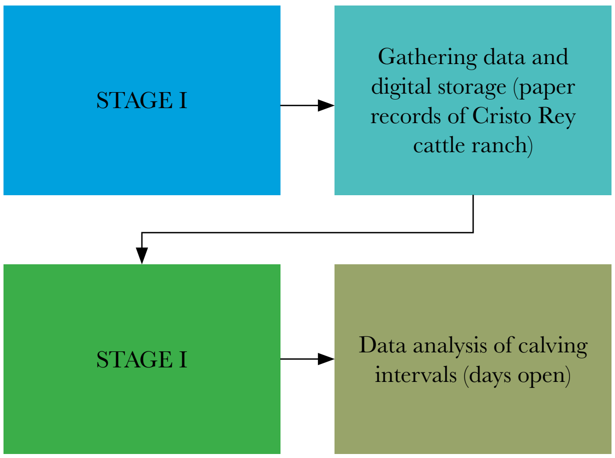

Figure 3. Work flowchart of the study conducted at Cristo Rey cattle ranch, San Andres Tuxtla (Veracruz) Mexico.

Stage 1. Collecting and classifying production records

Excel® spreadsheets with 15 years of records from the Cristo Rey ranch were compiled. Calving date and next conception date per cow were the data used to obtain the days open. Data were classified according to the latest calving date and the season in which that occurred. With the data, four groups of cows were categorized by calving in each season (winter, spring, summer, and autumn) to explore relationships between season and the number of days open for each cow.

Stage 2. Data analysis

With the data grouped by season, a descriptive analysis of the days open was done. We obtained the frequency distribution, then we performed an analysis of variance using InfoStat© to determine whether there was a difference among the days open in each group, then if that was the case, finding which group took the shortest time (fewer days open) until the next breeding.

RESULTS AND DISCUSSION

A total of 985 births were recorded over 15 years. During this time, cows averaged 240 days open as overall calving interval to subsequent breeding. This is a higher number than that reported by Ríos *et al.* (2020), 162 days open for Zebu×European cows under tropical conditions. However, the mode of the data is 160 days open. Another interesting fact is that some cows presented more than 500 days open. This finding is consistent with other studies indicating that long calving intervals have negative consequences on the productivity and profitability of livestock systems. According to Cerri *et al.* (2009), long calving intervals (greater number of days open) are associated with lower reproductive efficiency rates, which can lead to lost revenue due to lower calf production. In our case, prolonged periods without breeding could reflect problems in fertility or reproductive management. This circumstance requires attention to avoid significant economic costs (Table 1).

Only 15.8% of cows had less than 103 days open, which means less than four months passed before they bred again. Higher percentages were concentrated between 104 and 358 days open. There were 17 cows which took more than a year and a half (≥528 days open) to breed again. This pattern could suggest that, although most cows breed within a reasonable timeframe, a significant fraction of them face challenges that lengthen this period. Thus, a thorough review of reproductive management protocols is required, which could include among others, improving estrus synchronization, optimizing nutrition, and controlling diseases associated with reproduction (Figure 4).

The analysis of variance showed significant difference (F_c=2.614, p≤0.0003) in days open per season. With the results of the mean comparison test of days open, for the four groups according to season, it is observed that the group of cows that calved in winter presented the shortest calving interval ($\bar{x}$ =219 days open, p≤0.05). In contrast, cows that calved in autumn had longer calving intervals ($\bar{x}$ =263 days open; p≤0.05), tough these were statistically similar to the groups of cows that calved in spring and summer (Table 2).

Table 1. Descriptive statistics for calving interval of cows in the 'Cristo Rey' ranch, San Andres Tuxtla (Veracruz) México, 2009-2024 records.

Calving interval (days open)		
Mean	$\alpha_{\bar{x}}$	239.554
Standard Error of the Mean	$S_{\bar{x}}$	4.170
Median		225.500
Mode		159.500
Standard Deviation	s	130.895
Sample Variance	s^2	17133.753
Kurtosis		0.295
Skewness Coefficient		0.630
Class Interval		848
Lower Limit		18.500
Upper Limit		866.500
Sum		235961.500
Cows that calved	n	985
Typical error (uncertainty)	u	0.0174

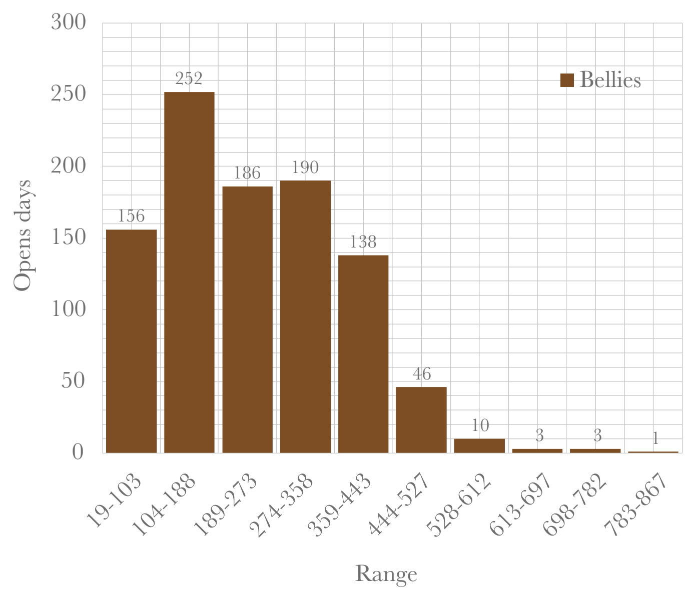

Figure 4. Frequency distribution of days open recorded over 15 years in San Andres Tuxtla, Veracruz. Source: Cristo Rey cattle ranch, 2009-2024 records.

Another important fact is that in winter occurred the most calving events, while summer was the season with the fewest. Seasonality in reproduction is a commonly observed phenomenon in many livestock systems. However, the low number of calving events recorded in summer could also indicate that those cows faced suboptimal prior seasonal conditions, which limited their ability to breed efficiently (Domínguez *et al.*, 1987).

Table 3 shows how next breeding by season reflect on calving by season. For example, autumn is the time with the fewest number of conceptions recorded, also with the greatest

Table 2. Seasonal difference in calving interval (days open) of cows of the 'Cristo Rey' ranch in San Andres Tuxtla, Veracruz, México (2009-2024).

Season	Mean	n	SEM	HSD
Winter	218.87	381	6.65	A
Spring	243.16	247	8.26	B
Summer	250.77	113	12.22	B
Autumn	263.01	244	8.31	B

Mean comparison test; n: cows that calved in season; SEM: Standard Error of the Mean; Honest Significant Difference, HSD=25.242 ($p \leq 0.05$); degrees of freedom, d.f: 981.

Table 3. Observed trend between calving (per season) and subsequent conception of cows in the 'Cristo Rey' cattle ranch, San Andres Tuxtla, Veracruz, Mexico (2009-2024 records).

Season	Calving	Mean of days open	Next conception	Season of next breeding
Winter	381	218.87	238	Spring-summer
Spring	247	243.16	392	Summer-autumn
Summer	113	250.77	245	Autumn-winter
Autumn	244	263.01	110	Winter-spring

number of days open, and this effect is reflected on calving in the following summer. With adequate monitoring, we can add up the days open of the season (in this example, autumn) then infer that those cows would breed again between winter and spring.

The fewer number of days open in winter may be due to the feed supplementation with sugarcane, ground maize, and mineral salt implemented by the ranch staff at the end of December. Therefore, this nutritional management may have an effect on reducing days open in cows (Pires *et al.*, 2011). Furthermore, for the geographic area where the study area is located, average 23 °C temperatures are reached in winter, and 49 mm of accumulated rainfall (CONAGUA, 2024), both maintaining conditions for pasture recovery.

Under the situation shown, we can observe that not all cows will calve in the same season, since their calving intervals (days open) determine their estrus and consequently the season of next calving (in a cyclical rhythm). Therefore, a cow will calve in different seasons throughout its productive life.

CONCLUSIONS

The results of this study highlight the importance of season on days open of cows at the Cristo Rey ranch. Days open (meaning shortest calving interval) during the winter season were statistically fewer than those during other seasons. Likewise, seasonal days open (in addition to genetic, nutritional, and health factors) influence the date of conception and, consequently, the date of calving.

Since each cow can breed and calve in different season, appropriate management during seasons of greater stress can help decreasing calving intervals and improve livestock productivity. Identifying those factors that influence on days open is crucial to improve the profitability and long-term sustainability of cattle production.

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Colegio de Postgraduados

Morphological characterization of native maize varieties (*Zea mays* L.)

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ABSTRACT

Objective: To characterize the traditional *milpa* agricultural system and evaluate the phenotypic variation of native maize ears in the coastal region of Oaxaca.

Design/methodology/approach: Thirty semi-structured interviews were conducted with local farmers, and morphological traits were evaluated in 100 ears from seven native maize types. Tests for normality (Shapiro-Wilk) and homoscedasticity (Kolmogorov-Smirnov) were performed, followed by ANOVA. Where significant differences were found, Tukey's test was applied ($\alpha<0.05$).

Results: The *milpa* is a polyculture system, and seven native maize types were documented. Significant differences were found in traits such as peduncle and ear length, number of kernel rows, and grain dimensions.

Findings/conclusions: The *milpa* is a system that harbors biological diversity, contributing to the conservation of genetic resources and food security.

Keywords: diversity, maize, phenotypic variation, traditional agricultural system.

Citation: García-López, D., Jacinto-Hernández, G., Pérez-Nicolás, M., & De la Rosa-Galindo, A. (2025). Morphological characterization of native maize varieties (*Zea mays* L.). *Agro Productividad*. https://doi.org/10.32854/ph0y8c80

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

Received: January 30, 2025.
Accepted: May 12, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 221-229.

INTRODUCTION

The *milpa* system is a traditional agricultural practice that has been used since pre-Hispanic times in Mesoamerica. It is characterized by the association of maize (*Zea mays* L.) with other crops such as common bean (*Phaseolus vulgaris* L.) and squash (*Cucurbita argyrosperma*) (Chávez-Servia *et al.*, 2011; Mateos-Maces *et al.*, 2016). This system is key to food security and the conservation of agrobiodiversity, as it allows for the coexistence of multiple species that provide agronomic and ecological benefits (Acosta-Díaz *et al.*, 2014).

In Mexico, maize represents a central element in rural culture and economy, with approximately 65 registered races, 59 of which are native. Of these, 35 are found in Oaxaca, establishing the state as a center of genetic diversity for the crop (Santillán-Fernández *et al.*, 2021; CONABIO, 2011). The *milpa* system is fundamental to food security and ecological sustainability, as it maintains soil fertility through practices such as the incorporation of organic matter and crop rotation (Marcial-Medina *et al.*, 2024). However, it faces various

threats, including the introduction of commercial hybrid maize seeds, intensive use of agrochemicals, and changes in land use, all of which jeopardize the conservation of native varieties and the autonomy of local farmers (Márquez-Sánchez, 2008; Moreno-Calles *et al.*, 2013).

In the coastal region of Oaxaca, Mixtec communities cultivate native maize and other useful species within *milpa* systems; however, their cultivation practices have not been documented. The objective of this study was to characterize the traditional agricultural system in Santiago Tetepec and to evaluate the phenotypic variation of native maize ears cultivated within this system, in order to contribute to the appreciation of traditional agriculture and to inform conservation strategies and breeding programs.

MATERIALS AND METHODS

Study Area

Santiago Tetepec (Figure 1) is located in the Jamiltepec district of Oaxaca. It is a Mixtec community with a warm sub-humid climate and annual precipitation ranging from 1,000 to 2,500 mm. Agriculture is the main economic activity, with maize, beans, and chili peppers being the principal crops (INEGI, 2020).

Characterization of the *milpa* system

A semi-structured interview was designed and applied to local farmers to document the *milpa* production cycle. The questionnaire included detailed sections on the crop's phenological stages, cultural practices, tools used, preferred maize types, yields obtained, and uses of the grain, among other aspects. It also included some sociodemographic

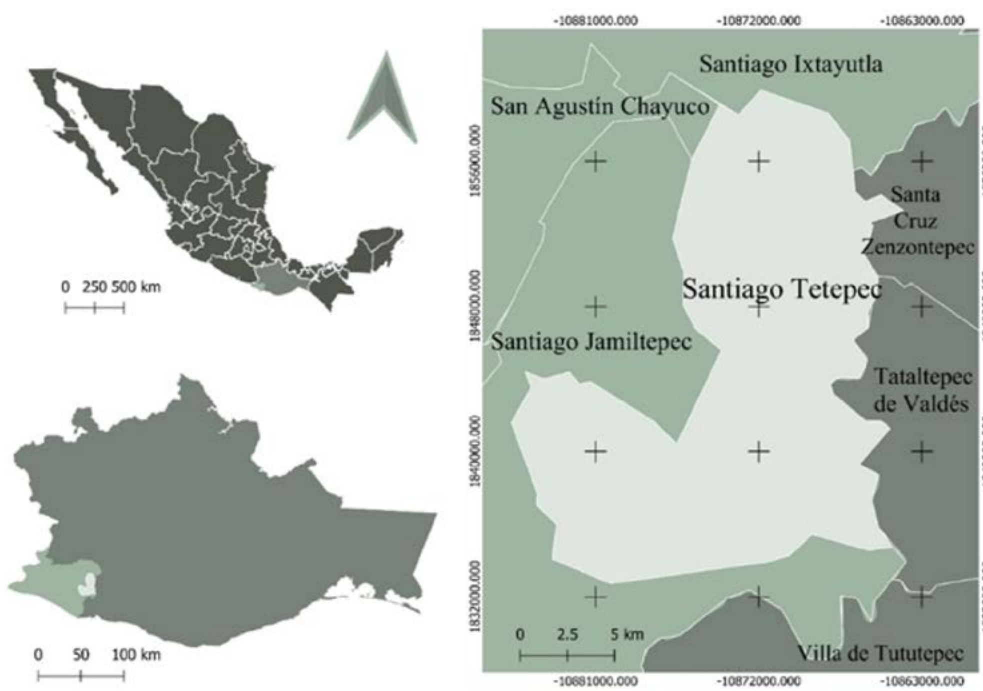

Figure 1. Geographic location of Santiago Tetepec, Oaxaca.

questions to gain insight into their work and educational background. The *milpas* of the interviewees were visited, and the information was complemented through participant observation, photographic documentation, and open-ended interviews.

Phenotypic characterization of maize

Ears from seven types of native maize were collected from the farmers interviewed in the community. An experimental plot was established in collaboration with a cooperating producer. The plot was divided into several subplots, each planted with 25 maize rows.

Planting was rainfed and carried out by making holes in the soil using a tool locally called *enduyo*. Four to five mixed seeds were placed in each hole, with a spacing of 1 m between rows. For weed control, two applications of Gramoxone® Super were made at different periods. Pest and disease control was carried out using agrochemicals. Fertilization was also chemical, with two applications of Yara® fertilizer: the first at the V5 growth stage and the second at the V10 stage.

Samples were taken from five central rows of each of the seven maize types. From each row, 20 plants were randomly selected, resulting in a total of 100 ears per maize type. Each collected sample was labeled and recorded, indicating the maize type, date, and row of origin. A total of 21 morphological traits were evaluated, of which 17 were quantitative and four qualitative. The number of ears per plant, the number and shape of the bracts, the color of the grain and cob, the number of rows, and the arrangement of the rows on the ear were recorded. A vernier caliper was used to measure the diameter of the cob, pith diameter, rachis diameter, peduncle diameter, and ear diameter at ¼, ½, and ¾ of its length. A ruler was used to measure the length of the peduncle and the ear. The length, width, and thickness of the grain were also recorded. Ear weight and the weight of 100 grains were obtained using a scale.

Statistical analysis

The information obtained from the interviews was systematized and analyzed using descriptive statistics. To evaluate phenotypic variability, normality tests (Shapiro-Wilks) and homoscedasticity tests (Kolmogorov) were applied, as well as ANOVA to determine significant differences among the measured variables. Tukey's test ($\alpha < 0.05$) was used for mean comparisons.

RESULTS AND DISCUSSION

Characterization of the traditional milpa agricultural system

The planting of native maize is a tradition among the inhabitants of the Mixtec community of Santiago Tetepec. It is an activity inherited from parents to children, as the maize is used for food preparation, ensuring their food security. Maize production is carried out under a polyculture system called *milpa* (*itu savi*), where other species are also established, such as common bean (*Phaseolus vulgaris* L.), squash (*Cucurbita argyrosperma* K. Koch), sesame (*Sesamum indicum* L.), hibiscus (*Hibiscus sabdariffa* L.), cassava (*Manihot esculenta* Crantz), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), cucumber (*Cucumis* sp.), chili pepper (*Capsicum annuum* L.), jícama (*Pachyrhizus erosus* (L.) Urb.), and bottle gourd (*Lagenaria* sp.). The maize types include short-cycle varieties, which take two to two and a half months, and long-cycle varieties, which take three to three and a half months. Various management activities are carried out in the cultivation, as shown in Figure 2.

The land preparation (slash-and-burn) —*Kañiyoo, cha'yayo yutu, Ka'miyoo chiin*

Land preparation begins between late April and early May and consists of slash, clear, and burn. Tall trees are cut with a chainsaw for firewood, while shrubs and grasses are removed with a machete and left to dry before burning. Currently, most producers only slash and burn, meaning they cut only shrubs and grasses, let them dry, and then burn them. To prevent uncontrolled fire spread, a firebreak is established by clearing vegetation along the edges of the area to be burned. The burning is carried out in the afternoon when winds are calmer. Subsequently, a minimum of three consecutive days of rain is awaited to ensure adequately moist soil for planting. Previous studies have documented this practice in different communities. Ayala-Enríquez *et al.* (2019) reported it in Santa Catarina, Morelos, including the use of harrows and fallow periods. Bastida-Francisca *et al.* (2024) mention it in the Mazahua agricultural calendar, where it begins in December, with fallow in January and harrowing in February. Carrera-García *et al.* (2012) describe a similar process in the Mazatec agricultural calendar, carried out with machetes and hoes.

Planting – *Tachiyoo*

It is carried out after the first rains and before noon. The tool used is known as *enduyo*, placing 3 to 4 maize grains per hole at a distance of one meter between rows

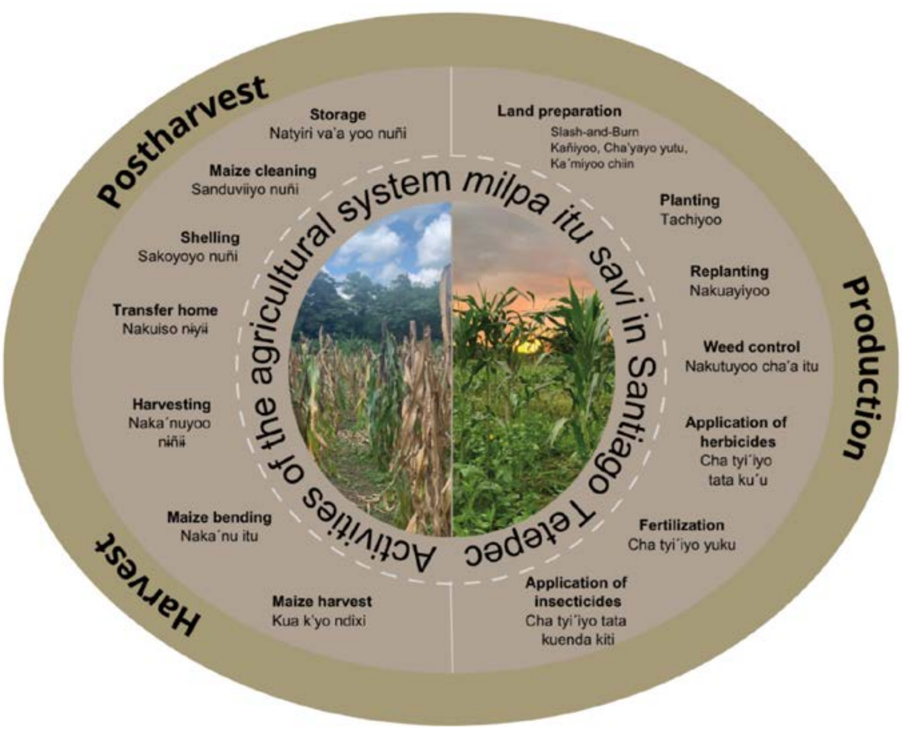

Figure 2. Agricultural activities carried out during the milpa production cycle.

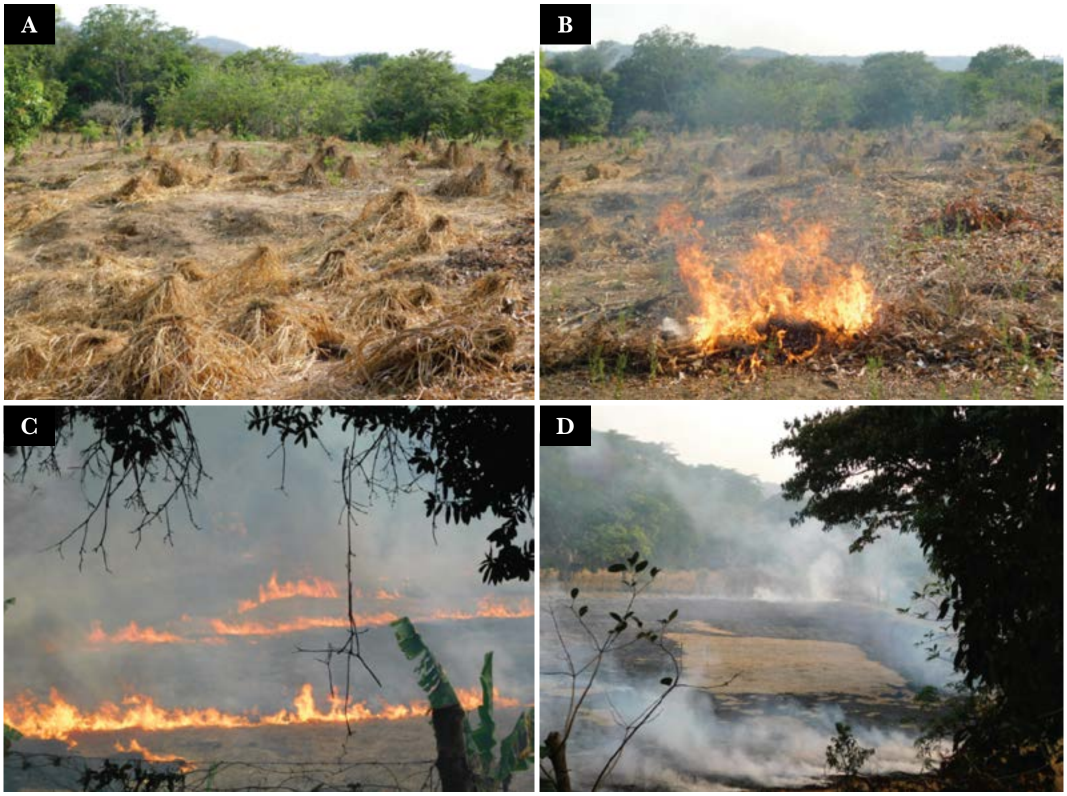

Figure 3. Land preparation. A) Slashing, B) Burning, C) Fire spreading, D) End of burning.

and between plants. Associated crops are planted on different dates. Ayala-Enríquez *et al.* (2019) documented that in Santa Catarina, Morelos, this activity is performed after eight consecutive days of rain, with a spacing of 70-80 cm between rows and one step between plants. Unlike the Mazahua agricultural calendar reported by Bastida-Francisca *et al.* (2024), where planting begins in March and April, the Mazatec calendar carries out planting in February and March (Carrera-García *et al.*, 2012).

Replanting – *Nakuayiyoo*

This is done three or four days after planting, in the spots where coleoptile emergence did not occur. Ayala-Enríquez *et al.* (2019) mention that this activity is carried out eight days after replanting in Santa Catarina, Morelos; and Bastida-Francisca *et al.* (2024) report that planting takes place in May.

Fertilization – Applying fertilizer – *Cha tyi´iyo yuku*

Fertilization is carried out using chemical products, the most commonly used being urea. The first application is made 15 days after planting, and the second when 50% of the plants are in male inflorescence. Ayala-Enríquez *et al.* (2019) agree with the use of chemical fertilizers; however, they also describe an accompanying activity called primera mano performed in the community of Santa Catarina, Morelos, which consists of loosening the soil.

Herbicide application – *Cha tyi´iyo tata ku´u*

Herbicides are applied using 20 L backpack sprayers; application doses depend on the recommendations from agrochemical store personnel, and some farmers perform a second application. In other regions such as Morelos (Ayala-Enríquez *et al.*, 2019), the State of Mexico (Bastida-Francisca *et al.*, 2024), and Oaxaca (Carrera-García *et al.*, 2012), manual weeding is performed using tools like the hoe and machete.

Insecticide application – *Cha tyi´iyo tata kuenda kiti*

The main pests affecting maize are the fall armyworm, which attacks the whorl, and the wireworm, which damages the roots. Daily monitoring is carried out, and insecticides are applied using backpack sprayers. In other regions, these pests are not controlled with agrochemicals (Ayala-Enríquez *et al.*, 2019; Bastida-Francisca *et al.*, 2024; Carrera-García *et al.*, 2012).

First harvest (Green maize) – *Kua k'yo ndixi*

The harvest of maize takes place when the kernels are still tender. They are consumed boiled or used to prepare tamales and tortillas made from fresh maize. This practice coincides with harvest dates reported in Santa Catarina, Morelos, and in the Mazahua agricultural calendar (Ayala-Enríquez *et al.*, 2019; Bastida-Francisca *et al.*, 2024). In Mazatec tradition, the green maize harvest occurs in August (Carrera-García *et al.*, 2012).

Maize bending – *Naka´nu itu*

This practice is carried out in October or early November to facilitate the drying of the maize and to protect it from pests and birds such as parakeets and grackles. Its implementation is not mentioned in Santa Catarina, Morelos, the State of Mexico, or Oaxaca (Ayala-Enríquez *et al.*, 2019; Bastida-Francisca *et al.*, 2024; Carrera-García *et al.*, 2012).

Post-harvest (Harvesting – *Naka'nuyoo nĩñĩ*; Shelling – *Sakoyoyo nuñi*; Cleaning – *Sanduviiyo nuñi*; Storage – *Natyri va'a yoo nuñi*)

Harvesting is the activity in which the maize ears are collected. It takes place in January and February, when the ears are dry enough to detach easily. Shelling is done at home, followed by cleaning (removal of plant residues), inspecting for weevils, and storing the grain in sacks, drums, or silos. In the Mazahua agricultural calendar, post-harvest occurs in October and November, while in Santa Catarina, Morelos, it is carried out in December, after the feast of Our Lady of Guadalupe (Ayala-Enríquez *et al.*, 2019; Bastida-Francisca *et al.*, 2024). In the Mazatec culture, harvesting takes place between September and October (Carrera-García *et al.*, 2012).

Phenotypic characterization of native maize ears

The variables cob peduncle diameter, number of husk leaves per ear, number of ears per plant, and kernel thickness showed no significant differences (Table 1). The variables ear diameter at ¼, ½, and ¾ length, cob (rachis) diameter, pith diameter, rachis diameter, and ear weight exhibited variability (Table 1). Morphometric variables of native maize from Santiago Tetepec, Oaxaca.

In terms of ear length, the Conejo and Amarillo types are similar to each other but differ from the other maize types. In comparison with the study by Sánchez-Hernández *et al.* (2015), where only collection number three from the community of Chilapa showed greater ear length than the other collections in the state of Tabasco, Cabrera-Toledo *et al.* (2019) found that among 18 populations of the Zapalote Chico race, the collection OAX-834 from the locality of Santiago Laollaga presented the greatest ear length compared to other collections from the Isthmus of Tehuantepec. In the study by Toxtle-Flores *et al.* (2023), among four groups formed, only one group exhibited shorter ear length among the types collected in the municipality of Coronango, Puebla. Similarly, in the work of Gonzáles-Martínez *et al.* (2020), which analyzed two groups and three subgroups, one group and one subgroup had shorter ear length and were significantly different from the rest, based on 98 maize populations from Tamaulipas.

In terms of the number of rows per ear, the Conejo type showed a significant difference compared to the Rojo type, and both differed from the other maize types. Guillen-De la Cruz *et al.* (2014) reported highly significant differences among 71 populations of native maize from the state of Tabasco. Cabrera-Toledo *et al.* (2019) found that only one collection from the locality of San Pedro Comitancillo showed a higher number of rows among the 18 populations of the Zapalote Chico race collected in the Isthmus of Tehuantepec. Similarly, in the study by Villalobos-González *et al.* (2019), only one accession had a higher average number of rows per ear among the thirteen collected in a community in the state of

Table 1. Morphometric variables of native maize from Santiago Tetepec, Oaxaca, Mexico.

Variables	p-value	Blanco A	Blanco B	Café	Rojo	Azul	conejo	Amarillo
Length of the peduncle	<0.0001	4.99 bc	5.55 ab	5.93 a	4.34 cd	5.22 b	4.12 d	3.90 d
Peduncle diameter	<0.0001	1.12 a	1.02 b	1.00 b	1.00 b	1.14 a	0.96 b	0.93 b
Length of the ear	<0.0001	17.32 a	16.23 bc	16.74 ab	15.66 c	15.93 bc	14.04 d	13.78 d
Ear diameter (1/4)	<0.0001	4.19 a	3.99 b	4.00 b	3.90 bc	3.99 b	3.96 bc	3.81 c
Ear diameter (1/2)	<0.0001	4.05 a	3.89 b	3.86 b	3.80 b	3.87 b	3.80 b	3.62 c
Ear diameter (3/4)	<0.0001	3.85 a	3.61 b	3.60 b	3.64 b	3.68 ab	3.59 b	3.35 c
Cob diameter	<0.0001	2.35 a	2.04 d	1.92 d	2.20 bc	2.41 a	2.20 c	2.32 ab
Pith diameter	<0.0001	0.62 a	0.50 bcd	0.47 d	0.49 cd	0.58 ab	0.56 abc	0.55 bc
Rachis diameter	<0.0001	1.05 a	0.88 c	0.87 c	0.88 c	1.01 ab	0.93 bc	0.94 bc
Number of rows	<0.0001	9.35 bc	8.61 de	8.72 de	8.25 e	9.84 ab	10.13 a	9.19 cd
Length of the grain	<0.0001	1.08 bcd	1.13 ab	1.18 a	1.12 abc	1.07 cde	1.05 de	1.02 e
Width of the grain	<0.0001	1.09 b	1.11 b	1.07 bc	1.15 a	1.03 cd	1.01 a	1.04 cd
Thickness of the grain	0.8181	0.41 a	0.41 a	0.41 a	0.40 a	0.40 a	0.40 a	0.40 a
Number of the bracts	<0.0001	9.69 a	9.78 a	10.28 a	8.55 b	8.47 b	10.16 a	8.27 b
Ear weight	<0.0001	132.97 a	121.87 a	125.39 a	102.83 b	102.49 b	93.03 bc	83.69 c
Weight of 100 grains	<0.0001	35.14 b	38.44 a	36.44 ab	36.29 ab	30.44 c	29.16 c	29.25 c
Number of ears	<0.0001	1.02 b	1.04 b	1.20 a	1.00 b	1.00 b	1.02 b	1.08 b

Campeche. In the work of Toxtle-Flores *et al.* (2023), one group exhibited a lower number of rows than the other groups collected in the community of Coronango, Puebla, Mexico.

Regarding grain width, the Conejo type showed a significant difference compared to the Rojo type; however, both types were similar to the other maize types. In comparison, Ángeles-Gaspar *et al.* (2020) reported that four accessions out of 23 collected in three localities of the municipality of Molcaxac, Puebla, exhibited greater grain width. Guillen-De la Cruz *et al.* (2014) also observed significant differences in native maize among 71 populations from the state of Tabasco. Similarly, Montes-Hernández *et al.* (2014) classified 18 populations of the Jala race and seven reference varieties into three groups based on differences in grain width in the municipality of Jala, Nayarit, Mexico.

The various studies conducted by different researchers in several locations across the State of Mexico report significant differences in morphological traits, primarily due to the climatic conditions under which native maize develops. Maize plants adapt to different climatic conditions and can either accelerate or delay their production cycle. The research carried out in Santiago Tetepec, Oaxaca, highlights the importance of the traditional milpa agricultural system as an integrated production model that combines polycropping, biodiversity conservation, and practices adapted to local conditions. In this Mixtec community, the milpa serves as both a means of subsistence and a reflection of the interaction between traditional knowledge and agrobiodiversity.

CONCLUSIONS

The milpas of Santiago Tetepec host a wide variety of useful species, with maize standing out as the main crop, accompanied by beans, squash, sesame, hibiscus, bush sweet potato, chili, and others. This system supports the coexistence of native maize varieties with both short and long growth cycles, which exhibit significant differences in phenotypic traits such as cob peduncle length, grain length and width, number of rows, and the weight of 100 grains. These differences highlight the genetic variability present in these plant genetic resources.

ACKNOWLEDGMENTS

We thank Acacio López Luengas for the translation into the Mixtec language and Leonardo Beltrán Rodríguez for his support with the statistical analysis.

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Colegio de Postgraduados

Topographic tetrazolium test in seeds of *Tillandsia ionantha* Planch.

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ABSTRACT
Objective: To develop a protocol proposal for the application of the tetrazolium test on *Tillandsia ionantha* Planch. seeds.
Design/methodology/approach: A factorial design was used, with the first factor corresponding to tetrazolium concentrations (0.1%, 0.5%, and 1%), the application or omission of preconditioning by soaking for 12 hours, and the presence or absence of physical scarification. This resulted in twelve treatments, each with three replicates of ten seeds per experimental unit. The seed coats were removed, and according to each treatment, seeds were soaked in sterile distilled water for 12 hours and subjected to physical scarification using water sandpaper. The seeds were then placed in plastic containers with lids at room temperature (23 °C) for 48 hours to assess staining percentage and topographic seed staining patterns.
Results: ANOVA (α=0.05) revealed that the only main factor capable of producing a statistically significant difference ($p \leq 0.05$) in seed staining —and therefore in viability detection— was seed preconditioning through 12-hour soaking. In contrast, neither scarification nor the tetrazolium concentrations tested had a significant effect on staining outcomes ($p > 0.05$).
Limitations on study/implications: The seeds of this species are very small, which makes them difficult to handle, and the seed coat sometimes prevents proper observation of the staining.
Findings/conclusions: To perform the test, the process will begin by removing the coma, followed by soaking the seeds for 12 hours.

Keywords: Bromeliad, germination, viability, protocol.

Citation: Vázquez-Flores, X., Valdez-Hernández, E.F., MataAlejandro, H., & Castañeda-Chávez, M. del R. (2025). Topographic tetrazolium test in seeds of *Tillandsia ionantha* Planch. *Agro Productividad*. https://doi.org/10.32854/bf7wd502

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

Received: February 04, 2025.
Accepted: May 11, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 231-238.

INTRODUCTION

To develop *ex situ* seed conservation programs aimed at mitigating the effects of illegal exploitation, it is necessary to understand the germination characteristics of wild plant species (Flores *et al.*, 2015). Germination is defined as the process that begins with seed imbibition —that is, the absorption of water— and ends with the emergence of the embryonic axis through the surrounding structures (Bewley *et al.*, 2012).

To determine whether a seed sample has the capacity to germinate, seed viability tests can be conducted. According to Schmidt (2007), these include techniques for visually

evaluating seeds and assessing at least some critical physiological processes. Among these techniques is the tetrazolium test, which is based on the topographic evaluation of a biochemical assay that identifies living embryonic structures —those responsible for enabling seed germination (Dadlani & Yadava, 2023).

The principle underlying the test is that dehydrogenases are a class of metabolic enzymes present in cells with active metabolism, which release hydrogen during reduction processes. This hydrogen has the ability to convert a pale yellow solution —containing either 2,3,5-triphenyltetrazolium chloride or 2,3,5-triphenyltetrazolium bromide— into a stable red-colored compound known as triphenyl formazan. Consequently, the production of red formazan serves as an indicator of dehydrogenase activity, which in turn reflects metabolic activity and, therefore, cellular viability.

Because tissue staining is localized, it is possible to distinguish between the living (red-stained) and dead (unstained) sections of the seed. In cases where necrotic (dead) tissue is present only on the surface of the cotyledons while the radicle stains normally, the seeds may still retain their viability. In contrast, even small areas of necrotic tissue in the essential region of the embryo generally indicate that the seed is unlikely to germinate (Schmidt, 2007).

Some of the advantages of this test, as noted by Dadlani & Yadava (2023), are that it can be considered an alternative to standard germination tests, and it helps assess both the physical and physiological condition of seeds by detecting structural alterations and damage caused by insects or post-harvest handling. Additionally, the test is simple to perform, does not require expensive equipment, and is not influenced by environmental factors. However, to obtain more accurate results in tetrazolium testing for a specific seed species, Mancipe *et al.* (2018) indicate that it may be necessary to adjust the test conditions. To ensure the success of the test, factors such as tetrazolium concentration, staining duration, temperature, and correct interpretation of the staining pattern must be considered. Therefore, the objective of this study was to propose a protocol for the application of the tetrazolium topographic viability test on Tillandsia ionantha seeds, including seed pretreatments such as soaking, physical scarification, and the use of different tetrazolium concentrations.

MATERIALS AND METHODS

The topographic evaluation using the tetrazolium test on *Tillandsia ionantha* seeds was carried out at the Plant Tissue Culture Laboratory of the Departamento de Fitotecnia at the Universidad Autónoma Chapingo. Seeds were provided by the University Center for Research and Conservation of Mexican Bromeliads (CUCIBROM, by its initials in Spanish) and were stored at an average room temperature of 20 °C in plastic containers with lids.

To facilitate seed handling, the plumose appendage (coma) of each seed was removed using fine scissors and tweezers. Twelve treatments were applied, half of which included soaking the seeds in sterile distilled water for 12 hours. Additionally, half of the seed samples underwent physical scarification using water sandpaper. Finally, ten seeds were placed in each plastic container with a lid, to which tetrazolium solution was added at concentrations of 0.1%, 0.5%, and 1%. The treatments described above are summarized in Table 1.

Table 1. Treatments applied to *Tillandsia ionantha* Planch. seeds established in the tetrazolium test assay.

Treatment	Soaking (12 hours)	Physical scarification	Tetrazolium
T1	Yes	Yes	0.1
T2	Yes	Yes	0.5
T3	Yes	Yes	1
T4	Yes	No	0.1
T5	Yes	No	0.5
T6	Yes	No	1
T7	No	Yes	0.1
T8	No	Yes	0.5
T9	No	Yes	1
T10	No	No	0.1
T11	No	No	0.5
T12	No	No	1

The variables evaluated were, on one hand, the percentage of seeds showing staining in the embryonic area and radicle, as well as the staining pattern present in the seeds. These variables were assessed 24 hours after the establishment of the bioassay. Each treatment had three replications with $n=10$ seeds per replication, and the experimental design corresponded to a factorial design. Statistical analysis was performed using Minitab® 18.1 software.

RESULTS AND DISCUSSION

The percentage of seeds showing staining in the embryo and radicle areas was evaluated. Analysis of variance ($\alpha \leq 0.05$) showed that only the soaking factor (12 h) had a statistically significant effect on seed staining ($p \leq 0.006$). The interactions Tetrazolium-Physical Scarification, Tetrazolium-Soaking, and Physical Scarification*Soaking did not show statistically significant effects ($p \geq 0.05$). Finally, the interaction of all three factors evaluated was not statistically significant ($p \geq 0.293$) for seed staining (Table 2).

The Pareto chart of standardized effects ($\alpha = 0.05$) (Figure 1) confirms the results of the analysis of variance (ANOVA), showing that the only critical factor affecting staining is the seed soaking treatment, as it exceeds the critical value (2.064).

The Main Effects Plot for seed staining (Figure 2) shows that there is a greater effect or response on staining when seeds undergo a 12-hour pre-soaking before applying tetrazolium. However, as mentioned in the ANOVA, different tetrazolium concentrations and the application or omission of physical scarification do not have a significant effect on staining. In the Main Effects Plot, it can be observed that the 0.1% tetrazolium concentration has a greater effect than the 0.5% and 1% concentrations. Additionally, it can be seen that not performing physical scarification has a greater effect on potential seed staining.

Table 2. Analysis of variance of the factors tetrazolium (TZ), physical scarification (PS), and soaking (SO). DF=degrees of freedom, SS=sum of squares, MS=mean squares.

Source	DF	SS	MS	Value F	Value p
Model	11	0.004244	0.000386	1.84	0.102
Lineal	4	0.002052	0.000513	2.45	0.073
TZ	2	0.000123	0.000061	0.29	0.748
PS	1	0.000063	0.000063	0.30	0.588
SO	1	0.001866	0.001866	8.92	0.006
Interaction 2 terms	5	0.001650	0.000330	1.58	0.205
TZ*PS	2	0.000108	0.000054	0.26	0.775
TZ*SO	2	0.001086	0.000543	2.60	0.095
PS*SO	1	0.000456	0.000456	2.18	0.153
Interaction 3 terms	2	0.000541	0.000271	1.29	0.293
TZ*PS*SO	2	0.000541	0.000271	1.29	0.293
Error	24	0.005022	0.000209		
Total	35	0.009266			

Statistically significant difference ($p \leq 0.05$).

Staining Topography

Regarding the staining topography of the seed due to the effect of tetrazolium, it was determined that the stained parts correspond to the cotyledon, hypocotyl, and root (Figure 3). Although physical scarification does not affect tetrazolium staining, it proves to be a useful technique to facilitate the observation of the internal staining pattern, since the seeds are very small and covered by the coma, making it difficult to see the staining pattern.

Staining pattern scale

Seven staining patterns were identified in the seeds, ranging from seeds with a complete absence of staining, staining in the root area, staining in the root and hypocotyl, staining in

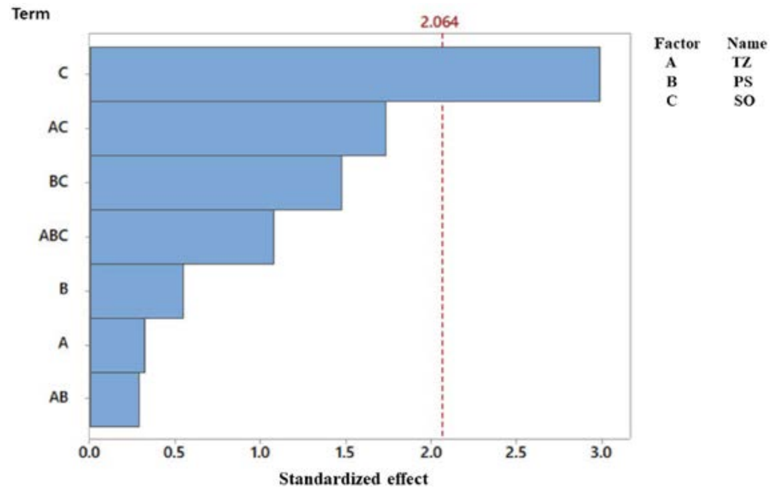

Figure 1. Pareto chart showing that only the seed soaking factor is a main effect. (Critical value=2.064, TZ=Tetrazolium, PS=Physical Scarification, SO=Soaking)

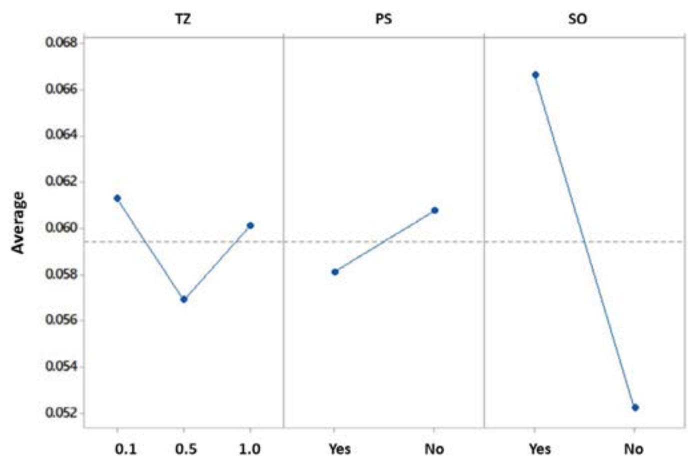

Figure 2. Main effects plot of seed staining in *Tillandsia ionantha* Planch. for the factors tetrazolium dose, physical scarification, and soaking. TZ=Tetrazolium, PS=Physical Scarification, SO=Soaking.

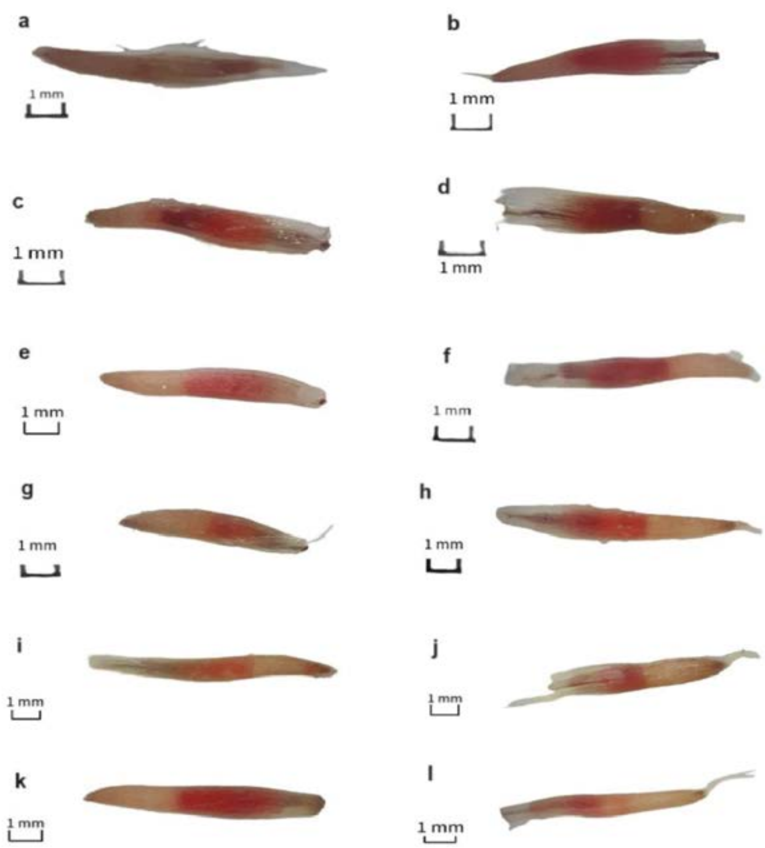

Figure 3. Staining pattern of *Tillandsia ionantha* Planch. seeds under different treatments. a) T1: Tetrazolium 0.1 N, with physical scarification and soaking; b) T2: Tetrazolium 0.5 N, with physical scarification and soaking; c) Tetrazolium 1 N, with physical scarification and soaking; d) Tetrazolium 0.1 N, without physical scarification and with soaking; e) Tetrazolium 0.5 N, without physical scarification and with soaking; f) Tetrazolium 1 N, without physical scarification and with soaking; g) Tetrazolium 0.1 N, with physical scarification and without soaking; h) Tetrazolium 0.5 N, with physical scarification and without soaking; i) Tetrazolium 1 N, with physical scarification and without soaking; j) Tetrazolium 0.1 N, without physical scarification and without soaking; k) Tetrazolium 0.5 N, without physical scarification and without soaking; l) Tetrazolium 1 N, without physical scarification and without soaking.

the cotyledon and hypocotyl, staining in the cotyledon and root, seeds with staining only at the cotyledon level, to seeds showing total staining of the embryonic area, which includes the root, hypocotyl, and cotyledon. The staining topography caused by tetrazolium indicates that seeds are viable when the staining in the embryonic area is darker. Likewise, when there is no staining or it is barely perceptible with pinkish hues and the staining is not uniform, the seed can be considered non-viable (Figure 4).

Based on the results obtained and in contrast to what was reported by Calderón (2019), who indicated that in *Espeletia* and *P. ledifolia*, the most important factor for staining was the concentration of tetrazolium —since when 1% tetrazolium was applied for 24 h, viability reached 76% and 80%, respectively— in the case of *T. ionantha* seeds, it was determined that the main factor affecting staining was the prior soaking of the seeds for 12 hours, rather than the tetrazolium concentration.

Regarding the anatomical structure of the seeds, Calderón (2019) indicates that in the cases of *H. goudotiana*, *C. buxifolium*, and *X. spiculifera*, which are characterized by having either a thick seed coat or abundant endosperm, the test must be applied directly to the embryos using tetrazolium at a 1% concentration. Additionally, the author mentions that for *G. anastomosans*, *P. prostrata*, and *V. floribundum*, which have a hard seed coat, it is necessary to cut the seed coat and increase the tetrazolium concentration to 1.5% in order to obtain viability values of up to 40%. However, *T. ionantha* seeds do not exhibit these anatomical characteristics, which facilitates the direct application of the tetrazolium test without the need to cut or physically scarify the seed coat to allow the solution to permeate the seed tissue.

In the study conducted by Elizalde (2014) with *H. perotensis*, it was determined that, similar to the seeds of *T. ionantha*, tetrazolium concentrations of 0.2% and 1% did not show statistically significant differences ($p>0.05$) in embryo staining, allowing for the identification and assessment of seed viability. In viability tests using tetrazolium on

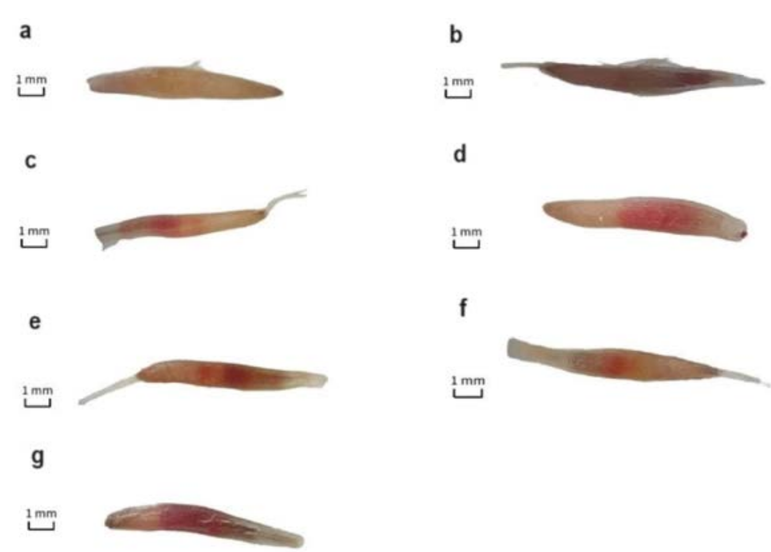

Figure 4. Staining patterns obtained from the different treatments, indicating the variety of areas within the seed that are capable of being stained by tetrazolium in *Tillandsia ionantha* Planch. seeds.

Hordeum vulgare L., authors such as Grzybowski *et al.* (2012) determined the usefulness of pre-conditioning seeds by directly immersing them in water. Unlike *T. ionantha* seeds, in barley it is sufficient to apply pre-conditioning for only four hours. They also concluded that effective tetrazolium concentrations for staining barley seeds are 0.1% or 0.5%, which aligns with the results obtained for *T. ionantha*, where seed staining also occurred at those concentrations. Mollo (2011) reported that seeds of *Hordeum muticum* and *Bromus catharticus* did not show sensitivity to the evaluated concentrations (0.1%, 0.5%, and 1%), and viability was observed at any of these doses, which is consistent with the behavior observed in *T. ionantha*.

Finally, it is important to highlight, as noted by Elizalde *et al.* (2017), that the application of the tetrazolium test depends on the evaluator's experience in detecting seed viability. In addition, it is necessary to develop specific protocols to assess viability in bromeliad seeds, which are poorly documented —partly, according to the authors, due to the notably small size of the seeds in this family.

CONCLUSIONS

The proposed protocol for the tetrazolium viability test in *T. ionantha* seeds should begin with the removal of the coma and soaking the seeds in sterile distilled water for 12 hours. Tetrazolium should be applied at a concentration of 0.1%. Physical scarification may be optionally included as a preconditioning step, solely to facilitate the visualization of staining patterns due to the small size of the seeds.

ACKNOWLEDGMENTS

We thank the Consejo Nacional de Humanidades Ciencia y Tecnología (CONAHCyT) for the scholarship granted for the completion of the master's studies (first author) and the postdoctoral stay (corresponding author), as well as the Plant Tissue Culture Laboratory at Universidad Autónoma Chapingo, where the experiment was conducted.

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Colegio de Postgraduados

Endogenous increase of proline in leaves of quelite (*Amaranthus hybridus* L.) through sun drying

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ABSTRACT

Objective: To evaluate the amount of proline in leaflets of quelite (*Amaranthus hybridus* L.) dried under sunlight.

Design/methodology/approach: Quelite plants were collected in Salvatierra, Guanajuato, Mexico, defoliated, and the leaves were exposed to two drying conditions: one group directly under the sun and another in the shade, for five days. The samples were ground, and 24 g of each were used to prepare aqueous extracts using a homemade percolator (T-fal® Heliora model). Proline content was determined in each extract.

Results: Statistically significant differences were found through ANOVA and Tukey's test, showing that sun drying was a better treatment compared to shade drying.

Limitations of the study/implications: The quelite plants used were exclusively collected from uncultivated sites; it is recommended to conduct comparisons in other growth environments.

Findings/conclusions: Sun drying is an effective technique to achieve an endogenous increase of proline in quelite plants, enhancing their quality for use as a nutritional or nutraceutical product, either in flour or extract form.

Keywords: Proline, *Amaranthus hybridus*, extracts, secondary metabolites, antioxidants.

Citation: Ortega-García, N., Pérez-Olvera, Ma. A., Raya-Pérez, J.C., & Aguirre-Mancilla, C.L. (2025). Endogenous increase of proline in leaves of quelite (*Amaranthus hybridus* l.) through sun drying. *Agro Productividad*. https://doi.org/10.32854/hgkhcm76

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

Received: February 15, 2025.
Accepted: May 14, 2025.
Published on-line: August 5, 2025.

Agro Productividad, 18(6). June. 2025. pp: 239-248.

INTRODUCTION

Plants contain phytochemicals, mainly categorized as secondary metabolites, including terpenes, cyanogenic compounds, glucosinolates, alkaloids, phenolic acids, and glycosides, among others. Most of these compounds possess antioxidant capacity, which has led to their inclusion in daily diets for health care purposes (Gasaly *et al.*, 2020; Santiago *et al.*, 2019; Slabbert & Krüger, 2014; Chagas *et al.*, 2022; Muscolo *et al.*, 2024; Buchanan *et al.*, 2015).

In Mexican cuisine and traditional medicine, the use and utilization of plant species are considered essential for dishes that hold great recognition in local gastronomy, as well as being highly valued by traditional medicine to produce remedies addressing health problems in rural populations (Muscolo *et al.*, 2024; Hernández *et al.*, 2022; Mateos *et al.*, 2020; Santiago *et al.*, 2019).

Quelites are plants that encompass more than 500 species, widely distributed in Mexico. Many of these species are considered undesirable in both agricultural and urban contexts, being categorized as weeds (Bang *et al.*, 2021; Mateos *et al.*, 2020; Santiago *et al.*, 2019; Linares & Bye, 2015; Rzedowski & Calderón, 2004).

In the municipality of Salvatierra, Guanajuato, Mexico, *Amaranthus hybridus* L. is known as quelite (Rzedowski & Calderón, 2004). This plant is considered highly nutritious, with its leaves being a good source of essential amino acids, carbohydrates, and mineral elements (Nwaogu *et al.*, 2006). Its importance for health is increasingly recognized as an alternative for treating diseases affecting the population, thanks to the abundance of secondary metabolites it contains (Bang *et al.*, 2021; Santiago *et al.*, 2019).

This plant serves both as food and medicine for humans and livestock and is often exposed to various types of stress, which promotes the production of phenolic compounds, polysaccharides, and both essential and non-essential amino acids, including proline. This underlines its importance as a nutraceutical food source (González *et al.*, 2019; Santiago *et al.*, 2019; Kumar & Goel, 2019; Zou *et al.*, 2019; Bielach *et al.*, 2017; Slabbert & Krüger, 2014).

Proline (Pro) is an amino acid classified as a primary metabolite and has the ability to alleviate the effects of oxidative stress. It is involved in the production of secondary metabolites and acts as an inducer for enzymatic and protein synthesis that counteracts the excess of reactive oxygen species (ROS). Moreover, proline remains the main indicator of the level of stress experienced by an organism (Perassolo *et al.*, 2013; Slabbert & Krüger, 2014; Hosseinifard *et al.*, 2022).

Recent studies have documented that the exogenous application of substances helps to complement the endogenous production of proline. This has allowed more detailed research on the use of proline in efficiently eliminating excess ROS at the cellular level (Pisoschi *et al.*, 2020; Ejiofor *et al.*, 2022; Chaudhary *et al.*, 2023; Muscolo *et al.*, 2024).

Other substances supplied exogenously that have recently gained increased use are secondary metabolites, especially those that prevent cellular damage caused by oxidative stress. These compounds are considered safer and less toxic for humans, as they are naturally found in a wide variety of plants (Gonçalves & De Souza, 2022; Lourenço *et al.*, 2019). Some experts argue that when a product becomes commonly used by people, sooner or later there will be a need to meet demand, generating the necessity to implement biotechnological processes or to seek strategies that enhance its productivity and quality (Narayani & Srivastava, 2017).

Due to the high adaptability and phytochemical content of the quelite plant, some of its chemical components can be utilized as inputs within the food processing and preservation sectors, the pharmaceutical industry, and the manufacture of beauty products, without neglecting the fresh consumption currently practiced by the population (Chaudhary *et al.*, 2023; Gielecińska *et al.*, 2023). The present study aimed to evaluate the amount of proline in leaflets of quelite (*Amaranthus hybridus* L.) dried under sunlight as a strategy to increase endogenous proline in the plant, thereby leveraging the benefits this amino acid offers by enhancing its nutritional and nutraceutical quality. This, in turn, brings benefits to society and positions the plant as a valuable input for the food industry and processing.

MATERIALS AND METHODS

Quelite plants were collected from lands adjacent to cultivated fields in the municipality of Salvatierra, Guanajuato, Mexico, specifically from the ejido San Nicolás de los Agustinos, at coordinates 20.252158° N latitude and −100.961503° W longitude. The plants were uprooted during the fruiting stage from three randomly selected sites and immediately taken to the laboratory for manual defoliation. Subsequently, the leaves were spread out on paper. Half of the sample was left in the shade, while the other half was exposed to sunlight for 5 days, uncovered during the nights (Figure 1).

The sample preparation was carried out using the dried leaflets, which were finely ground using a domestic coffee grinder. The ground material was then sieved to standardize particle size. Twenty-four grams of each sample were weighed using an Escali Primo electronic balance, model P115M, with a capacity of 0 to 5000 g.

The method used for proline determination was that of Bates (1973). A crude extract of each sample was prepared using a homemade percolator, a ®T-fal Heliora model. A total of 1.8 L of water was used to obtain 1.75 L of extract. For the extraction process, the quelite flour sample was placed on filter paper inside the percolator compartment. Then, 1.8 L of purified water was added, the device was turned on, and left running until the water was completely evaporated from the reservoir. The device was turned off once dripping into the collection container ceased completely.

Figure 1. Different types of drying of quelite leaflets (Left: leaflets exposed to shade drying; Right: leaflets dried directly under the sun).

To determine the amount of proline (Pro), a calibration curve was obtained using L-proline at 98.5% purity (SAFC TM) with a Bio-Rad xMark™ spectrophotometer at a wavelength of 520 nm. Subsequently, the Pro content of each quelite extract sample was quantified. For this, 600 μL of the crude extract was placed into a 2 mL Eppendorf tube, followed by the addition of 600 μL of sulfosalicylic acid. The mixture was shaken, then 400 μL of acid ninhydrin was added. After further mixing, 400 μL of glacial acetic acid was incorporated. The mixture was heated in a water bath for one hour, and the reaction was stopped by cooling the tubes on ice for 5 minutes. Then, 600 μL of toluene was added, and the mixture was vortexed. The supernatant was taken for analysis in the previously calibrated plate spectrophotometer at 520 nm. Two measurements were made for each extract, each with three repetitions (Figure 2).

The data obtained were analyzed using the statistical software Infostat version 2020. Initially, a normality check was performed using the Q-Q plot test. Once normality was confirmed, an ANOVA was conducted followed by Tukey's multiple comparison test with a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Absorbance data were converted to mg/L using the equation obtained from the previous calibration curve (Table 1). Here, MS1 and MS2 correspond to the sun-drying and shade-drying methods, respectively.

The proline content values of the quelite extracts were subjected to statistical tests (Infostat version 2020) to verify normality using the Q-Q plot test (Figure 3), with this information confirmed, an ANOVA was performed (Table 2) followed by Tukey's multiple comparison test with $p \leq 0.05$ (Figure 4). Statistically significant differences were found between the sun-dried quelite sample (MS1) and the shade-dried sample (MS2).

The sun-drying process of the quelite leaflets (MS1) is statistically considered the best treatment in terms of increasing Pro content, compared to shade drying. This is supported by various research studies involving this plant. Slabbert & Krüger (2014) clearly show

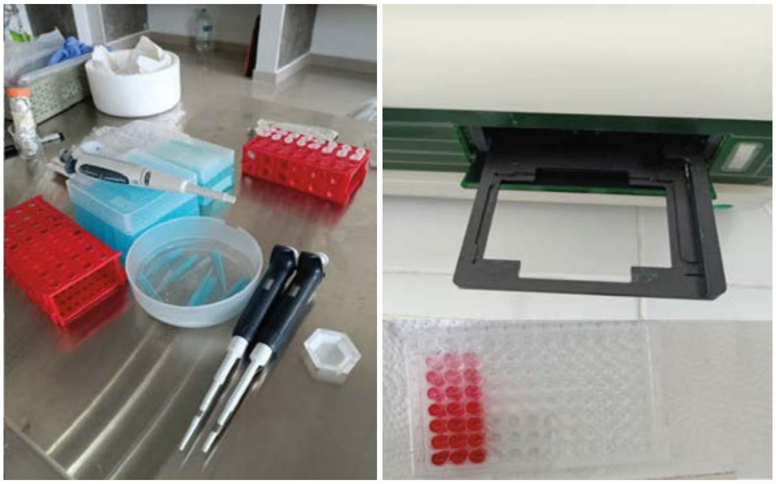


Figure 2. Equipment and materials used for the quantification of proline in quelite extracts.

Table 1. Absorbance data and proline content in mg/L of quelite extracts.

Treatment	Absorbance	Proline (mg/L)
MS1	3.548	33.792
MS1	3.643	34.758
MS1	3.714	35.479
MS1	3.617	34.493
MS1	3.610	34.422
MS1	3.480	33.101
mean	3.602	34.34083
MS2	3.324	31.516
MS2	3.377	32.054
MS2	3.328	31.556
MS2	3.270	30.967
MS2	3.397	32.258
MS2	3.303	31.302
mean	3.3331	31.608

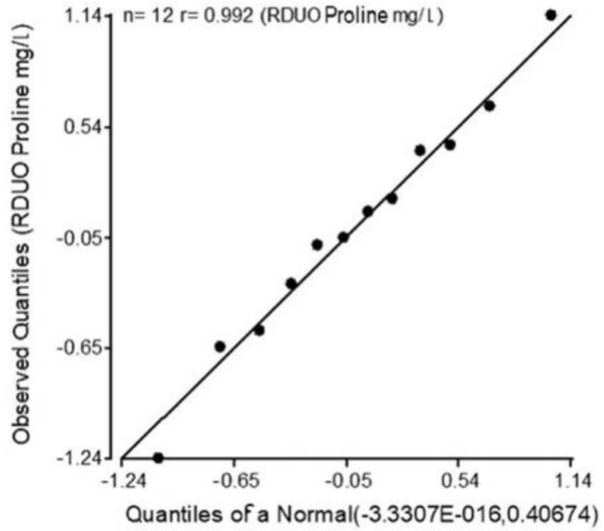
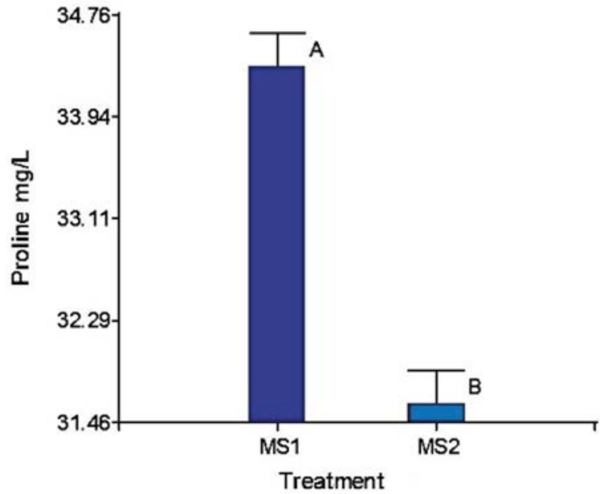


Figure 3. Normality analysis using the Q-Q plot method. (Source: Own data using Infostat software version 2020).

Table 2. Analysis of variance (Type III SS) of proline values obtained from quelite extracts considering the type of plant drying.

SV	SS	Gl	MS	F	p-value
Model	22.39	1	22.39	50.05	<0.0001
Treatment	22.39	1	22.39	50.05	<0.0001
Error	4.47	10	0.45		
Total	26.87	11			

N=12; R^2=0.83; Adjusted R^2=0.82; CV=2.03; p≤0.05.



Means sharing a common letter are not significantly different ($p \leq 0.05$).

Figura 4. Tukey's multiple comparison test for proline content in mg/L in quelite extract considering the type of plant drying (Source: Own data using Infostat 2020 software).

that when plants are exposed to a period of induced water stress, they produce a higher amount of Pro. Vwioko *et al.* (2018) found that when quelite plants were kept inside two chambers —one with NO_2 and another with SO_2— for one hour daily over three weeks, the amount of proline increased compared to the control, by more than 105% under SO_2 and by 74.8% under NO_2. Asadollahzadeh *et al.* (2023) determined over two consecutive years that in quelite plants subjected to different levels of water stress, the Pro content increased as the degree of stress increased (with a decrease in available water by 25%, 50%, 75%, and 87.5%, respectively).

The results show that quelite plants subjected to stress caused by cutting the plant, defoliation, and direct sun exposure significantly increased the amount of endogenous Pro, opening new possibilities for its use as a nutritional supplement for other organisms. This is supported by Dawood *et al.* (2014), who found that applications of exogenous Pro increase the level of endogenous Pro, enhancing antioxidant activity and alleviating the toxic effects of stress caused by seawater use in faba bean (*Vicia faba*) cultivation. It has also been demonstrated that adding Pro exogenously to the human diet increases stress tolerance and helps maintain redox homeostasis, allowing proper cell function in both cancers and conditions associated with inflammation and fibrosis (Vettore *et al.*, 2021).

Within the results of the present research, Pro reached average values of 34.34 mg/L for the sun-drying process and 31.61 mg/L (in both cases, the values transformed to the Proline content per gram of flour used for the extract, considering the amount of solvent, correspond to 0.002504 g/g of flour) for shade drying. These data are close to those found by Akubugwo *et al.* (2007) in quelites collected from cultivated fields in Nigeria, which were also sun-dried (Table 3).

Perassolo *et al.* (2013) mentioned that Pro promotes the production of secondary metabolites. Valdés *et al.* (2012) conducted experiments with oregano plants (*Lippia graveolens*) grown under four different conditions (commercial nutrient solution, water,

Table 3. Components of the quelite plant reported in research studies. (considering 100 g of dry weight sample).

Parameter evaluated	Akubugwo *et al*. (2007)	Asaolu *et al*. (2012)
Calorific value (kcal $100g^{-1}$)	268.92	
Moisture Content (%)	84.48	
Ash Content (%)	13.80	15.55
Crude Protein (%)	17.92	49.02
Crude Fibre (%)	8.61	8.05
Available Carbohydrate (%)	52.18	3.36
Crude Lipid (%)	4.65	14.02
Na (mg)	7.43	88.00
K (mg)	54.20	168.96
Ca (mg)	44.15	70.40
Mg (mg)	231.22	249.92
Fe (mg)	13.58	39.04
Zn (mg)	3.80	21.68
P (mg)	34.91	32.63
Mn (mg)		10.06
Ni (mg)		12.24
Cu (mg)		0.08
Ascorbic acid (mg)	25.40	
Proline (g of protein*)	3.43	
Alkaloid (mg)	3.54	
Flavonoid (mg)	0.83	
Saponin (mg)	1.68	
Tannins (mg)	0.49	
Phenols (mg)	0.35	

* The values transformed to the proline content per gram of sample used, considering the protein content, are 0.006147 g/g of sample.

moderate stress, and intermediate stress) and exposed to varying concentrations of NaCl, Fe^{2+}, and Cu^{2+}. They concluded that plants subjected to more stressful conditions caused by NaCl and these chemical ions showed greater accumulation of Proline in the roots and increased production of secondary metabolites, which was reflected in an enhanced production of essential oils (thymol and carvacrol). These studies support the idea that plants with higher concentrations of secondary metabolites possess greater antioxidant capacity. In the case of quelite, this is no exception, as several works have evaluated its antioxidant properties using the plant in extractions with different types of solvents, resulting in various types of extracts (ethanolic, ethyl acetate, methanolic, hydroacetonics, hexanoic, dichloromethane, and aqueous) (Ihezie *et al*., 2023; Bang *et al*., 2021; Ndukwe *et al*., 2020; Nana *et al*., 2012; Maiyo *et al*., 2009). These studies have focused on analyzing parameters that allow the detection of this vegetable's ability to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS (2,2'-azino-bis(3-

ethylbenzothiazoline-6-sulfonic acid) ammonium salt), total flavonoid content (TFC), or total polyphenol content (TPC) (Vázquez *et al.*, 2022; Bang *et al.*, 2021; Guija *et al.*, 2015), as well as reducing capacity, hydroxyl radical inhibition, and phosphomolybdate scavenging (Ndukwe *et al.*, 2020).

In 2021, Bang *et al.* demonstrated that this plant has an antioxidant capacity to scavenge the free radical DPPH that is equal to or greater than that of amaranth, commonly known as "alegría" (*Amaranthus hypochondriacus*). Additionally, it is known that hydroacetonic, methanolic, and aqueous extracts of quelite, besides their antioxidant power, exhibit high antimicrobial activity attributable to terpenoids, alkaloids, and saponins, effective against certain pathogens that affect humans. They also inhibit enzymes such as xanthine oxidase, whose excessive activity can cause liver and kidney damage (Bang *et al.*, 2021; Nana *et al.*, 2012). Similarly, it has been found that this plant contains a large amount of squalene, a compound with anticancer and antitumor properties, a discovery that positions it as an alternative in traditional medicine and the pharmaceutical industry with new nutraceutical benefits (Tang, 2020). All of the above reveals a broad potential for the quelite plant as a high-quality product and raw material, both for society and industry, since it was confirmed that the internal proline content can be increased by exposing the plant to greater stress while it is still alive. Finally, it is necessary to mention that this research raises questions to be explored in the near future, especially in fields of knowledge that may enhance the metabolic activity of this plant, which has been mistakenly labeled as a "weed."

CONCLUSIONS

Drying quelite plant material directly under the sun is recommended to increase the proline content in flour or extract, which can be used for nutraceutical products. In addition to proline, quelite plant material provides a large amount of antioxidants included in secondary metabolites, proteins, minerals, and ascorbic acid, which can also increase due to the content of this amino acid. The use of this plant, either directly as food or as a raw material for the food industry, presents great nutraceutical potential. It can be used fresh, dehydrated as flour, or as an extract.

ACKNOWLEDGMENTS

We thank SECIHTI for the support provided through the National Scholarship program. We also thank the Roque Technological Institute for the facilities to carry out the laboratory work, as well as Dr. Adriana Basilio for her support in adapting the proline determination technique.

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