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AGRO PRODUCTIVIDAD

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Sodium Chloride in the Germination and Initial Growth of Three Huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) Cultivars

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ABSTRACT

Objective: To determine the effect of sodium chloride (NaCl) and cultivar on germination and initial growth of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*).

Design/methodology/approach: A 3×5 factorial experiment in a completely randomized design was conducted. Three huauzontle cultivars (Mexico City, Puebla, and Tlaxcala) and five doses of NaCl (0, 75, 150, 225, and 300 mM) were evaluated. Each treatment had five replicates. The germination percentage was evaluated 24 h after sowing, plant height of the seedlings for 11 d, and the weights of fresh and dry biomass were determined at 11 d after germination. The data were analyzed using ANOVA, Tukey's test ($p \leq 0.05$), and a regression model was generated for the variable seedling height as a function of the time of exposure to NaCl.

Results: Seed germination began 24 h after sowing in the three cultivars at all NaCl concentrations evaluated. The germination percentage was reduced by 17.6% at 300 mM compared to the control without salinity. Seedling height was higher in the Mexico City and Tlaxcala cultivars at 75 and 150 mM compared to the control; in these treatments, growth was only inhibited with 300 mM NaCl. On the contrary, the Puebla cultivar showed a reduction in growth starting at 225 mM NaCl. The weights of fresh and dry biomass were only affected by the cultivar study factor, where Mexico City followed by Puebla had the highest means; both higher than Tlaxcala.

Limitations of the study/implications: This study only considers the germination and initial growth phases in the three huauzontle cultivars. Therefore, it is necessary to evaluate the effects of salinity in later phenological stages.

Findings/conclusions: The responses observed in huauzontle in germination and initial growth depend on the NaCl concentration and the cultivar. The germination percentage decreased only with the 300 mM NaCl dose with respect to the control. NaCl at doses of 75 and 150 mM NaCl promoted growth in the Mexico City and Tlaxcala cultivars. The Mexico City cultivar had the highest fresh and dry biomass weights, followed by Puebla and Tlaxcala.

Keywords: Amaranthaceae, Chenopodiaceae, underutilized crops, abiotic stress, salinity.

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INTRODUCTION

Huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) is a pseudocereal native to Mexico belonging to the Amaranthaceae family, Chenopodiaceae subfamily, which has been

consumed since pre-Hispanic times until today as a leaf vegetable [1,2]. It contains dietary fiber, proteins, essential amino acids, fatty acids, minerals, and phenolic compounds [3-5]. In Mexico, it is grown in Puebla, Tlaxcala, Guerrero, and Morelos, with average yields of 2 to 11 Mg ha^{-1} [6].

Huauzontle is an annual, dicotyledonous plant, adapted to adverse climatic conditions [7]. It is found in arid and semi-arid areas of Mexico; it has a taproot, stems with sympodial branches, alternate leaves with serrated edges, and seeds of various colors, from orange to red [8]. Its inflorescences are green and are consumed in their mature state [2].

Like many cultivated plants, huauzontle is exposed to various stressors during its growth and development [9]. Soil salinity stands out as one of these factors, characterized by the presence of dissolved salts such as NaCl, Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCl, and Na_2CO_3 [10,11].

The dominant ions in saline environments are Na^+ and Cl^-, and their high concentrations are toxic to seeds [12], so salinity represents a risk for plants, mainly in germination and in the seedling phase because it causes three types of stress: oxidative, ionic, and osmotic [13]. Osmotic stress affects germination, reduces the water potential of the medium, and influences water absorption, the development of embryonic organs, and the vigor of seedlings [14,15]. The effect of salinity on germination and emergence has been studied in cereals and pseudocereals. In quinoa (*Chenopodium quinoa* Willd.), germination was reduced to 50% with 50 mM Na_2CO_3 [16]. In sorghum [*Sorghum bicolor* (L.) Moench], the dose of 200 mM NaCl decreased germination to 57% [17].

The emergence of seedlings in a saline environment provides information to investigate the degree of sensitivity of seeds to this type of stress [18]. In the specific case of huauzontle, this aspect has not been studied and its level of tolerance to salinity in germination and in the initial growth phase is unknown. Therefore, the objective was to study the effect of the application of NaCl on the germination and initial growth of three cultivars of huauzontle.

MATERIALS AND METHODS

Plant material

Three cultivars of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) were collected from (i) Mexico City, in the municipality of Iztapalapa; (ii) Puebla, municipality of Texmelucan; and (iii) Tlaxcala, municipality of Panotla.

Treatment design and experimental design

Fifteen treatments were evaluated from a 3×5 factorial experiment. The first factor was the huauzontle cultivar with three levels: Mexico City, Puebla, and Tlaxcala. The second factor was the NaCl concentration: 0, 75, 150, 225, and 300 mM. The experimental units consisted of hinge-type plastic trays with 30 seeds each using peat as a substrate. Each treatment had five replicates, which were distributed in a completely randomized design.

Application of treatments

The seeds of each cultivar were immersed for 5 min in each of the NaCl concentrations. Thirty seeds were then sown in a tray with peat (experimental unit described above), a substrate that was previously saturated with 25 mL of each NaCl solution. In the following days of evaluation, 15 mL irrigations were applied with the corresponding NaCl solution. The minimum and maximum temperatures during the test ranged between 15 and 27 °C, with a relative humidity of 62.7%.

Variables evaluated

To obtain the germination percentage, the germinated seeds were counted (2 mm radicle emergence). The height of the seedling was measured with a graduated ruler, from the moment of germination until they had two true leaves (11 d). The fresh biomass of each seedling was determined on an analytical scale, and the dry biomass was obtained after drying the sample in a forced air circulation oven for 24 h at 70 °C.

Statistical analysis of the data

The results of germination, seedling height, and fresh and dry seedling biomass were analyzed for variance and the means were compared with the Tukey test ($p \leq 0.05$), using the statistical program Statistical Analysis Systems (SAS) 9.1®. In addition, regression models were performed using the method proposed by Volke [19], starting from an initial model based on the graphical relationship between the response variables and the study factors that showed a response trend until obtaining a model with the lowest mean square error (MSE).

RESULTADOS Y DISCUSIÓN

Germination

The germination of the seeds of the three evaluated cultivars of huauzontle began 24 h after sowing in all NaCl concentrations; although, in general, the germination time has a negative relationship with the level of salinity present in the medium [20,21]. This time is greater than that reported in quinoa seeds cv. Sajama, where germination was recorded at 6 h with 250 mM NaCl, the maximum germination (80%) was observed at 14 h [22]. On the contrary, in amaranth seeds (*Amaranthus caudatus* L.) Rojo genotype, germination began at 72, 96, 100, and 72 h with treatments with 0, 50, 75, and 150 mM NaCl; while in the Verde genotype, germination was recorded after 150, 168, 170, and 180 h for the same NaCl doses tested [23].

Figure 1 shows the appearance of seed germination under the different NaCl concentrations. Only the NaCl dose had significant effects on this variable; that is, there was no effect of cultivar or cultivar × NaCl interaction. Although there is no cultivar effect, it is pertinent to indicate that the Mexico City and Puebla cultivars reached 100% germination in the control and this decreased as salt stress increased, with values of 85.3 and 79.3% respectively with 300 mM NaCl. The Tlaxcala cultivar presented 100% germination with 75 mM NaCl and 93.3% in the control, while with 300 mM NaCl it recorded 76% germination.

The germination percentage decreased with increasing salinity; with 300 mM NaCl it was reduced by 17.6% with respect to the control (Figure 2). The ability of plants to survive germination and emergence is recognized as the main indicator of their tolerance

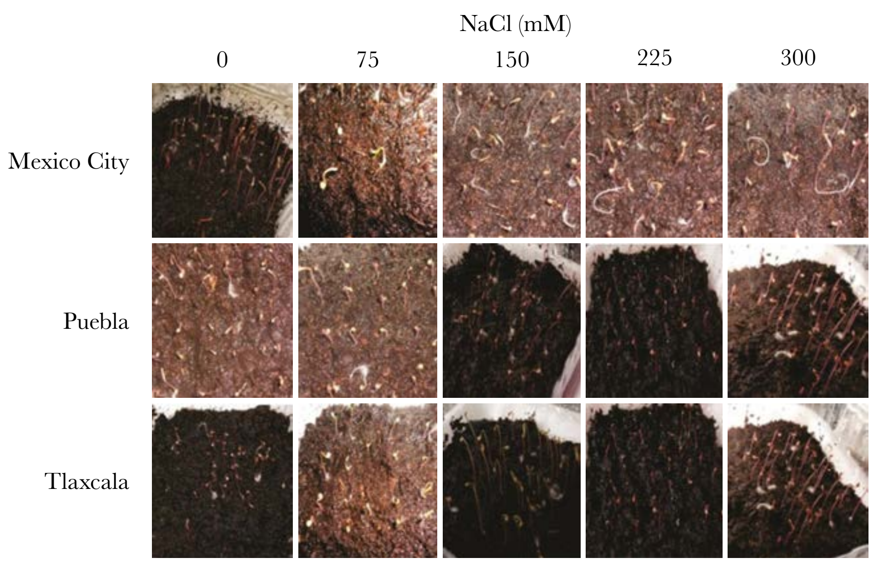

Figure 1. Germination in three cultivars of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) depending on treatment with different concentrations of sodium chloride (NaCl).

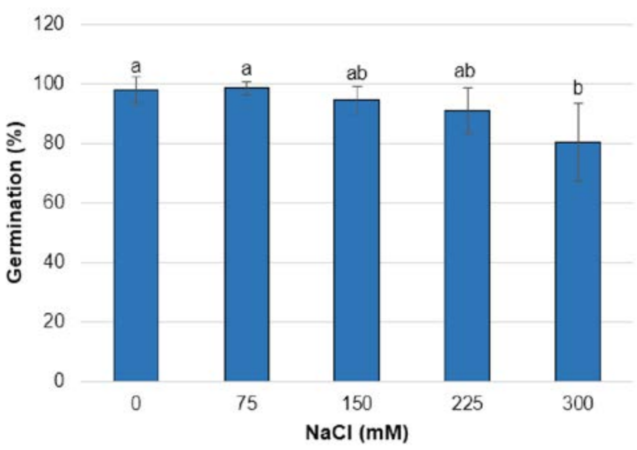

Figure 2. Germination percentage of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) seeds treated with different concentrations of sodium chloride (NaCl). Means ± SD with different letters indicate statistical differences (Tukey, $p \leq 0.05$).

to salinity [24,25]. Salt stress affects the water absorption capacity of the seed and hinders hydration and germination, which causes physiological stress, similar to that of drought [26,27]. Furthermore, if the amount of salt exceeds the tolerance level of the species, the germination process can be completely inhibited [28].

Seedling height

The presence of NaCl in the soil or substrate negatively affects plant growth. The magnitude of this effect depends on several factors, such as the species, stage of development, and NaCl concentration [29]. Likewise, growth delay is an adaptive mechanism for survival, which allows plants to combat salinity stress and depends on time [30]. In the evaluations of seedling height carried out every 24 h between 2 and 11 d, statistical significance was observed both for the main effects of the study factors and for the interaction effects. Figure 3 shows the results of seedling height at 10 d. It can be observed that, without salinity, the Puebla cultivar had the greatest height, followed by Mexico City and Tlaxcala. On the other hand, it is observed that the cultivars have differential responses to the salinity gradient. In the Mexico City and Tlaxcala cultivars, seedling height was increased with doses of 75 and 150 mM NaCl, compared to 0 mM NaCl. In contrast, the Puebla cultivar showed no differences in height in the range of 0 to 150 mM NaCl; doses equal to or greater than 225 mM NaCl inhibited growth.

To explain the behavior of the seedling height of the three cultivars as a function of NaCl concentration and time, the following regression model was used:

$$At = 0.78 + 0.248H + 1.107T - 0.05668T^2$$

PrF: 0.0001; CV: 19.69 %; R^2: 0.854

Where: *At*: seedling height in cm, $H=0$ Mexico City and Tlaxcala; $H=1$ Puebla; $T=$time (evaluation days).

This model indicates a negative effect of NaCl on the three cultivars (Figure 4); while, due to time, an increasing behavior was observed in the first six days of evaluation; subsequently, the growth rate decreased and stabilized at 10 d after germination. As previously indicated, it was observed that the growth of huauzontle seedlings during 11 d is different between cultivars; Mexico City and Tlaxcala behaved similarly (Figures 3 and 4a) compared to Puebla, which had greater growth inhibition due to salinity (Figures 3 and 4b).

The growth inhibition induced by NaCl is a commonly observed effect in several crops such as *Amaranthus caudatus* (L.), which showed a decrease in growth from 54 to 27% with 100 mM NaCl [23]; in sorghum, initial growth was affected with 200 mM NaCl [31].

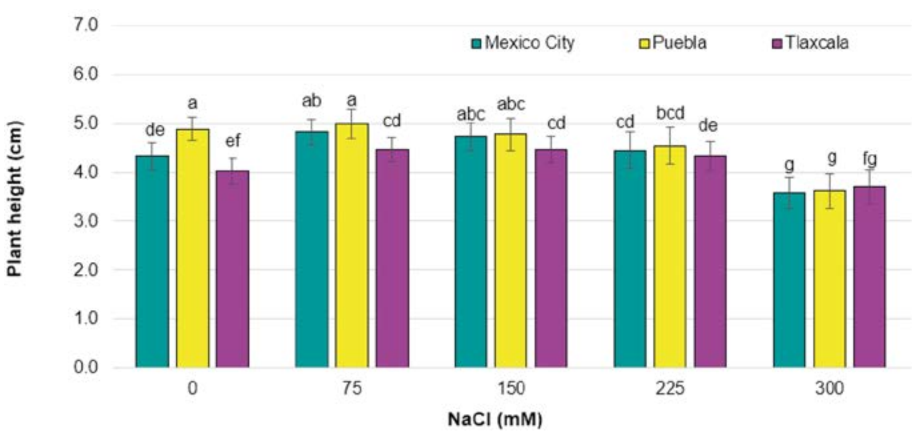

Figure 3. Seedling height of three cultivars of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*) treated with different concentrations of sodium chloride (NaCl). Means ± SD with different letters indicate statistical differences (Tukey, $p\leq 0.05$).

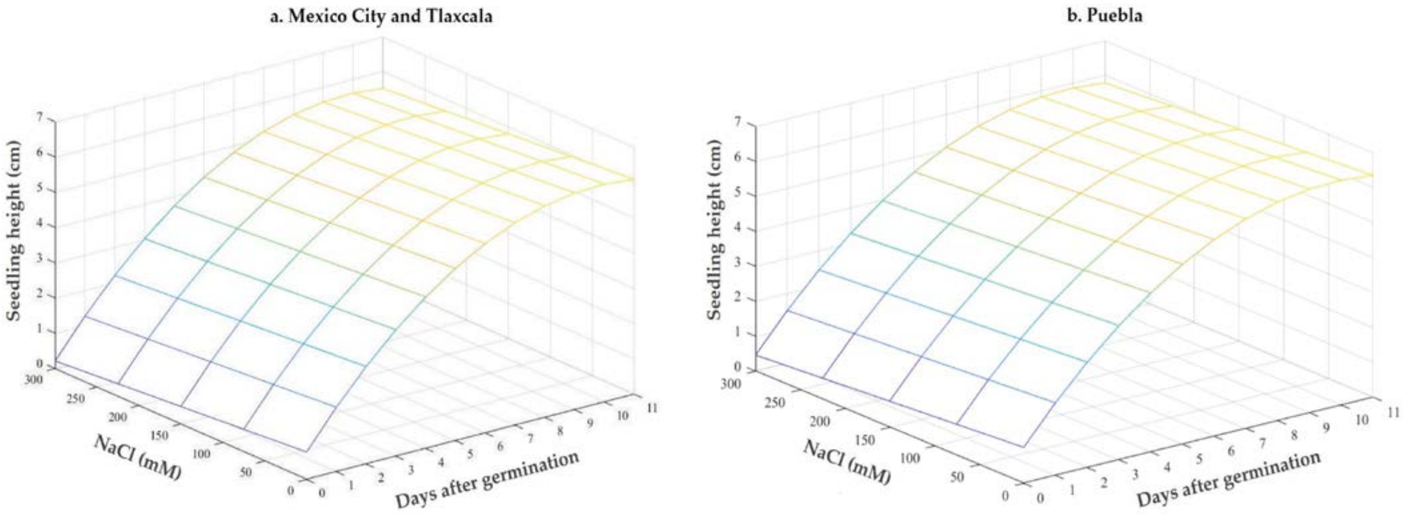

Figure 4. Effect of sodium chloride (NaCl) over time on seedling height in the cultivars Mexico City and Tlaxcala (a), and Puebla (b) of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*).

Fresh and dry seedling biomass. The weights of fresh and dry seedling biomass were only influenced by the cultivar study factor (Figure 5). The cultivars Mexico City and Puebla exceeded Tlaxcala by 10.9 and 9.7% in fresh seedling biomass (Figure 5a); as well as by 21 and 18.6% in dry seedling biomass (Figure 5b).

On the other hand, although there was no effect of the NaCl factor, it is pertinent to mention that the values of fresh biomass ranged from 0.854 to 0.795 g seedling^{-1} and corresponded to the concentrations of 75 and 300 mM NaCl, respectively. Meanwhile, with the doses of 75 and 0 mM NaCl, the highest and lowest dry biomass weights were obtained (0.032 and 0.027 g seedling^{-1}), respectively.

Regarding the interactions of the study factors, although they did not cause significant effects either, it is appropriate to indicate that the highest fresh biomass averages in the Mexico City cultivar were with 75 mM NaCl (0.934 g seedling^{-1}), in Puebla with 150

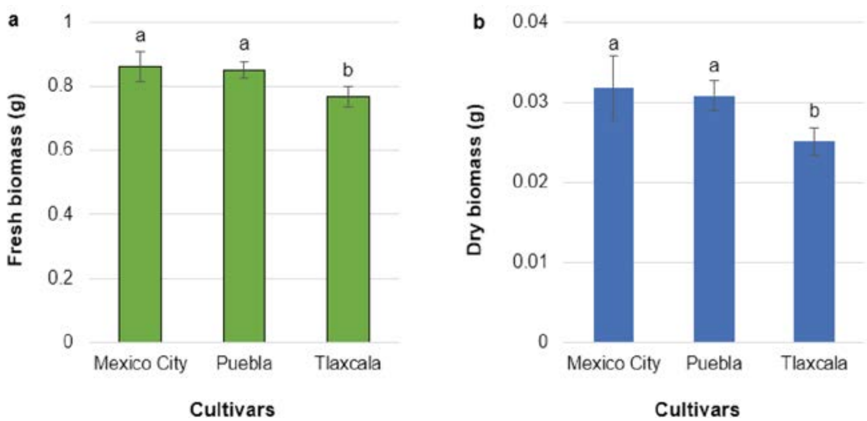

Figure 5. Fresh (a) and dry (b) biomass of seedlings of three genotypes of huauzontle (*Chenopodium berlandieri* subsp. *nuttalliae*). Means ± SD with a different letter in each subfigure indicate statistical differences (Tukey, $p \leq 0.05$).

mM NaCl (0.878 g seedling^{-1}), and in Tlaxcala with 225 mM NaCl (0.814 g seedling^{-1}). Likewise, the lowest means in the Mexico City cultivar were with 300 mM NaCl (0.792 g seedling^{-1}), in Puebla and in Tlaxcala in the treatment without salinity (0.802 and 0.744 g seedling^{-1}, respectively). On the other hand, the highest dry biomass in the Mexico City cultivar was with 75 mM NaCl (0.0396 g seedling^{-1}); while, in Puebla and Tlaxcala it was obtained with 300 mM with values of 0.0327 and 0.0284 g seedling^{-1}, respectively. Similarly, the Mexico City, Puebla, and Tlaxcala cultivars presented the lowest seedling weights with 300, 150, and 0 mM NaCl, respectively, with means of 0.0281, 0.0292, and 0.0217 g.

The decrease in plant dry weight due to increasing salinity may be caused by the interaction of osmotic effects and the presence of specific Cl^- and Na^+ ions, which reduce the photosynthetic activity of young leaves [32]. Moreover, the reduction in biomass with increasing salinity may be attributed to the negative effects of the increased energy needed to produce osmolytes during osmotic adjustment as the plant grows [33]. In *Amaranthus cruentus* (L.), the dry biomass weight of leaves decreased 27% with 50 mM NaCl, compared to the treatment without NaCl [34]. In *Amaranthus tricolor* (L.) with 50 and 100 mM NaCl the dry biomass weight decreased significantly [35]. In quinoa, the dry fresh biomass decreased 1.7 times with 300 mM NaCl [36].

As plants mature, they may develop greater tolerance to salinity, depending on the crop in question. Tolerance is not uniform across species or at all growth stages [30]. However, at vegetative and reproductive growth stages, salinity can affect growth the most [29].

CONCLUSIONS

The responses observed in huauzontle in germination and initial growth depend on the NaCl concentration and the cultivar. The germination percentage decreased only with the 300 mM NaCl dose with respect to the control. Seedling height increased by 75 and 150 mM NaCl in the Mexico City and Tlaxcala cultivars; on the contrary, Puebla shows significant inhibition in seedling growth starting at 225 mM NaCl. The Mexico City cultivar had the highest fresh and dry biomass weights, followed by Puebla and Tlaxcala.

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Agronomic Evaluation of Castor Bean Cultivation (*Ricinus communis* L.) Under Soil Moisture-Limiting Conditions

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ABSTRACT

Objective: To assess yield and growth responses of castor bean collections under soil moisture-restricted conditions.

Design/methodology/approach: The study was conducted in the municipality of Salinas de Hidalgo, San Luis Potosí. Twenty castor bean collections were evaluated under rainfed conditions and irrigation at 50% of crop evapotranspiration, with treatments arranged in randomized blocks.

Results: Significant differences (α=0.05) between collections were observed for variables such as stem diameter, plant height, number of clusters per plant, number of fruits per cluster, 100-seed weight, and aerial biomass. Regarding soil moisture levels, variables including stem diameter, plant height, number of branches, number of leaves, number of clusters, number of fruits per cluster, 100-seed weight, seed yield, and aerial biomass showed significant differences (α=0.05).

Limitations on study/implications: The results are based on a single production cycle.

Findings/conclusions: The Calvillo and Encarnación de Díaz collections showed the highest seed yields under rainfed conditions, with 385 and 326.7 g/plant, respectively, while the Luis Moya collection exhibited the highest yield under irrigation, with 680.7 g/plant. Seed yield was influenced solely by soil moisture, independent of collection origin. Collections under the most restrictive soil moisture condition (rainfed) flowered and fruited slightly earlier.

Keywords: Rainfed, biomass, yield.

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INTRODUCTION

Population growth, increasing energy demands, environmental changes, and the depletion of fossil fuels —as well as the growing difficulty in accessing fossil fuel reserves— have highlighted the need for society to seek alternative energy sources. Various countries have channeled efforts and public policies in this direction, exploring alternatives for energy generation for both domestic consumption and export (SAGARPA, 2009).

Mexico, with its great biodiversity and favorable climatic and geographic conditions, is a country rich in natural resources suitable for bioenergy production, which could enable it to achieve energy autonomy and support the development of the biofuel industry (ASA, 2014). One of these products is biodiesel, which has garnered considerable interest due to its beneficial properties (Vyas, 2010; Moreira *et al.*, 2022). Several bioenergy crops can be used for biodiesel production, including castor bean (*Ricinus communis* L.).

Castor bean is highly adaptable to diverse climatic conditions, with drought resistance being one of its most notable characteristics. However, to achieve high seed yields, it requires a minimum annual rainfall of 600 mm. The crop is susceptible to high relative humidity during the flowering and fruiting stages, which can lead to increased disease incidence. Excessive soil moisture also negatively affects its development. The ideal average temperature range for castor bean cultivation is 20 to 30 °C. The plant does not tolerate frost, and production can be impacted at temperatures above 38 °C. It thrives in deep, medium- to high-fertility soils that are loose, permeable, well-aerated, well-drained, with moderate to high nutrient levels, and a pH above 5.5 (Mazzani, 2007; Díaz-López *et al.*, 2020).

The oil extracted from castor bean can be used as a biofuel, which has increased interest in this plant (Cardona *et al.*, 2009). This biofuel may contribute to reducing petroleum-based fuel consumption, thereby decreasing negative environmental impacts (Sepúlveda, 2012). Additionally, cultivating castor bean presents an opportunity for development in degraded areas, enabling viable activities on currently unproductive lands (Mazzani, 2007; Díaz-López *et al.*, 2020). This plant also has applications in the production of medicines, cosmetics, plastics, fuel additives, and biopolymers, among other uses (Cardona *et al.*, 2009; Rocha *et al.*, 2022; Singh *et al.*, 2023).

Given castor bean's adaptability and the need to generate theoretical and practical research accessible to rural communities in the San Luis Potosí-Zacatecas Highlands, the objective of this study was to evaluate the performance of 20 castor bean collections under two soil moisture-limiting conditions. The agronomic evaluation considered morphological variables (plant height, stem diameter, number of branches, and number of leaves) and yield components (seed weight and number of clusters) to identify the best collections for potential use in future castor bean breeding programs.

MATERIALS AND METHODS

Castor Bean Collections and Experiment Site Location

The castor bean collections evaluated in this study originate from four states within Mexico (Table 1). The experiment was conducted in the locality of Diego Martín (22° 43.875' N, 101° 42.6825' W, 2096 m.a.s.l.), approximately 10 kilometers north of the town center of Salinas de Hidalgo, San Luis Potosí.

Treatments and Experimental Design

Two soil moisture conditions were applied. Treatment one (T1) involved irrigation at 50% of crop evapotranspiration (ETc). Treatment two (T2) involved rainfed conditions, with a recorded rainfall of 390.8 mm during the crop cycle.

Table 1. Geographic Distribution of Castor Bean Collections.

Sample No.	Estate	Municipality	Community	Altitude (amsl)
1	San Luis Potosí	Ciudad del Maíz	Francia Chica	1851
2	San Luis Potosí	Moctezuma	Moctezuma	2063
3	San Luis Potosí	Ciudad del Maíz	San Antonio	2003
4	San Luis Potosí	Capulines	Capulines	1830
5	San Luis Potosí	Mexquitic	Milpillas	1728
6	San Luis Potosí	Mexquitic	Las Moras	1773
7	San Luis Potosí	Venado	Venado	2083
8	San Luis Potosí	Mexquitic	Corte Primero	1955
9	San Luis Potosí	Salinas	Salinas	2083
10	San Luis Potosí	Mexquitic	Rancheria Guadalupe	1349
11	Aguascalientes	Tepezala	Tepezala	1851
12	Aguascalientes	San José de Gracia	San José de Gracia	2095
13	Aguascalientes	Aguascalientes	Fraccionamiento Soledad	1819
14	Aguascalientes	Calvillo	Calvillo	1936
15	Jalisco	Encarnación de Díaz	Encarnación de Díaz	1811
16	Jalisco	Villa Hidalgo	Villa Hidalgo	1920
17	Jalisco	Encarnación de Díaz	Tecuan	1971
18	Zacatecas	Orito	Orito	2399
19	Zacatecas	Noria	Noria	2040
20	Zacatecas	Luis Moya	Luis Moya	1971

The 20 collections under rainfed conditions were randomly assigned in blocks within one plot, while those under irrigation were arranged in another plot, with each collection replicated three times. Thus, each treatment comprised 60 experimental units (20 collections and 3 replications = 60 experimental units), with each unit consisting of four plants.

Planting

Planting was conducted in 2 kg plastic pots using a mix of sand, volcanic soil, and coconut fiber, with each substrate constituting 33.3% of the total. Seeds were sown at a depth of 2.5 cm, with daily morning watering for six weeks.

Land Preparation and Transplanting

In a 2,300 m^2 plot, soil cleaning and tillage were carried out to a depth of approximately 50 cm using a chisel plow.

Castor bean seedlings were transplanted 46 days after planting. Prior to transplanting, each seedling received 3 liters of water, with an additional 2 liters applied per plant after transplanting was completed. Plants were spaced 2 m apart in rows 1.5 m apart, resulting in a population density of 3,333 plants per hectare.

Cultural Practices and Irrigation

Throughout the crop development, pest control and weed removal were conducted. Half of the plants received supplemental water in addition to rainfall, delivered through a drip irrigation system. Crop evapotranspiration was estimated using the Penman-Monteith equation, with castor bean crop coefficients (Shuba *et al*., 2006) and data from a nearby meteorological station. Crop evapotranspiration (ETc) was calculated as:

$$ETc = ETo \bullet Kc$$

where: *ETc* is crop evapotranspiration; *ETo* is reference evapotranspiration and *Kc* is the crop coefficient for castor bean.

Evaluated Variables

a) Phenological Variables: Emergence (days from sowing to full exposure of cotyledons), flowering (days from sowing to the appearance of the first floral cluster), and physiological maturity (days from floral cluster emergence until 50% of the fruits in the clusters changed from green to brown).

b) Growth Variables: Stem diameter (measured at the stem base), plant height (from the stem base to the plant apex), number of branches, and number of leaves. These variables were measured biweekly, with one plant per experimental unit selected.

c) Production Variables: Number of clusters (panicles) per plant, number of fruits per cluster, number of seeds per fruit (capsules), and weight of 100 seeds. Fresh and dry biomass weights were also determined to estimate moisture content and produced biomass.

Harvest, Drying, and Biomass Determination

For dehiscent castor bean plants, clusters were harvested when 50% of the fruits changed color from bright green to dark green or brown. For indehiscent collections, fruits were harvested when they reached a brown color. The clusters and aerial biomass were transported to a glass greenhouse for drying, with clusters placed vertically in paper bags to allow sunlight penetration to the bag's base for uniform drying of plant material. The clusters were then placed in an oven at 30 °C for 72 hours to reach a 10% moisture level, while the remaining aerial biomass was dried at 70 °C for 72 hours.

Dry matter weight was determined using an electronic scale with a 10 kg capacity, and a precision balance was used to measure the weight of 100 seeds.

RESULTS AND DISCUSSION

Tables 2 and 3 present the average values for the variables days to fruit maturity, number of fruits per cluster, weight of 100 seeds, seed yield, and aerial biomass for the 20 castor bean collections under rainfed (Table 2) and irrigated (Table 3) conditions, along with each variable's measure of dispersion (SD=standard deviation). The collections are ordered according to the mean seed yield. The tables show that the collections with the highest yield values differed between rainfed and irrigated conditions; Calvillo had the highest yield under rainfed conditions, while the Luis Moya collection showed the highest yield under irrigation.

Phenological Variables

The variables days to seedling emergence, days to flowering, and days to fruit maturity for the 20 castor bean collections under both soil moisture conditions showed no significant differences ($\alpha = 0.05$). However, some general differences in mean values were observed; for instance, the Moctezuma and Venado collections were the earliest to mature under rainfed conditions.

Growth Variables

Only stem diameter and plant height ($\alpha = 0.05$) showed significant differences among the 20 collections (Figures 1 and 2). Figure 1 shows that the Luis Moya, Zac. collection recorded the largest stem diameter (4.5 cm) under irrigation, while the Salinas, S.L.P. collection had the largest diameter (3.8 cm) under rainfed conditions. The Tepezalá, Ags. collection displayed the smallest stem diameter (1.8 cm). These differences in stem diameter and plant height appeared early in the crop's development, specifically from the second

Table 2. Recorded Variables for 20 Castor Bean Collections Under Rainfed Conditions.

Community	Days to fruit maturity		Fruits per cluster		Weight of 100 seeds (g)		Seed yield (g/plant)		Biomass above ground (g/plant)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Calvillo, Ags.	190	2.65	29	9.8	29.7	2.04	385	437.6	366.6	390
Encarnación de Díaz, Jal.	196	12.7	62	34.7	29.2	2.14	326.7	259.7	455	296
Corte Primero, SLP.	200.5	7.78	64.3	48.3	16.38	2.1	189	195.6	233.3	242
Salinas, SLP.	206	0	36.6	3.7	27.5	0	161.8	90.7	873.3	770
Fracc. Soledad, Ags.	198.6	11.8	27	3.6	21.2	2.77	142.7	20.4	250	106
Villa Hidalgo, Jal.	197.6	7.37	25.6	12.6	25.7	2.35	132.4	107.6	136.6	94
Francia Chica, SLP.	205	0	79	54.0	16.59	1.61	123.4	97.1	368.3	154
Orito, Zac.	205	0	97.6	31.5	11.23	0.5	122.1	98.8	153.3	88
Luis Moya, Zac.	200	7.07	45.6	18.1	18.56	0.45	121.5	110.8	310	242
Moctezuma, SLP.	189.3	14.6	42.3	20.6	14.3	1.08	115.7	118	171.6	145
Tecuán, Jal.	194.5	13.4	84.5	28.9	11.24	1.42	114.4	70.7	175	86
Venado, SLP.	187.3	16.6	26.6	3.5	20.5	2.41	110.7	66.3	226.6	85
San José de Gracia, Ags.	200.3	7.37	70.3	22.5	14.8	1.51	94.8	34.5	170	48
Noria, Zac.	204.5	0.71	63	31.5	11.24	0.77	86	96	236.6	287
Milpillas, SLP.	196	12.7	72.6	15.0	11.22	0.18	73.7	85.7	100	108
Ranchería Guadalupe, SLP.	201	7.81	70.6	6.03	10.23	1.16	59	20.8	95	37
Capulines, SLP.	205.5	0.71	44.6	33.3	8.71	0.65	52.4	55.8	140	102
Tepezalá, Ags.	192	9.9	70.6	62.9	10.1	0.87	51.6	40.3	91.6	50
San Antonio, SLP.	205.6	0.58	41.3	22.9	16.89	0.3	32.2	19.8	166.6	83
Las Moras, SLP.	205.3	0.58	33	20	16.86	1.5	29.8	21	100	52

Table 3. Recorded Variables for 20 Castor Bean Collections Under Irrigated Conditions.

Community	Days to fruit maturity		Fruits per cluster		Weight of 100 seeds (g)		Seed yield (g/plant)		Biomass above ground (g/plant)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Luis Moya, Zac.	201	7.8	77.3	26.6	24.5	1.01	680.7	344.5	935	452
Venado, SLP.	200	8.3	63.3	29.5	20.1	2.01	433.4	529.9	673	579
Francia Chica, SLP.	199	9.9	96.3	17.2	22.8	2.7	371.7	290.2	558	307
Ranchería Guadalupe, SLP.	194	9.07	77.3	19.5	11.6	0.36	278.2	242.5	390	305
Villa Hidalgo, Jal.	195	14.1	34.6	3.06	22.49	1.67	275.6	145.1	528	230
Moctezuma, SLP.	193	10.1	50.6	15.2	17.8	0.58	271.5	219.4	555	384
Fracc. Soledad, Ags.	191	12.1	28.6	9.45	24.05	1.31	235	188	713	488
Calvillo, Ags.	197	11.9	23	4.36	30.7	2.52	229.8	152.8	221	100
Orito, Zac.	185	3.21	74.6	18.8	12.7	0.68	226.6	150.8	351	149
Capulines, SLP.	205	0.58	74	8.54	12.4	0.21	223	76.8	576	195
Milpillas, SLP.	191	12	87.3	52.6	10.7	0.42	193.1	110.3	300	229
Corte Primero, SLP.	195	8.8	81	22.3	13.9	1.68	191	264.2	228	231
Las Moras, SLP.	187	8.6	53.3	17.5	18.5	0.16	171.3	91.3	365	45.8
Tepezalá, Ags.	196	7.8	93.6	13.3	10.1	0.78	166.3	88.5	300	146
Encarnación de Díaz., Jal.	192	14.8	38.6	12.2	28.6	0.15	156.1	119.8	350	285
Tecuán, Jal.	206	0	104.5	9.19	13.6	0	150.8	43.3	420	301
Noria, Zac.	198	13	129	61.1	10.5	0.61	150.6	76.3	338	195
San José de Gracia, Ags.	205	0.58	103	20.2	14.3	0.24	146	21.1	203	60.4
Salinas, SLP.	205	0	40	6.56	32.6	0	145.6	42.5	565	353
San Antonio, SLP.	204	2.65	86.3	33.2	17.8	1.23	128.7	39.4	246	44.8

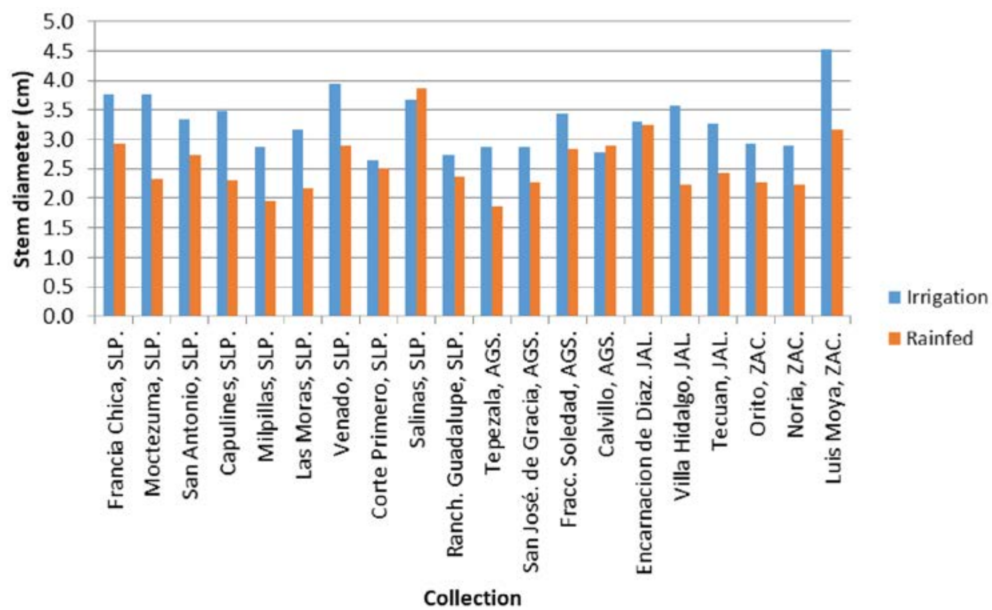

Figure 1. Stem Diameter of 20 Castor Bean Collections Under Two Soil Moisture Conditions.

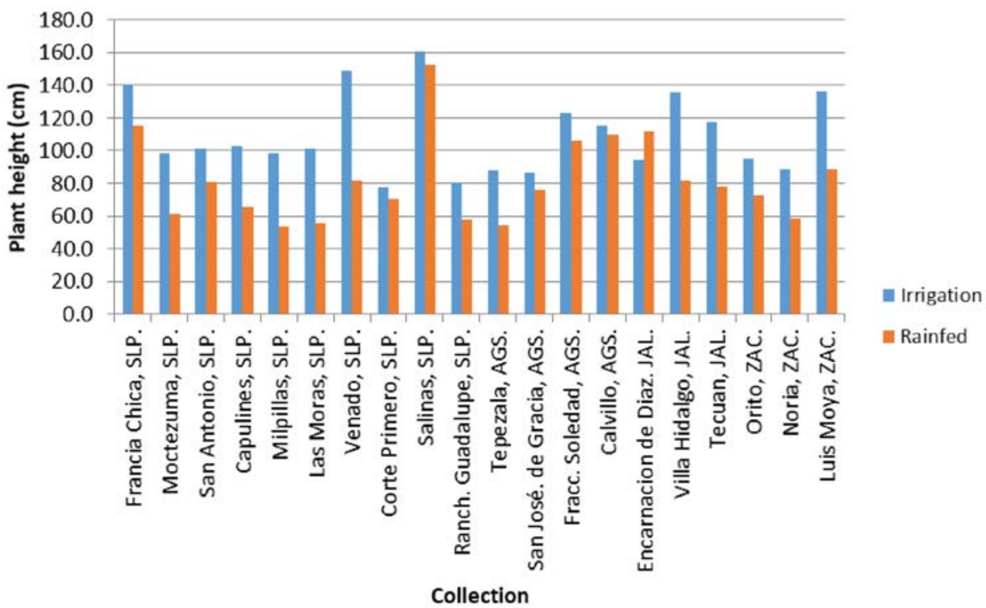

Figure 2. Plant Height of 20 Castor Bean Collections Under Two Soil Moisture Conditions.

sampling onward, and became more pronounced by the final sampling during the crop's developmental stage (sampling No. 11) (Figure 3).

Production Variables

The variables number of clusters per plant, number of fruits per cluster, and weight of 100 seeds showed significant differences ($\alpha=0.05$) both among collections and between soil moisture conditions (Figures 4 and 5). In contrast, yield displayed significant differences only between irrigation and rainfed conditions, not among collections. Additionally, Figure 5 shows that the 100-seed weight values for plants grown under

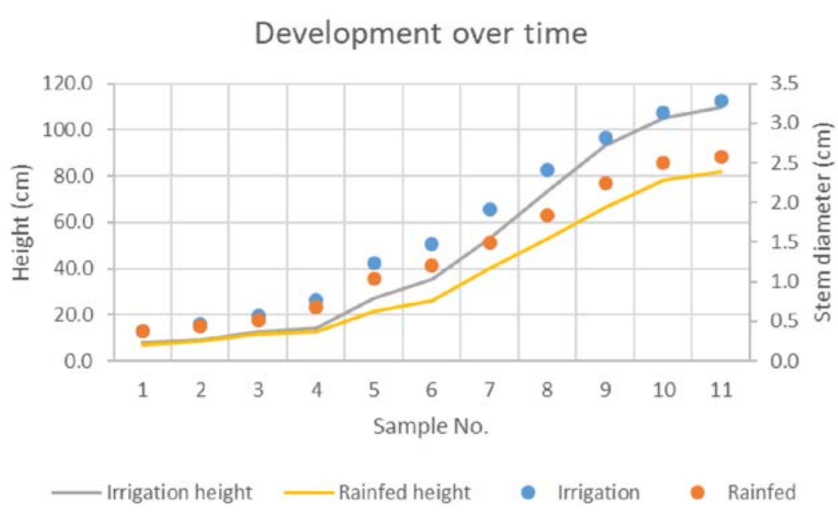

Figure 3. Castor Bean Development (Plant Height and Stem Diameter) Throughout the Crop Cycle Under Two Soil Moisture Conditions.

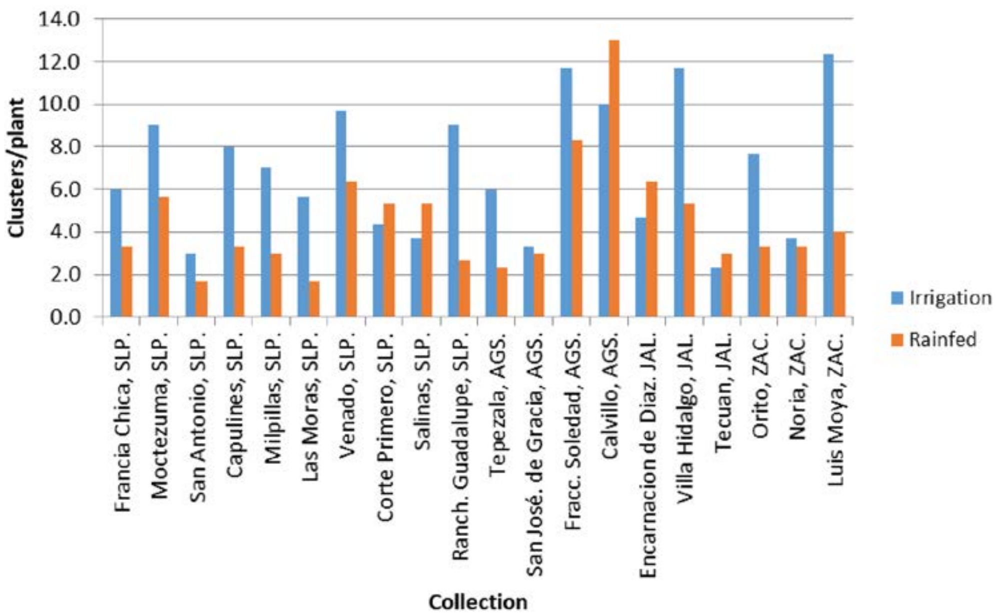

Figure 4. Number of Clusters per Plant for 20 Castor Bean Collections Under Two Soil Moisture Conditions.

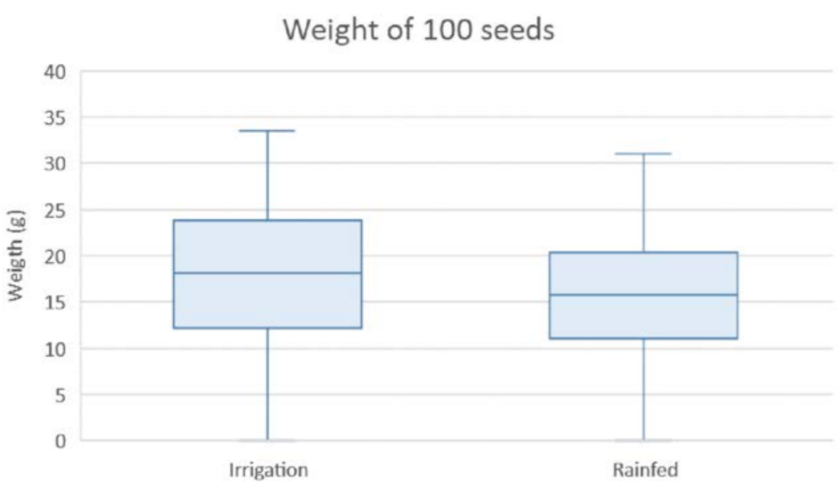

Figure 5. Weight of 100 Seeds for 20 Castor Bean Collections Under Two Soil Moisture Conditions.

rainfed conditions were more closely clustered around the mean and median compared to those grown under irrigation.

Figure 6 shows that collections grown under irrigation conditions had the highest seed yield (1078.0 g/plant for the Luis Moya collection). In contrast, the highest yield under rainfed conditions was not significantly lower, reaching 888.7 g/plant (Calvillo collection). Despite some experimental units showing zero yield, the highest yields under both rainfed and irrigated conditions were much higher than those reported by García-Herrera *et al.* (2019), who recorded 190.4 and 192.7 g/plant for the Calvillo and Venado collections, respectively, for the same site and soil type, but under unrestricted moisture conditions (full

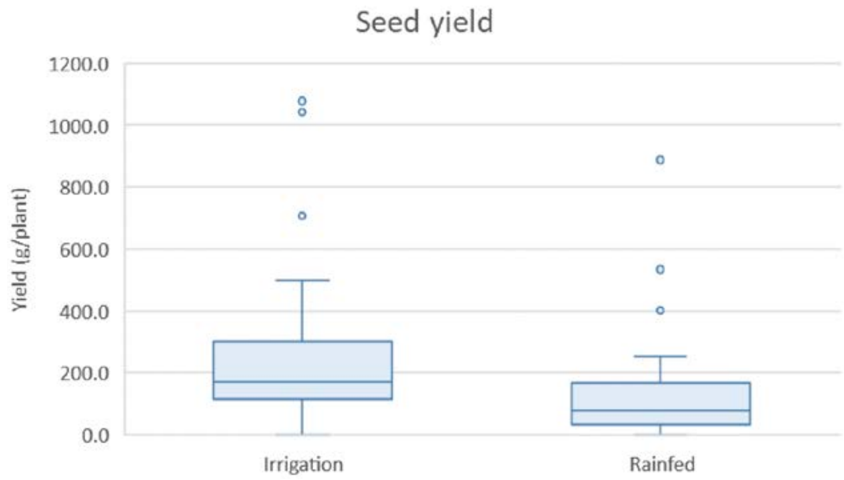

Figure 6. Seed Yield of 20 Castor Bean Collections Under Two Soil Moisture Conditions.

irrigation). Figure 6 also indicates that the yield of collections under irrigation exhibited slightly greater variation compared to those under rainfed conditions.

CONCLUSIONS

No single collection showed the highest values across all evaluated variables. Both the origin of the collection and soil moisture influenced the growth of the evaluated castor bean collections. Seed yield was affected only by soil moisture and not by the origin of the collection. The Calvillo and Encarnación de Díaz collections achieved the highest seed yields, attributed to the number of branches, number of fruits per cluster, and weight of 100 seeds. Collections grown under the most restrictive soil moisture conditions (rainfed) showed slightly earlier flowering and fruit maturation. Aerial biomass is considered an important variable due to its high production; the Salinas collection had the highest aerial biomass production, though it did not produce a high seed yield. Cultivating bioenergy plantations should be considered a productive option for farmers with marginal or idle lands.

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Colegio de Postgraduados

Imbibition of seeds of romerito (*Suaeda mexicana* Standl.) and purslane (*Portulaca oleracea*) in saline solutions and its effect on germination and initial growth

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ABSTRACT

Objective: To evaluate the effect of salinity sources and concentrations, as well as the interaction of these study factors on the increase in seed mass during imbibition, germination and initial growth of romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*) seedlings.

Design/methodology/approach: Seeds of romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*) from the agricultural area of Tláhuac and San Gregorio Atlapulco, Xochimilco, Mexico City were used. They were incubated for 3 h in 15 saline solutions, which resulted from the combination of three sources: NaCl, Na_2SO_4, and $CaCl_2$; and five concentrations: 0, 0.25, 0.50, 0.75, and 1.00 M. The seeds were incubated in Petri dishes at 28 °C in 80% relative humidity. The increase in seed mass gained after the imbibition period, germination percentage, and radicle and shoot length of the seedling were determined.

Results: The 0.25 M NaCl treatment increased germination in romerito; in contrast, doses higher than 0.25 M of $CaCl_2$ and Na_2SO_4 significantly reduced germination, as well as shoot growth. NaCl causes less inhibition of romerito radicle growth than the other sources. In purslane, the 0.25 M $CaCl_2$ treatment significantly reduced germination; but, it stimulated radicle growth. The Na_2SO_4 and NaCl sources at doses starting at 0.5 M inhibited germination in purslane.

Limitations of the study/implications: No certified germplasm was used for this study, since the seed was obtained directly from producers who maintain semi-domesticated native accessions.

Findings/conclusions: Romerito is tolerant to NaCl and Na_2SO_4, but sensitive to $CaCl_2$; this was also observed in purslane, although the ranges of tolerance to the concentration vary between salinity sources.

Keywords: Abiotic stress, growth, halophytes, salinity, seeds.

INTRODUCTION

Glycophyte and halophyte plants respond in a similar way to salinity during germination [1]. In some species, salt stress causes an inhibitory effect on germination, so the development of strategies that allow tolerance of salinity during this phase and in the early stages of growth is essential for plant survival [2]. Facing fluctuations in osmotic potential, salt concentration, and ionic species represents a high energy cost for the plant, which translates into decreased seed germination, prolonged dormancy, and decreased seedling size [3].

The effects and degree of affectation of salinity have been documented to vary from one species to another, according to the type of salinity and its concentrations. In peanut (*Arachis hypogaea*), for example, the increase in salt concentration caused a decrease in seedling emergence, as well as root elongation and plant length, and also decreased dry weight [4]. Similar effects were reported in the germination of pigeon pea (*Cajanus cajan*) seeds [5]. Likewise, in cabbage (*Brassica oleracea* var. *capitata* L.), sugar beet (*Beta vulgaris*), amaranth (*Amaranthus paniculatus*), and pak-choi (*Brassica campestris*), a reduction in germination, root and shoot length was observed [6]. In halophytic plants such as *Crithmum maritimum*, high salt contents delay the germination of their seeds; also, the previous imbibition of these in ascorbic acid improves their germination capacity during saline exposure [7]. In a study carried out on *Suaeda salsa* populations, the seeds belonging to a germplasm bank of an intertidal zone with concentrations of 0.6 to 35.1 ppt NaCl favored the germination of the seeds; however, when the seeds were transferred to a constant saline environment such as a Petri dish, the seeds collected from the same Place were affected by all saline concentrations causing a decrease in germination. The above suggests that even facultative halophyte species such as *S. salsa* are affected by salinity when it is constant [8].

In their natural environment, some seeds take advantage of periods of low salinity and high availability of low-salt water to soak and resist the effects of salinity. This is the case of fenugreek (*Trigonella foenum-graecum*), whose seeds that were soaked in distilled water prior to saline treatments and after being placed in these, where the percentage of final germination, germination speed, and root length increased, compared to those seeds that were not soaked [9]. In three varieties of beans (*Plaseolus vilvaris*) exposed to NaCl and Na_2SO_4, the latter salt decreased germination and inhibited seedling emergence compared to NaCl [10]. Carbonates are salts that also affect seed germination. In purslane (*Portulaca oleracea*), when evaluating the effect of $KHCO_3$, $NaHCO_3$, and $(NH_4)_2CO_3$ on germination and root and stem length, the 50, 100, and 200 mM concentrations of the three salts caused a decrease in the germination percentage as well as the root length and dry weight; while low doses (25 mM) slightly increased the stem length and fresh weight [11].

Although there is a gradient of resistance and adaptation to salinity [12], some plants adopt different seed forms, react differently to dormancy, or may contain different concentrations of abscisic acid (ABA); this combination of factors makes them better adapted to environmental changes and enhances their establishment [13]. In a study on adaptive strategies in the germination phase, it was found that *Suaeda physophora*, *Haloxylon ammodendron*, and *H. persicum* produce seeds without endosperm with a highly developed embryo and covered with a soft testa that facilitates their germination and rapid establishment in saline environments [1].

In Mexico, romerito (*Suaeda mexicana*) is a plant generally found in saline environments, although it can also colonize non-saline sites [14]. It has economic and nutraceutical value since it is part of the diet of Mexicans [15].

Purslane is a halophyte plant with C4 NAD-ME metabolism (NAD-dependent malic enzyme) with economic potential and wide consumption worldwide [16,17,18]. Studies on the effect of NaCl on its germination and early development indicate that the germination of its seeds is not altered at low concentrations ($<$150 mM NaCl), but at higher concentrations, a change in the concentration of several organic compounds such as chlorophyll and proteins [19], flavonoids [20], urea and pinitol [21] is evident, which give the plant a greater response capacity to stress.

Thus, the objective of this research was to compare the germination response of seeds and initial growth of two important halophyte plants in Mexico, romerito and purslane, exposed to different salts and concentrations.

MATERIALS AND METHODS

Plant material

Seeds of romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*) from collections from the agricultural areas of Tláhuac and San Gregorio Atlapulco, Xochimilco in Mexico City were used. These areas are semiarid with soils of the Terric Salic Anthrosol type [22], with a saturation extract pH between 7.69 and 8.44, which denotes its alkaline character due to the presence of CO_3^{2-} ions in addition to the electrical conductivity value of 34.19 dS m^{-1} [23]. The climate is humid temperate C(w2)(w)b(y'), considered the driest of the subhumid temperate climates with summer rains. Frosts usually begin in October and end in March. Rains occur mainly during summer and autumn; however, due to the relief of the area, there are also two climate subtypes: Cwb (plain and low slope region), and Cwc (temperate with cold winter) which corresponds to the highest areas [24].

Treatment design and experimental design

Two independent experiments were carried out for the species under study: romerito and purslane . In each essay, the main effects of two study factors were evaluated: salinity source ($CaCl_2$, NaCl, and Na_2SO_4) and salt concentration (0, 0.25, 0.50, 0.75, and 1.0 M), as well as their interaction, in a 3×5 factorial experiment with a completely randomized distribution. This resulted in 15 treatments in each experiment. The experimental unit for the treatments with romerito was a Petri dish with 20 seeds; while for purslane, it was a Petri dish with 50 seeds. Each treatment had three replicates. The salinity sources used were reagent grade Meyer® (Mexico City) brand and the solutions were prepared using distilled water.

Prior to applying treatments, groups of 20 romerito seeds and 50 purslane seeds were weighed to obtain their individual weight. Subsequently, the seeds of each experimental unit were incubated for 3 h in 3 mL of the corresponding solution according to the treatment, shaking for 1 min every hour. After the incubation time, the seeds were removed from the flasks, dried, and weighed again, then transferred to Petri dishes with medium-pore filter paper where they were placed, and 10 mL of distilled water was added. The Petri dishes were placed inside an incubation oven (Thermo Scientific, PR205075G, USA), at 28 °C and 80% RH.

Variables evaluated

Percentage increase in initial seed mass due to water absorption. It was determined in each experimental unit before and after imbibition according to Kaymakanova [10], using the formula:

$$PIMAW = \left[(W2 - W1) / W1 \right] \times 100$$

where: *PIMAW* is the percentage increase in initial seed mass due to water absorption; *W*1 is the weight of the seeds in each experimental unit before imbibition and *W*2 the weight of the seeds after imbibition.

Germination percentage. The germination period was 120 h (5 d) during which time the seeds were kept at constant temperature and hydration, and seeds were considered germinated when the radicle reached a length of 2 mm [6]. The evaluation was carried out every 24 h.

Stem and radicle length. In romerito, length measurements were made 10 d after the start of germination and in Purslane 8 d after the start of germination. The difference in days was due to the fact that the romerito seeds took longer to germinate.

Statistical analysis

With the data obtained in each species, analysis of variance and comparison of means test (Duncan, $P \leq 0.05$) were performed, using the SAS® software [25].

RESULTS AND DISCUSSION

Water absorption

Halophyte species have different adaptation strategies to salinity, so the germination response can be as diverse as the micro habitats in which they develop. Hence the increase in ionic concentration in the soil is closely linked to the increase in temperature and the decrease in the percentage of humidity [26]. Therefore, most species germinate when such concentrations are reduced, either by a decrease in temperature or by the arrival of rains diluting the concentration of salts.

In this study, the results of water absorption in romerito seeds showed a high coefficient of variation (77.6%), which explains why despite the differences between sources and saline concentrations evaluated, there were no statistical differences (Table 1). The percentage of water absorption in these seeds was 26.5 with NaCl, 18.5 with Na_2SO_4, and 15.3 with $CaCl_2$. Likewise, the percentages of increase in seed weight recorded with concentrations of 0, 0.25, 0.50, 0.75, and 1 M were 10.9, 22.7, 20.8, 25.4, and 20.6, respectively; these data show the positive relationship between salt concentration and water absorption in romerito.

In purslane, neither saline sources nor the interaction of saline source and concentration had any effect on the increase in seed mass due to water absorption during imbibition (Table 1). The increases in seed mass due to water absorption were 8.2, 7.7, and 6.4% in Na_2SO_4, NaCl, and $CaCl_2$, respectively. On the contrary, after a 3-h imbibition period, only the saline concentration significantly influenced water absorption in purslane seeds (Table 1; Figure 1).

Although the effect of the saline source was not significant in either species, the trends observed in the increase in seed mass due to water absorption during the imbibition phase allow us to affirm that in romerito the order of osmotic affectation by the saline source was $CaCl_2 > Na_2SO_4 > NaCl$, while in purslane it was $CaCl_2 > NaCl > Na_2SO_4$.

Contrary to what was observed in romerito, in purslane a negative relationship was observed between the saline concentration and water absorption as shown in Figure 1, with values of 15.3% in the control without salinity and approximately 4.4% with 1 M.

Both romerito and purslane are species that grow in environmental conditions with high abiotic stress [15,21], being exposed to salinity and water stress conditions, possibly one of their adaptive strategies is the production of seeds with permeable testas which facilitate imbibition in rainy conditions, which is combined with the decrease in the

Table 1. Main effects of source, salt concentration, and interaction on the increase in seed mass of romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*) due to water absorption during the imbibition phase.

Species	Salt source (SS)	Salt concentration (SC)	SS×SC
Suaeda mexicana	0.1463 ns	0.3749 ns	0.7710 ns
Portulaca oleracea	0.2828 ns	<0.0001 *	0.7583 ns

*Significant at 5 %; ns: non significant.

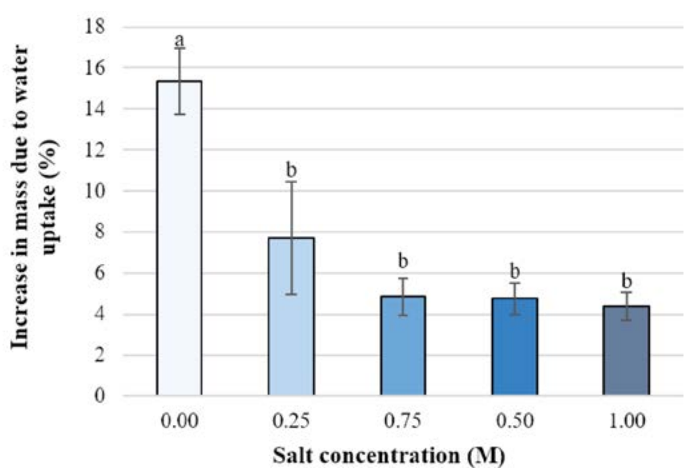

Figure 1. Water absorption in romerito (*Suaeda mexicana*) seeds after 3 h of imbibition in different salt concentrations. Means ± SD with different letters indicate significant statistical differences (Duncan, $P \leq 0.05$).

saline concentration in the soil. The morphology of the seeds of *Suaeda salsa* and *S. mexicana* is similar, so it is possible that both species develop the same adaptive strategies to salinity in response to short periods of rain [1,8]. On the other hand, *P. oleracea*, being a C4 species that can shit its metabolism to the CAM type [27], has the capacity to better tolerate water stress and salinity, so the permeability of its testa in saline conditions constitutes a representative factor of its adaptation to the environment.

Germination percentage

The germination percentage was evaluated for 120 h at 24-h intervals. In romerito, no significant effects of the interaction between the saline source and its concentration were observed in all evaluations; while, after 48 h, the effect of the saline source was significant and the main effect of the saline concentration was significant in all evaluations. On the contrary, for purslane in all samples, both the main and interaction effects were significant in its germination percentage (Table 2).

The main effects that were significant in romerito are shown in Figure 2. NaCl caused significantly greater germination than the rest of the sources evaluated (Figure 2a). On the other hand, increasing saline doses significantly reduced germination compared to the control, with the exception of the 1 M dose, which in all the samplings followed the control in germination (Figure 2b).

Purslane was less sensitive to sodium salts (NaCl and Na_2SO_4) than to $CaCl_2$; with the latter source, no germination was recorded starting from a concentration of 0.5 M (Table 3). It was also reported that only 50% of the seeds germinated in purslane at a concentration of 106 mM NaCl [28], that with concentrations of 200 mM NaCl germination was significantly reduced [19] and that with an electrical conductivity of 25 dS m^{-1} induced by NaCl, the germination percentage was reduced by up to 60% [29].

Table 2. Main effects of the source, saline concentration, and their interaction on the germination percentage of seeds of romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*).

Species	Germination time (h)	Salt source (SS)	Salt concentration (SC)	SS×SC
Suaeda mexicana	24	0.2218ns	<0.0001 *	0.6491 ns
	48	0.0249 *	<0.0001 *	0.5657 ns
	72	0.0349 *	<0.0001 *	0.5298 ns
	96	0.0121 *	0.0001 *	0.4460 ns
	120	0.0419 *	0.0022 *	0.6591 ns
Portulaca oleracea	24	<0.0001 *	<0.0001 *	<0.0001 *
	48	<0.0001 *	<0.0001 *	<0.0001 *
	72	<0.0001 *	<0.0001 *	<0.0001 *
	96	<0.0001 *	<0.0001 *	<0.0001 *
	120	<0.0001 *	<0.0001 *	<0.0001 *

*Significant at 5%; ns: non significant.

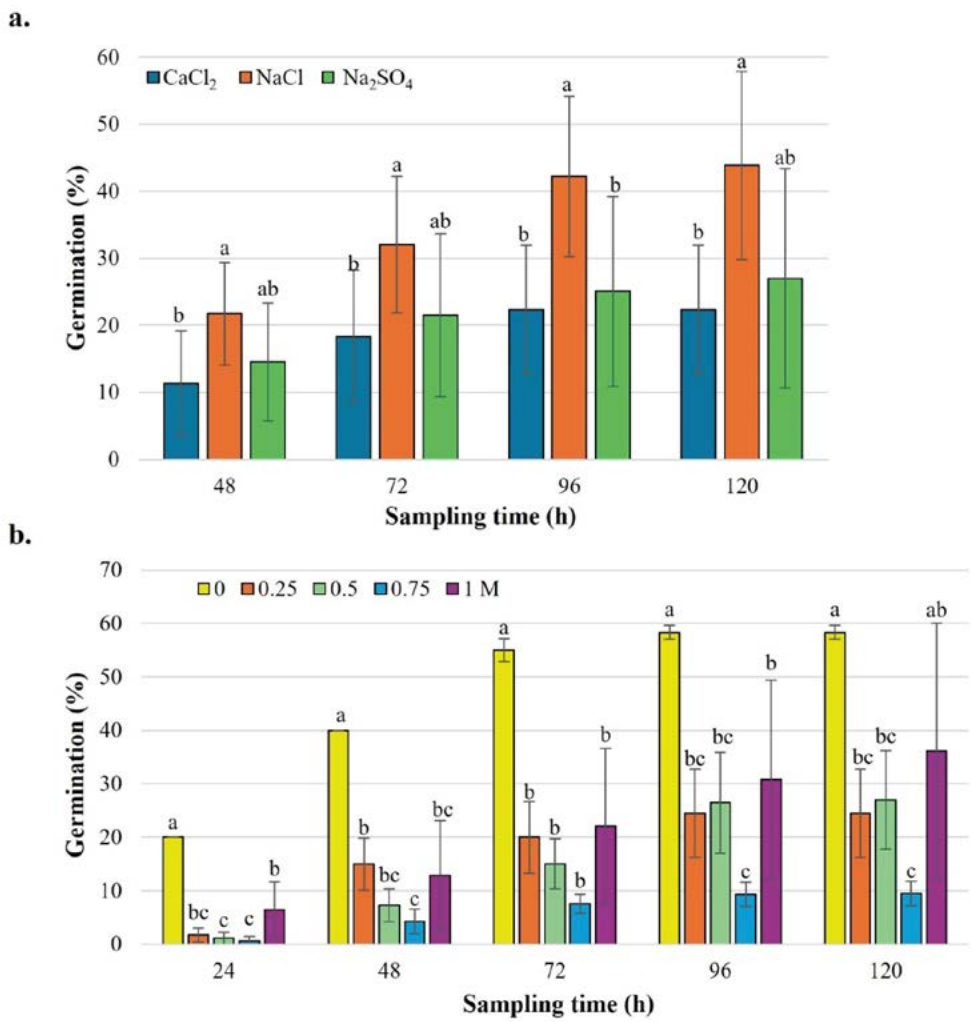

Figure 2. Germination percentage of romerito (*Suaeda mexicana*) seeds incubated for 3 h with different salt sources (a) and different salt concentrations (b). Means ± SD with a different letter in each figure and sample indicate significant statistical differences (Duncan, $P \leq 0.05$).

Stem and radicle length

Table 4 shows the significance values of the study factors and their interaction on stem and radicle lengths in romerito and purslane seedlings. It should be noted that, in both species, the saline concentration had significant effects, while the single effects of the saline source were only significant in purslane. Likewise, the effect of the interaction of the study factors is significant in both species.

Stem length in purslane seedlings was observed to be the variable that experienced the most changes according to their exposure to the evaluated treatments; therefore, it is considered the more sensitive crop (Figure 3). Regarding romerito, the stem growth was statistically lower than the treatment without salinity, with the saline sources $CaCl_2$ and Na_2SO_4 from a concentration of 0.25 M, while with NaCl this occurs from the concentration of 0.5 M (Figure 3a). It should be noted that the same phenomenon was observed in purslane seedlings (Figure 3b).

Table 3. Germination percentage of purslane (*Portulaca oleracea*) seeds incubated for 3 h with different salt sources and concentrations.

Salt source	Salt concentration (M)	Sampling time (h)				
		24	48	72	96	120
$CaCl_2$	0.00	88.00±0.50 a	97.00±0.87 a	97.00±0.87 a	97.00±0.87 a	97.00±0.87 a
	0.25	76.00±1.73 c	80.67±0.58 d	83.33±2.08 c	86.00±1.73 b	86.00±1.73 b
	0.50	0.00±0.00 g	18.00±0.00 g	27.33±4.04 f	33.33±8.02 e	39.33±9.24 e
	0.75	0.00±0.00 g	0.00±0.00 h	0.00±0.00 h	0.00±0.00 g	0.00±0.00 g
	1.00	0.00±0.00 g	0.00±0.00 h	0.00±0.00 h	0.00±0.00 g	0.00±0.00 g
NaCl	0.00	88.00±0.50 a	97.00±0.87 a	97.00±0.87 a	97.00±0.87 a	97.00±0.87 a
	0.25	85.00±0.50 b	93.00±0.50 b	93.33±0.29 ab	93.33±0.29 ab	93.33±0.29 ab
	0.50	25.67±0.29 e	25.67±0.29 f	52.67±1.15 e	52.67±1.15 d	53.33±1.53 d
	0.75	3.33±1.44 f	24.67±0.29 f	70.67±0.58 d	93.33±1.15 ab	93.33±1.15 ab
	1.00	0.00±0.00 g	0.00±0.00 h	19.33±6.25 g	24.33±2.02 f	24.33±2.02 f
Na_2SO_4	0.00	88.00±0.50 a	97.00±0.87 a	97.00±0.87 a	97.00±0.87 a	97.00±0.87 a
	0.25	86.67±1.15 ab	96.67±1.15 a	96.67±1.15 a	96.67±1.15 a	96.67±1.15 a
	0.50	48.33±1.44 d	84.00±1.73 c	88.67±1.15 bc	88.67±1.15 b	88.67±1.15 ab
	0.75	26.67±0.58 e	72.67±1.15 e	72.67±1.15 d	72.67±1.15 c	72.67±1.15 c
	1.00	0.00±0.00 g	0.00±0.00 h	0.00±0.00 h	0.00±0.00 g	0.00±0.00 g

Means ± SD with a different letter in each sample indicate significant statistical differences (Duncan, $P \leq 0.05$).

Table 4. Main effects of the saline source and concentration and their interaction on stem and radicle lengths in romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*) seedlings, from seeds incubated in different saline sources and concentrations.

Species	Variable	Salt source (SS)	Salt contentration (SC)	SS×SC
Suaeda mexicana	Stem length	0.0728 ns	<0.0001 *	0.0037 *
	Radicle length	0.0770 ns	<0.0001 *	0.0010 *
Portulaca oleracea	Stem length	<0.0001 *	<0.0001 *	<0.0001 *
	Radicle length	<0.0001 *	<0.0001 *	<0.0001 *

*Significant at 5%; ns: non significant.

Figure 4 presents the results obtained for radicle length based on the interaction of the study factors in both species. In general, it was observed that NaCl causes less inhibition of radicle growth. Although in particular, it was observed that in purslane the 0.25 M NaCl treatment stimulates it; compared to the rest of the treatments (Figure 4b). In the same way, $CaCl_2$ reduces radicle growth to a greater extent from 0.25 M, compared to the treatment without salt stress for both species (Figures 4a and 4b).

One of the effects of salt stress on seedlings is the reduction of stem length (Figure 3) and radicle length (Figure 4). In this experiment, it was observed that rosemary is more sensitive to the salt sources and concentrations evaluated, given that the inhibition of the growth of the radicle and the stem is of a greater magnitude than that recorded in purslane. Since salinity affects the growth of seedlings and delays the mobilization of reserve substances, cell division tends to be suspended, damaging the hypocotyls [10].

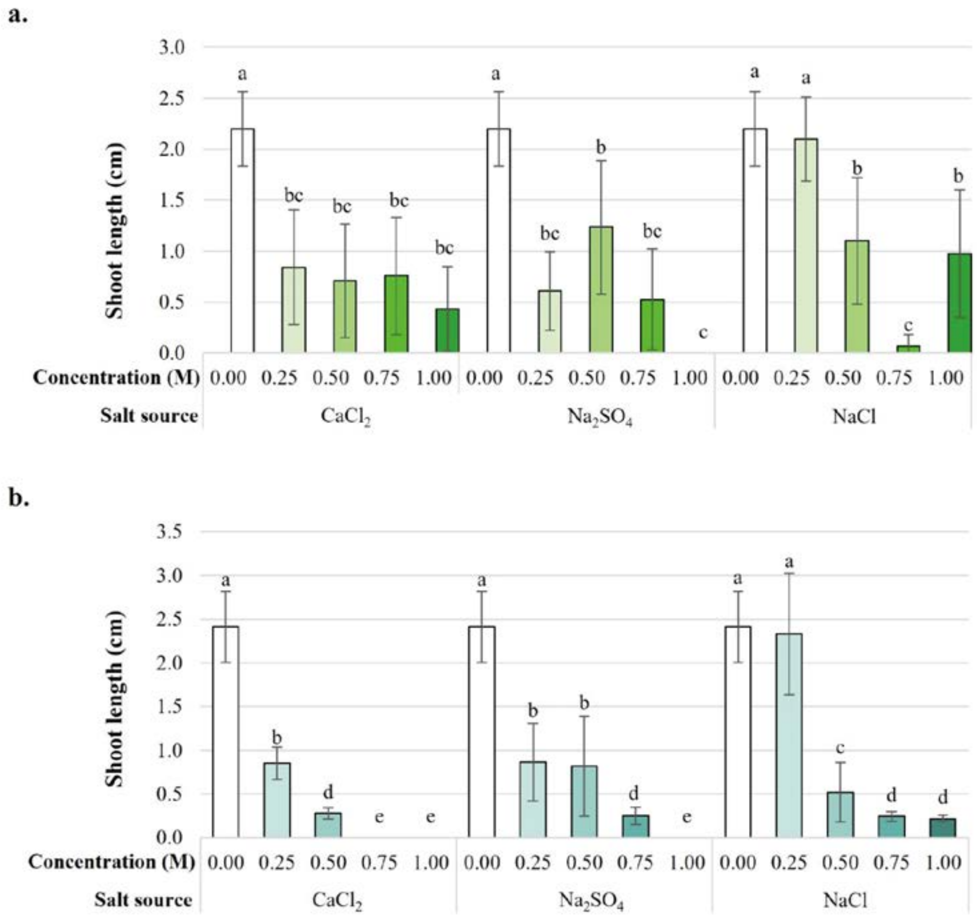

Figure 3. Stem length of romerito [*Suaeda mexicana* (a)] and purslane [(*Portulaca oleracea* (b)] plants from seeds incubated for 3 h with different salt sources and concentrations. Means ± SD with a different letter in each figure indicate significant statistical differences (Duncan, $P \leq 0.05$).

CONCLUSIONS

The germination percentage, and radicle and shoot length are germination variables that respond to the salinity source, concentration, and interaction of the source and concentration. Both romerito (*Suaeda mexicana*) and purslane (*Portulaca oleracea*) have different response mechanisms to the source and concentration gradient of salinity. Purslane is tolerant to sodium salts (NaCl and Na_2SO_4) but sensitive to $CaCl_2$; this is also observed in romerito, although the tolerance ranges vary from one concentration to another and from the type of salt with which it interacts. Both are species that have evolved in semiarid environments; their anatomical and morphological characteristics have allowed them to develop very efficient adaptation mechanisms, which allow their seeds to germinate and the initial growth of seedlings under constant salt concentrations.

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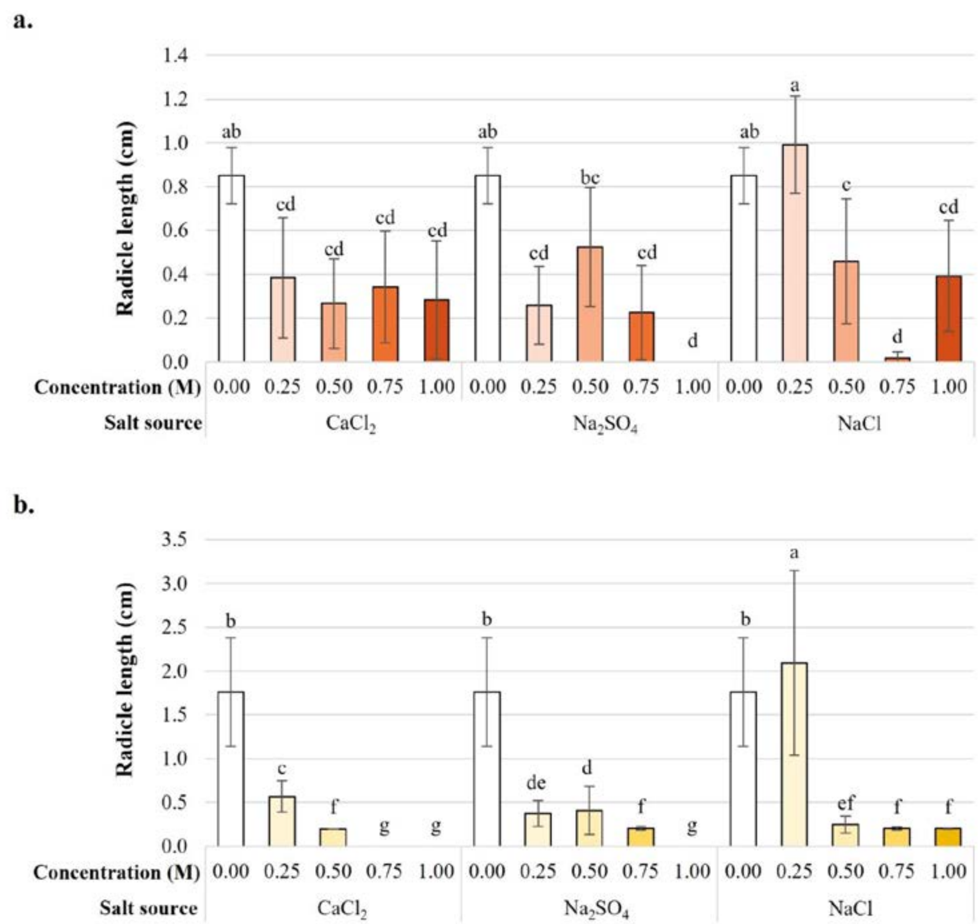

Figure 4. Radicle length of romerito [*Suaeda mexicana* (a)] and purslane [(*Portulaca oleracea* (b)] plants from seeds incubated for 3 h with different salt sources and concentrations. Means ± SD with a different letter in each figure indicate significant statistical differences (Duncan, $P \leq 0.05$).

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Colegio de Postgraduados

Design and implementation of an electronic system to monitor and record agroclimatic variables in greenhouses

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ABSTRACT

Objective: To describe the development of a low-cost electronic device that can monitor, track, record, store, export, and interpret climatic variables of greenhouse-produced crops.

Methodology: An Arduino UNO board was used, along with specific sensors, to measure the agroclimatic variables under study. A Raspberry Pi® 3B+ board was also used to store and export the recorded information. The previously assembled device was installed in a greenhouse located in San Agustín Calvario, municipality of Cholula, Puebla, from March to July. Finally, graphs were developed to interpret the variables according to the agroclimatic requirements of native cucumber.

Study Limitations/Implications: Greenhouses must have access to an electrical supply, regardless of their geographic location or the crop they produce.

Findings: A low-cost electronic system and device were successfully developed, allowing each sensor to monitor, record, store, and export the following agroclimatic variables: minimum and maximum temperature, relative humidity, carbon dioxide (CO_2) concentration, and light intensity in a greenhouse with electrical supply. Regarding data interpretation, graphs were developed for the agroclimatic variables in the greenhouse, based on the needs of the native cucumber plant.

Keywords: protected agriculture, weather station, hardware, software.

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INTRODUCTION

According to the November 2022 census of the United Nations, the global population reached 8 billion people, which involves an increasing demand for abundant, quality, and varied food [1]. Meanwhile, climate change has caused an increase in the demand for food, which has resulted in uncertainty regarding rainfed crops. Therefore, a search to increase the safety and volume of greenhouse food production has begun.

Simultaneously, recent technological advances have made it possible to replace the rigid structure of traditional systems with a more flexible one, supported by computing, conditioning circuits, data transmission, and data acquisition hardware and software [2]. One of the most significant technological advances in the evolution of electronics has been automation and agriculture is one of the areas it has had a major impact on [3].

Greenhouse crop production stands out among the techniques currently used in agricultural production. This recently-developed technique enables the cultivation of crops out of season and during the winter, without the risk of the plants dying due to low temperatures [4].

In order to address this situation, the crop area in protected agriculture must be increased to face decisive situations for crop growth —such as higher temperature, light intensity, relative humidity, and a greater water requirement—, all of which must be managed within optimal ranges [5]. Several efforts have been made to manage agroclimatic variables [6].

Consequently, the objective of this research was to design a low-cost electronic system for the recording, storage, and export of data on the agroclimatic variables with the greatest impact on crops grown in non-technified greenhouses that nonetheless have electric power.

MATERIALS AND METHODS

This section describes the main components used in the construction of the monitoring system. It also explains in detail the design and development of the software used to monitor the variables in the greenhouse, covering everything from the code to the connection of the components.

Design of the monitoring system and data collection

The goal of this project was to build a system that could monitor the agroclimatic variables that affect crops. For this purpose, a Raspberry Pi® 3B+ board was used as the interface. An Arduino UNO board was employed as data collection nodes. Both devices allow the acquisition and storage of data about various agroclimatic variables. The variables were selected based on their importance for the growth and development of crops during the operation of a greenhouse. The following variables were taken into consideration: maximum and minimum temperature, relative humidity, light intensity, and carbon dioxide concentration. Table 1 shows the measuring instruments used and their costs as of 2022.

Circuit Assembly

The circuit assembly is defined by the presence of a solderless breadboard. Jumper wires were used to connect the breadboard, the sensors, and the Arduino UNO board. Additionally, the sensor connections were made as follows: the signal pin of the DHT22 sensor was connected to the Arduino board's digital input module. The same procedure was made with the BH1750 sensor, which is also digital. The capacitive humidity sensor —which is an analog sensor— was connected to the analog input module. Finally, the two communication pins of the MH-Z19 sensor were connected to the digital inputs.

Table 1. Electronic components costs.

Equipment	Cost/Unit (USD)
DHT22 temperature and relative humidity Sensor	3.00
BH1750 light intensity Sensor	2.00
MH-Z19 CO_2 Sensor	40.00
Soil moisture Sensor %	10.00
Arduino board	27.00
Raspberry 3B+ board	35.00
Touch screen for Raspberry pi 3B+	12.00
Total	129.00*

*Prices in USD, calculated at an exchange rate of $20.00 MXN per dollar.

Figure 1. shows the sensors connected to the assembled circuit. The connection between the Arduino board and the Raspberry 3B+ board, which is done via a USB cable, is not included.

Programming phase

The programming environment used for the Arduino UNO board is found in the Arduino platform. Regarding the Raspberry Pi® 3B+ board, various programming languages are typically used, but Python was chosen for this study. The first part of the programming was done with the Arduino UNO board. The libraries for the sensors were included, because they are essential for their operation (Step 1, Figure 2). The Wire library was used for the BH1750 sensor, because it works through the I2C bus.

Meanwhile, the uart library was used for the MHZ19 sensor, because it works through the UART bus, which consists of two communication lines (TX and RX). Variables were declared to store the data obtained from each sensor (Step 2, Figure 2). The sensors were subsequently initialized (Step 3, Figure 2). In the final stage, the data obtained by the sensors was printed through the serial port. A 10-minute delay (sampling time) was defined. For the lines of code which manage data printing through the serial.print function, the values

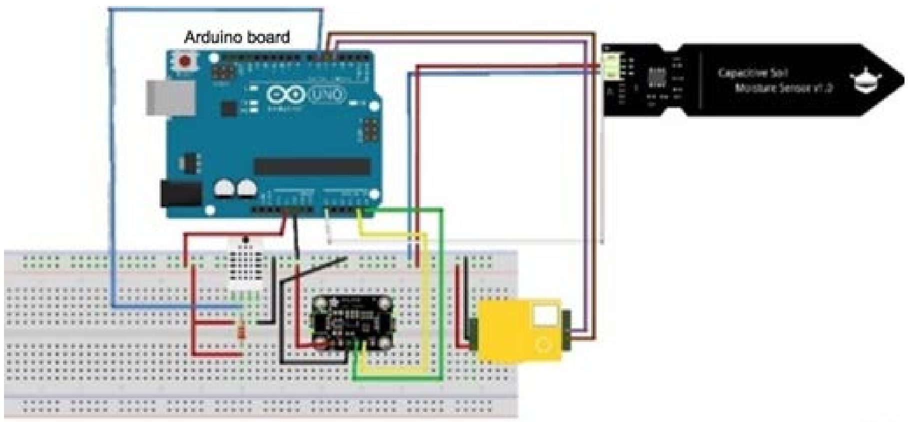

Figure 1. Sensor connections to the Arduino board.

Figure 2. Arduino Code.

are converted to String to avoid programming errors (Step 4, Figure 2). Once the code was implemented for the four sensors, a test was performed opening the serial port from the Arduino platform to verify data reception (Step 5, Figure 2).

Therefore, a Python script was developed within the system of the Raspberry Pi® 3B+ board to enable communication, as well as to store and export data obtained by the sensor network of the Arduino board. In the first lines of code, the following libraries were included: "serial," which establishes communication between Python and Arduino; "simplejson," necessary for the program's execution; and finally, "csv," which stores data in a CSV file (recognized by programs like Excel) (Step 1, Figure 3).

For the next part, the connection port and the variables used were specified. In this case, they were declared and, in turn, stored in the MyData variable (Step 2, Figure 3). A name was assigned to each variable, after they had been defined according to the definitions established in the Arduino program (Step 3, Figure 3). To finish the script, the following lines of code created a CSV file named "datos_sensores" (Step 4, Figure 3).

A test was conducted to verify the effectiveness of information storage on the Raspberry Pi® 3B+ board. Once the programming phase was completed, the system was installed in the greenhouse.

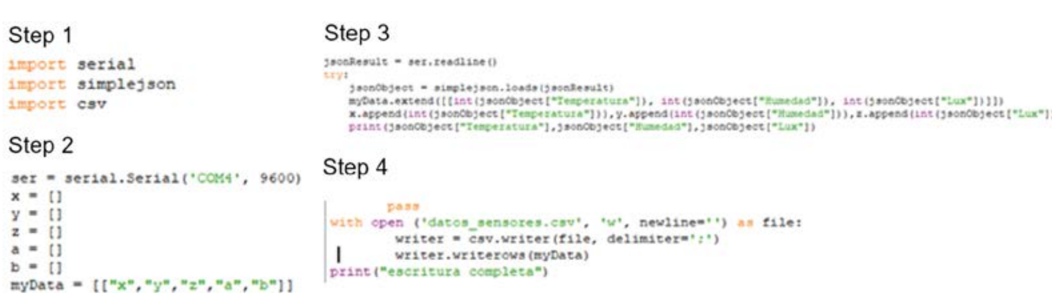

Figure 3. Python code.

Collection of agroclimatic variables

The monitoring system was installed in a greenhouse located in the community of San Agustín Calvario, in the municipality of Cholula, Puebla.

The monitoring was conducted for almost five months (from March to July). The equipment was installed in the middle of the greenhouse for better data recording [7] (Figure 4). Subsequently, the device was programmed to record the data of the variables every ten minutes. Given the large amount of information involved, the decision was made to analyze the recordings every hour, starting at 11:58 hours, identifying the optimal periods of each variable and the coldest and hottest hours of the day.

On the one hand, the hottest period of the day inside the greenhouse occurs between 11:00 and 15:00 hours. On the other hand, the coldest temperature occurs between 05:00 and 08:00 hours. Considering the optimal periods for each variable, graphs were developed based on monthly averages. Data was manually exported once a week using a USB drive. The data was transferred to the desktop and input into an Excel spreadsheet.

RESULTS AND DISCUSSION

This section presents the results obtained according to the information captured by the monitoring system about the agroclimatic variables.

Temperature

Temperature was recorded every hour and, from this data, the monthly average temperatures were calculated to obtain a representative value of the entire data universe. The collected information was used to develop the graph shown in Figure 5, illustrating the behavior of the "temperature" variable over the 5 months a horticultural crop would last; the resulting three stages describe the optimal and extreme conditions of greenhouse crops. The red and blue dotted lines show the optimum maximum temperatures of the day (28 °C) and the optimum minimum temperature of the night (15 °C), respectively (Figure 6). The lowest temperature of the day in the greenhouse is recorded at 05:58 hours. Consequently, the average minimum optimal temperature is not reached in any of the months and always remains below 15 °C. Nevertheless, March stood out with the

Figure 4. Installation of the monitoring system in the greenhouse.

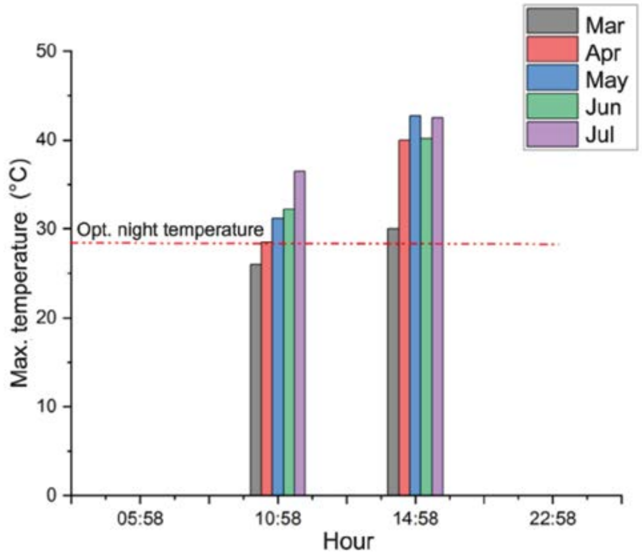

Figure 5. Maximum temperatures in the greenhouse.

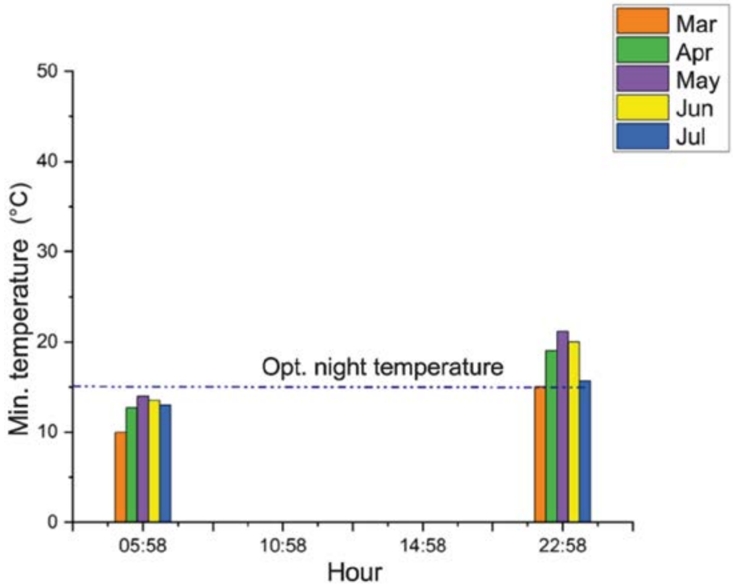

Figure 6. Minimum temperatures in the greenhouse.

lowest temperature (10 °C), followed by April (15 °C). Likewise, at 14:58 hours, all months exceed 28 °C, while at 10:58, only April falls within the optimal conditions [8]. Under these conditions, the decision should be made to lower the greenhouse temperature using a cooling system.

Relative Humidity

The relative humidity sensor shows the percentage of humidity in the environment (Figure 7). The graph is divided into three sections by two dotted lines. The blue dotted line shows the optimal relative humidity for crops during the night (90%). This condition is met

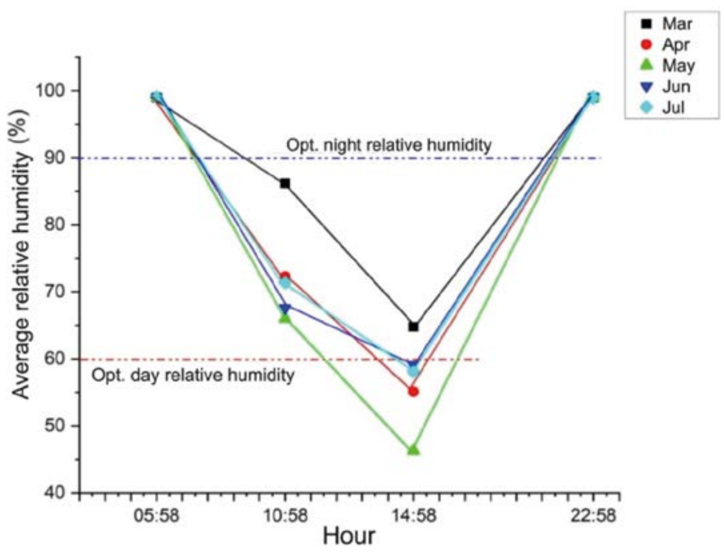

Figure 7. Relative humidity behavior.

in all months, as temperature does not affect it [9]. The red dotted line shows the optimal relative humidity during the day (60%). This condition is only met in June. In April, May, and July, the humidity falls below 60% due to the high temperatures recorded at 14:58 hours, when the maximum temperature is reached.

Light intensity

Figure 8 shows that all months meet an optimal light intensity, which is suitable for crops. Light intensity is clearly distributed throughout the day, with noticeable differences in March, when the days were shorter. For April, May, and June, light intensity distribution was affected by the daylight-saving time change in April, resulting in longer days. In July, light intensity was affected by the high frequency of afternoon storms.

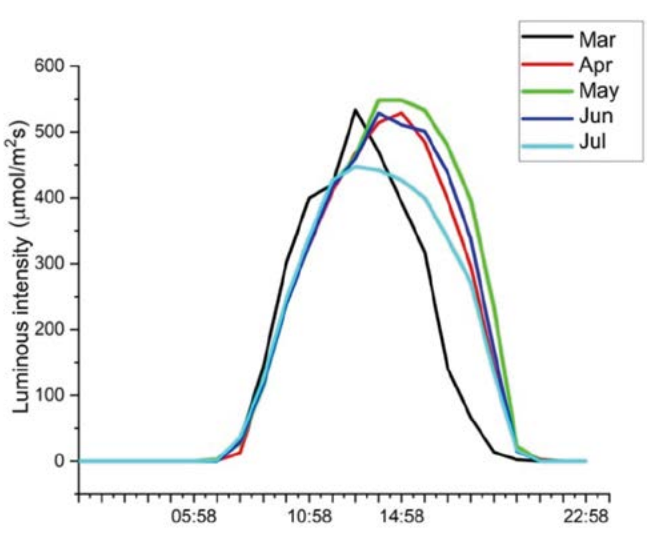

Figure 8. Behavior of light intensity in the greenhouse.

Carbon dioxide (CO_2)

This variable was measured in the greenhouse environment with an emissions sensor. As photosynthesis takes place, the plant absorbs the largest amount of CO_2. The inverse process occurs during the night: the plant no longer absorbs CO_2 and instead releases it into the environment [10].

Figure 9 shows a considerable increase in the concentration of CO_2 at night, expressed in ppm. The optimal CO_2 concentration in a greenhouse ranges between 600 ppm and 1,000 ppm [11]. In this case, the concentration fell below the 600-ppm threshold during the day and it exceeded 600 ppm at night. This is a very logical and normal phenomenon, because, during the day, the plant absorbs CO_2 and releases oxygen, while at night, the reverse process occurs.

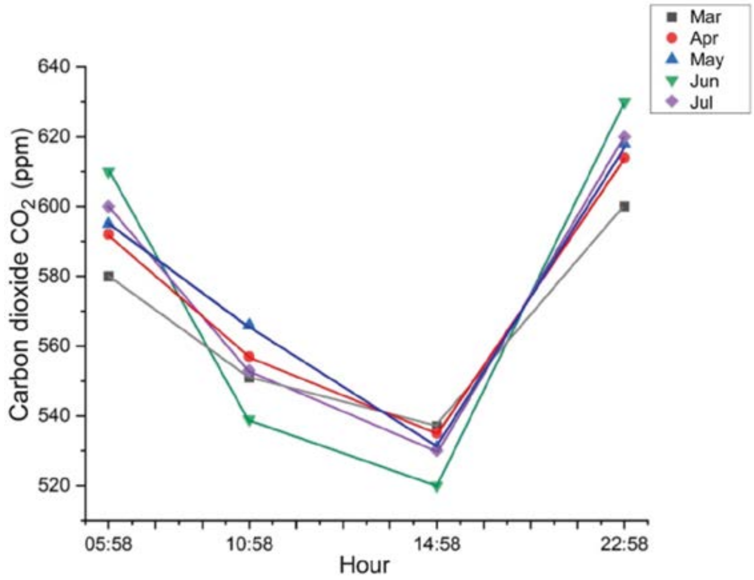

Figure 9. behavior inside the greenhouse.

CONCLUSIONS

The research team was able to design and build a low-cost electronic system and specific software for monitoring, recording, storing, and exporting agroclimatic variables that impact the development and production of crops in medium- and low-technology greenhouses. The graphs developed from the studied variables provided timely agroclimatological information that will be useful for decision-making in greenhouse crops.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Evaluation of different portions of the ImmuPlus® polyherbal additive fed to fattening lambs

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ABSTRACT

Objective: To verify if the ImmuPlus® polyherbal feed additive (*Tinospora cordifolia*, *Ocimum sanctum*, *Whitania somnifera*, *Andrographis paniculate*, and *Azadirachta indica*) improves the productive performance of finishing lambs.

Design/Methodology/Approach: Twenty Hampshire × Suffolk male lambs (initial live weight 33.6±1.9 kg) were distributed according to a completely randomized design. Treatments consisted of the inclusion of 0-1.4 g kg^{-1} of dry matter ImmuPlus® in the diets of lambs, for 35 days.

Results: The inclusion of the polyherbal mixture ($P<0.01$) improved average daily gain (ADG), final live weight (FBW), feed conversion (FC), dry matter (DM) intake; however, the said mixture did not modify the back-fat thickness or chop area ($P>0.05$).

Study Limitations/Implications: The large number of plants and bioactive compounds included in ImmuPlus® limits the identification of the components that modify the metabolism of lambs.

Findings/Conclusions: The inclusion of 1.4 g kg^{-1} dry matter of ImmuPlus® in the diet of lambs improved their productive performance.

Keywords: additive, alternative ingredients, productive performance, sheep.

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INTRODUCTION

There is an overall increase in the value of herbal products, as a result of their bioactive compound content (*e.g.*, saponins, tannins, essential oils, and flavonoids) that can positively modify ruminal fermentation, volatile fatty acids (VFAs), and methane production in the rumen (Patra and Saxena, 2010).

A wide variety of herbal products that provide benefits to livestock production is available in the market; however, many products do not include reliable information or

have not been validated. Consequently, the evaluation and validation of these products are fundamental. ImmuPlus® is a polyherbal feed additive (PFA) mixture, prepared with a combination of Indian plants, that include a concentration of antioxidants, polyphenols, and flavonoids (Khan *et al.*, 2020). The inclusion of ImmuPlus® in the diets of rabbits improved their productive performance (Pulido, 2018). Regarding bovines, it benefited their ruminal fermentation (Ponce, 2018; Razo-Ortiz *et al.*, 2020) and health (Das *et al.*, 2003). Finally, it improved the weight gain of goats (Roy *et al.*, 2003) and the IgG serum levels of dairy cattle that suffer from mastitis (Mukherjee *et al.*, 2010).

Supplementing the diets of fattening lambs with ImmuPlus® is an excellent option for the improvement of weight gain and feed conversion; additionally, it modifies the ruminal environment. Nevertheless, ImmuPlus® should be added in a 1 to 1.4 g kg^{-1} DM range (Razo-Ortiz *et al.*, 2020; Dorantes-Iturbide *et al.*, 2022), because higher doses can impact productive performance.

The benefits of the bioactive compounds of ImmuPlus® used to supplement livestock diets is an alternative for the improvement of the productive performance of fattening lambs. Therefore, the objective of this experiment was to verify and validate previous researches about the effect of the inclusion of different doses of ImmuPlus® in the productive performance and the characteristics of the carcass variables of fattening lambs.

MATERIALS AND METHODS

The experimental procedures complied with the ethical, biosecurity, and animal welfare standards of the Colegio de Postgraduados and the Mexican official standard NOM-062-ZOO-1999 (use of animals in experiments). The experiment took place in the Colegio de Postgraduados, Campus Montecillo, State of Mexico, located at 98° 48' 27" W and 19° 48' 23" N, at 2,241 m.a.s.l. The area has a 15.9 °C annual mean temperature.

A completely randomized design was used to distribute 40 male lambs (Hampshire×Suffolk), with a 33.6±1.9 kg of initial live weight. Table 1 shows that the treatments consisted of a dietary concentration of the ImmuPlus® (Nuproxa México, Querétaro, Mexico) polyherbal phytogenic: 0-1.4 g kg^{-1} DM were added to a basal diet that sought to obtain a 300 g daily weight gain (NRC, 2007). The lambs were housed in individual metabolic cages, with individual feeders and nipple drinkers. Before the experiment took place, the lambs were orally dewormed (5% Closantil®, 20 mg kg^{-1} LW) and were intramuscularly vaccinated against *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium sordelli*, *Clostridium perfringes*, *Pasteurella multocida* type A, *Pasteurella multocida* type D, and *Pasteurella haemolytica* (Bobact® 8, 2.0 Ml $lamb^{-1}$). The feed was provided at 08:00 and 15:00 h, to guarantee a 5-15% rejection. The lambs had *ad libitum* access to water and feed. The animals were subjected to a 7-day adaptation period to the experimental diets. The experiment lasted 35 days.

The following variables were evaluated: daily feed intake (DFI), average daily gain (ADG), feed conversion (FC), and final live weight (FBLW). The back-fat thickness and *longissimus dorsi* (chop) area were measured between the 12^{th} and 13^{th} ribs at day 35, using a SonoVet 600 real-time ultrasound (Medison Inc., Cypress, CA, USA), with a 7.5 Mhz transducer.

Table 1. Nutritional and ingredient composition of the experimental diets.

Ingredient (g kg^{-1})	Control	Immuplus
Corn	210.0	210.0
Sorghum	322.2	334.5
Soybean meal	116.8	117.1
Alfalfa hay	156.2	154.3
Oat staw	143.8	131.7
Cane molasses	40.0	40.0
Immuplus[†]	0.00	1.4
Common salt	1.0	1.0
Premix[¶]	10.0	10.0
Calculated nutritional composition (Base to DM)[§]		
Metabolizable energy (Mcal kg^{-1})	2.8	2.8
Crude protein (g kg^{-1})	147.0	147.0
Detergent acid fiber (g kg^{-1})	192.0	187.0
Calcium (g kg^{-1})	7.0	7.0
Phosphorus (g kg^{-1})	3.4	3.4

[†]Immuplus®, Nuproxa Mexico, Querétaro, Mexico. [¶]Composition of the pre-mixture per kg: 100 g phosphorous, 120 g calcium, 5 g iron, 1 g magnesium, 1.5 g copper, 1.2 g zinc, 0.5 g manganese, 0.5 g cobalt, 0.2 g iodine, 0.2 mg selenium, 50,000 IU vitamin A. [§] Values calculated following the recommendation of NRC (2007).

Statistical analysis

The Shapiro-Wilk and Levene tests were used to test the normality distribution and the homogeneity of variance of the variables. The data were analyzed with the GLM procedure of SAS 8.0 (2010, Statistical Analysis System Inc. Cary, NC, USA). Tukey's test was use to compare the means of the treatments ($P \leq 0.05$) resulting from the different levels of Immuplus®. The initial live weight was the covariable for weight gain and final weight.

RESULTS

Table 2 shows that the inclusion of 1.4 g kg^{-1} dry matter of ImmuPlus® in the diet improved final live weight, average daily gain, feed intake, and feed conversion ($P > 0.05$). However, the addition of ImmuPlus® did not modify ($P > 0.05$) the dorsal fat thickness and the chop area (Table 2).

DISCUSSION

Polyherbal mixtures usually include several molecules with one or more active substances or predominant nutrients, responsible for their biological effects (Frankič *et al.*, 2009). ImmuPlus® includes *Tinospora cordifolia*, *Ocimum sanctum*, *Whitania somnifera*, *Andrographis paniculata*, and *Azadirachta indica*, with a concentration of antioxidants, polyphenols, and flavonoids (Khan *et al.*, 2020).

Table 2. Dietary addition of Immuplus® on the productive performance of lambs (35 d).

	Immuplus (g kg^{-1} of DM)		**SE**	**P**
	0	**1.4**		
ILW, kg	33.25	33.96	0.60	-
FLW, kg	43.55^{b}	48.02^{a}	0.66	0.0002
ADG, kg	0.28^{b}	0.41^{a}	0.02	0.0002
Feed intake, kg	1.58^{b}	1.74^{a}	0.03	0.0008
Feed conversion	5.78^{a}	4.29^{b}	0.28	0.002
Dorsal fat thickness, mm	3.29	3.10	0.13	0.35
Chop area, mm^2	1118.70	1085.19	31.69	0.47

SE: standard error of the mean; ILW: Initial live weight; FLW: final live weight; ADG: average daily gain; DM: Dry matter. ^{a,b}Means with different literals are statistically different ($P \leq 0.001$).

O. sanctum has essential oils with antimicrobial properties, such as camphor, eugenol, and eucalyptol (Yamani *et al.*, 2016). *A. paniculata* has flavonoids and essential oils with immunomodulatory and anti-inflammatory properties (Hossain *et al.*, 2021; Kumar *et al.*, 2021). In addition, it modifies the ruminal metabolism of goats, improving nutrient digestibility (Yusuf *et al.*, 2017). *A. indica* has tannins, flavonoids, and essential oils with anti-inflammatory and antioxidant properties (Sarkar *et al.*, 2021) and it can be potentially used to feed lambs (50 mg kg^{-1} feed) (Webb *et al.*, 2022). In addition, it interacts with the rumen microbes and influences the ruminal fermentation pattern, inhibiting methane production and increasing feed digestibility (Akanmu *et al.*, 2020). *W. somnifera* has flavonoids, tannins, and alkaloids with antimicrobial and antifungal properties. This plant can modulate the immune response in chicks (Latheef *et al.*, 2013) and it is used as anthelmintic on sheep (Miaron *et al.*, 2005). *T. cordifolia* has alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarins (Upadhyay *et al.*, 2010). It modulates the immune response in chicks (Latheef *et al.*, 2013) and cows (Mukherjee *et al.*, 2010; Mallick and Prakash, 2011; Gupta *et al.*, 2016). In addition, it increases the antioxidant capacity and the cholesterol concentration in the semen (1 g kg^{-1} live weight) of rams, protecting the spermatozoa during cryopreservation (Jayaganthan *et al.*, 2013). Finally, it raises the antioxidant profiles in rats (Rawal *et al.*, 2004), helping to preserve membrane transportation and the fertilization capacity of the spermatozoa.

Supplementing the diets with some polyphenols acts as an antimicrobial agent against bacteria, protozoa, and fungi. Depending on the content (Huang *et al.*, 2018; Kalantar, 2018), type, and structure of the polyphenol, the said supplement can be beneficial or cause damage to feed consumption, nutrient digestibility, and growth (Waghorn, 2008).

The consumption of flavonoids in the diets of ruminants can potentially suppress methane production (Kalantar, 2018). Alhidary and Abdelrahman (2016) and Muqier *et al.* (2017) have proven that flavonoid supplementation increases weight gain and feed consumption in sheep. The flavonoid of some plants improves the immune response and the activity of antioxidant enzymes in blood serum (Hao *et al.*, 2020) and promotes the protein synthesis in sheep (Qin *et al.*, 2020).

Tannins can improve animal productivity (Huang *et al.*, 2018) and protein digestion. They also mitigate gas emissions produced during ruminal fermentation (Naumann *et al.*, 2017) and carry out antimicrobial and antiparasitic activities (Huang *et al.*, 2015; Malik *et al.*, 2017; Cipriano-Salazar *et al.*, 2018). Tannin supplementation improves protein digestibility, reduces energy loss resulting from the methane emission reduction, and increases the metabolic antioxidant capacity of lambs (Orzuna-Orzuna *et al.*, 2021a).

Essential oils improve the composition and functioning of the ruminal microbiota, the development of ruminal papillae in meat livestock (Zhang *et al.*, 2021), and the immune response (Wu *et al.*, 2021); in addition, they increase the VFA of the rumen (Zhang *et al.*, 2021).

Das *et al.* (2006) pointed out that ImmuPlus® is a non-toxic product that can be included in the diet of non-ruminant species, benefiting the animals. ImmuPlus® improved weight gain, feed consumption, and carcass yield in rabbits (Pulido, 2018). It improved ruminal fermentation and reduced the *in vitro* methane production of dairy cattle and sheep (Ponce, 2018; Razo-Ortiz *et al.*, 2020). In addition, ImmuPlus® increased the lgG levels in cows, helping to prevent and treat clinical mastitis (Das *et al.*, 2003). Finally, it has been used as antiparasitic in the diets of goats, improving their weight gain (Roy *et al.*, 2003).

Dorantes-Iturbide *et al.* (2022) pointed out that providing a low dose of ImmuPlus® (1 g kg^{-1} DM) to fattening lambs could promote feed conversion and increase the *longissimus* muscle area, consequently increasing their growth in the finishing stage; different results were obtained with 0, 2, and 3 g kg^{-1} DM of ImmuPlus®. Therefore, a low dose of ImmuPlus® can improve the productive performance of finishing lambs; however, high concentrations of this product reduce growth rate. In addition, the ImmuPlus® supplementation did not impact DM consumption, carcass yield, chemical composition of the meat, and health of lambs. The lack of changes in the consumption and the immune system variables suggest that the PFA did not have an impact on the welfare of the lambs.

Razo-Ortiz *et al.* (2020) evaluated different concentrations of ImmuPlus® and reported that a low dose of the product (1.4 g kg^{-1} DM) improved DWG and feed conversion, modified the ruminal environment (through pH reduction), and increased the total production of VFA and butyrate. In addition, it potentially altered the immune response of lambs. The quadratic response of the productive variables of lambs supplemented with ImmuPlus® (1, 2, and 3 g kg^{-1} DM) showed that the metabolites in the polyherbal mixture have a negative effect with $>$1.4 g/kg DM doses; nevertheless, the toxicology evaluations indicate they are safe for consumption (Das *et al.*, 2006). This hormetic response can be explained by the metabolites included in the PFA (Razo-Ortiz *et al.*, 2020).

CONCLUSIONS

The inclusion of ImmuPlus® improves the productive performance and the feed efficiency of fattening lambs.

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Colegio de Postgraduados

Low protein diets for pigs: where are we going?

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ABSTRACT

Objective: To update the trends of advantages and disadvantages of using low-protein diets (LPD) for fattening pigs, as well as providing information on the direction of LPDs in future research.

Approach: The advantages and disadvantages of reducing dietary crude protein (CP) in the diet with supplementation of synthetic amino acids (AA) have been reported in recent years, in order to generate a sustainable swine industry.

Results: The reduction of CP in diets for fattening pigs saves protein ingredients, decreases not only nitrogen excretion but also feeding costs, without affecting growth performance compared to traditional diets.

Implications: The good response of reducing the amount of CP in pig diets is not unique; there are also unwanted effects in the pigs' response to be considered. Therefore, the use of LPD for pigs needs further research to analyze the use of feed additives that maintain of improve the productive response and/or to avoid their negative effects on pork.

Conclusions: There are advantages and disadvantages of feeding pigs with LPD; however, there is the need to study the issue focusing on the reduction of the cost of feed and the protection of the environment from nitrogen excretion.

Keywords: Amino acids, Crude protein, Productive performance, Pigs.

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INTRODUCTION

In pig production, dietary crude protein (CP) content is reduced when the requirements for essential amino acids (EAA) and total nitrogen are met. In pigs, the dietary protein requirement is essentially to cover the amino acid (AA) requirements [1, 2]. In pig feed, the use of diets based on corn-soybean meal is frequent; however, due to the nature of corn, the lysine content is limited. Therefore, this traditional diet has high CP levels to meet the lysine requirements for pigs [3]. High protein diets (HPD) led to excesses of both essential amino acids (EAA) and nitrogen excretion in feces and urine. This not only reduces the efficiency of nitrogen use, but also leads to disadvantages: for example, protein fermentation in the large intestine damages intestinal health [2, 4].

Reducing dietary CP by 2 to 4 percentage units as recommended by the NRC (1998) and meeting requirements with synthetic amino acids (SAA) has been shown to increase nitrogen use efficiency, improve intestinal health, reduce feed costs and nitrogen excretion, without compromising pig growth and performance [5]. For example, in studies where LPDs were used with the addition of the four commercially available synthetic amino acids (L-lysine, DL-methionine, L-threonine and L-tryptophan), which are the first four limiting EAAs in standard corn-soybean meal diets, the productive response was similar [3]. The NRC (2012) nutrient requirement for pigs removed the recommendations for CP and replaced it with a total nitrogen requirement [6]. If the total nitrogen requirement from NRC (2012) is multiplied by the coefficient of 6.25, the CP level is 2 to 4 percentage units lower than that recommended in the previous NRC (1998) [2, 4]. However, one of the variables of greatest concern when pigs are fed LPD is the higher amount of carcass fat compared to those fed typical high protein diets (HPD) [7]. Therefore, the objective of this review was to update trends with the advantages and disadvantages of using low protein diets (LPD) for fattening pigs, as well as to provide information on the direction of LPD in future research.

APPROACH

Advantages:

Nitrogen excretion

Nitrogen excretion is the main criticism in pig production due to the negative impact on the environment, since manure contributes ammonia (NH_3) that damages ecosystems and generates unpleasant odor in farms [8]. The excess of other AAs in the standard diet in CP is broken down into nitrogen, and this surplus of nitrogen is excreted in urine as urea [9]S. An effective approach to decrease nitrogen emission is to reduce the CP content in the diet and add SAAs to cover the protein requirement of the pig. Based on the concept of ideal protein, the LPDs improve the efficiency of nitrogen utilization without affecting its digestibility and retention [10].

The results indicate that LPDs reduce water intake and urine urea nitrogen excreted [9]. Regarding plasma urea nitrogen, the main and final nitrogen product of protein catabolism, a lower concentration was also detected in pigs fed with LPDs compared to traditional protein diets [8, 11].

The odor of pig production units is bothersome to the neighboring human inhabitants. This odor is the result of anaerobic fermentation of indigestible proteins present in pig manure. Reducing CP in the diet decreases odor emission. It was reported that odor emission levels were significantly reduced by 31 and 33% for diets with 16 and 13% CP, respectively, compared to diets with 19% CP [2].

Recent studies [8, 11, 12] have been conducted to employ nitrogen sources more efficiently in pig production. First [1], the inclusion of α-ketoglutarate in a LPD with 17% CP improved productive performance in weaned piglets. Second, another nitrogen source that has been used to improve the response to a LPD is ammonia. With the addition of ammonia, nitrogen is rapidly absorbed but is poorly used for urea synthesis; however, with LPD for pigs it is used as a nitrogen source to increase the synthesis of Alanine, Citrulline and Glutamine, amino acids important for growth in pigs [8]. In sum, when the CP in the diet is reduced, pigs need an alternative nitrogen source to synthesize these metabolites, so future research will evaluate several nitrogen sources.

The intestinal microbiota is important in the digestive physiology and metabolism of pigs. Fermentation of undigested dietary proteins is associated with increased growth of coliform bacteria. Therefore, the source, quality and level of dietary protein influence gut microbial communities [11]. The influence of dietary CP levels on intestinal microbiota populations was studied in weaned piglets, since the bacterial composition structure of growing or finishing pigs was relatively stable. In a study with weaned piglets, a LPD decreased Enterobacteriaceae in feces [2,11].

Similar to the reduction of nitrogen excretion in pig farms, studies have also evaluated nitrogen sources with the aim of improving intestinal microbiota using LPDs. For example, the inclusion of α-ketoglutarate in a LPD improved gut microbiota not only by increasing the population of beneficial bacteria, *Bacteroides* and *Bifidobacterium*, but also by reducing the population of *Escherichia coli* and the amount of ammonia in the cecum. As a result, research to improve the intestinal microbiota with LPDs is expected to be a topic to develop in the near future [13].

Reduction of unpleasant odors

Crude protein (CP) in pig diets provides the animal with essential amino acids, nitrogen (N) and sulfur (S) for optimal growth and development. In a typical pig diet, due to the digestibility of feed nutrients in ingredients and the price of amino acids (AA), the CP content is often supplied in excess, which is excreted, estimated to be between 40 and 60% for N and between 50 and 75% for S in the diet [14, 15].

Excess nutrients are deposited in the feces and digested anaerobically. The main odors emitted during manure storage and transport off farms are: volatile fatty acids (VFAs); phenolic compounds; indole compounds; and volatile sulfur compounds (VSCs) [16,17]. The origins of these compounds in pig feed are related with the CP content. Consequently, reducing the loss of these nutrients in an economical manner is an important issue for the pig industry, since odor emissions are an environmental nuisance in rural communities [18].

Odor control technologies in animal production fall into four categories. First, management inside the farm (separation of urine from feces, adsorbents used as bedding additive, and indoor environment/manure surface spraying agent). Second, manure management (semi-permeable membrane coating, reactor composting, mulching and acidification). Third, end-of-pipe measures for air treatment (wet purifying of farm exhaust air and air biofiltration). Fourth, dietary manipulation (diet with low crude protein and enzyme additives in feed) [18]. Depending on availability and price, synthetic AAs are used to reduce CP levels in pig diets, without affecting productive performance [2].

The use of AAs to reduce CP is an example of innovative technology where pig producers do not need to upgrade facilities or change management practices, but only reformulate diets and use existing infrastructure to reduce emissions. Diets with lower CP content are effective in decreasing N excretion and NH_3 emissions but results in reducing odor/odorants in manure are inconsistent [19].

Early research focused on dietary impacts on fresh fecal and urine compositions, while later studies focused on dietary impacts of aged manure on air [20]. The level of CP in pig diets influences both manure properties and odor emissions. In sum, the chemical characteristics of manure from pigs fed low CP diets have lower manure pH, total solids, total N, and total S, with reduced concentrations of NH_3, VFA, and phenolic compounds. Emissions of NH_3, BCFA, phenolic compounds, and total odor are decreased in pigs fed LPDs. Emissions of NH_3, phenolic compounds, and total odor are reduced in pigs fed low CP diets [21,22]. Pigs fed a diet with low CP of 15% showed lower production of odorous compounds than the group of animals fed a 20% CP diet and this result is associated with changes in bacterial communities, especially their effect on protein metabolism [15].

Low protein diets used in pig feeding to reduce unpleasant manure odors is a novel topic, where good results have been observed when using a diet formulation for pigs under the concept of ideal protein; however, it is necessary to continue evaluating different sources and levels of protein in the diet, as well as the combination of these diets with the sources and levels of sulfur in pig feed [21,22].

Problems with pig farming in Yucatan: current conflict

Pork importing countries are: China, Japan, Mexico, South Korea, and the USA. The main exporting countries are: the European Union, USA, Canada, Brazil, and Mexico. According to the Agrifood and Fisheries Information Service (SIAP, 2021) [23], breeding of 18.8 million pigs was reported, with a production of 1,652,262 tons, which placed Mexico in 13th place in the world, with a value of 961 million dollars. The state of Yucatán is the third national producer of pork due to the large number of pig farms. There are all sizes, from very small to mega-farms with more than 30,000 pigs, many of which generate environmental and social problems; therefore, there is a great movement against pork production in the Yucatan Peninsula [24].

Arguments of the movement against pig farming in Yucatán

The Yucatan Peninsula is a place of great natural and cultural wealth, both characteristics preserved for many years thanks to the care of civil society. A general characteristic of the state of Yucatán is that it has no surface bodies of water (rivers or lakes). The scarcity of surface water is due to the fact that the soil in this region is shallow, resulting in the formation of underground rivers and cenotes. The Yucatan Peninsula is the richest reservoir of fresh underground water in Mexico. This resource is the source of drinking water for the different activities of its population.

During recent decades, pork production in Yucatán has increased. Pig farms are an important source of pollution, as pigs produce a huge amount of feces. The main pollutants are: ammonium (NH_4), nitrites (NO_2) and nitrates (NO_3). The farms do not comply with the maximum permissible limits of pollutants in residual discharges into water and national assets (NOM-001-SEMARNAT-1996 for the protection of aquatic life) [25], as established in NOM-127-SSA, which dictates the recommended limits for human use and consumption. Therefore, the movement against pork production in Yucatán proposes to adopt the international recommendation; that is, that there be one pig for each hectare of land, and not to issue more permits for the establishment of pig farms in certain municipalities [26].

Arguments of the pro-pig farming movement in Yucatán

Pork production in Yucatán is one of the most prestigious in the country, since it is recognized for its high genetic quality production. Yucatán is self-sufficient in pork production; it is the third largest national producer of pork. Yucatán is one of the entities in the country that supplies the largest export tonnage to countries such as China, Japan, South Korea, the United States, Canada and Chile, and also supplies the entire Mexican southeast, thanks to its high quality standards. In Yucatán there is international experience in the marketing of pork and in the management of farms, since it has a privileged location and it is a growing activity [27].

The millenary importance of pork in the state is transcendental in the gastronomy of the emblematic dishes of Yucatec cuisine; for example, cochinita pibil, which is a dish with added value for tourism. Pig farming in Yucatán is an important source of employment, since 7,000 families, who live mainly in rural areas, depend directly on this sector, which has registered sustained growth in recent years. Likewise, pig farms in Yucatán are not located in Natural Protected Areas (NPA). Livestock activity in the Yucatan Peninsula only represents 0.67% of the water discharge. The establishment of biodigesters is a common practice of pig farmers in Yucatán [23, 26, 27].

LPD in pigs: a real alternative for pig farming in Yucatán

The main issues to improve sustainability in pork production in Yucatán are to reduce nitrogen and phosphorus excretion into the environment [24, 27]. Because of the importance and extent of phosphorus excretion in pork production, this will be a pending issue for the authors for a future review. In this article, the problem was only mentioned in relation to nitrogen excretion, with a current issue in Mexico such as pork production in Yucatán [24, 26].

In the controversy in Yucatán, both sides, for and against pork production, have solid arguments to maintain their position. However, neither of them mentions the formulation of LPDs as a real and scientifically supported alternative to reduce nitrogen excretion in pork production in Yucatán [24, 26, 27].

Currently, five synthetic EAAs (lysine, methionine, threonine, tryptophan, and valine) are used, which allows nutritionists to formulate diets with low levels of crude protein. According to the review by Rocha *et al.* (2022) [28], there is a minimum level of CP in the diet that does not affect the productive performance of pigs, which is different for each stage of production. When the average daily gain and feed efficiency were considered, respectively, these levels were estimated to be 18.4 and 18.3% of CP in diets for weaned piglets. For growing pigs, 16.1 and 16.3% and for finishing pigs 11.6 and 11.4% of CP.

Reducing dietary CP is a solution to reduce N excretion and ammonia emissions, which are problems in pork production. According to Cappelaere *et al.* (2022) [29], the use of

LPD linearly reduces total N excretion, in urine, feces and ammonia emissions by 8.2, 10, 3.2 and 10%, respectively, per percentage point of CP reduction. Likewise, nitrogen efficiency in LPDs improves by 1.6% per percentage point of CP reduction in pig diets. Therefore, the positive impacts of CP reduction and the decrease in N excretion in pig production is a novel topic with a trend of continuous innovation to generate sustainable and practical production to solve current problems [12, 30].

The addition of AA to reduce the amount of CP is an example of innovative technology where pig farmers in the Yucatan Peninsula do not need to modernize facilities or change management practices, but only reformulate diets and use existing infrastructure to reduce emissions [28, 29] . The use of LPDs will help reduce pollution from nitrogen excretion, and will ensure sustainability in this area of Mexico. This will happen with the collaboration of producers, government, civil society and the scientific community.

Disadvantages:

Productive performance

Productive performance, which includes daily gain (DWG), daily feed intake (DFI), and feed efficiency (FE), is the first factor to evaluate LPDs and also a simple indicator to determine their performance [10, 31, 32]. Reducing CP by 3 percentage units in the diet within the NRC (1998) requirement, with the addition of the first four limiting amino acids (LAA), resulted in a similar productive performance compared to traditional growing-finishing pig diets [33]. However, conflicting results have been reported on the response to a reduction greater than 3 percentage units of CP. For example, productive performance was reported to be reduced when the level of CP decreased more than 3 percentage units [34].

To improve the productive response, it is necessary to include the following limiting AAs in typical corn-soybean meal diets: valine (Val) and isoleucine (IIe) [33]. When growing pigs (25-50 kg LW) are fed LPD, Val is more limiting than IIe, while Ile becomes the limiting AA for finishing pigs (75-125 kg) [3]. Reduction of more than 6 percentage units in the level of CP is possible when supplementing the diet with all EAAs without affecting the productive performance in pigs, implying that non-essential amino acids (NEAAs) were not limiting in LPDs for growing and finishing pigs [36]. Poor performance in pigs fed with very low protein diets is due to deficiency in intact protein or excess of free AAs. Therefore, the inclusion of protein-bound AAs (intact protein) was reported to be more effective in maintaining nitrogen retention and body protein homeostasis than free AAs [32,35]. Therefore, the use of LPDs is still an unfinished issue if the aim is to reduce CP by more than 4 percentage units. Future research indicates that the addition of di- and tri -peptides hydrolyzed from intact protein is a viable option to improve digestive enzyme activities and, therefore, productive performance.

Carcass quality

It is debatable whether LPDs added with SAAs affect carcass characteristics. The relationship between carcass weight and pre-slaughter live weight is not affected by the reduction of the dietary CP level. However, a constant increase in dorsal fat thickness and reduction in the *Longissimus* muscle area at slaughter was found with LPDs for growing and finishing pigs [3].

Three things are considered responsible for these results: 1) More energy is required for the excretion of excess AAs in a high protein diet. In contrast, LPDs are closer to the ideal AA ratio, so the energy needed for N excretion is lower; thus, there is more energy for adipose tissue synthesis. 2) The weight of the liver, intestine, kidney and pancreas increases in pigs fed with a high protein diet; as a result, energy requirements for maintenance increase. 3) The higher proportion of cereals in the LPD contributes a high content of available starch that is more efficient for fat deposition compared to AAs [7, 36].

To avoid the negative effects of LPDs on carcass characteristics, many studies have been carried out. First, the inclusion of some functional AAs with potential to reduce excessive body fat, for example: leucine, arginine, glutamine, glutamate and proline. Second, the system of formulating diets based on net energy to substitute digestible or metabolizable energy. Third, covering the nitrogen needs in the diet with sources of rapid absorption but low urea synthesis. Fourth, the use of growth promoters that reduce fat synthesis [36].

Where are we going?

Future research will focus on identifying and testing feed additives that maintain and/or improve the productive response of finishing pigs fed with LPDs supplemented with SAAs, such as phytobiotics, prebiotics, probiotics and enzymes, among others [10, 12, 32]. It will also focus on testing different sources of nitrogen/protein apart from the protein from the most commonly used (main) ingredients in pig diets. And finally, on the effect of both feed additives and different nitrogen sources on the microbial populations of the gastrointestinal tract of pigs and their productive response.

CONCLUSIONS

The practical application of LPDs is a recommended strategy in pig production, not only to reduce feed costs and nitrogen excretion but also to effectively improve intestinal health with a better microbiota. However, if one wishes to reduce CP by more than 3 or 4 percentage units, productive performance can be negatively affected. More research is required on the addition of EAAs in order not to affect the response of pigs. Likewise, it is necessary to use the net energy system in diet formulation, to provide this nutrient in amounts closer to the requirements of pigs, with which leaner carcasses could be obtained.

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Colegio de Postgraduados

Zinc oxide nanoparticles *vs.* Zn-EDTA in the growth and production of strawberry crops (*Fragaria×ananassa* Duch)

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ABSTRACT

Objective: The objective of this study was to evaluate and to compare the effects on growth between zinc oxide nanoparticles (ZnO NPs) and Zn-EDTA, nutrient concentration of zinc in leaf and fruit, and strawberry production.

Design/methodology/approach: Strawberry plants were used, established in plastic bags with a mixture of substrate, peat and agrolite. Two factors were evaluated: first, the concentration of ZnO NPs (100, 200, 500, 1000 mg L^{-1}), plus treatments with Zn-EDTA and a control; and the second, form of application (foliar and to the substrate). A completely randomized increased factorial design was used. Growth was measured, leaf and fruit zinc concentration was determined, the number of flowers and weight of fruits per plant, and the variables of firmness and total soluble solids of fruits were quantified.

Results: The results obtained indicated that the foliar application of the 200 mg L^{-1} dose of ZnO NPs caused the greatest plant height, as well as the highest number of flowers, crowns and fruits per plant, and the greatest production. The highest leaf zinc concentrations within the sufficiency interval were observed with the leaf application of 200, 500 and 1000 mg L^{-1} of ZnO NPs.

Findings/conclusions: The study allows inferring that the use of zinc oxide nanoparticles is an alternative source of fertilizer with respect to conventional Zn sources to improve growth, leaf zinc concentration and fruit production in strawberry.

Keywords: Nanotechnology, plant nutrition, zinc, *Fragaria×ananassa* Duch.

INTRODUCTION

Strawberry fruits (*Fragaria×ananassa* Duch) are among the most appreciated and consumed in the world due to their nutritional, organoleptic and nutraceutical properties (Liu *et al.*, 2018). Its cultivation is of great economic importance for Mexico, ranking fourth in worldwide production and as the main exporter of strawberry fruit to the United States (FAOSTAT, 2022).

As with any plant species, mineral nutrition through the application of agrochemicals is necessary to complement the natural fertility of the soil; however, in recent years, given the incorrect technical views or recommendations in a good number of production areas, there has been an indiscriminate use of chemical fertilizers, causing damage to production, the environment and human health (Davarpanah *et al.*, 2016). Therefore, it is necessary to look for alternatives to maintain productivity without affecting the quality of the fruit and, in turn, reduce environmental damage.

Nanotechnology applied to agriculture arises as an emerging technology focused on sustainable crop production, using various strategies (Lira Saldivar *et al.*, 2018). One of them is the production and use of nanometric-sized fertilizers, seeking to make the use of agrochemicals more efficient and sustainable (Singh *et al.*, 2018). Examples of these are carbon-based organic nanomaterials, nanostructured from zeolite as carriers for nitrogen, phosphorus and potassium, and nanofertilizers developed from metallic nanoparticles (NPs), such as zinc oxide, iron, copper, titanium dioxide and selenium (Singh *et al.*, 2018).

Specifically, zinc oxide nanoparticles (ZnO NPs) have gained great interest in the agricultural sector, since Zn plays a vital role in the physiological and anatomical responses of plants (Amezcua Romero and Lara Flores, 2017). The effects of the use of these nanoparticles on the growth and physiology of crops such as chickpea (*Cicer arietinum*), coffee (*Coffea arabica* L.) and chili pepper (*Capsicum annum*) have been documented (Pavani *et al.*, 2014; Rossi *et al.*, 2019; García-López *et al.*, 2019).

In order to verify the viability of ZnO NPs as a fertilization alternative, it is necessary to conduct comparative studies to determine the efficiency of nanofertilizers (ZnO NPs) versus that of conventional fertilizers (Zn-EDTA). Therefore, the objective of this study is to compare the effect of foliar and substrate application of ZnO NPs and Zn-EDTA on the growth, foliar zinc concentration, and production of strawberry.

MATERIALS AND METHODS

The study was conducted at the Experimental Fruit Field of the Montecillo Campus, Colegio de Postgraduados, located in Montecillo, Texcoco, Estado de México, from April 14, 2021, to March 2022. The experiment was carried out under a chapel-type greenhouse, with a 25% opacity milky plastic covering, surface area of 60 m^2, without controlled air conditioning; the average temperature conditions inside the greenhouse ranged between 15 and 23 °C (Figure 1), and the photosynthetically active radiation values ranged between 700 and 900 μmol m^{-2} s^{-1} measured at noon, except on cloudy days when radiation values decreased.

Short photoperiod strawberry plants of the 99.717 selection were used, developed at the National Institute of Forestry, Agricultural and Livestock Research (INIFAP), medium size, vigorous and medium-sized fruits, which were transplanted in 10 L black polyethylene bags, in a substrate prepared from a mixture of soil, peat and agrolite, in a 2:2:1 ratio, with pH of 5.9, electrical conductivity of 0.4 dS m^{-1}, organic matter of 33.1%, and good fertility (Table 1 and Figure 4).

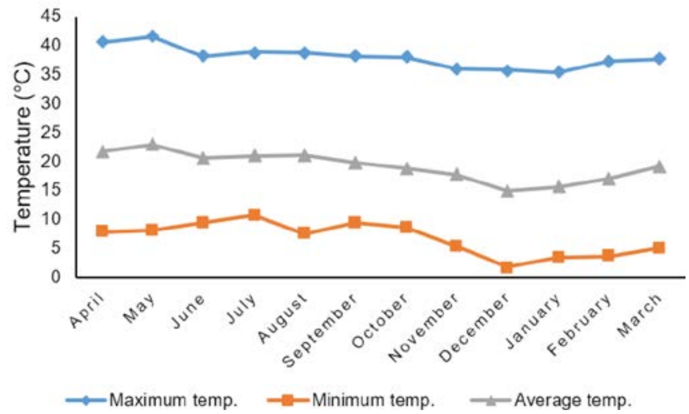

Figure 1. Record of average, maximum and minimum monthly environment temperatures during the experimentation period with greenhouse strawberry (April 2021-March 2022).

Irrigation was carried out using a pressurized drip system, by which fertigation was also carried out with Steiner's nutrient solution (1984) at 50%, with an EC of 1 dS m^{-1} and pH of 5.5, excluding the element zinc, while maintaining the required balance of anions and cations in the solution. Two applications of LOBI® 44 foliar fertilizer (2.5 g L^{-1}) were carried out to correct nitrogen deficiency, applied during growth and flowering.

The experiment used a completely randomized increased factorial design with eight replications, where the variation factors were: form of application (foliar and on substrate); and fertilization levels: dose of ZnO NPs (100, 200, 500 and 1000 mg L^{-1}) and Zn-EDTA (2.5 mL L^{-1} of water) as a conventional source of fertilization. The complete factorial design (2×5) was extended to accommodate a control, which consisted of not applying zinc, forming a 2×5+1 factorial; one plant per bag was considered as an experimental unit.

Zinc oxide nanoparticles (ZnO NPs) were used, provided by the Applied Chemistry Research Center (CIQA) in Saltillo, Coahuila, which have a polyhedral morphology and a diameter size between 20 to 40 nm (Figure 2).

Solutions with ZnO NPs and Zn-EDTA were prepared in deionized water and dispersed with a Branson 2510 sonicator for 30 minutes, in two 15-minute periods, separated by a

Table 1. Physicochemical analysis of the substrate prior to experimentation.

Chemical analysis													
pH	EC (dS m^{-1})	OM (%)	N - NH_4^+	N - NO_3^-	P	K	Na	Fe	Cu	Zn	Mn	Ca	Mg
			(ppm)									(meq 100 g^{-1})	
5.9	0.4	33.1	25	212	26	1.0	3.3	111	0.7	3	22	27	12

Physical analysis					
Total porosity %	Aeration porosity %	Moisture retention %	Bulk density Mg/m^3	Field capacity %	Permanent wilting point %
78	32	43	0.31	78	45

EC=Electrical conductivity, OM=organic matter, N-NH_4^+ =ammonium, N-NO_3^- =nitrate.

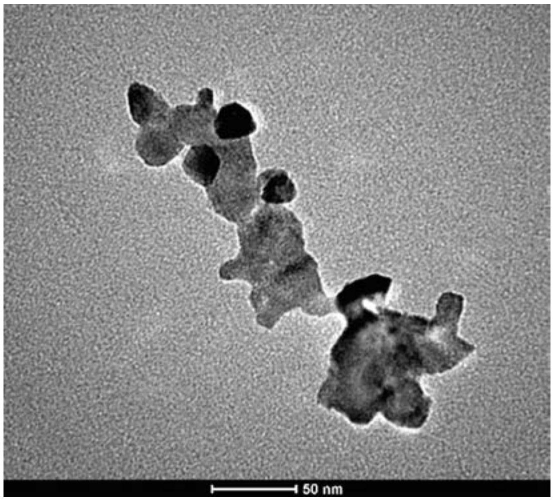

Figure 2. Morphology of ZnO NPs observed by transmission electron microscope (in bright field), Tecnai 2 Spirit brand, Thermo Fisher Fei Company, USA, operated at 120 keV. Electron Microscopy Unit at Colegio de Postgraduados (CP-UME), Montecillo, Texcoco, Mexico.

rest period of 5 minutes. A commercial adjuvant (DAP PLUS) was used for its application. Foliar treatments were applied by spraying 30 mL of solution per plant on the foliage and 30 mL of solution per bag on the substrate, every 15 days, starting on day 51 after transplant (June 4, 2021).

The variables under study were: a) **Plant height**, measured in centimeters at the end of the production cycle, using a tape measure from the base of the stem (crown) to the highest leaf of the plant. b) **Number of crowns per plant**, which were counted at the end of the production cycle, when they could be separated. c) **Number of flowers per plant**, by weekly counts to determine the total accumulated number of flowers during the production cycle. d) **Number of fruits per plant**, which was quantified periodically when the fruits reached harvest point (2/3 coloration); at the end of the cycle, the accumulated total was determined. e) **Fruit production per plant**, which was obtained by weighing all the fruits harvested during the experiment. f) **Zinc concentration in leaves and fruits per plant**, leaf samples (petiole together with leaflets) were taken during vegetative growth (June 14, 2021), flowering (November 22, 2021), and fruiting (February 16, 2022). The samples were washed with deionized water and dried in a forced air oven at 70 °C for 48 to 72 h, until a constant dry weight was obtained. Subsequently, each sample per plant was ground; then, 1 g of each pulverized sample was subjected to wet digestion with a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 2:1 (v/v) ratio. The liquid digest obtained was titrated with deionized water, from which samples were taken to be diluted and subjected to atomic absorption measurement with a GBC brand spectrophotometer, model SAVANTA PM, series FTSLA06. The fruit samples were analyzed in a similar way. g) **Fruit firmness**, measurements were carried out with a texturometer (Wagner, model FDV-30) with 8 mm conical strut; readings were taken on opposite sides at the equator of the fruit, thus obtaining the average value per plant. The data were expressed in Newtons (N). h) **Total soluble solids in fruits**, were determined in °Brix, using a digital refractometer model ATAGO-Pelette with a scale of 0 to 32%, using two drops of fruit juice per plant.

The data obtained were analyzed with generalized linear mixed models (GLIMMIX), and Tukey's means comparison was made ($P \leq 0.05$). The statistical analyses were performed with SAS software version 9.4.

RESULTS AND DISCUSSION

Plant height. Foliar application with 200 mg L^{-1} of ZnO NPs significantly increased the height compared to the control and the treatments with Zn-EDTA applied foliarly and on the substrate (Figures 3 and 4).

The increase in height caused by treatment with ZnO NPs may be due to the fact that zinc is involved in the formation of tryptophan, a precursor to the synthesis of auxins, associated with cell division and elongation, which stimulate growth in plants (Amezcua Romero and Lara Flores, 2017). Furthermore, nanometric-sized zinc particles can be absorbed by the plant (Rossi *et al.*, 2019) and penetrate more quickly than larger particles (Prasad *et al.*, 2012).

These results are consistent with those reported by Kumar *et al.* (2017), who found that strawberry plants treated with ZnO NPs plus FeO NPs showed greater growth. Similarly, the application of ZnO NPs increased the height of chickpea, habanero chili, coffee and tomato plants (Pavani *et al.*, 2014; García-López *et al.*, 2019; Rossi *et al.*, 2019; Pérez Velasco *et al.*, 2020).

Number of crowns, flowers and fruits. A significant increase in the number of crowns (NC) was found with the different concentrations of ZnO NPs applied foliarly and on the substrate, obtaining averages of up to 10 crowns $plant^{-1}$, with the 200 mL L^{-1} treatment being statistically different from the treatments with foliar Zn-EDTA and the control (Table 2).

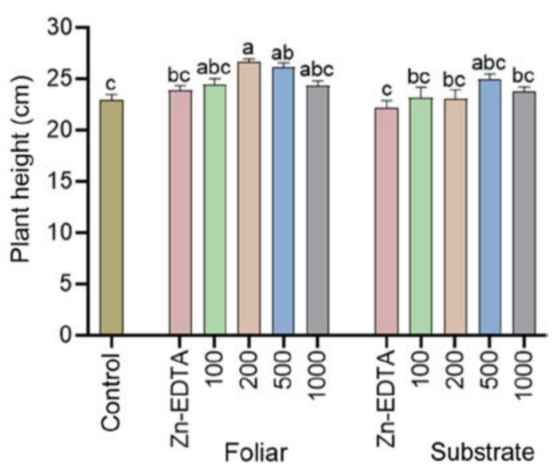

Figure 3. *Fragaria×ananassa* Duch. plant height grown under greenhouse conditions, treated with different doses of ZnO NPs and Zn-EDTA applied foliarly and on the substrate [Nanoparticle doses are expressed in mg L^{-1} and Zn-EDTA in mL L^{-1}; the vertical bars represent standard error (n=8). Bars with the same letter are statistically equal (Tukey, $P \leq 0.05$)].

Regarding the number of flowers (NFL), the concentration with 200 mg L^{-1} of ZnO NPs applied foliarly produced 113 flowers $plant^{-1}$ which was statistically different from the number of flowers produced with the foliar treatments and the Zn-EDTA substrate, as well as from the control, although not different from the applications of NPs to the substrate (Table 2 , Figure 4).

On the other hand, foliar application of ZnO NPs significantly influenced the number of fruits (NFR) per plant, positively correlating with the results obtained in case of number of flowers. Fruits increased with the foliar application of 200 mg L^{-1} of ZnO NPs, which was significantly different from the other treatments and the control (Table 2).

Table 2. Number of crowns, flowers and fruits per strawberry plant grown under greenhouse conditions, treated with different doses of ZnO NPs and Zn-EDTA applied foliarly and on the substrate.

Application	Treatments[3]	NC[4]	NFL	NFR
	Control	[2]6.6±0.18c[1]	93.2±4.18bc	75.9±9.00d
Foliar	Zn-EDTA	7.4±0.25bc	89.0±4.81c	76.5±5.18cd
	100	10.0±0.38a	107.4±11.69ab	91.6±4.66ab
	200	10.0±0.27a	113.0±10.38a	97.9±3.94a
	500	9.2±0.45a	97.0±11.95bc	91.6±14.04ab
	1000	9.1±0.44a	94.1±4.64bc	80.9±9.21bc
Substrate	Zn-EDTA	8.5±0.38ab	93.7±6.14bc	76.4±6.87cd
	100	9.1±0.29ab	99.4±5.03abc	77.1±7.25cd
	200	9.7±0.31a	98.5±7.13abc	81.4±14.91bc
	500	9.7±0.31a	100.9±5.32abc	80.5±6.82bc
	1000	9.0±0.50a	100.1±7.08abc	88.9±11.46abc

[1] The values followed by the same letter are not statistically different (Tukey, P≤0.05).
[2] Average values ± standard error.
[3] Nanoparticle concentrations are expressed in mg L^{-1} and Zn-EDTA in mL L^{-1}.
[4] NC=number of crowns; NFL=number of flowers; NFR=number of fruits.

Figure 4. Strawberry plants (*Fragaria*×*ananassa* Duch.) in development, showing in the image on the right the beginning of flowering which happened in the fall, given the short photoperiod habit of the selection under study.

The number of crowns in this crop is an indicator of vigor and rapid growth (Bish *et al.*, 2002) , since the crowns, as the growth center of strawberry, have the function of regulating the metabolic activities of the plant (Hancock *et al.*, 2008). A greater number of crowns correlates positively and significantly with plant growth, partly due to the fact that Zn participates in cell division (Rahman *et al.*, 2016) and that, as already mentioned, Zn assimilation is more efficient when nanometric-sized particles are used (Prasad *et al.*, 2012).

Zn is involved in the formation of flowers and fruits (Rahman *et al.*, 2016), since it is involved in genetic expression by being part of transcription factors related to the regulation of flower development (Amezcua Romero and Lara Flores, 2017). The increases in the number of flowers obtained are similar to those found by Kumar *et al.* (2017) and Mahmood and Al-Dulaimy (2021), agreeing that applications of zinc in nanometric size increase the number of flowers in strawberry plants.

Consequently, an increase in the number of fruits is also expected, due to more flowers (Amezcua Romero and Lara Flores, 2017). The results obtained coincide with those found by Davarpanah *et al.* (2016), García-López *et al.* (2019), Faizan and Hayat (2019), and Mahmood and Al-Dulaimy (2021), whose studies show that plants exposed to foliar application of ZnO NPs produce a greater number of fruits in pomegranate, chili pepper, tomato and strawberry, respectively.

Fruit production. Given the above results, plants exposed to foliar application with 200 mg L^{-1} of ZnO NPs showed a statistically different fruit production, compared to that obtained in the other treatments and the control (Figure 5).

Zn in nanometric size has greater assimilation, which facilitates the penetration capacity in the leaf (Prasad *et al.*, 2012). Treatments with different concentrations of nanoscale zinc oxide (ZnO NPs) leads to improved plant metabolism, increased growth, and increased fruit production (Davarpanah *et al.*, 2016). Thus, Faizan and Hayat (2019) found that foliar applications with 50 mg L^{-1} of ZnO NPs increase tomato yield; Subbaiah *et al.* (2016) point out that foliar applications with 400 mg L^{-1} of ZnO NPs

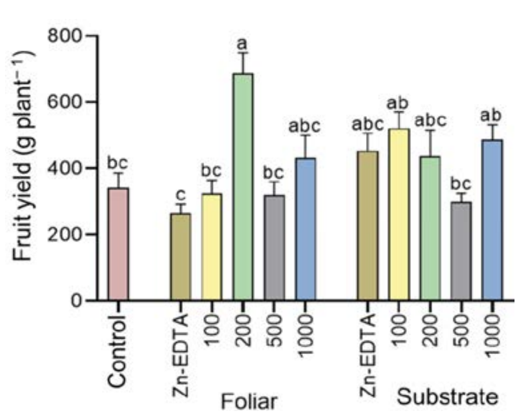

Figure 5. Fruit production of *Fragaria×ananassa* Duch. grown under greenhouse conditions, treated with different doses of ZnO NPs and ZnEDTA applied foliarly and on the substrate [Nanoparticle doses are expressed in mg L^{-1} and Zn-EDTA in mL L^{-1}. Vertical bars represent standard error (n=8). Bars with the same letter are statistically equal (Tukey, $P \leq 0.05$)].

increase corn yield; similar results were obtained in peanut and melon (Prasad *et al.*, 2012 ; Rivera-Gutiérrez *et al.*, 2021).

Zinc concentration in leaves. The results revealed that during vegetative growth there are no significant differences in foliar Zn concentration between treatments with ZnO NPs application and conventional fertilization treatments (Zn-EDTA), or without Zn application (Table 3). In the flowering stage, the control plants without Zn application had the lowest foliar concentration of Zn (18.83 μg g^{-1}), while the foliar treatments of 500 and 1000 mg L^{-1} of ZnO NPs reached the highest foliar concentrations of Zn (Table 3); however, it should be noted that these are excess foliar concentrations of Zn, as opposed to those determined for doses of 100 and 200 mg L^{-1}, which were within the range of Zn sufficiency (Becerril and Jaen, 2010) and are statistically equal to those observed in treatments with the highest doses of ZnO NPs. For the fruiting stage, plants treated with foliar and substrate doses of ZnO NPs had foliar concentrations of Zn significantly higher than the control (Table 3).

The results obtained confirm an increase in the concentration of zinc in strawberry leaves, in plants treated with ZnO NPs, which in principle indicates that the assimilation of Zn can be faster and more efficient by this method of application, and more so when nanometric-sized particles are used (Prasad *et al.*, 2012). These results agree with those found by Subbaiah *et al.* (2016) who point out that foliar applications with ZnO NPs increase foliar zinc concentration in corn, coinciding with what has been found in coffee and barley crops (Rossi *et al.*, 2019; Rajput *et al.*, 2021).

García-Gómez *et al.* (2020) indicate that high doses (>1000 mg L^{-1}) of ZnO NPs can be phytotoxic to plants and limit their yield; in this study the plants did not show symptoms of toxicity at concentrations of 500 and 1000 mg L^{-1} of ZnO NPs, even though the zinc

Table 3. Foliar zinc concentrations in strawberry plants grown under greenhouse conditions treated with different doses of ZnO NPs and ZnEDTA applied foliarly and on the substrate.

Application	Treatments[3]	Vegetative growth	Flowering	Fructification
		μg g^{-1} dry weight		
	Control	[2] 21.0±3.62a [1]	18.83±1.59c [1]	22.67±4.13b [1]
Foliar	Zn-EDTA	22.5±2.02a	21.83±6.93c	29.50±9.84ab
	100	21.5±2.31a	51.67±9.27abc	70.33±2.69ab
	200	32.0±6.65a	52.33±12.93abc	63.17±1.77ab
	500	31.6±5.35a	140.17±28.29a	106.00±197.8a
	1000	27.3±3.32a	125.00±18.05ab	103.33±32.49a
Substrate	Zn-EDTA	32.0±6.36a	23.67±0.44c	34.33±6.65ab
	100	22.3±0.73a	23.17±1.30c	34.50±3.55ab
	200	46.0±15.32a	21.83±1.30c	32.83±7.10ab
	500	26.0±4.62a	27.83±0.44bc	33.67±4.29ab
	1000	22.5±6.38a	24.50±3.61c	33.33±1.67ab

[1] The values followed by the same letter are not statistically different (Tukey, $P \leq 0.05$).
[2] Average values ± standard error.
[3] Nanoparticle concentrations are expressed in mg L^{-1} and Zn-EDTA in mL L^{-1}.

concentration was higher than the zinc sufficiency levels in leaf tissue, which according to Becerril and Jaen (2010), is 5-100 μg g^{-1} for strawberry crops.

Zinc concentration in fruit. The data showed an increase in the zinc concentration in the fruits with ZnO NPs and Zn-EDTA applications foliarly and on the substrate; however, there were no differences between treatments and the control (Figure 6).

Firmness and total soluble solids of fruits. The means comparison did not indicate significant differences between treatments for firmness, nor for total soluble solids of the fruit (Figure 7), although the values of both variables increased with the application of Zn.

Based on the results obtained, it is possible to predict that the use of ZnO NPs could be an alternative source of fertilizer, which, due to their size, is more efficiently absorbed than conventional Zn sources, and which could reduce possible losses when used in smaller quantities, with the consequent lower environmental impact in terms of pollution, in favor of improving the quality of crops.

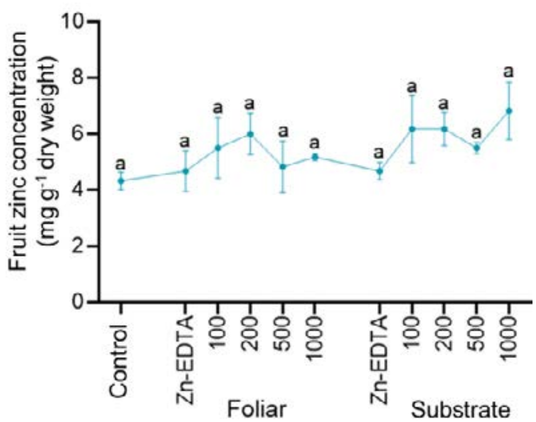

Figure 6. Zinc concentration in *Fragaria×ananassa* Duch. fruits from the effect of foliar or substrate applications of zinc oxide nanoparticles and Zn-EDTA. [Nanoparticle concentrations are expressed in mg L^{-1} and Zn-EDTA in mL L^{-1} . Vertical bars represent standard error (n=6). Bars with the same letter are statistically equal (Tukey, P≤0.05)].

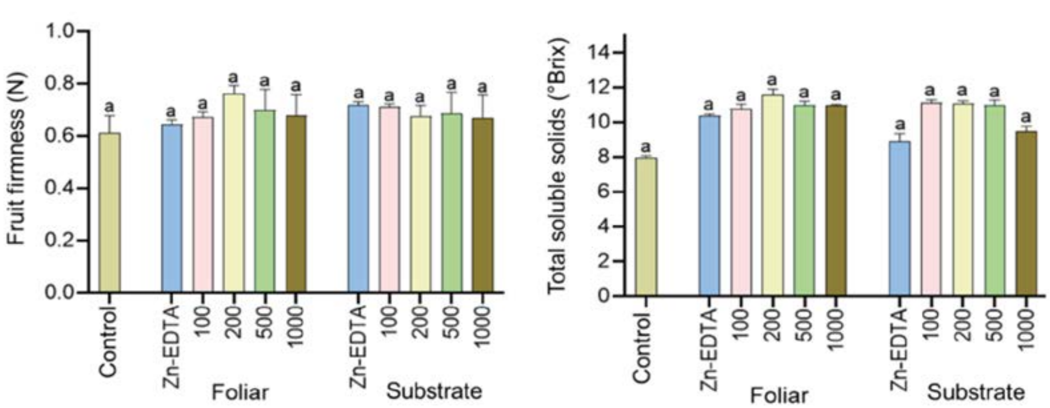

Figure 7. Fruit quality variables of *Fragaria×ananassa* Duch. grown under greenhouse conditions, treated with different doses of ZnO NPs and Zn-EDTA applied foliarly and on the substrate [Nanoparticle doses are expressed in mg L^{-1} and Zn-EDTA in mL L^{-1}. Vertical bars represent standard error (n=4). Bars with the same letter are statistically equal (Tukey, P≤0.05)].

The results obtained also demonstrate the potential of ZnO NPs applied foliarly to improve the growth and productivity of strawberry crops, since, in the case of Zn, it is required in smaller quantities and, in the vast majority of cases, its applications are complementary. Consequently, ZnO NPs can be an alternative to improve crop productivity, depending on the species and the concentration of nanoparticles applied. In the case of soil applications, it is necessary to carry out studies in field conditions to find out how Zn is distributed in the soil and to analyze its availability for the plant.

Overall, based on experimental evidence, nanometric fertilizers have great potential to improve the efficiency and sustainability of agriculture while reducing the use of conventional fertilizers, particularly in future precision agriculture systems. Therefore, it is important to continue researching and evaluating their safety and efficacy before their large-scale implementation, since it is also necessary to consider the potential risks, challenges, costs and profitability associated with their use, the appropriate regulation of their production and application, as well as the acceptance of this technology.

CONCLUSIONS

This study suggests that the application of ZnO NPs improves the development of strawberry plants with foliar dose applications of 200 mg L^{-1}, since they promoted plant growth and increased the number of crowns, flowers and fruits per plant, as well as fruit production.

High doses of nanoparticles can cause an increase in the leaf concentration of zinc, but they generate excess levels which could cause phytotoxicity, while doses of 100 and 200 mg L^{-1} promote levels within the sufficiency range.

The application of Zn fertilization positively affected the fruit quality variables studied.

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Colegio de Postgraduados

Characterization of the scientific production of *Moringa oleifera* Lam. for the period 2013-2023

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ABSTRACT

Objective: This study aims to map the scientific production of *Moringa oleifera* in the Scopus database during the period 2013-2023.

Design/methodology/approach: The bibliographic data of moringa publications was downloaded and data was characterized through a bibliometric analysis. The analysis identified key metrics, including the most productive countries, journals and authors with the highest number of publications and citations, topic niche, basic topics, emerging or declining topics, and motor topics.

Results: For the period 2013-2023,a total of 5680 documents were published, categorized as scientific articles (80.21%), bibliographic reviews (8.68%), conference papers (6.78%), book chapters (1.81%) and books (0.11%). Most of the research is embedded in areas such as: agricultural and biological sciences (21.92%), biochemistry, genetics and molecular biology (10.71%) and pharmacology, toxicology and pharmaceuticals (10.51%).

Limitations on study/implications: This study is limited to the Scopus database, which means that additional publications on *Moringa oleifera* from the 2013-2023 period may exist in other databases not included in this analysis.

Findings/conclusions: The bibliometric analysis provided insights into the scientific contributions related to *Moringa oleifera* and supports the development of research on more relevant and hot spots topics.

Keywords: moringa, research trends, bibliometrics.

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INTRODUCTION

Moringa oleifera Lam. is a species domesticated in India (Olson, 2017) and has spread into tropical and subtropical countries (Sanjay and Dwivedi, 2015). This plant is drought-resistance and can be grown in soils with low nutrient availability (Muhl *et al.*, 2013). Its high acclimation capacity and its nutritional, medicinal, forage, biofertilizer, bioinsecticide, flocculant, energy, and bioadsorbent potential have increased its global importance (Abd El-Hack *et al.*, 2018). The leaves contain proteins, lipids, carbohydrates, fiber, vitamins, minerals, and phenolic compounds (Gopalakrishnan *et al.*, 2016). The consumption of moringa leaves prevents various diseases associated with nutrient deficiencies (Matic *et al.*, 2018). Additionally, moringa is characterized by its content of isothiocyanates, chemical compounds with high medicinal potential (Fahey *et al.*, 2019). The properties of moringa make it an important resource in biotechnology, livestock, medical sciences, and the food industry (Brilhante *et al.*, 2017). Therefore, it has become a cost-effective food production or fortification option in developing countries (Razis *et al.*, 2014) and as a dietary supplement in developed countries. The plant's numerous properties have led various research centers to focus on its study. On the National Center for Biotechnology Information (NCBI) platform, 7,655 publications were identified, along with 6,722 in Scopus and 5,902 in ScienceDirect. In the last two decades, worldwide publications have increased significantly (Gupta and Ahmed, 2020). However, the existing information is outdated, and the trend in research topics may have shifted due to societal needs. This lack of updated information represents a limitation for the development of more accurate research. One tool that facilitates the classification of published information is the bibliometric analysis (Donthu *et al.*, 2021). This analysis involves the classification of publications indexed in various databases and measures the scientific contribution to a particular topic. It has the ability to identify countries, authors, and institutions with the highest number of publications (De Bellis, 2009). It groups research into various areas of knowledge and research topics, establishing trends in the field (Moya-Anegón *et al.*, 2007). Previous bibliometric analyses on moringa cover periods that do not provide updated information and are focused on just a single country (Gupta and Ahmed, 2020) or the type of documents published (George *et al.*, 2021), exclude information from 2020 up to date. Therefore, the aim of this research was to map the scientific production of *M. oleifera* in the Scopus database during the period 2013-2023.

MATERIALS AND METHODS

A bibliographic search was conducted in the Scopus database (http://www.elsevier.com/es-mx/solutions/scopus). The Scopus database was chosen because it contains a large number of journals with high impact factors. The search words used was: *Moringa oleifera*, and no additional filters were applied. This approach aimed to provide a general overview of *M. oleifera* across the various categories managed by Scopus for information classification. The search period ranged from January 2013 to August 12, 2023. A total of 5,680 published documents were identified. The retrieved bibliography was exported into a CSV (commas separated values) file containing all the information provided by the platform for each published document. Using the Bibliometrix package in R 4.2.0, the following variables were determined: number of publications per year, most published document types (scientific articles, reviews, books, book chapters, scientific notes, and other documents), areas with the highest number of publications, annual production in the most researched areas, countries with the most publications, journals with the highest number of publications, authors with the most publications and citations, and the most cited articles. Graphs for annual production, growth in the three most researched areas, and the most published document types were created using Sigmaplot version 10.0. The keyword network was created using the keywords assigned by the authors to their works, with the most frequent words represented as the largest nodes. A keyword network and the collaboration network between countries were developed using VOSviewer version 1.6.19. The thematic map was generated with the biblioshiny function of the Bibliometrix package in R version 4.2.0.

RESULTS AND DISCUSSION

The bibliometric analysis of moringa highlights the current state of research published on Moringa. Iftikhar *et al.* (2019) state that bibliometric analysis identifies the scientific impact of a particular topic.

Scientific Production Worldwide

Between 2013 and August 12, 2023, 5,680 documents were published, with an average of 516 documents per year and a growth rate of 9.73% anually. The years with the highest and lowest number of publications were 2022 and 2013, with 933 and 213 documents published, respectively (Figure 1). George *et al.* (2021) report 2,345 publications, considering only scientific articles and conference presentations. These researchers also identify an exponential increase in the number of publications. The increase in moringa production can be linked to the promotion by various international organizations, such as the FAO and WWO, regarding its multiple properties (Velázquez-Zavala *et al.*, 2016), as well as the identification and characterization of new phytochemicals in the plant (Chodur *et al.*, 2018). These facts motivate researchers to formulate new studies in various scientific fields to assess and validate its benefits.

Types of Documents Published

The types of documents in which the publications were classified were Research Article (80.21%), Review (8.68%), Conference Paper (6.78%), Book Chapter (1.81%), Book (0.11%), Scientific Note (0.26%), Short Survey (0.23%), Review presented at a conference (0.44%), and Undefined categories (1.48%). George *et al.* (2021) report only publications of scientific articles and conference presentations. This study integrated all the documents included in Scopus to provide a general overview of the topic.

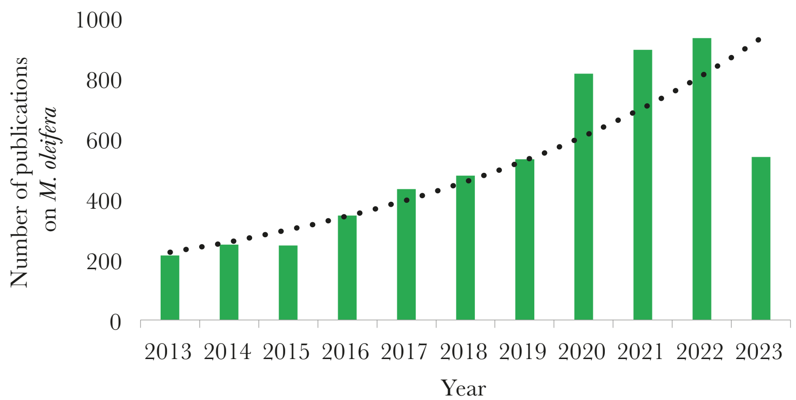

Figure 1. Global scientific production of *M. oleifera* published in Scopus from January 2013 to August 12, 2023. The research trend of *M. oleifera* is shown.

Research by Area of Knowledge

Moringa research has gained significant importance in various scientific fields. Each database defines different scientific categories or areas of knowledge, and each document is classified into one or more categories. Moringa publications in Scopus were classified into the following areas of knowledge: Agricultural and Biological Sciences (21.92%), Biochemistry, Genetics, and Molecular Biology (10.71%), Pharmacology, Toxicology, and Pharmaceuticals (10.51%), Medicine (9.27%), Environmental Science (8.90%), Chemical Engineering (11.70%), Engineering (4.55%), Materials Science (3.23%), Veterinary Science (2.54%), Immunology and Microbiology (2.40%), Energy (2.28%), Nursing (1.73%), Multidisciplinary (1.61%), and other Areas of Knowledge (8.64%). The areas of Agricultural and Biological Sciences; Biochemistry, Genetics, and Molecular Biology; and Pharmacology, Toxicology, and Pharmaceuticals have shown the greatest growth in the last 10 years (Figure 2). The Agricultural and Biological Sciences area ranks first, with the most frequent research focusing on topics such as chemical and organic fertilization, water stress, fruiting, morphological characterization, and agroforestry systems (Muhl *et al.*, 2013; Sarwar *et al.*, 2018; Ruiz *et al.*, 2021). Other topics in this category include the phenological and adaptive study of the species (Förster *et al.*, 2015). Due to its wide agro-climatic adaptability and rapid growth, moringa is considered a viable and low-cost alternative for animal feed (Su and Chen, 2020).

The areas of biochemistry, genetics, and molecular biology occupy the second place. In the field of biochemistry, publications have focused on proximate analysis, vitamin, mineral, amino acid, and fatty acid content in the leaves and seeds (Gopalakrishnan *et al.*, 2016; Dolma and Tashi, 2020). In the areas of genetics and molecular biology, molecular markers such as RAPD, SSRs, ISSRs, AFLPs, and biomarkers have been developed and widely used (Popoola *et al.*, 2014; Hassanein and Al-Soqeer, 2018)

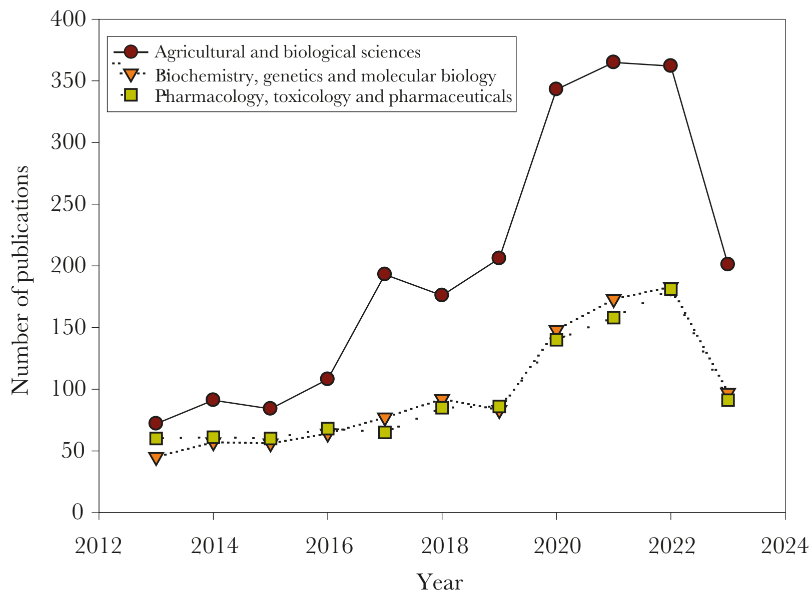

Figure 2. Annual growth of the three areas with the most research on *M. oleifera* in Scopus from January 2013 to August 12, 2023.

to determine the diversity, identity, and genetic structure of moringa populations in various countries (Hassanein and Al-Soqeer, 2018). These areas aim to understand the quantity and function of the genes in its genome (Yang *et al.*, 2015) and their genetic relationship with other species (Abdel-Hameed, 2015). These molecular tools represent an alternative for identifying genetic materials with particular attributes (Popoola *et al.*, 2014). Table 1 shows the countries with the highest number of published documents, with India being identified as the most productive. This high scientific output is due to the fact that moringa is native to India and has high morphological and genetic diversity (Olson, 2017). Mexico's scientific production has amounted to 213 publications, placing it in 11th worldwide position. Despite moringa being cultivated in various states of Mexico (Olson and Alvarado-Cárdenas, 2016) and exhibiting high morphological diversity (Ruiz *et al.*, 2021) and nutritional value (Velázquez-Zavala, 2016), this species is not considered a priority in Mexican research or for livestock or human nutrition. The South African Journal of Botany has published the highest number of documents in the past 10 years. Regarding researchers with the highest number of publications, Berga-Masco, R. holds the first place, while Anwar, F. is the most cited author. This indicates that Anwar, F. is a key figure in the field of moringa research at the international level in recent years. In Mexico, the most cited author is Olson, M. E., with 313 citations for the period 2013-2023.

Collaboration between countries

Figure 3 shows the collaboration network between countries regarding moringa publications. The Scopus database records 134 countries that have researched moringa. India has collaborations with 55 countries, including Saudi Arabia, Egypt, Pakistan, Malaysia, South Korea, South Africa, Italy, and Japan. Mexico has collaborations with 31 countries, with the most frequent being Pakistan, Egypt, India, the United States, and Nigeria. Collaboration between countries indicates the flow of genetic material between various regions and explore the large genetic reservoirs of moringa worldwide.

Each node represents a country. The size of the node refers to the total number of documents published. The width of the line represents the number of collaborations between two countries.

Table 1. Countries, journals, and authors with the highest number of *Moringa oleifera* publications in Scopus from January 2013 to August 12, 2023.

No.	Countries	NP*	Journals with the most publications	NP	Most productive authors	NP	Most cited authors	NC*
1	India	1172	South African journal of botany	106	Bergamasco, R.	84	Anwar, F	1736
2	Indonesia	481	Acta horticulturae	103	Napoleón, TH	44	Becker, K	1343
3	Nigeria	468	Iop conference series: earth and environmental science	98	Paiva, PMG	44	Ashraf, M.	1121
4	China	461	Aip conference proceedings	70	Coelho, LCBB	38	Wang, Y	1076
5	Egypt	446	Journal of ethnopharmacology	57	Tian, Y.	35	Zhang, Y	999

NP*: Number of publications; NC*: Number of citations.

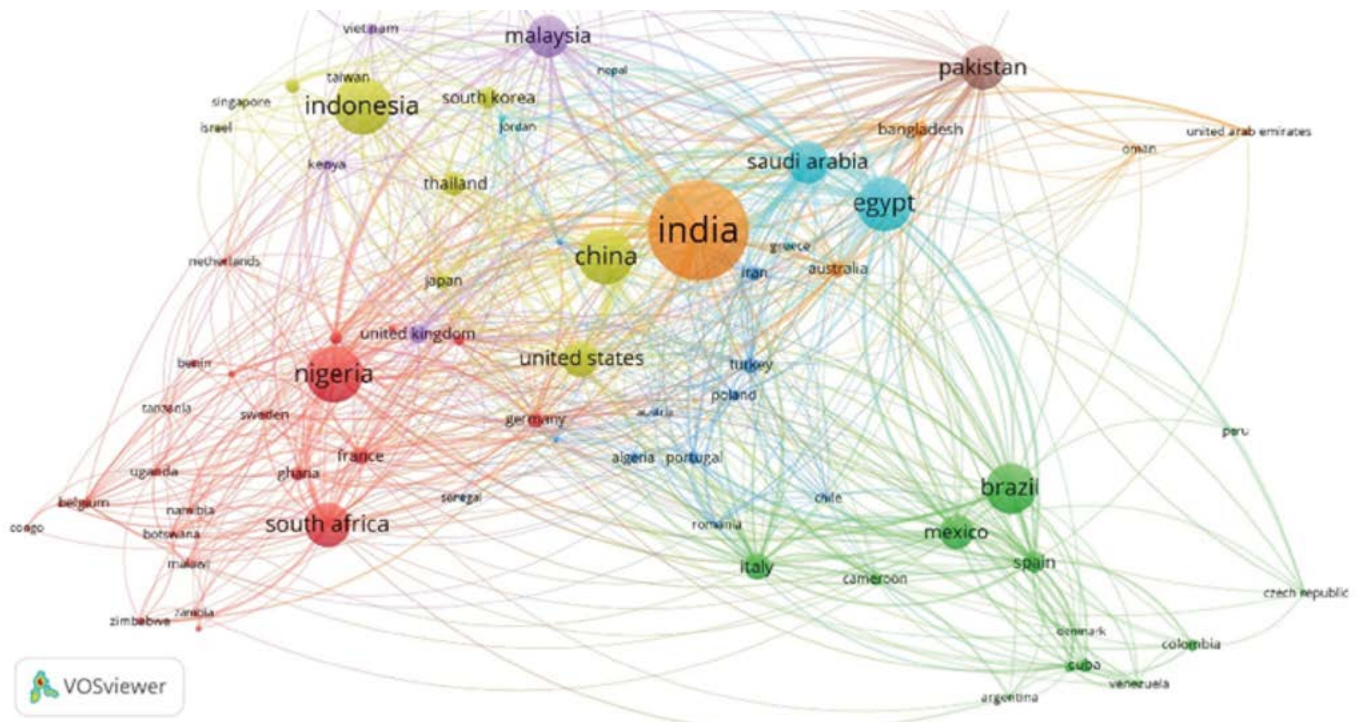

Figure 3. Collaboration between countries regarding *M. oleifera* publications in Scopus from January 2013 to August 12, 2023.

Of the 5680 publications on moringa, the publication by Rafieian-Kopaei M, 2014, in the *International Journal of Preventive Medicine* received a total of 573 citations, ranking first. The article by Gopalakrishnan *et al*., (2016) has 569 citations, ranking second. Bornmann and Hans-Dieter (2009) mention that the number of citations is proportional to the importance of the study and has a significant influence on readers and the development of future research.

Keywords and Trending Topics

A total of 11,799 keywords were identified by all authors, and 27,733 ID keywords were identified by the Scopus platform. The most frequent ID keywords were: *Moringa oleifera* (4141), plant extract (2062), antioxidants (930), plant chemistry (628), medicinal plant (503), animal experiments (485), plant seeds (467), and oxidative stress (447). Figure 4 shows the relationship between keywords and research frequency over the past 10 years. Cluster 1 includes topics related to performance and meat quality of animals fed with moringa. Cluster 2 groups topics related to flocculation and bioadsorption of potentially toxic elements. Cluster 3 includes topics from the fields of biochemistry and phytochemistry that study the medicinal properties of moringa.

There are 17 clusters, each represented by a different color. The size of each node represents the most frequent keyword. The lines connect the keywords, and the width of the lines shows the frequency of co-occurrence between two keywords. Figure 5 shows the thematic map that summarizes the 5680 published documents on moringa. The map groups the following sections: niche topics, basic topics, emerging or declining

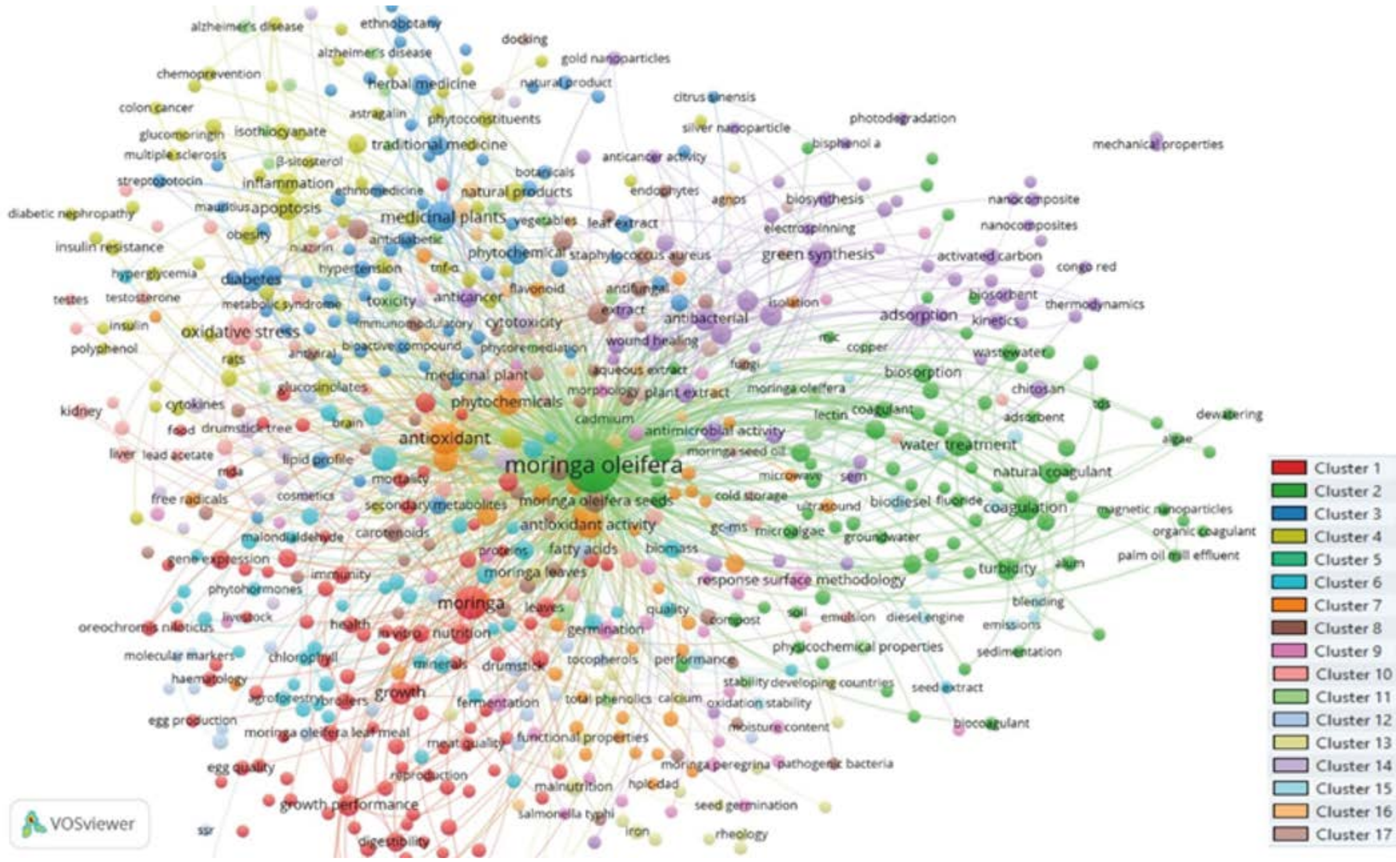

Figure 4. Network of most researched topics on *M. oleifera* in Scopus from January 2013 to August 12, 2023.

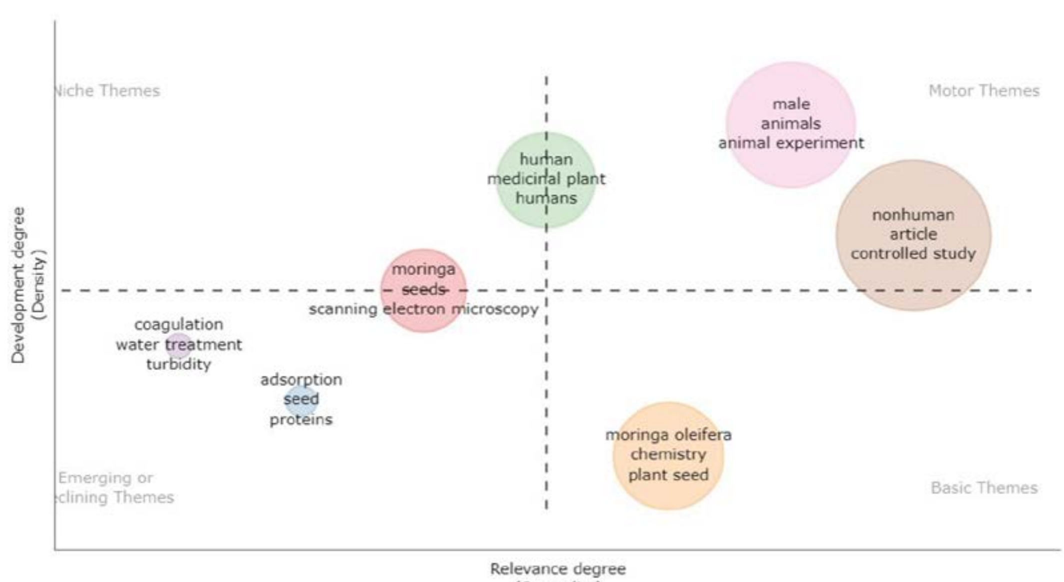

Figure 5. Thematic map of *M. oleifera* research published in Scopus from January 2013 to August 12, 2023.

topics, and core topics. Declining topics include flocculation and adsorption of toxic elements. Basic topics focus on the study of the chemical composition and antibacterial activity of the leaf.

The core topics are divided into two groups: the first focuses on experimentation with animals and oxidative stress, while the second addresses controlled studies on medicinal properties. This is because the leaf contains phytochemicals with antioxidant activity (Azlan *et al.*, 2023). Additionally, moringa contains unique chemical compounds that provide greater therapeutic properties (Chodur *et al.*, 2018) and help prevent cardiovascular and neurodegenerative diseases (Upadhyay *et al.*, 2015).

Moringa research in Mexico

In Mexico, a total of 168 documents were identified, placing the country in the tenth position globally. The individual production consists of 118 publications, with 50 publications being collaborative efforts with other countries. The countries that most frequently collaborate with Mexico are the United States, Spain, Egypt, Italy, Ecuador, and Pakistan. Although other research has been conducted in the country, these were not included in the analysis as only publications in Scopus were considered. This factor limits a more accurate understanding of the current situation of moringa in Mexico. Regarding the number of citations, 1,886 were identified, with an average of 11.22 citations per publication. Both nationally and internationally, an increase in moringa publications was identified in the Scopus database. However, publications not found within the platform could not be analyzed. Descriptive studies, physicochemical characterization, and secondary metabolite profiling have provided important information on moringa in recent years. Topics in the field of omics sciences will provide further information and increase scientific knowledge of the species.

CONCLUSIONS

Moringa has a high scientific production worldwide due to the multiple benefits it offers for human nutrition and health. The scientific contribution on moringa in recent years highlights its economic and ecological importance for the creation of sustainable agri-food systems. Bibliometric analyses help mapping research trends and serve as a foundation for developing research with a high degree of relevance.

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Colegio de Postgraduados

Analysis of macro and micro nutrients in six substrates and leaves for vanilla cultivation (*Vanilla planifolia* G. Jackson)

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ABSTRACT

Objective: To analyze the physicochemical properties and nutritional content in six substrates and the foliar content of macro and micronutrients in leaves of five phenological stages in vanilla.

Design/methodology/approach: Six substrates were evaluated, in which physicochemical properties and the content of total and available macro and micro nutrients were determined. The foliar content in leaves of five phenological stages in vanilla was also determined. The statistical analysis had a completely randomized design, the substrates and phenological stages were evaluated separately, with two repetitions per treatment.

Results: The substrates analyzed resulted in total amounts of macro and micro nutrients acceptable for good development of vanilla plants and, considering the foliar contents of the leaves, adequate management of the nutrition of the vanilla crop can be achieved.

Limitations on study/implications: The physicochemical analyses of the substrates present total amounts of nutrients for the proper development of vanilla plants. However, their availability must be improved.

Findings/conclusions: The foliar contents of macro and micro nutrients in vanilla leaves were different in the stages of plant development, with K being the one with the highest foliar content followed by N, P, Ca and Mg. Considering these foliar contents and the total and available nutritional contents in the substrates can contribute to adequate nutrition planning in vanilla cultivation.

Keywords: macronutrients, micronutrients, physicochemical properties, *Vanilla planifolia* G. Jackson.

INTRODUCTION

Vanilla (*Vanilla planifolia* G. Jackson) is distributed in tropical regions in Mexico and Central America, where the existence of 15 species of the genus *Vanilla* is recognized (Luis-Rojas *et al.*, 2020; Martínez-Monter *et al.*, 2022). It is used in the food and perfume

industry to obtain flavoring and aromatic substances, as well as in the field of medicine, because it has anti-mutagenic and anti-cancer properties (Arya *et al.*, 2021; Ramos-Castellá and Iglesias-Andreu, 2022). Vanilla presents a root system which develops superficially on substrates with large amounts of organic matter in decomposition (woody and leafy residues), product of pruning of trees and crops such as banana (Martínez-Monter *et al.*, 2022).

Nowadays, conventional commercial substrates are used, although these inputs increase the production cost (Santillán *et al.*, 2018). In addition to this, the substrates used generate environmental problems due to residue elimination; therefore, an alternative with low environmental impact is sought, such as local organic materials that are stable, of good quality, safe and which allow the adequate development of the crop. This will depend on the local agroindustry, which provides a diverse range of materials such as coconut fiber, wood chips, sawdust, and sugarcane bagasse (González-Chávez *et al.*, 2018; Martínez-Monter *et al.*, 2022).

Vanilla producers in Mexico use plant residues in their substrates, but there is no control over the nutrition levels, which is one of the important aspects to obtain good production, in addition to climate factors, substrate, management and age of the crop (de la Cruz *et al.*, 2014; González-Chávez *et al.*, 2018; Martínez-Monter *et al.*, 2022). Foliar analysis is a useful method to understand the nutritional status of a crop, since it assesses indirectly the fertility of the substrate or any culture medium. The substrates used currently for vanilla cultivation are elaborated empirically, and there are few studies related to nutrition of this crop in Mexico. Therefore, the objective of this study was to evaluate the concentration of total and available macro and micro nutrients in six different substrates, as well as the foliar content of macro and micro nutrients in leaves of five phenological stages of the vanilla crop. The hypothesis is that at least one of the substrates satisfies the nutritional needs in each of the phenological stages of the vanilla plant.

MATERIALS AND METHODS

Study site

The research was conducted in May, 2022, in the municipality of Tenampulco, Puebla, Mexico, located on geographic coordinates 20° 11' 52.23" N and 97° 22' 06.09" W, at an altitude of 240 m; with semi-warm climate and frequent summer rains (INEGI, 2005).

Vanilla substrates

In the elaboration of substrates for vanilla, available local materials from the study region were used, such as cattle manure, soil from the forest, banana leaves and stems (*Musa paradisiaca* L.), bamboo leaves (*Bambusa vulgaris* Vittata), and sawdust; the proportions of each component are presented in Table 1. The substrates chosen were organic materials, with the aim of not generating environmental problems and seeking stability and good quality in them for good growth of vanilla (González-Chávez *et al.*, 2018; Martínez-Monter *et al.*, 2022). For the analysis of total and available macro and micro nutrients, a sample of 1 kg was taken from each of the substrates, dried at room temperature, and sieved through a 2 mm mesh.

Table 1. Materials and proportions used for vanilla substrates.

Material	Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6
	%					
Manure	50	40	30	30	20	30
Soil	15	30	30	40	20	10
Leaf 1	25	20	30	20	50	50
Leaf 2	5	5	5	5	5	5
Sawdust	5	5	5	5	5	5

Sub=substrate; Leaf 1=banana leaf; Leaf 2=bamboo leaf.

Foliar analysis

The leaf samples were obtained from five phenological stages of vanilla, days after sowing (DAS): 1) Growth stage, from 0 to 240 DAS, 2) Before production, from 241 to 360 DAS, 3) In production, from 361 to 600 DAS, 4) Before flowering, from 601 to 660 DAS, and 5) During flowering, from 661 to 730 DAS. Sampling was conducted in 10 plants and three leaves were taken from each, for a total of 30 leaves for each of the phenological stages. The leaf samples were washed, dried, ground, sieved, and stored in plastic bags for their study (Silva and Uchida, 2000). Both analyses were carried out in the Laboratory of the Centro de Investigaciones en Ciencias Microbiológicas (ICUAP), Puebla.

The physicochemical characterization was conducted in each of the substrates: pH with method AS-02, electric conductivity (AS-18), organic matter (AS-07 from Walkley and Black), and apparent density with the AS-03 method of the NOM-021-RECNAT-2000 (SEMARNAT, 2000). With the AS-25 method of the NOM-021-RECNAT-2000, the total N of the substrates was evaluated. Meanwhile, the total macro-elements (P, K) and micro-elements (Na, Ca, Mg, Fe, Mn, Zn and Cu) in the substrate were determined with the calcination method. The available P in the substrate was determined with the ammonium fluoride method. Meanwhile, the available elements in the substrate (K, Na, Ca, Mg, Fe, Mn, Zn, and Cu) were determined with the ammonium acetate extraction method, and the total leaf elements (P, K, Na, Ca, Mg, Fe, Mn, Zn and Cu) with the microwave digestion method (SEMARNAT, 2000).

Statistical analysis

Six treatments were obtained for the substrates and 5 treatments for the phenological stages of vanilla. The substrates and the leaf samples had two repetitions per treatment. The experimental unit was 34.7 g for the substrates (physicochemical characteristics, total and available elements); and 0.4 g for the phenological stages. The normal distribution of the observations was carried out with a significance of $p \leq 0.05$, and they were analyzed with a completely randomized design. Analysis of variance (ANOVA) was conducted with the data from the substrates, and another ANOVA with the phenological stages of vanilla. In each ANOVA, Tukey's means comparison ($p \leq 0.05$) was added. The analyses were carried out with the statistical software SAS® version 9.0.

RESULTS AND DISCUSSION

The analysis of variance for the physicochemical variables, the content of macro and micro elements in the substrates and leaves were highly significant ($p \leq 0.05$), with the exception of the physicochemical AD variable and nitrogen in foliar analysis.

The physicochemical analysis showed that the pH in the six substrates ranged between 9.50 and 8.28 (Table 2), being higher in substrate five. In general, the six substrates can be considered to be alkaline, and these results differ from what was reported by Reyes-López (2021), due to the materials used for substrate manufacturing. On the other hand, Ortiz *et al.* (2022) mentioned that the pH in the organic substrate should range from 5.5 to 6.8. Likewise, Barbaro *et al.* (2019) recommend for a substrate to have a slightly acidic pH, in order for nutrients to be available. On the other hand, Montoya-Jasso *et al.* (2021) mentioned that alkaline composts are useful because they contain lower proportion of exchangeable hydrogen ions, high contents of calcium, magnesium and sodium. However, considering the NMX-FF-109-SCFI-2007, the pH of substrates 1, 2, 3, and 4 fall within the range established by that norm, since it establishes a range of 5.5 to 8.5.

Regarding the EC, it was found that it varied between 7.76 ds m^{-1} in substrate two to 3.38 ds m^{-1} in substrate three, and these differences were statistically significant ($p \leq 0.05$). Merino and Rodríguez (2018) mention that, in a substrate of optimal range, EC should range between 1.2 and 3.5 ds m^{-1}, considering that this range fulfills these values only in substrate three. In OM, there are also significant differences ($p \leq 0.05$), with substrate one being the one with highest amount of OM of 26.02%, and substrate three with the lowest value of OM of 17.88%, due to the amount of manure used. However, in most of the substrates in this study there are low percentages of OM, except for substrate one, since an adequate percentage in substrates is from 25 to 30%. However, the NMX-FF-109-SCFI-2007 establishes a range of 20 to 50%, with which most of the substrates would be within that range. No statistically significant differences were found in AD. However, these values can be considered high; Montoya-Jasso *et al.* (2021) mention that the AD in substrates should be lower than 0.60 Mg m^{-3}.

Regarding the analysis of total and available macro elements N, P, and K, they were statistically different ($p \leq 0.05$), with substrate four being the one that presented the highest

Table 2. Physicochemical variables evaluated in the substrates used.

Treatments	pH	CE (ds m^{-1})	MO (%)	Da (Mg cm^{-3})
Substrate 1	8.31 b	6.63 bc	26.02 a	0.73 a
Substrate 2	8.45 b	7.76 a	20.48 bc	0.75 a
Substrate 3	8.28 b	3.38 e	17.88 c	0.77 a
Substrate 4	8.28 b	5.47 d	20.59 bc	0.75 a
Substrate 5	9.50 a	7.14 ab	24.05 ab	0.76 a
Substrate 6	9.01 ab	6.19 c	20.49 bc	0.73 a
DMS	0.97	0.65	3.94	0.07

pH=hydrogen potential; CE=electric conductivity; MO=organic matter; Da=apparent density. Values with the same letter in the lines are statistically equal according to Tukey's means test with $p \leq 0.05$. DMS=Minimum Significant Difference.

values of nitrogen, because more soil was added, which possibly already contained nitrogen; on the other hand, substrate three presented the lowest values of N because it was the one where less amount of soil and banana leaves were used. The nitrogen content varies depending on the components of the substrate, since manure can increase the amount of nitrogen between 0.53 and 1.25% (Aguiñaga-Bravo *et al.*, 2020). On the other hand, the NMX-FF-109-SCFI-2007 establishes a total nitrogen range of 1 to 4%, where the six substrates comply with this norm.

Regarding phosphorus and potassium, the total and available values presented broad differences, possibly due to the pH found in the substrates, which being alkaline decrease the availability of nutrients. Potassium presented higher availability in all the substrates compared to phosphorus (Table 3). González-Chávez *et al.* (2018) proposed a range of 10 to 1000 ppm of total phosphorus in substrates. The composition of substrates determined the total and available amount of macro elements, since potassium presented higher total and available amount in substrates 5 and 6 because they have a higher amount of banana leaves (Table 1).

When it comes to total and available micro elements, similar to macro elements, values were observed with broad difference (Table 4), in relation to Na, Ca and Mg; Ca was the one that presented highest availability, followed by Mg and Na. Likewise, the highest values of available Ca and Mg was found in substrate five, which is due to the banana leaf, since it has higher percentage in the composition of this substrate. From these results, it can be inferred that banana leaf can be an element to consider in the manufacture of organic substrates, since it contributes a good amount of potassium, calcium, magnesium and copper.

Regarding Fe, Mn, Zn and Cu, no defined pattern was observed when it comes to total and available amount, because there is no coincidence between these amounts in the same substrate. However, it is observed that Mn presents higher values of availability in the six substrates, followed by Zn, Fe and Cu. In this sense, Gayosso-Rodríguez *et al.* (2018) mention that the optimal levels of Mn in organic substrates should be higher than 0.02 ppm.

Table 3. Total and available macro elements present in the substrates.

Treat.	Nitrogen	Phos. (T)	Phos. (D)	Pota. (T)	Pota. (D)
	ppm				
Subs. 1	7250 a	5297.62 c	14.46 b	6950.00 d	2123.15 c
Subs. 2	7950 a	6344.95 a	5.12 f	8150.00 c	2283.20 b
Subs. 3	4800 b	4261.90 d	13.21 c	6150.00 e	1583.55 d
Subs. 4	8250 a	4202.17 d	19.28 a	6350.00 e	2162.25 c
Subs. 5	7400 a	5702.38 b	11.66 d	10500.00 b	2895.35 a
Subs. 6	7150 a	5225.98 c	9.04 e	11050.00 a	2805.45 a
DMS	1194	206.53	0.63	344.68	92.98

Treat.=Treatment; Phos.=Phosphorus; Pota.=Potassium; T=total element; D=available element; ppm=parts per million; Subs.=Substrate. Values with the same letter within rows are equal according to Tukey's test at $p \leq 0.05$. DMS=Mínima Significativa Diferencia.

Table 4. Total and available micro elements in substrates.

Treat.	Na (T)	Na (D)	Ca (T)	Ca (D)	Mg (T)	Mg (D)	Fe(T)	Fe(D)	Mn(T)	Mn(D)	Zn(T)	Zn(D)	Cu(T)	Cu(D)
	ppm													
Sub 1	5450.0 a	290.0 b	51500.0 c	7414.4 ab	15550.0 b	1822.9 bc	2972.0 f	16.6 d	210.9 d	51.9 d	140.5 c	27.5 b	24.2 b	7.6 c
Sub 2	4600.0 b	380.0 a	55000.0 b	6612.9 bc	18650.0 a	2673.6 ab	3441.5 e	14.3 e	165.1 e	23.2 f	193.0 a	23.3 c	27.7 a	6.9 d
Sub 3	2350.0 d	171.5 d	40500.0 e	6612.9 bc	9750.0 f	2552.1 ab	4519.0 b	23.4 a	291.1 a	79.1 a	122.5 e	21.5 cd	15.4 e	9.5 a
Sub 4	3600.0 c	208.0 cd	44500.0 d	7815.2 a	12050.0 e	1093.8 c	4321.5 c	21.4 b	280.6 b	49.3 e	131.5 d	29.6 a	19.1 d	6.6 d
Sub 5	5200.0 a	238.0 c	54500.0 bc	8416.6 a	13700.0 c	3524.3 a	3896.0 d	17.2 d	254.3 c	57.2 c	164.0 b	20.7 d	27.1 a	9.1 b
Sub 6	3550.0 c	202.0 cd	59500.0 a	6212.1 c	13100.0 d	3524.3 a	4781.0 a	20.6 c	289.0 a	59.6 b	161.5 b	18.3 e	21.4 c	7.6 c
DMS	444.9	46.9	3446.8	1029.7	513.8	1279.6	9.3	0.6	5.4	0.7	5.1	0.9	0.6	0.4

Treat=Treatments; Sub=substrate; Na=sodium; Ca=calcium; Mg=magnesium; ; Sub = Substrate; Fe = iron; Mn = manganese; Zn = zinc; Cu = copper; T=total element; A=available element; ppm=parts per million. Values with the same letter in the lines are equal according to Tukey's test at $p \leq 0.05$. DMS=Minimum Significant Difference.

In some studies of substrates for vanilla cultivation, Martínez-Monter *et al.* (2022), when elaborating bokashi with forest soil, sawdust, corn stubble, sugarcane bagasse, bovine manure, carbon, ash, yeast and unrefined brown sugar, found higher amount of calcium (6.88 g Kg^{-1}), followed by nitrogen (1.58 Kg^{-1}), and potassium (1.39 Kg^{-1}); likewise, with pH of 8.4 and EC of 3.3, this substrate had a positive effect in the formation of roots and shoots in vanilla cuttings. Osorio *et al.* (2014) mention that there is a high significant interaction between the substrate and fertilization on the growth of vanilla plants, and report that when there is substrate, coconut fiber was added and produced high foliar contents of P, K, Cu, Mg and Mn, and when wood fragments were added it produced high contents of N and Ca. Some of these results agree with those found in this study and differ in others because of the components that were used to manufacture the substrates.

In foliar analyses of macro elements, it was found that the plants present a higher foliar content of potassium, followed by nitrogen and phosphorus (Table 5); these results agree with what was reported by Carrillo-González and González-Chávez (2016), who mention that K was the nutrient that accumulated most in a vanilla plant and calcium increased its concentration through time. On the other hand, Diez *et al.* (2015) report that the highest foliar content was found in Ca, K, N, and P. The nutritional demands in other species such as corn demand more nitrogen and can become a limitation in its production (Flores-Sánchez *et al.*, 2019). In stage T3 (production), there was higher foliar content of phosphorus, and in stage T1, higher amount of foliar potassium. In a study to differentiate orchids, Martínez-Monter *et al.* (2022) report values of phosphorus between 400 and 7800 ppm and of potassium from 17400 to 42600, and these ranges agree with what was found in this study.

Regarding sodium, calcium and magnesium (Table 6), a higher foliar content of calcium was found, followed by magnesium and sodium. Sodium is found in higher amount in stage T3, calcium in stage T5, and magnesium in stage T1. Vargas-Hernández *et al.* (2021) report that calcium is one of the main nutritional elements in vanilla, since it is required for the structure of cell walls and for vigor in the plant; Mg is accumulated with time, per day it accumulates 0.26 g/plant and per month 1.04 g/plant; these amounts make Mg one of the important elements because it helps photosynthesis, improves growth, and helps the health of vanilla plants.

Table 5. Total macro elements in the foliar analysis of vanilla.

Treatments	Nitrogen (%)	Phosphorus	Potassium
		ppm	
T1	1.15 a	3695.30 b	43245.70 a
T2	1.20 a	2970.70 c	35246.50 bc
T3	1.27 a	4999.50 a	31996.80 d
T4	1.14 a	3985.10 b	36496.50 b
T5	1.14 a	2680.90 c	34246.60 c
DMS	0.15	411.03	2103.40

ppm=parts per million. Values with the same letter in the lines are equal according to Tukey's test at $p \leq 0.05$. DMS=Minimum Significant Difference.

Table 6. Total micro elements in the foliar analysis of vanilla.

Treatments	Sodium	Calcium	Magnesium	Iron	Manganese	Zinc	Copper
	ppm						
T1	983.28 b	41339.50 c	3009.69 a	267.70 a	16.45 e	79.95 a	9.85 a
T2	816.59 b	36549.30 d	2018.12 e	112.75 d	29.95 d	53.45 c	9.85 a
T3	1349.87 a	29652.90 e	2428.09 b	179.95 b	33.50 c	43.20 e	6.65 b
T4	849.92 b	45541.70 b	2281.43 d	83.85 e	46.50 b	46.50 d	6.55 b
T5	816.56 b	49709.80 a	2336.43 c	130.15 c	53.50 a	59.45 b	9.85 a
DMS	193.82	947.58	27.72	5.93	0.86	0.55	0.28

ppm=parts per million. Values with the same letter in the lines are equal according to Tukey's test at $p \leq 0.05$. DMS=Minimum Significant Difference.

The micro elements were required by the plants depending on the stage of development; in the growth stage (T1), the plants presented higher foliar content of iron, copper and zinc (Table 6), before production (T2) only Cu, during production sodium, and during flowering (T5) calcium, magnesium and copper.

Osorio *et al*. (2014) report that the nutritional needs for vanilla growth under greenhouse production are 1.95% for N, 0.20% for P, 3.90% for K, 2.42% for Ca, and 1.75% for Mg. In this sense, Carrillo-González and González-Chávez (2016) found that the optimal ranges of vanilla nutrients were 1.10% to 2.43% for N, 0.16% to 2.34% for P, 2.34% to 5.64% for K, 0.48% to 2.64% for Ca, and 0.12% to 0.77% for Mg; in some of these values they agree and in others they differ from what was found in the present study because of the conditions in which the experiments were conducted.

In general, the results obtained in the substrates of this study for vanilla ought to be considered to lower the pH to slightly acidic in order to increase organic matter, which could be achieved by adding soil from the forest and pieces of wood in decomposition. On the other hand, the apparent density should be lowered, which could be achieved by adding sand or other materials such as perlite, agrolite or tezontle. Regarding the macro and micro nutrient contents found in the different substrates, it was found that all of them contain the total amounts that the plants require in their different development stages, although they should be more available for the plants to assimilate.

CONCLUSIONS

The foliar contents of macro and micro nutrients in vanilla leaves were different in the development stages of the plants, with K being the one of highest foliar content followed by N, P, Ca, and Mg. Considering these foliar contents and the total and available nutritional contents in the substrates can contribute to an adequate planning of nutrition in vanilla cultivation.

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Colegio de Postgraduados

Effect of thermal popping treatment on Toluqueño creole popcorn maize (*Zea mays* L. cv. Palomero Toluqueño): Changes in physical and structural properties

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ABSTRACT

Objective: To assess the effect of popping thermal treatment on the physical and structural properties of Toluqueño creole popcorn maize (*Zea mays* L. cv. Palomero Toluqueño)

Design/methodology/approach: Toluqueño creole maize and commercial used as a control, were subjected to three popping treatments: hot oil, hot air, and microwave. Popping yield, textural properties, and structural features were determined.

Results: The results showed that the treatment by hot air in commercial maize showed the highest pop yield (71.88%), while the microwave treatment was the best for Toluqueño maize (12.93%). The best textural characteristics for both types of maize were obtained after applying the hot air treatment, resulting in soft, low rubbing, and good chewing popcorns. The microstructural analysis on raw Toluqueño maize showed an intergranular space, which was related to a less compaction grade and consequently less hardness, meanwhile, the microwave popcorns showed the lowest gelatinization degree.

Limitations on study/implications: High resolution and improved methodologies to observe the starch grain and components distribution could have resulted in a better description of the effect of thermal treatment on the corn grains.

Findings/conclusions: Hot air treatment was the best process to obtain a good quality popcorn. The popping yield was related to humidity, hardness, grain shape, intergranular space, and the popping process.

Keywords: Popcorn maize (*Zea mays* L. cv. Palomero Toluqueño), expansion yield, textural properties, structural properties.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most consumed cereals in the world. In Mexico, maize has a very important sociocultural value, and it is a basic food in the diet (Serna-Saldivar, 2021). There are several maize varieties, each one with distinctive genetic characteristics. Maize is used as a raw material to obtain flours, tortillas or snacks (popcorns) among other products. The morphological, botanical, and genetical features have been used as a guide to its classification. From this classification, it is possible to find the group of "indigenous varieties" named popping varieties in which the Toluqueño popcorn maize (*Zea mays* L. cv. Palomero Toluqueño) is included (Bautista-Ramírez *et al.*, 2020). This type of corn has smaller grains than other varieties. Also, it has specific composition and botanical unique features making it suitable to prepare popcorns (Sweley *et al.*, 2013).

The starch contained in the endosperm is moisten and it acquires a gelatinous consistency when heated, which is known as gelatinization (Wang *et al.*, 2017). During it heating process, the pericarp acts a barrier to contains the moisture, leading to a pressure accumulation up to the point of rupture and explosion of the grain (Sweley *et al.*, 2013). In the process, the water is evaporated, passing through of the protein matrix of the grain and intercellular spaces, reaching the required pressure to swell, expanding the grain, and blowing up (García-Pinilla *et al.*, 2021). The fast expansion of the grains in the surrounding air cools the starch, leading a spongy structure of low-weigh knows as popcorn (García-Pinilla *et al.*, 2021).

Two main factors have an important effect on the popping process to obtain good quality popcorns: moisture and pressure. The moisture content determines the quantity of grains that blown up as well as the expansion volume (Sweley *et al.*, 2013). According to Cañizares *et al.* (2020), the moisture content desirable to obtain an optimal volume of blown up is from 11.39 to 12.91%, when expanded without oil and it is between 10.21 and 11.73% when expanded using oil. Regarding to the pressure, there is a significant increase in the mean size of grain and expansion volume as the pressure is reduced inside of the grain (Quinn *et al.*, 2005). The physical and texture features of the corn grains could be the affected by the heat transference medium. In this sense, the most common ways to supply heat to prepare popcorns are microwave, hot air, and immersion in hot oil. Each one of these methods result in different textural and microstructural features in the popcorn. Nowadays, through microscopy and image analysis is possible to describe and evaluate the structure of grains (Rojas-Candelas *et al.*, 2022) and the obtained products such as popcorns (García-Pinilla *et al.*, 2021).

Bautista-Ramírez *et al.* (2018), after studying the geographical distribution of the Palomero Toluqueño maize, pointed out that the local producers decided not to continue the production of this crop so, this breed could disappear if a feasible strategy is not implemented to encourage its conservation and use. As the Palomero Toluqueño is a popping corn, it is important to evaluate its popping yielding as well as its expansion volume (formation and size of the popcorn) to determine if it can compete with commercial brands. At our best knowledge, there are not studies about applying different thermal process to obtain information that may contribute to alternative uses to preserve this local variety. In this sense, the objective of this work was to assess the effect of thermal treatment on the physical and microstructural properties of commercial and Toluqueño popcorn maize (*Zea mays* L. cv. Palomero Toluqueño).

MATERIALS AND METHODS

Area and sample collection: Popcorn maize Toluqueño (TM) was grown and collected in the winter of 2018 in San Marcos Tlazalpan, Morelos, State of Mexico. The commercial maize (CM) was purchased in a local market. The vegetable oil used for the popping process was 1,2,3 brand (La Corona, S. A de C. V.).

Preparation of popcorns. Three methods were used to prepare popcorns with the two types of maize: microwave (MW), hot oil (HO), and hot air (HA). A borosilicate glass container (Ecolution EKPRE-4215, International Inc., Florida, USA) was used to prepare popcorn by microwaves (Hamilton Beach Brands Holding Company, model HB-P70J17AL-V2C, Virginia, USA). To prepare popcorns by hot oil, a popcorn maker machine (Hamilton Beach model 73302, Hamilton Beach Brands Holding Company, Virginia, USA) was used. Popcorns by hot air were prepared using a popcorn maker machine (Hamilton Beach model 73400, Hamilton Beach Brands Holding Company, Virginia, USA). For all treatments, 26 grams (equivalent to 140-176 maize grains) of Toluqueño or commercial maize samples were used to prepare popcorns. The temperature during popping was measured using a thermocouple TM500 (Extech Instruments, Massachusetts, USA). The temperature was registered each 30 seconds until 2.5 minutes.

Popping yield. The popping yield was measured after 1 and 2 minutes of the respective thermal treatment. The popcorns obtained were grouped according to similar shape and size. Four categories were used, using numbers of 0, 1, 2, 3, and 4. The zero category was for the grain without popping, 1 and 2 were for intermedium popping, and 3 and 4 to describe the popcorns with desirable features (higher volume and complete popping). This classification is based on the different popcorn shapes produced on the popping process and is based on the degree of opening of the grain. Figure 1 shows the different categories assigned to commercial and Toluqueño maize.

Figure 1. Categories assigned to commercial and Toluqueño maize.

Category	Description	Image	Image
0	Maize grains without popping.		
1	Popcorn grains fractured, leading to observe a small part of starch. They are hard and small.		
2	Maize grains not completely popping, less had than category 1, but more starch exposed.		
3	A crispy popcorn maize, acceptable size for consumers.		
4	A popcorn with the starch completely exposed, soft, fragile, the highest size respect to the other categories.		

Morphometric characteristics. The size (perimeter and area) of raw and popped corn were measured by image analysis using the Image J program (National Institutes of Health, Bethesda, MD, USA). Images of samples were obtained using a digital camera from a smartphone Galaxy M31 with a camera of 64 megapixels (Samsung Electronics, Suwon, South Korea). Then the images were converted in black and white images for further analysis.

Moisture content. The moisture content in raw and popped corn was determined using a thermobalance (Moisture Analyzer MB21; Ohaus Corp, Pine Brook, NJ, USA.) following the pre-established program of drying at 130 °C for 10 minutes. For all measurements, the samples were grounded in a coffee grinder (KitchenAid BCG111OB; Whirlpool Corporation, Benton Harbor, USA).

Heating rate kinetic. The temperature during the different thermal treatments for both maize samples was measured using a 12-channel Thermocouple Data Recorder Model TM500 (Extech Instruments, Massachusetts, USA). Measurements were carried out since the 00:00 minute progressing every 30 seconds up to 2.5 minutes.

Determination of texture parameters. The texture analysis was performed in a texture analyzer (TA-XT plus; Stable Micro Systems, Godalming, Surrey, UK). A cylindrical probe (stainless steel SMS P/2 of 2 mm) was used to determine the textural parameters in raw corn. The hardness was measured using a compression test, penetrating 25% of depth at a crosshead speed of 7 mm/s. Each grain measured individually on the maximum diameter and always in the same way for all grains.

For the popcorn samples, a TPA test of double compression was carried out using an aluminum compression probe of 75 mm (SMS P/75) to compress 50% of the initial height at a crosshead speed of 2 mm/s. Individual popcorn were analyzed. In all cases, the parameters obtained were hardness, fractureability, elasticity, cohesiveness, gumminess, chewiness, and resilience.

Color. The color of all samples was determined using a colorimeter (Konica Minolta CR-400/410, New Jersey, USA). CIELab scale was used to evaluate color.

Microstructure analysis. The microstructural analysis allowed observing the structure of Toluqueño and commercial popcorn maize. The analysis was performed in a stereoscope (Carl Zeiss, Oberkochen, Baden-Wurtemberg, Germany). Central parts of grains were cut, and cross sections were obtained using a scalpel. The samples were stained with lugol to observe the starch granules at 10x and 40x.

Statistical analysis. All measurements were carried out in triplicate and quantitative data are presented as mean $\pm$ standard deviation. The data were statistically treated by ANOVA and the Tukey test (SigmaPlot V.11.0. Systat Software Inc, San Jose, CA, USA). $p \leq 0.05$ values were considered significantly different.

RESULTS AN DISCUSION

Figure 2 shows the popping yield for commercial maize (CM) after 1 and 2 minutes of the three methods of preparation (hot air (HA), hot oil (HO), and microwave (MW)). One minute was insufficient to pop as the heat transfer was low, resulting in a poor popping yield (in all cases, lower than 3.8%). MW was the most effective (3.8%) because the high energy

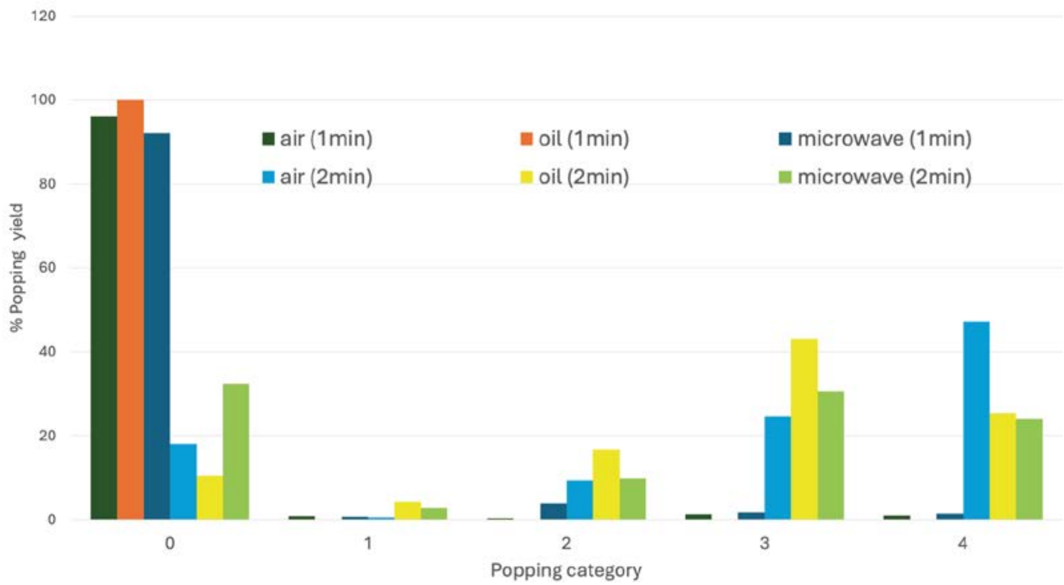

Figure 2. Popping yield for commercial maize after 1 and 2 minutes of treatments.

emitted by microwave radiation promotes the vibration of water molecules resulting in a rapid gelatinization of starch and popping. In the method by HO it is inferred that it takes energy to heat the oil (heat transfer medium) before starting the popping process, then it is the less effective method. The results for treatment of 2 minutes were surprisingly different. The HO and HA treatments showed more than 40% of grains popped in the 3 or 4 levels (Figure 1). Specifically, HA showed the highest popping yield in 3 and 4 levels (71.88%), while HO showed 68.56% and only 54.14% for MW.

HA reached the highest temperature in a short time and the air is in direct contact with the grains. When oil is the heat transmitter, it takes more time to reach the optimum temperature for popping. In the case of MW, the heat affects individually the grains. Koutchma (2022) reported that variations in electrical conductivity in microwaves causes non-uniform internal heating. Also, it is probably that, as the high microwave energy penetrates the grains, part of the moisture evaporates during the popping process resulting in an incomplete popping. The mechanism of heat transfer is different in all treatments. In the process by hot air both conduction and convection phenomena are involved. In the case of hot oil, convection drives the process (Rani *et al.*, 2023); while radiation is involved in the MW popping process.

Figure 3 shows the popping yield for Toluqueño creole maize (TM) after treatment for 1 and 2 minutes in the three preparation methods. No popping was registered after 1 minute in all treatments. Considering that levels 3 and 4 are the optimum, MW and HA treatments showed the best popping yield with 12.93% and 11.96%, respectively. In HO treatment only 5.68% of the grains reached the optimum levels for consumption.

Results showed that the popping yield in TM was almost six times lower compared to CM, regardless of the treatment used for popping. These differences are attributed to their genetical characteristics. Also, it is possible that genetic improvements have been

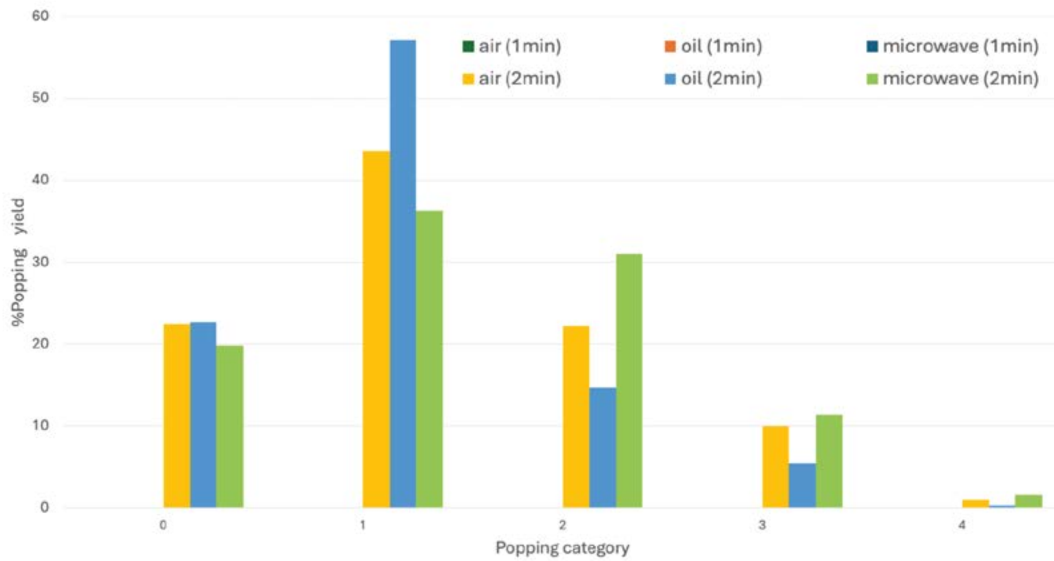

Figure 3. Popping yield for Toluqueño creole popcorn maize after treatment for 1 and 2 minutes.

implemented in commercial varieties to obtain the best popping yield characteristics (Divya, 2024).

Table 1 shows the size characteristics (area, perimeter) for the different categories of CM and TM. It is possible to observe that the area as perimeter is higher in TM.

The higher size in TM could explain its lower popping rate, because more energy is required to pop this variety. Also, the grain form may contribute to popping, thus CM was the most likely to pop because a more uniform heat transfer could be expected due to its more round form.

Table 2. Shows the total area in 100g of CM and TM popcorns for the different thermal treatments. The total area in CM was higher in all cases, but the treatment with HA for CM presented the highest among treatments. In the case of TM, the HA and MW treatments presented higher values compared to the HO treatment.

Moisture content in maize grains influences directly the quality of popcorns, because it affects the popping (García-Pinilla *et al.*, 2021). In this work, CM presented a higher moisture content (10.61±1.04%) than TM (8.42±0.29%), which is related to the popping.

Table 1. Size characteristics (area, perimeter) for category of popping in commercial and Toluqueño maizes.

Category	Commercial maize		Toluqueño maize	
	area (cm^2)	perimeter (cm)	area (cm^2)	perimeter (cm)
0	$0.27±0.03^a$	$1.94±0.17^a$	$0.70±0.04^a$	$3.52±0.20^a$
1	$0.49±0.06^b$	$2.66±0.18^b$	$0.91±0.04^b$	$4.30±0.19^b$
2	$1.34±0.08^c$	$5.31±0.15^c$	$1.22±0.04^c$	$4.47±0.17^b$
3	$1.99±0.28^d$	$6.98±0.35^d$	$2.61±0.11^d$	$8.12±0.14^c$
4	$3.22±0.46^e$	$9.47±0.31^e$	$4.58±0.33^e$	$11.02±0.70^d$

* Results are presented as means±SD (n=3).
** Values with different letters in the same column indicate significant difference (p≤0.05).

Table 2. Total area in 100g of popcorn commercial and Toluqueño maize.

Category	Commercial maize			Toluqueño maize		
	oil (cm^2)	air (cm^2)	microwave (cm^2)	oil (cm^2)	air (cm^2)	microwave (cm^2)
0	2.81±0.32[a]	4.87±0.32[a]	8.76±0.32[a]	15.94±1.00[a]	15.75±0.99[a]	13.92±0.87[a]
1	2.07±0.23[b]	0.34±0.23[b]	1.41±0.23[b]	52.06±2.37[b]	39.69±1.81[b]	33.10±1.51b
2	22.42±1.29[c]	12.53±1.29[c]	13.25±1.29[c]	17.85±0.53[c]	27.05±0.81[c]	37.85±1.13[c]
3	85.71±12.28[d]	48.93±12.28[d]	60.70±12.28[d]	14.15±0.58[d]	25.88±1.06[d]	29.54±1.21[d]
4	81.85±11.28[d]	152.12±11.28[e]	77.73±11.28[e]	1.15±0.08[e]	9.31±0.66[e]	7.34±0.52[e]
Total area	194.87±18.71[a]	218.79±18.71[b]	161.85±18.71[c]	101.14±4.57[d]	117.68±5.33[e]	121.75±5.25[e]

* Results are presented as means±SD (n=3).
** Values with different letters in the same column indicate significant difference (p≤0.05).
*** Values of total area with different letters in the same line indicate significant difference (p≤0.05).

As moisture content increases, optimum popping conditions are reached. In this sense, the optimum moisture content to obtain the maximum popping volume is around 12%. A lower moisture content will result in an insufficient vapor pression during heating and the popping will not take place. High moisture content will result in a collapse of the pericarp at a lower vapor pressure than required for the popping to occur (Sweley *et al.*, 2013). As TM had a low moisture content compared to CM, a less popping yield was reached.

Table 3 shows the moisture content for the different treatments for CM and TM. There is a general trend to increase the category of popping when the moisture content is lower.

In TM there is a decrease of moisture content in the category 4 for the oil treatment, but no significant differences were observed in the other treatments. These results are related to the area values of popcorn, because in CM there is a major expansion with respect to its initial size, while the total area reached in TM was lower. Then, it can be inferred that as the exposed area increases, there is a higher loss of moisture immediately after popping. On the other hand, in general, the moisture content in samples treated with oil was lower compared to the other treatments in both varieties which could be attributed to two phenomena: a) the continuous evaporation of water due to the heat transfer and, b) the decreasing evaporation driven by the diffusion of water from the internal structure to the surface of the grain.

Table 3. Moisture content (g/100g) of commercial and Toluqueño maize grains.

Category	Commercial maize			Toluqueño maize		
	oil	air	microwave	oil	air	microwave
0	4.51±0.08[a]	7.06±0.25[a]	7.00±0.04[a]	3.64±0.23[a]	5.51±0.80[a]	5.60±0.26[a]
1	3.80±0.06[b]	7.70±0.10[b]	6.36±0.04[b]	3.11±0.18[b]	4.86±0.16[a]	4.69±0.08[b]
2	2.42±0.03[c]	5.38±0.13[c]	6.07±0.56[c]	3.30±0.09[c]	5.54±0.09[a]	5.20±0.25[c]
3	2.59±0.11[d]	5.59±0.07[d]	5.13±0.44[d]	3.53±0.04[d]	6.68±0.23[b]	5.34±0.11[a]
4	3.27±0.10[d]	4.65±0.18[e]	4.74±0.46[e]	2.91±0.08[e]	5.60±0.14[a]	5.76±0.16[a]

* Results are presented as means±SD (n=3).
** Values with different letters in the same column indicate significant difference (p≤0.05).

Figures 4 shows the heating kinetic for the three treatments (HO, HA and MW) for both maize varieties. HA reached the highest temperatures in less time (heating rate) compared to HO and MW treatments. In the first minute, CM reached 182 °C (2.136 °C/s), while TM had 143.8 °C (2.02 °C/s). The differences in heating rate can be explained by the grain geometry, since the higher area of TM reduced the air circulation power and the heat transfer.

In MW, the heating rate was 0.815 and 1.163 °C/s for CM and TM, respectively which could explain the higher popping yield for TM. According to Proctor (2018), although the microwaves penetrate evenly throughout the product, the distribution of the electric field is not even throughout the irradiated material. Thus, the energy is not dispersed homogeneously since the distribution of the electrical field will depend on the geometry and the dielectric properties of the food. Apparently, the geometry of the maize grain induces a better behavior in microwave heating.

In HO for CM the heating rate was 0.27 °C/s, while for TM was 0.53 °C/s. Both are lower than the others because the oil must be heated first taking a longer time compared to HA and MW. Also, this explains the low rates of popping obtained with HO. The temperature of the heating medium is one of the important factors for the quality of popcorn, as it plays an essential role in the popping process. The temperature, area, and geometry of heating also play an important role in the popping of maize. Reaching high temperature in a short time will result in fewer grains without popping (Subramani *et al.*, 2023).

The texture analysis of the raw grains showed that CM is harder (319.176±60.203 N) than TM (234.997±27.97 N). According to Gao *et al.* (2024), the hardness of maize grain is related to the floury endosperm (soft) and glassy (hardness), which depends on size, morphology, compaction degree of the starch granules, and the proteinic matrix. These parameters could explain the popping yield observed in TM, since it is less hard, its pericarp is soft, and the intern pressure in the grain during cooking is low resulting in a low level of popping. Also, it is inferred that there is a less compaction of starch granules and high quantity of floury endosperm contributing to a low popping yield.

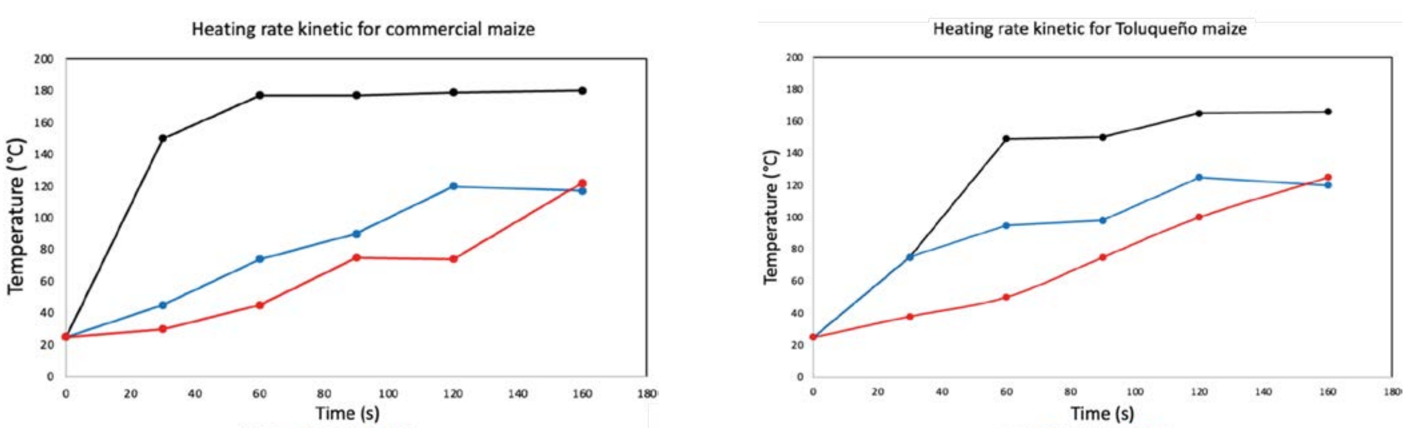

Figure 4. Heating rate kinetic for commercial maize and Toluqueño maize.

Table 4 shows the results for the texture analysis for both varieties of popcorns. The hardness of TM prepared with HO (273.658±87.469 N) and MW (283.975±79.021 N) treatments was higher compared to the HA treatment (184.121±73.889 N). Similar behavior was observed for CM; however, the values were low for all its treatments compared to TM. Results indicate that CM popcorns are softer and have a better texture. It seems like HA promoted a fast popping of maize grains promoting the release of starch which could explain their lower hardness. On the other hand, popcorns prepared with HO suggest that oil affects positively the texture, since it is smoother compared to those obtained by MW process.

Fracturability results show that CM popcorns are more brittle compared to TM. CM with HA required less force to break up (8.60±6.10), while TM by microwave was harder to break up (34.80±13.10). The elasticity results showed the highest value (0.33±0.038) in TM with HO treatment and it was significantly different to elasticity of CM. These values obtained after HA and MW treatments of CM and TM samples were no significantly different. The cohesiveness values were low, but the treatment with HA did not show significant differences in CM and TM. The highest values of gumminess obtained for popcorn were observed in TM prepared under HO (98.22±54.71) and HA (57.0±32.98), while that the lowest value was found in the MW treatment (10.17±4.13). In CM, the lowest value obtained was observed in HA (14.25±6.83), while no significant differences was found between HO and MW treatments. Chewiness values of popcorns from TM prepared with HO and HA indicate that a higher force is needed to disintegrate than that of MW. Texture analysis is of great importance to determine the acceptability and quality of foods. According to Sweley *et al.* (2013), the most desirable texture parameters in maize popcorns are associated with soft and crispy characteristics. Those described as

Table 4. Texture analysis of popping maize (category 4).

Texture parameter	Maize variety	Thermal treatment		
		Oil	Air	Microwave
Hardness (N)	CM TM	103.90 ± 41.20^a 273.70 ± 87.50^b	62.10 ± 25.40^a 184.10 ± 73.9^b	112.05 ± 0.05^a 284.1 ± 79.00^b
Fracturability (N)	CM TM	17.20 ± 5.40^a 21.40 ± 4.60^a	8.60 ± 6.10^a 13.80 ± 1.60^a	15.35 ± 11.9^a 34.80 ± 13.10^b
Elasticity	CM TM	0.28 ± 0.04^a 0.33 ± 0.03^b	0.21 ± 0.05^a 0.22 ± 0.05^a	0.23 ± 0.032^a 0.23 ± 0.05^a
Cohesiveness	CM TM	0.28 ± 0.03^a 0.34 ± 0.09^a	0.23 ± 0.03^a 0.29 ± 0.06^a	0.26 ± 0.02^a 0.35 ± 0.01^b
Gumminess	CM TM	29.79 ± 15.58^a 98.22 ± 54.71^b	14.25 ± 6.83^a 57.01 ± 32.98^b	29.36 ± 9.16^a 10.17 ± 4.13^b
Chewiness	CM TM	8.57 ± 5.23^a 31.29 ± 16.03^b	3.19 ± 2.26^a 13.40 ± 9.68^b	6.60 ± 2.29^a 2.45±1.27b
Resilience	CM TM	0.11 ± 0.02^a 0.19 ± 0.06^b	0.85 ± 0.01^a 0.15 ± 0.04^b	0.11 ± 0.01^a 0.19 ± 0.04^b

* CM: commercial maize; TM: Toluqueño maize.
** Values with different letters in the same column per texture parameter indicate significant difference ($p\leq0.05$).

undesirable characteristics need to be chewed for longer and tend to stick to the teeth. Therefore, TM popcorns produced by HA had the most desirable characteristics.

Table 5 shows the color values for raw maize and those after three different thermal treatments. The L value for raw CM (58.95±1.58) was lower compared to TM (64.81±1.83). Positive b* values indicate a yellow color. In this way, CM had a higher value (28.01±0.12) compared to TM (26.20±1.49). The statistical analysis indicated a significant difference in the b* value of raw maize samples. In the CIELab scale, a* value in raw materials refers the red color. CM had a significantly more intense red color (7.72±0.10) that TM (6.03±1.07). In general, L values were higher after the thermal treatments because of an increased lightness. Popcorns prepared with HO showed the lowest L values with a significant difference between other samples. The possible reason explaining these results is the remaining oil in the samples. A significant decrease in a* y b* values was observed in the popcorns obtained by CM and TM. As mentioned before, those values correspond to yellow and red color. The color is mainly due to carotenoids presents in samples and, when it was exposed to thermal treatments, oxidation processes were promoted. However, a drastic decrease in b* value was observed in TM indicating that the pigments in this grain are more susceptible to thermal treatments.

Differences in size, form, pericarp thickness, endosperm distribution, and degree of compaction are observed in Figure 5. The differences between grains have an important effect on their behavior during popping by the different methods, in addition to their physical and chemical characteristics. The pericarp thickness of CM was higher compared to TM. This is in line with the findings of Bautista-Ramírez *et al.* (2019), who evaluated the thickness of the pericarp of CM and TM reporting values of 0.54 μm and 0.9 μm, respectively.

Figure 6 shows the microstructure of popping maize (category 1 and 4) for CM and TM. Is possible to observe that it is A notably higher number of starch granules with defined shape was observed in the category 1 of both types of maize for the three heating treatments indicating a smaller gelatinization degree during the popping process. Furthermore, greater intergranular space is noticed in TM, which may be related to a low compacting degree which could be associated with lower values of hardness in the grain without popping (Table 4). For category 4, a higher expansion degree was observed in starch granules after HA and MW treatments although they kept well-defined structures. HO treatment showed a major gelatinization evidenced for larger starch granules. TM

Table 5. Color parameters for commercial and Toluqueño maize grains.

System CIE	Raw maize		Popping grains					
	CM*	TM*	Commercial maize **			Toluqueño maize**		
			oil	air	microwave	oil	air	microwave
L	58.95±1.58[a]	64.81±0.08[b]	96.10±5.49[a]	99.56±8.65[h]	100±2.33[v]	89.76±2.82[b]	97.97±11.21[h]	95.99±2.26[w]
a*	7.72±0.10[a]	6.03±0.06[b]	1.46±0.52[a]	1.46±1.00[i]	1.21±0.19[x]	0.91±0.98[a]	1.45±0.56[i]	1.51±0.31[x]
b*	28.01±0.12[a]	26.20±0.03[b]	25.98±2.14[a]	25.89±0.70[j]	23.00±3.08[y]	10.37±2.07[b]	8.43±1.16[k]	8.38±1.81[z]

* Values with different letters in the same file (per maize type) indicate significant difference (p≤0.05).

** Values with different letters in the same file per treatment (oil, air, microwave) indicate significant difference (p≤0.05).

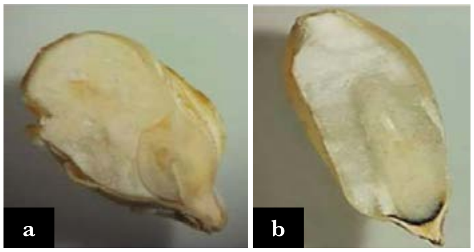

Figure 5. Microstructure from a) commercial maize and b) Toluqueño maize.

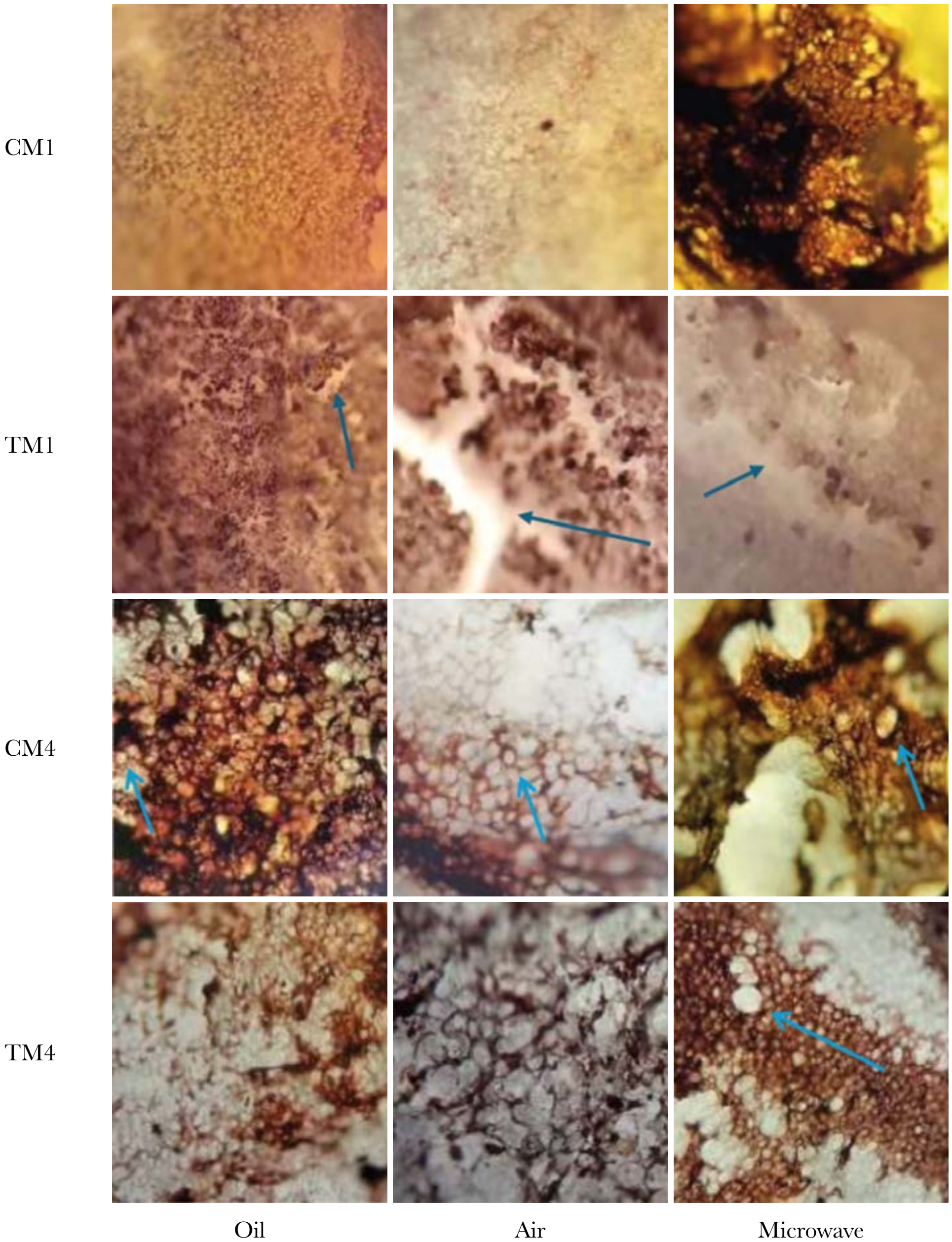

Figure 6. Microstructure of commercial and Toluqueño maize grains.

showed a notable swelling and deformation of the granule after treatment with HO and HA opposite to the behavior observed in the MW treatment suggesting a lower degree of gelatinization. These results can be explained by the higher hardness values observed in popcorn obtained after microwave treatment. To confirm the degree of gelatinization it is recommended to carry out further analyses regarding this parameter.

CONCLUSIONS

Results showed that TM had a lower popping yield compared to CM irrespective of the heating method which is related to its larger size, lower moisture content, and grain hardness. The HA method allowed obtaining the best results for popping yield and texture of popcorns in both types of maize grains; however, TM have a lower quality than those prepared from CM. Microstructure analysis allowed observing different intergranular spaces and degrees of gelatinization; nevertheless, more studies are needed to conclude on this. This study provides important information regarding the behavior of the TM after different thermal treatments.

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Colegio de Postgraduados

Valorization of prickly pear pericarp (*Opuntia albicarpa* Scheinvar) through aerobic fermentation

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ABSTRACT

Objective: To determine the change in the composition of 'Blanca cristalina' cactus prickly pear's pericarp (*Opuntia albicarpa* Scheinvar) subjected to aerobic fermentation with *Saccharomyces cerevisiae*.
Design/methodology/approach: The pH, total soluble solids, moisture, ashes, total carbohydrates and crude protein were determined on cactus prickly pear's pericarp before and after being fermented by *S. cerevisiae*. Data were compared through a paired t test.
Results: Significant difference ($p<0.05$) was found in the total soluble solids, carbohydrate content and crude protein content after the fermentation process. Total soluble solids and carbohydrates content both decreased from 12.67±0.58 °Brix to 6.33±1.53 °Brix and from 7.43±1.4% to 0.83±0.06%, respectively. Meanwhile, crude protein content increased from 0.47±0.42% to 8.87±1.02%.
Limitations on study/implications: Non-certified commercial yeast was used in this study, so the product obtained of process must be used for animal feeding, and for human food the process must be modified.
Findings/conclusions: The fermentation process described in this work is an alternative, to increase the protein content of cactus prickly pear byproducts, such as the fruit pericarp, making it possible to be used as an animal feeding with high nutritional quality.

Keywords: byproducts, carbohydrates, protein.

INTRODUCTION

The cactus prickly pear (*Opuntia* spp.) is distributed across various arid and semi-arid regions of Mexico (Torres-Ponce *et al.*, 2015). In 2022, approximately 69,600 hectares were reported as dedicated to the cultivation of *Opuntia* spp. for forage, vegetable cactus pads (nopalitos), and fruit (tunas) (SIAP, 2024). Prickly pear production accounted for nearly 65% of this area (45,000 hectares), positioning Mexico as the world's leading producer of cactus prickly pears (SIAP, 2024).

The largest percentage of land dedicated to prickly pear production is concentrated in the states of México (35%), Zacatecas (26%), and San Luis Potosí, with 2,900 hectares (5%). In terms of production volume, the State of México (35%), Puebla (27%), and Zacatecas (22%) contributed 84% (372,925.09 t), generating an economic revenue of 1.672 billion pesos (SIAP, 2024).

In the Potosino-Zacatecano high plateau region, cactus prickly pear production is a highly significant economic activity. However, this process can generate residues, by-products, and waste, such as pericarp and glochids (Marí-Campos *et al.*, 2024). The pericarp, which constitutes the non-edible part of the fruit, represents approximately 45% to 50% of the total weight and is generally discarded, creating an environmental problem. Therefore, it is essential to explore alternatives for its utilization (El-Beltagi *et al.*, 2023).

Fermentation may represent an alternative for the valorization of cactus prickly pear residues (Derabli *et al.*, 2022) to obtain bioactive compounds (Coronado-Contreras *et al.*, 2023), owing to their high content of fermentable carbohydrates (Carpena, 2023). Solid or semi-solid fermentation of biomass has been used to increase protein content in grasses (Hu *et al.*, 2013) and in cactus pads intended for livestock feed (Flores-Hernández *et al.*, 2019), making it a potential alternative protein source with lower resource consumption (Cortés-Chamorro *et al.*, 2024).

However, the increase in protein content largely depends on the microorganisms used. For instance, fermented cactus pads with *Aspergillus niger* Tiegh achieved a crude protein (CP) concentration of 12% (Oliveira *et al.*, 2001), while fermentation with *Saccharomyces cerevisiae* Meyen ex E.C. Hansen resulted in a 10% increase (Araújo *et al.*, 2008) and 26% CP (Araújo *et al.*, 2005). In this context, the present study aimed to determine the changes in the nutritional composition of 'Blanca Cristalina' cactus prickly pear (*O. albicarpa* Scheinvar) pericarp subjected to aerobic fermentation with *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Study Sites

The cactus prickly pear fruit were collected from communal land in the locality of La Palma Pegada, Salinas de Hidalgo, San Luis Potosí, located at 22.717147° N, −101.802674° W. The predominant climate is dry temperate, with an average temperature of 18.7 °C and precipitation of 319 mm (INEGI, 2024).

Plant Material

Pericarp of 'Blanca Cristalina' cactus prickly pear (*O. albicarpa* Scheinvar) was used, collected at the ripe stage. The fruit were manually harvested and transported to Laboratory 2 of the Coordinación Académica Región Altiplano Oeste of the Universidad Autónoma de San Luis Potosí.

Preparation and Fermentation of Prickly Pear Pericarp

The fruit were divided into three groups, and the pericarp was manually removed and crushed to obtain a liquid sample. The crushed pericarp was fermented using commercial yeast (*S. cerevisiae* lyophilized, Mauripan® at 1%) (Day *et al.*, 2018). The yeast was activated following the methodology of Catacora (2022). The fermentation process was carried out for six hours at 25 °C with an agitation speed of 200 rpm, with resting intervals of 30 minutes.

Response Variables

pH and Total Soluble Solids

The pH was measured using a digital potentiometer (Oakton®), and the total soluble solids (TSS) content was determined using a digital refractometer (Hanna® HI96801).

Determination of moisture

The moisture content was determined according to the method proposed by the AOAC (Association of Analytical Communities) 1990. A 5 mL sample of the crushed material was placed in porcelain capsules at constant weight and dried at 90 °C for 3 hours in a Binder® GmbH oven. The process was performed in triplicate, and the moisture content was calculated using the following expression (equation 1):

$$M(\%) = 100 * (W_w - W_D) * W_D^{-1} \quad (1)$$

where: W_w is the weight of the wet sample (g) plus the weight of the empty (dry) capsule (g); W_D is the weight of the tray (g) plus the weight of the dry sample (g).

Determination of Ashes

The ash percentage was determined according to the method proposed by AOAC 1990. For this, 5 mL of the sample were placed in crucibles at constant weight and incinerated at 500 °C for 50 minutes, then placed in a muffle furnace at 550 °C for 2 hours, and finally placed in a drying oven at 90 °C for 1 hour. The weight of the samples was measured on a Nimbus® ADAM analytical balance.

Determination of Carbohydrates

The carbohydrate content was determined based on the standard (NMX-F-312, 1978), which involves the direct determination of reducing and total carbohydrates in food. In a 50 mL Erlenmeyer flask, 1 mL of each of the solutions (A and B) of Fehling's reagent (measured with a volumetric pipette) was added, along with 5 mL of distilled water and one drop of methylene blue indicator. The burette was filled with a 1.0% glucose standard solution. The Fehling solution mixture was heated to boiling on a magnetic stirrer plate, and then the titration was performed.

The amount of carbohydrates was obtained using the following equation:

$$\text{Fehling's reagent titre} = (\text{mL of 1\% glucose}) \times (\text{Concentration of the same})$$

Determination of Crude Protein

The percentage of crude protein was determined according to the method proposed by AOAC 1990. For this, 1 g of sample was weighed and placed in a flask. Then, 2 g of copper sulfate, 10 g of anhydrous sodium sulfate, 25 mL of sulfuric acid, and glass beads were added. The flask was then placed in the digester and heated carefully at low temperature until all the material was carbonized. The temperature was gradually increased until the solution became completely clear and maintained for 30 minutes.

The sample was cooled, and 400 to 450 mL of water was added to completely dissolve the sample. Then, 3 zinc granules and 50 mL of 1:1 sodium hydroxide solution were added. The flask was connected to a distillation system, to which a 500 mL Erlenmeyer flask containing 50 mL of boric acid and a few drops of the Shiro Tashiro reagent as an indicator was previously placed at the exit of the condenser. Once the sample was distilled, the content of the receiving flask was titrated with 0.1 N hydrochloric acid. The nitrogen present in the sample, expressed as a percentage (%), was calculated using the following equation:

$$N(\%) = V * N * 0.014 * 100 * m^{-1} \quad (2)$$

Where: N is the percentage (%) of nitrogen; V is the volume of hydrochloric acid used in the titration, in cm^3; N is the normality of hydrochloric acid; m is the mass of the sample in g; 0.014 is the milliequivalent of nitrogen.

The percentage (%) of protein was obtained by multiplying the percentage of nitrogen by the corresponding factor.

Data Analysis

The pH, total soluble solids, moisture, ash, carbohydrate, and protein variables of the tuna pericarps, before and after fermentation, were compared using a paired t-test (α=0.05). The analysis was performed using the R programming language® 4.2.2 under the RStudio® interface RStudio 2023.09.1+494.

RESULTS AND DISCUSSION

The non-fermented cactus prickly pear pericarps and the fermented cactus prickly pear pericarps showed no significant difference in pH (p=0.065), ash (p=0.888), and moisture (p=0.27) (Table 1). In contrast, there were significant differences in the crude protein content (p=0.002), with the fermented tuna pericarps having a value 18 times higher than that of the non-fermented tuna pericarps. This increase in protein was accompanied by a 50% reduction in total soluble solids (p=0.034) and an 89% reduction in carbohydrates (p=0.015).

Table 1. Composition of prickly pear pericarp before and after aerobic fermentation.

	pH	TSS (°Brix)	Ash (%)	Protein (%)	Carbohydrates (%)	Humidity (%)
Before	5.68 ± 0.29^a	12.67 ± 0.58^a	2.44 ± 0.07^a	0.47 ± 0.42^a	7.43 ± 1.40^a	92.54 ± 0.59^a
After	5.05 ± 0.04^a	6.33 ± 1.53^b	2.38 ± 0.69^a	8.87 ± 1.02^b	0.83 ± 0.06^b	94.68 ± 0.11^a

The protein content (8.87%) achieved after 6 hours of fermentation with *S. cerevisiae* was similar to that obtained in the fermentation of prickly pear pericarp with *Aspergillus niger* (9.1%) and *Ryzopus* sp. (8.6%), although with these two latter microorganisms, it was after a fermentation period of 192 hours (Carvalho-Do Santos *et al.*, 2015). Other studies have achieved protein levels of up to 33% in the fermentation of nopal with the addition of external nitrogen (Flores-Hernández, 2019). This study did not include an external source of nitrogen, which could explain the lower protein percentage; in this regard, Hu *et al.* (2012) suggest adding 2.5% external nitrogen to improve protein increase.

The total carbohydrate content decreased, which is consistent with other studies, due to the action of microorganisms on cellulose and hemicellulose (Carvalho-Do Santos *et al.*, 2015). Although there is limited information on the fermentation of prickly pear pericarp, in the case of nopal fermentation, a metabolizable energy of 2.3 to 2.67 Mcal*kg^{-1} has been obtained, and non-fibrous carbohydrates decreased from 48.9% to 26.4% (Flores-Hernández, 2019).

The ash content did not show any difference after the fermentation process, which is consistent with the findings of Flores-Hernández *et al.* (2019) during nopal fermentation. Due to the fermentation time, there were also no changes in pH or moisture content.

The application of a fermentation process to the tuna pericarp allows for the utilization of this waste to obtain a product that can be reintroduced into animal or human feed due to its high protein content. It could also become an alternative protein source with lower natural resource consumption, making it necessary to conduct studies on protein quality. Implementing this process at low technological levels could help reduce the environmental impact of tuna by-products.

CONCLUSIONS

The fermentation of 'Blanca Cristalina' cactus prickly pear pericarp (*Opuntia albicarpa* Scheinvar) with *Saccharomyces cerevisiae* for 6 hours at 25 °C with agitation at 200 rpm increases the crude protein content to 8.87%, reduces carbohydrate content to 0.83%, and total soluble solids to 6.33 °Brix, without affecting pH or ash content. This microorganism achieved the protein increase in very short times compared to the time required by other microorganisms to achieve similar results, and without the use of external sources. This makes this technology an alternative for utilizing tuna waste, either for animal or human feed, with the potential for improved yields.

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Socio-environmental Study and Ecological Footprint in the Mixtec Community of El Calvario

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ABSTRACT
Subject: Currently, it is very difficult for communities to receive funding for their development, as they often lack efficient evaluations or significant impact to support such funding. Therefore, it is vitally important for research to help make these communities visible by applying instruments that describe their situation. In this sense, two of the most important tools are the socio-environmental diagnosis and the ecological footprint, which are rarely determined in various communities.
Methodology: To determine this, Likert-type surveys and interviews with key stakeholders were conducted to identify areas for improvement within the community.
Results: The most relevant findings were the need to pave the road leading to the community, which hinders marketing and increases the cost of its products and/or the residents' labor. Another issue is the management, disposal, and elimination of waste. The greatest waste identified is the bagasse from agave, which must be valorized. Additionally, efficient water collection systems are needed, and finally, there is a lack of medicines and an available family doctor.
Implications: As a result of this study, various strategies are proposed to provide continuity to the areas of opportunity identified in the El Calvario community.
Conclusions: It is important to listen to the community's context so that they become the promoters of change, without harming the environment and nature.

Keywords: Ecological footprint, socio-environmental, interviews and key actors.

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INTRODUCTION

The socio-environmental study is a tool used to provide a diagnosis through instruments, techniques, and methodologies to propose solutions to the social, economic, and environmental problems identified in the study (Armenta *et al.*, 2012). On the other hand, the ecological footprint is an environmental indicator of the impact a society has on its surroundings, considering the resources required and the waste generated for the maintenance of that society's production and consumption model (Rees and Wackernagel, 2000). The Mixtec community of El Calvario began its agricultural activities with the cultivation of poppy, a crop that was later discontinued to avoid violence among the residents. Currently, they are dedicated to the cultivation and use of *Agave cupreata*, as well as the production of fruits (peach, guava, avocado), some vegetables (lettuce, cilantro, radish, green beans), and cereals (corn), the making of some rustic wooden utensils, and the production of artisanal mezcal. The community has an ejido of 840 hectares located south of the city of Chilpancingo de los Bravo, Guerrero, and is home to around 120 families, 90% of whom speak indigenous languages, particularly Mixtec. Access is via dirt roads, which make it difficult to obtain basic services and sanitation (López *et al.*, 2021). Due to its location, the community has experienced slow development in all its sectors. By conducting these studies, we will be able to identify areas of opportunity to improve and/or correct, with the aim of developing strategies to preserve its natural environment, promote economic development, and improve the social well-being of all community members.

MATERIALS AND METHODS

Community Approach: The El Calvario community was selected due to the connection with one of its residents, who shared that few people are interested in the sustainable and enduring development of the community. In this context, they mentioned that in the past, they were involved in poppy cultivation, but for various reasons, they no longer grow illicit plants and now focus on other agricultural crops of interest, without harming their surroundings. This is why the socio-environmental diagnosis is conducted and the ecological footprint is determined.

Socio-environmental Diagnosis: To conduct the socio-environmental diagnosis, key stakeholders were identified in order to carry out interviews and surveys with the residents of the El Calvario community. Beforehand, the purpose of the questions was explained, and informed consent was obtained to protect the identity and data of each respondent, based on the Helsinki Declaration. Both the surveys and interviews included questions about the origins of the community, geographical description and location, environmental aspects of the region, population density, education, health, religion, customs, traditions, and the main economic activities (Adapted from Armenta *et al.*, 2012).

Ecological Footprint: To conduct the ecological footprint diagnosis, Pareto analysis (80/20 rule) was used (Izar and González, 2004), applying Likert surveys to 20% of the population. The main needs for each community member's subsistence were strategically selected. The surveys consisted of closed-ended questions, which were later weighted to identify areas of opportunity and areas of deficiency within the community. The categories included in the surveys are: sensitivity and participation, physical-natural environment, natural systems, basic services, mobility, and waste management.

RESULTS AND DISCUSSION

An approach was made to the El Calvario community for the application of surveys to the residents and key stakeholders of the community for the socio-environmental diagnosis and ecological footprint assessment (Figure 1).

Socio-environmental Diagnosis: Through the application of surveys, a qualitative description was then made for each category in the socio-environmental diagnosis. The results obtained are shown in Table 1.

The areas that show the greatest need for attention are primarily those related to mobility, as it enables access to basic products and the marketing of their goods and/or performance of work. According to Armenta *et al.* (2012), a socio-environmental study

Figure 1. Interaction with the community in its environment.

Table 1. Description of the ecological footprint diagnosis in the El Calvario community.

Category	Description
Community origins	The residents indicate that the community arose from a landslide that occurred in Metlatónoc belonging to Tlapa de Comonfort, which led them to migrate to the El Calvario community on February 14, 1976 and settle in that new settlement.
Description and geographic location	Orographically, the community is considered a mountain range, with a temperate climate and oak-pine vegetation predominating. The roads are not paved so the location is difficult to access, the water resource is supplied through springs and in the case of air, the residents consider it to be of good quality due to the abundance of native vegetation.
Environmental aspects of the region	The perception of environmental degradation is minimal since the number of inhabitants is small, however, they affirm that there are firewood extraction areas that can cause some type of damage by constantly using this resource.
Population density	The population is made up of adult women and men, children and a few older adults. Its composition is 52% women and 48% men.
Education	The maximum degree of education is primary since there is a strong tendency to abandon studies to join the agricultural field.
Health	There is a health center without medical personnel.
Religion, customs and traditions	They are Catholic, but they are governed by signs of the moon and some animals.
Economic activity	Family farming and the production of artisanal mezcal.

must include political issues, environmental education, strategies for strengthening the local economy, and community participation. This study is a general diagnosis, focused on seeking development strategies to improve these areas. Furthermore, the aim is to generate community participation and to include public policy topics in order to bring resources to the community that will enable its development. Other important aspects to highlight are those outlined in the socio-environmental diagnosis by Cabral *et al.* (2022), which emphasizes that one of the most critical issues for communities in environmental matters is the transfer of scientific knowledge and contributing to the dualism of conservation *vs.* development.

In this regard, the care of natural resources must be of vital importance to everyone. With the results presented, it was possible to assess that despite the productive activities in the community, there is no significant deterioration, and development would come with management after the development of strategies. González *et al.* (2019) highlights other aspects, such as the socio-environmental conflicts threatened by mega-projects involving mining, oil extraction, infrastructure construction for industrial expansion, housing, and agrochemicals, regardless of environmental degradation, population displacement, and the depletion of natural resources for their purposes. This particular issue must be of vital importance for the El Calvario community, as its development should be focused on sustainable development. Regarding the ecological footprint diagnosis: With the results obtained from the ecological footprint survey, a description was made for each category (Table 2), and then they were weighted to establish the significant impacts (Table 3).

Table 2. Description of the ecological footprint diagnosis in the El Calvario community.

Category	Description
Sensitivity and participation	In terms of participation, the community is governed by uses and customs and sensitivity; it is considered respectful of the coexistence between its members.
Physical-natural environment	They comment on minimal environmental damage due to the extraction of natural resources, and the landscape remains in good condition, but the dependence on the use of firewood for cooking and to produce artisanal mezcal is emphasized.
Natural systems	The soil is clayey with a high amount of organic matter for agriculture, the climate is predominantly temperate and agroforestry activity is practiced with wood species and with the implementation of crops for self-consumption.
Basic services	Access to basic services is considered unstable. The water is distributed to the community through a nearby spring, the drainage service is supplied through registers without connection to drainage systems, there is an electricity service which must be paid in the nearest community (Llanos de Tepoxtepec), finally, in terms of food, the community produces beans, pumpkin and corn, but there are complications in buying food from the capital and there is no transportation.
Waste management	There is no adequate management of waste generated by the community since the most used practice for its elimination is burning it. In terms of sanitary discharges, there is no specific area for its treatment due to the lack of service. drainage, on the other hand, in the existing mezcaleras the bagasse is thrown into the open air.
Socioeconomic aspects	The main economic sector is agriculture and the production of artisanal mezcal, the population lives in wooden houses and very few in concrete. In social welfare, everyone indicates that they have what is necessary to survive.

Table 3. SWOT Matrix for the El Calvario Community.

Strengths	Opportunities
• Participation and organization in the community, production of artisanal mezcal and maguey plantations	• Marketing of mezcal, rejection of bagasse, and designing an ecotourism model
Weaknesses	**Threats**
• Lack of road to the community, medical service and waste reuse	• Unfair prices in the sale of mezcal, lack of maguey for production, deficiency in marketing channels

With the results obtained for each category, a weight of 0.25 was applied to the total number of questions (Likert surveys) within the diagnosis, on a scale from 1 to 5, where 1 indicates a critical impact, signaling an area that requires immediate intervention, and 5 indicates an area with an adequate level of organization and development. The evaluated categories were: physical-natural environment, sensitivity and participation, natural systems, basic services, mobility, waste management, and socio-economic aspects, with significant impacts of 4, 5, 4, 1, 1, 1, and 3, respectively. Once the surveys were completed and the methodology used was weighted, it was identified that the areas needing improvement are as follows: mobility, basic services, and waste management. This analysis had a great deal of community involvement, as it clearly reflected the concerns of the residents, as well as the potential strategies to be followed for the community's development.

On the other hand, Páez (2000) describes the inclusion of energy consumption, per capita income, and the human development indicator in the study for a better evaluation. In this sense, the present paper did not delve into these aspects, as the goal was to create an overview that describes the situation of each area of the community, and once evaluated, to seek strategies to improve these areas. It is also important to mention that our sampling included interviews with key actors, who are of vital importance for the community's development, as they are the ones who can bring about an ideological change. Macedo *et al.* (2024) mention in their ecological footprint analysis for the life cycle, corporations, and cities that it is important to understand the reasons behind the accelerated pace of production and consumption in society, so that we can accurately quantify the degradation of natural resources and identify the waste generated within a given population. This will allow for the migration to sustainable technologies to create a sustainable life. Finally, the application of indices will also be of vital importance for measuring the different areas of opportunity. However, the best tool to counteract environmental deterioration will be environmental planning, where all levels of government and the community provide an early warning system for the preservation of natural resources and the potential utilization of waste.

To develop a better analysis, an Ishikawa diagram (Figure 2) (Ishikawa K., 2013) and a SWOT matrix (Ponce Talancon, 2006) were created for the community in question, as described in Table 3.

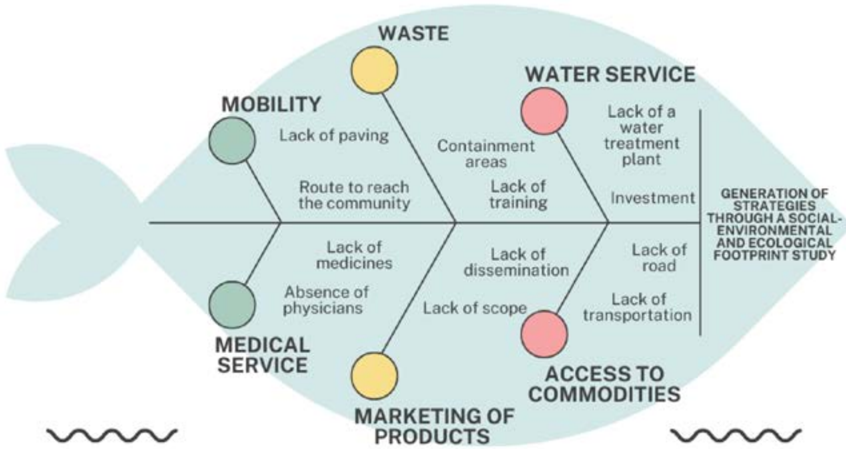

Figure 2. Cause-and-effect diagram for the addressed community.

CONCLUSIONS

Based on our study, several strategies are proposed to continue addressing the opportunities identified in the El Calvario community. These strategies begin with improving mobility conditions by seeking funding to pave the 12 kilometers connecting the community to the "Autopista del Sol." This will lead to numerous benefits, such as facilitating the commercialization of products to the city of Chilpancingo and surrounding communities, access to basic services like supply inputs, and revaluing their potential waste to create new environmentally friendly products. Another strategy to be developed is the potential for the community to create a tourist route with a restaurant, mezcal tasting, and outdoor activities. This would provide economic benefits, ensure access to quality health services and education, and, lastly, the construction of dry toilets that, using organic waste, could generate compost for use as fertilizer. There is a strong commitment to continue preserving natural resources and strengthening new initiatives for the sustainable development of the community and social well-being.

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Colegio de Postgraduados

Quality and postharvest life of tomato fruit (*Solanum lycopersicum* L.) produced under saline stress conditions

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ABSTRACT

Objective: To determine the post-harvest physiological behavior and quality changes of tomato fruit (*Solanum lycopersicum* L.) grown under saline stress conditions.
Design/methodology/approach: Tomato fruit quality variables (firmness, total soluble solids, titratable acidity and pH), along with respiration rate and weight loss were assessed at harvest and after seven days of storage at room temperature. These parameters were compared between fruit grown under saline stress (250 mM sodium chloride) and non-stressed control fruit.
Results: Tomato fruit grown under saline stress exhibited higher ($p<0.05$) total soluble solids (6.92±0.22 °Brix) and titratable acidity (0.39±0.03%), compared to the control fruit. No significant differences were observed in fruit firmness (13.01 N) or pH (5.86), at harvest time and after storage. The respiration rate decreased in both groups, from 30.77 mL CO_2 kg^{-1} h^{-1} at harvest time to 17.70 mL CO_2 kg^{-1} h^{-1} after storage; however, weight loss was not affected (11.50%).
Limitations on study/implications: Soil sampling in the production area, to measure the fruit quality and its post-harvest physiological behavior are needed on a larger scale.
Findings/conclusions: Saline stress increases the total soluble solids and titratable acidity, but does not affect the firmness, pH, weight loss and respiration rate of tomato fruit at harvest time and after storage at room temperature.

Keywords: Respiration rate, room storage, sodium chloride, saline stress.

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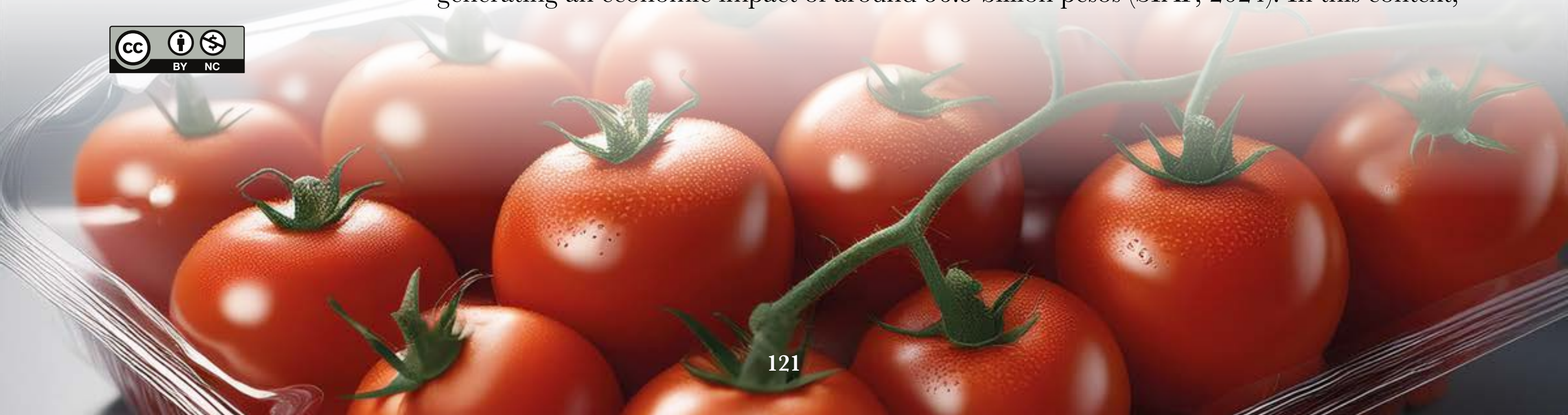
INTRODUCTION

In Mexico, the cultivation of tomato (*Solanum lycopersicum* L.) is carried out for both domestic consumption and export, primarily to North American countries (García-Estrada *et al.*, 2022). In 2023, more than 49,000 hectares in Mexico were dedicated to tomato production at varying technological levels, yielding approximately 3.6 million tons and generating an economic impact of around 36.5 billion pesos (SIAP, 2024). In this context,



San Luis Potosí ranked as the second state with the highest economic benefits derived from tomato cultivation (SIAP, 2024).

In arid and semi-arid regions, soils with high salinity levels are common (Bacha *et al.*, 2017), necessitating improvements in agricultural productivity (Wu *et al.*, 2022). Under these conditions, tomato cultivation is feasible despite it being a glycophyte (Hasegawa *et al.*, 2000); however, salinity can negatively impact its yield (Amador, 2022).

Various studies have shown that saline stress affects the physiology of tomato plants, reducing vegetative growth rate, root development, the number of fruit per plant, and consequently, yield (Singh *et al.*, 2011; Amjad *et al.*, 2014; Zhang *et al.*, 2016; Naeem *et al.*, 2020; Aguis *et al.*, 2022). It also induces other morphological and biochemical responses (Raza *et al.*, 2016; Bacha *et al.*, 2017), which are closely linked to the genotype and phenological stage of the plant (Alam *et al.*, 2021). However, subjecting the crop to moderate levels of water and saline stress can improve fruit quality (Ripoll *et al.*, 2016; Wang *et al.*, 2023), increasing the content of total soluble solids, titratable acidity, carotenoid levels, and lycopene content (Goykovic and Saavedra, 2007). Nevertheless, studies on the postharvest behavior of tomato fruit are scarce (Aguis *et al.*, 2022).

In the Potosino-Zacatecano Highlands region, soils and water with high salinity levels can be found, which may cause saline stress in tomato crops. Therefore, the objective of this research was to determine the postharvest physiological behavior and quality of tomato fruit (*Solanum lycopersicum* L.) produced under saline stress conditions.

MATERIALS AND METHODS

Study site and plant material

For this research, tomato fruit (*Solanum lycopersicum* L.) of the saladette type were used, produced under saline stress conditions in a greenhouse at the Experimental Area of the Coordinación Académica Región Altiplano Oeste of the Universidad Autónoma de San Luis Potosí, located in Salinas de Hidalgo, San Luis Potosí (22° 38' 28.5" N, 101° 42' 10.0" W).

Experimental design

The postharvest behavior of tomato fruit produced under saline stress conditions was determined in comparison to a control. For this, 15 tomato plants were irrigated with a solution of 250 mM sodium chloride (NaCl) every 72 hours for three weeks. The experimental unit consisted of five fruit and three replications. Under a completely randomized design with the following treatments: 1) Control at harvest, 2) Control after storage, 3) Saline stress at harvest, and 4) Saline stress after storage.

Response variables

The following quality variables were evaluated: firmness (N), total soluble solids (TSS) (°Brix), pH, and titratable acidity (% citric acid), as well as physiological variables including respiration rate (mL CO_2 kg^{-1} h^{-1}) and weight loss (%). These variables were measured in tomato fruit produced under saline stress and in control plants, both at harvest and after seven days of storage at room temperature.

Fruit firmness was measured using a GY-4 fruit penetrometer (Generic®, BW28) with a 3 mm diameter cylindrical probe, taking two opposite readings in the equatorial region (Al-Dairi *et al.*, 2021). The total soluble solids content was measured using a digital refractometer (Hanna®, HI96801, Japan). pH was measured with a digital potentiometer (OAKTON, pH700), and the titratable acidity was determined by titration, expressed based on citric acid (Flores *et al.*, 2007).

The respiration rate was determined using a closed system according to Pérez-López *et al.* (2014). For this, the fruit were weighed on a balance (OHAUS Scout®, H-7294) and placed inside a hermetic chamber of 3620 mL for 60 minutes. The change in CO_2 concentration was measured with an infrared CO_2 logger (DATALogger, Extech Instruments®, model CO210). The weight loss was determined according to the following equation.

$$wl(\%) = 100 * (w_0 - w_1) * (w_0)^{-1}$$

where: wl is the weight loss (%); w_0 is the initial weight of the fruit (g), and w_1 is the final weight of the fruit (g).

Data analysis

An analysis of variance and a mean comparison using the Tukey method ($\alpha=0.05$) were performed for the quality variables and respiration rate, and the assumptions of normality (Shapiro-Wilk) and homogeneity of variance (Bartlett's test) were verified. Weight loss was compared using a t-test ($\alpha=0.05$). The analysis was conducted in the R programming language (R-project® 4.3.3) using the RStudio® 2023.12.1 interface.

RESULTS AND DISCUSSION

In the analysis of variance, the assumptions of normality ($p>0.60$) and homogeneity of variance ($p>0.15$) were met. No significant difference ($F=1.493$, $p=0.289$) was found in the fruit firmness between the two salinity levels, both at harvest (13.54 N on average) and after storage (12.61 N on average) (Table 1). According to Wu *et al.* (2022), salinity stress conditions do not affect the firmness of these fruit. According to Naeem *et al.* (2020), fruit firmness increases under salinity conditions between 60 and 90 mM of NaCl; however, this behavior is related to the age of the plants (Botella *et al.*, 2001), which could explain the results of this study.

Table 1. Change in the quality variables of tomato fruit (*Solanum lycopersicum* L.) produced under different saline stress conditions.

Saline stress	Evaluation time	Firmness (N)	TSS (°Brix)	pH	Acidity (%)
Control	Harvest	14.42 ± 1.31^{a}	6.35 ± 0.14^{b}	6.20 ± 1.02^{a}	0.30 ± 0.04^{b}
Control	Storage	12.16 ± 0.08^{a}	6.32 ± 0.26^{ab}	5.59 ± 0.71^{a}	0.34 ± 0.04^{ab}
250 mM NaCl	Harvest	12.66 ± 1.31^{a}	6.95 ± 0.21^{a}	5.93 ± 0.70^{a}	0.38 ± 0.01^{a}
250 mM NaCl	Storage	13.06 ± 2.04^{a}	6.89 ± 0.28^{a}	5.71 ± 0.23^{a}	0.41 ± 0.04^{a}

* Means whith the same superscript whitin each column, have not significative difference ($\alpha=0.05$).

The TSS content of the fruit showed a significant difference ($F=6.598$, $p=0.015$) between salinity levels. Fruit produced under high salinity conditions exhibited higher TSS content (6.94 °Brix). According to Wang *et al.* (2023), the increase in total soluble solids content is related to the use of saline water for irrigation. Similarly, Wu and Kubota (2008) state that TSS is directly related to electrical conductivity; higher salinity levels result in higher TSS, with values similar to those found in this study (5.20-6.20 °Brix). This behavior can be explained by the reduction in water content in the fruit (Wu *et al.*, 2022) and the accumulation of calcium, potassium, and chloride ions (Safdar *et al.*, 2019).

On the other hand, an increase ($F=6.556$, $p=0.015$) in titratable acidity was observed, rising from 0.30% to 0.41% in fruit produced under saline stress (Table 1). This behavior contrasts with the findings of Li *et al.* (2022), who report that saline stress reduces the content of organic acids and vitamin C in tomato fruit. However, this change in acidity percentage did not affect the pH ($F=0.999$, $p=0.441$). The fruit exhibited a reduction in respiration rate ($F=9.642$, $p=0.005$), decreasing from 30.77 mL CO_2 kg^{-1} h^{-1} at harvest to 17.70 mL CO_2 kg^{-1} h^{-1} after storage. However, no significant differences were found between fruit produced under saline stress (Table 2). After storage, no significant difference in weight loss were observed ($p>0.05$), with an average loss of 11.51% (Table 2). This result is higher than that reported by Villarreal-Romero *et al.* (2002) for long shelf-life tomatoes after 20 days of storage at 20 °C. The observed difference is attributed to relative humidity conditions and the cultivars used.

Table 2. Changes in physiological variables of tomato fruit (*Solanum lycopersicum* L.) produced under different saline stress conditions.

Saline stress	Evaluation time	Respiration rate (mL CO_2 kg^{-1} h^{-1})	Weigth loss (%)
Control	Harvest	31.00 ± 1.73^a	—
Control	Storage	18.42 ± 5.89^b	11.65 ± 0.13^a
250 mM NaCl	Harvest	30.54 ± 5.49^a	—
250 mM NaCl	Storage	16.98 ± 1.89^b	11.38 ± 3.39^a

* Means whith the same superscript whitin each column, have not significative difference ($\alpha=0.05$).

Tomato fruit produced under saline stress conditions show improvements in certain quality variables without affecting physiological processes such as respiration rate and weight loss. This prolongs their shelf-life under ambient temperature conditions, potentially increasing the commercialization period in local markets without the need for additional preservation technologies.

CONCLUSIONS

Salt stress increases the total soluble solids content and acidity percentage but does not affect firmness, pH, weight loss, or respiration rate in saladette tomato fruit (*Solanum lycopersicum* L.) at harvest or after 7 days of storage at room temperature.

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Colegio de Postgraduados

Antifungal effect of the edible coating added with cinnamon essential oil (*Cinnamomum verum*) on the shelf life of strawberries (*Fragaria×ananassa*)

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ABSTRACT

Objective: to develop an edible coating with cinnamon essential oil (CEO) to prolong the useful life of strawberries.

Design/Methodology/Approach: We obtained CEO by the distillation method with water and steam, determining the minimum inhibitory concentration through an *in vitro* test with the agar diffusion technique. Also, we determined antifungal activity *in vivo*.

Results: CEO, obtained by the distillation method with water and steam, showed yields of 1.2±0.3% per extraction cycle. In turn, the in vitro test on the minimum inhibitory concentration that CEO presented against the fungus *Rizhopus stolonifer* using the agar dilution technique showed that its growth was completely inhibited at a concentration of 500 ppm. However, the antifungal activity *in vivo* was less effective due to the complex host-antifungal-pathogen system added to the temperature factor. The study at room temperature (27 °C) showed growth of the fungus *Rizhopus stolonifer* on the strawberries with the coating at 100 and 300 ppm on day 3 of treatment. On the contrary, at refrigeration temperature (8 °C), the strawberries coated with the different concentrations of CEO remained in good condition for a longer time, as proved by visual appearance without loss of color which is the appearance of freshness, added to a visually attractive shine.

Limitations/Implications of the study: using cinnamon essential oil (CEO) as an antifungal agent in edible coatings could provide an effective and natural alternative for strawberry preservation. This would potentially impact post-harvest practices. To implement CEO applications, combined with refrigeration, can help to reduce fungal growth and to extend the shelf life of strawberries.

Findings/Conclusions: a synergy of temperature and CEO concentrations should be used to slow the growth of *Rizhopus stolonifera*, to prolong the life time of strawberries, maintaining their appearance without loss of color, which is the appearance of freshness, adding a visually attractive shine.

Keywords: *Rizhopus stolonifer*, edible coating, cinnamon essential oil.

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INTRODUCTION

The strawberry (*Fragaria×ananassa*) is a plant belonging to the Rosaceae family. Currently it is considered as a gourmet fruit. Its importance lies on the content of several components such as vitamins E and C, tannins, flavonoids, anthocyanins, catechin, quercetin, kaempferol and organic acids, such as citric, malic, and ellagic, among others. These compounds exert antioxidant properties and help to reduce the risk of cardiovascular

events [1]. However, it is a non-climacteric fruit, highly perishable, with a reduced post-harvest shelf life.

The shelf life of fresh strawberries in refrigeration (0 to 4 °C) is usually about five days. Therefore, conservation constitutes a problem for producers and the industry due to the high speed at which metabolic processes occur. Mainly, a high respiratory rate and the lack of an external barrier to limit the water retention. Other characteristics that limit conservation are the low resistance to mechanical damage, and microbiological attack by fungi —especially *Botrytis cinerea* and *Rizhopus stolonifer*—, causing significant losses during transportation and marketing, and reducing its attributes about flavor, aroma and texture; affecting commercial quality [2].

Food industry is trying to satisfy consumer demands, and it has promoted the development and design of new technologies that allow maintaining and obtaining products with characteristics similar to fresh foods and with a useful life comparable to processed products [5], thus increasing shelf life and offering safe products.

Various food preservation technologies —some of which have been used for a long time— protect fruits from alteration by microorganisms that can be inhibited by refrigeration, reduction of water activity, acidification, and modification of the container atmosphere by non-toxic treatments. Thermal packaging in plastic coatings, surface processing (waxes and paraffin), refrigeration and edible coatings (EC) based on different materials [3].

Concerning ECs, lipids, resins, and polysaccharides such as chitosan have been used for their formulation and design due to their antimicrobial properties. Furthermore, applying antimicrobial agents could prevent or delay the microbial deterioration of food indicates that essential oils such as cinnamon have antimicrobial and antioxidant properties, so they can be considered as a natural preservative. Several studies have also been done on the antifungal and antioxidant properties of essential oils, such as extracts of oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.), coriander (*Coriandrum sativum* L), onion (*Allium cepa* L.), garlic (*Allium sativum* L.) and cinnamon [4].

One of the most effective antifungal agent is cinnamon leaf essential oil (CEO) due to its high antifungal power attributed mainly to its main compound, eugenol, which has a high capacity to stabilize and neutralize free radicals. Its high capacity to inhibit lipid peroxidation induced by reactive oxygen species has been demonstrated [5], so the composition of HCA gives its antifungal and antioxidant properties with the potential to be used as a post-harvest treatment.

Due to the above, the aim of the current study was to determine the antifungal effect of edible coating added with cinnamon (*Cinnamomum verum*) essential oil on the shelf life of strawberries (*Fragaria×ananassa*).

MATERIALS AND METHODS

Cinnamon (*Cinnamomum verum*) and strawberry (*Fragaria×ananassa*) were obtained from the local market of San Felipe del Progreso, Estado de México. Glycerol (glycerin USP, Azumex, Lot: OBBP76909), tween 80 (Sigma-aldrich), carboxymethylcellulose (NF ingredients, Lot: NFCMC1000), PDA agar (MCD LAB,7041, LOT 70414G053), and mycrodin disinfectant were also obtained from the local market.

Extraction of cinnamon (*Cinnamomum verum*) essential oil

Cinnamon essential oil (CEO) was extracted according to the methodology reported by Rodríguez & Real (2012) [6] with some modifications. For the distillation technique with water and steam, 250 g of starting material was used of each 2 L of purified water; the sample was placed on the separation grid, and then the distiller lid was placed. Subsequently, the hoses were placed in a container with 15 L of water (one hose was connected to the water pump, and the other was left free).

The essential oil extractor was placed on an electric grill, and then the grill and the water pump were connected to the electric power to obtain the essential oil. At the extractor outlet, a beaker with a capacity of 2 L was placed to collect the decanted hydrolate. The oil obtained was stored in amber bottles, and the extraction yield was determined based on Equation 1.

$$Yield\,(\%) = \frac{Volume\ obtained\ (mL)}{Sample\ quantity\ (g)} * 100 \qquad \text{Equation 1}$$

***In vitro* assay**

The minimum inhibitory concentration (MIC) was performed by dilution in agar. PDA agar was prepared according to the manufacturer's instructions, and a final concentration of 1% (v/v) of Tween-80 was incorporated into the agar medium after autoclaving to improve solubility in cinnamon essential oil (CEO). A series of double concentrations of cinnamon oil was prepared, ranging between 100,300,500 and 600 ppm. Three repetitions were done per treatment in addition to the control. The fungus *Rhizopus stolonifer* was planted in the centre of the Petri dishes as discs with 7 mm diameter taken from a colony with 16 days of culture [7]

Spore collection and quantification

Spores of fungus *Rhizopus stononifer* were collected from Petri dishes with 16 days of growth, 7.7 mg were weighed and added to 100 mL of sterile water. The solution was stirred at 800 rpm for 45 minutes. Subsequently, a 1:100 dilution was made. A Neubauer chamber was used to calculate the concentration of spores using the four largest squares of the chamber. The sample (suspension of the spores) was applied to the edge of the coverslip, which will enter by capillarity into the space between the slide and the coverslip, filling the chamber. Finally, it was observed under a microscope with a 40X objective, and the spore concentration was calculated according to Equation 2.

$$Concentration\,/\,mL = \left(\frac{Number\ of\ spores}{4}\right)(Dilution\ \ factor)(10{,}000) \qquad \text{Equation 2}$$

Solution preparation

A solution was prepared following the methodology by Mamani & Hernán (2019) [8], with some modifications. 0.4 g of 0.5% carboxymethylcellulose, 1.2 mL of glycerol, 0.8 mL of 1% Tween 80, 76.8 mL of sterile distilled water, and CEO in different concentrations

(100, 300, 500, 700, 900 ppm) were used. Subsequently, the solutions were mixed at 800 rpm for 30 min.

Preparation of edible coatings by immersion

Strawberries were selected by visual examination; those that showed physical damage were discarded (bruises, softening, degradation or rot, visible fungi or non-homogeneous color), and disinfection was made. Fruits were immersed in a solution of purified water with disinfectant micron according to the manufacturer's instructions (amount of disinfectant and disinfection time); fruits were then drained and dried thoroughly.

The edible coating was added according to the methodology reported by Van *et al.* (2023) [9] with some modifications; the strawberries were immersed in the coating for 10 seconds and placed on racks to drain the excess. Subsequently, we applied three sprays of the solution containing the fungus *Rhizopus stononifer* per fruit (0.60 mL), and they were stored in transparent trays hermetically closed and covered with plastic wrap. Tests were done at room temperature (Ta) (27 °C), by three treatments (100, 300, 500 ppm) with two replicates each and compared to the control. They were inspected in several days (1, 3, 4, 5 and 6) to monitor visual changes in the fruits. On the other hand, strawberries at refrigeration temperature (Tr) (8 °C) were monitored during 16 continuous days.

Experimental design

Experimental data was analyzed using SPSS 13.0 (Statistical Package for the Social Sciences, IBM SPSS, USA). Mean values were calculated and reported as mean ± SD (n=3). One-way analysis of variance followed by the least significant difference test was used on the *in vitro* and *in vivo* antifungal activities of cinnamon essential oil ($p<0.05$).

RESULTS AND DISCUSSION

CEO Extraction yield

The isolated oil fraction of cinnamon presented a pale yellowish to greenish appearance with a strong aroma, with an extraction yield of 1.2±0.3% per extraction cycle. This agrees with the findings of Kamaliroosta *et al.* (2012) [10] with values of 1.3% of the total weight. However, regarding the extraction cycles of cinnamon essential oil, 15 mL were obtained per 2 kg of raw material. Juarez (2015) [11] reported 6 mL of essential oil from 1 kg of dry cinnamon bark, obtaining a high extraction yield using the same extraction technique in this study. Nonetheless, they differ from those obtained by Vargas, (2019) [12] with a variation of 0.4%. Possibly, the differences are related to the geographical position, the cultivation, the variety of cinnamon, the harvest time, the discrepancy of the method, the climatic and environmental conditions, or the extraction method.

***In vitro* assay**

The minimum inhibitory concentration (MIC) presented by cinnamon oil against the *Rizhopus stolonifer* fungus tested by the agar dilution method is shown in Figure 1. Control showed significant growth on the first day with a growth halo of 18.1±0.03 mm in diameter, and complete growth on day 3 with a diameter of 80.2±0.1 mm and spores since the fourth day. The 100 ppm treatment showed a behavior similar to the control, with a growth halo of 8.6±0.01 mm in diameter and a difference compared to control of 9.5 mm. According to the values obtained (Table 1), we deduced that MIC in these *in vitro* assays is 500 ppm; growth of *Rizhopus stolonifera* was not observed.

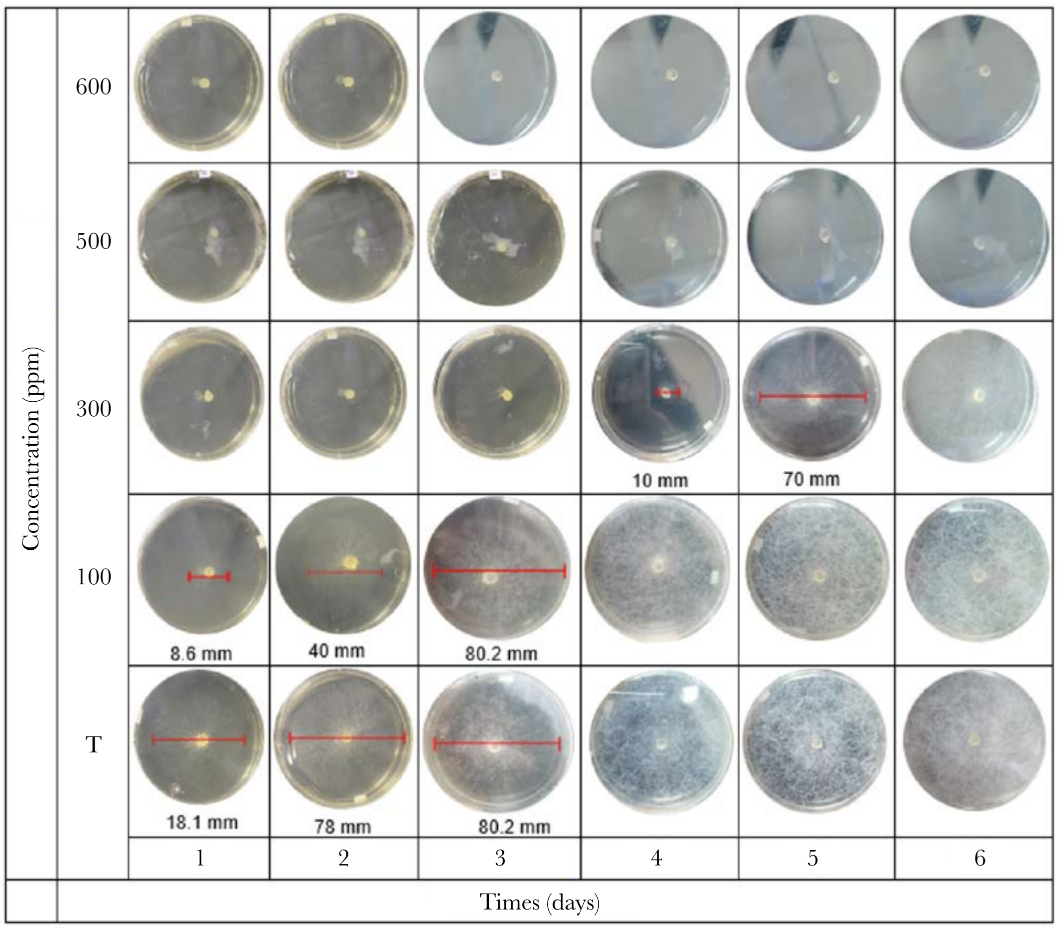

Figure 1. Minimum inhibitory concentration of cinnamon (*Cinnamomum verum*) essential oil on the growth of *Rhizopus stolonifer*.

Table 1. Growth inhibition zone in terms of concentration of cinnamon essential oil.

CEOC (ppm)	**Inhibition zone diameter (mm)**					
	Day 1	**Day 2**	**Day 3**	**Day 6**	**Day 8**	**Day 10**
0.0	18.1 ± 0.03^{a}	78.0 ± 1.03^{b}	80.2 ± 0.1^{b}	80.2 ± 0.09^{b}	80.2 ± 0.09^{b}	80.2 ± 0.09^{b}
100	8.6 ± 0.01^{c}	40.0 ± 0.8^{d}	80.2 ± 0.09^{b}	80.2 ± 0.09^{b}	80.2 ± 0.09^{b}	80.2 ± 0.09^{b}
300	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	10.0 ± 1.01^{c}	70.0 ± 1.03^{d}	80.2 ± 0.09^{b}
500	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}
600	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}

CEOC, Cinnamon essential oil concentration; Well diameter 10 mm. Different letters show significant difference, expressed as growth inhibition diameter.

Values obtained are similar to those by Ochoa *et al.* (2012) [13] who reported that cinnamon essential oil had an antifungal effect against *Fusarium* spp. at a concentration of 300 μg mL^{-1} (300 ppm) on the other hand Xing *et al.* (2010) [14] showed that higher concentrations of cinnamon oil in combinations with clove oil were more effective in inhibiting the growth of *Aspergillus flavus*. While Tzortzakis (2009) [15] reported complete inhibition of spores at concentrations of 500 ppm, which results similar to those obtained in this study.

In vivo antifungal activity

Results of the *in vitro* assays showed that CEO had less antifungal activity when it was tested on fruits at the same concentrations. This is related to the complexity of host-antifungal-pathogen system. Besides, Abdolahi *et al.* (2010) [16] reported that this observation is associated with the alteration of actions in the assay, or structural changes such as hydrolysis, degradation and polymerization of fruits under *in vivo* conditions.

The effects of edible coatings on strawberries —previously infected with *Rhizopus stolonifer*— and the control stored at room temperature (at 27 °C) are shown in Table 2 and Figure 2, respectively. The statistical analysis revealed that at all concentrations of cinnamon essential oil tested, strawberries without the edible coating (control) did not differ significantly ($p>0.05$) from strawberries coated with a concentration of 100 and 300 ppm since the third day of storage, where 100% of the strawberries showed *Rhizopus stolonifer* growth (Table 2). Therefore, the use of low concentrations in the edible coating will not have an antifungal action in strawberries. On the other hand, at the concentration of 500 ppm, a loss of firmness was observed since day 3; and a spread of fungal infection since day 5, with 50.00±1.5% of infected fruits. Overall, growth rates increased with temperatures close to the optimum.

On the other hand, when the study was repeated at low temperatures, there was a significant difference. The control showed growth up to fourth day with a growth of

Table 2. Percentage of coated strawberries and uncoated strawberries (control) artificially infected with *Rhizopus stolonifer* and stored at room temperature (27 °C) and refrigeration (8 °C).

Infected strawberries (%) at 27 °C					
CEOC (ppm)	**Day 1**	**Day 3**	**Day 4**	**Day 5**	**Day 6**
500	0,00±0.0% a	0,00±0.0%a	0,00±0.0% a	50,00±1,5%b	50,00±0,00%b
300	0,00±0.0% a	0,00±0.0%a	100,0±0,0%c	100,0±0,0%c	100,0±0,0%c
100	0,00±0.0% a	0,00±0.0%a	100,0±0,0%c	100,0±0,0%c	100,0±0,0%c
Control	96,67±3,1%d	100,0±0,0%c	100,0±0,0%c	100,0±0,0%c	100,0±0,0%c
Infected strawberries (%) at 8 °C					
CEOC (ppm)	**Day 1**	**Day 4**	**Day 8**	**Day 12**	**Day 16**
500	0,00±0.0%a	0,00±0.0% a	0,00±0.0%a	0,00±0.0%a	0,00±0.0% a
300	0,00±0.0 %a	0,00±0.0 % a	0,00±0.0 %a	0,00±0.0 %a	0,00±0.0 % a
100	0,00±0.0%a	5,02 ±0.9%b	13,33±5,77%c	50,00 ±0,0%d	60,00±0,0%e
Control	0,00±0.0 %a	15,33±2,52%c	30,00 ±0,00%f	90,00±0,0% g	96,67±2,89%h

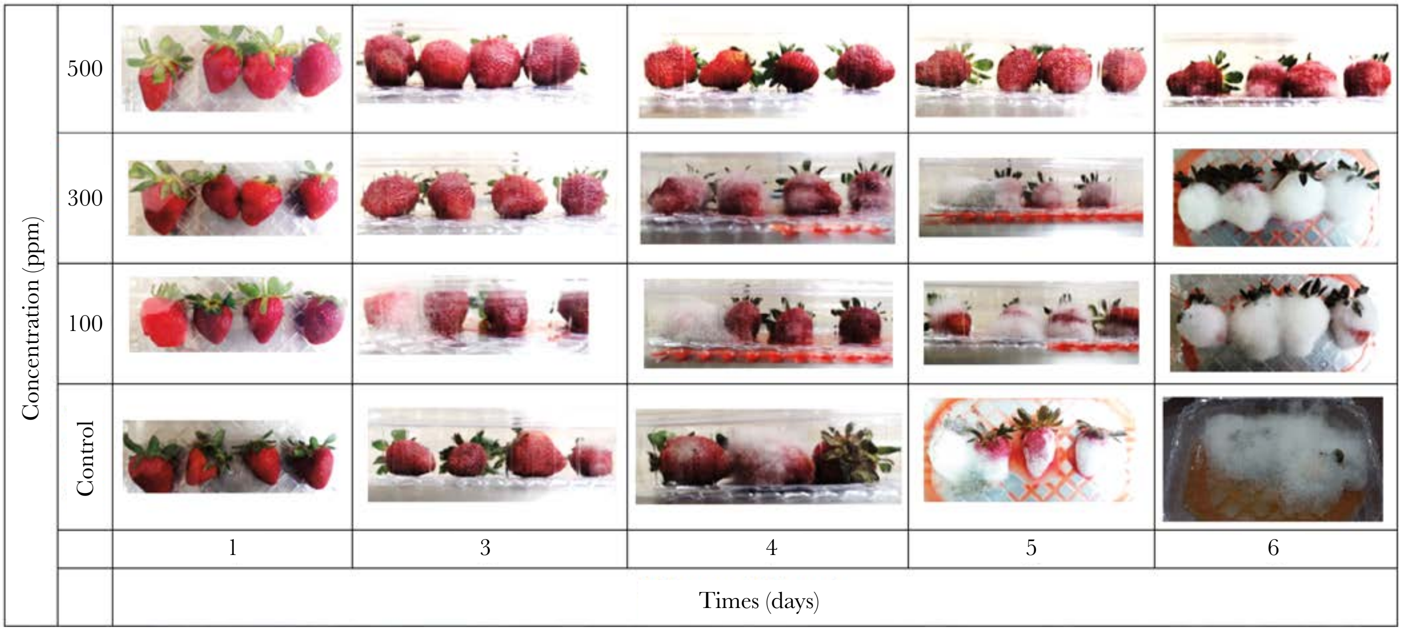

Figure 2. Effect of edible coatings on strawberry (*Fragaria*×*ananassa*) at room temperature (27 °C).

15.33±2.52%, while on the last day of the study (day 16), the infected fruits increased to 96.67±2.89%. Showing that the severity of the infection decreased starting at 100 ppm, and no growth at 300 ppm. This is an evident effect caused by temperature, that was also reported by Win *et al.* (2007) [17], who mentioned that the antimicrobial activity of essential oils in fruits and vegetables is benefited by a decrease in storage temperature, a higher concentration, and a decreased pH of the food.

While Ojagh *et al.* (2010) [18] demonstrated that cinnamon extract completely inhibited the mycelial growth of the fungi *Colletotrichum musae*, *Fusarium* spp. and *Lasiodiplodia theobromae* at 5.0 g L^{-1}, in banana *in vitro*. Furthermore, Tzortzakis (2009) [15] demonstrated that previously exposing the tomato fruit to 500 ppm of cinnamon for three days, then inoculated it with fungi, reduced the development of lesions caused by *Colletotrichum coccodes* and *Botrytis cinerea*.

According to the results, at low temperatures (8 °C), the percentage of strawberries infected with *Rhizopus stolonifer* and control showed significant differences ($p<0.05$). Vega-Ríos *et al.* (2014) [19] reported that during fruit storage at low temperatures, there is a decrease in physiological processes and a weakening of the pathogenicity of fungi. This is similar to the observation found in this study at temperatures of 8 °C, where a lower percentage of infection was observed, compared to those strawberries stored at temperatures of 27 °C. With 100, 300 and 500 ppm of CEO there was inhibition of *Rhizopus stolonifer*; we can say that there is a direct relationship between the treatment at refrigeration temperatures and the MIC In addition, a significant change could be observed in terms of the appearance of the strawberry, as it is shown in Figure 3; since the coated strawberries maintained good visual appearance without loss of color, with appearance of freshness, plus a visually attractive shine.

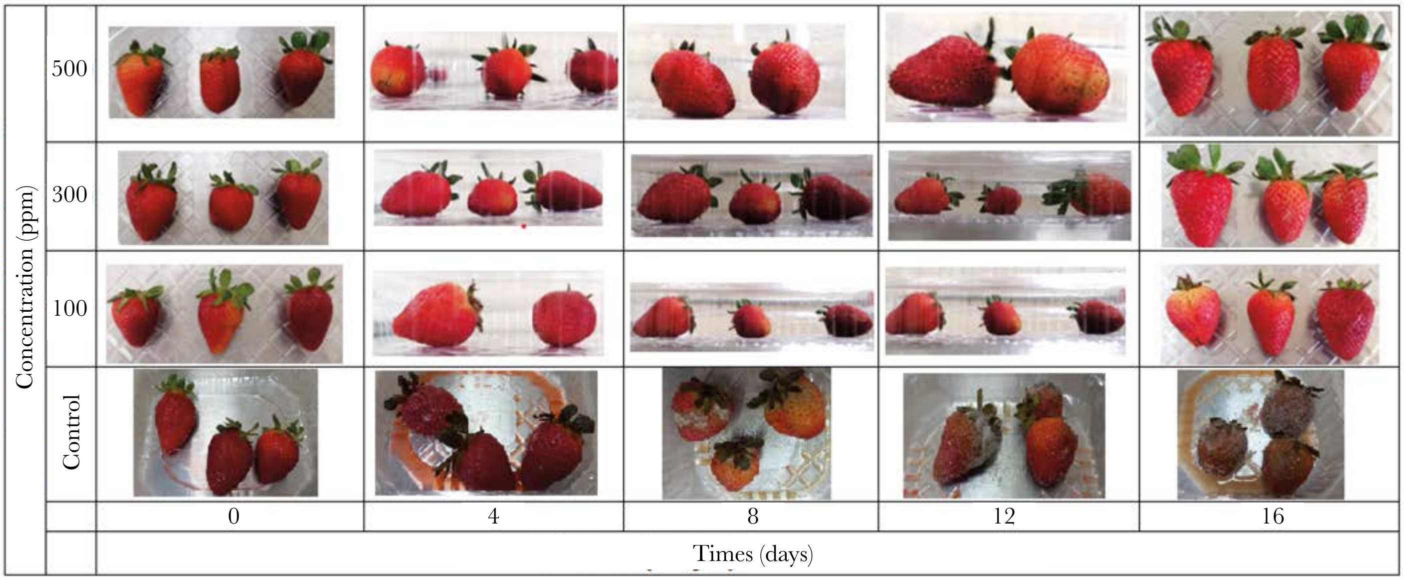

Figure 3. Effect of edible coating on strawberries (*Fragaria*×*ananassa*) at refrigeration temperature (8 °C).

The previous effect can be attributed to CEO's features; its constituents and hydrophobic properties, since they penetrate the cell membranes of fungi, altering their cellular structure and increasing permeability. Accordingly, dos Santos *et al.* (2012) [20] reported that permeability causes a leak of exhausted ions and other cellular contents, causing fungi cells death. In addition to the above, *Rhizopus* rot is related to temperature, with a maximum fungi growth at 27 °C, similar to what was found in this study.

CONCLUSIONS

The study demonstrated that cinnamon (*Cinnamomum verum*) essential oil effectively inhibits the growth of *Rhizopus stolonifer* both *in vitro* and *in vivo*. The effectiveness of the antifungal action was highly dependent on the essential oil concentration and storage conditions. At room temperature (27 °C), low concentrations (100 and 300 ppm) failed to prevent the proliferation of the fungus, while a concentration of 500 ppm significantly reduced growth up to the third day. However, greater effectiveness of the coating was evident at refrigeration temperatures (8 °C), where coated strawberries showed a notable reduction in fungi infection even after 16 days of storage.

These findings highlight the importance of the synergy between storage temperature and essential oil concentration in controlling *Rhizopus stolonifer* infections. Also preserving the freshness appearance of strawberries, combined effects could extend their shelf life. The use of edible coatings with cinnamon essential oil is a promising strategy for post-harvest preservation of fruits, especially under refrigeration conditions.

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Colegio de Postgraduados

Biomass Incorporation into Degraded Soils and its Effect on the Productivity of Common Bean (*Phaseolus vulgaris* L.)

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ABSTRACT
Objective: to evaluate the effect of incorporating biomass in degraded soils, and fertilization, on yield of common bean plants grown in the semiarid highlands of northern México.
Design/Methodology/Approach: from 2019 to 2022, experiments were established in different soil conditions in Durango (México). Pinto Saltillo (PS) and Negro San Luis (NSL) common bean cultivars were planted in three fertilization treatments, 1: chemical, 2: organic and 3: foliar sprayings. Data were recorded for days to flowering and physiological maturity (days after sowing, DAS), reaction to diseases, yield, and weight of 100 seeds. The analysis of variance was performed in a completely randomized design, with a factorial arrangement, and a partial yield-stability analysis was also included.
Results: the PS cultivar showed precocious flowering (38 DAS) and intermediate maturity (91 DAS); while NSL showed late flowering (43 DAS) and maturity (101 DAS). In most of the conditions, the absence of anthracnose and rust was observed, as well as intermediate and generalized levels (5 and 6 on the CIAT scale) of common bacterial blight (CBB) with low influence on the results obtained. The biological cycle of NSL was longer than PS, influencing its response to the fertilization treatments evaluated. However, yield results were statistically similar among common bean cultivars and fertilization treatments.
Limitations/Implications of the study: leaf biomass incorporated into the soil is a natural and sustainable method for common bean production; although only two common bean cultivars were included in the study.
Findings/Conclusions: soil-incorporated biomass and foliar fertilizer sprayings could be considered as natural low-cost inputs, both related to increased common bean yield in Durango (México).

Keywords: yield, agronomic management, sustainability.

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INTRODUCTION

In Durango and other states of the semi-arid highlands of México, physical and chemical degradation of agricultural soils is observed, which aggravates the production problems of common bean plants [1]. Soil degradation is expressed in the low content of organic matter

and limited availability of nutrients, such as potassium, iron and manganese, mainly due to the moderately high pH values (>7.9). The current condition of soils is combined with irregular rainfall, presence of pathogenic organisms, practices of subsistence agriculture and few use of agricultural inputs, which are present in the semi-arid highlands of México. All of this results in the low yield of common bean [2]. In this region, farmers apply chemical fertilizer (granulated and liquid) only when an acceptable amount of rainfall (>200 mm) is recorded from sowing until flowering of the common bean plants. This is due to the high risk of loss of investment in limited humidity conditions, which cause intermittent water stress in the plant.

The indiscriminate use of chemical fertilizers was related to the contamination of water tables, mainly due to the high mobility of some nutrients such as nitrogen that favors the formation of molecules such as nitrites (NO_2) and nitrates (NO_3) [3] that are carcinogenic [4-6]. These molecules are extracted during the pumping of well water and can cause health problems in the human population [7]. In addition, the transport of these molecules in water currents during heavy rains facilitates their accumulation in terrestrial water bodies and in the sea [8]. This causes health damage to macro- and microorganisms that inhabit water currents, river banks and dams; as well as on beaches and the open sea, which represents considerable ecological damage. The use of chemical fertilizer produces a momentary and costly increase in soil fertility, but shows inefficiency during its storage, application and use by plants.

The need to incorporate biomass has been established to increase grain yield in common bean plants for a long time, by reducing the problems caused by drought and low soil fertility. Biomass incorporation must be combined with the genetic improvement of common bean and other efficient and agroecological strategies to implement sustainable production of this legume in northern México.

The common bean varieties developed in Durango show high levels of potential yield, that is, the yield recorded when they are cultivated under irrigated conditions. Nevertheless, under rainfed conditions, the productive capacity of the common bean shows to be lower. Pinto Saltillo and Negro San Luis are the most popular common bean varieties for planting in the semi-arid highlands, due to their adaptation in most of the producing areas of the Mexican states of Durango and Zacatecas. The use of the Environmental Index is one of the techniques used to study the adaptability and stability of a variety yield under different evaluation conditions [9]. The technique consists of the linear regression of the yield of each variety at a specific site, in relation to the average of all varieties at that site. The objective was to evaluate the effect of biomass incorporation into degraded soils and fertilization on the yield of common bean plants grown in the semi-arid highlands of northern México.

MATERIALS AND METHODS

During the spring-summer cycles from 2019 to 2022, six experimental and commercial plots were established in different locations in the common bean-producing area of the state of Durango, México. Thus, six soil conditions which define growth environments were established at the planting sites, Durango 2020, Durango 2021, Durango 2022, La

Soledad (Canatlán) 2022, Poanas 2022 and La Purísima (Cuencamé) 2022. Two varieties of common bean (Pinto Saltillo and Negro San Luis) were planted and treatments 1: chemical fertilization, 2: organic fertilizer and 3: foliar application were evaluated. The experimental plot, for each variety, consisted of 20 rows of 100 m in length and 0.81 m of separation (1600 m^2), for a total area of about 1 ha. Chemical fertilization was based on technical recommendations for irrigated common bean cultivation in Durango [10], which include the 35-50-00 dose for nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O). The dose was obtained by mixing 109 kg of diammonium phosphate (DAP: 18-46-00) and 37 kg of urea (46-00-00); as well as with the calibration of the fertilizer canisters. The application was mainly carried out at the time of the first cultural work (weeding), which was made in stage V_3, related to the deployment of the first leaflet.

The application of biomass and compost (organic treatment) consisted of the incorporation of 6 Mg ha^{-1} of crushed maralfalfa (*Pennisetum* sp.) biomass and other experiments, established at a commercial level, in which compost mechanically incorporated into the soil was used. In the foliar fertilization treatment, a combination of liquid products (6 L ha^{-1} UAN 32®+5 L ha^{-1} FertigroP® or 1.1 L ha^{-1} of Bayfolán®) was applied during the pre-flowering stage (R_5), which was carried out by means of manual spraying equipment. Insecticide (dimethoate or spinetoram) was applied up to four times, to control the mexican bean beetle (*Epilachna varivestis*) and the pod weevil (*Apion* sp.). The weed control was achieved through two mechanized weedings, an application of herbicide (Fomesafén=Flex®) and two manual weedings made with a hoe. In Durango (2020, 2021 and 2022) and in La Soledad, of Canatlán municipality, a gravity supplemental irrigation was applied to avoid water stress in the common bean plants in order to obtain a greater expression of the genetic yield potential.

The number of days to flowering, reaction to diseases, days to physiological maturity, yield and weight of 100 seeds were evaluated. Days to flowering and physiological maturity [11] were counted in days after sowing (DAS). When the common bean varieties reached the phenological stage of grain filling (R_8), the reaction to the diseases with the highest incidence in Durango was evaluated; anthracnose (*Colletotrichum lindemuthianum*), rust (*Uromyces appendiculatus* var. *appendiculatus*) and common bacterial blight [*Xanthomonas axonopodis* (syn. *campestris*) pv. *phaseoli*]. The evaluation used the scale ranging from 1 to 9, proposed by the International Center for Tropical Agriculture-CIAT [11]. On this scale,

Table 1. Soil Characteristics in six conditions of evaluation of the effect of biomass incorporation on the yield of two common bean varieties in Durango, México.

Environment	Soil Type	[1]O. M.	N	P	K	pH
Durango, 2020	Clay Loam	Medium	High	[2]M. L.	Very High	8.2
Durango, 2021	Clay Loam	Very High	Low	Low	Very High	8.3
Durango, 2022	Clay Loam	High	Medium	M. L.	Very High	8.4
La Purísima, 2022	Sandy Loam	M. L.	Low	M. H.	M. H.	8.1
La Soledad, 2022	Loamy Sand	M. L.	Very Low	M. L.	High	8.3
Poanas, 2022	Sandy Loam	M. L.	Low	M. H.	High	8.6

[1]O.M.: organic matter content, N: nitrogen, P: phosphorus, K: potassium; [2]M. L.: moderately low content, M. H.: moderately high content.

common bean varieties classified in categories 1 to 3 are considered as resistant; 4 to 6, intermediate; and between 7 and 9 are susceptible to pathogenic microorganisms.

After maturity, the yield and weight of 100 seeds were determined in each of the varieties and treatments under study. The useful plot (8.1 m^2) consisted of two 5 m furrows 0.81 m apart, with five replications. The manually harvested plants were sun-dried, mechanically threshed and then the grain was cleaned with a sieve and electric blower for the removal of impurities. The cleaned grain was weighed on a digital scale with an accuracy of 0.1 g, for the estimation of the yield (kg ha^{-1}). The weight of 100 seeds (g) was determined in each of the samples obtained in the field, by collecting a random sub-sample of 100 whole and well-developed grains.

Field data were used in the analysis of variance to identify the outstanding variety or fertilization treatment. The yield and weight of 100 seeds were analyzed based on a completely randomized design, with factorial arrangement (varieties×fertilization treatments) and five replications. The comparison of means was performed with Tukey's test ($p \leq 0.05$). SAS© version 9.4 was used to perform both, the analysis of variance and multiple comparison of means, as well as the analysis of adaptability and yield stability.

RESULTS Y DISCUSSION

The Pinto Saltillo common bean variety showed precocity to flowering, with an average of 38 days after sowing (DAS) ranging from 31 to 48 DAS. The maturity of this variety was intermediate (91, 86-96 DAS) [12], through the environmental conditions defined by the study; when compared with Negro San Luis, which showed late flowering (43, 35-52 DAS) and maturity (101, 91-112 DAS) (Table 2). In most years there was an absence of symptoms of anthracnose and rust; and only in 2022, in the sites of La Purísima and

Table 2. Variables evaluated in two common bean varieties grown under six production conditions and three fertilization treatments in Durango, México.

Environment	Variety	[1]DF	A	R	B	DPM
Durango, 2020	Pinto Saltillo	41	1	1	5	96
Durango, 2021	Pinto Saltillo	39	1	1	6	90
Durango, 2022	Pinto Saltillo	48	1	1	6	95
La Purísima, 2022	Pinto Saltillo	31	2	1	6	90
La Soledad, 2022	Pinto Saltillo	36	1	1	6	86
Poanas, 2022	Pinto Saltillo	34	2	1	6	90
	Average	38				91
Durango, 2020	Negro San Luis	48	1	1	5	106
Durango, 2021	Negro San Luis	46	1	1	5	112
Durango, 2022	Negro San Luis	52	1	1	5	108
La Purísima, 2022	Negro San Luis	35	1	1	6	95
La Soledad, 2022	Negro San Luis	42	2	1	6	91
Poanas, 2022	Negro San Luis	38	1	1	5	94
	Average	43				101

[1]DF=days to flowering, A=anthracnose, R=rust, B=common bacterial blight, DPM=days to physiological maturity.

Poanas, very light symptoms (2, in CIAT scale) of anthracnose were recorded in the case of Pinto Saltillo (Table 1). In the case of Negro San Luis, similar levels of anthracnose symptoms were observed in La Soledad, Durango. In the case of common bacterial blight, intermediate (5, in CIAT scale) to generalized (6, in CIAT scale) levels of the disease were recorded [11], for both varieties under all environmental conditions under study.

The results showed that the prolonged duration of the biological cycle of the Negro San Luis variety influenced the use of each of the fertilization treatments included in the study. The influence of pathogenic microorganisms was low and, in the case of common bacterial blight, it presented similarity in both varieties through environments, so the effect on the results obtained was reduced.

Statistically equal results were recorded for grain yield between fertilization treatments and common bean cultivars (Figure 1). Only slight (non-significant) differences were observed between common bean cultivars for grain yield among plant nutrition systems and higher values (1929 to 2156 kg ha^{-1}) were recorded for Negro San Luis. This was mainly due to biomass incorporation into the soil combined with a late maturity (115 DAS), which allowed this variety to take advantage of the benefits of the organic matter incorporated. Among the benefits of incorporating organic matter are the increased fertility and nutrient availability; as well as the improvement of soil structure, moisture retention capacity and microbial activity [13].

In the case of Pinto Saltillo cultivar, it showed a shorter range of grain yield with values between 1853 and 1917 kg ha^{-1} related to intermediate maturity (96 DAS). The response of this variety was lower in the organic treatment, due to the shorter biological cycle and this makes it necessary to recommend biomass incorporation before planting, so that all the benefits from the organic matter activity can be obtained.

In each Environmental Index (EI), larger, although not significant, increases in yield were recorded in soils treated with biomass incorporation, mainly in the late-season variety (Negro San Luis) (Figure 2). The highest yield values in Negro San Luis were observed in the organic treatment with values ranging from 915 kg ha^{-1} in the least productive environment, up to 3498 kg ha^{-1} in the site with the highest level of productivity. Foliar application of liquid fertilizer caused low yields in Negro San Luis

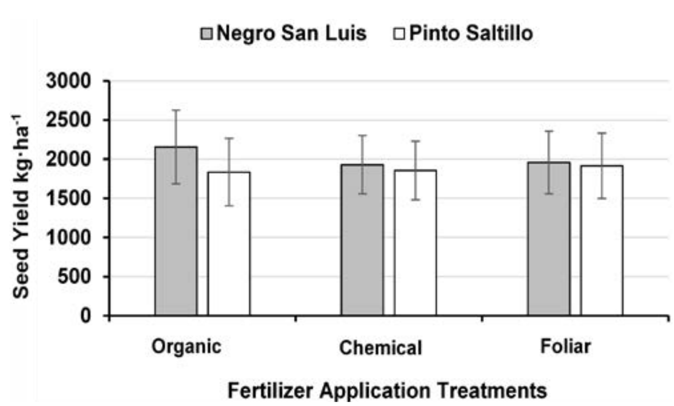

Figure 1. Effect of fertilization treatments on the average yield of two varieties of common bean, grown in six soil conditions (environments) in the state of Durango, México.

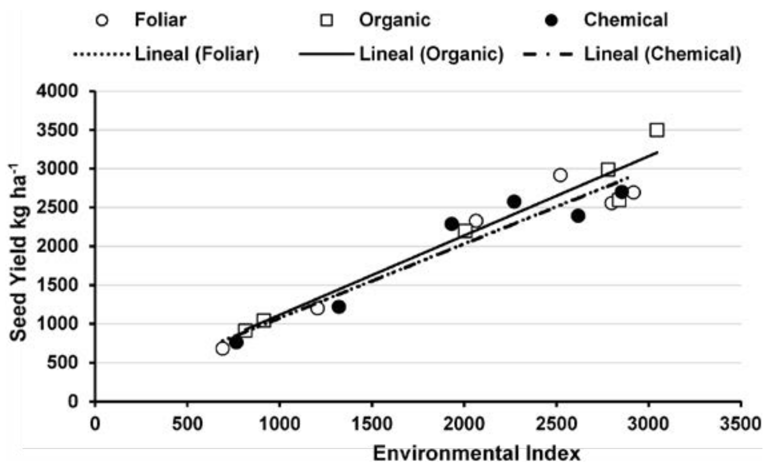

Figure 2. Effect of fertilization treatments on the yield of the Negro San Luis variety (NSL) of common bean, grown at six soil conditions (environments) in the state of Durango, México.

(687 to 2695 kg ha^{-1}); however, it was statistically equal to that obtained with the use of granulated chemical fertilizer (766 to 2700 kg ha^{-1}). Soil-incorporated biomass should be considered as a low-cost and sustainable system related to increased yield in the semi-arid highlands of México.

It is necessary to evaluate the date of early application of the biomass (before planting) to favor the efficient use of organic matter by common bean plants, whose varieties present different number of days to physiological maturity. The biological cycle of common bean is relatively short (86 to 120 days), which limits the use of nutrients from decomposing the natural biomass, such as that obtained from Maralfalfa grass (*Pennisetum* sp.).

The highest yield values in Negro San Luis were observed for the organic treatment, with values ranging from 915 kg ha^{-1} in the least productive environment, up to 3498 kg ha^{-1} in the site with high productivity. Foliar application of liquid fertilizer caused low yields in Negro San Luis (687 to 2695 kg ha^{-1}); however, it was statistically similar to those obtained by using chemical fertilizer (766 to 2700 kg ha^{-1}). It is necessary to evaluate the date for early application of the biomass (before planting) to achieve the efficient use of organic matter by common bean plants that present different number of days to physiological maturity.

In Pinto Saltillo, the same yield response was observed between fertilization treatments, with values ranging from 691 kg ha^{-1} in the least productive environment, up to 3138 kg ha^{-1} in the site with high productivity, which was achieved with foliar application of liquid fertilizer (Figure 3). It was observed that the biomass incorporated into the soil favored Pinto Saltillo at a lower level, compared to Negro San Luis. This is mainly due to the late moment of application, and the intermediate biological cycle that limited the benefits of the incorporation of organic matter into the soil. Similar results were obtained in previous studies with common bean, in which the application of organic matter reached yields (1.82 to 1.98 Mg ha^{-1}) statistically equal to the treatment with chemical fertilization [14]. In other studies, increased biomass accumulation was observed in broccoli plants with distinct types of organic fertilization [15].

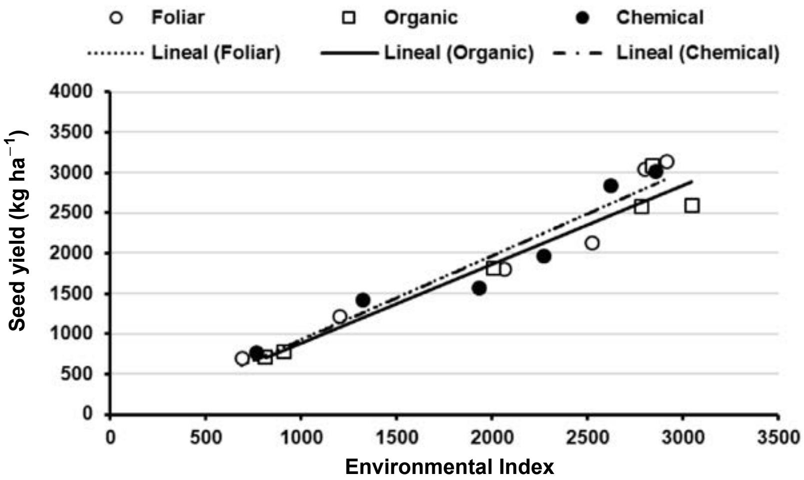

Figure 3. Effect of soil biomass application on the yield of the Pinto Saltillo variety of common bean, grown under six soil conditions (environments), in Durango, México.

CONCLUSIONS

Biomass incorporation into degraded soils, during the post-emergence of seedlings, had a positive effect statistically equal to that obtained with chemical fertilization, on the grain yield of common bean, mainly in the late-maturing variety (Negro San Luis). Liquid fertilizer favored the yield of the improved variety of intermediate maturity (Pinto Saltillo), although the influence was statistically equal to the use of granular chemical fertilizer and the use of organic fertilizer.

Common bean varieties responded differently to fertilization treatments; which is derived from their geographical and genetic origin, as well as from duration of their biological cycle. That is why it is necessary to adjust the timing, and methods of application of biomass and organic fertilizer before sowing, to optimize their positive effect on common bean yield. The goal is to strengthen sustainability in the cultivation of common bean plants, under the soil conditions of the semi-arid highlands of México.

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Colegio de Postgraduados

Microhabitat of Totolcozcatl mushroom [*Entoloma abortivum* (Berk. & Curtis) Donk, Basidiomycotina] for restoration in the cloud forest

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ABSTRACT

Objective: to characterize the microhabitat and requirements of *Entoloma abortivum*, which is considered as a wild fungus (known as Totolcozcatl, in Nahuatl), and as a non-timber forest resource in the cloud forest.

Design/Methodology/Approach: *E. abortivum* is used for food (as a mushroom); it has ecological, cultural and economic importance for rural communities. Their non-regulated collection has led to depletion and over-exploitation in the mountanous cloud forest of Xaltepuxtla (Puebla) Mexico. Habitat characterization of the fungus, which it requires for development and reintroduction is presented. Sensors for temperature and relative humidity were placed in each site determined in the field visits, at the depth of emergence of the carpophores, in order to record these microclimate variables. These sensors were used before and during the whole period of development of the fungus in two seasons, 2016-2017 and 2017-2018; pH and substrate temperature sensors were also used.

Results: the surrounding plant composition, site-specific shade density and soil-substrate chemical characteristics were described. The emergence of carpophores occurred during the winter when the microenvironmental temperature was recorded between 15.8 and 17.5 °C, relative humidity between 64%-67%, shade density 93.6%. In the substrate, temperatures fluctuated between 8.5 and 14 °C in the same period; the pH of the substrate, between 5.0-5.5; Chemical characteristics and base-type of the bamboo stem, leaf litter; tillers from decaying grasses, and rotten pieces of jonote are other conditions for the development of this fungus.

Limitations/Implications of the study: female collectors identified and delimited the emergency sites of the fungus in the field visits. The capabilities of these female collectors are based on morphological traits, and they reach identification up to the level of species.

Findings/Conclusions: this is the first site-specific characterization of the native habitat of the Totolcozcatl mushroom (*Entoloma abortivum*).

Keywords: emergency period, substrate, microclimate.

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INTRODUCTION

The wild mushroom "Totolcozcatl" (*Entoloma abortivum*) is a species of great nutritional, ecological, cultural and economic importance for the rural communities of Xaltepuxtla (Puebla), Mexico. This use has led to resource depletion and overexploitation

in the cloud forest. It is known that this fungus only emerges in the winter season and its propagation by conventional means has not been successful. Since it is evident that it requires particular ecological conditions, such restrictions have made it impossible to adequately restore it (Mateo, 2018). The reproduction of wild fungi in natural conditions is determined by the biology of each taxon and some ecological characteristics such as number of trees in the site, height, normal diameter, canopy cover, and average age of the trees. It is important to have information, as precise as posible, on temperature, average monthly and annual precipitation, which correspond to the period considered for sampling (Arteaga y Moreno, 2006; Velasco *et al*., 2010). In addition, Lorenzana (2008) recognized the importance of evaluating the characteristics of the soil microhabitat, such as pH, soil texture class, organic matter contents, nitrogen, phosphorus, potassium and cation exchange capacity. The assessment of the optimal ecological conditions for fungi development is decisive in the restoration process of edible mushrooms (Sözbir *et al*., 2015; Villanueva-Jiménez *et al*., 2006).

In addition to considering ecological factors, the technique for *E. abortivum* reproduction is another conditioning factor for restoration. Research by Mateo (2018) showed that *E. abortivum* can be isolated and reproduced by the context method, and that the malt-bran medium is the most viable for the induction process in terms of mycelial growth and hyphae production (Buendía-Espinoza *et al*., 2020). This is particularly important because this fungus is prone to be parasitized (aborted); in this condition spore isolation is not possible. So, research is important because it provides information on a little-known species whose restoration is urgent in ecosystems where extinction appears as imminent. The objective of this research was to characterize the ecological conditions of the microhabitat where the Totolcozcatl fungus (*Entoloma abortivum*) develops, in order to select key sites for propagation in the cloud forest of Xaltepuxtla, Puebla.

MATERIALS AND METHODS

The research was carried out in the community of Xaltepuxtla, Puebla; at the Ocotitla farm with an area of 40 ha (Ruíz, 2016). On the property there are remnants of severely disturbed cloud forest, predominantly dedicated to the production of local ornamental plants, such as "chima" (*Chamaecyparis lawsoniana*), myrtle, rhododendron and *Cedrela* sp. The area is located between the extreme coordinates 20° 11' 23.06" N, 97° 58' 53.03" W; and 20° 10' 57.124" N, 97° 57' 30.836" W; at an altitude of 1280 m. The climate (A)Cb(fm)(e)gw" semi-warm humid with rainfall all year round, the driest month rainfall greater than 40 mm, with less than 18% winter rainfall related to total average precipitation. The average annual temperature ranges between 18 and 24 °C, extreme weather, the hottest month before June (Ganges-type temperature march), and intra-summer drought.

The type of vegetation in the remnants corresponds to cloud forest species, with abundance of *Liquidambar* sp., *Platanus* sp., *Pinus patula*, tree-ferns of the genus *Cyathea*, *Heliconia* sp., locally called papatla leaf or apatla which is endemic to the area. Due to anthropocentric activity in the middle herbaceous stratum there is abundance of

Pteridium aquilinum (ocopetate), *Rhododendron simsii*, (azalea) and 'chima', *Chamaecyparis lawsoniana*; an some Bambusae individuals. The herbaceous (middle) stratum is composed of multi-purpose, medicinal and ornamental shrubs. In the lower stratum, vegetables, ornamental plants, short-cycle crops such as chili, tomato, radishes, coriander, and small aromatic plants, where 'papalo' (*Porophyllum ruderale*) predominates (López, 2013).

Natural distribution areas of *Entoloma abortivum*

The participation of commoners (community members), both 'medieros' and owners, is key to the process of restoration of depleted plant species; as well as to forest conservation and its resources (Durston and Miranda, 2002). Given the importance of Totolcozcatl mushroom for the community, female collectors who can identify the fungus up to the species level, participated in field trips to identify the areas where it develops. Field visits for delimiting aproximate areas were made in June 2016, before the period in which the mushrooms begins to emerge. According to the collectors, this occurs at the end of November or beginning of December, and occasionally it extends until February. Four of the sites identified by the collectors were chosen because of greater fungal emergence (Table 1).

Microclimate conditions at the sites

Once the sites were georeferenced and delimited, a HOBO Pro v2l sensor was installed in each. This sensor was placed in the centre of the site at ground level, in order to record temperature, relative humidity; maximum, minimum, and average dew point, with a recording frequency every 15 minutes. This information began to be recorded before fungi emergency to identify precisely the microclimatic requirements of this fungus. In addition, an instrument for soil studies (model SK-300B) was placed to measure pH and temperature. Monitoring of maximum, minimum and average ambient temperature and relative humidity at all sites was performed at 15-day intervals with a hygrothermograph. The canopy coverage density was obtained with a concave density meter with direction to the four cardinal points. These records were averaged and expressed as a percentage of visible sky, *i.e.* canopy opening.

Site-specific soil collection and chemical analysis

Twenty days after the emergency period, samples were taken from the substrate-soil where the fungi developed. Blocks of approximately 15×25×2.5 cm were extracted, stored in plastic bags and transported in a cooler. They were then refrigerated for processing and analysis. In the laboratory each block was cut into four sections; in one

Table 1. Location of the sites where *Entoloma abortivum* emerges.

Coordinates	Site 1	Site 2	Site 3	Site 4
N	20° 11' 12.1"	20° 11' 15.8"	20° 11' 15.4"	20° 11' 04.8"
W	097° 57' 42.5"	097° 57' 51.5"	097° 57' 51.4"	097° 57' 47.8"
Altitude	1215 m	1252 m	1251 m	1229 m

of those the moisture content was determined; the rest of the fractions of each sample were also dried in the oven at the same temperature for grinding. The determinations included organic matter (Walkley and Black); inorganic N, available P (Olsen technique); exchangeable K, Ca and Mg (ammonium acetate at neutral pH); Fe, Mn Cu, Zn (extracted with DTPA); B (extracted with $CaCl_2$); and S (by turbidimetry) according to the methodologies indicated in the Mexican Standard NOM-021-RECNAT-2000 (SEMARNAT, 2002).

Once the emergency sites were identified, data were taken from the place of collection, date, common names of the nearest trees, photographs illustrating characteristics of the habitat of the species, color, size and shape. Once the emergence period of the Totolcozcatl fungus began, specimens in the reproductive phase were collected. The specimens placed in aluminum foil were transported in a cooler to the laboratory of the Institute of Ecology, for taxonomic and phenotypic characterization (Mateo, 2018).

RESULTS AND DISCUSSION

Two forms of occurrence of the fungus were identified at the sites, most of the fruiting bodies in the form of an "aborted" white carpophore that lacks the well-formation of lamellas, and the shape of a typical agaricoid fruiting body with a stipe, as indicated by Lindner *et al.* (2001).

Description of the Sites where *Entoloma abortivum* emerges

For sites Two and Three, the description is generalized because they have a similar composition and structure, due to the proximity between them. It is also necessary to specify that only in these two sites, the emergence of the fruiting bodies of the species of interest was recorded.

Site One. The upper layer in the vegetation structure is composed of sweetgum (*Liquidambar styraciflua*); the middle stratum is made up of specimens of tree ferns (Pterophyta). In the lower stratum there are ornamental plants of 'chima' (*Chamaecyparis lawsoniana*), and the shrubby fern (*Nephrolepis exaltata*). The substrate is a mixture of leaf litter of the plant species identified on site, with pieces of wood, both decomposing (Figure 1).

Figure 1. Vegetation structure on monitoring Site One.

Sites Two and Three. They were very close, only separated by a drainage stream where water derived from a spring runs. In both sites, the upper and middle stratum is bamboo vegetation (*Guadua* sp.), better known by the people as "Otate"; in site Two, there is also a specimen of sweetgum. The lower stratum is made up of a kind of tiller grass. The substrate where the fungi develop is predominantly bamboo leaf, with the presence of sweetgum litter, decomposing wood debris, a 'jonote' trunk (*Heliocarpus appendiculatus*), and rotten tillers of grass (Figure 2).

Site Four. The vegetation is diverse because it is a remnant of the forest that exists in the place, *i.e*. the vegetation that once predominated in the locality. The upper stratum presents specimens of sweetgum and poplar (*Platanus* sp.); the middle stratum is made up of tree ferns, also, citrus and vines species. In the herbaceous stratum we found fern (*Nephrolepis* sp.), selaginels, cycads, some palms and grasses (Figure 3). The substrate is decomposing leaf litter, combined with a large amount of dead plant material.

Figure 2. Conditions of Site Two and Site Three.

Figure 3. Site Four location.

The identification of the plant species that complement the microhabitat of the fungus is important because the leaf litter is what generates a substrate conducive to the reproduction of the fungus. Therefore, it is essential to know the plant composition that is present. As indicated by Velasco *et al.* (2010), the production of wild mushrooms under natural conditions is determined by the conformation of the tree dimension present in the stands where they develop. As well as by the edaphic and climatological elements of their habitats. The average diameter and average age of the trees are also important ecological features (Arteaga y Moreno, 2006).

What was observed also coincides with Villanueva *et al.* (2006) in proving that the lower density of trees and decrease in the humus layer impacts the development of macroscopic sporomes. Another parameter of importance in the characterization of the habitat was the average shade density, in site Two, this was 94.54%; and in site Three, 92.72%. With these results we can affirm that *E. abortivum* requires considerable tree cover for its production, which coincides with what was identified by Velasco *et al.* (2010) regarding that the total fungal production depends on the number of trees on site, and the canopy cover they provide to the site.

Substrate at the emergence sites

The substrate where the fungus emerged was basically composed of bamboo leaves and decaying wood; base of bamboo stalks, decaying tillers from grasses, and on a rotten section of jonote (*Heliocarpus appendiculatus*); all of them mixed with the soil. Díaz (2002) also described the species growing at the base of trees, among leaf litter or on decaying wood. As a sapoptroph species, *E. abortivum* obtains its nutrients from the decomposition of dead organic matter (Montañez, 2013). Similar observations were recorded by Watling (1974) and Huffman *et al.* (2008) in deciduous forests. The chemical analysis of the substrates where the fungus emerged is summarized in Table 2.

As it can be seen, a common denominator in both sites is that fungus requires high contents of organic matter in the soil, high level of K and S; and adequate of

Table 2. Chemical properties of the substrate-soil in the emergence sites of *Entoloma abortivum*.

	Site Two										
	MO	Ca	Mg	K	S	P	Zn	Mn	Fe	B	Cu
	%	me 100 g^{-1}			mg kg^{-1}						
$\bar{X}$	12.7	9.53	1.78	1.27	20.4	5.8	9.8	49.7	70.2	2.4	0.78
σ^Z	2.66	8.83	0.50	0.25	12.4	7.9	5.4	35.3	36.1	1.1	0.57
Level	high	medium		high	high	medium	adequate				marginal
	Site Three										
$\bar{X}$	9.0	6.72	1.48	1.27	12.5	3.3	2.3	6.3	119.1	2.1	0.35
σ^Z	1.41	8.73	0.29	0.41	7.3	2.3	0.42	2.0	16.6	0.63	0.09
Level	medium			high	high	low	adequate				marginal

ZN=10.

micronutrients Zn, Mn, Fe and B. According to Olfati and Peyvast (2008) high K content in the substrate (6200-7920 mg per 100 g DM) is essential for the production of fruiting bodies of *Pleurotus ostreatus* var. sajor caju. Detailed research in regard to the valuation of substrates in the production of *Pleutorus ostreatus* and *P. cystidious*, indicates that the content of Ca, Fe, Mn and Zn is strongly related to the biological efficiency of *Pleurotus ostreatus* and *Pleurotus cystidiosus* (Ha *et al.*, 2015). Substrate requirements for *E. abortivum* have not yet been reported. Due to mushroom cultivation is a multifactorial process (Krupodorova y Barshteyn, 2015), the data obtained in this research should be considered in the preparation of a culture medium for *E. abortivum*, since these requirements are unique for each species of fungus.

Emergence period and climate requirements of *Entoloma abortivum*

The averages of the temperature and micro-environmental relative humidity data recorded at the two sites, during the two monitoring periods on the emergence of the fungus, indicated that its requirements fluctuate between 15.8 °C to 17.5 °C temperature, and 64 to 67% relative humidity. In the case of the substrate, in order to emerge the species needs temperature conditions between 8.5 °C and 14 °C, and a neutral to slightly acidic pH (5.0 to 5.5). Figures 4 and 5 show the temperatures recorded in the substrate during the period of emergence of the fungus in the collection sites, in which the requirements for temperature drop, that the species demands to be able to emerge, can be observed.

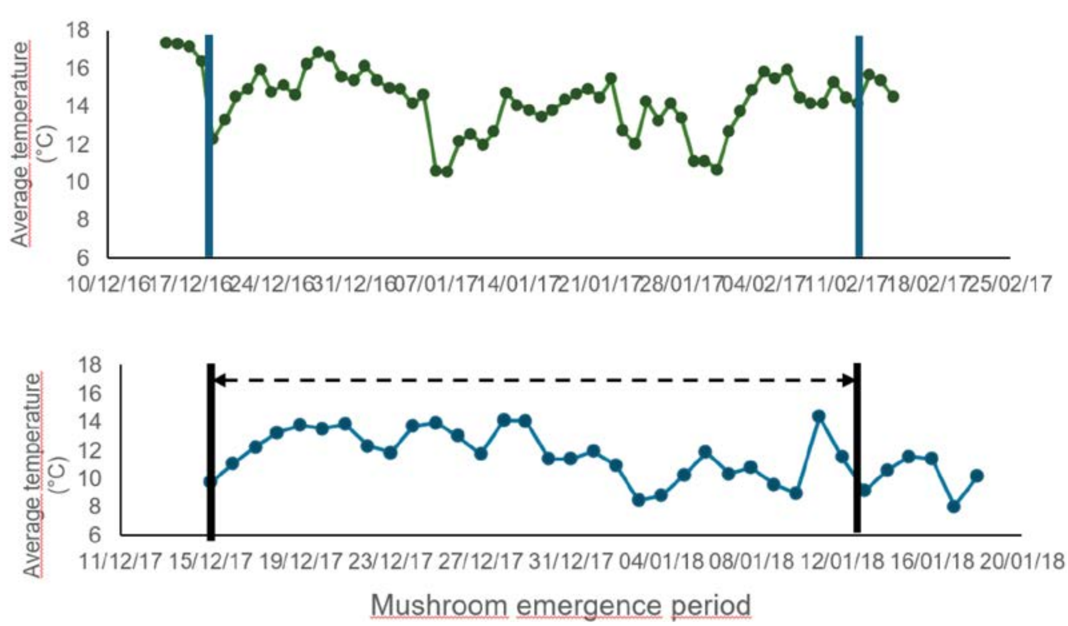

Figure 4. Microenvironmental temperature and emergence period of *Entoloma* at site two (years 2016-2017 and 2017-2018).

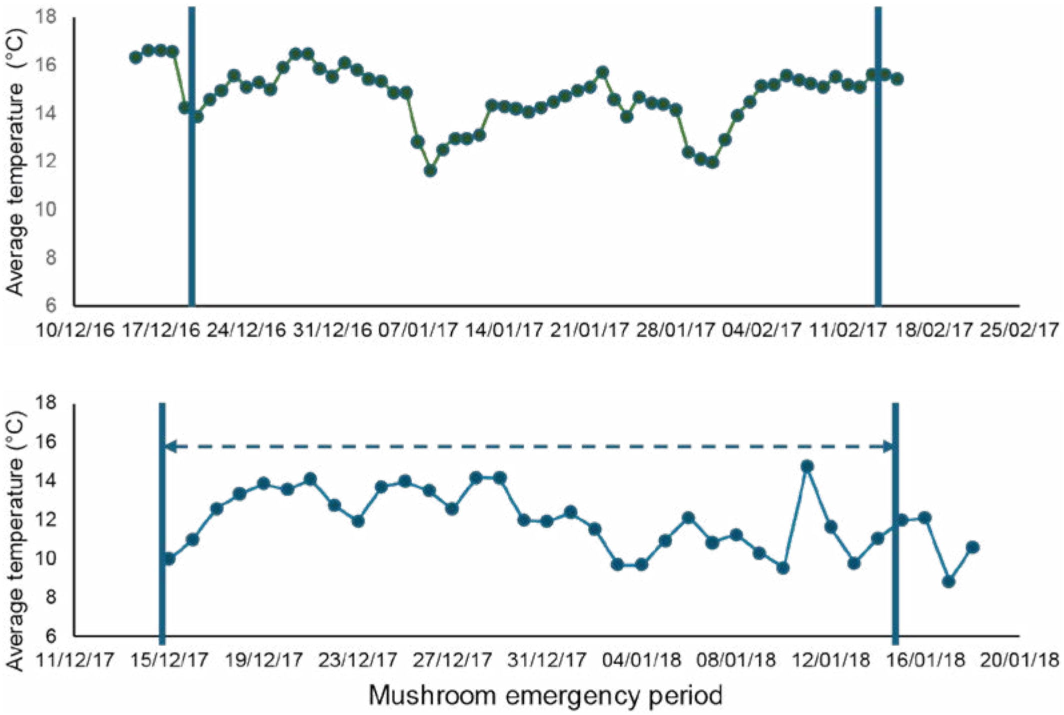

Figure 5. Micro-environmental temperature and emergence period of *Entoloma abortivum* at site Three (2016-2017 and 2017-2018).

CONCLUSIONS

The reintroduction of the wild fungus *Entoloma abortivum* known as Totolcozcatl, in the mountain cloud forest in the community of Xaltepuxtla, (Puebla) Mexico, requires the location of microhabitats with a shade percentage of almost 100%, and high relative humidity (64 to 67%). Other requirements, linked to the emergence period of this fungus (December-February), are low microenvironmental temperatures (15.8-17.5 °C) and substrate temperatures (8.5-14 °C). Materials derived from the base of the bamboo stem, as well as its leaf litter, as well as tillers from decaying grasses and rotten pieces of jonote (*Heliocarpus appendiculatus*), which provide slight acidity (pH 5.0-5.5) and adequate nutritional conditions are other restrictions for this fungus development.

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Colegio de Postgraduados

Strategic planning with a production chain approach to identify training needs in the agrifood sector

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ABSTRACT

Objective: to generate a strategic plan, with a production chain approach, that allows identifying the training needs in the agrifood sector in Quintana Roo.

Design/Methodology/Approach: the study was developed in three methodological stages 1) A workshop to explore the training needs through participatory action, in order to obtain a relational framework of those institutions in the sector in which the trainees interact, aiming to identify and prioritize the training needs in a double-entry matrix; 2) Design of the training program, using as input the needs identified in the exploratory workshop; 3) Implementation of the training, with an ex ante and ex post evaluation of each topic to verify that the training was effective.

Results: the main problems detected were marketing (28.8%), production (25.9%), supply of inputs and services (25.3%), transformation (12.2%), and consumption (7.1%). Likewise, a relational framework of 25 institutions that provide support to the different links identified in the production chain. The median of achievement (correct scores) before training was 8.92, and 10.81 afterwards.

Limitations/Implications of the study: the capacity of the course was limited by the available resources, both facilities and financial. However, it is important to implement a mixed modality to reach a greater number of people.

Findings/Conclusions: strategic planning with a production chain approach is a useful tool to focus ad hoc training needs, since 70.9% of professionals improved their skills on crops of interest.

Keywords: production chains, training, tropical fruits trees.

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INTRODUCTION

The agricultural sector in Mexico faces some challenges to overcome, including low levels of productivity, limited access to consumer markets, an unfavorable background of plant and animal sanitary conditions, high risk of the activity, as well as insufficient financing. To face these problems, we propose to guarantee the insertion of scientific and technological research production as innovation processes to those prioritized production chains. To this end in the state of Quintana Roo, strategic planning with a production chain approach was implemented to identify training needs aimed at both traditional and alternative crops. For those traditional crops, coconut (*Cocos nucifera* L.) and citrus trees; as well as other crops with potential for industry and health benefits, that represent an alternative for adverse agro-ecological zones, such as dragon fruit (*Hylocereus* spp.) and soursop (*Annona muricata* L.) (Esquivel y Araya, 2012; García y Quiroz, 2010).

In 2022, 41 984 hectares (ha) were established in the state of Quintana Roo with these crops, of which 2141 ha were coconut, 1813 ha of dragon fruit and 4657 hectares with citrus trees, 68.9% lemon, 30.2% orange, and 0.9% tangerine (SIAP, 2022). Although there are no official statistics on the cultivation of soursop, it is of interest both to public policy agencies and to local producers, because it has alleged health benefits and positive effects on glycosidic metabolism, and against cancer (Sosa *et al.*, 2022).

The assumption is that development of knowledge and skills, among those trainees contributing to the generation of value, in the chains of interest and the guiding axis of participatory action, both conform a useful methodology to understand the processes of formal and community teach & learning processes in order to improve social existing practices. Capacity development focuses on the solution of community problems with context analysis, processes evaluation, and categorization of priorities (Almaguer-Kalixto *et al.*, 2024). Thus, the aim of this study was to generate a strategic plan with a production chain approach that allows identifying the training needs in the agrifood sector in Quintana Roo (Mexico).

MATERIALS AND METHODS

To generate the strategic plan and to identify training needs, a descriptive, cross-sectional, and participatory study was implemented with a production chain approach. Which made it possible to identify the sequence of dependent and related activities, that is, the necessary 'links' to successfully bring a product to its destination, through the development of a training program that will allow this purpose to be achieved. To this end, it was considered that each link is the set of groups of actors in the production chain, which work in related economic activities (Padilla y Oddone, 2016).

Three methodological stages were defined: 1) a workshop to gather training needs; 2) development of an ad hoc training program to the identified needs; 3) implementation of the training program.

To collect the information, 1) two participatory workshops were held (October 24 and 25, 2023), with the attendance of 38 professionals specialized in the agricultural sector. The information collected was, on the one hand, to form a relational framework of the institutions that promote activities in the sector; and on the other hand, the demands or training needs for each of the links: suppliers of inputs, production, storage, transformation and marketing of each of the crops of interest: coconut, citrus trees, dragon fruit and soursop. This set of problems or training needs was prioritized, by link and by crop, through the use of a double-entry matrix; for this purpose, a tool designed in Excel® was used to facilitate the compilation and analysis of data.

2) Development of the training program. The identified and prioritized needs were used as input to design a training program, in which the needs were considered by link for each crop chain, trying to influence as far as possible, the sustainable development goal (SDG 4.7) on acquiring theoretical and practical knowledge to promote sustainable development (Naciones Unidas, 2018).

3) Implementation of the training program. The professionals in the sector who participated in the workshop on exploring needs, in addition to other actors in the agrifood sector were trained by implementing the program developed. An *ex ante* & *ex post* evaluation of each topic taught was implemented to verify that the training was effective. That is, if the achievement among the course attendees improved with the training. To this end, a structured questionnaire of closed questions was designed for each topic, a total of 45 issues that were applied before and after the training. For the statistical analysis of this dataset, Wilcoxon's non-parametric test was used to test the hypothesis of equality between two population medians for related samples (an error $p \leq 0.05$ was set) in SPSS© version 23.

RESULTS AND DISCUSSION

Workshop to gather research needs

There was active participation and dialogue among the attendees from eight Mexican agencies: Ministry of Agriculture– AGRICULTURA (37%), Secretariat of Agricultural, Rural and Fisheries Development in Quintana Roo– SEDARPE (29%), Plant Health Committee (11%), Secretariat of Economic Development– ECONOMÍA (5%), Agrifood and Fisheries Information Service– SIAP (5%), National Service of Health, Safety and Agrifood Quality– SENASICA (5%), the municipality (5%) and the city council (3%). In total, a relational framework of 25 institutions in which actors interact was identified, then classified according to their purposes in seven areas, which could also promote cooperation and collaboration between entities to empower rural inhabitants by contributing to SDG 17 (Naciones Unidas, 2018). (Table 1; Figure 1).

The essence of strategic planning is the identification of opportunities and threats that, together with other relevant data, provides the basis for decision-making (Palacios, 2020). In this study, it was identified that most of the problems were categorized as follows: marketing (28.8%), production (25.9%), supply of inputs and services (25.3%), transformation (12.2%), and consumption (7.1%) (Table 2 to Table 5). Marketing is one of the critical nodes in

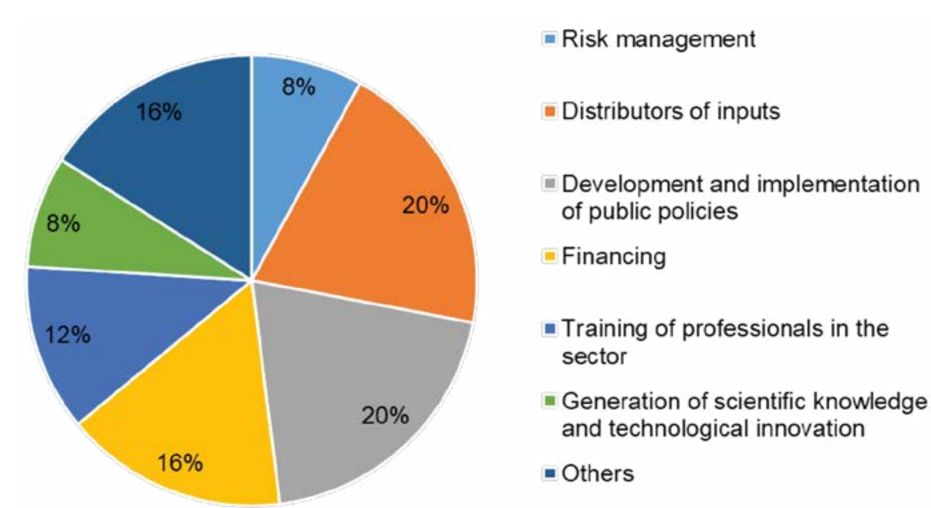

Figure 1. Classification of institutions according to their purposes.

Table 1. Relational framework of the identified institutions supporting the production chains of interest.

Classification	Institutions	Acronyms
Risk management	Federal Commission for the Protection against Sanitary Risks	COFEPRIS
	National Insurance Institution	AGROASEMEX
Distributors of inputs	Agricultural Solutions	AGRISOL
	Agrochemicals “El Inge”	
	Agro veterinary “Huimanguillo”	
	“La Anita” from Chetumal	
	Agrochemicals from the Field	
Development and implementation of public policies	Ministry of Economy, National Institute of Social Economy	INAES
	Ministry of Rural Agricultural Development and Fisheries of the State	SEDARPE
Financing	Ministry of Agriculture and Rural Development (SADER) and Ministry of Welfare	SEBIEN
	Trusts Established in Relation to Agriculture	FIRA
	Risk Sharing Trust	FIRCO
	Mexican Popular Fund and SMG Fund S.C. DE A.P. DE R.L. DE C.V.	
Training of professionals in the sector	Technological Institute of the Mayan Zone	ITZM
	Felipe Carrillo Puerto Higher Technological Institute	TNMFCP
	Mayan Intercultural University of Quintana Roo	UIMQROO
Generation of scientific knowledge and technological innovation	National Institute of Forestry, Agricultural and Livestock Research	INIFAP
	Quintanarroense Council of Science and Technology	COQCYT
Others	Ministry of Economic Development	SEDE
	Federal Consumer Protection Agency	PROFECO
	Mexican Institute of Industrial Property	IMPI
	State Committee for Plant Health of Quintana Roo	CESAVEQROO

agriculture, therefore, the adoption of marketing strategies, the creation of new products, and the search for selling points are answers in a continuous improvement program of agricultural development (Mendoza *et al.*, 2019).

Development of the training program

Jiménez *et al.* (2020) stated that a strategy is the result of the sum of values, understanding of the importance of the parties involved, analysis of internal resources and competencies, the opportunities of the sector and the related industry. As well as a tool to achieve quality standards and improve performance, that also facilitates the implementation of tactical and

Table 2. Problems found on the Dragon fruit production chain.

Link	Problematic
Supplier of inputs and services	Lack of certified supplier of vegetative material (cuttings of white and red species), lack of technical assistance for the efficient application of inputs and crop management, lack of accessible financing, lack of services for soil analysis, no equipment and infrastructure to provide benefit.
Production	Lack of organization of producers to make consolidated purchases of inputs. Lack of availability of suppliers close to the production locations. Lack of knowledge of the nutritional needs, management and phytosanitary calendar of the crop. Lack of specialized technical assistance, low production volume and quality. Low production densities. Lack of product safety. Lack of updating of the technological package. Lack of training in pruning and fertilization.
Transformation	Lack of knowledge of the different options for transforming the product. Underutilization of the infrastructure for transformation. Lack of technical assistance in the production of by-products and derivatives (jams, liqueurs, dehydrated products). Lack of increasing the gastronomic offer of pitahaya.
Marketing	Lack of knowledge of market niches for processed products (with benefit). Lack of knowledge of official regulations for the processing, presentation, packaging and marketing of products. Lack of knowledge of the market, poor organization for marketing. Lack of knowledge of official guidelines for selecting the characteristics of the export product. Lack of a marketing strategy, advertising campaigns. Lack of designation of origin, low sales prices. Lack of formalization of purchase/sale trade. Underutilization of storage infrastructure. Lack of distribution channels and lack of public policies that encourage the marketing of products and derivatives.
Consumption	Lack of knowledge of consumer tastes and preferences, beneficial properties of the product, little availability of information on the benefits of consuming pitahaya and derivatives, insufficient information on the benefit of its consumption, few points of sale at fairs.

Table 3. Problems found on the Soursop production chain.

Link	Problematic
Supplier of inputs and services	Lack of certified silver and organic inputs, lack of a specialized soil and water laboratory, scarce financing services, lack of technical development of the irrigation system, lack of public policy oriented to cultivation, lack of equipment and inputs for its processing.
Production	Lack of specialized technical advice throughout the production process (pests and diseases, post-harvest, safety), lack of a local technological package.
Transformation	Lack of specialized equipment and inputs for processing, lack of traceability of the origin of the product.
Marketing	Lack of marketing channels, lack of training for distribution on the cold chain, lack of presentation in the market, lack of safety management and lack of promotion of products and derivatives.
Consumption	High product prices, lack of knowledge of the offer, limited commercial diversification, lack of consumer culture.

operational strategies to achieve goals (López & De la Garza, 2020). Likewise, considering that higher education is one of the main indicators to measure the level of development, by promoting efficiency and economic competitiveness (Urcid-Puga & Rojas, 2020), a training program was designed that addressed the following topics: strategy for production

Table 4. Problems found on the Coconut production chain.

Link	Problematic
Supplier of inputs and services	Lack of organization of producers for consolidated purchases, lack of certified walnut suppliers, lack of phytosanitary services.
Production	Lack of training on the technological package, lack of knowledge of the nutritional needs of the crop, lack of soil analysis to determine fertilization doses, lack of technical assistance and support for the comprehensive management of the crop, lack of knowledge of phytosanitary management, lack of own capital for investment, lack of accessible financing.
Transformation	Low availability of equipment and infrastructure to add value to production, lack of training for product transformation, lack of knowledge of official regulations for processing, presentation, packaging and marketing of products with benefit.
Marketing	Lack of knowledge of market niches for processed products (with benefit). Lack of knowledge of the market, lack of organization of producers for marketing. Lack of collection centers in the main production areas. Low production volume for agroindustry. Lack of a packing plant. Lack of marketing strategies for positioning products and identifying marketing channels. Lack of regulation for certification of authenticity of production by origin, culture, history and social impact, lack of a business plan for community companies. Lack of sales training.
Consumption	Lack of knowledge of consumer tastes and preferences.

Table 5. Problems found on the Citrus trees (lemon, orange, and tangerine) production chain.

Link	Problematic
Supplier of inputs and services	Lack of availability of certified seedlings and nurseries. Lack of certified mother gardens with buds and stalks, lack of soil, water, plant and phytosanitary laboratory services. Lack of financing services, shortage of phytosanitary specialists for national and international mobility certification as well as specialists for obtaining derived products. Insufficient supply of specialized inputs, technical services and irrigation systems, high cost of freight for inputs.
Production	Lack of updating of the technological package. Lack of knowledge of the appropriate doses of fertilization, low crop density because of diseases, efficient phytosanitary management (pruning and management calendar). Shortage of irrigation infrastructure. Lack of training in the integral management of the crop. Low availability of labor. Lack of organization of producers. Lack of knowledge of the processes for production certification. Lack of knowledge of the procedure for well authorization. Lack of knowledge of the advantages of high-density production. Lack of regulation of certified plants.
Transformation	Lack of agro-industry. Shortage of machinery to process and transform production. Lack of benefit to production (juices, liquors, oils, extracts, preserves, cosmetics, etc.). Absence of micro-enterprises to obtain products and derivatives. Lack of training for the transformation of raw materials. Lack of knowledge of good practices for obtaining products. Lack of knowledge of regulations on processes for agro-industry and lack of financing for benefit of production.
Marketing	Lack of collection centers in the main producing areas. Lack of packing plants for the national and international markets. Lack of a distinctive seal or registered trademark that reflects the identity of the product. Lack of knowledge about the traceability of production. Lack of knowledge of the market for products and derivatives. Lack of marketing strategies for positioning, promotion, entry into niches and new markets. Lack of post-harvest transportation. Lack of public policies that encourage the consumption of local products. Lack of selection, waxing and packaging of the product. Lack of guaranteed prices. Lack of organization of producers for marketing. Lack of logistics for product transportation.
Consumption	High variability in purchase price, low product quality for local consumption, poor promotion of acceptance of derived products.

planning towards agricultural sustainability, value-added strategies and transformation of production.

Implementation of the training program

The training course was held from February 26 to 28, 2024, with the participation of 134 professionals from the sector. The median achievement before the course was 8.92 correct scores, and 10.81 afterwards. Of the trainees, 70.90% increased their achievement score after the training, 12.69% of the cases remained at the same score, and 16.42% decreased their score. The statistic value was significant ($Z=-7.246$; with a p-value$=0.0005$); therefore, it was concluded that there was a significant difference in the median achievement after training.

In the agriculture sector, training is necessary to acquire updated knowledge and to replace obsolete or harmful techniques for the environment; to supply food to the population in quantity and quality; to venture into new markets and to preserve natural resources for future generations (Valencia-Benítez *et al.*, 2023).

CONCLUSIONS

Strategic planning with a production chain approach resulted in a useful tool to increase the knowledge of 70.90% of the participants, thus contributing to the strengthening of capacities on crops of interest for the agricultural and economic development of the state of Quintana Roo (Mexico).

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Colegio de Postgraduados

Wildfire analysis in the Cobos-Parga Hydrographic Basin, Aguascalientes, through satellite images: impacts and solutions

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ABSTRACT

Objective: Detect and analyse forest fires happening in the Cobos-Parga Hydrographic Basin.

Design/methodology/approach: For this analysis, the methodology that was followed ranges from the extraction of satellite images to the calculation of the Normalised Burn Ratio, thus enabling the detection of combusted zones. All this work was done thanks to the use of Geographic Information Systems.

Results: Frequent occurrence of fires was found in the area, mainly of anthropogenic origin and registered mainly from January to May. The increasing trends in annual precipitation and extreme maximum temperature are also evident in the study area. Therefore, climatic conditions do not exhibit a clear correlation between them and fires, supporting the idea that forest fires are mostly caused by anthropogenic reasons.

Limitations on study/implications: Finally, it is suggested that the CPHB is not suitable for the construction of real estate complexes, since it is estimated to be a zone of high fire risk.

Findings/conclusions: This highlights the necessity of establishing protection measurements that could control illegal activities, as well as monitoring fires and along with it, guarantee the biological and cultural preservation of the area.

Keywords: Aguascalientes, Cobos Forest, Fires, NBR, Remote sensing.

INTRODUCTION

Fire is a key ecological factor that is part of the natural dynamics of some ecosystems, with benefits such as the reproduction of certain species and soil fertilisation (Pérez-Verdín *et al.*, 2013). However, currently, the vast majority of wildfires have an anthropogenic origin (SEMARNAT, 2018), triggered both in accidental and intentional ways (Vaiciulyte *et al.*, 2023). This, along with higher and higher temperatures, as well as more and more recurrent and prolonged droughts (Roy *et al.*, 2021), can overcome the regeneration

capacity of these ecosystems against this phenomenon (Mastachi-Loza *et al.*, 2024), even for species well adapted to it (Azpeleta-Tarancón *et al.*, 2023).

Some of the negative consequences of recurrent wildfires are biodiversity loss, soil erosion, global warming, the expansion of invasive species and affections to human health (Pérez-Verdín *et al.*, 2013; Cruz-Núñez *et al.*, 2014). Therefore, forest fires have impacts on the environment, as well as on society and economy (Mastachi-Loza *et al.*, 2024).

One of the countries with greatest susceptibility to these phenomena is Mexico (Cruz-Núñez *et al.*, 2014), since it has ecosystems found under high anthropogenic pressure and characterised by a marked precipitation seasonality, both of which favour ignition (Azpeleta-Tarancón *et al.*, 2023). Moreover, due to ongoing climate change and natural growth of its population, particularly in medium and small-sized cities, it is foreseen that the risk of this type of events in Mexico will increase (Vaiciulyte *et al.*, 2023), including natural areas of high ecological value (Ressl *et al.*, 2009). In spite of this, these phenomena are not being investigated with the urgency they deserve.

There are some detailed studies in certain Mexican states like Puebla (Carrillo-García *et al.*, 2012), Durango (Pérez-Verdín *et al.*, 2013), Jalisco (Cerano-Paredez *et al.*, 2015), State of Mexico (Gutiérrez-Martínez *et al.*, 2015) and Nuevo León (Roy *et al.*, 2021). Nevertheless, this type of studies do not exist for Aguascalientes, even though it is the 13th Mexican state with greatest number of wildfires in relation to its area, reporting 335 events between 2010 and 2020 (Cruz-Núñez *et al.*, 2014). Furthermore, on account of its dense urbanisation, it is considered a fire-prone area, since in this entity, more than 10% of these phenomena are registered in the so-called wildland-urban interface (Vaiciulyte *et al.*, 2023). This evidences the great pressure that the city of Aguascalientes exerts over the natural landscape.

An example of such threatened landscapes is located at the south of the state of Aguascalientes, in the municipalities of Aguascalientes and El Llano, and is known as the Cobos-Parga Hydrographic Basin (CPHB). It is an area of great ecological and cultural value, which is part of the state strategy for the protection and conservation of natural resources, contributing with ecosystem services such as climate regulation, flood mitigation and aquifer recharge, among others (SEGGOB, 2021a). However, this high richness is now at severe risk, as numerous real estate complexes have been proposed for this zone (M. Vázquez, personal communication, 12th of August 2023).

This is why it is important to preserve the ecological integrity of this area and minimise the risks that threaten it, for example, wildfires. To do that, a series of wildfires that occurred in this area between 2010 and 2021 are analysed through satellite images and Geographic Information Systems (GIS), along with associated climate data. The general objective of this investigation is to provide information about the wildfire problematic in the CPHB in Aguascalientes, Mexico. Specific objectives are, on the one hand, to analyse a series of wildfires that took place in the study area for the 2010-2021 period and determine the vegetated areas affected by them and, on the other hand, to establish the potential relationship between climate data and wildfires occurred in the CPHB. It is hypothesised that wildfires happening in the CPHB make this area inviable for the construction of real estate complexes.

MATERIALS AND METODS

Study area

The study area is the CPHB, which is situated at a latitude of 21° 48' 47.7"- 21° 49' 25.6" N and a longitude of 102° 15' 23.9"-102° 14' 16.1" O (Figure 1), and has a surface area of 20,962 ha. This zone captures a large amount of water every year, being an important zone for aquifer recharge and a supply source for the Parga Dam (SSMAA, 2019). The Programme for the Local Ecological Management of the Municipality of Aguascalientes 2016-2040 (SEGGOB, 2021b) classifies this area under the "protection" category, within the Environmental Management Unit number 32 "Cobos" (SEMADESU, 2016).

Figure 2 represents the climate graph for the municipality of Aguascalientes, whose data was obtained from the weather station "Aguascalientes", with code 1030 from the

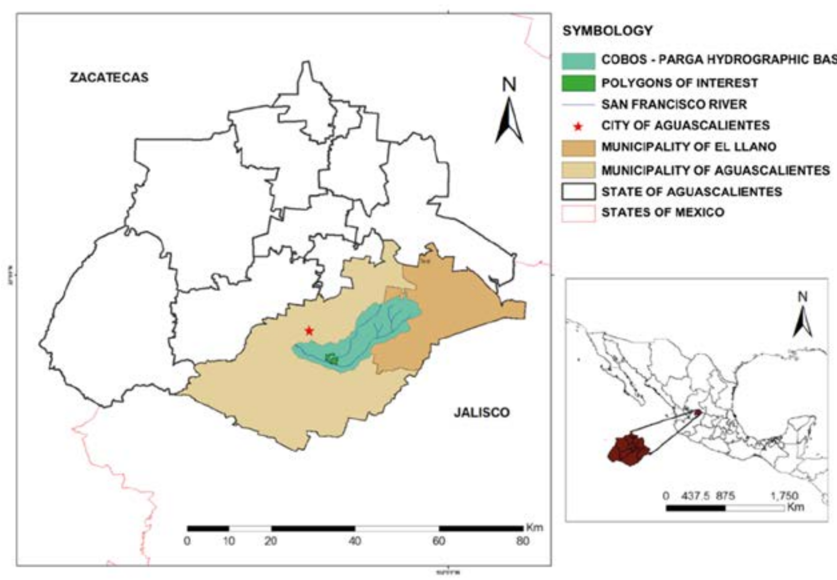

Figure 1. Geographic location of the study area in the city of Aguascalientes, Mexico.

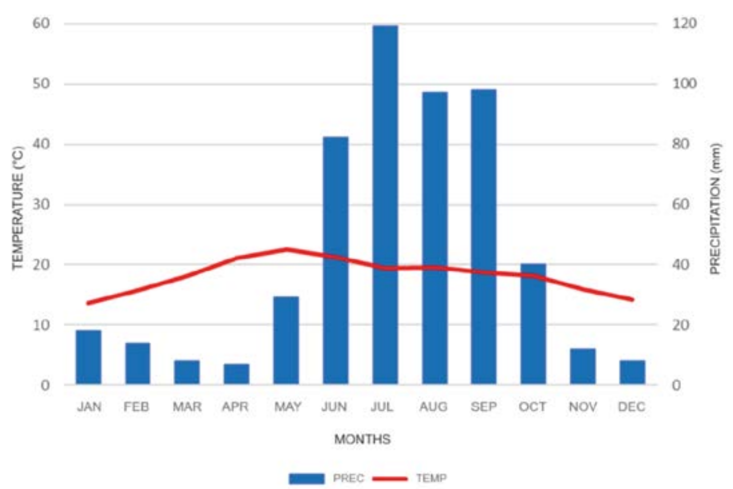

Figure 2. Climate graph of the municipality of Aguascalientes, Mexico (1948-2021).

National Weather Service (SMN, 2024). As can be observed, climate is predominantly temperate semi-arid, BSh according to the Köppen classification system modified by García (2004). Thus, lowest temperatures are recorded in winter, the moment with lowest insolation, whereas maxima are registered in late spring-early summer, when insolation is high and summer storms have not initiated yet. Regarding precipitation, Aguascalientes has eight months of aridity (October-May) that give rise to a markedly dry season, which highly contrasts with the wet season (June-September), in which precipitation is both abundant and intense, in the form of convective storms. Also, it is noteworthy that interannual precipitation variability is very high, with a coefficient of variation higher than 50%, since rainfall regime may undergo alterations induced by El Niño Southern Oscillation (Cerano-Paredes *et al.*, 2015).

On the other hand, Figure 3 represents the temporal evolution of climate. As can be appreciated, although precipitation registers important interannual oscillations, an upward trend can be inferred, both annually (Figure 3A) and in the dry season (Figure 3B). As for temperature maxima (Figure 3C), it exhibits peaks between 1948-1955, 1997-2008 and 2017-2020, and it also displays an upward trend. This means that, in this municipality, precipitation and temperature maxima are getting higher and higher over time.

The intense aridity of Aguascalientes favours the development of vegetation adapted to water scarcity, with most of the CPHB covered with thorny shrub represented by mesquites (*Prosopis* sp.), huisaches (*Vachellia farnesiana*), prickly pear cacti (*Opuntia* sp.), gatuño (*Mimosa monancistra*) and cane cholla (*Cylindropuntia imbricata*). According to the Sustainability, Environment and Water Secretary of Aguascalientes (SSMAA, 2019), another important soil use is temporal agriculture, although, currently, that use is barely present and is gradually being replaced by the recolonisation of native natural grasslands. Due to natural wildfire occurrence in this ecosystem, enhanced by precipitation seasonality, vegetation is also adapted to this element, which increases wildfire risk (SEGGOB, 2021a). Regarding wildlife, the CPHB has a wide variety of mammals, birds, reptiles and amphibians, highlighting mud turtles (*Kinosternon* sp.) or mountain tree frogs (*Dryophytes eximius*), both of them highly vulnerable to fire occurrence (Mastachi-Loza *et al.*, 2024).

Finally, it is important to point out that, despite having low population density, the area is not exempted from anthropogenic threats, like brick factories, debris dumping, uncontrolled livestock grazing or the entrance of unauthorised people, who occasionally vandalise the property (SEGGOB, 2021a).

WORKFLOW

The present investigation was carried out in several phases (Figure 4), which are detailed hereafter.

PHASE 1: Delimitation of the study area

For the delimitation of the study polygon, QGIS software version 3.28.11 - Firenze (QGIS, 2023) was used, and different geospatial layers were accessed through various sources (Table 1).

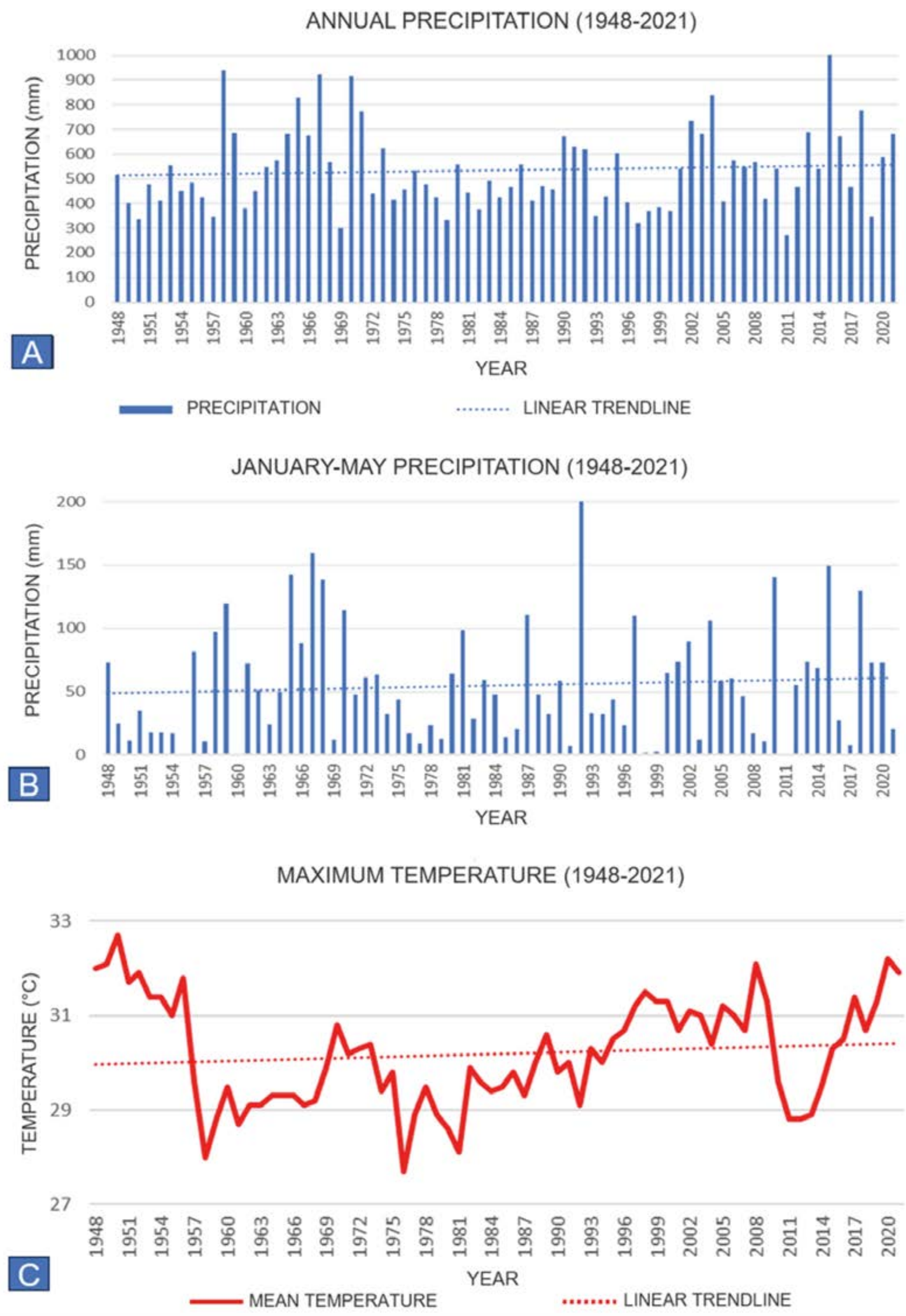

Figure 3. Temporal evolution of climate in the municipality of Aguascalientes (1948-2021): annual precipitation (A), January-May precipitation (B), maximum temperature (C).

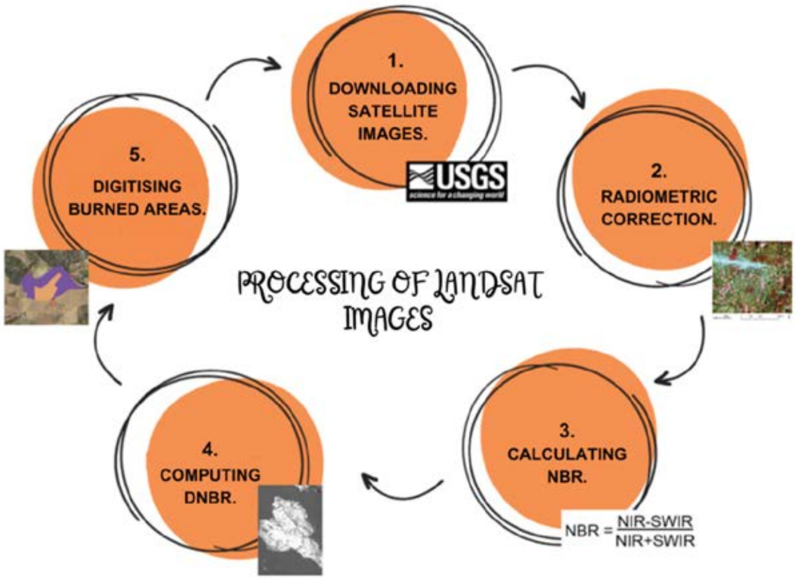

Figure 4. Conducted workflow for the processing of satellite images.

Table 1. GIS layers used during this investigation.

Layer	Type	Resolution	Source
Elevation	Raster	15×15 m	INEGI (2023)
States	Vector	N/A	CONABIO (2023)
Municipalities	Vector	N/A	CONABIO (2023)
Satellite images	Raster	15×15 m	USGS (2023)
Wildfires	Vector	N/A	CONAFOR (2023)
Rivers	Vector	N/A	CONABIO (2023)

PHASE 2. Information about forest fires occurred within the study area

The next step involved using the shapefile of wildfires in the Mexican territory reported by CONAFOR (2023), to select those events coinciding with the study area (Figure 1), adding up a total of 45 events between 2010 and 2021 (Table A1).

PHASE 3. Downloading satellite imagery

Later on, satellite images from Landsat missions 7, 8 and 9 were downloaded from the EarthExplorer portal by the US Geological Survey (USGS, 2023), corresponding to bands 5 (Near Infrared) and 7 (Short-Wave Infrared), together with the MTL file.

PHASE 4. Radiometric correction

Radiometric correction was conducted in order to reduce non-desired artifacts due to atmospheric effects or differential light caused by the time of the day, the place on Earth or the relief (Palazón-Fernando, 2006). To do that, the Semi-Automatic Classification Plugin from QGIS was utilised (2023).

PHASE 5. Computing the Normalised Burn Ratio (NBR)

Finally, the Normalised Burned Ratio (NBR) was calculated in grey levels (Formula 1). As can be observed, this algorithm exclusively focuses on the infrared region, where burned vegetation has a more distinctive spectral signal (Keeley, 2009). The final step involved computing the Differenced Normalised Burn Ratio (DNBR), that is to say, the difference between the satellite image before and after the event, which makes burned areas stand out, facilitating subsequent digitising. This index has been successfully applied in other investigations about wildfires in Mexico (Ressl *et al.*, 2009).

$$NBR = \frac{NIR - SWIR}{NIR + SWIR}$$

Formula 1. Algorithm used in the calculation of NBR, where NIR=Near Infrared (*i.e.* Band 5) and SWIR=Short-Wave Infrared (*i.e.* Band 7).

RESULTS AND DISCUSSION

A total of 120 wildfires were registered within the CPHB, mainly from January to May, with March, April and May being the months with highest occurrence (31.67%, 25.83% and 20%, respectively), that is to say, spring months coinciding with the end of the dry season (Figure 2), albeit with a lower percentage compared to the findings by Cerano-Paredes *et al.* (2015) for the state of Jalisco. In turn, Gutiérrez-Martínez *et al.* (2015) also detected a peak in wildfire occurrence in spring for the state of Mexico, although, in their case, maximum was registered in April.

The reason why wildfires concentrate in spring responds to climatic reasons. Indeed, during the wet season, a large amount of biomass is accumulated, which gets dehydrated during the dry season and, due to high temperatures, becomes more fire-prone (Minnich *et al.*, 1994). As for the study years, 2019 recorded a greater number of wildfires and these affected larger areas, with more than half of the hectares of the time series combusted during this specific year. Conversely, the 2011-2015 period did not present any wildfire. All reported fires were superficial, since prevailing shrubland does not constitute closed arboreal formations.

It must be underlined that CONAFOR (2023) identified 45 wildfires in the zone (Table A1), whilst the present study detected 120 events, *i.e.* 272% more. Likewise, based on the methodology of the present study, 16% of the basin is found to have fire presence, a figure that doubles that of CONAFOR (2023), whose total burned area was only 8% of the catchment.

This could be due to the fact that CONAFOR (2023) excludes non-forested surfaces from burned areas. However, dominant thorny shrubland and grassland of the study area (SEGGOB 2021a) could have been left out from this classification. Thus, it is considered that the methodology presented in this investigation fits better with the dynamics of the study area.

Similarly, the main wildfire occurrence reasons in the CPHB were analysed (Figure 5). To do that, data from CONAFOR (2023) were obtained, because of the lack of this type of information about burned areas additionally detected in this research. As can be observed, almost 2/3 of the fires were intentionally triggered, followed by 16% of unknown reasons and 9% owing to negligence (landfill burning or cigarettes, among others):

On the other hand, Figure 6A illustrates the 120 fire events that took place in the study area for the 2010-2021 period, whereas Figure 6B displays a zoomed in sector of high interest. It can be concluded that most of the wildfires in the CPHB are concentrated in the medium and low areas of the basin. Furthermore, more than half of them are located in the surroundings of the conurbation area of the city of Aguascalientes, which was also pointed out by Mastachi-Loza *et al.* (2024) for the State of Mexico, an entity that, just like Aguascalientes, is subject to great urban pressure.

Figure 5. Main causes of wildfires in the CPHB.

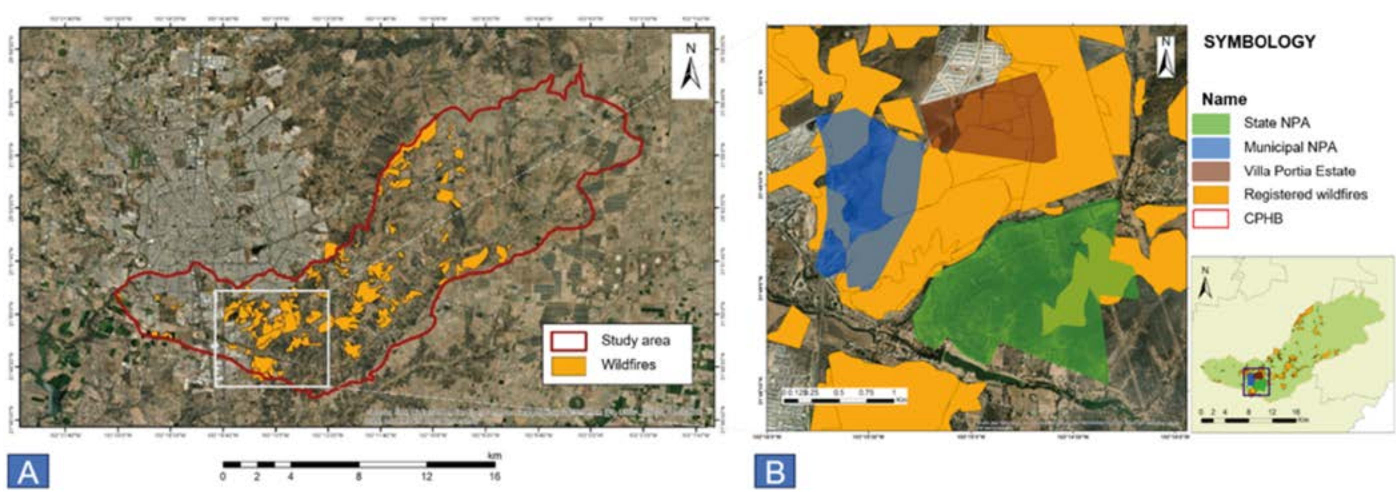

Figure 6. Map of identified wildfires in CPHB: general overview (A) and zoomed in view with polygons of interest (B).

In addition, as shown in Figure 6B, several events were registered close to the state Natural Protected Area (NPA) and especially, the municipal NPA, which facilitated the protection of the latter (SSMAA, 2019). In spite of this, both NPAs only comprise a small portion of the CPHB and they are separated from each other, thereby evidencing the necessity of a green infrastructure that could connect them, for instance, an ecological corridor. Related to this, the polygon where the Villa Portia Estate is proposed exhibits an even greater number of wildfires, which makes it inviable for construction.

Additionally, temporal analysis of precipitation (Figures 3A and 3B) shows that this parameter has been increasing over time, both annually and in the dry season, when almost all the events are recorded. Similarly, maximum temperature (Figure 3C) also exhibits an upward trend over time. The selection of this parameter, rather than mean monthly temperature, is justified by the fact that fires are enhanced by extreme climatic phenomena. This means that, even if temperature increase could favour the occurrence of fires in the last years, precipitation would act in the opposite sense, inhibiting this type of events. Therefore, climatic factors do not seem to have a clear correlation with respect to wildfires. Instead, this supports the hypothesis that wildfires have an anthropogenic origin, which coincides with the analysis presented in Figure 5.

The former hypothesis is corroborated while analysing the geographical centres of wildfires per year (Figure 7), where it can be appreciated that these are located close to the city centre of Aguascalientes or even inside it in 2016. This agrees with the findings by Pérez-Verdín *et al.* (2013), who detected that the mean centre of wildfires in the state of Durango got closer and closer to its capital. Temporally, it must be underlined that, since 2017, wildfires are moving further and further west (except for 2019), that is to say, towards the urban area of Aguascalientes, staying just a few metres away from the location proposed for the Villa Portia Estate in 2021. This evidences the great pressure that this zone is

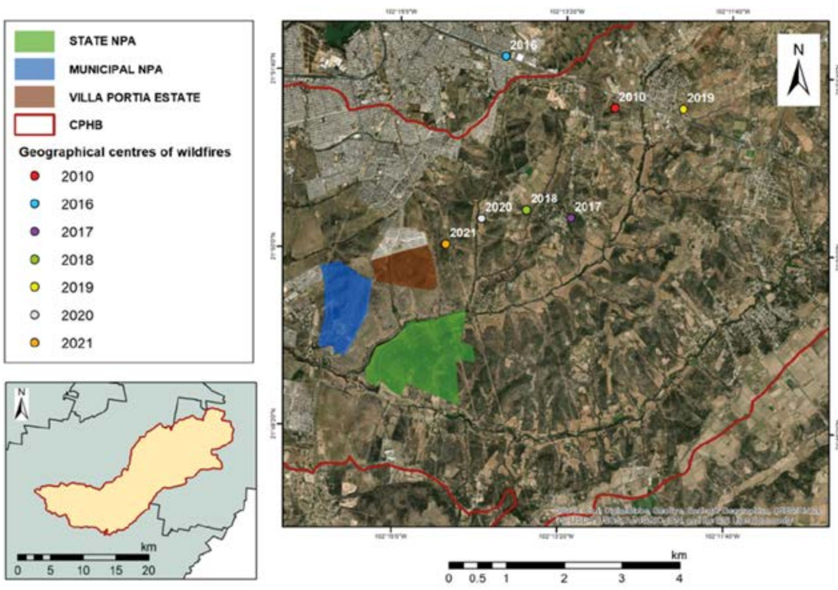

Figure 7. Mean centre of wildfires occurred in the CPHB per year.

currently suffering from urban expansion, with recent real estate complexes being built in the area, like Lunaria (SSMAA, 2019). Moreover, since the area is located at the wildland-urban interface, wildfires can suppose an alarming risk for its inhabitants (Vaiciulyte *et al*., 2023).

Consequently, it can be stated that the CPHB is severely threatened by fire, primarily of anthropogenic origin, which is why it is necessary to monitor, through GIS, the occurrence of fires in this zone. This material would provide useful tools for decision-makers, which would help real estate management and the protection of natural and cultural resources at the CPHB.

For that, visitor control and surveillance of the area should be intensified, so as to avoid these events in a valuable area that is still undergoing intense vandalism (M. Vázquez, personal communication, 10th of September 2024). In addition, with ongoing climate change, drought events are expected to be more and more recurrent and prolonged in the future (IPCC, 2023).

In this situation, it is noteworthy that the basin acts as an area of aquifer recharge and drinkable water supply for the city, so the occurrence of human-induced fires and the construction of real estate complexes would negatively impact water quality and quantity, putting water security of the inhabitants of Aguascalientes at risk (SSMAA, 2019). Again, this highlights the urgent necessity to protect this space.

To do that, studies about physical and chemical soil properties in the area are projected, which would permit the identification of optimal areas for the installation of permanent water bodies. These would guarantee the quality of water, as well as the survival of sensitive organisms like amphibians and reptiles, such as the mountain tree frog (*Dryophytes eximius*), mud turtles (*Kinosternon* sp.) or the western black-tailed rattlesnake (*Crotalus molossus*). Simultaneously, these protected areas would function as ecological corridors between the state and municipal NPAs, acting as conservation and genetic exchange zones and being subject to protection figures that would effectively regulate the activities that could potentially threaten them.

CONCLUSIONS

The Cobos-Parga Hydrographic Basin presents multiple wildfires during the 2010-2021 period, affecting state and municipal NPAs, as well as the area proposed for the Villa Portia Estate, which makes it inviable for the construction of real estate complexes. On the other hand, since climate does not exhibit a clear correlation with wildfire occurrence, these are of anthropogenic origin, with threats such as vandalisation or urban expansion, which evidences the necessity of environmental education and protection of the zone. The results of the present study can support decision-making that guarantees the correct functioning and sustainable exploitation, through protection figures, of the precious natural resources and ecosystem services offered by the CPHB.

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Table A1. Cronología de incendios forestales ocurridos en el área de estudio, todos de tipo superficial. Modificado de CONAFOR (2023).

DATE	CAUSE	BURNED AREA (Ha)
22/04/2010	Unknown	2.50
11/01/2016	Intentional	1.24
22/01/2016	Negligence	31.72
04/03/2016	Intentional	24.11
06/03/2016	Others	6.80
30/03/2016	Others	97.38
18/02/2017	Intentional	15.00
02/03/2017	Intentional	8.00
03/03/2017	Intentional	4.57
04/03/2017	Unknown	51.40
23/03/2017	Intentional	11.14
02/04/2017	Intentional	33.00
18/04/2017	Negligence	92.14
06/03/2018	Intentional	2.29
06/03/2018	Intentional	8.32
09/03/2018	Intentional	3.79
19/03/2018	Negligence	21.01
21/03/2018	Intentional	21.46
22/03/2018	Intentional	2.59
22/03/2018	Intentional	6.14
03/04/2018	Intentional	12.91
09/04/2018	Intentional	9.57
16/04/2018	Intentional	72.53
26/01/2019	Unknown	35.39
26/01/2019	Unknown	1.47
02/02/2019	Intentional	8.24
03/02/2019	Unknown	2.96
19/02/2019	Intentional	29.42
07/03/2019	Negligence	80.65
10/03/2019	Intentional	48.27
11/04/2019	Intentional	238.40
01/05/2019	Intentional	44.71
06/05/2019	Unknown	31.18
11/05/2019	Controlled	39.86
13/05/2019	Unknown	218.43
24/03/2020	Intentional	12.44
25/03/2020	Controlled	1.67
21/04/2020	Controlled	3.03
19/05/2020	Intentional	20.26
24/05/2020	Intentional	14.14
04/01/2021	Intentional	1.16
13/03/2021	Intentional	5.37
16/03/2021	Intentional	159.49
17/03/2021	Intentional	8.75
18/04/2021	Intentional	4.15

Colegio de Postgraduados

Yield of Cushaw squash (*Cucurbita argyrosperma* H.) with organic fertilizers

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ABSTRACT

Objective: to evaluate different doses of composted poultry manure and leachate applied to soil and foliage as organic fertilizers on the yield and yield characteristics of Cushaw squash (also known as Silver-seed gourd, and less frequently as creole squash).

Design/Methodology/Approach: ten treatments (T) were tested in four doses, 2.5, 5, 7.5, and 10 tons per hectare (Megagrams, Mg ha^{-1}) of composted poultry manure (C) applied to the soil at the beginning of crop cultivation, and leachates (L) at 50% applied to the foliage. A completely randomized design was used, with 15 replicates (fruits) per treatment.

Results: significant effects of compost and leachate treatments on the yield of Cushaw squash fruit were found.

Limitations/Implications of the study: it is suggested to evaluate more production cycles in order to identify the best treatments in further studies.

Findings/Conclusions: the combination of composts and leachates applied to the soil can be an alternative source of fertilization in the cultivation of Cushaw squash in Tabasco.

Keywords: poultry manure, compost, leachates, yield.

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INTRODUCTION

China is the first producer of squash worldwide with 7 325 193 tons (Megagrams, Mg) followed by Ukraine 1 097 780 Mg and Mexico occupies the 7th place with 729 201 Mg (FAO, 2022). One of several varieties grown in Mexico is the Cushaw squash (*Cucurbita argyrosperma* H.) which at domestic scale is grown in 17 states in the country. Campeche is the first producer (11 622 ha), followed by Guerrero (7867 ha); Tabasco is at the third place (7610 ha), then Zacatecas (6609 ha). These four Mexican states are the largest producers; the rest of states in Mexico grow squash in smaller quantities and smaller areas. In Mexico, squash cultivars are planted in different environments; frequently abusing soil because of an excessive use of agrochemicals to obtain production.

The addition of organic matter has the ability to improve soil properties. Leachate contains nutrients; also, microorganisms fix atmospheric nitrogen, releasing phytohormones to protect crops against plant pathogens, and control the pest population. The technological management of Cushaw squash is very variable. Differences are observed in a very wide range of production systems. Due to the lack of a fit-to-all technological set of strategies there are problems in each operation needed for this crop. So, there is an opportunity to transfer appropriate innovations to the local production system (Barrón & Rodríguez, 2020). In current agriculture, the intensive use and abuse of synthetic agrochemicals, such as fertilizers and biocides, has caused an ecological imbalance with the consequent degradation of ecosystems (Espinosa-Ramírez & Santiago-Mejía, 2023). The objective was to evaluate different doses of composted poultry manure applied to the soil, and leachate in foliar application, assessing their effects on the agronomic component and yield characteristics of Cushaw squash.

MATERIALS AND METHODS

Experimental site

This study is part of a research project that began in the spring-summer 2023 cycle with a trial of composts and manure leachate, which continued in the autumn-winter cycle of that same year (2023) in the fields of Campo Experimental Huimanguillo (INIFAP); located in the Huimanguillo-Cardenas road (17.847711, −93.396465 UTM), at 20 m altitude.

Soil and climate of the experimental site

The type of climate is warm humid (Am), classified by García (1964), which defines the conditions of the study *in situ*. The average maximum temperature was 32.8 °C and the minimum, 23.3 °C; rainfall was recorded as 585.8 mm during crop growing cycle (Figure 1).

The soil of the site is Fluvisol with a sandy-clay loam texture with a moderately acidic 5.8 pH, an average content of organic matter 3.2% and average total nitrogen 0.12%; phosphorus, 9.85 mg kg^{-1} and potassium 0.25 cmol kg^{-1}; As well as calcium 9.98 cmol kg^{-1}, magnesium 1.4 cmol kg^{-1}; and high contents copper and zinc (10.11 and 5.95 mg

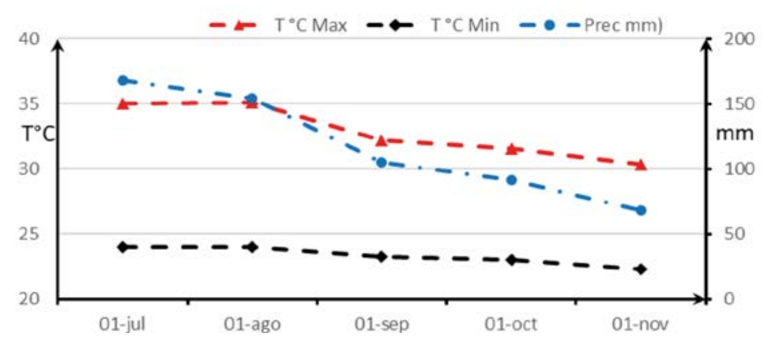

Figure 1. Rainfall (mm), maximum and minimum temperature (°C) during the spring-summer 2023 crop cycle. Source: Barrón F. S. (2023). Data from the climatological station of the C. E. Huimanguillo, Tabasco, Mexico.

kg^{-1}, respectively); an iron content 32.3 mg kg^{-1} that is considered medium, and low in manganese 0.98 mg kg^{-1}.

The laboratory analyses of the compost (C) and leachate (L) applied that summarize the nutrients provided to the squash are shown in Table 1 and Table 2, below.

The chemical fertilization was divided into two applications, the first was supplied 9 days after planting with 152 kg of diammonium phosphate (18-46-00); the second at 31 days with a mixture of 71 kg of urea plus 75 kg of potassium chloride, to attain a 60-75-45 formulation. In both applications the fertilizer was deposited around the plants, then covered with soil to minimize airborne losses.

Treatments and experimental design

Ten treatments were tested including the chemical (commercial) and zero controls (000); four doses 2.5, 5, 7.5, and 10 Mg ha^{-1} of composted poultry manure (C) and leachate (L). The size of the experimental plot was 30 m×40 m (width×length) with 10 rows of plants separated by 3 m each, where treatments were applied (Table 3). The arrangement was a completely randomized experimental design with 15 samples (fruits) per treatment. For treatments with composted poultry manure (C), it was mixed with the soil on the same date as the first application of the chemical fertilizer (this is, 16

Table 1. Chemical analysis of poultry manure composted (C).

pH	CE dS m^{-1}	MO	N	P mg kg^{-1}	K	Ca	Mg	Na	CIC	Fe	Cu	Zn	Mn
		%			cmol (+) kg^{-1}					mg kg^{-1}			
8.2	2.15	63	2.66	47.2	2.63	7.49	2.35	0.61	55.41	214.66	77.05	15.0	13.04

Table 2. Chemical analysis of manure leachate (L).

pH	CE dS m^{-1}	N	P	K	Ca	Mg	Na	Fe	Cu	Zn	Mn
		%						mg kg^{-1}			
7.9	2.40	1.29	2.70	1.70	2.29	0.71	0.17	131.06	49.24	10.28	10.81

Table 3. Treatments applied of compost (C) to the soil (S) and 50% leachate (L) to the foliage (F).

Treatments: Compost (C) and leachate (L) at 50 %	Abbreviation
T_1 Compost (C) 10 t ha^{-1}+leachate (L) to the soil (S)	TC10LS
T_2 Compost (C) 10 t ha^{-1}+leachate (L) to the foliage (F)	TC10LF
T_3 Compost (C) 7.5 t ha^{-1}+leachate (L) to the soil (S)	TC7.5LS
T_4 Compost (C) 7.5 t ha^{-1}+leachate (L) to the foliage (F)	TC7.5LF
T_5 Compost (C) 5 t ha^{-1}+leachate (L) to the soil (S)	TC5LS
T_6 Compost (C) 5 t ha^{-1}+leachate (L) to the foliage (F)	TC5LF
T_7 Compost (C) 2.5 t ha^{-1}+leachate (L) to the soil (S)	TC2.5LS
T_8 Compost (C) 2.5 t ha^{-1}+leachate (L) to the foliage (F)	TC2.5LF
T_9 Chemical control, (60-45-75) N-P-K fertilizer	TQm
T_{10} Control (000)	T000

days after sowing). Manure leachate at 50% concentration were supplied to the soil and foliage in three applications, the 1st at 23 days; the 2nd at 34 days, and the 3rd at 45 days after sowing.

Variables analyzed and statistical analysis

Fruit weight (g), each fruit was weighed on a scale (Electronic Compact Scala SF-400A, USA). Equatorial diameter of the fruit (cm), it was obtained from the most prominent middle part of the fruit. Polar diameter (cm), it was taken from the base of the middle part to the stem with a measure tape. Fruit shape index, it was obtained by the relationship:

$$Shape\ index = polar\ diameter / equatorial\ diameter\ (dimensionless)$$

Pulp thickness (mm), fruits were divided by their equatorial diameter, once the seed was extracted from the pulp, the thickness was measured in five fruits with a caliper (Truper®, Mexico). Seed weight (g) was measured as dry matter for each sample with the same balance; correlations were of seed weight versus polar diameter and equatorial diameter of the fruits.

In order to measure these variables, a randomized sample was collected with 20 replicates in each row of the 10 treatments. Then, with data an analysis of variance and a DMS test of means ($p \leq 0.05$) were done. Statistical analyses were performed on SAS®, version 9.3.

RESULTS AND DISCUSSION

Fruit weight (g)

The fruit weight variable did not show significant differences among treatments (DMS, $p \leq 0.05$). A trend from higher to lower value of fruit weight was observed in the foliar application treatments, which allows us to infer that the plant has the best response to foliar application of the treatments (Figure 2). It is worth mentioning that in cycles previously evaluated, the weight of squash fruits showed the same trend to the foliar applications of leachate. Villalobos *et al.* (2017) reported, in regard to the weight of Cushaw squash, 1600

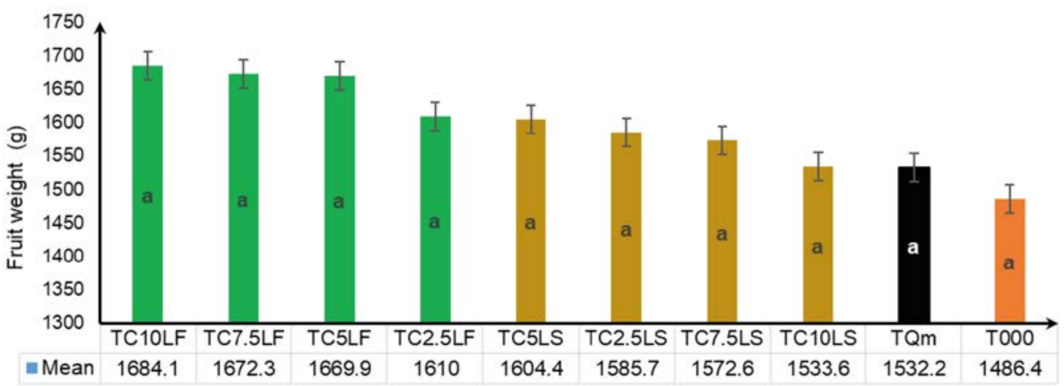

Figure 2. Average weight (g) of Cushaw squash fruits under combinations (on soil and foliar application) of fertilizer treatments; composted poultry manure and leachate at 50%.

g a value similar to the T2.5CLF and T5CLS treatments in our study,1640±99.20 EE and 1604±84.4 EE g, respectively. In another study, Tucuch-Haas *et al.* (2022) found no significant differences in the fruit weight of four varieties of Cushaw squash: Chihuahua, Aguascalientes, Yucatan and Norteno with an average of 1697.2 g; although the Yucatan genotype presented a greater number of fruits and a greater number of seeds with marketing quality. Ruelas *et al.* (2015), from 16 accessions of *C. argyrosperma*, reported an average fruit weight of 1060 g, comparable to the T10CLF treatment in our study, which was the highest (1684.10±111 g), the superior value by 62.94%. Whereas Cerón (2010) obtained an average fruit weight of *C. argyrosperma* H. of 710.13 g.

Pulp thickness (mm)

Figure 3 shows the effects of the different treatments on pulp thickness; significant differences were found among treatments (DMS, p≤0.05). The TQm presented fruits with a higher value with 25.20±1.50 mm, a significant difference of 24.6% compared to the T2.5CLS (19.00±1.92 mm), the latter value was the lowest (Figure 3). Regarding the other treatments, in this variable they were statistically equivalent, except the T7.5CLF (19.80±1.85 mm) when compared to the chemical control TQm (25.20±1.16 mm). In this variable, some results coincide with those reported by Tucuch-Haas *et al.* (2022), for the varieties of Cushaw squash Chihuahua, Aguascalientes, Yucatan and Norteno that reached 23.2, 22.6, 16.2 and 14.9 mm of pulp thickness, respectively.

Fruit polar diameter (cm)

Figure 4 shows the variable polar diameter of the fruit; there were no significant differences among treatments (DMS, p≤0.05) although the best response of this variable was obtained with the T5CLF treatment (51.00±1.25 cm), 4% larger than TQm (48.95±0.96 cm). Although it was not significant, all treatments improved this variable. Tucuch-Haas *et al.* (2022), in four varieties of Cushaw squash (*C. argyrosperma*), found an interval between 50.81 and 56.34 cm; compared to our study, TQm and T5CLF treatments coincided within that range (48.95, 51.00 cm) tough smaller and with no statistical difference.

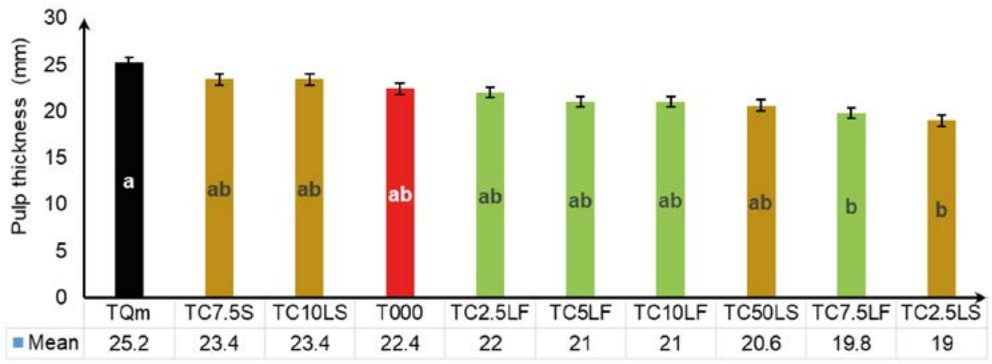

Figure 3. Pulp thickness (mm) of Cushaw squash fruits under combinations (on soil and foliar application) of fertilizer treatments; composted poultry manure and leachate at 50%.

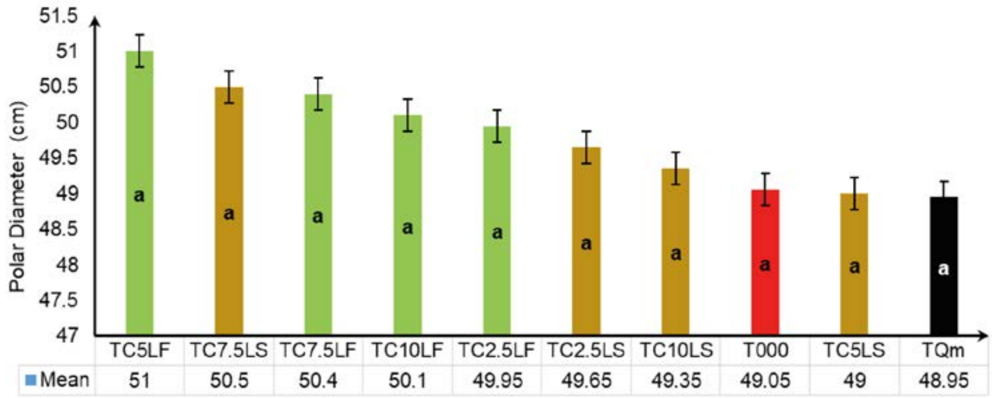

Figure 4. Average polar diameter (cm) of Cushaw squash fruits under combinations (on soil and foliar application) of fertilizer treatments; composted poultry manure and leachate at 50%.

Fruit equatorial diameter (cm)

Results obtained in the equatorial diameter variable of Cushaw squash fruit can be observed in Figure 5. There were significant differences among treatments (DMS, $p \leq 0.05$); the T7.5CLF with 53.80±1.12 cm exceeded that of the chemical control (TQm 51.10±1.20 EE) by 5%, and the zero control (T000) by 2.7%; this latter recorded an intermediate value (52.30±1.23 cm). The T10CLS (50.40±1.20 cm) showed the lowest record in the equatorial diameter variable of Cushaw squash fruit, compared to this the other treatments were better.

Tucuch-Haas *et al.* (2022), when evaluating four varieties of this squash, Norteno, Yucatan, Aguascalientes and Chihuahua, found fruit diameters 61.85, 60.08, 42.43 and 44.11 cm respectively, with significant differences. Villalobos *et al.* (2017) reported a fruit diameter of 50 cm in Cushaw squash that was lower when compared against those 53.80±1.12 cm of the highest value of T7.5CLF treatment in our study. Whereas Ruelas *et al.* (2015), found in *C. argyrosperma* an average fruit width of 13.0 cm, a value well below those obtained in this study. Lira-Saade (1995), in their morphological characterization of *C. argyrosperma* reported a range 14-25 cm in fruit diameter.

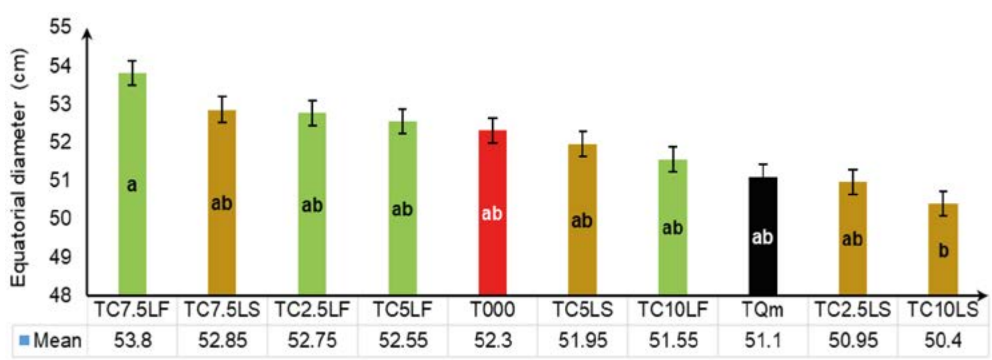

Figure 5. Equatorial diameter (cm) of *Cucurbita argyrosperma* fruits under combinations (on soil and foliar application) of fertilizer treatments; composted poultry manure and leachate at 50%.

Fruit shape index

Statistical differences among treatments (DMS, p≤0.05) were presented, as it is shown in Figure 6. Trends compared to control (T000), whose average was lower (0.93±0.01) in relation to the average of T10CLS treatment (0.98±0.01). This may be due to a lack of nutrients which affects the development of the fruit. The shape index of the polar diameter/equatorial diameter ratio according to the morphology (shape) of the fruit, a value below the unit (<1) was recorded. From which it is interpreted that fruits are oval or oblong (Lira-Saade, 1995). This index is obtained with the dimensions of fruit diameters (equatorial and polar), whose expression is polar D/equatorial D.

This ratio is an index to qualify the shape of the fruit, which should ideally have an equal ratio (1:1) in its dimensions, corresponding to the volume of a sphere. Overall, the indices obtained were greater than the unit (1), which indicates that the shape is slightly elongated to the fruit poles, thus giving it an ellipsoidal shape. Lira-Saade (1995) reported that the fruit shape of *C. argyrosperma* can be of several types; globose, oblate, ovoid, piriform, or clubs-form. In lemon, another type of fruit which is more homogeneous shape in lemon, García (2015) obtained index values in a range of 1.07 to 1.23 in fruits of Persian lemon grafted in *C. volkameriana*.

Seed weight (g)

Results of the seed weight variable are described in Figure 7; there were significant differences among treatments according to the test (DMS, p≤0.05). The best treatment was T10CLF with 68.78±4.35 g, surpassing control T000 (54.50±4.78 g) by 20.7%; with intermediate values in T2.5CLF, T10CLS and TQm treatments, 64.83±3.33, 62.47±4.49 and 62.39±4.75 g, respectively. This is, no statistical differences among those, but all of them statistically lower than T10CLF. Regarding the zero control (T000), it was surpassed by all the treatments under study.

FAO (2022) highlighted the importance of seeds for human sustenance and their genetic potential, improved over time, selected by producers for self-consumption and for sale, to improve production despite environmental problems. García and Dzul (2017) reported

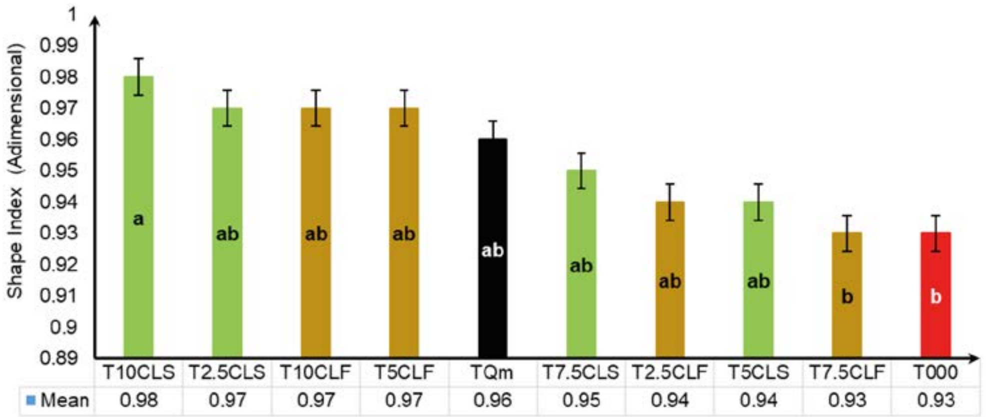

Figure 6. Fruit shape indices (dimensionless) of *Cucurbita argyrosperma* under combinations (on soil and foliar application) of fertilizer treatments; composted poultry manure and leachate at 50%.

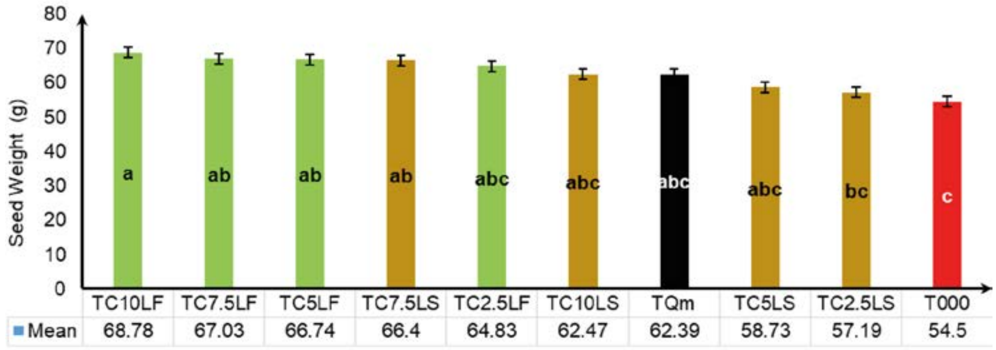

Figure 7. Average seed weight (g) per Cushaw squash fruit under combinations (on soil and foliar application) of fertilizer treatments; composted poultry manure and leachate at 50%.

70.09 g of seeds per fruit of Cushaw squash, those fruits were of flattened shape and fruit weight of 1728 g, values similar to those obtained with highest treatment tested here (T10CLF, 68.78 g of seeds per fruit, and 1684.10 g fruit weight). Ayvar *et al.* (2008) reported that with periodic application of chemical fertilizer to the soil, 59 g of dry seed per fruit can be reached, which significantly influences the number of fruits and the yield of dry seed, when fertilizers are supplied at the time of sowing, and then at intervals of 20 days.

Tucuch-Haas *et al.* (2022) reported that the Yucatan, Norteno, Chihuahua and Aguascalientes varieties can reach 66.54, 54.15, 24.63 and 18.4 g of dried seed per fruit respectively, those values are very similar and slightly higher than those obtained in this research. In addition, Villalobos *et al.* (2017), as a reference, reported 28 g as the weight of 100 seeds of good quality from fruits of Cushaw squash.

In this variable, it was possible to observe the effect of the application of leachate to the leaves (foliar application) with a slightly higher tendency on the application to the soil in this cycle, as it is shown (Figure 7). This may be due to the leachate effect, in addition to the residual effect of the poultry manure incorporated into the soil in the three cycles prior to cultivation. These results coincide with various reports mentioning that, as a characteristic effect of organic fertilizers applied to the soil. Where the soil acts as a nutrient reservoir and organisms continue to function by degrading organic matter and releasing nutrients for the next crop cycle (Sánchez, 2021). In other crops, as the application of leachate in maize at 55 days, in a dose of 500 L ha^{-1} originated the maximum biomass yield (37.5 Mg ha^{-1}), and in sorghum crop (4.8 Mg ha^{-1}) of grain yield (Patishtan *et al.*, 2023).

Results shown above, in the different variables can be taken as evidence of the goodness of these products. Improvements in growth and increased yields of Cushaw squash fruits could be due, in part, to a significant increase in soil microbial biomass following the application of compost to the soil in combination with leaf leachate, leading to the production of hormones or humates (humic deposits) in the compost and leachate that act as regulators of plant growth plus nutrient elements suppliers (Mkhabela *et al.*, 2020). Organic matter improves growth by lowering the pH of the rhizosphere, resulting in a better solubilization of nutrients, thus increasing availability to plants.

In addition, organic matter is a key source of minerals such as N, P, and K that improves soil structure and water-holding capacity (De los Santos *et al.*, 2022). The TC10LF treatment (10 Mg of compost+50% leachate, both applied to the soil) seems to favor nitrogen assimilation because to the presence of potassium and phosphorus (Table 1 and Table 2), which are essential elements for an optimal response to nitrogen (Alegbe *et al.*, 2020). Likewise, the synthesis of photosynthetic compounds is improved by increasing growth hormones and amino acids. These findings are in close agreement with the previous results obtained by Kaur and Rattan (2021) and Almudhafar and Obaid (2024) when studying the effect of the application of organic fertilizers in the cultivation of common squash (*Cucurbita pepo*), compared to chemical sources.

Correlations of seed weight vs. polar and equatorial fruit diameter

A correlation analysis of seed weight versus the variables of polar and equatorial diameter was made for characterizing the fruits; these results are presented in Table 4.

A trend to a higher correlation (r^2) of seed weight with fruit equatorial diameter was observed in all treatments. An example is observed in the scatter plots of Figures 8A and 8B, which shows the highest correlation of seed weight in T5.0CS treatment, both to fruit polar diameter (r^2=0.74) and to fruit equatorial diameter (r^2=0.79); contrasting with the T5.0CF treatment (r^2=0.19 and r^2=0.00, for those same variables).

Table 4. Ratio of seed weight versus polar and equatorial diameter of Cushaw squash fruits.

Treatment	Diameter	
	Polar	Equatorial
TQm	y=0.1607X+37.29, R^2=0.75	y=0.2009X+37.38, R^2=0.63
T000	y=0.138X+39.88, R^2=0.46	y=0.1544X+42.83, R^2=0.47
TC2.5LF	y=0.2111x+35.94, R^2=0.54	y=0.2747x+34.54, R^2=0.76
TC2.5LS	y=0.2009X+37.21, R^2=0.41	y=0.2027x+38.76, R^2=0.65
TC5LF	y=0.1614x+40.51, R^2=0.19	y=0.0174x+51.17, R^2=0.00
TC5LS	y=0.1592x+38.78, R^2=0.74	y=0.1650x+41.15, R^2=0.79
TC7.5LF	y=0.0854x+45.17, R^2=0.11	y=0.0684x+49.50, R^2=0.04
TC7.5LS	y=0.0464x+48.38, R^2=0.01	y=0.2171x+39.36, R^2=0.27
TC10LF	y=0.1288x+40.11, R^2=0.48	y=0.1954x+36.81, R^2=0.63
TC10LS	y=0.1561x+39.01, R^2=0.55	y=0.1358x+40.76, R^2=0.49

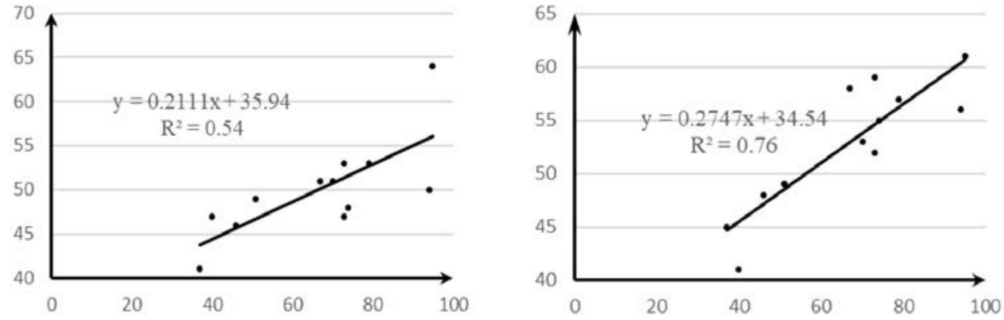

Figure 8. Regression plots between seed weight and the diameters (A: polar, and B: equatorial) of Cushaw squash fruits.

Overall, it appears that r^2 values were separated almost in half as slightly higher or slightly lower treatments, above and below the regression line. The statistics were obtained adjusted to a linear model (Figure 8A and 8B). These results contrast with those reported by Garcia and Dzul (2017), who found values up to r=0.90 in the correlation of the amount of dry seed per fruit of Cushaw squash; although that correlation was established against the weight of the fruits.

CONCLUSIONS

Composts incorporated to the soil, and leachates applied to the soil and to the foliage had effects on vegetative variables and fruit characteristics of Cushaw squash. The best treatments evaluated were combinations of composted poultry manure on the soil (5, 7.5 and 10 Mg ha^{-1}) plus the application to the soil and foliar of leachate at 50%.

When those combinations were supplied, the largest size of fruits, greater values of polar and equatorial diameters, as well as greater fruit weight were observed. The highest yield values of dry seeds of Cushaw squash were obtained with the treatments TC10LF, TC7.5LF, TC5LF and TC7.5LS, compared to the chemical control (TQm) and to the zero (T000) control.

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Colegio de Postgraduados

Effect of sanitizers on the sensory shelf-life of chia (*Salvia hispanica* L.) microgreens

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ABSTRACT

Objective: To compare the efficacy of different sanitizing agents in extending the sensory shelf life and maintaining the quality of chia (*Salvia hispanica* L.) microgreens in terms of overall quality, color, rot development and off-flavor.

Design/methodology/approach: Different parametric models (gamma, exponential, Weibull and log-logistic) were evaluated to determine the most appropriate one to analyze the sensory shelf life of treatments with sanitizing agents. The functions proposed by Guillermo Hough were used to calculate the sensory shelf life for each treatment. Finally, the Kruskal-Wallis test was used to compare the effects of the different sanitizing agents.

Results: Survival rates of microgreens varied according to the sanitizing agent used. On day two, sodium hypochlorite showed a survival rate of 80%, which decreased with time. On the other hand, colloidal silver reached a survival rate of 90% on day two, while calcium oxide saturated solution showed a survival rate of 85% in the same period. The control revealed a rate of 65% by day two, a lower percentage than the previous ones.

Limitations on study/implications: In this study, three sanitizers (colloidal silver, sodium hypochlorite and saturated solution of calcium oxide nanoparticles) were evaluated and other possible treatments or combinations of treatments that could be effective in preserving the quality and sensory shelf life of chia microgreens were not considered.

Findings/conclusions: The study revealed that colloidal silver treatment was the most effective in extending the sensory shelf-life of microgreens, with an estimated shelf life of 1.78 days at 10% rejection, followed by chlorine and saturated calcium oxide solution.

Keywords: microgreens, disinfectants, postharvest, shelf life, models.

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INTRODUCTION

Microgreens are gaining popularity in modern nutrition due to their high concentration of bioactive compounds. These plants are young and fragile vegetables with limited post-harvest life. Their optimal consumption period is usually between 7-21 days after harvest (Kyriacou *et al.*, 2016).

Since microgreens have young tissues at the time of harvest, they are highly susceptible to deterioration which is associated to a stress reaction more than to natural aging (Xiao *et al.*, 2016). Previous research has identified that, once they are cut, both pre- and post-harvest treatments, as well as packaging materials and packaging in a modified atmosphere, are determining factors of the shelf- life of microgreens (Kyriacou *et al.*, 2016).

The use of sodium hypochlorite in post-harvest washing has been shown to be beneficial for the visual quality and microbiological safety of microgreens, such as broccoli, by keeping them in optimal condition at 5 °C. Although it has been studied how the oxygen permeability of the packaging material affects the levels of CO_2 and O_2, this does not seem to have a significant impact on the shelf-life of the products. However, it has been confirmed that the temperature at which microgreens are stored is the most critical factor in preserving their freshness and prolonging their shelf-life (Ghoora *et al.*, 2020).

Sensory analysis supports the evaluation of food quality, allowing the identification of signs of deterioration and taking measures to preserve its integrity (Mondino *et al.*, 2023). The aim of the study was to determine the effect of different sanitizers on the sensory shelf-life of chia microgreens based on overall quality, rot development, color and unpleasant aroma.

MATERIALS Y METHODS

Materials

The sanitizers used in the study were: 1) sodium hypochlorite (Cloralex, Alen del Norte, S.A. de C.V. Mexico), 2) colloidal silver (Microdyn® Alen del Norte, S.A. de C.V, Mexico) and 3) calcium oxide nanoparticles (CONP, Oxical® Cal de Alta Pureza, Mexico) with a particle diameter of 90 nm and 95% purity.

Methods

Chia microgreen production

The production of chia (*Salvia hispanica*) microgreens was carried out in the facilities of the Facultad de Ciencias Biológicas y Agropecuarias of Universidad Veracruzana using a micro tunnel with a transparent plastic cover. Trays of 55 cm×28 cm×6 cm were used for microgreen growing. Sand was used as a substrate up to a height of 2.5 cm (approximately 1.30 Kg of substrate per tray). A total of 9 g of chia seeds were sown per tray. The seeds were distributed on the substrate and kept in darkness using a black plastic layer for three days to allow the seed germination (Kou *et al.*, 2013). After germination, the seedlings still in autotrophic condition were fed with a Soluponics® Standard A+B Universal hydroponic solution from Inverfarms Hidroponics (Mexico) applied daily by subirrigation until harvest.

Preparation of saturated calcium oxide solution

The calcium oxide saturated solution was prepared as follows: 100 g of CONP was mixed in 500 mL of distilled water and stirred for 30 min. It was then left to stand for 24 h and the precipitate was removed. The pH of the saturated solution was measured with a Thermo Scientific™ Orion™ 3-Star Plus potentiometer (USA). Subsequently, a portable refractometer HI96801 HANNA® (USA) was used to determine the percentage of soluble solids.

Harvest and storage of chia migrogreens

Microgreens were harvested using previously sterilized gloves and scissors. Microgreens were cut once they reached a height of 7 to 10 cm. After harvesting, they were treated with the sanitizers: 1) chlorinated water was prepared at a concentration of 200 ppm using sodium hypochlorite (Villenas *et al*., 2010); 2) saturated solution of calcium oxide nanoparticles and 3) commercial disinfectant for vegetables (Microdyn® colloidal silver) at a concentration of 1 mL L^{-1} according to the manufacturer›s instructions. The immersion time of the microgreens in the sanitizers was 15 min. Additionally, a control group without sanitizing treatment was included in the study. After sanitizing, microgreens were dried on absorbent paper towels and placed in disposable 11 cm hinged dome-type polystyrene containers (code RB9X9, Reyma, Mexico) and stored at 4 °C. Storage was carried out for 5 days (Dayarathna *et al*., 2023).

Determination of sensory shelf-life

To assess the sensory shelf life of chia microgreens, an evaluation was conducted with n=8 panelists who had prior experience with vegetable consumption. An information session introduced and defined the attributes under consideration, including overall quality, rot development, color, and unpleasant aroma (Mondino *et al*., 2023). Participants rated their acceptance using a binomial scale (yes/no) and then assessed the intensity of each quality characteristic (overall quality, rot development, color, and unpleasant aroma) on a 5-point verbal scale (excellent, good, regular, bad, and very bad). Sample evaluations were performed every 24 hours in Current Status Data mode (Araneda, 2008).

Statistical analysis

The deterioration of microgreens was modeled using the acceptance data and different parametric models were employed: gamma, exponential, Weibull, and log-logistic. To identify the best model, the highest log-likelihood value of the regression was used. The sensory shelf-life values for each treatment were estimated using the function described by Hough (2010) for survival analysis in a Current Status Data design. The comparison among treatments based on the quality data (overall quality, rot development, color, and unpleasant aroma) measured on an ordinal scale was performed with the Kruskal-Wallis test.

RESULTS AND DISCUSSION

pH and soluble solids of the saturated solution of calcium oxide nanoparticles

The saturated solution of calcium oxide nanoparticles resulted with pH values of 12.1 and 0.5% soluble solids. However, this compound has been scarcely used in a nanometric form, so there is limited information on the subject (Roy *et al*., 2011).

Evolution of deterioration of microgreens

Figure 1 shows the survival probabilities of microgreens treated with the different sanitizers from day zero to day four. Sodium-hypochlorite-treated microgreens decreased their survival rate to 80%. Those treated with colloidal silver reached 90% on day two, whereas those treated with saturated lime solution had an 85% survival rate on day two. The control treatment had a rate of 65% for day two. Storage temperature is crucial, with optimal ranges between 1 and 10 °C to maintain product quality (Priti *et al.*, 2022). However, other elements such as relative humidity, packaging material quality, and initial microbial load also play significant roles. These findings underline the importance of integrated cold chain management and hygienic practices to extend the shelf-life of these perishable products. The study conducted by Xiao *et al.* (2014) examined the postharvest preservation of radish microgreens, concluding that the optimal storage temperature is 1 °C. Furthermore, they indicated that the use of a chlorine solution at a concentration of 100 mg/L as a washing method considerably decreases the amount of microorganisms present (0.5 log CFU g^{-1}). However, they observed that the amount of microorganisms tends to increase after one week of storage.

Determination of sensory shelf-life

The shelf-life estimation using various sanitizers at various acceptance levels (90, 75, and 50%) is shown in Table 1. The treatment with saturated lime solution at 90% acceptance presented an estimated shelf life of 1.68 days. Calcium is essential to improve the nutritional quality and extend the postharvest life of fruits and vegetables (Aghdam *et al.*, 2012). Colloidal silver at 90% acceptance had a superior performance compared to chlorine and saturated lime solution with a shelf life of 1.78 days. As expected, the estimation in the control treatment at 90% acceptance resulted in a value of 1.42 days since no external factor was applied that could affect the shelf-life of the microgreens.

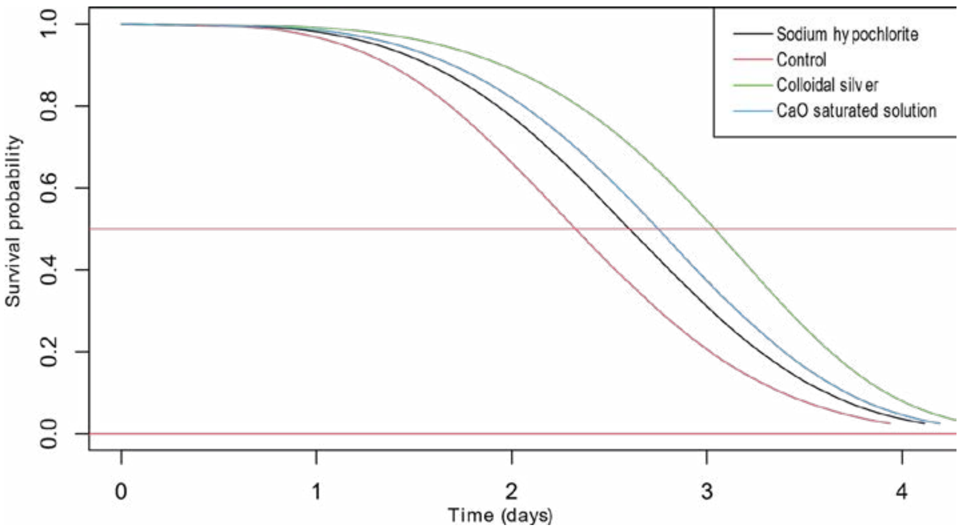

Figure 1. Evolution of microgreen survival probabilities for different treatments modeled with a Weibull function.

Table 1. Shelf life evaluation with different sanitizing agents using a Weibull model at various acceptance levels*.

	Percent Acceptance	Shelf-Life (days)	Lower ci (days)	Upper ci (days)	Serror
Sodium hypochlorite	90	1.63	1.25	2.11	0.22
	75	2.12	1.74	2.59	0.22
	50	2.68	2.27	3.16	0.23
Control	90	1.42	1.08	1.86	0.20
	75	1.85	1.49	2.29	0.20
	50	2.33	1.94	2.80	0.22
Colloidal silver	90	1.78	1.38	2.29	0.23
	75	2.32	1.91	2.83	0.23
	50	2.93	2.47	3.48	0.26
CaO saturated Solution	90	1.68	1.30	2.17	0.22
	75	2.19	1.80	2.67	0.22
	50	2.76	2.34	3.26	0.23

Lower ci=lower confidence interval; Upper ci=Upper confidence interval; Serror=standard error.

Colloidal silver (Microdyn®) is widely used in Mexico (Rangel-Vargas *et al.*, 2017). In their study with drug-resistant *Salmonella serotypes* (M-RSs) isolated from cilantro, they found that extracts from hibiscus flower calyx can be as effective as colloidal silver for the control of these pathogens.

Data analysis using the Kruskal-Wallis test indicated that consumers did not detect differences among treatments in terms of overall quality, rot development, color and unpleasant aroma, but they did detect deterioration in these variables over the days (p-value <0.05).

CONCLUSIONS

The deterioration of microgreens was adequately modeled with a Weibull function. The difference between treatments was significant. Colloidal silver extended the sensory shelf-life of microgreens up to 1.78 days whereas the saturated solution of calcium oxide nanoparticles up to 1.68 days at 90% acceptance. The panelists were able to identify the deterioration of microgreens over time. The use of sanitizing agents is essential to maintain the quality of microgreens during their postharvest period.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Agricultural Backyard Production in the Food Security Framework: A Case Study of a Microregion of Chicontepec Veracruz, Mexico

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ABSTRACT

Objective: This study evaluated the impact of agricultural backyard production on the well-being of producing families in the context of food security in a Microregion of Chicontepec Veracruz, Mexico, highlighting the substantial economic advantages of this practice.

Design/methodology/approach: Through probabilistic sampling, a structured survey on availability, accessibility, and nutrition was designed and applied to backyard-producing families. A multinomial logistic regression model was employed to analyze the relationship between a dependent variable and a set of independent variables.

Results: In the microregion of study, backyard production is distributed in 55.4% of fruit trees, 25.4% of regional crops, and 19.2% of vegetables. The two crops that add the most to the basic basket are corn and Chichimeken beans. 98% of producers estimate that backyard production (livestock and agriculture) saves the family economy 10% to 50% of its costs.

Limitations on study/implications: Knowledge of the backyard production of the microregion under study will allow the development of municipal policies that will link producers with the local market.

Findings/conclusions: Backyard production has been shown to contribute significantly to food security since different fruit trees, vegetables, and local crops substantially contribute to the household economy through sales and self-consumption.

Keywords: Food self-sufficiency, high-priority development communities, responsible consumption and production.

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INTRODUCTION

The rural workforce in Mexico is concentrated in the countryside, where social reproduction has compelled the development of adaptable techniques (Jaramillo-

Villanueva *et al.*, 2017). Backyard production is an informal activity that draws from the expertise and experience of indigenous people and peasants (López *et al.*, 2012). By providing rural families and communities with goods and services that meet their fundamental requirements, backyard agriculture helps to ensure food security (FAO, 2019; Hernández *et al.*, 2010). Food during periods of scarcity, savings, and revenue are produced by this practice (Jaramillo-Villanueva *et al.*, 2017). According to definitions, food security is when people have timely and continuous access to food —quantity and quality— for their biological needs and adequate consumption, ensuring a well-being that supports their nutritional development (FAO, 2019). The three fundamental pillars supporting food security are access to, availability of, and sufficient intake of food (FAO, 1996).

Food security and poverty are linked. Twenty-three percent of Mexicans are food insecure, 53% of Veracruz residents experience food insecurity, and 61.8% of the population is poor, with 17.7% living in extreme poverty and 44.1% in moderate poverty (CONEVAL, 2021).

Because backyards are important as productive systems and because they help low-income families in the state of Veracruz be self-sufficient and eat well, studies that quantitatively evaluate backyard activities within the context of food security in areas where poverty, marginalization, and cultural diversity are commonplace are crucial. The findings will aid in formulating public policy initiatives to address poverty.

Studies conducted in the states of Puebla and Veracruz highlight the following research approaches: backyard production as a survival strategy (López *et al.*, 2012), backyard production's economic significance and its connection to food security in high-priority development communities (Jaramillo-Villanueva *et al.*, 2017; Urquía-Fernández, 2014), and backyard production's ability to boost the local economy (Sánchez-Galván *et al.*, 2019, 2020).

In this context, the following inquiries served as the foundation for this study: What savings do backyard farmer families realize from this activity in their economy? What additional benefits do your products offer in terms of food security and your well-being? According to the theory above, families who engage in backyard production greatly benefit from increased food availability, accessibility, and quality.

MATERIALS AND METHODS

Study area. Chicontepec, the municipal seat of the homonymous municipality, is a significant location for this study. It is located northwest of Veracruz, Mexico, and is part of the Chicontepec microregion under study. This microregion includes the communities of Ahuimol, Akichzintla, Alashcuatitlan, Ateneo, Cuatzapotl, Tecuapa, and Tenexaco. These communities represent diverse rural settings, each with unique challenges and opportunities for agricultural backyard production (Figure 1).

Field work. The research methods used were interviews, structured questionnaires, and observation. Through in situ observation, data regarding backyard production methods and products, areas set aside for this purpose, and conventional agricultural techniques were gathered.

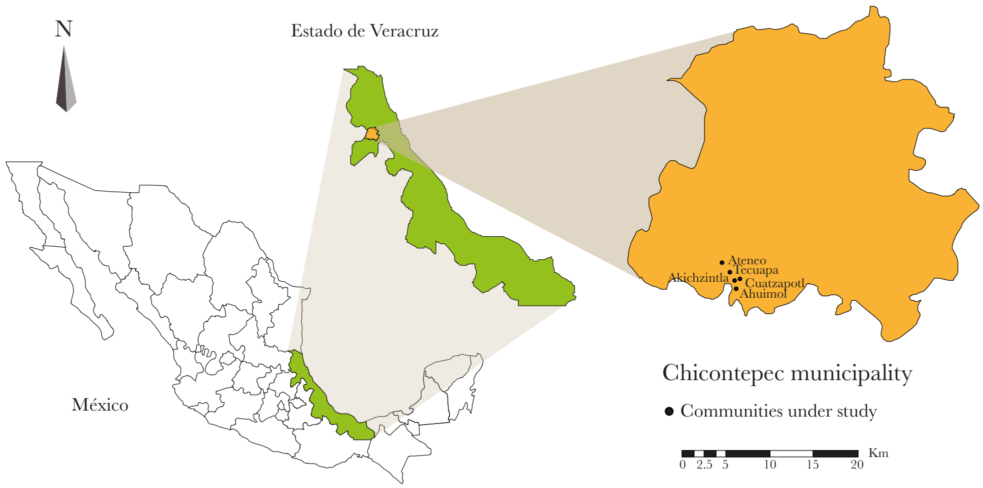

Figure 1. Study location. Source: own elaboration based on data obtained from INEGI (2010, 2016 and 2019). They are prepared in QGIS (2021) Desktop 3.10.8. Microregion designed in (Bautista-Santos *et al.*, 2021).

Survey design, construction, and implementation. A structured survey was created and implemented to gather data on product types (fruit trees, vegetables, backyard animals), accessibility (product sales, savings to the family economy from the product sale, average monthly income), and nutritional aspects (product variety, consumer products). Using data from the most recent population and housing census, a probabilistic random sampling was used, considering the number of families in the studied communities (INEGI, 2010). The household leader was regarded as the head of the peasant domestic group or the backyard producer (González *et al.*, 2014). Cronbach's alpha statistical method was used to validate the data (Tuapanta Dacto *et al.*, 2017). A case study of households in backyard farming and residing in a Chicontepec, Veracruz microregion was investigated as part of the research methodology (Bautista-Santos *et al.*, 2021).

One hundred forty-three backyard producers were surveyed, accounting for 36% of the study population and 246% more of the sample size. The above is according to the maximum variance equation Eq.1 (Gil & Zarate Lara, 2012). Each family was taken as a production unit. Total families (401): Ahuimol (192), Akichzintla (45), Alasxcuatitlan (36), Atenol (41), Cuatzapotl (20), Tecuapa (50) and Tenexaco (17).

$$n = \frac{NZ^2_{\alpha/2}pq}{Nd^2 + Z^2_{\alpha/2}pq} \qquad \text{Eq. 1}$$

Where n=sample size; N=population size (N=401); $Z^2_{\alpha/2}$ =value of Z distribution tables ($Z^2_{\alpha/2}$ =2.6896); p=proportion of the population with a binomial characteristic, $q=1-p$ (pq=0.25); d^2=maximum desired absolute error (set as a fraction of p) (10%) (d^2=0.01).

This study employed a multinomial logistic regression model to analyze the relationship between a dependent variable and a set of independent variables. This model is instrumental in understanding the impact of multiple factors on a definite outcome, which in this case is the well-being of the producing families. The model, constructed with the IBM SPSS statistics 25 statistical packages, estimates the probability of a multinomial event represented by the dependent variable $\left(\widehat{Y}_i\right)$ based on a series of discrete or continuous predictor or prognostic variables $\left(X_i\right)$. Model validation utilized the maximum likelihood ratio and Cox and Snell's R square. The Pearson correlation matrix was also used to determine the degree of correlation among the independent variables Eq.2. This rigorous analysis was crucial in drawing meaningful conclusions from the data collected.

$$\widehat{Y}_i = Pr\left(Y=1/X\right) = \frac{1}{1+e-\left(\widehat{\beta}_0+\widehat{\beta}_i X_i+\ldots\widehat{\beta}_n X_n\right)} \qquad \text{Eq. 2}$$

Where: $\widehat{Y}_i$ =Probability of producing regional crops (i=0 (not making anything), 1 (producing corn and/or beans)); e=Base of the natural logarithm; $\widehat{\beta}_0, \widehat{\beta}_i$ =Intercept and estimators of the independent variables (X_i); and X_i=Independent variables (X_1=vegetable production; X_2=fruit tree production).

RESULTS AND DISCUSSION

In the study area, the primary backyard production is fruit trees (55%), and in that exact order are regional crops (25%) and vegetables (20%) (Table 1). Backyard production (agricultural and livestock) contributes 15% to the family economy since this percentage is for sale. In this savings area, regional crops are the most destined for sale (18%). Of all production, fruit trees are the one that contributes the most to self-consumption (92%) (84%, 82%, and 87% for vegetables, regional crops, and livestock, respectively). In regional crops, 75% of production is corn (52%) and chichimekel bean (23%), while vegetable production is dominated by cilantro (24%) and mint (23%). The data confirm that the production of these last two products dominates the area's agricultural economy (*e.g.*, corn: 86% consumption and 14% for sale; chichimekel beans: 82% consumption and 18% sale) (Basurto *et al.*, 2018). Production is carried out in an approximate area of 1.19 hectares, one of the largest compared to others reported (*e.g.*, 520% more than in Puebla) (González *et al.*, 2014; López *et al.*, 2012). Those surveyed say 95% of agricultural and livestock products are obtained without fertilizers or processed foods. This represents a comparative advantage in terms of savings in food production and quality.

The fruit tree production as the main product obeys the social policy promoted by the federal government in its "sembrando vida" (sowing life) project (https://www.gob.mx/bienestar/acciones-y-programas/programa-sembrando-vida). The production of fruit trees requires minimal investment, a benign climate, accessibility, and availability of land, which makes it the state with the largest fruit-growing area in the country (Cruz-Delgado *et al.*, 2013).

The investment is more significant for regional crops than the other two activities (fruit trees and vegetables) (Olvera-Hernández *et al.*, 2017). The order of importance of backyard

Table 1. Main backyard products and by-products.

Product (%)	By-product (%)	%	Self consumption %	Sale %
Fruit trees (55.4)	Avocado creole (*Persea americana* var. Mexicana)	6	92	8
	lime/lemon (*Citrus limon*)	9	91	9
	tangerine (*Citrus reticulata*)	5	100	0
	mango (*Mangifera indica*)	11	94	6
	orange (*Citrus sinensis*)	15	88	12
	banana (*Musa paradisiaca*)	15	87	13
	others	38	94	6
Average			92	8
Vegetables (19.2)	cilantro (*Coriandrum sativum*)	24	70	30
	mint (*Mentha spicata*)	13	83	17
	cassava (*Manihot esculenta*)	7	92	8
	chayote (*Sechium edule*)	6	88	12
	garlic (*Allium sativum*)	3	100	0
	pumppkin (*Cucurbita*)	4	100	0
	sweet potato (*Ipomoea batatas*)	6	68	32
	papatla (*Heliconia schiedeana*)	8	62	38
	epazote (*Dysphania ambrosioides*)	4	100	0
	others	27	77	23
Average			84	16
Regional crops (25.4)	Corn (*Zea mays*)	52	86	14
	chichimenkel bean (*Phaseolus vulgaris*)	23	82	18
	creole chili (*Capsicum annuum*)	9	74	26
	pemuche (*Erythrina coralloides*)	6	92	8
	others	10	77	23
Average			82	18
Livestock	poultry (*Gallus gallus domesticus*)	68	86	14
	bovine (*Bos taurus*)	24	90	10
	pig (*Sus scrofa domesticus*)	8	85	15
Average			87	13

Source: Own elaboration based on information collected in fieldwork.

production coincides with (López *et al.*, 2012; López *et al.*, 2013) in the states of Puebla and Hidalgo; these authors conclude that corn and beans are food bases for rural communities. Rainfed corn and chichimekel beans are two foods that provide the body with the primary sources of calories and proteins of non-animal origin (GOB-MEX, 2017). In the region, these two plants contain a very particular syncretism that permeates the maintenance of the social fabric since their agricultural cycle is closely related to the diet of this Huasteca population (Basurto *et al.*, 2018).

Backyard livestock farming involves raising birds, horses, cattle, pigs, goats, sheep, and cattle (Jaramillo-Villanueva *et al.*, 2017). Backyard animals complement the protein intake since 87% is for self-consumption (availability), and the remaining (13%) is for sale (accessibility).

For the construction of the logistic model, 111 data were considered. The fit shows a lower maximum likelihood ratio compared to that which only includes the intercept (β_0). This is corroborated by the likelihood ratio, which indicates a very significant difference ($p \leq 0.001$) between including all variables in the model versus not including any. That is, the variables contribute to explaining the backyard production of regional crops (corn and/or beans). The Pseudo R^2 of Cox Snell and Nagelkerke suggests that between 22% for the first and almost 25% for the second variation in the dependent variable is explained by the independent variables included in the model. Although this value is not high, in social research, it is considered acceptable (Cruz-Huerta *et al.*, 2015), providing a solid foundation for our findings. Additionally, Pearson's goodness of fit indicates no correlation between the independent variables by showing values close to zero. The estimated multinomial logistic model generally presents a satisfactory goodness of fit (Table 2).

Table 2. Results of the multinomial logistic model.

A) Summary of processing of nominal variables cases			
Variable	**Valor nominal**	**n**	**Marginal (%)**
Regional crops (Y_i)	0 no crops	12	10.9
	1 corn and/or beans	90	81.8
	2 other	8	7.3
Vegetables (X_1)	0 no crops	68	61.8
	1 some crop	42	38.2
Fruit tres (X_2)	0 no fruit tree	31	61.8
	1 orange and/or mangoes and/or banana	72	65.5
	2 other	7	6.4

B) Likelihood ratio tests			
Variables	**X^2**	**g.l.**	**p**
Vegetables (X_1)	25.656	2	0.672
Fruit trees (X_2)	8.8893	4	0.031

c) The goodness of fit of the model				
Adjustment criteria		**Likelihood ratio**		
Model	**Log. of likelihood -2**	**X^2**	**g.l.**	**p**
Intersection only	606.017	64.716	33	0.001
Final	541.301			
		Goodness of fit		
Pseudo R cuadrada		Parson	708	0.104
Cox and Snell 0.223		755.641		
Nagelkerke 0.246		Desvianza	708	1
			541.301	

Source: elaboration with data collected from a survey and outputs from the logistic regression model on a database previously created in Microsoft Excel 2001.

The model indicates that the probability of producing corn and/or beans and other products is only significant in fruit trees ($p<0.05$) (Table 3). That is to say, the probability of producing corn and/or chichimenkel beans is 3.51 times greater if there are no fruit trees and 2.9 times more significant if oranges and/or mangoes and/or bananas are produced. Regional crops can be produced without fruit tree production (see Parameter Estimation Table). Considering the variables above, this can establish public policies to promote the production of fruit trees or corn and/or beans.

Regarding the economic component, families earn income through the sale of surplus production; they save money and time by avoiding purchasing products that families consume and produce (Olvera-Hernández *et al.*, 2017). Backyard production is significant for the family economy (contribution to the basic basket). 98% of producers indicate a saving of between 10 and 50% due to backyard production (agriculture and livestock) (Table 4).

Backyard produce is an essential resource for the economy of families living in high-priority development communities. The minimum monthly income (in July 2019 pesos) for a rural community fluctuates between 141.71 and 356.84 USD (GOB-MEX, s. f.); these

Table 3. Matrix of coefficients of significant independent variables ($p<0.05$).

Regional crops		B	Significance (<0.05)	Exp(B)
Corn and/or bean	Intersection	19.128	.000	
	[vegetables=0]	.182	.777	1.199
	[vegetables=1]	0	.	.
	[fruittrees=0]	−17.151	.000	3.561
	[fruittrees=1]	−17.347	.000	2.927
	[fruittrees=2]	0	.	.
Others	Intersección	17.095	.000	
	[vegetables=0]	.893	.403	2.441
	[vegetables=1]	0	.	.
	[fruittrees=0]	−17.842	.000	1.78
	[fruittrees=1]	−18.466	.	9.553
	[fruittrees=2]	0	.	.

Source: Own elaboration. IBM SPSS Statistics software package (V. 25.0, 64-bit Edition) on a database previously created in Microsoft Excel 2001.

Table 4. Perception of respondents about saving the family economy on backyard production.

Variable	Interval (%)	Frequency	(%)
Perception of contribution to the family economy through backyard production	between 10 and 25	104	73
	between 25 and 50	36	25
	between 50 and 75	3	2
Average monthly income (USD/month)	$\bar{x}$ =216.86* d.s=77.73*		

Source: own elaboration.
* 1 USD=19.05340 Mexican pesos. Average exchange rate during July 2019 (BANXICO, 2019).

data coincide with the average income reported by respondents of 216.86 USD per month on average: 37% of the salary considered by Below the poverty line, this is 592.55 USD (CONEVAL, 2021). The income coincides with studies in other countries' rural regions, especially in the State of Mexico in the Mazahua and Otomi and forest communities (Rodríguez-Zúñiga *et al.*, 2023).

CONCLUSIONES

In the resilient system study region, 55.4% of backyard production is distributed among fruit trees, 25.4% among regional crops, and 19.2% among vegetables. Chichimeken beans and corn, the backbone of the basic basket, demonstrate this resilience. Based on vegetables and fruit trees, it is possible to predict the production of regional crops probabilistically.

98% of producers indicate that backyard production (agricultural and livestock) saves the family economy between 10% and 50%. This is significant since the average monthly income of producers is around 37% less than the salary considered below the poverty line. Backyard agricultural and livestock products are crucial in improving food availability: 86% for self-consumption, while accessibility (product sales) is around 14%. The nutritional part of backyard production is that most are organic, representing a competitive advantage. Carry out regional market studies (development of production and demand functions) supported by a detailed analysis of investment costs and their benefits; likewise, determine the nutritional value and traditional intake of backyard production.

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Colegio de Postgraduados

Application and Effects of Rare Earth Elements in Agricultural Production Systems

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ABSTRACT

Objective: To review the history of rare earths and their practical applications, as well as to identify the effects of some rare earths on crops.

Design/methodology/approach: We performed an exhaustive review of the scientific literature related to the history of rare earth elements (REE), their chemical characteristics, composition in the Earth's crust, and uses in industry, as well as of the effects of the light rare earth elements (LREE) on higher plants. The most relevant articles on the aforementioned topics of interest were then selected, analyzed, and discussed.

Results: In recent years, the industrial and technological use of REE has increased significantly. Their use in the automotive, aeronautical, and space industries, in medicine, in renewable energies, and in electronic and military technology is resulting in the accumulation of these elements in the environment and their bioavailability for crops. Importantly, REE have been reported to have both stimulatory and inhibitory effects on biological systems, including plants.

Limitations on study/implications: While some REE like lanthanum (La) and cerium (Ce) have been extensively studied, others have been scarcely explored and, therefore, little information has been published on them in the international literature.

Findings/conclusions: The use of REE in technology, combined with poor waste management and recycling, cause contamination in soil and water, allowing REE bioavailability in plants. Further studies are needed to identify beneficial effects of REE in the face of biotic or abiotic stress factors.

Keywords: Lanthanides, Biostimulants, phytotoxicity, LREE, HREE.

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INTRODUCTION

Rare earth elements (REE) include the 15 lanthanides (elements from atomic number 57 to 61), in addition to scandium (Sc) and yttrium (Y); these have become critical elements today due to their high demand and use in renewable energy techniques, low recycling rates, and possible scarcity in the future [1,2]. Based on their atomic weight, these elements are divided into light rare earths (LREE) and heavy rare earths (HREE) as shown in Table 1. These minerals are widely used, as they are useful in the automotive, aerospace, medical, renewable energy, electronics, and military industries. For the automotive industry alone, in the area of production of electrical units, an increase in demand of 22% (315 Mg of REE) is expected globally for the period 2018 to 2030 [3,4].

Table 1. Classification and properties of rare earth elements [1].

Element	Symbol	Atomic number	Atomic mass (g mol^{-1})	Oxidation states	Electronegativity (Pauling scale)
Light Rare Earth Elements (LREE)					
Scandium	Sc	21	44.9	+3	1.36
Lanthanum	La	57	138.9	+3	1.10
Cerium	Ce	58	140.1	+3, +4	1.12
Praseodymium	Pr	59	140.9	+3	1.13
Neodymium	Nd	60	144.2	+3	1.14
Promethium	Pm	61	147	+3	1.13
Samarium	Sm	62	150.3	+2, +3	1.17
Europium	Eu	63	151.9	+2	1.2
Heavy Rare Earth Elements (HREE)					
Yttrium	Y	39	88.9	+3	1.22
Gadolinium	Gd	64	157.2	+3	1.2
Terbium	Tb	65	158.9	+3	1.2
Dysprosium	Dy	66	162.5	+3	1.22
Holmium	Ho	67	164.9	+3	1.2
Erbium	Er	68	167.2	+3	1.2
Thulium	Tm	69	168.9	+3	1.25
Ytterbium	Yb	70	173	+2, +3	1.1
Lutetium	Lu	71	174.9	+3	1.2

Contrary to what their name suggests, so-called rare earths are relatively abundant in the Earth's crust. China has the largest REE reserves and therefore has ample market control. Globally, by 2022, REE reserves were estimated at 130 million Mg (Figure 1). Of this volume, China holds about one third with 44 million Mg, followed by Vietnam, Brazil, Russia, the United States, and Australia [5,6]. Since 2019, China has accounted

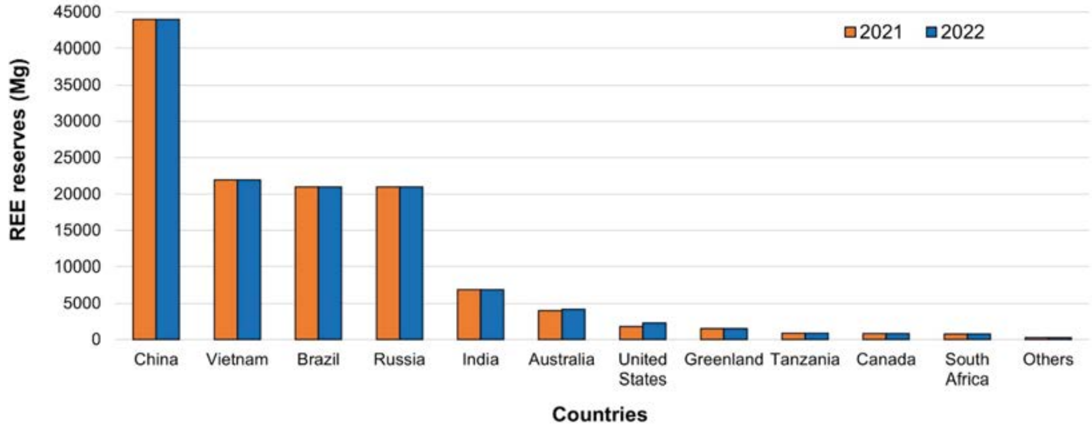

Figure 1. Ranking of countries with the largest reserves of rare earth elements (REE) [5].

for slightly more than 80% of the world production of REE, and by 2022, its share of their extraction and separation of these rose to 210 000 Mg, 190 850 Mg of which were LREE. REE extraction in the United States, Australia, Burma, and Thailand was 43 000, 18 000, 12 000, and 7 100 Mg, respectively [3,6].

REE reserves in North America are estimated to be 3.6 million Mg in the United States and more than 14 million Mg in Canada. REE are mainly extracted from bastnaesite mined in Mountain Pass, CA, and from monazite from the southeastern United States [6]. Mexico has some REE reserves, although there is little or no commercial extraction. For example, the Cerro de Mercado mine in Durango is considered among the main deposits of fluorapatite ore [$Ca_5(PO_4)_3F$], which contains high amounts of Nd, up to 0.45% by weight of neodymium oxide (Nd_2O_3) [7]. Similarly, fluorapatite and some associated minerals can contain up to 3 000 mg Ce kg^{-1}, 2 000 mg La kg^{-1}, and 500 mg Nd kg^{-1}, as well as, Y, Gd, and Pr in concentrations of less than 500 mg kg^{-1} [8].

In Guanajuato, Mexico, the average concentration of some REE in the soil is 103 mg Ce kg^{-1}, 38 mg La kg^{-1} and 41 mg Nd kg^{-1}. For the other REE, concentrations are less than 8 mg kg^{-1}. In water, Ce concentrations range from a few nanograms per liter to 0.5 mg L^{-1}. Elements such as Tb, Ho, Tm, and Lu are found at levels below the detection limit [9]. Even with these data, REE mining is not an economically important activity in Mexico, although their existence makes it necessary to study them for different applications. This paper provides an exhaustive review of historical aspects of rare earths and their practical applications, and also identifies the effects of some rare earth elements on crops.

HISTORY OF THE DISCOVERY OF RARE EARTHS

In 1787, the Swedish chemist Carl Axel Arrhenius (1757-1824) found a dark colored mineral that he named "Ytterby heavy stone" in the village of Ytterby, Sweden [10]. By 1792, the Finnish chemist Johan Gadolin (1760-1852) received a sample of such a mineral from Arrhenius and discovered that 38% of it was a new element he called ytterbia, which was later known as yttria; in 1794, the substance Gadolin was discovered and published with the name of yttrium oxide, and it was considered the first element of the so-called isolated "rare earths" [11].

In 1803, the Swedish chemists Jöns Jacob Berzelius (1779-1848) and Wilhelm Hisinger (1766-1852), as well as the German chemist Martin Heinrich Klaproth (1743-1817), simultaneously analyzed an ore from the Bastnäs iron mine in Sweden. Berzelius and Hisinger called it waxy oxide, while Klaproth called it terre ochroite. Later, Berzelius renamed the compound ceria or cerite, which was eventually changed to "cerium," after the dwarf planet Ceres [12].

In 1839, the Swedish chemist Carl Gustaf Mosander (1797-1858) showed that ceria was a complex composition of "earths," from which he separated an oxide he named lanthana, today known as lanthanum [13]. In 1843, Mosander separated yttria and discovered three other compounds, including erbium and terbium, the latter named after Ytterby, Sweden. In 1878, the Swiss chemist Jean Charles Galissard de Marignac (1817-1894) discovered ytterbium by separating it from the rare earth erbia [14].

In 1879, the Swedish chemist Lars Fredrik Nilson discovered scandium by spectral analysis of gadolinite. The name scandium was derived from his country's name, Scandinavia [15]. In the same year, the Swedish chemist Per Teodor Cleve (1840-1905) discovered thulium by obtaining a sample of erbia free of iterbia and scandia, which he dissolved into three constituents: erbia, holmia, and thulia. Thulium was so named because Thule was the ancient name for Scandinavia. In the same year, the Swiss chemists Jacques-Louis Soret (1827-1890) and Marc Delafontaine (1837-1911) studied holmium by spectroscopy. However, it was Cleve who separated it, naming it after Holmia, the Latin name for the city of Stockholm, although all three were credited with the discovery of the element [14].

The Swiss chemist Jean Charles Galissard de Marignac discovered samarium in 1853, and in 1886 he extracted gadolinium, a name he coined in honor of Johan Gadolin, the initiator of rare earth studies. The French chemist Paul Émile Lecoq de Boisbaudran (1838-1912) isolated samarium in 1879 from samarskite earth [16]. In 1885, the Austrian Baron Carl Auer Freiherr von Welsbach (1858-1929) discovered praseodymium and neodymium from the splitting of didymium, which he obtained from residues left over from the extraction of cerium and lanthanum [14].

In 1886, Lecoq de Boisbaudran separated dysprosium from dysprosium oxide dissolved in acid and ammonia. In 1889, the English chemist William Crookes (1832-1919) separated and made the first references to europium, so called in Europe, but it was the French chemist Eugène-Anatole De-marçay (1852-1903) who, in 1901, by crystallization sequence of samarium and magnesium nitrate, isolated europium [18].

The French chemist Georges Urbain (1872-1938) published in 1907 that he had discovered lutetium, named after Lutetia, the ancient name of Paris. In 1908 von Welsbach reported having isolated the same element, calling it cassiopium, a name used in German-speaking countries for some years. Both scientists found it as an impurity of ytterbium [19]. Between 1913 and 1914 the Danish physicist Niels Henrik David Bohr (1885-1962) and the English physicist and chemist Henry Gwyn Jeffreys Moseley (1887-1915) established that the so-called rare earths were composed of 15 elements [20].

In 1947, the American chemists Charles DuBois Coryell (1912-1971), Jacob Akiba Marinsky (1918-2005), and Lawrence Elgin Glendenin (1918-2008) proved the existence of promethium (named after the titan Prometheus) after analyzing the ashes of a nuclear reactor [18]. This concludes the search for rare earth elements, which began in 1787 [10]. The above events are depicted in Figure 2, as an abbreviated timeline.

EFFECTS OF LREE ON CROP PLANTS

Lanthanum (La)

It is a metallic, malleable, ductile, and soft element, found in nature in combination with cerium and other elements such as neodymium or yttrium, forming minerals such as monazite and bastnasite. Regarding its percentage in the Earth's crust, it occupies the twenty-eighth place (0.0032%), its abundance in soil is approximately 32 mg kg^{-1} and its extraction is a challenge for mining [19-24].

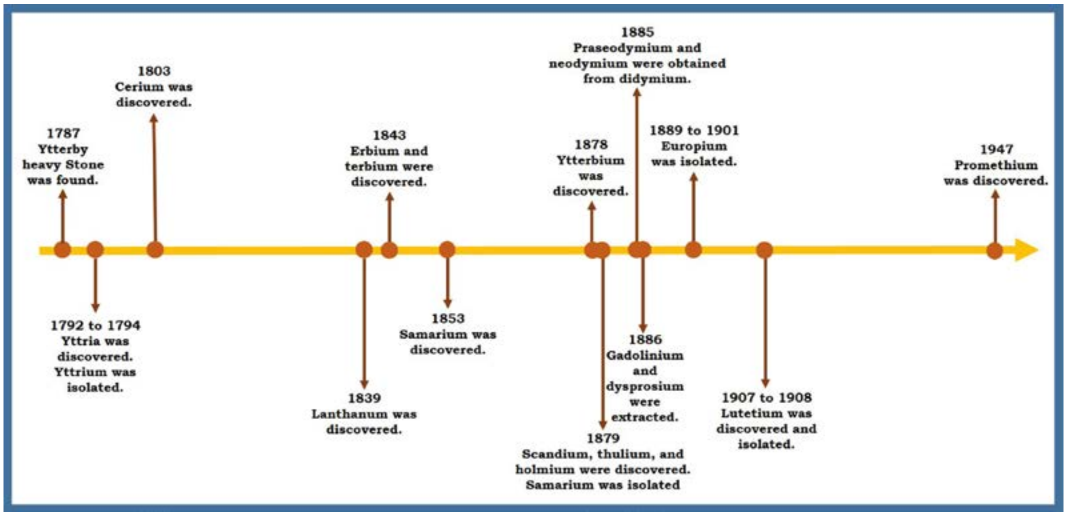

Figure 2. Timeline of the discovery of rare earth elements.

In two varieties of lisianthus (*Eustoma grandiflorum*), Mariachi Blue and Echo Lavanda, the application of lanthanum in the nutrient solution at concentrations of 0, 10, 20, and 30 μM delays the onset of flowering, flower opening, full flowering, and senescence, and only the 10 μM dose increases flowerpot life in both varieties [25]. With the addition of 40 μM La to the preservative solution of 15 varieties of tulip (*Tulipa gesneriana*), higher concentrations of total soluble sugars in petals and total soluble proteins in leaves, increases in fresh weight of stems, and longer vase life were found; it also improves the concentrations of biomolecules [26]. In switchgrass (*Panicum virgatum* L.) seedlings, the addition of La (10 μM), abscisic acid [ABA (10 μM)], and the combination of both (La+ABA) improved metabolic activity and root elongation, and increased leaf chlorophyll content [20].

Cerium (Ce)

Cerium (Ce) is the most abundant REE in the Earth's crust (0.0043%); its concentration in soils ranges from 20 to 68 mg kg^{-1}. It is used in the ceramics and glass industries [19,23,27,28]. Its main source is monazite. The countries with the largest number of Ce mines are China, USA, Brazil, India, Sri Lanka, and Australia [23].

Beneficial effects of Ce have been demonstrated in some crops. For example, in common bean (*Phaseolus vulgaris*), treatments with 50, 100, and 200 mg Ce kg^{-1} increase stomatal conductance; doses with 200 mg Ce kg^{-1} alter the homeostasis of the antioxidant defense system and lipid peroxidation in roots and increase the mineral content of the pods [29]. In tomato (*Solanum lycopersicum*) cv. Bonny Best exposed to doses of 0, 50, and 250 mg Ce kg^{-1}, it increased fruit dry weight and lycopene content, and suppressed wilting caused by *Fusarium* [30]. In maize (*Zea mays*) seedlings treated with Ce (0, 200, 400, 600, 1000, and 2000 mg kg^{-1}) and Cd (0 and 0.5 mg kg^{-1}), it was observed that Ce affects Cd absorption and translocation. In addition, it was found that Ce partially controls the content of B, Mn, Ni, Cu, Zn, Mo, and Fe in roots and shoots, and affects root anatomy [31].

Praseodymium (Pr)

Praseodymium (Pr) is a light metal with high corrosion resistance when exposed to the environment. It ranks 42^{nd} in abundance in the Earth's crust (0.00095%) and has an average concentration of 9.5 mg kg^{-1} in soil. It is obtained from minerals such as monazite and bastnasite [23,24,32-33].

There are few studies on the effects of Pr on plants. In the medicinal plant danshen (*Salvia miltiorrhiza*), doses with 100 μM Pr induced greater root length and improved chlorophyll content, antioxidant enzyme activity, and production of secondary metabolites [34]. In maize (*Zea mays*) cv. Xindan 29, under Cd stress (0, 50, 100, 100, 150, and 200 mg L^{-1}) and treated with 10, 30, and 90 μM Pr per day, Pr improved maize tolerance to Cd, through the regulation of enzymatic activities in the regeneration and biosynthesis pathways of ascorbate and glutathione [35]. In trumpet lily (*Lilium longiflorum*) treated with praseodymium nitrate [$Pr(NO_3)_3$], the activity of peroxidase (POD), catalase (CAT), and four enzymes in the ascorbate-glutathione cycle increased, in addition to soluble sugar and proline content, resulting in better quality and longer vase life [36].

Neodymium (Nd)

With 0.0038% of the total Earth's crust (38 mg kg^{-1}), Nd ranks 27^{th} among the elements contained in it [37]. Although Nd is not naturally free in the environment, electronic, automotive, and medical waste containing Nd can contaminate soil and water, affecting the environment and making this REE bioavailable.

This element has different effects on plant morphology, physiology, and biochemistry, and these depend on the form of application, phenological stage, and edaphoclimatic conditions in which the plant develops [38]. For example, in sicklepod (*Cassia obtusifolia*) seeds treated with 3 mg Nd L^{-1}, improvements in germination and seedling growth were observed [39]. In seedlings of lettuce (*Lactuca sativa*) cv. Rhodenas, Nd (40 and 60 μM) increased plant height, as well as leaf and root length [40]. Likewise, in lettuce cv. Ruby Sky, doses of 2,855 and 5,770 mg Nd L^{-1} increased plant height, leaf area, root volume and whole plant fresh biomass weight, in addition to significantly increasing leaf concentration of N, P, and K [41]. In sugarcane (*Saccharum* spp.) seedlings, the addition of 100 and 150 μM Nd to the nutrient solution improved growth and nutrient concentration [42].

Promethium (Pm)

Promethium (Pm) is an element without stable isotopes, which can be found in the Earth's crust in small quantities (4.5×10-20 mg Pm kg^{-1}). Marketable Pm comes from nuclear reactors and is obtained from fission products of uranium, thorium, and plutonium [23,24,43]. It is required in the phosphor industry, in Liquid Crystal Display (LCD) lamps, in the construction of laser beams, in long-life miniature atomic batteries, and in aperture calipers, the last used especially in the aerospace area [23,43].

Its effect on plants has not been extensively studied due to its low occurrence or bioavailability in soils. In podzols and sierozem soils with a concentration of 0.37 MBq

147Pm kg^{-1}, the accumulation of this element in higher plant tissues behaved as follows: barley grain < wheat grain < pea seeds < potato tubers < radish roots < sugar beet roots, indicating that Pm tends to lodge in lower parts [44].

Samarium (Sm)

Samarium (Sm) is a slightly soft, silver-colored metal that is separated and purified by ion exchange. Its abundance in the Earth's crust is 7.9 mg kg^{-1} and is found mainly in monacite. The main mines of extraction are in the United States, Brazil, India, Sri Lanka, and Australia. Worldwide, 700 Mg of Sm are produced per year [21, 23, 45]. It is used in the technology industry and may be present in some commercial phosphate fertilizers [45]. The bioavailability of Sm depends on the source and chemical characteristics of the soil (pH, texture, and organic matter content). The over-accumulation of Sm causes toxicity in both plants and mammals [45-47]. In wheat (*Triticum aestivum*), the 2 500 μM Sm dose induced a hormetic effect on growth and dry weight increases [48].

With the data presented above, there is evidence that six of the 17 REE exhibit beneficial effects in some crops, in diverse agronomic, physiological, and biochemical attributes that can contribute to increasing quality and yield indicators, as well as improving responses to biotic and abiotic stress factors. The effect of more REE remains to be explored in order to decipher their hormetic curves and determine the application ranges for their best use. The potential offered by these elements is encouraging in the face of the global challenges imposed by increased demand for food for a growing population, in restrictive environments aggravated by global climate change.

CONCLUSIONS AND PROSPECTS

As a consequence of the rise in REE use in various industries, their accumulation levels in the environment have increased. This means that various organisms in ecosystems are exposed to these elements, which makes it necessary to study them and determine their hormetic curves. In this review it has become clear that the REE La, Ce, Pm, Pr, Nd, and Sm can have some beneficial effect on plants, which makes them excellent candidates for the development of technologies that allow their use as inorganic biostimulants. Future research on sources, formulations, doses, frequency of application, and genotypes that show better responses will help to expand their use in improving yield indicators, quality and responses to limiting environmental factors.

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