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oak

species for their use as biofuels

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

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The level of nutrition received during the post-weaning development period influences the reproductive behavior of Pelibuey rams in adulthood

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ABSTRACT

Introduction: Post-weaning nutrition plays a pivotal role in the reproductive development of sheep; however, its long-term effects during adulthood remain insufficiently studied.

Objective: To assess the long-term impact of post-weaning diets that induced varying average daily gains (ADG) on the reproductive performance of adult Pelibuey rams.

Design/Methodology/Approach: Twenty-eight Pelibuey rams were evaluated after being subjected to nutritional regimens aimed at achieving high (240 g/d), medium (209 g/d), or low (144 g/d) ADG during the post-weaning development phase (up to 40 weeks of age). Thereafter, all animals were maintained on a standardized diet from 18 to 30 months of age. Evaluations included body measurements, testicular and seminal characteristics, sexual behavior, and seminal plasma concentrations of zinc, α -glucosidase, and fructose.

Study Limitations/Implications: The lack of hormonal profiling constrains the physiological interpretation of the mechanisms involved and limits extrapolation to field scenarios.

Results: Rams with low ADG were able to recover body and testicular dimensions and exhibited seminal quality comparable to their counterparts; nonetheless, they demonstrated reduced mating efficiency, requiring a greater number of attempts per ejaculation. Conversely, rams with high ADG exhibited lower progressive motility, potentially associated with testicular oxidative stress.

Findings/Conclusions: Post-weaning undernutrition may exert lasting effects on sexual behavior in adulthood, despite the eventual normalization of body and seminal parameters. Promoting post-weaning ADG within the range of 200–240 g/d is recommended to optimize reproductive performance in hair sheep production systems.

Keywords: Sexual behavior, seminal plasma, reproductive performance, Pelibuey sheep, post-weaning nutrition



INTRODUCTION

Sheep production in Mexico is carried out under intensive, semi-intensive, and extensive systems, with marked differences in feed availability and quality. In intensive systems, specialized breeds are kept in confinement and fed high-energy diets designed to accelerate weight gain and growth. In semi-intensive systems, grazing is combined with limited supplementation, while in extensive systems (the most common), grazing with poor supplementation often leads to nutritional deficiencies and undernutrition (Herrera-Haro *et al.*, 2019; Secretaría de Agricultura y Desarrollo Rural, 2024). In this context, the reproductive efficiency of rams becomes a critical factor, as in extensive systems a single male can service numerous females, thus determining the productivity of the flock and, consequently, the economic profitability of producers (Macedo & Arredondo, 2008; Notter, 2012).

The use of efficient rams is essential to ensure a high number of effective matings, optimal semen quality, and sustained fertility. In hair breeds such as Pelibuey, which is widely distributed in Mexico, males represent a strategic genetic resource due to their hardiness, heat adaptability, and productive relevance in meat systems (Aguilar-Martínez *et al.*, 2017; Chay-Canul *et al.*, 2019; García-Chavez *et al.*, 2020; Castañeda-Hidalgo *et al.*, 2021). Understanding the factors that affect their reproductive performance is therefore a priority to optimize productivity in these systems. Among the most influential factors is nutrition during the post-weaning period, a stage during which growth rate, gonadal maturation, and the onset of puberty are determined. The availability and quality of nutrients modulate the secretion of metabolic and reproductive hormones that directly impact gonadal function (Castellano *et al.*, 2011; Kenny *et al.*, 2018). Recent studies confirm that both early undernutrition and overfeeding can have persistent effects on testicular morphology, semen quality, and sexual behavior in ruminants (Nazari-Zonouz *et al.*, 2022; Masudul *et al.*, 2024; Imik *et al.*, 2024; Keogh *et al.*, 2025; Giaretta *et al.*, 2025). The physiological mechanism by which early undernutrition impacts adult sexual behavior has been linked to neuroendocrine programming of the hypothalamic-pituitary-gonadal axis, reducing the secretion of GnRH and LH, which may alter testosterone production (Cardoso *et al.*, 2015; 2020) and the activation of hypothalamic circuits involved in the expression of libido and sexual behavior (Duittoz & Kenny, 2023). These early modifications may persist into maturity, conditioning reproductive behavior even after body recovery. In a previous study with Pelibuey lambs, it was observed that a high post-weaning average daily gain (ADG; 240 g/d) advanced puberty and improved testicular parameters compared to a low ADG (144 g/d) (Vargas *et al.*, 2018). However, it is still unknown whether these post-weaning nutritional differences exert a residual long-term effect, particularly on semen quality and sexual behavior in adult rams, two variables of high practical relevance for extensive systems. The aim of this study was to evaluate the long-term effects of three post-weaning nutritional plans, which generated high, medium, and low ADG, on body and testicular characteristics, semen quality, and sexual behavior in adult Pelibuey rams. The hypothesis proposed that low post-weaning ADG compromises reproductive performance in adulthood, while medium or high ADG allows for adequate reproductive development.

MATERIALS AND METHODS

General conditions

The study was conducted with the approval of the Institutional Subcommittee for the Care and Use of Experimental Animals (SICUAE) of the National Autonomous University of Mexico (SICUAE.DC-2020/2-2, 06/22/2020).

The study took place at the facilities of the National Research Center for Physiology and Animal Breeding, INIFAP (Colón, Querétaro, Mexico, 20° 43' N, 100° 15' W). A total of 28 Pelibuey rams (initial age: 18 months; initial weight: 78.4±0.4 kg) were used, originating from a previous study (Vargas *et al.*, 2018), in which they had been randomly assigned to three ADG treatments: high (240 g/d), medium (209 g/d), and low (144 g/d). Briefly, from weaning (60 days of age) until 40 weeks of age, they were offered diets formulated to achieve the target ADG, adjusting the levels of metabolizable energy and crude protein (Table 1).

Table 1. Experimental diets offered to male Pelibuey lambs from weaning to 40 weeks of age. Initial requirements¹ and diet characteristics.

Group	Expected ADG (g)	DM (kg)	Mcal/kg	CP ² (%)	ADG obtained (g)
High	>250	0.80	2.89	15.5	240
Medium	150 a 200	0.72	2.83	14.3	209
Low	<100	0.64	2.75	12.8	144

¹ Estimated requirements for male lambs weighing 15 to 20 kg of live weight (LW).

² Containing 40% bypass protein.

ADG=Average daily gain, DM=Dry matter, Mcal=Megacalories, CP=Crude protein.

The present study focused on the evaluation of animals from 18 to 30 months of age. This age range was selected because it corresponds to the reproductive adult stage in hair sheep, when rams have already reached stability in sperm production and full sexual capacity (Notter, 2012). During this stage, all rams were offered a maintenance diet (11.1% CP and 2.54 Mcal ME/kg DM), consisting of a concentrate (73% sorghum grain, 7.8% DDGS, 7.9% oat hay, 7.8% molasses, 2.2% calcium carbonate, and 1.3% microminerals) and corn silage, supplied at 700 g and 4 kg/animal/day, respectively, with ad libitum access to water. The content of microminerals (especially zinc, copper, and selenium) was not determined, although it was assumed to be adequate based on the composition tables of the ingredients used. It is worth noting that the number of animals (n=28) was defined by the availability of the experimental herd, and the groups continued to be designated as High (n=9), Medium (n=9), and Low (n=10), referring to the treatment received during the post-weaning stage. Prior to the start of the experiment, a basic clinical and reproductive examination was performed to rule out reproductive or infectious diseases that could interfere with the results.

Body and testicular morphometry

Every two months, live weight and body measurements were recorded, including height at the rump and height at the withers, using a somatometric ruler or measuring

tape (Teletape, Ketchum, Ontario, Canada), as well as body length and thoracic perimeter, measured with a flexible tape. Scrotal circumference (SC) was assessed using a flexible measuring tape, and testicular diameter and length were measured with a Vernier caliper. Additionally, the body mass index (BMI) was calculated (Tanaka *et al.*, 2002; 2012).

Evaluation of semen samples

Semen evaluation was conducted at 22, 25, 28, and 30 months of age, using samples collected via artificial vagina and analyzed by microscopy in their fresh state. Prior to each sampling, one semen sample per day was collected over three consecutive days to deplete and homogenize the sperm reserves in the extratesticular duct system. The following parameters were assessed: ejaculate volume (ml), sperm concentration (spermatozoa/ml), mass motility (MM; scale 0 to 4), and the percentages of progressive motility (PM), live spermatozoa, and morphologically normal spermatozoa, following WHO guidelines adapted for sheep (Ávalos *et al.*, 2018).

Determination of seminal plasma components

At 22 months of age, after two consecutive days of semen collection using an artificial vagina, a semen sample was obtained, centrifuged at 1,000 g for 10 minutes at 5 °C. The sperm-free seminal plasma was decanted and stored at –20 °C until analysis.

The methods used to measure zinc, fructose, and neutral α -glucosidase in seminal plasma followed the procedures documented by the World Health Organization (Lu *et al.*, 2010). Zinc measurement was based on the formation of a complex between zinc and 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol (5-Br-PAPS), which absorbs light at a wavelength of 560 nm; a commercial zinc kit (catalog number MAK032, Sigma-Aldrich) was used for this determination. Fructose was measured by heating the sample to 50 °C at a low pH (2.8), allowing the formation of a complex with indole that absorbs light at 470 nm. Neutral α -glucosidase activity was determined by its ability to convert synthetic glucopyranoside into p-nitrophenol, which, upon reaction with sodium carbonate, absorbs light at a wavelength of 405 nm (Lu *et al.*, 2010). The content of these components was expressed as concentration per milliliter and total per ejaculate.

Sexual behavior and mating capacity

Sexual behavior was evaluated every three months by presenting estrus-induced females, treated with estradiol benzoate (0.02 mg/kg; Forestro) three days prior to testing. The ethogram was conducted during a 5-minute observation period and included courtship variables: number of foreleg elevations, anogenital sniffing events, and Flehmen responses, as well as copulatory variables: latency to first mounting attempt, frequency of mounts without ejaculation, latency to first ejaculation, and refractory period (time from first ejaculation to the next mounting interaction). These parameters were used to calculate indicators of copulatory efficiency: mating capacity (frequency of mounts with ejaculation) and mating efficiency (total number of mounting interactions [attempts, mounts with and without ejaculation] divided by the number of ejaculations). This description is provided

to facilitate interpretation, even though the ethogram has been previously validated (Price *et al.*, 1991; Perkins & Roselli, 2007). All observations were performed by evaluators who were blinded to the treatment of the animals.

Statistical analysis

The response variables measured at different ages were analyzed using a completely randomized design (CRD), applying the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA) for repeated measures over time, with the “repeated” statement, the option sub=animal (group), and an autoregressive (1) covariance structure within the animal. Seminal plasma variables were analyzed as a CRD using the GLM procedure of SAS, which included the fixed effect of treatment. The assumptions of normality and homoscedasticity were verified using the Shapiro-Wilk and Levene tests, respectively.

To meet the assumptions of ANOVA, semen quality variables (volume and sperm per ejaculate) and sexual behavior variables measured in time units (seconds) were transformed using $\log N(y+1)$ and/or $\log N(y)$. For mass motility and the number of courtship and copulatory behaviors, the transformation $\sqrt{y+0.5}$ was applied. Percentage variables, expressed as proportions (p), were transformed using the arcsine \sqrt{p} function. After analysis, data were back-transformed to real values for tables and figures. Mean comparisons were performed using Tukey’s test, with statistical differences considered at $P < 0.05$ and trends at P-values between 0.05 and 0.1.

RESULTS AND DISCUSSION

Body measurements

Daily weight gain and body weight

Data in Table 2 and Figure 1 show a significant group \times age interaction for ADG ($P=0.01$) and body weight ($P=0.001$). At 18 months, the High group had a greater weight (82.5 kg) than the Medium (76.6 kg) and Low (74.2 kg) groups. Between 20 and 24 months, no significant differences were detected among groups, suggesting a stabilization phase. From 26 months onward, the High group again showed an advantage over the Low group ($P=0.03$). Thus, the group effect was significant at early and late stages, but not during the intermediate period; the age effect showed a progressive recovery pattern across all groups. At 30 months, body weights were: High 89.0 kg, Medium 82.7 kg, and Low 84.2 kg. Between 18 and 22 months of age, ADG was higher in the Low group, but from 24 months onward, it was similar among all three groups.

Table 2. Evolution of average daily gain (g) from 18 to 30 months of age in adult Pelibuey rams subjected to different post-weaning nutritional levels.

Nutritional level	Age (months)						
	18	20	22	24	26	28	30
High	12 \pm 2 ^a	7 \pm 2 ^a	3 \pm 2 ^a	3 \pm 2 ^a	54 \pm 2 ^a	39 \pm 2 ^a	53 \pm 2 ^a
Medium	6 \pm 2 ^a	7 \pm 2 ^a	3 \pm 2 ^a	3 \pm 2 ^a	26 \pm 2 ^a	55 \pm 2 ^a	70 \pm 2 ^a
Low	68 \pm 2 ^b	48 \pm 2 ^b	10 \pm 2 ^b	4 \pm 2 ^a	36 \pm 2 ^a	30 \pm 2 ^a	43 \pm 2 ^a

^{a, b} values within the same column without a common letter differ significantly ($P < 0.05$).

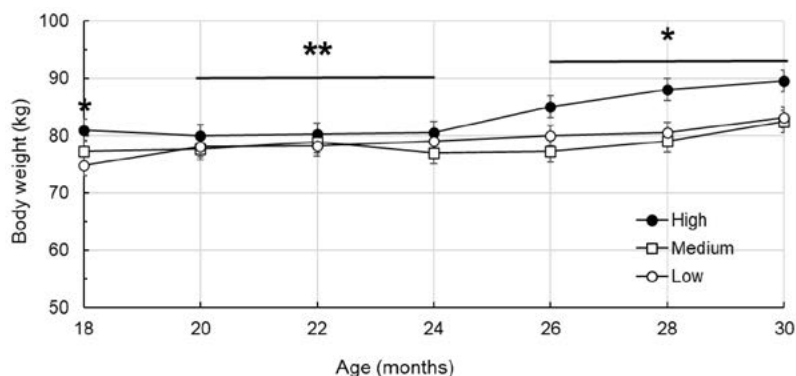


Figure 1. Evolution of body weight in adult Pelibuey rams subjected to different post-weaning nutritional levels. *Months in which Low and Medium are not different from each other ($P>0.05$) but differ from High ($P<0.05$). **High, Medium and Low do not differ from each other ($P>0.05$).

Body morphometry and body mass index

The group \times age interaction tended to be significant for body length ($P=0.07$). Initially, the High and Medium groups showed greater values than the Low group; however, this difference disappeared by 26 months of age. Table 3 presents the results of body measurements. The group effect was significant for rump height ($P=0.003$), thoracic perimeter ($P=0.04$), height at the withers ($P=0.001$), and body length ($P=0.025$), while BMI showed no differences among groups. The age effect was not significant for these variables, indicating stability during adulthood, except for the compensatory adjustment observed in the Low group.

Testicular measurements

The group \times age interaction was not significant for any of the testicular variables, while the group effect was significant for testicular diameter, which was greater in the Low group compared to the High group ($P=0.03$), and showed a trend for scrotal circumference ($P=0.07$; Table 3). However, this finding may be attributed to a compensatory effect in

Table 3. Average body and testicular measurements of Pelibuey rams from 18 to 30 months of age, which experienced three levels of average daily gain during the post-weaning stage (High, Medium, and Low; means \pm SE, across the age).

	Nutritional level			P
	High	Medium	Low	
Rump height (cm)	79.2 \pm 0.6 ^a	76.1 \pm 0.6 ^b	77.0 \pm 0.6 ^b	0.012
Thoracic perimeter (cm)	100.9 \pm 0.9 ^a	97.4 \pm 0.9 ^b	99.6 \pm 0.9 ^{ab}	0.032
Height at the withers (cm)	79.5 \pm 0.6 ^a	76.5 \pm 0.6 ^b	78.2 \pm 0.6 ^a	0.027
Body length (cm)	80.9 \pm 0.7 ^a	79.4 \pm 0.7 ^{ab}	78.1 \pm 0.7 ^b	0.010
Body mass index	13.0 \pm 0.2	12.7 \pm 0.2	12.9 \pm 0.2	0.71
Scrotal circumference (cm)	33.5 \pm 0.5 ^b	34.1 \pm 0.5 ^{ab}	35.1 \pm 0.5 ^a	0.042
Testicular length (cm)	12.2 \pm 0.2	12.0 \pm 0.2	12.1 \pm 0.2	0.98
Testicular diameter (cm)	6.6 \pm 0.1 ^b	6.8 \pm 0.1 ^{ab}	7.0 \pm 0.1 ^a	0.045

^{a,b} Different letters in the same row indicate significant differences ($P<0.05$).

gonadal growth following nutritional recovery, although it does not necessarily reflect improvements in semen quality or sexual behavior.

The age effect was significant for both testicular length ($P < 0.001$) and diameter ($P = 0.02$), which increased with sexual maturation.

Semen quality

The group \times age interaction tended to be significant for sperm concentration ($P = 0.06$; Table 4). Regarding the group effect (Figure 2), progressive motility was lower in the High group (57.2%) compared to the Medium (67.5%) and Low (68.5%) groups ($P = 0.05$). Progressive motility increased with age (67.0% at 18 months *vs.* 73.7% at 30 months; $P < 0.001$). Figure 2 shows that the percentage of normal spermatozoa was slightly but significantly higher in the Low group (99.3%) compared to the High (98.7%) and Medium (98.9%) groups ($P = 0.03$). Thus, the group effect revealed relevant differences in motility and morphology, while the age effect indicated a progressive improvement in motility.

Table 4. Sperm concentration (millions/ml; means \pm SE) of Pelibuey rams that experienced three levels of average daily gain during the post-weaning stage (High, Medium and Low; means \pm SE).

Age (months)	Nutritional level		
	High	Medium	Low
22	^x 3,080 \pm 320 ^b	^x 2,580 \pm 320 ^b	^y 3,940 \pm 330 ^a
25	^x 2,780 \pm 320	^{xy} 3,070 \pm 320	^x 3,030 \pm 330
28	^{xy} 3,370 \pm 320 ^{ab}	^x 2,850 \pm 320 ^b	^y 3,730 \pm 300 ^a
30	^y 3,380 \pm 320	^y 3,560 \pm 320	^y 3,440 \pm 300

Group \times age interaction ($P = 0.063$).

^{a,b} Values within a row without a common letter indicate significant differences ($P < 0.06$).

^{x,y} Values within a column without a common letter indicate differences ($P < 0.06$).

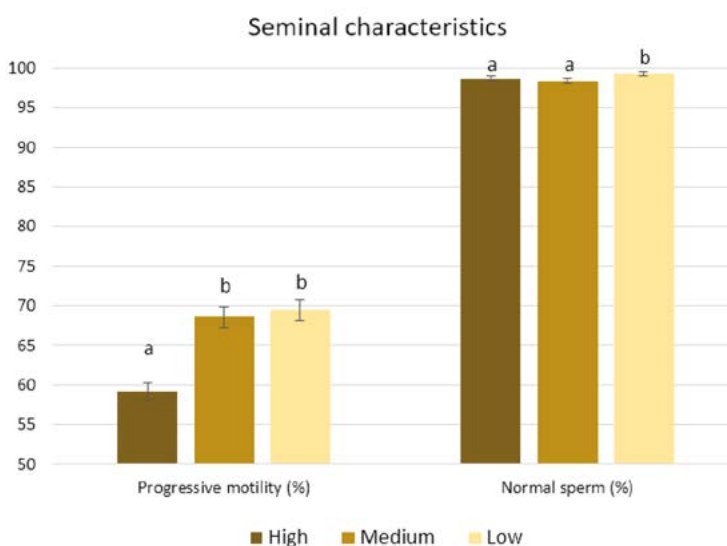


Figure 2. Progressive motility and normal spermatozoa in Pelibuey rams that experienced three levels of average daily gain during the post-weaning stage (High, Medium and Low; mean \pm SE).

Concentration of seminal plasma components

No group × age interaction was detected. There were also no significant differences among groups in seminal plasma concentrations of zinc, fructose, or α-glucosidase, nor in total semen volume (Table 5). The relatively low zinc values may be related to the fact that the maintenance diet was not specifically formulated to optimally meet zinc requirements, which limits interpretation.

The age effect was not analyzed since measurements were performed at a single time point (22 months).

Sexual behavior and service capacity

Table 6 presents the results of sexual behavior. No group × age interaction was observed. Regarding the group effect, the High group recorded fewer mounting attempts than the

Table 5. Concentrations of zinc, fructose, and α-glucosidase in seminal plasma of Pelibuey rams that experienced three levels of average daily gain during the post-weaning stage (High, Medium, and Low; means ± SE).

	Nutritional level			P
	High	Medium	Low	
Seminal plasma concentration				
Zinc (mg/ml)	0.49±0.02	0.54±0.02	0.48±0.02	0.12
Fructose (mg/ml)	6.2±2.3	7.2±2.1	5.4±2.8	0.26
Neutral α-glucosidase (μmol/ml)	90.3±30.8	79.6±30.1	72.2±30.8	0.34
Total content in ejaculate				
Zinc (mg)	0.40±0.05	0.37±0.05	0.33±0.05	0.64
Fructose (mg)	5.6±2.1	5.7±2.0	4.3±2.6	0.46
Neutral α-glucosidase (μmol)	71.2±11.8	61±50.1	50.6±33.1	0.46

Table 6. Sexual ethogram variables of Pelibuey rams that experienced three levels of average daily gain during the post-weaning stage (High, Medium, and Low; means ± SE).

	Nutritional level		
	High	Medium	Low
Courtship behaviors			
Flehmen (n)	0.8±0.2	0.7±0.2	1.2±0.3
Forelimb elevations (n)	3.0±0.8	3.6±0.8	3.9±0.9
Anogenital sniffing (n)	7.2±0.8	8.1±0.8	8.1±0.8
Copulatory behaviors			
Mount attempts (n)	2.1±1.0 ^a	3.5±1.0 ^{ab}	5.2±1.1 ^b
Mounts without ejaculation (n)	2.1±0.3 ^a	1.7±0.4 ^a	3.2±0.4 ^b
Service capacity (Mounts with ejaculation, n)	2.5±0.3	2.3±0.3	2.6±0.3
Latency to first copulatory interaction (s)	32.8±7.3	30.0±7.2	17.1±7.7
Latency to first ejaculation (s)	48.5±11.2	54.8±11.1	44.7±11.8
Refractory period (s)	86.7±11.6	104.1±11.5	71.6±12.1
Mount/ejaculation ratio (n)	3.3±0.8 ^a	4.3±0.8 ^{ab}	6.4±0.8 ^b

^{a,b} Values within a row without a common letter indicate differences (P<0.05). n: number of events, s: seconds.

Low group ($P=0.03$), while the Low group showed more mounts without ejaculation than the High and Medium groups ($P=0.01$). The High group had fewer interactions per ejaculation compared to the Low group ($P=0.05$). The age effect was not significant for these variables, indicating stable behavior during adulthood.

Our hypothesis was that male sheep receiving three post-weaning diets that produced high, medium, and low ADG during growth and development would exhibit compromised long-term reproductive performance in adulthood. The results of this study showed that differences in body weight and morphometry associated with post-weaning nutritional management tended to diminish in adulthood, reflecting a partial recovery capacity in animals that had lower ADG (Low group). However, this recovery was not complete across all reproductive variables, as sexual behavior and certain seminal parameters still bore traces of early nutritional management.

The higher body weight observed in the group with greater post-weaning ADG confirms that a high nutritional plane promotes somatic growth, as previously reported by López *et al.* (2010) and Lourençon *et al.* (2023) in hair sheep breeds. Nevertheless, the fact that the Low group reached similar body values to the High group by 26 months of age indicates the presence of compensatory growth mechanisms, which are highly correlated with body weight and ADG (De Jesús *et al.*, 2013; da Silva *et al.*, 2015; da Silva-Souza *et al.*, 2019). In this regard, it is noteworthy that despite its disadvantage during development, the Low group was able to recover body dimensions within a normal range once nutritional conditions improved. This plasticity suggests that animals can partially restore body size under improved conditions, although such recovery does not necessarily translate into equivalent improvements in reproductive efficiency, as reflected by subsequent limitations in sexual behavior.

In testicular morphometry, the greater diameter observed in the Low group should be interpreted cautiously, as lower ADG during sexual development does not necessarily indicate superior gonadal development. Recent studies have shown that testicular response to nutrition depends on the interaction between dietary plane and body condition (Ghorbankhani & Souri, 2014; Rosales-Nieto *et al.*, 2021). Moreover, scrotal circumference has been shown to correlate highly with body weight ($r=0.83$; Espitia-Pacheco *et al.*, 2018; Chacon *et al.*, 2019), and findings in Pelibuey sheep by Campos-Frías *et al.* (2023) report a strong positive correlation ($r=0.89$). Therefore, the variations in ADG and weight observed in the Low group during the evaluated period likely contributed to the restoration and enlargement of their testicular size. Although this may reflect compensatory growth following nutritional recovery, the increase did not represent a clear reproductive benefit, as it was not accompanied by improvements in sexual behavior or seminal characteristics.

In semen quality, the High group exhibited lower progressive motility, classified as “regular” (40-59% of spermatozoa with progressive motility; Ávalos *et al.*, 2018), whereas sperm from the Low and Medium groups showed “good” values (60-79%). The reduced progressive motility in the High group could be associated with potential testicular oxidative stress induced by a more energy-rich diet during sexual development, consistent with findings by Johnson *et al.* (2020) in bulls subjected to enhanced prepubertal nutrition,

as well as studies reporting damage to the sperm membrane (Xu, 2025) and reduced progressive movement (Pintus, 2021; Wang, 2025). Conversely, the higher proportion of morphologically normal sperm in the Low group may be related to a more balanced and efficient metabolism, despite their initial growth disadvantage. Additionally, motility has been shown to increase gradually and linearly with SC size (Avellaneda *et al.*, 2006; Pabón-Quevedo & Pulido-Medellín, 2021), and in both cattle and sheep, SC has been positively correlated ($r=0.51$, $P<0.04$) with the percentage of progressively motile sperm (Pérez-Osorio *et al.*, 2014). Thus, the better motility observed in the Low and Medium groups may be due to their larger testicular size compared to the High group.

In seminal plasma, zinc concentrations in all three groups were low relative to values reported in other breeds (0.56 to 1.79 mg/ml; Juyena & Stelletta, 2012), which may have affected sperm motility, given the positive correlation between seminal zinc and sperm motility (Mahsud *et al.*, 2013). These zinc levels likely reflect deficiencies in the maintenance diet, as it was not formulated to optimize micromineral intake, limiting the interpretation of its impact on fertility. As for fructose concentrations in seminal plasma, the High and Medium groups showed higher values than those reported by Juyena & Stelletta (2012). These elevated levels may have contributed to reduced motility, as fructose concentrations have been negatively correlated with motility (Lewis-Jones *et al.*, 1996). Additionally, fructose synthesis is stimulated by testosterone (Juyena & Stelletta, 2012), and it has been suggested that seminal fructose concentration may depend on the spermatozoa's ability to utilize it (Giaretta *et al.*, 2025). Therefore, as reproductive hormones such as testosterone or LH were not measured, the results do not allow for a direct link between this metabolite and the endocrine regulation of spermatogenesis and sexual behavior. Future studies should include hormonal profiling to strengthen conclusions.

Sexual behavior was the variable most sensitive to post-weaning nutritional plane. Differences among groups suggest that post-weaning ADG can influence libido and mating efficiency, as reported by Kumar *et al.* (2015), Joshi (2022), and Zaobi *et al.* (2025). Despite recovering body and testicular size, the Low group exhibited lower copulatory efficiency, requiring more attempts to achieve ejaculation compared to the High group, which required fewer interactions. There are very few reports on the effect of nutrition on sexual behavior in sheep; existing studies indicate that undernutrition or feed restriction compromises libido (Parker & Thwaites, 1972; Zaobi *et al.*, 2025) and mating capacity (Kumar *et al.*, 2015). Although sexual behavior is complex and influenced by various factors (Petherick, 2005), the effect of nutrition during postnatal stages has been studied in animal models such as rodents (Laus *et al.*, 2011), showing that inadequate diets and consequently low ADG during early life compromise proper brain development and function, leading to behavioral alterations (Dorantes-Barria, 2021). This may explain how post-weaning nutritional plane affects the neuroendocrine programming of the hypothalamic–pituitary–gonadal axis, which regulates libido expression and mating capacity (Martin *et al.*, 2010; Gurule *et al.*, 2024). These effects are long-lasting and severe, as previously reported (Neu *et al.*, 2007; Laus *et al.*, 2011; Dorantes-Barria, 2021). Thus, even though nutritional recovery in the Low group allowed for compensation in body

dimensions, sexual function remained impaired, which has a more substantial practical impact on fertility than morphological differences.

Overall, the results suggest that in extensive systems, where supplementation is often limited, implementing post-weaning feeding strategies that ensure gains of 200 to 240 g/d in lambs may enhance reproductive performance in males without incurring the risks associated with overfeeding.

The present study was conducted under controlled feeding and management conditions, which allowed for the isolation of the effect of the post-weaning nutritional plane. However, these conditions may differ from the practices of extensive production systems, which could influence the observed responses. Additionally, the sample size and duration of follow-up limit the extrapolation of results to other sheep breeds or different agroclimatic environments. Future research is recommended to include evaluations under field conditions and across various production systems, as well as to incorporate a greater racial diversity and longer follow-up periods. This would allow for validation and broader applicability of the findings, taking into account the genetic, environmental, and management variations present in sheep production at the regional and national levels. Overall, the results highlight that post-weaning nutrition has differential and persistent effects on the reproductive biology of Pelibuey sheep. While body variables tend to recover with age, sperm motility and copulatory efficiency are more susceptible to the effects of early nutritional restriction or excess. Thus, a moderate nutritional plane that ensures ADG between 200 and 240 g/d emerges as the most effective strategy for optimizing semen quality and sexual behavior, contributing to improved reproductive efficiency in extensive systems, where ram fertility determines production profitability.

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Participation of women and men in the post-harvest of native corn in rural communities

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ABSTRACT

Objective: To analyze the post-harvest practices carried out by women and men for the conservation of native maize in Tlaxcala, and to identify the limitations they face throughout the process.

Design/Methodology/Approach: The study employed a mixed-methods approach, combining qualitative techniques (participant observation and workshops) with quantitative data collection (structured questionnaire). A survey was administered to 117 individuals across two communities.

Results: Post-harvest practices for conserving native maize are largely similar between women and men in the studied communities of Tlaxcala. However, women play a more prominent role in tasks such as shelling, drying, selection, and transformation for household consumption. The knowledge underlying these practices stems from traditional knowledge systems that have evolved over time. Despite this, both women and men face several limitations in executing these practices.

Findings/Conclusions: The primary barriers to effective post-harvest conservation of native maize particularly for women include limited access to training, tools or modern technologies, financial resources, labor, time, and physical capacity.

Keywords: post-harvest management, native maize, food security.

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INTRODUCTION

Maize (*Zea mays* L.) has been a staple in the diet of numerous families across Latin America, owing to its versatility as a multifunctional grain. It serves as a fundamental food for household consumption, a key input in animal feed, a source of income, and a central element of cultural identity for many communities. In rural areas, maize represents a cornerstone within a wide array of family and community strategies, where its production, processing, and conservation are of vital importance (Esponda & Galindo, 2024). Peasant agriculture accounts for 80% of the land dedicated to maize cultivation and contributes approximately 60% of total maize production. Native maize varieties predominate in these systems and play a fundamental role in ensuring household food security (Guzmán, 2021). Both women and men actively participate in maize production and post-harvest conservation practices. While agricultural activities are crucial for obtaining the grain,



post-harvest management practices are equally important, as their proper implementation helps reduce losses, ensure grain availability for self-consumption, and, in some cases, generate marketable surpluses (Hodges & Stathers, 2013; Tefera *et al.*, 2011). Post-harvest losses are linked to contamination risks stemming from abiotic (physical, chemical, and mechanical) and biotic (insects and microorganisms) factors. In this context, implementing safe practices throughout the entire chain from production to consumption is essential. In Mexico, post-harvest maize losses range from 20% to 40% in tropical regions, 10% to 20% in subtropical zones, and up to 10% in arid areas (García-Lara *et al.*, 2010). In the state of Tlaxcala, there are no specific records of post-harvest losses of native maize nor of gender-differentiated participation in such practices. Therefore, the objective of this study is to analyze the participation of women and men in the post-harvest conservation of native maize and to identify the main limitations they face in this process.

METHODOLOGY

The study was conducted in two Indigenous communities in the state of Tlaxcala, Mexico: Guadalupe Tlachco, in the municipality of Santa Cruz Tlaxcala (19° 12' 42" N, 98° 05' 24" W), and San Felipe Cuauhtenco, in the municipality of Contla de Juan Cuamatzi (19° 18' 48" N, 98° 07' 19" W). Both communities cultivate native maize varieties with high cultural, productive, and nutritional value.

A mixed-methods approach was adopted, integrating both qualitative and quantitative methods. The qualitative component included exploratory visits, participant observation, and participatory workshops involving both women and men affiliated with the peasant organization Grupo Vicente Guerrero (GVG), which promotes agroecological practices as well as the production and conservation of native maize. The workshop in Guadalupe Tlachco included eight women and six men, while the workshop in San Felipe Cuauhtenco included five women and one man. These workshops focused on identifying and analyzing native maize production practices, post-harvest management, and uses in both communities.

For the quantitative component, a structured survey was administered to women and men from the two localities. In Guadalupe Tlachco, the sampling frame was based on the National Agrarian Registry (RAN, 2023) list of ejidatarias and ejidatarios, which was later verified with the local ejido committee. In San Felipe Cuauhtenco, the list of beneficiaries of the fertilizer program served as the sampling base. The sample size was determined using the maximum variance formula, with a 95% confidence level and a 10% sampling error. Once the sample size was established for each community, a proportional stratified sampling approach was used to ensure representation of both women and men in each population stratum. In total, 117 surveys were conducted: 76 in Guadalupe Tlachco (25 women and 51 men) and 41 in San Felipe Cuauhtenco (17 women and 24 men). The instrument used was a structured questionnaire comprising the following sections: 1) personal and socioeconomic characteristics; 2) agricultural and livestock activities; 3) uses and post-harvest management of native maize; and 4) gender-differentiated participation in management practices. The data collected were processed using descriptive statistics in Microsoft Excel and the Statistical Package for the Social Sciences (SPSS), version 24.

RESULTS AND DISCUSSION

General characteristics of the surveyed population

The surveyed population in both communities belongs to a rural context. In Guadalupe Tlachco, women reported an average age of 66 years, while men had a mean age of 68 years. In contrast, San Felipe Cuauhtenco showed a relatively younger population, with an average age of 55 for women and 57 for men.

Regarding educational attainment, the overall average level of schooling was six years, indicating that most respondents had at least completed primary education. Notably, in San Felipe Cuauhtenco, 12% of women and 13% of men reported having completed university studies. As for marital status, a low proportion of married individuals was observed in both communities. In Guadalupe Tlachco, only 16% of women and 51% of men reported being married; in San Felipe Cuauhtenco, these percentages were 29% and 49%, respectively. Although many did not report being in a formal legal union, they described themselves during the interviews as widowed or divorced. In this context, it was common for interviewees to rely on support from daughters, sons, or daughters-in-law within extended family households.

With respect to family structure, an average of three children per family was identified in both communities. Only 9.4% of the total surveyed population reported having no offspring, and few cases of large families with up to eleven children were recorded. In terms of economic activities, women in Guadalupe Tlachco primarily engage in domestic work, with limited participation in agricultural tasks. Men mostly work as day laborers, though they also engage in the cultivation of native maize. In San Felipe Cuauhtenco, women also dedicate time to domestic and agricultural work, with some involved in textile-related labor. Men in this community combine farming on their own plots with jobs in construction and textile manufacturing.

The native maize cropping system

In both communities, the predominant agricultural system is polyculture, which includes various species such as common bean (*Phaseolus vulgaris*), squash (*Cucurbita* spp.), oat (*Avena sativa*), wheat (*Triticum aestivum*), and fava bean (*Vicia faba*), with maize (*Zea mays* L.) serving as the central crop in local production systems (Table 1). This approach reflects a traditional model based on diversification, characteristic of many agricultural areas

Table 1. Percentage of crops cultivated by women and men in the study communities.

Crop	Guadalupe Tlachco		San Felipe Cuauhtenco	
	Women n=25 %	Man n=51 %	Woman n=17 %	Man n=24 %
Corn	100	100	100	100
Bean	32	31	76	46
Beans	28	33	53	27
Pumpkin	24	33	35	10
Oats	8	16	0	5
Wheat	4	8	0	2

in Tlaxcala, where agrobiodiversity is actively promoted through complex agroforestry systems or milpas, commonly referred to as metepantles (Vincent-Fequiere *et al.*, 2024). Crop diversification and the use of various native maize varieties are part of family strategies aimed at mitigating both environmental and socioeconomic risks. Moreover, this practice ensures the availability of a broader range of food products for household consumption, thereby strengthening food security in peasant production units (Sánchez *et al.*, 2017).

Both communities are characterized by the continued cultivation and conservation of native maize varieties, among which white, yellow, blue, and cacahuacintle maize stand out. To a lesser extent, other varieties such as tepite, pink maize, and arrocillo are also cultivated. The land area allocated to each variety primarily depends on land availability and preferences related to their culinary and cultural uses.

The average area dedicated to white maize cultivation in both communities is 1.4 hectares, with no significant differences between women and men in terms of the land sown. While most respondents indicated that they maintain a diversity of native varieties (blue, yellow, cacahuacintle, pink, arrocillo, and tepite), the land area allocated to these varieties does not exceed one hectare. These are mainly grown for their complementary value in the family diet and for specific uses such as nixtamalization, ceremonial or medicinal purposes, among others. It is worth noting that there is a marked preference for native varieties. It is estimated that approximately 90% of the agricultural area in the state of Tlaxcala is planted with native maize, predominantly from the conical race group (Ramírez & Guevara, 2017). In terms of yields, an average of 1.7 t ha⁻¹ was reported for white maize, 1.0 t ha⁻¹ for yellow maize, and values below 1.0 t ha⁻¹ for blue and cacahuacintle maize. Varieties such as pink maize, tepite, and arrocillo show yields below 0.1 t ha⁻¹. Although these yields are relatively low, the primary purpose of their cultivation is to meet household food needs in both quality and quantity. In some cases, surpluses are used to feed backyard animals or are sold locally, generating income that helps cover family expenses such as food (meat, sugar, coffee), clothing, footwear, medicines, or agricultural inputs for the next planting cycle. The low productivity observed in recent years has been attributed to various factors, including changes in rainfall patterns, mid-season droughts, hailstorms and heavy rains, poor soil fertility, pest and disease incidence, and rising production costs (Vega, 2022).

Uses and consumption of maize

In the studied communities, maize is highly valued not only for its nutritional role but also for its cultural significance. Both women and men recognize that the use of maize varies depending on kernel color. White maize is preferred for making tortillas, while other varieties such as blue, tepite, arrocillo, and pink maize are primarily used in traditional foods like tamales, burritos, and atoles. Some informants also indicated that colored maize varieties possess medicinal properties. In the specific case of cacahuacintle maize, its use is almost exclusively reserved for preparing pozole. In the community of San Felipe Cuauhtenco, a different perception of yellow maize was observed, as it is mainly designated for animal feed due to the belief that its consumption by humans could cause intestinal problems. Regarding weekly family maize consumption, a difference was noted between the two communities. In Guadalupe Tlachco, the average consumption was 14.5 kg per

family, while in San Felipe Cuauhtenco it was 10.8 kg. This variation may be related to the larger average family size in Guadalupe Tlachco. Concerning the supply of maize produced during the 2022 agricultural cycle, most families reported having met their annual food needs. In Guadalupe Tlachco, 80% of respondents stated that production was sufficient, while 20% reported otherwise. In San Felipe Cuauhtenco, 83% reported sufficient production and 17% indicated they did not meet their yearly requirements. Among those who did not achieve self-sufficiency, the average duration of their maize reserves was approximately seven months. In such cases, families resorted to purchasing grain or tortillas to supplement their consumption.

Post-Harvest practices for native maize and the participation of women and men

Post-harvest practices carried out by women and men comprise two phases (Figure 1). Phase 1 includes activities performed in the field, while Phase 2 involves those conducted at home. These practices are the result of a body of traditional knowledge built and transmitted across generations over time.

Phase 1: Post-harvest activities in the field

In the first phase, traditional practices such as *dobla*, *amogotado*, and *engavillado* are less frequently used than in the past, when they were widespread. *Dobla* is a Mesoamerican technique consisting of bending the maize stalk below the ear once the grain has reached physiological maturity. Its purpose is to accelerate drying in the field, prevent bird damage, and protect the ear from moisture, thus reducing the risk of rot. Producers who still use this method reported that they only implement it under favorable weather conditions specifically when there is no rain and the maize can be left in the field longer. Although it is effective in preserving grain quality during storage (Centurión *et al.*, 2016), it is highly labor-intensive and involves both women and men. Sun drying maize in the field, either standing or laid on the ground, is a common traditional practice aimed at reducing moisture content and preventing fungal damage. It is widely practiced in both communities by women and men alike. *Amogotado* involves cutting maize plants at ground level, laying them out, and then forming them into a conical structure. This technique enhances ear ventilation, reduces

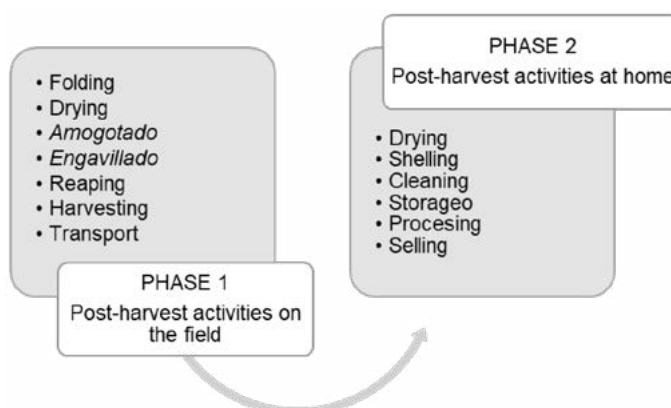


Figure 1. Post-harvest phases in the study communities carried out by women and men.

moisture, and prevents rot, while also allowing the leftover stalks (zacate) to be used as livestock fodder. This practice is performed exclusively by men. Another traditional method is *engavillado*, which consists of cutting the stalks with a sickle and stacking them in bundles with ears attached. This facilitates field drying, eases transportation, and allows the plant residues to be reused as animal feed. *Segado* (cutting of stalks for fodder) also forms part of this phase, though it is now less common and is still practiced by some men. One of the most representative activities of this phase is *pizca* or harvesting, which involves cutting the ears from the plants. This can be done manually using *pizcalones*, puncturing tools, or by hand, as well as mechanically with harvesters or threshers. In both communities, harvesting shows a strong female involvement. In Guadalupe Tlachco, all respondents reported harvesting manually. In San Felipe Cuauhtenco, 100% of the women harvest manually, while 83% of the men do so, and only 17% use mechanical methods. Women in this community explained that mechanization is not suitable for them because their plots are small, machinery is not adapted to their conditions, and it leaves too many impurities in the grain, making cleaning difficult. Finally, the transport of the harvest to the home marks the end of this phase. Maize is transported in sacks or bundles using various means: personal trucks, rented vehicles, carts, or even on foot. In Guadalupe Tlachco, some women with plots close to their homes carry the maize in wheelbarrows or on their backs, demonstrating their active role in all stages of the process.

Phase 2: Post-harvest activities at home

In this second phase, one of the key activities identified by participants is drying the ears at home. This involves exposing the ears to sunlight to ensure proper moisture loss and prevent mold or rot. Some people choose to harvest maize before it is fully dry in the field due to fear of theft, which makes it necessary to complete the drying process at home. In Guadalupe Tlachco, drying is typically done in patios or on rooftops. Women expressed a preference for patio drying, as it requires less physical effort than carrying ears to rooftops. Patio drying also allows them to monitor the maize more easily during unexpected rain or weather changes, giving them greater control over the process. A subsequent step is shelling, which involves separating the kernels from the cobs. This process may be done manually using *oloteras*, stones, or bare hands, or with mechanical shellers. In both communities, mechanical shelling is the predominant method; however, its use varies by gender and equipment availability. As shown in Figure 2, manual shelling is more common among women, who often opt for this method for economic or practical reasons particularly when dealing with small quantities or lacking machinery. In contrast, men tend to use mechanical shellers more frequently, either their own or shared, especially when working with larger volumes. These activities reflect not only a gender-based division of labor but also a system of practical knowledge passed down through generations. Decisions regarding how and where to dry or shell the maize are shaped by both material factors (such as access to technology, physical characteristics of the home or plot) and symbolic ones (social construction of gender roles and ownership of technical-traditional knowledge).

Once shelling is completed, the next post-harvest step is maize cleaning, performed by both women and men, though more frequently by women. This process aims to remove

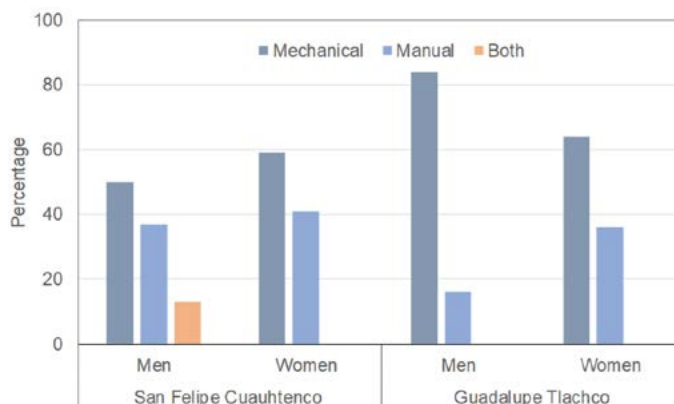


Figure 2. Shelling method in the study communities.

impurities such as stones, husk fragments, dust, and pests. Cleaning is often conducted concurrently with shelling using traditional tools like sieves, sifters, and, in some cases, winnowing.

After cleaning, the maize is stored a critical stage in post-harvest management, as it involves preserving the grain for several months while ensuring it remains free from mold, pests, and contaminants. Both women and men participate in storage, utilizing various containers and structures: woven polypropylene sacks, hermetic plastic bags, plastic buckets, hermetic silos, traditional granaries (*trojes*), barrels, as well as purpose-built rooms and storage facilities. In both communities, over 50% of respondents prefer woven sacks. However, women in San Felipe Cuauhtenco reported broader knowledge of post-harvest technologies such as hermetic bags and silos and noted the continued, albeit limited, use of traditional granaries, contrasting with practices in Guadalupe Tlachco. Two main approaches to grain preservation during storage were identified: conventional and agroecological. The conventional method includes using lime and chemical products such as aluminum phosphide. In Guadalupe Tlachco, 52% of women and 51% of men reported using aluminum phosphide, typically applying two tablets per 50 kg of stored grain. Although effective against pests at all life stages, the compound is extremely toxic, releasing phosphine gas upon contact with moisture posing serious health risks to humans and animals (Rodríguez, 2022). Agroecological methods were mentioned by fewer than 10% of respondents in Guadalupe Tlachco, while in San Felipe Cuauhtenco, 29% of women and 21% of men reported using alternative pest-control methods involving plants and fruits with insecticidal properties. These include castor (*Ricinus communis*), prickly poppy (*Argemone munita*), ragwort (*Senecio cinerarioides*), fruits such as hawthorn (*tejocote*), and vinegar. These alternatives hold strong potential for storage pest management, though further research is needed to develop appropriate formulations, dosages, and safe application protocols.

Following storage, the grain is processed for household consumption a process managed exclusively by women. They handle every step: preparing the *nixtamal* (*nixcon*), milling the maize, and producing traditional foods. Women possess in-depth knowledge of the culinary properties of each maize variety and demonstrate specialized processing skills

(Sarmiento *et al.*, 2017). Men acknowledge their limited involvement at this stage, typically only assisting with placing the nixtamal to cook. The sale of maize the final activity in the post-harvest cycle is predominantly handled by men. Grain sales generate additional family income to cover food, medicine, clothing, or agricultural inputs for the next planting season. Typically, maize is sold locally to neighbors or tortilla producers. In San Felipe Cuauhtenco, men view maize as a commercial product starting from its elote stage, while women value it primarily for culinary use. In Guadalupe Tlachco, although few women sell grain directly, they find opportunities through by-products such as tamale leaves or cobs for crafts. Maize in poor condition (discolored, rotten, or broken) is sold as animal feed, though at substantially lower prices. Notably, several women voiced concerns about lacking decision-making autonomy, as men typically control maize sales.

Limitations faced by women and men in the post-harvest process

Peasant strategies involving crop diversification offer ecological, agronomic, and nutritional benefits (Bartra *et al.*, 2014); however, they are constrained by factors such as soil degradation, shifting rainfall patterns, pest and disease pressure, and rising production costs (Guzmán, 2021; Vega, 2022). Yet, post-harvest grain conservation challenges have received limited attention in the literature. Participants in the study communities reported various constraints encountered during native maize post-harvest management. Table 2 summarizes the ten main post-harvest practices, perceived limitations, and their relative importance, differentiated by community and gender. Notably, in several activities both women and men shared similar concerns.

In both communities, labor shortage was identified as the principal constraint during harvest (pizca), driven by rural youth migration to non-agricultural economic sectors significantly reducing available field labor (Vega, 2022). Harvest transport was hindered by high freight costs, affecting both genders equally. For grain drying, limitations varied. In San Felipe Cuauhtenco, both women and men cited lack of physical space for drying. In Guadalupe Tlachco, women highlighted unstable weather especially rainfall that interrupts

Table 2. Constraints faced during the post-harvest process.

Limitations	Community	Sex	order of importance	Activities														
				Harvesting	Transport	Drying	Shelling	Cleaning	Selection	Storage	Transfer to the place of sale	Selling	Processing					
	San Felipe Cuauhtenco	Women	1															
			2															
			3					*				*		*				
		Men	1															
			2															
			3															
	Guadalupe Tlachco	Women	1														*	
			2															
			3					*										
		Men	1															
			2															
			3													*		

Limitations

1. Lack of labor
2. High freight costs
3. Lack of space
4. Climate chance
5. Lack of time
6. Lack of implements / machinery
7. Lack of transportation
8. Lack of buyers
9. Lack of markets
10. Lack of knowledge
11. Lack of training
12. Competition with hybrids
13. Distance
14. Health problems
15. Poor grain quality
16. Not allowed

outdoor drying as the primary barrier, while men prioritized lack of time due to balancing farm work with off-farm income generation.

Regarding shelling, women in both communities identified the lack of mechanical tools as the key obstacle on average, around 40% still shell maize manually. Men again emphasized time constraints as their main barrier. Cleaning and grain selection techniques, storage, transport for sale, commercialization, and grain processing also faced shared limitations across communities and genders. Time scarcity was the most reported issue for cleaning and sorting. Storage was limited by inadequate space. Transport was restricted by high freight costs. Selling maize faced low market demand and few buyers. Finally, grain processing was hindered by lack of time especially for women, who traditionally manage this task. In summary, while limitations vary by post-harvest practice, perceptions between women and men did not differ significantly. Nevertheless, addressing the most frequently cited constraints time scarcity, labor shortages, inadequate storage spaces, and high transport costs is crucial. Native maize production and conservation are threatened by these multiple constraints. Nonetheless, maize remains a central resource not only nutritionally, but also socially and culturally. Its management is closely tied to peasant knowledge, family structure, and community dynamics, all of which help sustain traditional agricultural systems. The use and conservation of native varieties represent a fundamental peasant strategy for securing food supply and preserving local genetic diversity (Guzmán, 2021).

CONCLUSIONS

Post-harvest practices carried out by women and men in the communities of Guadalupe Tlachco and San Felipe Cuauhtenco follow a similar structure, organized into two phases: the first, conducted in the field, and the second, in the domestic sphere. While both genders actively participate in both stages, greater female involvement was observed in the second phase, which includes activities such as shelling, cleaning, storage, and maize processing for household consumption.

The differences in levels of involvement between women and men are closely linked to the structural limitations that women face in their daily lives. Among these are the work overload resulting from their multiple roles, unequal access to productive resources, information and technical training, as well as limitations regarding community organization and the physical capacities required for certain tasks. Addressing these barriers could serve as a strategic starting point to strengthen local food security by improving post-harvest conservation processes of native maize and recognizing the key role of women in this work.

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Bovine mastitis: Prevalence and economic losses

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ABSTRACT

Objective: To determine the prevalence of mastitis and estimate the economic losses caused by the disease in dual-purpose livestock units in Cuajinicuilapa, Guerrero, Mexico.

Design/Methodology/Approach: A total of 261 cows were sampled, encompassing 1,025 mammary quarters. The California Mastitis Test (CMT) was used for diagnosing mastitis, and economic losses were estimated based on milk production loss according to the severity of the disease.

Results: The overall prevalence was 42.9%, with 40.2% corresponding to subclinical mastitis and 2.7% to clinical mastitis. Among the quarters sampled, 80% tested negative, 17.5% showed subclinical mastitis, 0.7% presented clinical mastitis, and 1.8% were classified as blind quarters. Economic losses associated with mastitis ranged from USD 0.248 to USD 1.253 per day.

Study Limitations/Implications: The lack of record-keeping in the Livestock Production Units (LPU) studied hindered the estimation of additional costs related to the presence of mastitis.

Findings/Conclusions: The prevalence of mastitis in dual-purpose LPUs in Cuajinicuilapa, Guerrero, was lower compared to other regions of the country. Subclinical mastitis was more frequent, leading to economic losses across all herds due to reduced income from milk sales. These losses often go unnoticed by producers, given the asymptomatic nature of subclinical cases.

Keywords: Subclinical mastitis, clinical mastitis, prevalence, milk production.

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INTRODUCTION

Bovine mastitis is a multifactorial disease whose manifestation depends primarily on pathogens, the efficacy of the cow's udder immune defenses, environmental risk, and the interaction of factors such as breed, poor hygiene, and inadequate milking practices. These aspects collectively contribute to a decrease in both milk production and quality (Pérez *et al.*, 2019a; Ruiz *et al.*, 2016). This disease is considered the most significant challenge facing the global dairy industry in terms of economic losses. Losses of up to 35 billion pesos have been reported (Acosta *et al.*, 2017), attributed to reduced milk yield, treatment costs, discarded milk due to antibiotic contamination, and recovery time for affected animals (Vissio *et al.*, 2015). Mastitis manifests in both clinical and subclinical

forms, with the latter being the most frequent and economically impactful (Fernández *et al.*, 2012). This pathology can be diagnosed through the measurement of inflammatory components and pathogenic agents present in milk (Ruiz-García & Sandoval-Monzón, 2018). Various diagnostic methods are available, with the most common being the California Mastitis Test (CMT) and Somatic Cell Count (SCC), both of which are conducted in the field prior to each milking session (Tommasoni *et al.*, 2023). Diagnosis is critical, as somatic cells in milk, comprising approximately 75% leukocytes and 25% epithelial cells (Ruiz-García & Sandoval-Monzón, 2018), representing the udder's first line of defense against mastitis-causing pathogens (Pereyra *et al.*, 2014). Consequently, elevated SCC levels serve the primary indirect indicator of subclinical mastitis. In Mexico, studies on the prevalence and economic impact of this disease are limited, and the findings vary across geographic regions. In Sonora, a prevalence of 65.80% was identified (Pinelli-Saavedra *et al.*, 2022). In Veracruz, mastitis prevalence was reported at 40.52% (León-García *et al.*, 2021), while in the central region of Yucatán, it reached 53% (Pech *et al.*, 2007). However, there are areas such as the Costa Chica region of Guerrero specifically the municipality of Cuajinicuilapa where no studies have been conducted on mastitis prevalence. This municipality ranks fifth in milk production within the state and first in the Costa Chica region, with a total output of 4,704,560 liters (SIAP, 2024), making it one of the main economic activities for small-scale producers in the area. The closest studies have been conducted in the Tierra Caliente region, where a clinical mastitis prevalence of 20.50% was reported (Olivares-Pérez *et al.*, 2015), and in Marquelia, Guerrero, where subclinical mastitis prevalence reached 45.9% (Muñoz *et al.*, 2012). Given that no data exists for Cuajinicuilapa on the prevalence or economic losses associated with this disease, the present study aimed to determine the prevalence of bovine mastitis and the associated economic losses in a region where milk production holds considerable socioeconomic importance (SIAP, 2023).

MATERIALS AND METHODS

The study was conducted in eight localities within the municipality of Cuajinicuilapa, Guerrero, Mexico: El Pitayo, San Nicolás, Montecillos, El Quizá, Comaltepec, Buenos Aires, and El Júcaro (Figure 1). A total of 15 Livestock Production Units (LPUs) were studied, selected based on convenience and their willingness to participate in the study. All lactating cows from each herd were included, resulting in the sampling of 261 cows and a total of 1,025 mammary quarters.

The study was conducted during the rainy season (June to September). Each Livestock Production Unit (LPU) was visited during milking hours, between six and seven in the morning. A questionnaire was administered to each producer, which included data on each lactating cow (identification, breed, age, number of calvings, physiological status, origin, economic value, and date of last calving), as well as information on the LPU (location, land area, herd composition, feeding, health management, equipment, external inputs, labor, and costs). For sample collection, each teat of the cow's udder was disinfected with a 0.5% iodine-based solution. Immediately after, each quarter was stripped and subjected to the California Mastitis Test (CMT). The reading and interpretation of the test were carried out

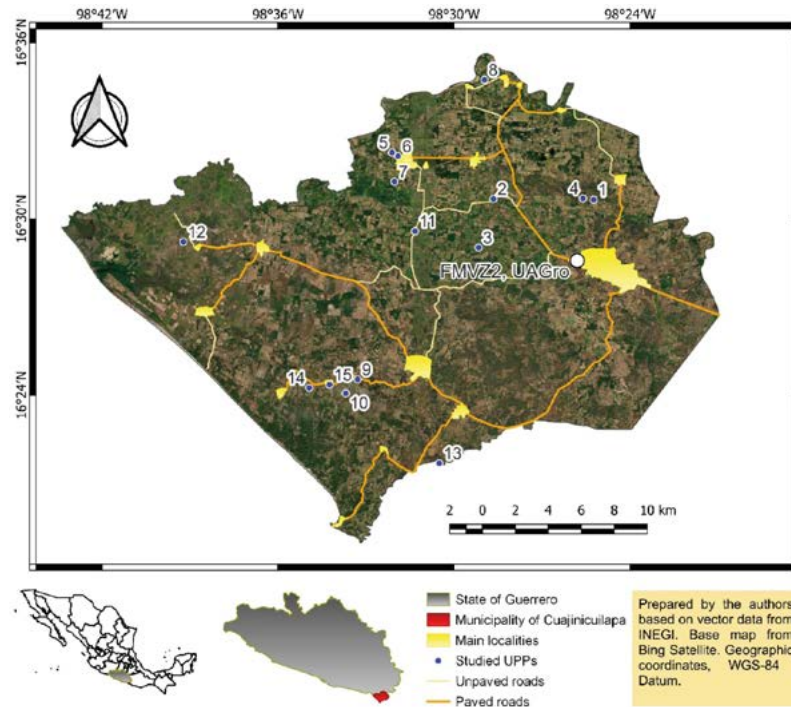


Figure 1. Location of the studied LPU.

according to the criteria described by Bedolla *et al.* (2007). To estimate the economic losses caused by clinical and subclinical mastitis, the method proposed by Fort (1985) was used, as described below:

$$X = \frac{100 \times N \times P}{100 \times N - (aA + bB + cC + dD + eE)}$$

Where: X = The ideal herd milk production (in the absence of mastitis), P = Total herd production (liters), N = Number of quarters sampled, $a = 0.103$ (Milk production reduction factor for quarters with grade 1 mastitis), $b = 0.159$ (Reduction factor for grade 2 mastitis), $c = 0.228$ (Reduction factor for grade 3 mastitis), $d = 0.058$ (Reduction factor for quarters with traces), $e = 0.515$ (Reduction factor for quarters with clinical mastitis); A = Number of quarters with grade 1 mastitis, B = Number of quarters with grade 2 mastitis, C = Number of quarters with grade 3 mastitis, D = Number of quarters with traces, and E = Number of quarters with clinical mastitis. Once X is calculated, the volume of milk lost due to mastitis is obtained by subtracting P . The economic data are expressed in U.S. dollars, using the average exchange rate for 2021, which was 20.27 Mexican pesos per dollar (Government of Mexico, 2021).

Data were recorded in Microsoft Excel, and descriptive statistics were obtained. The prevalence (P) of mastitis was calculated by dividing the number of positive cases at the time (N) by the total population studied (T) (Fajardo-Gutiérrez, 2017).

$$P = \frac{N}{T(100)}$$

RESULTS AND DISCUSSION

During the study, a total of 261 lactating cows were recorded —242 crossbred with Swiss breeds and 19 with Zebu. The average number of lactating cows per herd was 23.3, ranging from seven to 37 cows. In 14 herds, milking was performed manually, while only one used mechanical milking. In general, the producers and their families carried out most of the activities within the Livestock Production Unit (LPU); only four out of the 15 producers had a permanent employee.

Feeding was primarily based on grazing, with occasional supplementation of corn stover and commercial mineral salts. Reproductive management was conducted through the rotation of breeding bulls. Health management included vaccination against paralytic bovine rabies and blackleg, as well as deworming for gastrointestinal parasites and administration of B-complex vitamins. In 93.3% of the herds, no sanitary practices were implemented during the milking process, and none of the studied herds used any diagnostic method for mastitis control. Out of the 261 cows sampled, 112 tested positive for either subclinical or clinical mastitis, representing a mastitis prevalence of 42.9%. Among the cows diagnosed with some form of mastitis, 104 were crossbred with Swiss breeds and eight with zebu breeds. The highest prevalence was observed in LPU 8, where 100% of the cows were diagnosed with subclinical mastitis, while LPU 9 had the lowest prevalence, at 15% (Table 1).

Table 1. Number of lactating cows per production unit, herd-level mastitis prevalence, and cows with mastitis by breed cross.

LPU	Total number of cows	Prevalence %	Mastitis according to caste (N°)	
			Swiss/bred cows	Caste cows/zebu
1	12	67	7	1
2	7	71	4	1
3	13	46	6	-
4	24	67	16	-
5	12	42	3	2
6	19	32	6	-
7	11	55	6	-
8	7	100	6	1
9	20	15	3	-
10	37	43	15	1
11	20	30	6	-
12	31	23	7	-
13	17	71	10	2
14	12	50	6	-
15	19	16	3	-
Total	261	42.9	104	8

When determining quarter-level prevalence (Figure 2), it was observed that, out of the 1,025 mammary quarters sampled, 80% tested negative. Subclinical mastitis was found in 17.5% of the quarters, while clinical mastitis was present in 0.7%. Additionally, 1.8% of the quarters were classified as blind.

All 15 herds studied experienced economic losses attributable to the presence of clinical or subclinical mastitis. The LPUs with the highest milk losses corresponded to herds with the greatest incidence of subclinical mastitis. These losses ranged from 3.07 to 3.63 liters of milk per day, representing USD 1.074 to 1.253, based on the price per liter paid to the producer (Table 2).

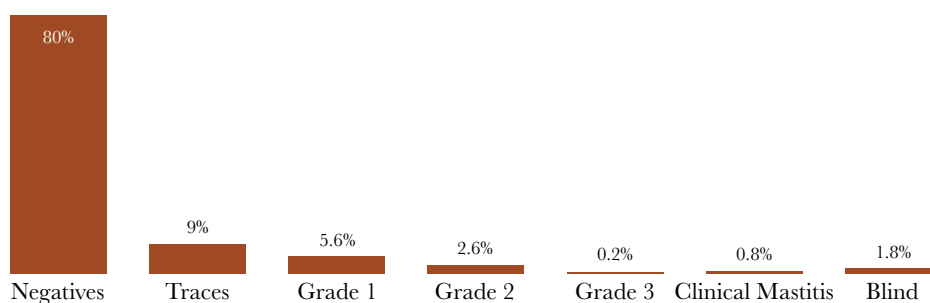


Figure 2. Prevalence of mastitis in sampled cows in the municipality of Cuajinicuilapa, Guerrero, Mexico.

Table 2. Estimated economic losses due to reduced milk production associated with mastitis in each production unit.

LPU	Milking cows	Liters of milk per LPU	Loss of milk production (liters) by type of mastitis				Economic Loss (\$/day) *	Estimated loss % of milk
			Grades 1, 2 and 3	Traces	Clinic	Total		
4	24	135.9	2.11	0.84	0.68	3.63	1.253	2.6
8	7	53.4	1.64	1.43	-	3.07	1.211	5.44
1	12	67	2.32	0.27	0.74	3.33	1.149	4.74
10	37	168.6	1.34	1.17	0.6	3.11	1.074	1.81
13	17	80.92	0.69	1.28	-	1.97	0.680	2.38
9	20	97.07	0.4	0.22	1.29	1.91	0.659	1.93
2	7	46.6	1.28	0.22	-	1.5	0.518	3.12
6	19	145.6	0.96	0.47	-	1.43	0.493	0.97
12	31	119.6	0.82	0.11	0.5	1.43	0.493	1.18
14	12	50.83	0.43	0.62	-	1.05	0.362	2.02
11	20	121.5	0.71	0.36	-	1.07	0.316	0.87
5	12	58.3	0.7	0.15	-	0.85	0.293	1.44
7	11	31.13	0.29	0.2	0.4	0.89	0.263	2.16
3	13	34.8	0.67	0.09	-	0.76	0.262	2.14
15	19	69.6	0.19	0.06	0.47	0.72	0.248	1.02

LPU: Livestock Production Unit.

* It was obtained by multiplying the volume of the loss (liters) by the price of milk paid to the producer (0.35 to 0.40 USD).

On the contrary, the herds with the lowest milk loss were those with the fewest cases of subclinical mastitis. Clinical mastitis was detected in seven of the studied LPU, in eight quarters and seven animals in total (Table 2).

The mastitis prevalence found in this study was 42.9%, which is lower than that reported in various studies (Pinelli-Saavedra *et al.*, 2022; Pérez-Morales *et al.*, 2022; León-García *et al.*, 2021; Aguilar *et al.*, 2014; Muñoz *et al.*, 2012), despite the fact that the study was conducted during the rainy season a period considered conducive to the presence of mastitis (Mora *et al.*, 2015; Pérez-Morales *et al.*, 2022). These results are similar to those reported by Muñoz *et al.* (2012) in Marquelia, Guerrero, who found a prevalence of 45.9%, although they did not specify the time of year. In the study by Pérez-Morales *et al.* (2022), no significant seasonal differences were reported when comparing mastitis prevalence in two different periods of the year in Sonora. The authors reported a higher prevalence during the rainy season (June-September), at 64.9%, compared to the present study. However, in winter (December-February), they reported a prevalence of 65.7%, without attributing this difference to any specific cause. Therefore, future studies in the Costa Chica region of Guerrero should consider at least two seasons to provide evidence of potential seasonal variations in mastitis prevalence. One of the predisposing factors associated with mastitis is breed. In the Costa Chica region, the American Swiss breed is most commonly used for crossbreeding and genetic improvement due to its dairy aptitude. Mora *et al.* (2015) stated that crossbreeding with American Swiss results in high susceptibility to mastitis, which could partly explain why, in this study, the highest number of subclinical mastitis cases occurred in cows crossbred with American Swiss (Table 2). It has been reported that the Holstein breed is more susceptible to subclinical mastitis than Jersey and American Swiss breeds (Santivañez-Ballón *et al.*, 2014), which contrasts with findings by Alvarado *et al.* (2019), who indicated that the Simmental breed is the most susceptible, followed by Brown Swiss and Holstein. Similarly, in the present study, crossbreeding with European breeds showed a higher proportion of subclinical mastitis. However, this study does not allow us to conclude that Swiss crosses are more susceptible, as a specific study with a larger sample of zebu-crossed animals is required. When analyzing mastitis prevalence by quarter, 80% tested negative and 17.5% tested positive. These results are very similar to those reported by Trujillo *et al.* (2011) in Antioquia, Colombia, where 19.9% of quarters tested positive for mastitis in seven herds. Compared to the present study, they found a slightly higher proportion of grade 1 and 2 cases, a lower proportion of grade 3 and trace cases, but a higher rate of clinical mastitis. In this study, no clear trend was observed that would attribute the presence of subclinical mastitis to a specific cause or factor. While climatic conditions, cow breed, and milking method may influence mastitis occurrence, it is evident that improved sanitary management during milking could reduce disease prevalence. Diagnostic testing can also help identify causative factors and guide better prevention strategies. Moreover, all LPU showed economic losses related to mastitis. These losses ranged from USD 0.248 to 1.253 per day, with estimated milk volume losses ranging from 0.87% to 5.44%. The highest losses occurred in four herds (1, 4, 8, and 10), and in three of these (1, 4, and 10), both clinical and subclinical mastitis were present. These four herds experienced milk

losses of 3.11 to 3.63 liters per day, corresponding to daily economic losses ranging from USD 1.074 to 1.253. LPU 8 recorded the highest economic loss despite the absence of clinical mastitis, with more quarters affected by subclinical mastitis, resulting in a daily milk loss of 3.07 liters equivalent to USD 1.211 per day. These losses are lower than those reported by Calderón *et al.* (2012) in 15 dual-purpose herds in Córdoba, Colombia, where milk production losses ranged from 4.39% to 14.58%. In another study conducted in 19 herds in Argentina, economic losses from clinical mastitis were reported at USD 230.36 for cows and USD 178.09 for heifers, with a prevalence of 2.5 cases per 100 cows (Richardet *et al.*, 2016), adjusted to September 2021 prices using INPC data. These findings show that although clinical mastitis is less frequent, its economic impact is more severe due to the symptomatic nature of the condition. In the present study, clinical mastitis prevalence was 2.7%, with daily milk losses per herd ranging from USD 0.13 to 0.44, and a total milk loss across all herds of USD 1.61 excluding treatment costs. These comparisons indicate that losses vary depending on mastitis prevalence in the herd and the extent of glandular and systemic impact on the affected cow, ultimately influencing milk yield and resulting income losses. However, the selling price of milk must also be considered. In the present study, economic losses were higher than those reported in previous research, mainly due to the rising price of milk in recent years. From 2003 to 2019, the price increased from USD 0.23 to USD 0.39 per liter (SIAP, 2019), meaning that the economic impact of mastitis today is significantly greater. Although some herds experience greater losses due to subclinical mastitis-related milk reduction, the economic impact is not uniform. In this study, milk losses due to mastitis accounted for 0.87% to 5.44% of total herd production. Herds with the highest percentage losses were those producing less than 67 liters of milk per day, which could mean a greater impact on the income of the producer and their family. Therefore, it is essential to consider implementing a support program in these LPUs aimed at the diagnosis, prevention, and control of subclinical mastitis, as for some, the milk loss could represent nearly 6% of their total income.

CONCLUSIONS

The prevalence of mastitis in dual-purpose systems in Cuajinicuilapa, Guerrero, was lower (42.9%) than in other regions of the country, despite the absence of sanitary management during milking and the lack of diagnostic methods for mastitis control. Subclinical mastitis was more frequent, accounting for 40.2% of cases, and was present in all herds studied. The estimated milk reduction due to mastitis resulted in economic losses across all herds, caused by decreased income from milk sales losses that largely go unnoticed by producers, primarily because the condition manifests subclinically.

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Growth and development of *Tabebuia rosea* (Bertol.) DC. with controlled release fertilizers in the nursery

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ABSTRACT

Objective: To understand the effect of controlled release fertilizers on the growth and development components of *Tabebuia rosea* (Bertol.) DC.

Design/methodology/approach: In this research, a randomized block design with seven treatments and 50 replicates was used. Three treatments consisted of substrate with different mixtures of controlled release fertilizers (CRFs) at three concentrations each, of 4, 5 and 6 kg m⁻³ plus a control (without fertilization).

Results: The use of controlled release fertilizers such as Multicote™ Agri 8 and Basacote® Plus 9M demonstrated greater growth and development in pink trumpet seedlings on the components of plant height, basal stem diameter, aerial biomass, root biomass, among others. Of the seven treatments presented in this study, the concentrations of 5 and 6 kg m⁻³ proved to be the best nutritional alternative, which will result in a higher survival rate in the field.

Limitations on study/implications: Due to the loss of undisturbed specimens, it is becoming increasingly difficult to find trees with seed production capabilities.

Findings/conclusions: Multicote™ Agri 8 in concentrations of 5 and 6 kg m⁻³ is the best nutritional alternative for optimal growth and development of *Tabebuia rosea* (Bertol.) DC. It can be considered essential for reforestation in urban and rural areas of the region, and due to its floral beauty, which is part of culture in the state of Tabasco.

Keywords: Pink trumpet, Growth, Development, Quality Indices.

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INTRODUCTION

The species *Tabebuia rosea* (Bertol) DC., known as pink trumpet, is distributed in tropical areas; in Mexico, it is found from Tamaulipas to the Yucatan Peninsula, and in the Pacific gradient from Sinaloa to Chiapas (Herrera, 2015; Jasso-Mata, 2018). The beauty of its flowers is one of the qualities of this species, although its main use is the wood which is used for the elaboration of furniture and houses (Pennington and Sarukhán, 2005; Jasso-Mata, 2018).

Unfortunately, the demand for wood from this species has increased, and trees that are found in natural habitats are used mostly clandestinely and have not been entirely documented, so this species could be threatened. This is a concern that has been reported

since the 1940s (Benítez *et al.*, 2002; Jasso-Mata, 2018). The success of germination, establishment and survival of plants in a natural environment are determined by multiple biotic factors such as density, pests, diseases, pathogens, and abiotic factors such as availability of light, water, temperature, and nutrients in the soil (Harms and Paine, 2003; Pérez-Hernández *et al.*, 2011). Presently, these factors have been affected by the transformation of native forests into agricultural, livestock, industrial and urban areas, contributing to the degradation and disappearance of rain forests and ecosystems (Lanly, 2003). Therefore, the propagation of native species is necessary for the enrichment, reforestation and restoration of areas affected; unfortunately, the reforestation plans implemented in the American tropics are scarce in native species, since they promote mainly fast-growth exotic species, such as eucalyptus, teak, beechwood and acacia, creating monospecific forests with the aim of production of wood and cellulose. These forests are poor in biodiversity, since they displace native plants and animals (Pérez-Hernández *et al.*, 2011). Controlled release fertilizers favor the growth of nursery plants primarily because they do not leach in equal proportion to conventional fertilizers during irrigation, which are constant under this type of management (Reyes-Castro *et al.*, 2020). Currently, controlled release fertilizers are used under nursery conditions to produce small trees for commercial plantations of teak (Escamilla-Hernández *et al.*, 2015) and mahogany (Domínguez-Liévano *et al.*, 2023), among other species. However, they are not commonly used for species such as those in this study. Therefore, the objective of this research was to understand the effect of controlled release fertilizers (CRFs) on the components of growth and development, and quality indices of *Tabebuia rosea* (Bertol.) DC., in the nursery stage.

MATERIALS AND METHODS

The study was carried out in Colegio de Postgraduados Campus Tabasco, located on km 3.5 of the Cárdenas-Huimanguillo highway, Tabasco. According to Köppen's classification, the climate that predominates in the region is Am(g)w" which is characteristic of the humid tropics, where there is a total annual precipitation of 2,324 mm, with dry months like March and April (with less than 50 mm per month), and other rainy ones like September and October (up to 400 mm per month). The mean annual temperature is 26 °C, with the lowest temperatures found in the winter months and the highest ones in the summer (Palma-López *et al.*, 2007).

Species under study

It presents rapid growth with heights that range from 25 to 30 m and average diameter of 1 m; it develops in a large variety of habitats, primarily in soil with good drainage; the range of development of this species varies from 0 masl to 1200 m, with temperatures that fluctuate between 10 and 30 °C and have a mean annual requirement of 500 mm of rain (Flores and Marín, 2010).

Experimental design, treatments and study variables

The seeds were collected considering trees with adequate silviculture characteristics, and for this purpose visits were carried out in different plots of the alluvial plain of Tabasco.

The seeds were placed to germinate in a sowing bed of 1 m by 0.5 m with a height of 3 cm, and substrate made up of soil, peat moss and agrolite (2:1:1). The moisture was kept constant throughout the entire germinative stage; when a height of 5 cm was reached, the plants were moved to nursery bags of 5×6×22 cm height, with an approximate capacity of 500 g of substrate per bag.

A completely randomized block design with seven treatments and fifty repetitions was used, where six treatments are made up of substrates with different doses of two controlled release fertilizers (CRFs): Multicote™ Agri 8 (11.22.09+4 MgO+0.1 Boron) and Basacote® Plus 9M (16-8-12) at three concentrations each, of 4, 5 and 6 kg m⁻³ plus a control (without fertilizer); these CRFs have been recommended and used in nurseries for forest species. Then, they were placed for 3 months under shade mesh (75%).

Moisture of the seeds

A total of 100 pink trumpet seeds were selected, from which their fresh weight was obtained; they were stored in paper bags and introduced into a drying stove at 72 °C until constant weight; the value of moisture was obtained through a rule of thirds (Quinto *et al.*, 2009).

Weight estimation of 100 seeds

Three repetitions of 100 seeds were randomly selected, which were weighed in an analytical scale (Ohaus® AR0640) with precision of 0.001 g (Quinto *et al.*, 2009).

Estimation of percentage of germination

Because of the characteristics of the seeds of this species, sowing in seedbeds is recommended (Rueda-Sánchez *et al.*, 2014; Orozco *et al.*, 2019). A germination test without scarification with three repetitions of 100 seeds was carried out. Once the seeds were sown in germination beds, the percentage of germination was estimated 30 days after sowing (DAS), through the following formula:

$$\% \text{ of germination} = \frac{\text{Total of seeds germinated} \times 100}{\text{Total of seeds sown}}$$

Morphological variables and quality indices

Four plants from each study treatment were randomly selected. In the seedlings selected for destructive sampling, the aerial and root system were washed with water to eliminate the particles of substrates, leaving them free of impurities. A compound sample for each treatment was carried out where the following variables were determined (Sáenz *et al.*, 2010; Rueda *et al.*, 2012): a) plant height (cm), with a ruler calibrated in centimeters, from the base of the stem to the apex; b) diameter of the root crown (mm), with a digital vernier calibrator series QL-V of 0-150 mm/0-6 in, resolution 0.001 mm, and accuracy of ±0.02 mm; c) dry weight and fresh weight of aerial biomass of leaves and stems (g); d) dry weight and fresh weight of root biomass (g⁻¹). The aerial and root biomass were placed in paper bags, to dry in a Shel® Mod stove. CE5F of forced circulation at 70 °C for 72 h; then, they

were weighed on an analytical scale (Ohaus® AR0640) with precision of 0.001 g. With data from these variables, the quality indices of the plants were calculated: robustness index (RI), dry aerial biomass/dry root biomass ratio (DAB/DRB R), and Dickinson quality index (DQI) (Dickson *et al.*, 1960; Prieto *et al.*, 2003), using the following formulas:

Height/diameter ratio of the root crown or robustness index (RI).

$$RI = \frac{\text{Height (cm)}}{\text{Diameter of root crown (mm)}}$$

Dry aerial biomass/dry root biomass ratio (DAB/DRB R).

$$DAB / DRB R = \frac{\text{Dry aerial biomass (g)}}{\text{Dry root biomass (g)}}$$

Dickinson Quality Index (DQI).

$$DQI = \frac{\text{Total dry weight of the plant (g)}}{\frac{\text{Height (cm)}}{\text{Diameter of root crown (mm)}} + \frac{\text{Dry aerial biomass (g)}}{\text{Dry root biomass (g)}}}$$

Where the results obtained will be compared in relation to the value ranges of Table 1.

Nutritional characteristics

When the nursery stage finished, a plant per repetition of each of the seven treatments was chosen randomly, which was separated into aerial biomass and root biomass, with the aim of quantifying the nutrients Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), and Magnesium (Mg). Each of the samples obtained were stored in paper bags and dried in a stove at a temperature of 65 °C for 72 hours, ground and pulverized in a sieve mill (Yinda®-YDGS-200) with a sieve of 1.0 mm diameter. A compound sample of four plants for each of the treatments was conducted. The methods to analyze the nutrients

Table 1. Values that qualify the quality of the plant with normal growth in a forest nursery.

Variable	Type of plant	Quality		
		Low	Medium	Hight
Height (cm)	Broadleaf	<12.0	12.0-14.9	≥15.0
Diameter (mm)	Broadleaf	< 2.5	2.5-4.9	≥ 5.0
Robustness Index (IR)	Broadleaf	≥ 8.0	6.0-8.0	< 6.0
R BSA / BSR	All	≥ 2.5	2.0-2.4	< 2.0
Dickson Index (ICD)	All	< 0.2	0.2-0.4	≥ 0.5

*DAB/DRB R=Dry aerial biomass and dry root biomass ratio.

Source: Data cited by Rueda *et al.* (2012) and Sáenz *et al.* (2010) with contributions from Santiago *et al.* (2007); Conafor (2009); and Escamilla-Hernández *et al.* (2015).

were: Semimicro-Kjeldahl (Bremer, 1965) for N, the method described by Alcántar and Sandoval (1999) for P and K, and finally the extracts were quantified in the Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer 400). To compare the concentrations of nutrients from the dry and root biomass, the intervals recommended by Drechsel and Zech (1991) and Jacobs and Landis (2014) were used, where they describe the nutrients for healthy plants of tropical species. Macronutrients: (N=1.50-3.50%, P=0.10-0.25%, and K=0.60-1.80%, Ca=0.50-2.5%); Micronutrients: (Mg=35-250 ppm).

Statistical analyses

The values obtained in the response variables evaluated were subjected to the Shapiro-Wild normality tests, and Bartlett homogeneity of variances, to verify the hypotheses of normality and homoscedasticity of the observations. Then, a variance analysis was conducted and, in the cases where significant effects from the treatments were detected, a multiple means comparison test was carried out via Tukey's method ($p < 0.05$), with the statistical software R Studio 3.4.3. for Windows 11 from Microsoft Corporation (2024).

RESULTS AND DISCUSSION

Weight (g) of 100 seeds

An average of 43,951 seeds per kg was found, equivalent to 2.487 g for every 100 seeds. Because of the factors present during their growth and development, and their genetic diversity, these data presented great variability in comparison to those presented by Quinto *et al.* (2009), who obtained an average of 40,209 seeds per kg, less than what was reported in this study.

Moisture in the seeds

A fresh weight of 2.487 g was obtained and a final value of 2.260 g; through a rule of thirds the value of moisture obtained was 9.1%. This content was higher than what was presented by Quinto *et al.* (2009), where a moisture content of 8.74% was obtained.

Germination and sowing of seeds

There was 98% of germination, and this value was obtained 12 days after sowing. This result is similar to what was observed in Santiago de Tuxtla, Veracruz, by Quinto *et al.* (2009), where 99% of germination was found. Ríos-García *et al.* (2018) obtained 100% of germination from *T. rosea* and *T. donnell-smithii* when sowing them after collection, with a decrease of 20% of germination at twelve months of storage. The ageing of seeds is a factor that decreases their viability, being of utmost importance to understand the viability to determine the period when they can be conserved without significantly affecting the capacity for germination and managing to achieve successful propagation (Vázquez *et al.*, 1999; Ríos-García *et al.*, 2018).

Growth and development of pink trumpet seedlings

Table 2 shows the monthly results of monthly diameter and height of pink trumpet seedlings in nursery stage. In the first month of growth and development, the Multicote (4

kg m⁻³) treatment shows the best results compared to the other treatments; in the second month, the Multicote (5 and 6 kg m⁻³) and Basacote (4 kg m⁻³) treatments show they are statistically equal in height; and for the variable of basal diameter, the Multicote (5 and 6 kg m⁻³) treatments showed the highest values. At three months old, the pink trumpet seedlings showed three statistically outstanding treatments, which were Multicote (5 and 6 kg m⁻³) and Basacote (6 kg m⁻³), with a height of 30.42, 33.37 and 33.00 cm, respectively; they both enter into the category of high quality according to Orozco *et al.* (2010), Rueda-Sánchez *et al.* (2014), and Orozco *et al.* (2019). These same authors show that the high quality of the basal diameter are values ≥ 5 where the Multicote (6 kg m⁻³) treatment showed a diameter of 5.25 mm, which was statistically equal to what was obtained with Multicote (6 kg m⁻³) with 4.94 mm, Multicote (4 kg m⁻³) with 4.30 mm, and Basacote (6 kg m⁻³) with 4.72 mm; these diameters entered into the category of average quality. In addition, these results of plant height obtained by treatments with CRFs plus the control were higher than those found by Rueda-Sánchez *et al.* (2014) in the Cepraf forest nursery in Nayarit, which at the same age of three months obtained an average height of 20.2 cm and a basal diameter of 5.0 mm, the last being outperformed by the Multicote (6 kg m⁻³) treatment, and corroborated by Orozco *et al.* (2010) and Orozco *et al.* (2019). In addition to this, in the same state, in the nursery of San Agustín, they obtained a final height of 11.0 cm (low quality) at the age of three months and a basal diameter of barely 4.2 mm, which qualifies as average quality.

Quality Indices of seedlings

When the nursery stage ended, the pink trumpet showed a robustness index (RI) under the Multicote (4 and 5 kg m⁻³) treatments, and average with the others (Table 3) (Rueda-Sánchez *et al.*, 2014), contrary case to what was found in the San Agustín forest nursery, where despite having lower plant heights, they obtained a high quality index with a value of 2.7 (Rueda-Sánchez *et al.*, 2014). For the dry aerial biomass and dry root biomass ratio, only the control shows high quality, followed by the Basacote (4 and 6 kg m⁻³) treatments,

Table 2. Growth and development of *Tabebuia rosea* (Bertol.) DC. seedlings in nursery stage, in a period of 3 months.

Treatments (kg m ⁻³)	Plant height (cm)			Basal stem diameter (mm)			
	1 st month	2 nd month	3 rd month	1 st month	2 nd month	3 rd month	
Multicote	4	6.87 a	12.00 bc	26.27 b	1.66 a	2.95 ab	4.30 abc
	5	6.37 ab	14.06 ab	30.42 a	1.52 ab	3.20 a	4.94 a
	6	5.46 abc	15.00 a	33.37 a	1.36 abc	3.28 a	5.25 a
Basacote	4	5.37 bc	9.20 d	22.7 b	1.43 abc	2.14 c	3.10 d
	5	4.62 c	10.13 cd	24.33 b	1.33 abc	2.53 bc	3.73 bcd
	6	4.68 c	11.26 cd	33.00 a	1.16 bc	2.91 ab	4.72 ab
Control	0	4.62 c	9.73 cd	23.24 b	1.12 c	2.24 c	3.40 cd
Average		5.43	11.62	27.62	1.37	2.75	4.21
CV		25.46	18.67	13.72	25.72	19.71	24.82
Pr(>F)		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

*CV=Coefficient of Variation.; Pr(>F)=Significance Value; NS=No Significance; <.0001=Highly Significant, Tukey p \leq 0.05.

Table 3. Quality Indices obtained by *Tabebuia rosea* (Bertol.) DC.

Treatments (kg m ⁻³)		<i>Tabebuia rosea</i> (Bertol.) DC.					
		IR	Quality	R BSA. BSR	Quality	ICD	Quality
Multicote	4	8.04	Low	2.90	Low	0.47	Medium
	5	8.62	Low	3.37	Low	0.80	Hight
	6	6.65	Medium	3.22	Low	0.87	Hight
Basacote	4	6.79	Medium	2.10	Medium	0.52	Hight
	5	6.98	Medium	3.12	Low	1.00	Hight
	6	7.27	Medium	2.10	Medium	0.77	Hight
Control	0	6.41	Medium	1.82	Hight	0.52	Hight
Average		7.25		2.56		0.71	
CV		18.22		34.18		37.82	

* RI=Robustness Index; DAB/DRB R=Dry Aerial Biomass and Dry Root Biomass Ratio; DQI=Dickson Quality Index; CV=Coefficient of Variation.

with average quality; the remaining treatments were of low quality. These data were similar to those found in the San Agustín, San Juan and Cepraf nurseries (Rueda-Sánchez *et al.*, 2014). The Dickinson quality index proves to be more representative to determine the quality of this species; only the Multicote (4 kg m⁻³) treatment presents average quality, compared to the other treatments with high quality, including the control. These results are similar to those obtained by Orozco *et al.* (2010), Rueda-Sánchez *et al.* (2014), and Orozco *et al.* (2019).

Morphological values of pink trumpet seedlings

Table 4 shows the morphological values recorded in this species, where Multicote (5 kg m⁻³) presents the greatest height of all the treatments, although it was statistically similar to all the treatments except for Multicote (4 kg m⁻³) and the control, with the latter being of lower height; the coefficient of variation was 13.24%. In the variable stem height, there was no statistical difference between treatments; the general mean was 8.85 cm, the minimum value 8.50 cm was observed in the control, and the highest was 9.62 cm for the Multicote (4 kg m⁻³) treatment. Regarding the basal diameter, there was no statistical difference between treatments. However, Basacote (6 kg m⁻³) presented the largest basal diameter numerically with 6.63 mm, with the control being the one with smallest diameter (4.88 mm). Statistically significant differences were found for number of leaves, with the highest values being for Basacote (4 and 6 kg m⁻³) and the lowest for Basacote (5 kg m⁻³). The variable of fresh weight of leaves presented a statistically significant difference, with the highest value being for Basacote (6 kg m⁻³) with 4.05, and the lowest for the control with 2.02. Regarding the dry weight of leaves, there was no statistically significant difference between treatments. The fresh and dry weights of roots presented statistically significant differences with coefficients of variation of 35.72 and 35.09%, respectively, and in both cases the highest value was found in Multicote (6 kg m⁻³) with 5.25 g and 1.75 g, respectively, and the lowest in Multicote (4 kg m⁻³) with 1.67 g and 0.52 g.

Table 4. Morphological values of *Tabebuia rosea* (Bertol.) DC.

Treatments (kg m ⁻³)	Hight (cm)		Diameter (mm)		Number of leaves per plant	Leaves (g)		Stem (g)		Root (g)		Root length (cm)	
	Plant	Stem	Basal stem	Root collar		Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight		
Multicote	4	49ab	9.62a	5.23a	5.28a	8.75ab	2.75 ab	0.72a	3.75b	1.17ab	1.67b	0.52b	24.50a
	5	61.00a	8.75a	5.98a	6.36a	9.75ab	2.85 ab	0.75a	5.65ab	1.82ab	3.12ab	1.20ab	26.25a
	6	60.50a	7.87a	6.22a	6.31a	8.50ab	2.32 b	0.75a	5.90ab	2.05a	3.60ab	1.17ab	22.25a
Basacote	4	60.50a	9.25a	6.21a	6.11a	12.00a	2.72 ab	0.70a	6.42ab	2.05a	4.02ab	1.72a	27.75a
	5	57.50a	9.00a	6.05a	6.31a	7.50b	2.60 ab	0.87a	5.92ab	2.12a	3.50ab	1.27ab	24.50a
	6	58.25a	9.00a	6.63a	6.9a	12.00a	4.05 a	0.70a	7.69a	2.42a	5.25a	1.75a	24.75a
Control	0	36.25b	8.50a	4.88a	5.11a	9.50ab	2.02 b	0.62a	2.82b	0.52b	2.00b	0.55b	28.00a
Average		54.71	8.85	5.89	6.05	9.71	2.76	0.73	5.45	1.73	3.31	1.17	25.42
CV		13.24	25.04	14.99	14.76	19.12	26.74	40.23	30.12	33.7	35.72	35.09	20.39
Pr(>F)		<.0001	NS	NS	NS	0.0159	0.0293	NS	<.0001	<.0001	<.0001	<.0001	NS

* CV=Coefficient of Variation.; Pr(>F)=Significance Value; NS=No Significance; <.0001=Highly Significant, Tukey p≤0.05.

The control presented the greatest root length, value that contrasts with what was found by Martínez-Nevárez *et al.* (2023), who found greater root length with higher concentration of CRF. Concerning the plant height, Orozco *et al.* (2010) obtained an average height of 22.5 cm and average basal diameter of 4.4 mm in two-month-old pink trumpets; the plants were sown in tubes of 170 cm³ capacity. Rueda-Sánchez *et al.* (2014) found a plant height of 25.4 cm in three-month-old pink trumpets, which is lower than what is presented in Table 4, and a basal diameter of 5.1 mm, slightly lower than the general mean obtained in this study (5.30 mm).

Nutritional values of pink trumpet seedlings

The Multicote (5 kg m⁻³) treatment yields the highest content of nitrogen in aerial and root biomass (Table 5), while the control shows the lowest values of concentration of this element. For phosphorus (P), Multicote 8M (6 kg m⁻³) shows the highest content in aerial biomass, and this value is slightly higher than the optimal reported by Jacobs and Landis (2014). Something interesting found in this species is that the Basacote (5 kg m⁻³) treatment yielded the highest contents of K in aerial and root biomass with 2.37% and 1.97%, respectively; both values are slightly above the optimal reported by Jacobs and Landis (2014). The values of nitrogen, phosphorus and potassium are similar to those reported by Orozco *et al.* (2010) for pink trumpets, obtaining concentrations of 1.63%, 0.36% and 2.17%, respectively.

Calcium proves to be the nutrient of greatest presence compared to the others, in the seven treatments. Basacote (6 kg m⁻³) presented the highest concentration both for aerial and for root biomass, with 7.79% and 5.68%, respectively. These concentrations are above the optimal recommended by Jacobs and Landis (2014). Regarding magnesium, the

Table 5. Nutritional values of *Tabebuia rosea* (Bertol.) DC.

Treatments (kg m ⁻³)	Biomass	Nutritional content					
		N (%)	P (%)	K (%)	Ca (%)	Mg (%)	
Multicote	4	Aerial	0.97	0.20	2.10	7.25	0.26
	5	Aerial	2.50	0.25	2.13	6.00	0.29
	6	Aerial	1.12	0.26	1.89	5.49	0.26
Basacote	4	Aerial	1.32	0.24	2.22	7.36	0.34
	5	Aerial	1.20	0.21	2.37	6.28	0.36
	6	Aerial	1.49	0.24	1.94	7.79	0.32
Control	0	Aerial	0.91	0.23	0.94	7.79	0.32
Multicote	4	Radical	0.55	0.13	1.14	2.66	0.27
	5	Radical	1.24	0.18	1.51	3.07	0.19
	6	Radical	0.62	0.14	1.11	2.53	0.26
Basacote	4	Radical	0.61	0.18	1.26	2.43	0.21
	5	Radical	0.99	0.28	1.97	4.08	0.74
	6	Radical	0.78	0.26	1.55	5.68	0.74
Control	0	Radical	0.60	0.27	1.34	5.54	0.46

Basacote (5 kg m⁻³) treatment showed the highest values, with 0.36% in aerial biomass and 0.74% in root biomass. Rueda-Sánchez *et al.* (2014) found similar values in total biomass. For potassium, the values ranged between 0.9% for the control treatment and 2.37% for the Basacote (5 kg m⁻³) treatment. The contents of N, P and K in this study were similar to those found by Rueda-Sánchez *et al.* (2014).

CONCLUSIONS

The use of the controlled release fertilizer, Basacote, in concentrations of 5 and 6 kg m⁻³ favored the growth and development of pink trumpets in the nursery stage in a better way, which is reflected in the better quality indices of the plant.

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Effect of different concentrations of chia (*Salvia hispanica* L.) on the productive variables of Creole hens and internal characteristics of the egg

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ABSTRACT

Objective: To evaluate the effect of chia seed inclusion on productive variables and internal characteristics of eggs from Creole hens.

Methodology: Sixty 24-week-old Creole hens were selected, and five concentrations of whole chia seed (*Salvia hispanica* L.) 0, 5, 10, 15, and 20% were evaluated, incorporated into a diet consisting of corn, soybean meal, corn starch, vitamins, and minerals. The analyzed variables included feed intake (g), egg production (g), feed conversion (g/g), egg mass (g), egg weight (g), Haugh units, yolk percentage, albumen percentage, yolk color, and eggshell thickness (mm).

Results: Significant differences ($p \leq 0.05$) were found only in average egg weight, Haugh units, and yolk color.

Study limitations: This research focused on Creole hens; future studies could compare these results with commercial strains or other breeds to validate and broaden the findings.

Conclusions: The addition of whole chia seed to the diets increased egg weight and improved Haugh units compared to the control diet, without affecting the productive variables in Creole hens.

Keywords: chia, hens, Creole, egg.

INTRODUCTION

The composition of hen eggs is highly valued for its content of proteins, lipids, vitamins, and minerals, making it a fundamental food in the human diet. However, despite its high nutritional value, egg consumption has declined in recent decades due to concerns related to its cholesterol and saturated fatty acid content (Simopoulos, 2002; Ayerza & Coates, 2000). In this context, chia seeds represent an interesting alternative due to their high



oil (13.5-34.4%) and protein (18-26.5%) content (Weber *et al.*, 1991). Nevertheless, their use in the diet of laying hens has been scarcely explored. Currently, at the Colegio de Postgraduados, a breeding and improvement program for a flock of Creole hens is being conducted, whose nutritional requirements for energy and protein have recently been determined (Matus-Aragón *et al.*, 2021). Therefore, the aim of the present study was to evaluate the effect of chia seed inclusion in the diet on productive variables and internal egg characteristics in Creole hens.

MATERIALS AND METHODS

Study location

The experiment was conducted at the poultry facilities of the Colegio de Postgraduados, Montecillo Campus, located in Texcoco, State of Mexico (19° 29' N, 98° 53' W; 2,247 masl) (García, 2004). The poultry house features natural ventilation, regulated by lateral curtains.

Experimental animals

A total of 60 Creole hens, 24 weeks old, with an average initial weight of 1866 ± 36.8 g, were used. The birds were housed individually in cages measuring $37 \times 43 \times 30$ cm (height \times length \times width), with free access to feed and water. Animal management was conducted in accordance with the guidelines of the Animal Welfare Committee of the Colegio de Postgraduados (COBIAN/008/24; COLPOS, 2016).

Proximal analysis of chia

Chia seeds (*Salvia hispanica* L.), sourced from a supplier in Cholula, Puebla, were analyzed at the Nutrition Laboratory of the Universidad Autónoma Chapingo. The proximal composition was as follows: dry matter 94.9%, ash 4.22%, crude protein 23.00%, ether extract 32.21%, crude fiber 9.39%, and nitrogen-free extract 26.1%.

Experimental diets

Five diets were formulated with whole chia seed inclusion at levels of 0, 5, 10, 15, and 20%, based on corn, soybean meal, corn starch, vitamins, and minerals. These treatments were designated as T0, T5, T10, T15, and T20. The composition and calculated analysis of the diets are shown in Table 1.

The linolenic acid content of the diets (%) was: 3.08, 19.84, 24.76, 36.39, 39.09 according to the order of the treatments.

Response variables

The productive variables included feed intake (g), egg production (g), feed conversion (g/g), and egg mass (g). Data were recorded weekly. The internal egg characteristics evaluated were egg weight (g), Haugh units, yolk percentage (%), albumen percentage (%), yolk color expressed as DSM color fan intensity, and eggshell thickness (mm); these data were measured during three periods (14, 49, and 84 days).

Table 1. Composition (%) and calculated analysis of the experimental diets.

Ingredients	T0 ¹	T5	T10	T15	T20
Yellow corn	29.23	37.52	44.61	49.92	54.05
Starch	20.00	15.00	10.00	5.00	0.00
Chia seed	0.00	5.00	10.00	15.00	20.00
Soybean meal	12.80	12.33	12.27	18.29	11.49
Corn gluten	2.60	1.87	1.09	0.80	3.38
Wheat bran	12.17	5.40	0.00	0.00	0.00
Canola meal	12.00	12.00	11.20	0.00	0.00
Fine calcium carbonate	4.38	4.38	4.38	4.46	4.44
Coarse calcium carbonate	4.37	4.37	4.37	4.37	4.37
Dicalcium phosphate	1.22	1.15	1.09	1.14	1.12
Soybean oil	0.44	0.20	0.20	0.20	0.20
Common salt	0.33	0.34	0.34	0.35	0.35
Vitamin and mineral premix ²	0.30	0.30	0.30	0.30	0.30
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10
DL-methionine	0.07	0.05	0.04	0.06	0.02
L-lysine HCl	0.00	0.00	0.00	0.03	0.17
Total	100.0	100.0	100.0	100.0	100.0
Calculated analysis					
Dry matter	91.10	90.80	90.50	90.10	90.10
Ash	12.60	12.50	12.50	12.40	12.30
Crude protein	16.00	16.00	16.00	16.00	16.00
Ether extract	2.30	3.70	5.30	6.90	8.60
Crude fiber	3.40	3.40	3.40	3.00	3.30
Linoleic acid	1.00	1.10	1.50	1.80	2.20
Calcium carbonate	3.70	3.70	3.70	3.70	3.70
Available phosphorus	0.35	0.35	0.35	0.35	0.35
Na	0.18	0.18	0.18	0.18	0.18
Cl	0.24	0.23	0.23	0.24	0.27
K	0.72	0.67	0.64	0.68	0.57
EMAn ³ (kcal/kg)	2600	2600	2600	2600	2600
Digestible amino acids					
Lysine	0.66	0.63	0.60	0.58	0.55
Methionine	0.31	0.28	0.26	0.24	0.21
Methionine + cystine	0.54	0.50	0.47	0.41	0.37
Threonine	0.48	0.45	0.43	0.39	0.34
Tryptophan	0.15	0.14	0.13	0.12	0.09
Isoleucine	0.54	0.51	0.48	0.46	0.41
Valine	0.63	0.59	0.56	0.52	0.46

¹ T0, T5, T10, T15, and T20: indicate the percentage concentration of chia seed included in the diet.

² Contribution per kg of feed: vitamin A, 12,000 IU; vitamin D₃, 5,000 IU; vitamin E, 85 IU; vitamin K₃, 3 mg; vitamin B₁, 3 mg; vitamin B₂, 8 mg; vitamin B₃, 62 mg; vitamin B₆, 4 mg; vitamin B₁₂, 0.017 mg; pantothenic acid, 14.5 mg; folic acid, 1.8 mg; biotin, 0.2 mg; Zn, 100 mg; Mn, 120 mg; I, 1.25 mg; Se, 0.3 mg; Cu, 15 mg; Fe, 80 mg. ³ AMEn: Apparent Metabolizable Energy corrected for nitrogen retention.

Experimental design

The experiment was conducted following a completely randomized design. The statistical model used was as follows:

$$Y_{ijk} = \mu + T_i + E_k + T_i * E_k + \varepsilon_{ijk}$$

Where: Y_{ijk} =Response variable measured in the j -th replicate ($j=1-12$) of the i -th treatment ($i=1-5$) during the k -th period ($k=1-12$ weeks for productive variables and $k=1-3$ for internal egg characteristics); μ =Common population constant; T_i =Effect of the i -th treatment, $i=1-5$; E_k =Effect of the k -th period, $k=1-12$ or $k=1-3$; $T_i * E_k$ =Interaction effect between treatment and period; ε_{ijk} =Random error effect associated with the i -th treatment, the j -th replicate, and the k -th period.

Data were analyzed using the MIXED procedure of the SAS statistical software (SAS Institute Inc., 2013), with period evaluated as a repeated measure. For the repeated measures analysis, four covariance structures were tested, and the most appropriate was selected based on Akaike's and Schwarz's Bayesian Information Criteria.

Each treatment (experimental diet) was administered to 12 hens, each considered an experimental unit.

The adjusted means obtained were compared using Tukey's test with an $\alpha=0.05$.

RESULTS AND DISCUSSION

Productive variables

Throughout the 12-week study, the productive variables evaluated feed intake per hen, egg production, feed conversion, egg mass, and laying percentage showed no significant differences between treatments ($p>0.05$), both over the course of the experiment and in the overall average (data not shown). The only exception was feed intake in week 12, where hens fed the diet containing 20% chia seed had significantly lower consumption ($p\leq 0.05$) compared to the other treatments.

Average egg weight

Figure 1 shows the trend in egg weight over the 12-week period. Significant differences ($p\leq 0.05$) were observed between treatments in most weeks, except for weeks 3 and 8. On average for the experimental period, the diets with 10% and 15% chia inclusion promoted greater egg weight. These treatments were significantly superior to the control, as well as to the treatments with 5% and 20% chia. Notably, the treatment with 20% chia seed showed values similar to the control in most of the weeks evaluated.

Internal egg characteristics

The average egg weights used to evaluate these variables were 48.3, 49.4, 50.7, 51.3, and 50.3 g for the treatments with 0%, 5%, 10%, 15%, and 20% chia, respectively. Regarding Haugh units, during the first period, the diet with 5% chia showed values similar to the control treatment, while the 20% chia diet yielded comparable results to

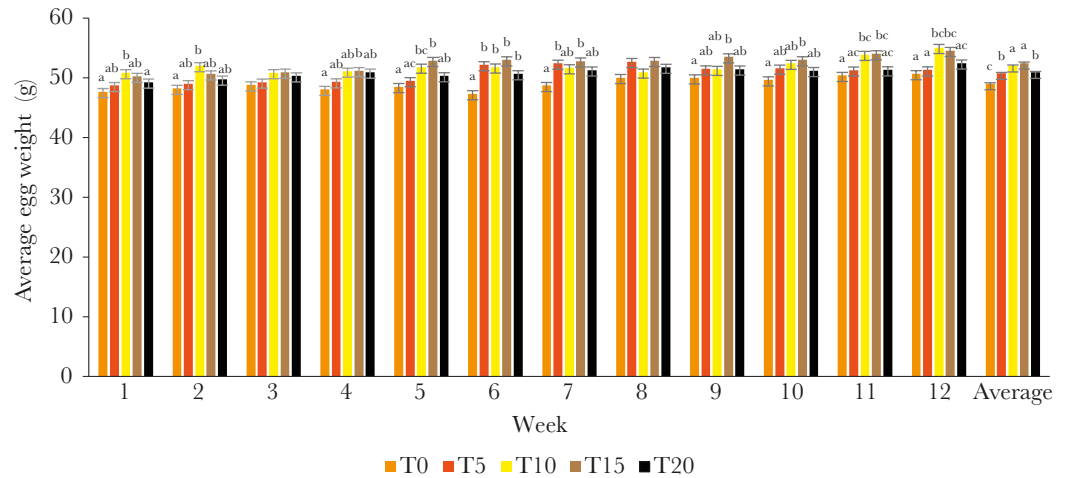


Figure 1. Average egg weight (g) of Creole hens fed for 12 weeks with different concentrations of chia. The numbers 0, 5, 10, 15, and 20 indicate the level of chia inclusion (%) in the diet. ^{abc}Means with different superscript letters within each week indicate significant differences ($p \leq 0.05$).

the 5% group. The diets with 10% and 15% chia did not differ from each other, but were significantly higher than those with 0% and 5% chia. In periods two and three, no significant differences were observed among treatments ($p > 0.05$). However, the overall average across the three periods indicated that all chia-inclusive diets significantly outperformed the control in this variable. Concerning yolk color, during the first period, the 20% chia diet produced the most intense yolk color compared to the control. No significant differences were found among treatments in periods two and three; however, the overall average showed that only the 20% chia treatment significantly increased yolk color intensity ($p \leq 0.05$) (Figure 3).

Regarding yolk percentage, albumen percentage, and eggshell thickness, no differences were observed due to treatment effects or in the overall average ($p > 0.05$) (Table 3).

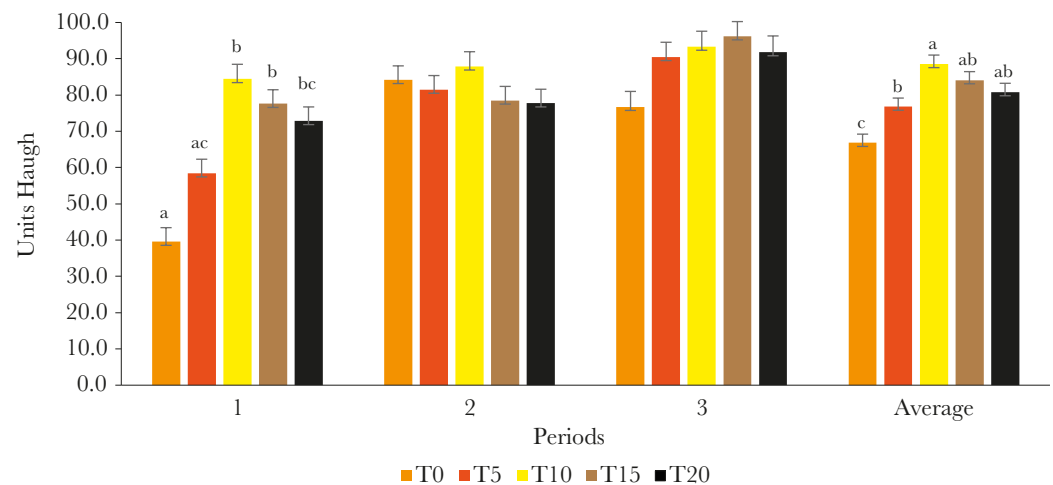


Figure 2. Haugh units of eggs from Creole hens fed diets with different concentrations of chia. The numbers 0, 5, 10, 15, and 20 indicate the level of chia inclusion (%) in the diet. ^{abc}Means with different superscript letters within each period indicate significant differences ($p \leq 0.05$).

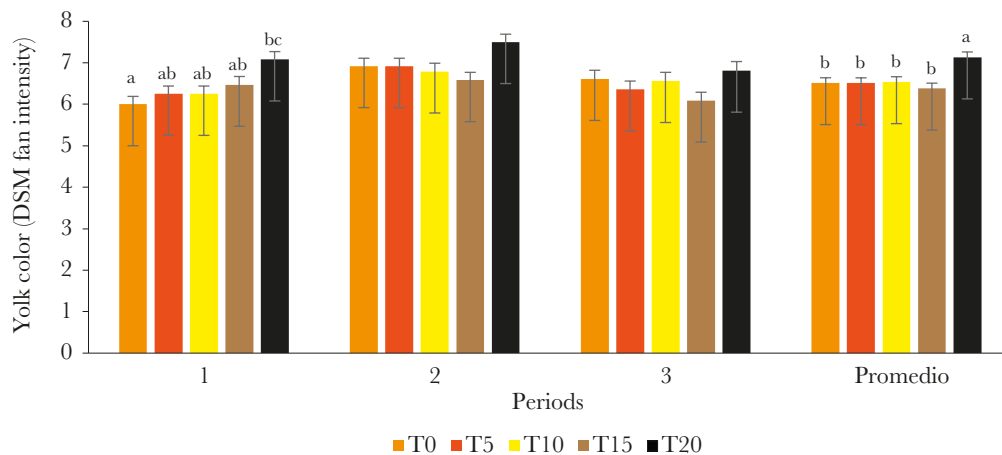


Figure 3. Yolk color expressed as DSM color fan intensity in eggs from Creole hens fed diets with different concentrations of chia. The numbers 0, 5, 10, 15, and 20 indicate the level of chia inclusion (%) in the diet. ^{abc}Means with different superscript letters within each period indicate significant differences ($p \leq 0.05$).

Table 2. Internal egg characteristics of Creole hens fed diets with different concentrations of chia.

	Treatment						Period				P>F		
	T0	T5	T10	T15	T20	EE	1	2	3	EE	Treatment	Period	Treatment × Period
Haugh Units	66.82 ^c	76.80 ^b	88.55 ^a	84.09 ^{ab}	80.79 ^{ab}	2.39	66.57 ^c	81.96 ^b	89.72 ^a	1.78	0.0001	0.0001	<.0001
Yolk (%)	35.62	34.62	35.21	34.67	35.64	0.85	33.39 ^b	36.16 ^a	35.92 ^a	0.61	0.8521	0.0001	0.0459
White (%)	51.64	52.00	51.05	52.23	51.38	1.01	50.34 ^b	50.26 ^b	54.40 ^a	0.75	0.9306	0.0001	0.2815
Yolk Color	6.51 ^a	6.50 ^a	6.53 ^a	6.38 ^a	7.13 ^b	0.12	6.41 ^b	6.94 ^a	6.49 ^b	0.08	0.0018	0.0001	0.2263
Shell Thickness (mm)	0.32	0.34	0.33	0.32	0.32	0.01	0.33	0.34	0.33	0.00	0.4562	0.0415	0.1113

The numbers 0, 5, 10, 15, and 20 indicate the level of chia inclusion (%) in the diet. ^{abc}Means with different literals in each row indicate significant differences ($p \leq 0.05$). SE: Standard error.

Proximal analysis

The results of the proximal analysis of chia seed conducted in this study partially align with those reported by Ayerza and Coates (1999), revealing a higher protein content (23% *vs.* 17%) and lower crude fiber content (9.4% *vs.* 22.1%), while the ether extract was similar (32.2% *vs.* 32.8%).

The average results for feed intake, egg production, egg mass, feed conversion, and laying percentage were similar throughout the 12-week study. This is attributed to the diets having comparable contents of apparent metabolizable energy, crude protein and digestible amino acids, with the exception of the 20% chia diet, which had a lower concentration of amino acids. Average egg weight was higher in hens fed diets containing 10% and 15% chia, followed by those of 20% and 5%, and lowest in the control group (T0). This effect

is linked to the higher content of linoleic and linolenic acids in the chia diets, compounds that, along with amino acids, directly influence egg size (Scott *et al.*, 1976). These findings partially agree with those of Ayerza and Coates (2001), who reported no differences in egg production, yolk weight, or albumen weight; however, no significant differences in egg weight were observed in their study. It is worth noting that this discrepancy may stem from the statistical test used, as Ayerza and Coates employed Duncan's multiple range test, while the present study used Tukey's test. In a later study, Ayerza and Coates (2002) reported that the yolk-to-egg weight ratio was lower in white hens compared to brown hens, regardless of chia inclusion level in the diet. Similarly, Salazar-Vega *et al.* (2009) observed an increase in egg weight with higher chia proportions in the diet of Babcock hens, findings that align with those of the present study.

Eisen *et al.* (1962) indicated that Haugh units are a reliable indicator of internal egg quality, as they reflect albumen height. In the present study, hens fed chia-supplemented diets produced eggs with higher Haugh units compared to the control group. Yolk and white percentages showed similar trends across treatments. Regarding yolk color, an increase in intensity was observed toward the end of the study, measured using the DSM color fan scale, with the most notable enhancement in diets with higher chia inclusion. In contrast, eggshell thickness showed no significant differences among treatments.

CONCLUSIONS

The inclusion of whole chia seed in the diets of Creole hens significantly increased egg weight and improved Haugh units compared to the control diet, without negatively affecting the productive variables evaluated. These results indicate that chia can be used as a functional ingredient in the feeding of Creole hens, contributing to the improvement of internal egg quality without compromising productive performance.

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Effect of chemical fertilization and worm leachate on tomato crop production

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ABSTRACT

Objective: To evaluate the effect of organic and inorganic fertilization on the physiological and productive variables of tomato-mate crops grown under greenhouse conditions.

Design/methodology/approach: A completely randomized design was used with four treatments: T1 (100% worm leachate), T2 (65% worm leachate + 35% nutrient solution), T3 (50% worm leachate + 50% nutrient solution), and a control (100% nutrient solution). The response variables evaluated were plant growth, yield, and fruit size.

Results: Treatment T3 produced the highest yield at 171.6 t ha⁻¹, being significantly superior to the other treatments in fruit size and weight, while T1 showed nutritional limitations in growth and development.

Findings/conclusions: The combination of worm leachate and nutrient solution (T3) reduced the use of chemical fertilizers by 50%, promoting more sustainable agriculture while maintaining high productivity levels.

Keywords: *Solanum lycopersicum* L, organic fertilization, yield, sustainability.

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INTRODUCTION

The intensive production of tomato has led to a strong dependence on chemical fertilization due to its immediate effectiveness in supplying essential macro- and micronutrients. However, a significant proportion of these fertilizers is lost to the environment through leaching or runoff, reducing their availability to plants (Benbi, 2013). This excessive use can also compromise food safety and quality, for example, through nitrate accumulation in plant tissues (Ye *et al.*, 2020). In recent years, the rising costs of synthetic fertilizers and their adverse effects on soil health have renewed interest in organic alternatives. Likewise, the importance of preserving organic matter in agricultural systems has been increasingly recognized, prompting the search for more sustainable and economically viable inputs to meet crop nutritional needs. One promising option is the use of liquid organic materials, such as manure extracts or compost leachates (Preciado-Rangel *et al.*, 2011). In this context, organic agriculture has demonstrated its ability to produce high-quality food without compromising soil or environmental health, thus advancing toward more sustainable systems (Yadav *et al.*, 2013). Among these inputs, vermicompost stands out; its leachate contains bioactive compounds that enhance

economically viable inputs to meet crop nutritional needs. One promising option is the use of liquid organic materials, such as manure extracts or compost leachates (Preciado-Rangel *et al.*, 2011). In this context, organic agriculture has demonstrated its ability to produce high-quality food without compromising soil or environmental health, thus advancing toward more sustainable systems (Yadav *et al.*, 2013). Among these inputs, vermicompost stands out; its leachate contains bioactive compounds that enhance



nutrient availability, stimulate plant development during critical growth stages, and accelerate the formation of flower buds and fruits (Eyheraguibel *et al.*, 2008; Rodríguez-Fernández, 2017). However, organic fertilization alone is often associated with lower yields, making chemical fertilization still necessary to sustain significant production levels. In this regard, integrated nutrient management is proposed as a viable strategy not to eliminate the use of chemical fertilizers entirely, but to reduce their application by combining them with organic sources (Ye *et al.*, 2020). Based on this, the present study aims to evaluate the effect of the combined application of organic and inorganic fertilizers on physiological variables and fruit yield in tomato crops under greenhouse conditions.

MATERIALS AND METHODS

The experiment was conducted during the spring-summer 2023 cycle in the greenhouse of the Colegio de Postgraduados, Montecillo Campus, located in Texcoco, State of Mexico. The greenhouse is equipped with zenithal ventilation, a polycarbonate cover, and lateral walls with anti-aphid mesh. The saladette-type tomato variety ‘Cid F1’, of indeterminate growth, was used. Sowing took place on February 24, 2023, and transplanting on April 12, 2023, when seedlings had four to five true leaves. The substrate consisted of a 50% mix of sheep manure compost and 50% tezontle, placed in 12 L bags, one plant per bag, with drainage holes at the base. Irrigation was applied via a drip system with self-compensating drippers (8 L/h), controlled by a programmable digital timer with eight events. Nutrient management included two main sources. The first was Steiner’s nutrient solution (1984), applied at a concentration of -0.036 MPa during the initial phase and adjusted to -0.072 MPa during the vegetative and reproductive stages. The second source was worm leachate, applied at a rate of 2 L diluted in 20 L of water. An analysis of this fertilizer, conducted at the laboratory of the Universidad Autónoma Chapingo, indicated concentrations of $8,800$ mg L⁻¹ of potassium, 294.5 mg L⁻¹ of organic carbon, and 222.5 mg L⁻¹ of sodium as the main nutritional components. A completely randomized design (CRD) was used for this study, with four treatments consisting of different combinations of Steiner’s nutrient solution (1984) and worm leachate, applied to tomato crops in a hydroponic system. The planting density was three plants per square meter, arranged in a three-row pattern. The concentrations used in each treatment are detailed in Table 1.

Agronomic variables

Plant growth was evaluated using four randomly selected plants per treatment, which were marked and measured for height every eight days, from transplanting until the

Table 1. Specification of the combination of worm leachate with Steiner’s nutrient solution (1984).

Treatments	Nutrient solution combinations
T1	100% worm leachate
T2	65% worm leachate + 35% nutrient solution
T3	50% worm leachate + 50% nutrient solution
TES	100% nutrient solution

formation of the tenth cluster. Yield was also determined in four randomly selected and tagged plants per treatment, monitored throughout the crop cycle. Harvested fruits at maturity were weighed, and their equatorial and polar diameters were measured using a digital caliper. These measurements were conducted on all clusters, and at the end of the experiment, fruits were classified according to the Mexican standard NMX-FF-031-1997-SCFI. The data were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test ($p \leq 0.05$), employing the R statistical software, version 4.3.2 (2023-10-31 ucrt).

RESULTS AND DISCUSSION

Growth and development

The growth and development of the crop followed an exponential pattern over the 108 days after transplanting (DAT), a behavior that was similar across the four treatments evaluated (Figure 1). Plant height exhibited a well-defined vegetative phase up to 65 DAT, after which a slight deceleration in growth was observed, associated with the onset of flowering. From that point forward, growth remained stable until 108 DAT, marking the end of the vegetative stage. No significant differences in height were found among treatments T2, T3, and the control; however, treatment T1 showed slower and significantly lower growth. This outcome is attributed to the fact that nutrition supplied solely by worm leachate was insufficient to meet the physiological demands of tomato cultivation under intensive conditions. These findings partially align with those reported by Reyes-Hernández *et al.* (2023), who observed no significant differences in plant growth at 90 DAT between those treated with organic fertilizers and those fertilized with Steiner's solution. In contrast, Torres *et al.* (2018) reported that organic fertilization favored vegetative development, with significantly greater plant heights compared to chemical fertilization. Such differences may

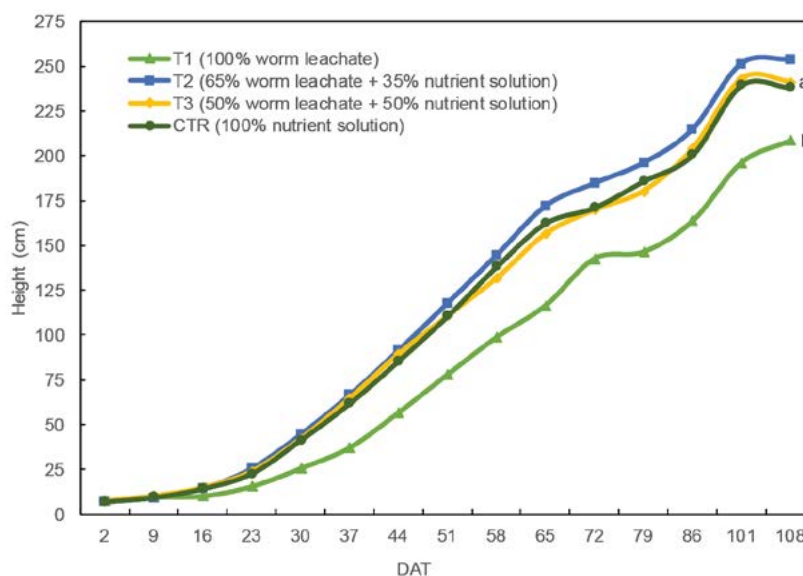


Figure 1. Average height of tomato plants (cv. Cid F1) under four organic and inorganic fertilization treatments over 108 days after transplanting.

be attributed to the capacity of certain organic fertilizers to more evenly meet the crop's nutritional requirements.

Yield

The effect of the treatments on yield is shown in Figure 2. Treatment T3 produced the highest yield, reaching 17.16 kg m^{-2} , a value significantly greater than those of the other treatments. In comparison, T3 surpassed T1, T2, and the control by 6.21 kg m^{-2} , 1.10 kg m^{-2} , and 2.76 kg m^{-2} , respectively. When extrapolated to a per-hectare basis, the yields were 109.5 , 160.0 , 171.6 , and 144.0 t ha^{-1} for T1, T2, T3, and the control, respectively. These values exceed those reported by Estrada-Arellano *et al.* (2023) and Cervantes-Vázquez *et al.* (2024), who achieved yields of 95.22 and 87.3 t ha^{-1} using bovine manure fertilization. However, the yield obtained with T3 was slightly lower than those reported by Mendoza-Pérez *et al.* (2020) and Ruelas-Islas *et al.* (2022), who recorded 19.65 and 19.98 kg m^{-2} , respectively, using chemical fertilization exclusively. These results suggest that the combination of worm leachate and nutrient solution may represent a viable and competitive alternative to the exclusive use of chemical fertilizers.

Fruit size

The classification of fruit size is shown in Figure 3. Treatment T3 achieved the best results, recording the highest percentage of medium and large fruits, meeting national and international standards for commercial distribution. Additionally, T3 had the lowest proportion of small and extra-small fruits compared to the other treatments. These differences were statistically significant, as indicated in Table 2. When comparing T2 with the control, no significant differences were found in the extra-small, medium, and large size categories; however, differences were observed in the small and extra-large sizes. In contrast, treatment T1 was significantly inferior across all size categories, with a high proportion of fruits classified as small. These results indicate that the combination of organic and inorganic fertilizers (T3) significantly improves the commercial size of

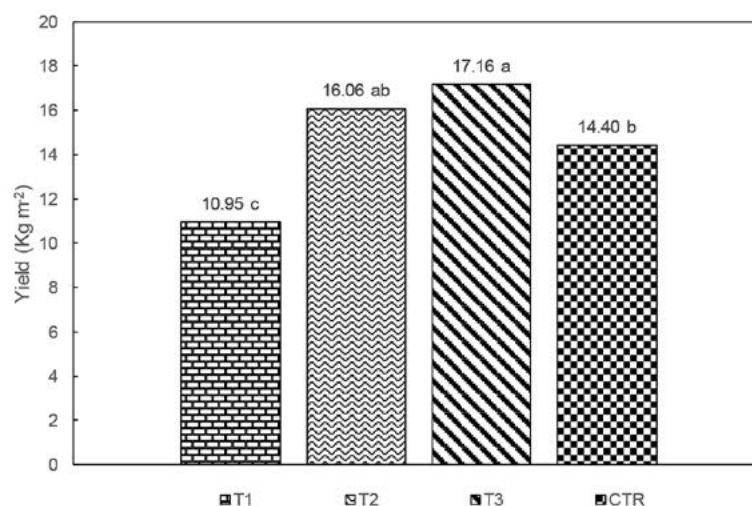


Figure 2. Average tomato yield (cv. Cid F1) under four fertilization treatments.

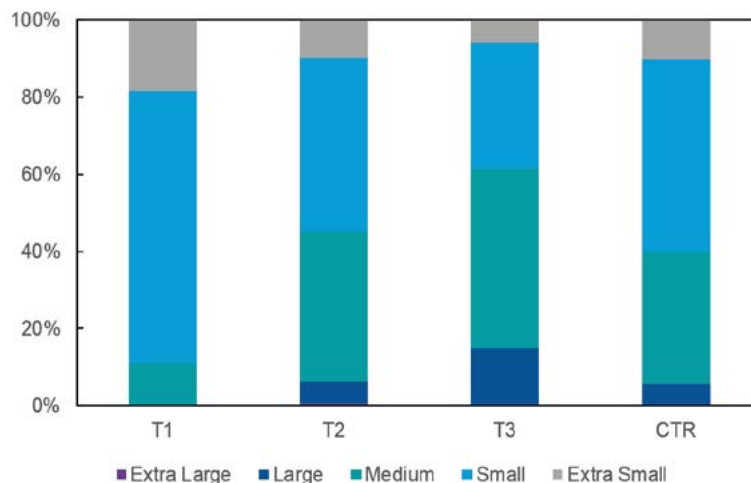


Figure 3. Percentage distribution of tomato fruits (cv. Cid F1) by commercial size under different fertilization treatments.

Table 2. Fruit size classification.

Treatments	Extra small (%)	Boy (%)	Medium (%)	Big (%)	Extra Large (%)
T1	18.40 a	70.52 a	10.85 c	0 c	0.24 b
T2	9.77 b	45.31 c	38.48 b	5.86 b	0.59 a
T3	5.70 c	32.70 d	46.84 a	14.56 a	0.21 b
TES	10.02 b	50.11 b	34.12 b	5.76 b	0 c

Means that do not share a letter are significantly different (Tukey $p \leq 0.05$).

the fruits by reducing the proportion of undesirable calibers. The findings surpass those reported by Martínez-Sías *et al.* (2020) and Estrada-Arellano *et al.* (2023), who used only organic fertilization in tomato cultivation.

CONCLUSIONS

The exclusive application of organic fertilizer (T1) did not result in significant improvements in the agronomic variables evaluated, leading to low yield. Therefore, it is concluded that worm leachate, used as the sole nutrient source, does not meet the nutritional requirements necessary for optimal tomato development. In contrast, the combination of organic and inorganic fertilization significantly improved agronomic variables, with treatment T3 standing out. This strategy allows for a reduction of up to 50% in the use of inorganic fertilizers when aiming to maximize yield, or up to 65% when seeking to maintain an acceptable yield under a more cost-effective approach. This reduction not only represents substantial savings in chemical inputs but also promotes more sustainable and balanced agricultural production in the long term.

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Horticulturists perceive that arbuscular mycorrhizal fungi improve the nutrition of chili plants

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ABSTRACT

Objective: To classify 19 horticulturists based on their perception concerning knowledge (human capital, HC) about the use of arbuscular mycorrhizal fungi (AMFs) and their effects on chili production plots along the agricultural strip region in the state of Zacatecas.

Design/methodology/approach: A questionnaire with 16 statements on technical-productive issues and on the use of AMFs belonging to the HC was designed and applied to 19 chili producers within the Calera, Morelos, Villa de Cos, and Zacatecas municipalities. The data were subjected to a principal components analysis (PCA). A multivariate cluster analysis (CA) was also performed to classify the horticulturists involved through standardized variables and to ratify the groups evidenced by the PCA.

Results: PC1 and PC2 explain 54% and 12.8% of total variance, respectively. The orthogonal plane defined by these PCs identified three groups of horticulturists. The CA allowed visualization of the same three groups as appreciated by the PCA.

Limitations on study/implications: The sample is relatively small because in fact there are still few horticulturists that use AMFs.

Findings/conclusions: The group integrated by the four most productive horticulturists perceives that AMF use (*Trichoderma harzianum* and *Bacillus subtilis*) allows improving chili plant nutrition, soil aeration, and phosphorus plant absorption.

Keywords: Human capital, Principal Components Analysis, Multivariate Cluster Analysis, Likert scale.

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INTRODUCTION

The use of agrochemical products in agricultural production systems contributes to the emission of carbon dioxide (CO₂) (Vermeulen *et al.*, 2012), which affects biodiversity, and the environment (Steffen *et al.*, 2015). The interest in minimizing the use of these products leads to substituting them with microbial inoculants. In general, many microorganisms catalogued as beneficial have the capacity to increase food production when they are used adequately (Singh *et al.*, 2020).

In this sense, the soil microbiota lead between 80% and 90% of edaphic biotic processes (Bajsa *et al.*, 2013). Specifically, arbuscular mycorrhizal fungi (AMFs) form symbiotic associations with plant roots and exchange nutrients such as phosphorus, and nitrogen (Scott, Kiers and West, 2025). Sadhana (2014), Jamiolkowska *et al.* (2018), and Parihar *et al.* (2020) have documented that AMFs improve some soil properties such as water retention, nutrient availability, and carbon, nitrogen and phosphorus cycles. In addition, AMFs induce stress tolerance and resistance to attacks from disease (Klironomos, 2003; Chen *et al.*, 2018). Recently, Scott, Kiers, and West (2025) described that, when a plant is attacked by an herbivore, it can send a signal to other plants connected to the same mycorrhiza network that regulates its defense mechanisms; this translates into the hypothesis that attacked plants produce an alert signal to other plants to prevent damage.

In the case of sweet chili pepper (*Capsicum annum* L.) cultivation, the inoculation with AMFs during seedling production can cause an increase in the concentration of phosphorus in the soil solution and its fast absorption through a larger absorbent surface of the root system due to the mycelium hyphae, which at the same time has positive effects on the yield and quality of the fruits (Franczuk *et al.*, 2023). For example, sweet chili pepper plants inoculated with AMFs presented more fruits, had fruits with thicker pericarp and more mass, leaves with more chlorophyll, stem with greater diameter, root system with more mass, and aerial part with more mass than that corresponding to plants without inoculum (Franczuk *et al.*, 2023). In addition, inoculation with AMFs increases nearly 4 times the production of capsaicin and dihydrocapsaicin in *C. annum* compared to the control (without inoculation) (Canpolat and İşlek, 2023).

In general, the multiple benefits evidenced with a scientific basis are translated into important ecological services (Chen *et al.*, 2018). Consequently, the selection of the best inoculum of AMFs should be carried out for each crop (Rouphael *et al.*, 2015). However, the decision that the farmer makes is a function of the products (AMF complexes) available on the market and of the knowledge that the grower has in that regard. Nevertheless, information about the knowledge, experience and disposition that growers have to adopt the practice of using AMFs in chili production is absent from the literature. Without a doubt, this type of information could be useful to classify the horticulturists, to design strategies for the development of their skills, and to transfer effective technology and innovations. Therefore, the aim was to classify 19 horticulturists based on their perception regarding knowledge (human capital, HC) on the use of arbuscular mycorrhizal fungi (AMFs), and their effects on the chili pepper (*Capsicum annum* L.) production units along the agricultural strip region in the state of Zacatecas, Mexico.

MATERIALS AND METHODS

A questionnaire with 16 statements (appreciated in Table 2) about technical-productive aspects that belong to human capital (HC) was designed and applied to 19 chili pepper growers from the municipalities of Calera, Morelos, Villa de Cos, and Zacatecas. The statements were designed based on the Likert scale and five levels of perception (Likert, 1932). The levels were the following: Totally disagree; Partially disagree; Neither disagree nor agree; Partially agree; Totally agree. The scale allowed us to evaluate the perception

of horticulturists on the use of AMFs in chili production reliably. The level of reliability of the information generated was estimated through the Cronbach's Alpha. A principal components analysis (PCA) was conducted to determine the statements that explained most of the variance of the data matrix. The principal components (PCs) facilitated the identification of the variables (statements) that explained the structure of each component to perform the ordination of the involved growers. A multivariate cluster analysis (CA) was carried out to group the involved horticulturists through standardized variables and to ratify the groups evidenced through the PCAs. The analyses were carried out with the Minitab® 16 software.

RESULTS AND DISCUSSION

The first two PCs explain 66.8% of the total variation of the data matrix (Table 1). The PC1 explains 54% of the total variance and the PC2 12.8%. The PC1 is a compound variable defined by statements CH3, CH4, CH5, CH6, CH7, CH8, CH9, CH10, CH11, CH12, and CH14 (Table 2); this suggests that horticulturists know that AMFs induce root development, homogeneous plant growth, product quality, reduction in the amount of used chemical fertilizers, enrichment of the soil with microorganisms, improvement of soil aeration, improvement of plant nutrition, promotion of tolerance to hydric and nutritional stress, resistance to diseases, reduction of soil contamination caused by chemical fertilizers, and incorporation of nutrients to the soil solution and its faster absorption. The PC2 is dominated by statements CH1, CH2, CH13, CH15 and CH16; this means that some horticulturists perceive that the application of AMFs is a simple task, germination improves with their use, more soil aggregates formed, decreases of loss of phosphorus in the soil through lixiviation, and the application of nitrogen, sulfur, copper and zinc is reduced.

In the orthogonal plane defined by PC2 and PC1, three groups of horticulturists are identified (Figure 1). The first group is made up of survey respondents 10, 12, 14, 18, and 20, who disagree with the statement that the use of AMFs allows for the reduction of used chemical fertilizer; they use complexes of *Glomus intraradices* and *Trichoderma harzianum*. The second group is made up of survey respondents 1, 4, 5, 7, 8, 9, 11, 16, 17, and 19; this group is characterized by the perception that the application of AMFs is a simple task, and the soil is enriched with microorganisms when AMFs are used; these horticulturists disagree with the idea that soil aggregation is improved; the complexes that they use involve *Glomus intraradices*, *Trichoderma harzianum*, and *Bacillus subtilis*. For their part, the

Table 1. Eigenvalues, proportion of variance explained, and accumulated variance explained of the first two Principal Components (PCs) corresponding to 16 statements of human capital about the use of arbuscular mycorrhizal fungi according to the perception of 19 horticulturists.

	PC1	PC2
Eigenvalue	8.642	2.042
Proportion of Variance Explained	0.540	0.128
Accumulated Variance Explained	0.540	0.668

Table 2. Coefficients of correlation (loadings) between the first two Principal Components (PCs) and the 16 statements or variables of human capital on the use of arbuscular mycorrhizal fungi (AMF) according to the perception of 19 horticulturists.

Variable		Principal Component	
		CP1	CP2
HC1	I am convinced AMF application is simple.	0.194	0.377
HC2	I know that use of AMF improves germination.	0.002	-0.537
HC3	I know the effect of AMF on roots.	0.307	0.034
HC4	I know plants grow in an homogeneous form when using AMF.	0.258	-0.081
HC5	I perceive plant products improves their quality when using AMF.	0.318	-0.025
HC6	I know use of AMF allows diminishing chemical fertilizers needs.	0.251	0.235
HC7	I know use of AMF enriches soil microbiota.	0.296	0.051
HC8	I know use of AMF improves soil aeration.	0.248	0.037
HC9	I know use of AMF improves plant nutrition.	0.290	0.119
HC10	I know use of AMF promotes plant hydric and nutrient stress tolerances.	0.295	-0.103
HC11	I have observed use of AMF provides plant disease resistance.	0.281	-0.032
HC12	I know use of AMF reduces soil contamination by chemical fertilization effect.	0.231	-0.147
HC13	I know use of AMF increases soil aggregation formation.	-0.172	0.404
HC14	I perceive use of AMF improves nutrients soil incorporation and plant absorption.	0.298	0.125
HC15	I perceive use of AMF reduces soil phosphorus losses.	0.196	-0.331
HC16	I know use of AMF reduces nitrogen, sulfur, copper and zinc fertilization needs.	0.166	0.407

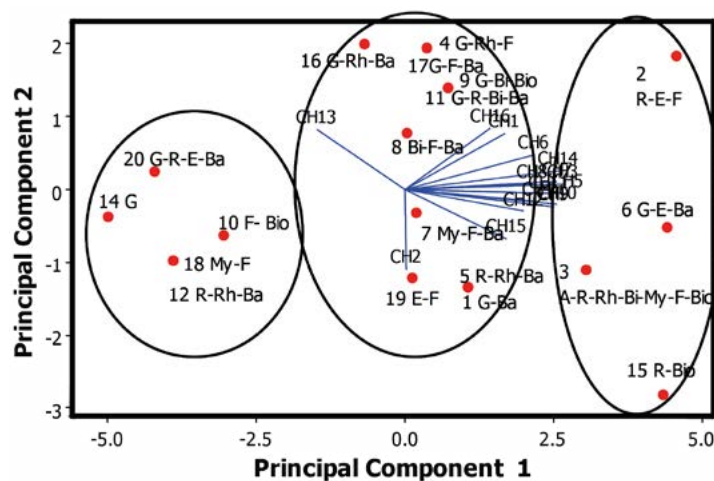


Figure 1. Position of the 19 horticulturists and the 16 statements of human capital (CH) on the use of arbuscular mycorrhizal fungi in the orthogonal plane defined by the first two extracted Principal Components.

third group is integrated by the rest of the horticulturists (2, 3, 6, and 15), who perceive that plant nutrition improves with the use of AMFs; they use complexes of *Trichoderma harzianum*, and *Bacillus subtilis*.

The CA through the Ward's method and the Manhattan distance at a similarity = -47.99 allowed us to ratify the three groups (Figure 2), previously evidenced through the PCAs. The first group of 5 horticulturists disagrees with the statement that the use of AMFs allows the reduction of used chemical fertilizer. This result differs from what was pointed out by Chen *et al.* (2018), who noted that the application of AMFs contributes to decreasing the amount of fertilizers incorporated into the soil without affecting the yield; this could be attributed to the fact that AMFs release phosphatases to hydrolyze the phosphate of the organophosphorus compounds (Marschner, 2011); if this process takes place, the productivity of the crops can improve (Smith *et al.*, 2011). Likewise, the AMFs are important to increase the absorption of ammonium and micronutrients that have scarce mobility in the soil, such as copper and zinc (Smith and Read, 2008).

The second group is made up of 10 growers and characterized because they perceive that the application of AMFs is a simple task and that with their use, the soil is enriched with microorganisms. This result agrees with what is described by Larkin (2008), and Vestberg *et al.* (2011), who describe that the density and diversity of AMFs tend to increase; this could be attributed to the rotation of crops have a significant impact on the populations and activities of the AMFs (Rouphael *et al.*, 2015).

The third group is made up of 4 horticulturists; they perceive that with the use of AMFs the plants' nutrition improves. This result agrees with that described by Chen *et al.* (2018), who pointed out that AMFs contribute to the nutrition of plants regarding the absorption and translocation of mineral nutrients; this could be attributed to the fact that AMFs interfere with the phytohormonal balance of host plants and influence the development of plants (bioregulators) (Rouphael *et al.*, 2015).

In general, the perception of the involved horticulturists, segmented into three groups, confirms that the application of AMFs is a simple task, and that with their use the soil is enriched with microorganisms. They use complexes that involve at least two of the species *Glomus intraradices*, *Trichoderma harzianum*, and *Bacillus subtilis*, which has allowed them to understand that they can reduce the use of chemical fertilizers, and improve the nutritional

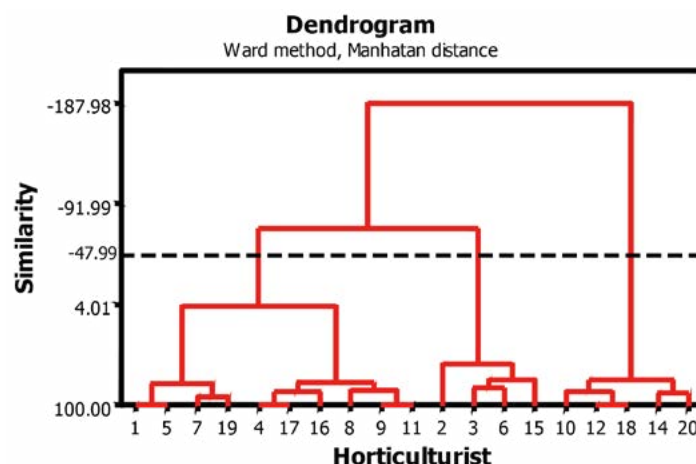


Figure 2. Dendrogram of the 19 horticulturists when considering their perception on human capital regarding the use of arbuscular mycorrhizal fungi based on 16 statements.

condition of plants with the intention of increasing their chili pepper (*C. annum*) yield and quality. In other words, chili pepper growers from the agricultural strip region in Zacatecas have knowledge in accordance with the scientific knowledge generated regarding the use of AMFs as inoculum, particularly what was described by Klironomos (2003); Smith and Read (2008); Sadhana (2014); Chen *et al.* (2018); Jamiolkowska *et al.* (2018); Parihar *et al.* (2020); Franczuk *et al.* (2023) and Scott, Kiers and West (2025), among others.

CONCLUSIONS

The four most productive horticulturists (2, 3, 6, and 15) perceive that the use of AMFs (*Trichoderma harzianum*, and *Bacillus subtilis*) improves the nutrition of the chili pepper plants, the aeration of the soil, and the absorption of phosphorus. In addition, they also perceive that, with the use of AMFs, production costs decrease from minimizing the use of inorganic fertilizers as sources of nitrogen, phosphorus, sulfur, copper, and zinc. Therefore, joint work among horticulturists, technical advisors, public servants, and input suppliers on the use of AMFs in chili production is advisable; this can contribute to the improvement of soil conservation programs with an integral approach to develop capacities regarding the use of AMFs.

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Phytochemicals, antioxidants, and oxidative stress in ruminant reproduction

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ABSTRACT

Objective: To review the importance of phytochemical compounds with antioxidant capacity in improving various reproductive processes in ruminants.

Methodology: A review of scientific literature was conducted to identify phytochemical compounds reported to positively influence ruminant reproduction, as well as to analyze the concepts of antioxidants and oxidative stress and their impact on reproductive function.

Results: Research has demonstrated that natural antioxidants found in certain plants contain various phytochemical compounds that strengthen the immune system. These compounds possess antibacterial, antiseptic, tonic, and antioxidant properties that help reduce oxidative stress, improving outcomes during gestation and parturition. Additionally, they enhance the vitality of newborns.

Limitations: Although promising results have been reported, limited research exists on certain phytochemicals with antioxidant properties that may benefit ruminant reproduction.

Conclusions: Phytochemicals with antioxidant properties neutralize free radicals, restoring balance in the endocrine, metabolic, and immune systems, and reducing oxidative stress on reproductive function.

Keywords: Oxidative stress, antioxidants, phytochemicals, reproduction.

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INTRODUCTION

In animals, the reproductive process involves the generation of free radicals (FR); however, when harmful endogenous or exogenous stimuli increase FR levels beyond the body's antioxidant capacity, oxidative stress occurs. This condition can trigger multiple cellular dysfunctions, adversely affecting fertility and reproductive performance in both females and males (Mayorga, 2015). The body possesses antioxidant defense systems composed of enzymes and other molecules whose role is to neutralize FR. Antioxidants are classified as enzymatic and non-enzymatic. The former represent the first line of endogenous defense, while the latter are primarily derived from the diet. Unlike enzymatic



antioxidants, non-enzymatic ones lose their antioxidant capacity after participating in redox reactions and must be constantly replenished. Notable examples include vitamins E and C, retinol, β -carotenes, uric acid, albumin, pyruvate, polyphenols, and flavonoids (Gumbao, 2015). In recent years, the use of natural products as additives and supplements in animal feed has increased, both to improve productive efficiency and to replace synthetic drugs and additives. Some of these products have antioxidant properties that may enhance the endogenous antioxidant system. Dietary antioxidant compounds play a crucial role in mitigating oxidative stress. The plants used to obtain these products contain active ingredients widely employed in traditional human and veterinary medicine (Carro *et al.*, 2006). These bioactive compounds, known as secondary metabolites, serve ecological functions in plants, including protection against ultraviolet radiation, adaptation to water stress, defense against herbivores, and attraction of pollinators through chemical signaling (Greathead, 2003; Silva *et al.*, 2021). It has been reported that plants may contain between 20 and 80 phytochemicals at varying concentrations, mainly monoterpenes and sesquiterpenes. However, aliphatic hydrocarbons, acids, alcohols, and esters are also present in smaller amounts (dos Santos *et al.*, 2019). These phytochemicals confer antioxidant, antibacterial, antifungal, and tonic properties (Lahlou, 2004).

Oxidative stress and reproduction

Oxidative stress is defined as an imbalance between the generation of free radicals (FR) and the body's ability to eliminate them, disrupting redox homeostasis (Sies *et al.*, 2017). Free radicals are small, unstable, and highly reactive molecules due to the presence of unpaired electrons; they diffuse easily and act as intermediaries in various biochemical reactions (Daenen *et al.*, 2019). The main types of radicals include reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Miller *et al.*, 1990). Under physiological conditions, FRs play crucial roles in host defense, cellular signaling, and biosynthetic processes. However, under internal or external adverse stimuli, their production may increase excessively. If not neutralized promptly, their accumulation can exceed physiological levels, leading to structural and functional cellular damage, such as disruptions in signaling pathways, metabolic imbalances, genetic mutations, and alterations in protein structure (Rahal *et al.*, 2014). In reproductive terms, oxidative stress can impair critical processes, including oocyte maturation, ovarian steroidogenesis, ovulation, embryonic implantation, blastocyst development, luteolysis, and corpus luteum maintenance (Leal *et al.*, 2011). It has also been linked to reproductive disorders such as infertility, spontaneous abortion, and premature membrane rupture (Mora *et al.*, 2019). In ruminants, blastocyst hatching from the zona pellucida occurs approximately seven days before implantation and is associated with increased ROS production (Aurousseau *et al.*, 2006). From conception through day 140 of gestation, a progressive increase in ROS has been observed, promoting cellular apoptosis and potentially halting embryonic development (Massman *et al.*, 1999; Moreira da Silva *et al.*, 2010). During early pregnancy, the intrauterine environment is considered hypoxic. Initially, the placenta relies on histotrophic nutrition and acquires oxygen through maternal circulation, thereby generating a concentration gradient between the mother and the fetus. The impact of oxidative stress in this context depends on the degree of oxygen

tension change and the effectiveness of the placental antioxidant system. Therefore, oxidative stress may have either physiological or pathological effects depending on specific conditions (Leal *et al.*, 2011). In ruminants, maintaining an adequate antioxidant status before and during gestation is crucial for optimal placental development and function, which helps reduce embryonic mortality, facilitate uncomplicated deliveries, and ensure neonatal vitality (Chavatte-Palmer *et al.*, 2008).

Antioxidants

Cells possess defense mechanisms that transform free radicals (FR) into less toxic or even harmless compounds. This cellular protection against FR includes the prevention of their formation, the inhibition of their propagation, and the repair of the damage they cause (González *et al.*, 2000). A second level of protection is provided by antioxidants, which neutralize FR and suppress their harmful activity within the cell. These antioxidants are classified into two major groups: enzymatic and non-enzymatic. Enzymatic antioxidants comprise three main systems: superoxide dismutase, catalase, and glutathione peroxidase. These enzymes are synthesized endogenously and can neutralize free radicals, interrupting the lipid peroxidation chain reaction due to their ability to donate hydrogen ions (Márquez, 2001) efficiently. Non-enzymatic antioxidants, on the other hand, function by binding to FR, displacing them from critical cellular sites to compartments where their effects are less damaging, or converting them into less reactive radicals. Key compounds in this group include α -tocopherol, β -carotene, ascorbic acid, glutathione, urate, flavonoids (Lawrence & Bendich, 1987), and various phenolic compounds (Burdock, 2005).

Phytochemicals

In addition to endogenous defense mechanisms, a wide variety of natural molecules with antioxidant properties can strengthen the body's antioxidant system. Among these, phytochemicals stand out—bioactive compounds found in seeds, cereals, fruits, vegetables, leaves, roots, spices, and herbs (Skerget *et al.*, 2005). These compounds play a key role in protecting plants against various biotic and abiotic stresses, including pathogens, herbivores, ultraviolet radiation, water stress, and salinity. Phytochemicals encompass a wide range of compounds, including carotenoids, phenols, flavonoids, stilbenes, coumarins, tannins, alkaloids, nitrogenous compounds, and organosulfur compounds (Hall & Cuppett, 1997) (Figure 1). Their importance in animal production has grown due to their well-established antioxidant activity. Oxidative stress induced by free radicals has been linked to numerous diseases and reproductive disorders. In this context, plant-derived products with antioxidant capacity, such as plant extracts, have been proposed as promising natural additives for use in ruminant nutrition.

Phytochemicals with antioxidant activity can act through various mechanisms, depending on the reaction system and the nature of the radical or oxidant involved (Prior *et al.*, 2005). The effectiveness of these compounds is often expressed as antiradical activity or IC₅₀, defined as the concentration of antioxidant required to reduce the radical's absorbance by 50% relative to the initial value (Floegel *et al.*, 2011). This value is commonly used to estimate the radical-scavenging capacity against compounds such as 2,2'-azino-

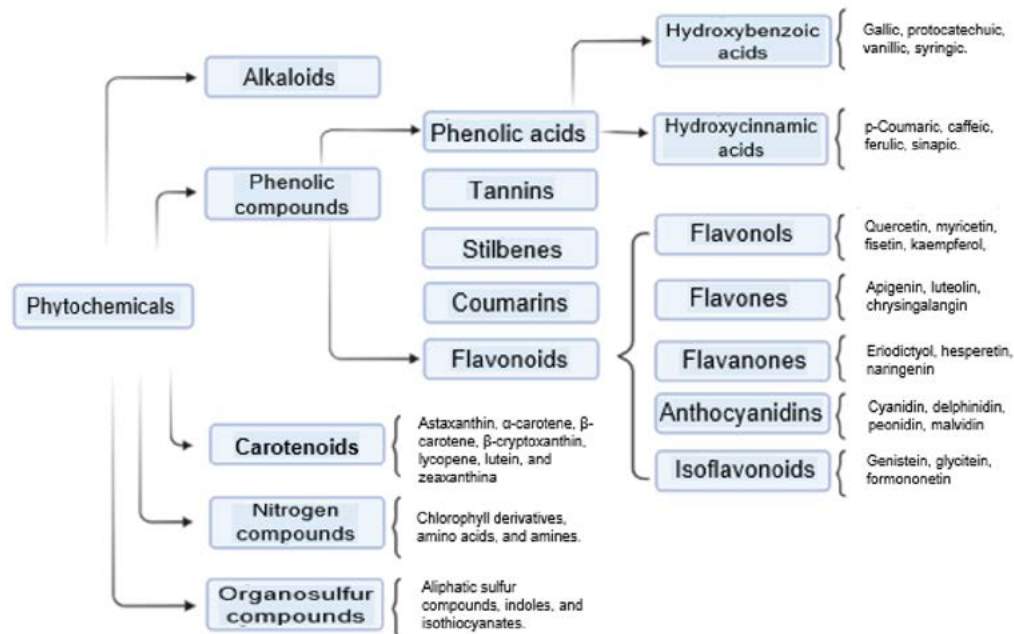


Figure 1. Leading groups of natural phytochemicals (Liu, 2003).

bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS • +) or 2,2-diphenyl-1-picrylhydrazyl (DPPH •), comparing it to standard antioxidants. However, the antioxidant capacity of plant products also depends on the extraction method and the concentration of phytochemicals present (Castillo *et al.*, 2007). León *et al.* (2015) reported higher antioxidant activity in the essential oil of *Plectranthus amboinicus* (French oregano) obtained through conventional hydrodistillation compared to that obtained through microwave-assisted hydrodistillation. When evaluating three methods to estimate antioxidant capacity (DPPH •, ABTS • +, and ORAC), the lipophilic ORAC method yielded the highest antioxidant activity values, highlighting a result of 682,952.60 μmol trolox/100 g of sample, significantly higher than that of many oils, fruits, and vegetables (Apak *et al.*, 2007; Rautenbach & Venter, 2010). Similarly, Muñoz *et al.* (2007) (Table 1) analyzed the chemical composition and antioxidant capacity of six plant species: thyme, common oregano, wild oregano, Castilian oregano, creeping oregano, and marjoram. All essential oils evaluated exhibited high levels of thymol and carvacrol, with antioxidant capacities comparable to those of standard synthetic antioxidants, such as BHA, BHT, and α -tocopherol, as measured by the ABTS • + radical cation scavenging assay.

Arango *et al.* (2012) evaluated the antioxidant activity of the essential oil of *Lippia origanoides* (EO) using spectrophotometric methods based on DPPH • and ABTS • + radicals. The effective concentrations (EC50) at 50% values obtained for the EO and BHT (butylated hydroxytoluene, a reference synthetic antioxidant) were 5.58 and 1.33 mg/mL, respectively. In contrast, the Trolox Equivalent Antioxidant Capacity (TEAC) values were 0.33 mg/ml for the EO and 0.68 mg/ml for BHT. These results suggest that wild EO exhibits a significant ability to stabilize free radicals, comparable to that of synthetic antioxidants and even superior to other essential oils from the same species. Plant-based

Table 1. Total Antioxidant Activity (TAA) values for reference substances and essential oils with high thymol and carvacrol content, based on ABTS radical cation scavenging capacity.

Substance	TAA (mmol Trolox kg ⁻¹ SE) Averages
α -Tocopherol	2590 ± 33
BHT	990 ± 31
BHA	6900 ± 487
Wild orégano	2040 ± 21
Castile orégano	670 ± 24
Thyme	890 ± 32
Common orégano	800 ± 24

Total Antioxidant Activity (TAA); mmol Trolox kg⁻¹ - Test substance (Muñoz *et al.*, 2007).

products rich in phenylpropanoid-type compounds, such as those found in species of the *Lippia* genus, have demonstrated antioxidant performance superior to many conventional essential oils. Various assessment methods indicate that their antioxidant capacity may be up to five times greater than that of commonly used synthetic compounds in the food and pharmaceutical industries, such as BHT and α -tocopherol (Stashenko *et al.*, 2014).

Antioxidant phytochemicals in reproduction

Naturally, the body has developed an antioxidant system to protect itself when there is an imbalance between free radicals (FR) and antioxidants. When this balance shifts toward an excess of reactive oxygen species (ROS), oxidative stress occurs. Free radicals play a significant role in pathological processes that affect the female reproductive system, influencing various physiological functions—from oocyte maturation and fertilization to embryonic development and gestation (Agarwal *et al.*, 2005). The fetus and placenta are protected by an antioxidant defense system that prevents lethal effects caused by high rates of FR production. Glutathione (GSH) production and metabolism are crucial for preventing gestational pathologies, not only due to its ability to scavenge free radicals but also for its role in maintaining intracellular redox balance. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) form a group of specialized enzymes that defend against ROS (Aurousseau *et al.*, 2006). However, when external factors weaken the antioxidant system, reproductive performance may be negatively affected. Atlagich (2006) administered antioxidant vitamins C and E to pregnant ewes as a preventive measure against hypobaric hypoxia. A hypoxic environment triggers oxidative stress, evidenced by increased oxidative damage and reduced antioxidant capacity. Morphological changes observed in the placenta revealed it as the most affected organ, resulting in reduced nutrient transport efficiency to the fetus, growth retardation, and impaired postnatal development. Supplementation with vitamins C and E improved redox balance and reversed fetal growth and placental morphological differences. Furthermore, supplementation during lactation enhanced lamb development.

According to Lizárraga *et al.* (2017), supplementation with minerals (Cu: 50 mg, Zn: 200 mg, Mn: 50 mg, and Se: 25 mg) along with vitamins A and E (3,150 IU and 250 IU, respectively), administered to cows during intravaginal device placement, prevented a drop in antioxidant capacity and the oxidative damage typically associated with the physiological state. This strategy proved useful in mitigating the detrimental effects of oxidative stress on fertility and preventing reduced conception rates in reproductive protocols. Nieto (2009) evaluated the effects of supplementing 10% and 20% distilled rosemary (DRL) and thyme leaves (DTL), as well as 3.7% and 7.5% fresh thyme leaves (FTL), to pregnant ewes during gestation, parturition, and lactation. The quality of fresh lamb meat was analyzed under modified atmosphere packaging and aerobic cooking conditions, with storage at 4 °C. Diets containing FTL reduced lipid and pigment oxidation, improved the sensory attributes of the meat, and limited the growth of spoilage microorganisms in fresh cuts. Regarding the role of β -carotene in the endocrine and reproductive axis of goats, it has been shown that this molecule regulates ovarian activity both during the reproductive season and in anestrus, and modulates the secretion of growth hormone (GH) in adult females and luteinizing hormone (LH) in yearlings (López, 2021). In synchronization protocols, antioxidant phytochemical supplementation in bovine females has been shown to have positive effects, enhancing reproductive potential and improving outcomes in reproductive biotechnologies such as follicular aspiration and in vitro embryo production (Brem *et al.*, 2001; Agudelo & Medina, 2022). Madureira *et al.* (2020) found that Holstein cows with higher plasma concentrations of beta-carotene exhibited greater fertility and fewer gestational issues. β -carotene, as an antioxidant phytochemical, helps protect uterine and embryonic cells from lipid peroxidation, promoting embryo implantation and survival. It also prevents inactivation of the cytochrome P450 enzyme, which is responsible for converting cholesterol into hormones, thereby enhancing corpus luteum size and increasing serum progesterone levels. Overall, phytochemicals with antioxidant potential have proven to be an excellent alternative for stabilizing abrupt changes in metabolic, endocrine, and immune systems during reproduction in both females and males, significantly reducing oxidative stress across various reproductive stages.

CONCLUSIONS

The use of plant-based products, such as phytochemicals with antioxidant potential, in ruminant nutrition represents a promising alternative for reducing oxidative stress conditions that occur during conception, gestation, and parturition, affecting both the dam and the offspring. Studies on the antioxidant activity of these compounds have demonstrated their potential as powerful natural antioxidants, with the ability to enhance various reproductive aspects in ruminant animals.

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Empirical analysis of agricultural crop diversification in Irrigation District 053, State of Colima

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ABSTRACT

Objective: To examine the potential of crop diversification as a risk management strategy in the 053 State of Colima Irrigation District during the 1997/98-2021/22 period.

Design/methodology/approach: The relationship between yield, price, and income risk levels and the degree of diversification in annual agricultural portfolios for the autumn-winter (A-W), spring-summer (S-S) cycles, and perennial crops was analyzed. Diversification was measured using the Herfindahl Index (HI). Risk was interpreted as the instability of the variables, represented by the standard deviation and calculated using Markowitz's mean-variance methodology.

Results: It was found that agricultural portfolio diversification is a useful strategy to reduce price risk in both agricultural cycles, as well as yield and income risk in perennial crops (significantly positive correlations). However, it proved unfavorable for managing these latter two risks in the S-S cycle (considerably negative correlations). It was also observed that in all three crop groups, income risk is primarily determined by price variation (market risk).

Study limitations/implications: The results lose validity when the elements of the variance-covariance matrix in the irrigation district change. The analysis can be complemented by calculating the optimal (analytical) portfolio for each crop group.

Findings/conclusions: It is concluded that, in order to contribute positively to income stability in the irrigation district, special attention should be given to managing price fluctuations (market risk) across the three crop groups and to increasing diversification, particularly in perennial crops.

Keywords: agricultural portfolios, Herfindahl index, risk management.

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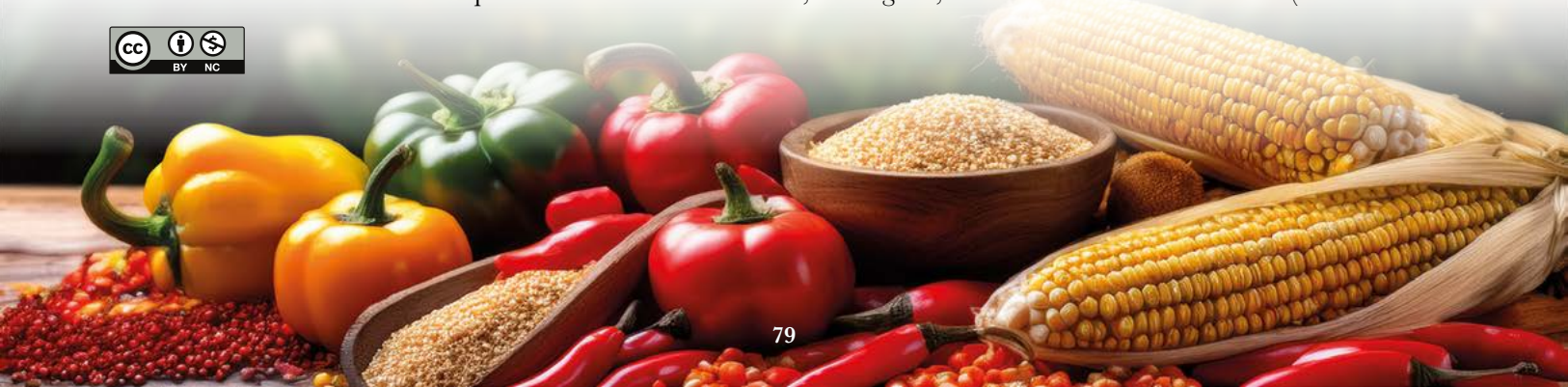
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INTRODUCTION

Globally, the agricultural sector experiences significant annual losses and damages in production due to climatic, biological, and environmental factors (United States



Department of Agriculture [USDA], 2021; Food and Agriculture Organization of the United Nations [FAO], 2021). Disruptions in production (yields) and market conditions (prices) caused by these factors lead to pronounced income instability for producers, thereby compromising their economic, productive, and social stability (Duncan & Myers, 2000), as well as the sustainability and prosperity of the agri-food sector, particularly in countries with higher poverty levels (FAO, 2023). In this context, and considering that globally, the majority of people living in extreme poverty (around 80%) reside in rural areas of developing countries and rely primarily on agriculture for their livelihood (FAO, 2021), the implementation of policies, mechanisms, and strategies that offer greater certainty, stability, and economic resilience to producers becomes a top priority (Solano-Alonso *et al.*, 2021). Crop diversification is considered a logical, feasible, and necessary alternative for mitigating risks and uncertainty in farming operations (Deshpande *et al.*, 2007; Khanam *et al.*, 2018; Mango *et al.*, 2018). However, in Mexico, empirical studies clearly demonstrating its benefits remain scarce. The aim of this research was to explore the potential of crop diversification as a risk management mechanism in the 053 Irrigation District, State of Colima, during the 1997/98-2021/22 period. Additionally, trends in diversification, income risk, and expected income in the district were analyzed, and the historical minimum income risk portfolio was identified for the autumn-winter and spring-summer cycles, as well as for perennial crops. The results of this analysis are expected to serve as a reference for producers in the district and neighboring regions to make more efficient allocations of their productive resources.

MATERIALS AND METHODS

Data

The data used for this analysis were obtained from the agricultural statistics section of the irrigation districts, published annually by the National Water Commission (CONAGUA) in Microsoft Excel format. From this dataset, information corresponding to the 053 Irrigation District in the State of Colima was extracted, covering the 25 available agricultural cycles (1997/98-2021/22). The variables considered were: a) area planted (ha), b) yield (tons/ha), and c) rural average price (thousand MX\$/ton). Based on variables b and c, a fourth variable was generated: d) income (thousand MX\$/ha), calculated as the product of yield and rural average price.

Both the rural average price and income were deflated and expressed in real terms using the National Consumer Price Index (INPC%) provided by the Economic Information Bank of the National Institute of Statistics and Geography (BIE-INEGI), with 2018 as the base year.

The degree of diversification of annual agricultural portfolios was measured using the Herfindahl Index (HI), mathematically defined as:

$$IH = \sum_{i=1}^n p_i^2 = \sum_{i=1}^n \left(\frac{S_i}{S_T} \right)^2 \quad (1)$$

Where p_i is the proportion of the area planted with the i -th crop that makes up the agricultural portfolio, defined as the area under each crop (S_i) between the total cultivated area ($S_T = \sum_{i=1}^n S_i$). Of course $\sum_{i=1}^n p_i = 1$ (Mukherjee, 2012).

The IH takes values between zero and one ($0 < IH < 1$). A value close to zero ($IH \rightarrow 0$), indicates a relatively high level of diversification ($i \rightarrow \infty$), while a value close to unity ($IH \rightarrow 1$) implies a relatively low level of diversification ($i \rightarrow 1$). The unit ($IH = 1$) evidently means perfect specialization ($i = 1$) (Malaisamy, 2021; Kaur *et al.*, 2021). The reciprocal of the IH (IH^{-1}) reflects the number of crops on which an agricultural portfolio is concentrated.

Expected Income and Risk

The expected income and risk levels of the annual agricultural portfolios were calculated using Markowitz's (1952) mean-variance model. The expected income was determined using the following expression ($E[\underline{I}_P]$) (Avilés Cano *et al.*, 2006).

$$E(\underline{I}_P) = p_1 E(I_1) + p_2 E(I_2) + \dots + p_n E(I_n) = \sum_{i=1}^n p_i E(I_i) \tag{2}$$

With the following conditions: $0 \leq p_i \leq 1$ and $\sum_{i=1}^n p_i = 1$; and $E(I_i)$ defined as (Díaz-Carreño *et al.*, 2007):

$$E(I_i) = \underline{I}_i = \frac{1}{m} \sum_{j=1}^m I_{ij} \tag{3}$$

For its part, the income risk of the portfolios was represented by the variance of their generated income ($Var[\underline{I}_P]$) (Díaz-Carreño *et al.*, 2007), calculated as:

$$\sigma_P^2 = Var\left(\sum_{i=1}^n p_i E(I_i)\right) = \sum_{i=1}^n \sum_{j=1}^n p_i p_j \sigma_{ij} \tag{4}$$

Provided that $i = j$, σ_{ij} represents the variance of the income of each of the crops that make up the portfolio and when $i \neq j$, σ_{ij} is the covariance of income between crop i and crop j (Avilés Cano *et al.*, 2006).

The above equation can be rewritten as:

$$\begin{aligned} \sigma_P^2 &= Vp_{n,1}' \cdot \Omega_{n,n} \cdot Vp_{n,1} \\ &= [p_1 p_2 \dots p_n] \cdot [\sigma_{11} \sigma_{12} \sigma_{21} \sigma_{22} \dots \sigma_{1n} \dots \sigma_{2n} \dots \sigma_{n1} \sigma_{n2} \dots \dots \sigma_{nn}] \cdot [p_1 p_2 \dots p_n] \end{aligned} \tag{5}$$

Where $Vp_{n,1}$ is the vector of shares of each crop, $Vp_{n,1}'$ is the aforementioned vector but transposed; and $\Omega_{n,n}$ is the variance and covariance matrix of income $[\sigma_{ij}]$ among the crops in the portfolio.

The solution to equation five was performed in Microsoft Excel using the following function:

$$\sigma_p^2 = MMULT(TRANSPONER(Vp_{n,1}), MMULT(\Omega_{n,n}, Vp_{n,1})) \tag{6}$$

RESULTS AND DISCUSSION

Crop diversification analysis

Figure 1 shows the degree of diversification in the annual agricultural portfolios observed in the 053 Irrigation District, State of Colima, during the analysis period (1997/98-2021/22).

As shown, the annual portfolios of perennial crops are generally the most diversified (or least concentrated), with a virtually stable trend over the analyzed period. According to the average Herfindahl Index value (HI=0.1994), the area planted with perennial crops is, on average, distributed among five crops. In contrast, the least diversified (or most concentrated) portfolios correspond to the spring-summer (S-S) cycle. Based on the average HI for these portfolios (0.3692), the planted area is concentrated, on average, in 2.7 crops. Moreover, the standard deviation of the HI in this cycle (13.13%) indicates higher variability in crop diversification over time compared to the autumn-winter (A-W) and perennial cycles, which show standard deviations of 10.93% and 1.84%, respectively. The degree of diversification in A-W portfolios typically falls between the values observed for the other two groups, with an average HI of 0.3247, suggesting that the planted area is, on average, distributed among three crops. It is worth noting that the HI of the cyclical crop portfolios (A-W and S-S) shows an increasing trend over time ($HI \rightarrow 1$), reflecting a progressive concentration of the planted area in fewer crops—in other words, a clear trend toward specialization.

Analysis of expected revenue and revenue risk

F-I Cycle

Figure 2 presents the expected income ($E[L_p]$) and income risk levels (σ_i) of the annual agricultural portfolios observed in the FY cycle.

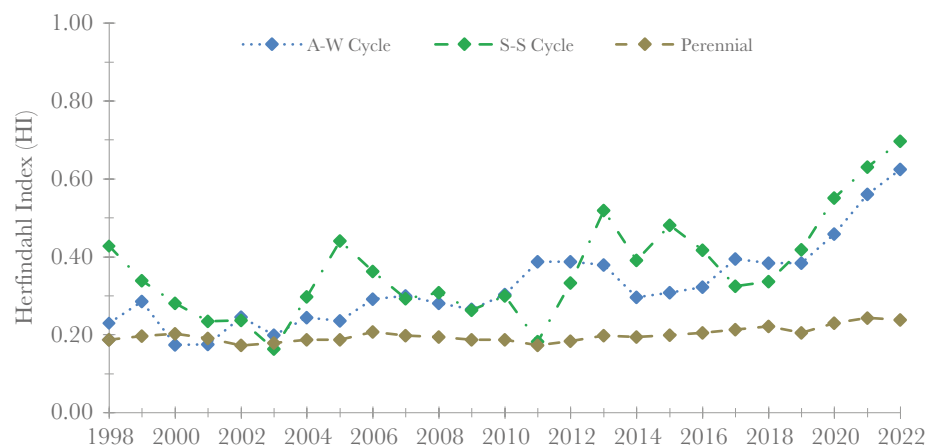


Figure 1. Herfindahl Index of the 053 Irrigation District, State of Colima (1997/98-2021/22).

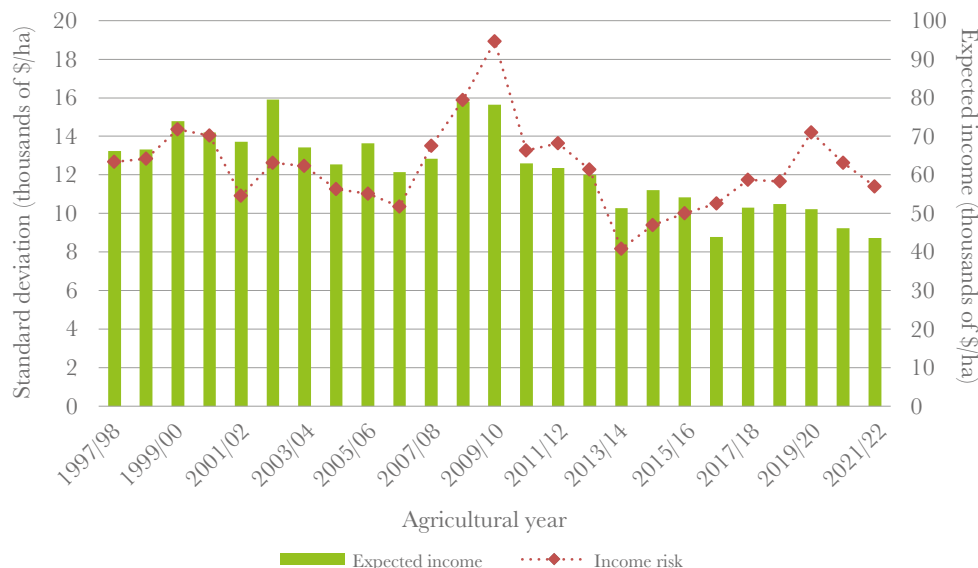


Figure 2. Expected income and income risk in the O-I cycle of DR 053.

As can be observed, the expected income and income risk level of the agricultural portfolios corresponding to this cycle show a moderately positive association, with a correlation of 57.81%. Additionally, a slight downward trend is noted over time. The minimum income risk portfolio for this cycle corresponds to the 2013/14 agricultural year, with its characteristics presented in Table 1.

P-V Cycle

As shown in Figure 3, the expected income and income risk of agricultural portfolios in the P-V cycle exhibit a strong positive correlation (96.28%) and a clear downward trend over the analyzed period. The minimum income risk portfolio identified in this cycle

Table 1. Portfolio of minimum risk of entry in the O-I cycle of DR 053 Edo. Colima.

Agricultural year	Assets	Surface (ha)	Pi (%)	Risk (σ_i)	$E(I_p)$	IH
2013/14	Chile	175	7.13	8.166	51.361	0.297
	Tomato	9	0.37			
	Sweet Corn	1,109	45.21			
	Forage Corn	5	0.20			
	Grain Corn	697	28.41			
	Melon	106	4.32			
	Cucumber	96	3.91			
	Watermelon	27	1.10			
	Forage Sorghum	90	3.67			
	Grain Sorghum	91	3.71			
	Tomato	48	1.96			
	Total	2,453	100.00			

Note: P_i =share; σ_i =standard deviation of income (thousands of \$/ha); $E(I_p)$ =expected income (thousands of \$/ha).

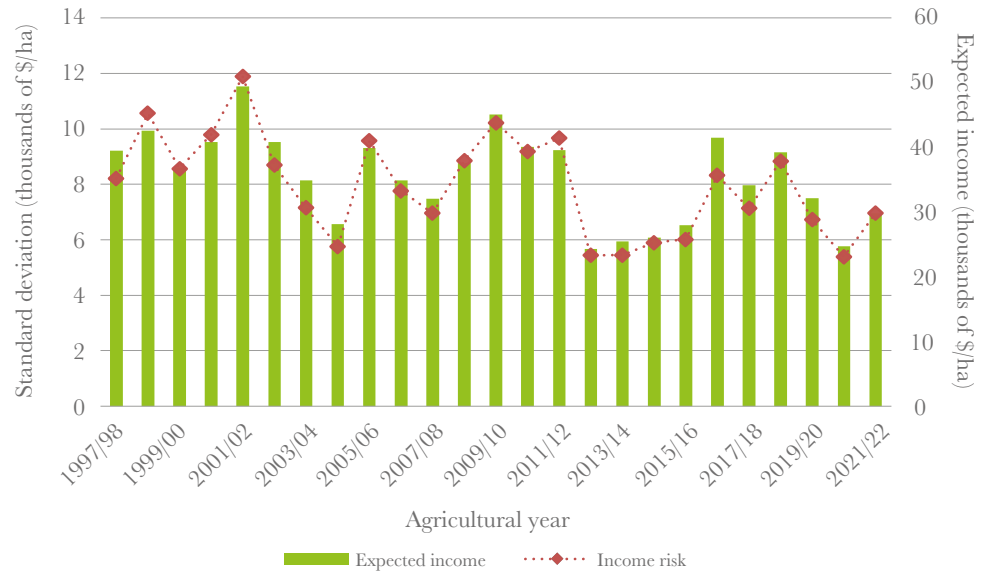


Figure 3. Expected income and income risk in the P-V cycle of DR 053.

corresponds to the 2020/21 agricultural year, the specific characteristics of which are detailed in Table 2.

Perennial crops

As shown in Figure 4, the expected income and income risk of agricultural portfolios for perennial crops display a strong positive correlation (97.77%) and a marked upward trend over time. The minimum income risk portfolio for this group of crops corresponds to the 1998/99 agricultural year, with its components presented in Table 3.

Correspondence analysis between risks and diversification

O-I Cycle

According to the correlations between yield risk (σ_r), price risk (σ_p), income risk (σ_i), and the HI of the annual agricultural portfolios for the O-I cycle in DR 053, State of Colima (see Table 4), greater crop diversification ($IH \rightarrow 0$) tends to significantly reduce

Table 2. Minimum income risk portfolio in the P-V cycle of DR 053, Colima State.

Agricultural year	Assets	Surface (ha)	Pi (%)	Risk (σ_i)	$E(I_p)$	IH
2020/21	Rice	678	78.75	5.390	24.695	0.630
	Sweet corn	54	6.27			
	Grain corn	40	4.65			
	Cucumber	20	2.32			
	Forage sorghum	45	5.23			
	Grain sorghum	24	2.79			
	Total	861	100.00			

Note: P_i =share; σ_i =standard deviation of income (thousands of \$/ha); $E(I_p)$ =expected income (thousands of \$/ha).

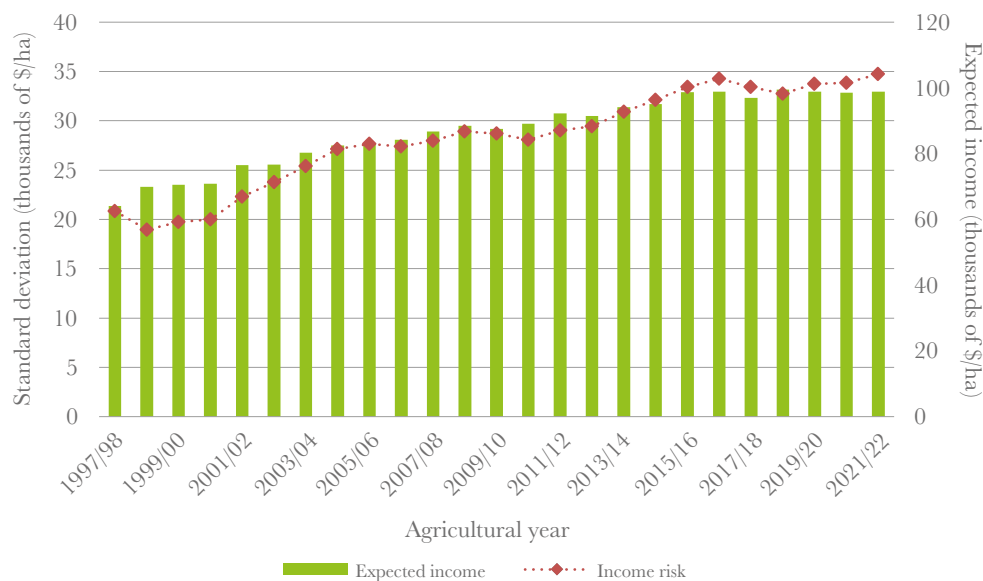


Figure 4. Expected income and risk of entry in perennial crops in DR 053.

Table 3. Minimum entry risk portfolio in perennial crops in DR 053, Colima State.

Agricultural year	Assets	Surface (ha)	Pi (%)	Risk (σ_i)	$E(I_p)$	IH
1998/99	Sugarcane	1,309	7.46	18.952	69.934	0.197
	Coconut tree	761	4.34			
	Associated fruit trees	36	0.21			
	Soursop	82	0.47			
	Lime	20	0.11			
	Lemon	6,338	36.13			
	Associated lemon	3,045	17.36			
	Mango	464	2.65			
	Associated mango	72	0.41			
	Orange	140	0.80			
	Other crops*	83	0.47			
	Papayo	88	0.50			
	Banana	1,227	6.99			
	Associated banana	2,037	11.61			
	Tamarind	123	0.70			
Others**	1,717	9.79				
	Total	17,542	100.00			

Note: P_i =share; σ_i =standard deviation of income (thousands of \$/ha); $E(I_p)$ =expected income (thousands of \$/ha). *Grouped by CONAGUA, 2023. **Grouped by the author; includes blueberry, Bermuda grass, associated forages, associated soursop, lychee, associated orange, other forages, other grasses, associated papaya, and jackfruit.

price risk (positive correlation of 78%). However, this diversification slightly increases the variability of yields and incomes, as indicated by the negative correlations of 10.9% and 10.7%, respectively.

Additionally, comparing risks (measured by the coefficient of variation [CV]) (see Figure 5), it is clear that income risk in this agricultural cycle is determined to a greater extent by price fluctuations (positive correlation of 89%); although it also corresponds considerably positively with yield uncertainty (correlation of 64%).

P-V Cycle

According to the correlation matrix between risks (measured as standard deviation) and the HI for this agricultural cycle (see Table 5), crop diversification ($IH \rightarrow 0$) in the P-V

Table 4. Correlations (%) between risks and diversification in the F-I cycle (1997/98-2021/22).

	Crop yield (σ_r)	Price (σ_p)	Income (σ_i)	IH
Crop yield (σ_r)	100.00	26.63	93.91	-10.88
Price (σ_p)	26.63	100.00	29.49	78.13
Income (σ_i)	93.91	29.49	100.00	-10.74
IH	-10.88	78.13	-10.74	100.00

Note: σ_r , σ_p and σ_i are the standard deviations of the expected (average) yield (ton/ha), price (thousands of dollars/ton) and income (thousands of dollars/ha) of the annual agricultural portfolios.

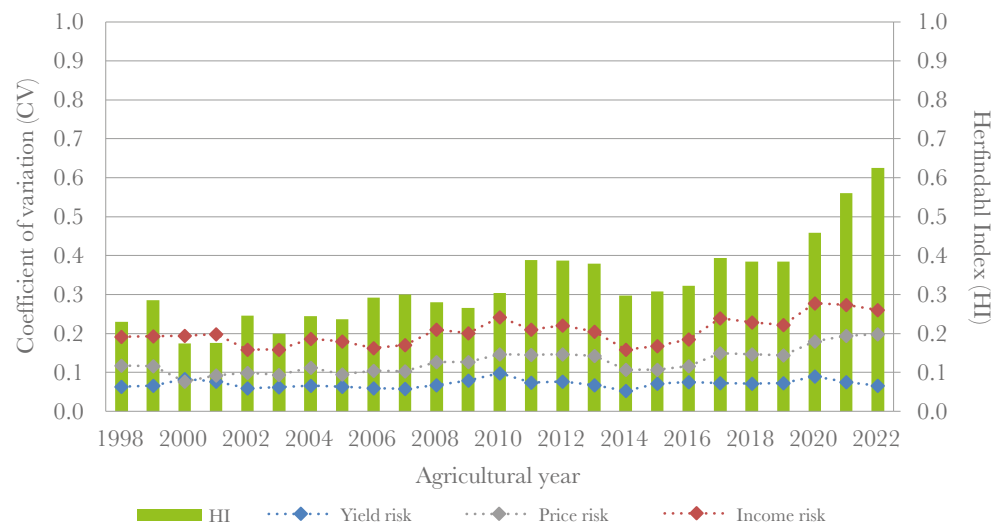


Figure 5. Risks and diversification in the F-W cycle (1997/98-2021/22).

Table 5. Correlations (%) between risks and diversification in the P-V cycle (1997/98-2021/22).

	Crop yield (σ_r)	Price (σ_p)	Income (σ_i)	IH
Crop yield (σ_r)	100.00	-78.70	83.01	-64.47
Price (σ_p)	-78.70	100.00	-56.48	80.07
Income (σ_i)	83.01	-56.48	100.00	-62.44
IH	-64.47	80.07	-62.44	100.00

Note: σ_r , σ_p and σ_i are the standard deviations of the expected (average) yield (ton/ha), price (thousands of dollars/ton) and income (thousands of dollars/ha) of the annual agricultural portfolios.

cycle considerably reduces price risk (positive correlation of 80%), but it also significantly increases the variability of yields and incomes, as indicated by the negative correlations of 64% and 62%, respectively).

Similarly, comparing risks (measured by the CV) (see Figure 6), it is evident that in this agricultural cycle as well, price risk is the main driver of income variation. However, income risk maintains a certain degree of positive association with the dynamics of yields (correlation of 30%).

Perennial crops

According to the correlation matrix between the three types of risk analyzed (measured as standard deviation) and the HI (see Table 6), it is observed that diversification of perennial crops ($IH \rightarrow 0$) moderately reduces yield risk and income risk (positive correlations of 67% and 61%, respectively).

Reviewing the trends and correlations between risks (measured by the CV) (see Figure 7), it can be inferred that, as with cyclical crops, income risk in perennial crops stems mainly from price uncertainty and is highly associated with the variation in agricultural yields (positive correlation of 81%).

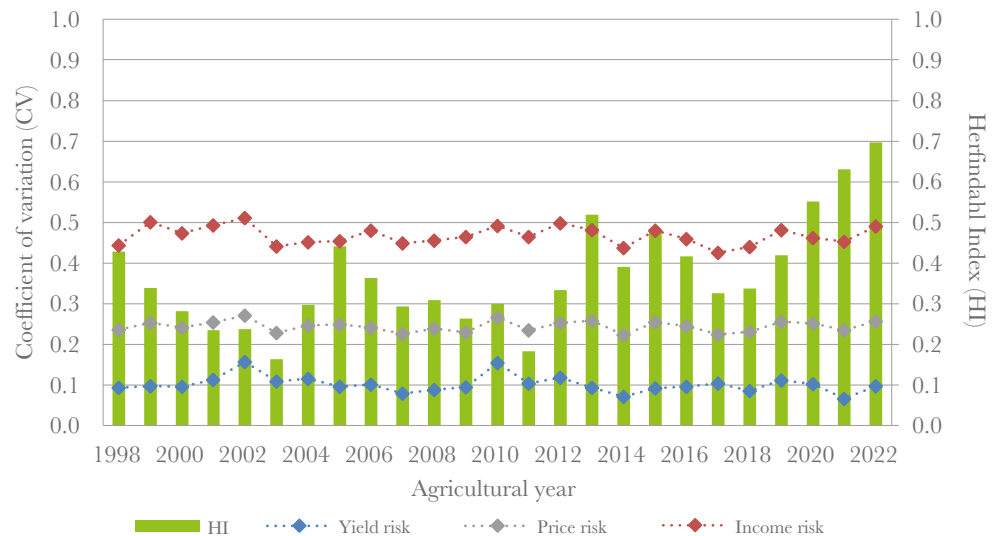


Figure 6. Risks and diversification in the P-V cycle (1997/98-2021/22).

Table 6. Correlations (%) between risks and diversification in perennial crops (1997/98-2021/22).

	Crop yield (σ_r)	Price (σ_p)	Income (σ_i)	IH
Crop yield (σ_r)	100.00	-37.79	91.68	66.67
Price (σ_p)	-37.79	100.00	-52.76	-5.78
Income (σ_i)	91.68	-52.76	100.00	60.92
IH	66.67	-5.78	60.92	100.00

Note: σ_r , σ_p and σ_i are the standard deviations of the expected (average) yield (ton/ha), price (thousands of dollars/ton) and income (thousands of dollars/ha) of the annual agricultural portfolios.

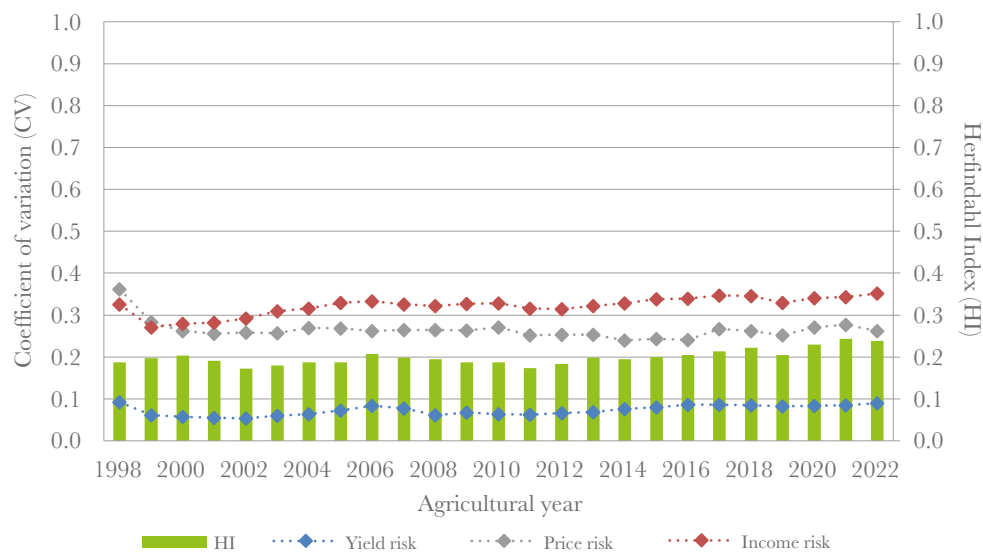


Figure 7. Risks and diversification in perennial crops (1997/98-2021/22).

CONCLUSIONS

The diversification analysis revealed that annual portfolios of perennial crops were generally the most diversified, with an almost flat HI trend over the analyzed period. In contrast, the HIs of agricultural portfolios in the O-I and P-V cycles showed an increasing trend ($HI \rightarrow 1$), indicating a clear movement toward specialization in both cycles. A moderate positive association was observed between average income and risk level in the O-I cycle portfolios (correlation of 58%) and a very strong correspondence in the P-V cycle (correlation of 96%), both with a declining trend over time. In perennial crop portfolios, the correlation was also highly positive (98%) but showed a distinctly upward trend. The analysis of the relationship between different types of risk and the HI indicated that greater diversification ($IH \rightarrow 0$) is an effective strategy for significantly reducing price risk in both agricultural cycles, as well as yield and income risks in perennial crops. However, in the P-V cycle, this strategy is less favorable for managing yield and income risk. Additionally, it was found that in all crop groups both cyclical and perennial income risk is mainly driven by price variability (market risk). This suggests that to strengthen income stability in the district, priority should be given to managing price fluctuations and promoting greater diversification, especially in perennial crops. The identified historical minimum income risk agricultural portfolios correspond to the 2013/14 (O-I cycle), 2020/21 (P-V cycle), and 1998/99 (perennial crops) agricultural years, representing a viable alternative for mitigating income instability in the district.

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Chayote (*Sechium edule* var. *virens levis*) cultivation and environmental factors

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ABSTRACT

Objective: The objective of this study was to identify the factors that influence chayote production through a biophysical and descriptive characterization.

Design/methodology/approach: The methodology consisted of a biophysical characterization based on the analysis of land use and vegetation mapping, soil type, climate, relief, and altitudinal range. The descriptive analysis focused on cultivation practices, agricultural techniques, adaptation to different biophysical conditions, pests, diseases, and socioeconomic factors that affect production.

Results: The results show that in the municipality of Coscomatepec, chayote occupies 80% of the agricultural land. Currently, the crop is grown under suitable conditions of climate, slope, and altitude; however, it faces challenges related to water availability. Chayote is drought-sensitive and requires irrigation. In some cases, the irrigation method is inadequate, increasing the risk of disease, especially in clay soils. Additionally, the lack of phytosanitary control and technological lag hinder the crop's competitiveness.

Limitations/implications: It is necessary to deepen the economic analysis of irrigation-related costs by evaluating their impact on crop yield and profitability.

Findings/conclusions: To improve production and address current challenges, it is recommended to implement efficient irrigation systems, construct water catchment infrastructure to reduce costs, enhance drainage in clay soils, and promote preventive phytosanitary control to ensure crop sustainability.

Keywords: agricultural practices, production, irrigation, phytosanitary control

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INTRODUCTION

Chayote (*Sechium edule* var. *virens levis*) is a species of great economic importance in Mexico. Nationally, approximately 3,500 hectares are cultivated, positioning the country as the world's leading producer of this species, accounting for 53% of the global market share (Martín, 2014). The central region of the state of Veracruz stands out as the primary production area (Barrera-Guzmán *et al.*, 2022), generating significant income both from domestic sales and exports, thereby contributing to improved living standards for producers (Cadena-Íñiguez *et al.*, 2010). This crop is mainly cultivated in family orchards and small properties (Guevara *et al.*, 2014). However, chayote production faces a high risk of loss due to various factors, including limited technical management, adverse climatic conditions,



the presence of pests and diseases, high production costs, and poorly designed institutional policies (Alvarenga *et al.*, 2007). All these factors directly affect the economic stability of producers. Therefore, it is essential to understand the biophysical characteristics associated with chayote cultivation to be better prepared to meet current and future challenges. One of the most effective strategies for identifying the factors that directly influence chayote production is conducting a biophysical and descriptive characterization. This approach allows for a comprehensive understanding of the production context and the optimization of crop management. Biophysical analysis involves the study of abiotic elements such as soil type, climatic conditions, water resource availability, and topography (Nkiaka *et al.*, 2024). In contrast, the descriptive analysis focuses on aspects directly related to the crop, including agricultural techniques, adaptability to different biophysical conditions, presence of pests and diseases, and certain socioeconomic factors affecting production.

The information derived from these analyses is crucial for decision-making in the integrated management of the crop (Ruiz-García *et al.*, 2020). Given the agricultural importance of chayote in the region, it is essential to carry out an analysis that identifies the key factors influencing its production, particularly in one of the most significant areas: the central micro-watershed of the municipality of Coscomatepec, Veracruz. This study aims to provide a detailed description of the biophysical conditions and the cultivation system, offering useful tools for decision-makers to sustain and enhance productivity, thereby ensuring the crop's viability in the medium and long term.

MATERIALS AND METHODS

Study area

The study area is located in the central zone of the municipality of Coscomatepec, in the central region of the state of Veracruz (Figure 1). It corresponds to a micro-watershed situated within the Jamapa River basin. The average annual temperature is 17.5 °C, with minimum values of 12 °C and maximums of 21 °C, and an average annual precipitation of 1,750 mm.

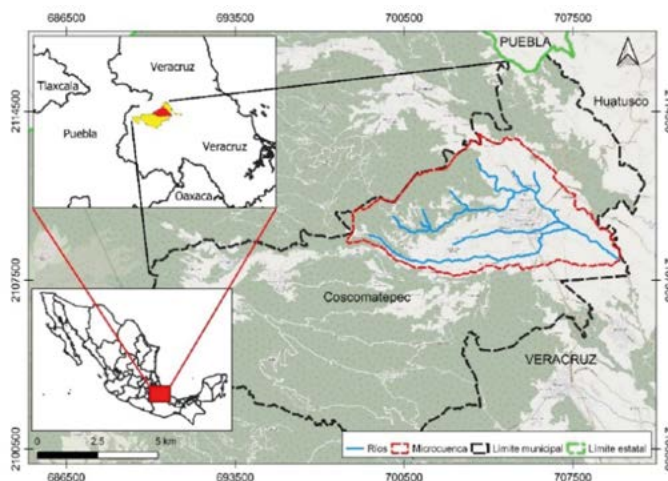


Figure 1. Geographical area of the study. Prepared by the authors using information from INEGI and CONABIO.

Biophysical characterization

For the biophysical characterization analysis, specific cartography was developed for the following aspects: land use and vegetation, soil type, relief, and altitudinal range. Additionally, official data on climate, temperature, and precipitation were incorporated.

Data and information sources

Land use and vegetation cartography was created through supervised classification based on SENTINEL satellite images (USGS, 2023). Climatic data, including precipitation and average annual temperature, were obtained from layers provided by CONABIO. Slope and elevation data were derived from processing the Digital Elevation Model (DEM) by INEGI (2023). The soil map was developed using sampling results, local producers' knowledge, and the INEGI (2014) edaphological dataset.

Land use and vegetation cartography

To accurately determine and quantify the areas where chayote cultivation is established, a supervised classification was carried out. This cartographic process involved field sampling to define control points in cultivated zones. Classification began with preprocessing SENTINEL-2 imagery using a false color band composite (bands 4, 3, and 2) via the Semi-Automatic Classification Plugin (SCP) in QGIS (Congedo, 2021). Recognition polygons identified five dominant land cover classes besides chayote: urban, pine forest, avocado, maize, and livestock production areas.

Soil type cartography

To enhance accuracy in the distribution of soils within chayote cultivation zones, a specific soil type cartography was developed, as soil conditions directly impact crop growth, yield, and health. Following the methodology of Ortiz *et al.* (1990), sampling consisted of 15 composite samples distributed across upper, middle, and lower areas of the plots. This process was supported by local knowledge to delineate soil boundaries. Subsequently, with laboratory data on physical and chemical characteristics pH, electrical conductivity (EC), field capacity (FC), permanent wilting point (PWP), texture, bulk density (BD), porosity (P), and color a new taxonomic soil classification was performed in accordance with the World Reference Base for Soil Resources (FAO, 2014).

Slope and altitudinal range cartography

The analysis of slope and altitudinal range helps identify factors directly related to cultivation, such as water distribution, soil erosion, drainage type, and climatic variability. This cartography was generated through DEM processing. Slope categories were defined within the ranges of 0-2%, 2-4%, 4-8%, and 8-15%, while altitudinal ranges were classified in 250-meter intervals.

Descriptive characterization

The descriptive characterization aimed to understand the features of the cultivated variety, agricultural practices, and water and soil requirements, as well as climatic factors

affecting crop development. Information was gathered following the methodology of Ortiz and Gutiérrez (1999), through field visits with 15 key informants selected for their deep knowledge of chayote cultivation management within the community. This method integrates local and technical knowledge to refine management techniques, minimize risks from pests, diseases, or nutritional deficiencies, and optimize resource use. The information generated supports local-level planning and decision-making, enabling small producers to adapt their practices to current environmental conditions.

RESULTS AND DISCUSSION

Cultivated variety

In the study area, the most commonly cultivated variety is white chayote (*Sechium edule* var. *virens levis*). This variety is prevalent in the region due to its strong adaptation to local climatic and edaphic conditions, in addition to its high demand in both local and national markets (Nataren-Velázquez, 2021).

Land Use and vegetation

According to the supervised classification, agricultural land use predominates, covering 38.6% (1,480 ha), followed by temperate pine forest at 35% (1,342.1 ha), and areas designated for livestock production at 14.4% (Figure 4). Specifically within the agricultural area, the dominant crop is chayote, occupying 1,032.2 ha (69.7%), followed by maize at 21.7% and avocado at 8.6% (Figure 2).

Climate

According to the literature, chayote requires an average annual precipitation between 1,500 and 2,000 mm, well distributed throughout the year, and temperatures ranging from 18 to 25 °C, with tolerable minimums down to 10 °C (Cadena *et al.*, 2005; Cadena *et al.*, 2006). In the study area, 72.8% of chayote is established in a temperate-humid climate; 97.5% of the crop is located in zones with average annual temperatures

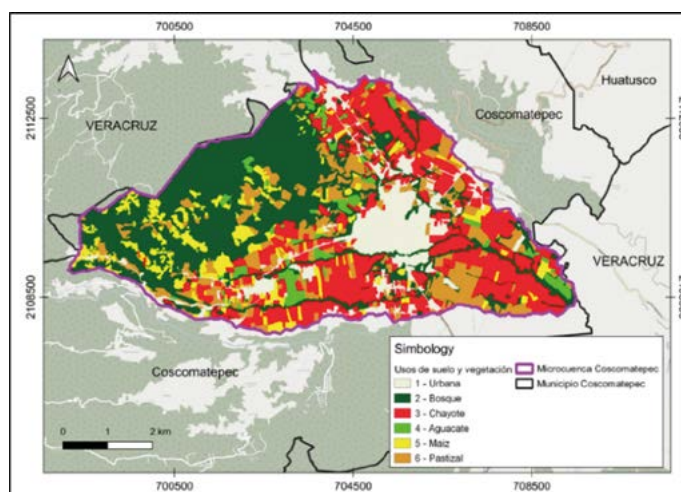


Figure 2. Geographic location of the areas cultivated with chayote. Own elaboration.

of 17 °C (minimum of 10 °C and maximum of 24 °C), and 99.9% grows in areas with an average annual precipitation of 1,750 mm. These conditions fall within the optimal range for chayote development, allowing for high-quality production without requiring significant management adjustments. However, thermal variations particularly low temperatures and periods of drought remain persistent challenges for production in this region.

Slope and altitudinal range

The general slope reclassification analysis in the micro-watershed yielded the following values: 0-2% (63.4%), 2-4% (9.1%), 4-8% (23.3%), and 8-15% (4.1%) (Figure 3a). Regarding altitudinal range, the 1,500-1,750 meters above sea level (masl) interval predominates, covering 48.5% of the area, followed by the 1,250-1,500 masl range, with 21.5% (Figure 3b). Although chayote cultivation can occur between 300 and 2,800 masl, its optimal altitude is between 500 and 1,500 masl (Montecinos *et al.*, 2019). In the study area, 94.8% of the crop is located between 1,250 and 1,750 masl. Additionally, 91.1% is established on nearly level surfaces, with slopes ranging from 0 to 2%. This topography facilitates agronomic management, reduces production costs, improves accessibility, and enables more efficient irrigation control, mitigating issues associated with excessive surface runoff and minimizing the risk of water erosion.

Soil type

According to the analysis based on taxonomic classification, three soil types were identified within the micro-watershed. The dominant soil type is Humic Andosol (AnHu), covering 56.8% of the area, followed by Orthic Acrisol (AcOr), accounting for 24.3% (Figure 4). These results provide greater precision in the delineation and distribution of soils, which is essential for optimizing agricultural management and promoting sustainable cultivation planning.

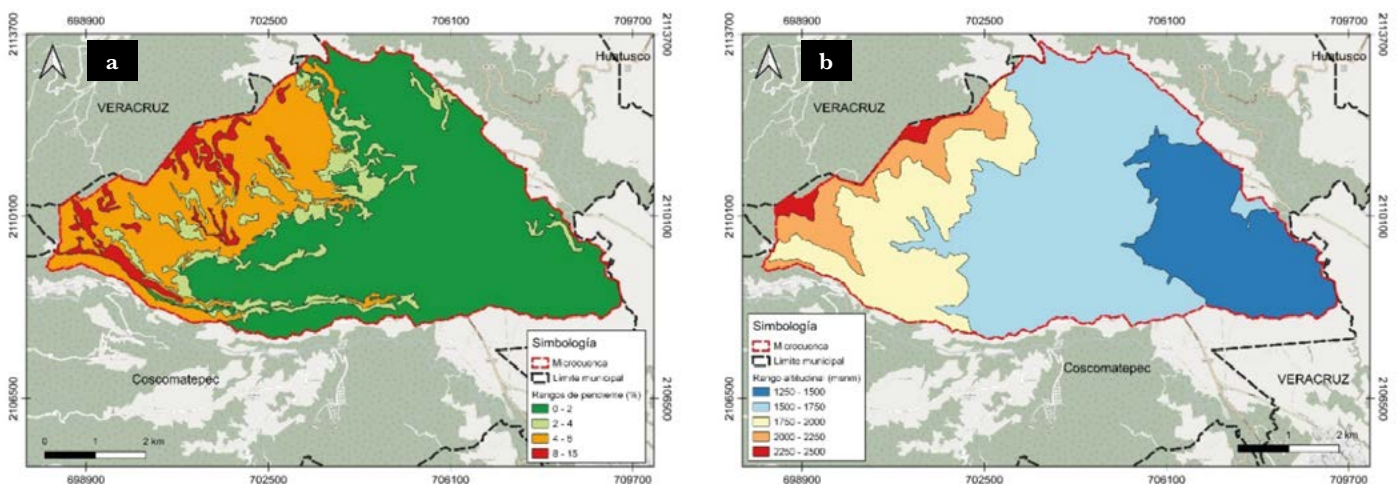


Figure 3. Slope (a) and altitudinal range (b) in the central Coscomatepec micro-basin. Prepared by the authors, based on information from INEGI (2013).

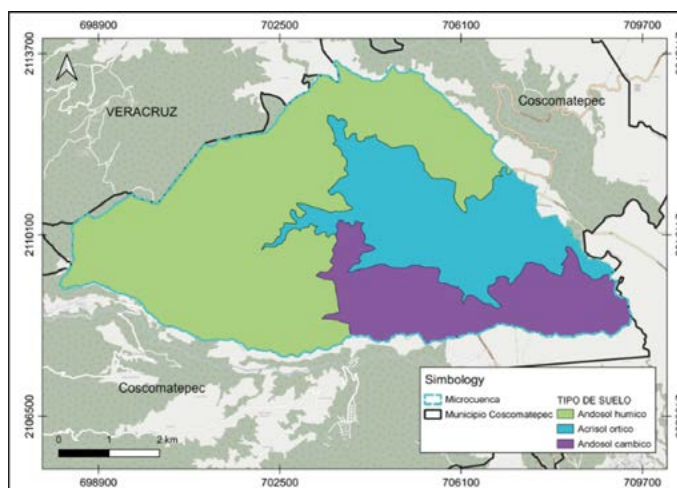


Figure 4. Soil types in the central micro-watershed of Coscomatepec. Source: Own elaboration.

Studies have shown that chayote thrives in acidic to slightly acidic soils (pH 4.5 to 6.5), well-drained, and rich in organic matter (Cadena *et al.*, 2005), preferably with a loamy-sandy texture (Cisneros-Solano, 2016). Within the micro-watershed, 44.3% of chayote is cultivated in Humic Andosol soils, followed by 33.6% in Cambic Andosol and 22.1% in Orthic Acrisol. Humic Andosol presents optimal characteristics for crop development (pH 5.2, loamy-sandy texture, depth, good drainage, and high organic matter content), although it is prone to erosion if water resources are not properly managed. On the other hand, crops established in areas with Orthic Acrisol face greater challenges due to its higher acidity (pH 4.7) and low fertility (Table 1). Additionally, its clay texture, combined with poor water management and inadequate drainage, creates conditions favorable for pathogen development.

Descriptive characterization

The chayote plant is classified as a perennial herbaceous vine with tuberous roots and a high production of stems featuring abundant branching (tendrils) (Rincón *et al.*, 2020). This variety exhibits a rapid growth rate, reaching harvest maturity between 18 and 21 days (Avendaño-Arrazate *et al.*, 2010). Each plant produces between 150 and 300 fruits during its productive cycle, with a productive lifespan in the region ranging from 3 to 5 years. In the study area, it is cultivated at an average density of 2,500 plants per hectare, with a

Table 1. Average surface area and physical and chemical properties by soil type.

Soil Type	Surface %	Physical and chemical properties						
		pH	C. E. (dS m ⁻¹)	D. A. (G cm ⁻³)	C. C. %	P. M. %	P %	Texture
Humic Andosol	44.3	5.2	0.18	0.91	58.7	34.8	61.3	Sandy Loam
Andosol Cambico	33.6	4.9	0.18	0.99	49.3	30.4	58.0	Loam
Acrisol Orthic	22.1	4.7	0.14	1.00	47.7	33.2	58.6	Clay Loam

spacing of 2 to 3 meters between plants. During the rainy season harvest (June-October), the average yield is 30 to 50 tons per hectare, while during the dry season (November-May), yields range from 20 to 30 tons per hectare. Regarding crop establishment and maintenance, producers indicate that the main limitation to productivity is water availability, as chayote is highly susceptible to drought. This is consistent with its high water content, close to 90% (Cruz-León & Querol-Lipovich, 1985). Depending on the duration of the dry season, the crop requires approximately 900,000 to 3 million liters of water per hectare (Dzib *et al.*, 1993). According to Cisneros-Solano (2016), the dry period (November-May) averages seven months per year, creating a deficit of about 35% of total annual precipitation in the water balance. To meet water demands during the dry season and mitigate frost damage, producers rely on water trucks sourced from the same micro-watershed. Water requirements account for 35% to 40% of total production costs, making it the main input. On average, three supplemental irrigations are applied per week, totaling approximately 840,000 liters of water during the dry months. Irrigation is typically performed using hoses through basin flooding, which represents inefficient water management. This excess moisture often causes plant death due to the development of pathogenic fungi (Cadena *et al.*, 2001; Montecinos *et al.*, 2019), especially in clay soils. These issues are more frequent in the 22% of the crop grown in Acrisol soils, due to their clay texture and poor drainage, which create anaerobic conditions favorable to the development of fungi, particularly *Phytophthora capsici*, the causal agent of chayote wilt in the region (Olguín Hernández *et al.*, 2013). Regarding pests, producers report problems with red spider mites (*Tetranychus urticae*) and other mites (Nataren-Velázquez, 2021). Additionally, 50% of producers have reported damage from severe hailstorms in the past three years, and 40% have experienced frost damage in recent production cycles. A deficiency in management practices has been identified in the study area, as 60% of producers do not implement preventive phytosanitary control (Cadena *et al.*, 2016). There is also a lack of a fertilization plan adapted to the crop's phenological stages (Rincón *et al.*, 2020). Furthermore, a technological lag persists due to the limited adoption of new agricultural techniques that could enhance productivity and competitiveness (Rincón *et al.*, 2020). There is also an absence of business culture, equipment, infrastructure, market access, and productive association schemes that would ensure crop profitability (Cadena *et al.*, 2016). To improve chayote production, it is recommended to implement drip or micro-sprinkler irrigation systems that optimize water use, reduce the incidence of fungal diseases, and minimize frost damage. The construction of water catchment infrastructure, such as ponds or small dams, would help reduce water stress and dependence on water trucks, thereby lowering costs. Additionally, installing moisture sensors would support precise irrigation decisions. In clay soils (Acrisol), it is necessary to improve drainage through ditches, channels, or soil tillage to remove excess moisture and prevent pathogen-related diseases. Preventive phytosanitary control should be encouraged, especially early detection of *Phytophthora capsici* and the application of appropriate fungicide treatments. The use of biological control agents such as phytoseiid mites (Phytoseiidae) is also recommended for the natural control of red spider mites and other pests. It is essential to establish a stage-based fertilization plan, install anti-hail netting, adopt new production technologies, and provide training to producers to optimize crop management and ensure profitability.

CONCLUSIONS

Chayote is a crop of economic importance in Mexico; therefore, conducting biophysical and descriptive studies in the central micro-watershed of Coscomatepec is essential to optimize its management, enhance productivity, and ensure long-term sustainability. The region exhibits climatic conditions that are close to ideal for crop development, favoring high-quality production. However, temperature and precipitation variations pose significant challenges. While Humic Andosol offers optimal conditions, Orthic Acrisol presents limitations due to its higher acidity, low fertility, clayey texture, and poor drainage, which increase the risk of disease. The main constraint to production is water availability, given the crop's high susceptibility to drought. The reliance on irrigation and its inefficient use particularly in clay soils elevate the risk of fungal diseases. To improve chayote production, it is recommended to implement efficient irrigation systems, develop water catchment infrastructure, enhance drainage in soils with physical limitations, and strengthen preventive phytosanitary control.

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Adaptation practices and challenges of the milpa intercropped in fruit trees (MIAF) system to cope climate change

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ABSTRACT

Objective: To describe and analyze the main practices and challenges associated with the Milpa Intercropped in Fruit Trees (MIAF) system in response to the climate variability due to climate change (CC) in the MIAF Module of San Andrés Tuxtla, Veracruz.

Methodology: A climatological analysis of the study area was conducted, along with a review of adaptation practices to climate change and the conceptual framework of the milpa system, climate change, and adaptation, using the Scopus platform. Review metrics and a bibliometric analysis were generated using VOSviewer.

Results: In the 1970s, maximum temperatures were lower as compared to the 1950s and 1980s. Four adaptation strategies implemented in the MIAF system in response to climate change were identified, focused on water management, biodiversity, agronomic practices, and the functionality of those strategies. A total of 12 relevant documents published between 2018 and 2022 were found, comprising 75% scientific articles, 16.7% essays, and 8.3% book chapters. The publications comes from Mexico, the United States, the United Kingdom, and Philippines. The predominant thematic areas were environmental sciences (30.4%), social sciences (26.1%), and agriculture (13.0%).

Conclusions: The study reveals a limited amount of literature on the subject. Furthermore, the MIAF system faces critical challenges, including the need to adapt traditional agricultural practices to increasingly extreme climatic conditions, the transfer of knowledge and adaptive technologies to rural communities, and the potential decline in crop yields due to fluctuations in temperature and precipitation.

Keywords: temperature, precipitation, terraces, soil, water.

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INTRODUCTION

The Milpa Intercropped in Fruit Trees (MIAF) system was initially developed in 1988 by researchers from INIFAP and the Colegio de Postgraduados, incorporating the Live Wall Terrace (LWT) technology, which consists of *Gliricidia sepium* L. hedgerows reinforced by a runoff filter designed to impede free-flowing water and delineate strips for annual crops, gradually leading to terrace formation (Cortés-Flores *et al.*, 2007). After an



average of eight years, this technology proved to be effective for erosion control, retaining approximately 2 tons per hectare per year, and significantly reducing soil loss compared to traditional tillage systems, which can result in up to 146 tons per hectare per year of soil loss (Francisco *et al.*, 2006). In another study based on five years of observation, the average runoff coefficient decreased from 30% to 15% under both traditional tillage and LWT treatments. In this context, water erosion also entails the loss of organic matter and nutrients contained within sediments and surface runoff caused by rainfall. The organic matter, nitrogen, and phosphorus content in sediments collected from LWT systems was higher than that of traditional systems. However, due to the greater volume of sediment in traditional systems, overall nutrient loss was significantly higher (Turrent, 1995). Organic matter loss reached values of 600 and 3,400 kg ha⁻¹, while potassium, calcium, and magnesium base losses through runoff were between 37 and 66 kg ha⁻¹ for both treatments. In terms of productivity, the LWT system achieved higher annual maize yields (4.95 t ha⁻¹) with acceptable yield stability, whereas the traditional tillage system had lower productivity (4.12 t ha⁻¹) and less stable yields (Turrent, 1986). Continuous maize cultivation across 13 cycles and terrace formation led to a slight decline in soil fertility, reflected in a pH reduction of 0.43 units, a decrease in organic matter by 0.56%, and reductions in exchangeable potassium and calcium by 0.15 and 3.08 c mol kg⁻¹ of soil, particularly in the upper parts of the terrace. Building on this experience, INIFAP and the Colegio de Postgraduados developed the MIAF system in 2003 as a technological evolution for small-scale production units. This system sought to recover the core elements of the traditional milpa paradigm while integrating principles from classical agronomic science. MIAF combines strategies and components designed to control soil erosion and improve soil quality, and it is considered a viable alternative for smallholders to enhance household income (González & López, 2023). The system consists of three intercropped species: a fruit tree (epicrop), maize (mesocrop), and beans or another edible legume (undercrop), in an intensive agronomic interaction that optimizes land use spatially and temporally (Pérez & Martínez, 2022). It is also regarded as a multi-objective or multipurpose technology aimed primarily at increasing net income and family employment, protecting soil against water erosion, fostering synergistic interactions among component crops to improve natural resource use, and increasing carbon capture. It is important to note that the system is inherently complex, requiring a range of technical skills grounded in agronomic knowledge of the soil-plant-atmosphere relationship. Furthermore, climate change presents a significant challenge for tropical fruit production not only under the MIAF system but also in commercial plantations in temperate and tropical climates as it poses a risk of yield reduction, fruit damage, and even threatens the productive viability of certain crops (IPCC, 2019; FAO, 2021). Given these concerns, the objective of this research was to describe and analyze the primary practices and challenges that the MIAF system faces in response to climate change variability. MIAF was originally proposed as a sustainable alternative for hillside agriculture, offering benefits such as erosion control, improved soil quality, and enhanced biodiversity. However, climate change introduces a new threat to the system's viability, making it imperative to assess its potential impacts and develop effective adaptation strategies.

MATERIALS AND METHODS

Study area

The MIAF Module, officially named the “Research and Technology Transfer Module for Productive and Conservationist Agriculture Dr. Antonio Turrent Fernández” (MIAF-INIFAP Module), is located in the community of Axochio, municipality of San Andrés Tuxtla, Veracruz, at an altitude of 60 meters above sea level, with coordinates 18° 20' 0.5" N and 95° 17' 57.7" W. Los Tuxtlas region is characterized by a warm sub-humid climate with summer rainfall (Aw2) (García, 1981), and annual precipitation ranges between 1,500 and 2,000 mm. Geomorphologically, the area consists of rolling hills with slopes ranging from 10% to over 20%. The soils have developed through alluvial sediment deposition and volcanic activity (Comisión del Papaloapan, 1972).

Information search

This phase involved a state-of-the-art analysis focused on the conceptual triad MILPA-CLIMATE CHANGE-ADAPTATION. The search was conducted on the Scopus platform, filtering by title, abstract, and keywords.

Information analysis

A metric analysis was carried out considering publication year, country, language, discipline, and document type. Additionally, a bibliometric analysis was conducted using VOSviewer, a software tool developed by the Center for Science and Technology Studies (CWTS) at Leiden University, specifically designed to visualize bibliometric networks (Van Eck & Waltman, 2010). This tool was used to generate visual maps that represent bibliographic connections among the documents retrieved from the Scopus database (Guallar *et al.*, 2020).

Considerations for comprehensive assessment of climate change impact on the MIAF system

Assessing the impact of climate change on the MIAF system is essential to ensuring its long-term viability and supporting the adaptation of hillside agriculture to changing climatic conditions (IPCC, 2021). This type of approach provides a comprehensive framework for evaluating impacts, identifying adaptation strategies, and monitoring the performance of the MIAF system within the context of climate change, as outlined below (FAO, 2022).

RESULTS AND DISCUSSION

Historical Behavior of Climatic Variables in the Region of Axochio, San Andrés Tuxtla, Veracruz

Climate change poses a significant challenge to tropical fruit production worldwide, as variations in temperature, precipitation, climate patterns, and extreme weather events can adversely affect several agricultural factors, including yield and fruit quality, pest and disease incidence, water availability, and pollination processes. In this context, Figure 1 illustrates the behavior of maximum and minimum temperatures from 1951 to 1981,

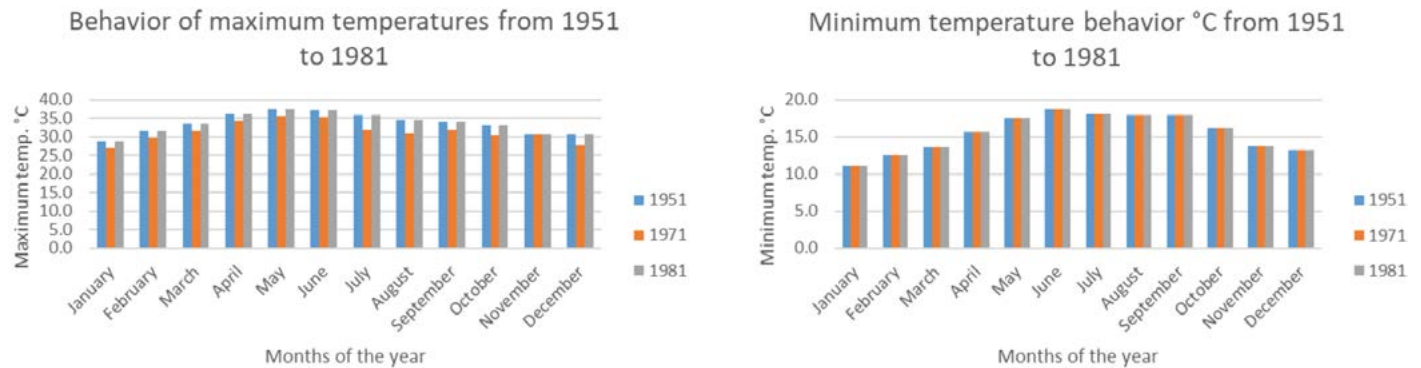


Figure 1. Behavior of maximum temperatures (left side) and minimum temperatures (right side) based on data from the Sihupan station, municipality of San Andrés Tuxtla, Veracruz.

based on data recorded at the National Meteorological Service (SMN) station located in the community of Sihupan, municipality of San Andrés Tuxtla, Veracruz. Notable variations in maximum temperatures were observed in 1951 and 1981 when compared to the 1971 data. However, with respect to minimum temperatures, no significant changes were recorded in any of the years analyzed.

Muller *et al.* (2011) report that elevated temperatures can reduce flowering, pollination, and fruit filling, ultimately leading to lower yields. Additionally, high temperatures may impair fruit development and quality by causing premature ripening, reduced size and flavor, and increased susceptibility to disease. Likewise, Lobell and Burke (2010), in their study of changing temperature, precipitation, and humidity patterns, found that these variations can promote the proliferation of pests and diseases, increasing both their incidence and severity. Certain pests and pathogens are particularly sensitive to specific climatic changes, which can alter their geographic distribution and life cycles. Regarding precipitation trends, Figure 2 illustrates the behavior of this climatic variable, showing that from August to November in the years 1951 and 1981, precipitation levels were

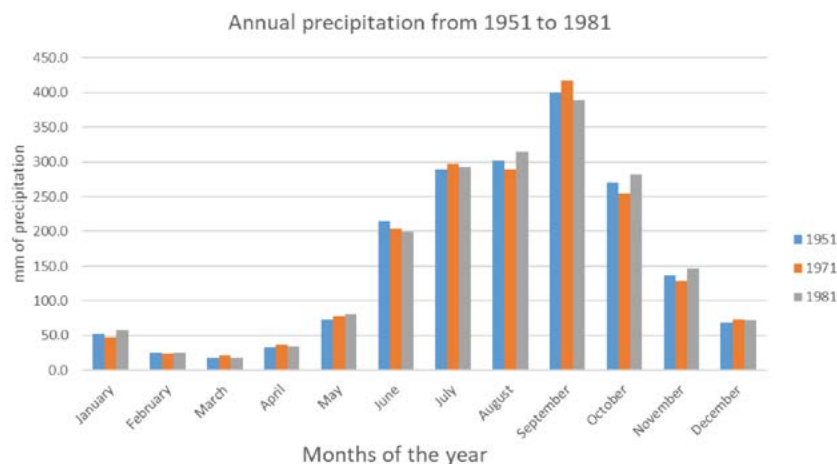


Figure 2. Precipitation patterns from 1951 to 1981 based on data from the SMN station in Sihupan, San Andrés Tuxtla, Veracruz.

notably higher as compared to 1971. These years recorded greater rainfall in the region. In this context, Giuliani and Zwane (2016) identified that water stress induced by climate change primarily resulted from rising temperatures and increased evapotranspiration, along with altered precipitation patterns. This led to greater water stress in crops, negatively affecting their growth, development, and productivity. Water scarcity, in turn, limited agricultural output, especially in arid and semi-arid regions. Finally, Aizen *et al.* (2009) revealed that climate change can significantly affect the distribution, abundance, and activity of pollinators such as bees and bats. This has direct implications for the pollination of tropical fruit crops, compounded by biodiversity loss and pesticide use both additional factors contributing to pollinator decline.

At the center of the diagram is the MIAF system, surrounded by a series of adaptation strategies grouped into four key categories: water management, biological diversity, management practices, and functional strategy roles. The diagram highlights principal practices such as crop diversification, the use of resilient varieties, rainwater harvesting, and agroforestry, among others, showing how they interconnect to support adaptation efforts.

This framework serves as a key tool for understanding and addressing the challenges posed by climate change globally. Moreover, it offers not only a comprehensive approach for tackling these challenges in agricultural and rural systems, but also acts as a bridge between theoretical understanding and practical implementation promoting sustainable and scalable solutions.

Sustainable water management includes techniques such as rainwater harvesting, drip irrigation, and soil moisture conservation through mulching or vegetative ground cover. Mahato (2014) and Ravishanker *et al.* (2013) found these practices effective in enhancing crop resilience in regions vulnerable to climate change, as demonstrated in studies conducted in Sub-Saharan Africa and South Asia.

Crop diversification and agroforestry systems involve integrating drought- or pest-resistant crops, intercropping different varieties, or implementing agroforestry systems that strengthen the resilience of agricultural systems. This approach helps stabilize farmers' incomes and preserve biodiversity (Tiamiyu *et al.*, 2017; Mugagga *et al.*, 2019).

Genetic improvement and crop selection focus on developing varieties that tolerate climate-related stress, such as drought or salinity critical strategies for preserving plant and animal species. Research by Hu *et al.* (2017) and Waongo *et al.* (2015) emphasizes the importance of improving seeds and germplasm to cope with climate change.

Soil conservation practices such as terracing, live barriers, crop rotation, and organic fertilization are not only effective in reducing erosion but also in enhancing soil fertility and quality. These techniques have been validated in highly agriculture-dependent countries like Ethiopia and Nigeria (Wolka & Zeleka, 2017).

Integration of climate-smart technologies involves implementing climate monitoring, early warning systems, and digital tools for crop management, enabling farmers to make informed, proactive decisions that reduce negative impacts. According to FAO (2013) and De Pinto *et al.* (2020), Climate-Smart Agriculture (CSA) encompasses practices that enhance agricultural productivity while reducing greenhouse gas emissions.

Capacity building and infrastructure development entail training farmers in resilient practices and investing in climate-adaptive infrastructure such as flood-resistant rural roads, while strengthening the response capacity of farming communities (Asrat & Simane, 2018).

Livelihood diversification encourages engagement in non-agricultural activities such as small enterprises or agricultural insurance schemes designed to reduce economic risk identified as key strategies for ensuring food security under extreme climatic conditions (Fosu-Mensah *et al.*, 2010).

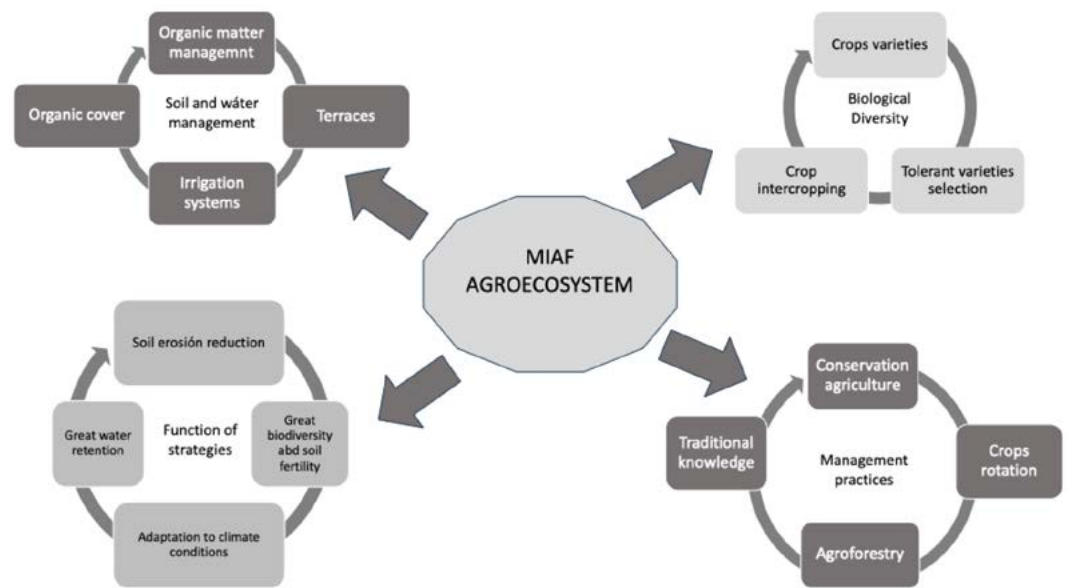


Figure 3. The Milpa as a climate change adaptation strategy practice. Source: Self elaboration.

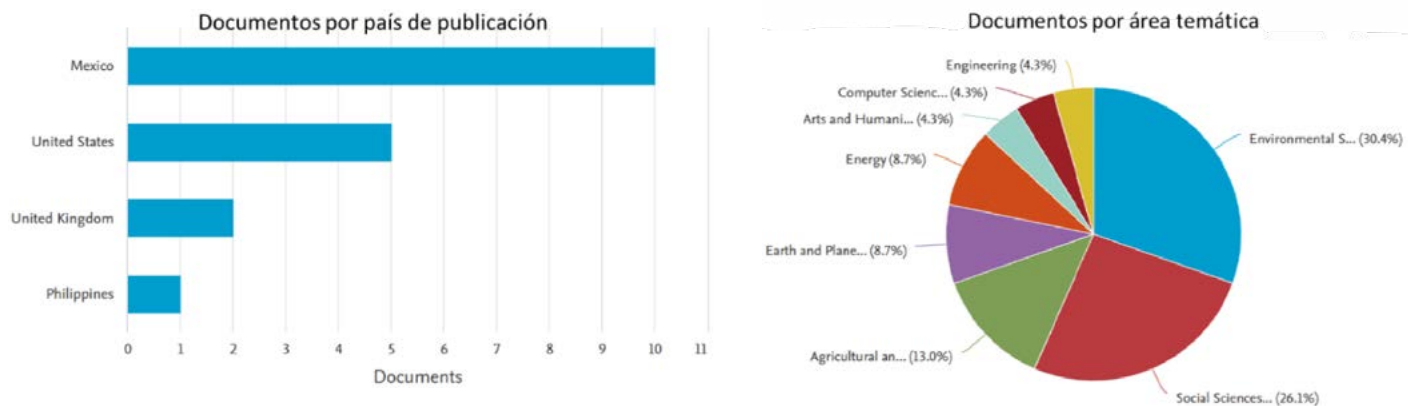


Figure 4. Documents by country of publication (left side) and documents by thematic area (right side) related to the concepts of milpa, climate change, and adaptation.

The following presents the results of the bibliometric analysis of the concepts Milpa-Climate Change and Adaptation, conducted using the Scopus database. A total of 12 documents were identified, published between 2018 and 2022 (2018: 1 article; 2020: 3 articles; 2021: 6 articles; and 2022: 2 articles). Of these, 75% were scientific articles, 16.7% were essays, and 8.3% were book chapters. The main findings from the identified metrics are summarized below.

Figure 5 illustrates the relationships identified among the keywords related to milpa, climate change, and adaptation. A strong interconnection is observed among these terms and the countries associated with them.

Main adaptation practices to climate change

Table 1 outlines a range of adaptation strategies designed to support producers working within the MIAF system in addressing the challenges posed by climate change (CC) and ensuring the sustainability of their production systems. It is essential to emphasize that climate change adaptation requires a comprehensive approach that integrates multiple strategies and actively involves all stakeholders in the fruit value chain. According to Siam *et al.* (2019) and Salazar *et al.* (2018), various adaptation practices have been developed to help producers of tropical fruit trees increase the resilience of their orchards and maintain sustainable production.

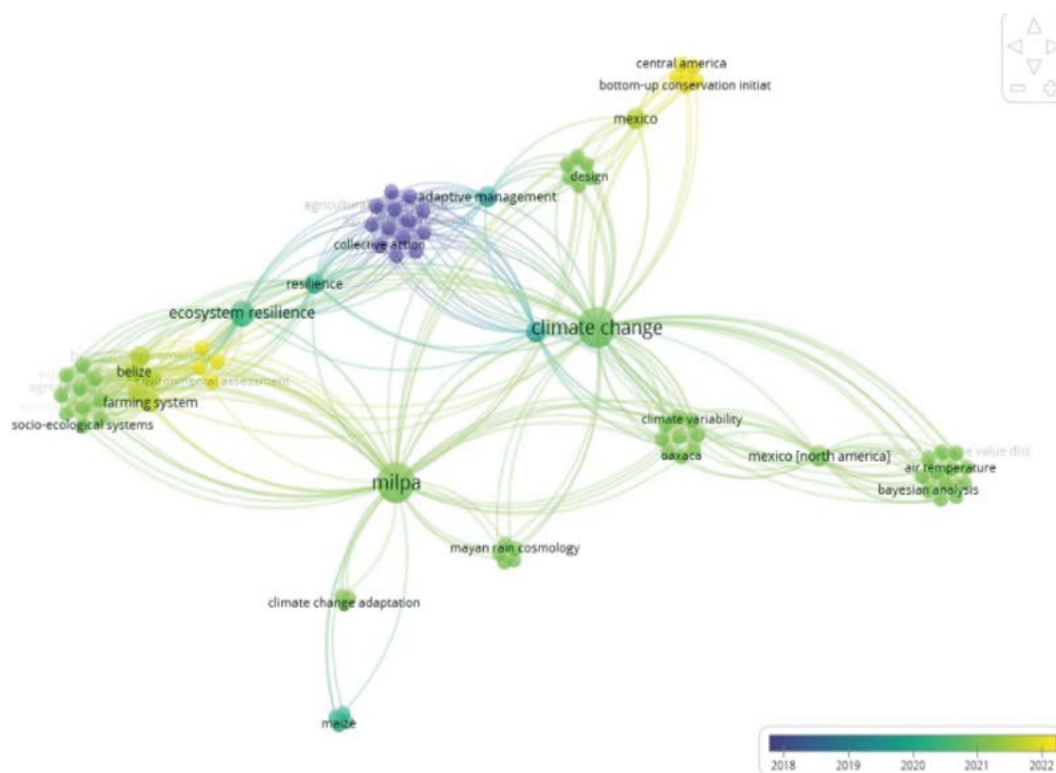


Figure 5. Nodes and links of the bibliometric analysis generated with the keywords milpa, climate change and adaptation.

Table 1. Adaptation strategies within the MIAF system to address climate change challenges.

Adaptation Practices for the MIAF System	Description
Selection of resistant varieties	<ul style="list-style-type: none"> • Use of stress-tolerant varieties: Selecting cultivars resistant to abiotic and biotic stress factors associated with climate change such as increased temperatures, drought, salinity, flooding, pests, and diseases is essential for adaptation. An example is the use of drought-tolerant mango varieties, which has proven to be an effective strategy for improving water productivity and yield under dry conditions (Mekuria <i>et al.</i>, 2015; Salazar <i>et al.</i>, 2018). • Diversification of fruit tree varieties: Incorporating varieties with different maturation periods and stress tolerance levels helps mitigate the risk of total crop loss due to extreme weather events. For instance, the diversification of rambutan varieties has shown to enhance orchard resilience to climate change in Thailand (Siam <i>et al.</i>, 2019). • Prioritization of research and development: Emphasizing the breeding of new varieties with higher climate resilience and encouraging the exchange of genetic material across regions are critical to broadening the available genetic base and strengthening adaptation capacity.
Water management	<ul style="list-style-type: none"> • Improve irrigation efficiency through drip or micro-sprinkler systems to reduce water use and enhance water productivity, especially under drought conditions. According to FAO (2020), “Drip irrigation has proven to be an effective strategy, reducing water consumption by 40% and increasing banana yield by 20% under drought conditions.” • Rainwater harvesting during the rainy season serves as an alternative water source for irrigation during the dry season, reducing reliance on scarce surface or groundwater sources. For example, Mekuria <i>et al.</i> (2015) found that rainwater harvesting increased water availability for irrigating fruit trees by 50% in areas with seasonal rainfall. • Implement soil management practices that improve moisture retention and reduce erosion, such as the application of organic fertilizers, mulching, and minimum tillage. • Utilize treated wastewater for irrigation when it meets appropriate quality standards, providing an additional water source while promoting resource reuse.
Soil management	<ul style="list-style-type: none"> • Improve soil quality by adopting agricultural practices that enhance soil health, such as adding organic matter, reducing tillage, and using organic fertilizers. These practices increase the soil’s water-holding capacity, improve plant nutrition, and boost drought resilience. According to Jones <i>et al.</i> (2017), compost application has been shown to increase soil water retention by 15% and mango yield by 10%. • Soil conservation through the implementation of techniques such as vegetative cover and terracing helps reduce erosion caused by heavy rainfall, maintaining soil fertility and productivity. Pinho <i>et al.</i> (2014) reported that vegetative cover effectively reduced soil erosion by 50% and increased pineapple yield by 15%.
Crop management	<ul style="list-style-type: none"> • Establish plantations in areas with microclimates more favorable to climate change, such as shaded slopes or zones with greater water availability. • Implement practices to capture rainwater and/or retain soil moisture. • Adjust planting and harvesting dates according to shifting climatic patterns. • Prune trees to improve ventilation and sunlight penetration, reducing the incidence of disease. • Apply fertilizers and pesticides efficiently and sustainably, following technical recommendations and using low-impact environmental products (Junqueira <i>et al.</i>, 2017). • Establish a monitoring system to assess the impact of climate change on the MIAF system over time. • Evaluate the effectiveness of implemented adaptation strategies and make adjustments or modifications as needed.

Table 1. Continues....

Adaptation Practices for the MIAF System	Description
Crop diversification	<ul style="list-style-type: none"> • Include other fruit species in production systems that are more tolerant to environmental stress conditions. • Combine fruit trees with other crops such as legumes or cereals to enhance soil fertility and reduce dependence on external inputs. • Establish agroforestry systems that integrate fruit trees with timber species and other vegetative components.
Capacity building	<ul style="list-style-type: none"> • Train tropical fruit producers in practical techniques for climate change adaptation. • Promote the exchange of information and experiences between producers and technical experts. • Encourage research and development of appropriate technologies for climate change adaptation in tropical agriculture. • Develop public policies that support the implementation of adaptation strategies in the fruit production sector.
Research and development	<ul style="list-style-type: none"> • Invest in research and development of new technologies for climate change adaptation in tropical agriculture • Encourage collaboration among researchers, extension agents, and producers to develop solutions tailored to local conditions • Prioritize research on the impacts of climate change on different tropical fruit species • Evaluate the effectiveness of various adaptation strategies in different contexts; use climate models to project future changes in variables such as temperature, precipitation, and evapotranspiration in MIAF-implemented areas • Incorporate crop impact models to assess the effects of climate change on the yields of MIAF crops (maize, beans) (CIMMYT, 2021; Morris & Scholes, 2022) • Identify and evaluate potential adaptation strategies for MIAF, such as selecting stress-tolerant varieties, implementing soil and water management practices, and diversifying crops • Analyze the economic and social feasibility of proposed adaptation strategies • Ensure farmer participation in the evaluation and adaptation process • Consider hydrological models to assess the impact of climate change on soil erosion and water availability

CONCLUSIONS

The MIAF system is a resilient alternative to cope climate change, combining practices such as crop diversification, water management, and soil conservation to enhance agricultural productivity. However, it still faces challenges, including adaptation to extreme climates, technology transfer, and potential reductions in crop yields. Strategies such as the use of resistant varieties, rainwater harvesting, agroforestry, and soil conservation are essential to strengthen its resilience. In this context, it is crucial to promote research, train producers, and develop public policies that support the adaptation of MIAF to climate change. Climate change adaptation practices offer multiple advantages for tropical agriculture by helping producers increase the resilience of their cropping systems, improve productivity and crop quality, and ultimately contribute to food security and the sustainable development of agroecosystems.

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Diagnosis of the causal agent of fruit necrosis in tabaquero pepper (*Capsicum annuum* L.)

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ABSTRACT

Objective: To identify the causal agent of fruit necrosis in tabaquero pepper (*Capsicum annuum*).

Design/methodology/approach: Since the planting and harvesting season of tabaquero pepper was suspended in 2022, a habanero pepper plantation exhibiting symptoms similar to those observed in tabaquero pepper was selected for pathogen isolation. Morphological and pathogenic characterization of the fungus associated with these lesions, as well as with foliar spots on the plants, was carried out. Additionally, molecular identification techniques using the 18S molecular marker were implemented to confirm the causal agent of anthracnose in this crop.

Results: The results indicate that the fungus belonging to the genus *Colletotrichum*, specifically the species *C. truncatum* (syn. *C. capsici*), may be the causal agent responsible for fruit and leaf necrosis in tabaquero pepper plantations in Macuspana, Tabasco, during the 2021-2022 season. Therefore, this research provides relevant information for the development of effective phytosanitary management and control strategies in current plantations.

Study limitations/implications: Greenhouse infrastructure conditions and climatic variables may have influenced the parameters obtained during the study. Additionally, the complete molecular identification of the selected fungi at the species level was limited due to the high costs associated with this process.

Findings/conclusions: Pathogenicity tests and the expression of symptoms in pepper fruits indicate that the fungus *Colletotrichum truncatum* may be the causal agent of fruit anthracnose and leaf spot in tabaquero pepper plantations in the municipality of Macuspana, Tabasco, Mexico. However, the need to implement more precise diagnostic techniques, such as microscopy and molecular analyses, is emphasized in order to detect the presence of the fungus at early stages of infection. This would not only improve phytosanitary control measures but also support the design of integrated management strategies aimed at reducing the spread of the pathogen in high-value commercial crops.

Keywords: *Colletotrichum*, Koch's postulates, pathogenicity, molecular, fungi.

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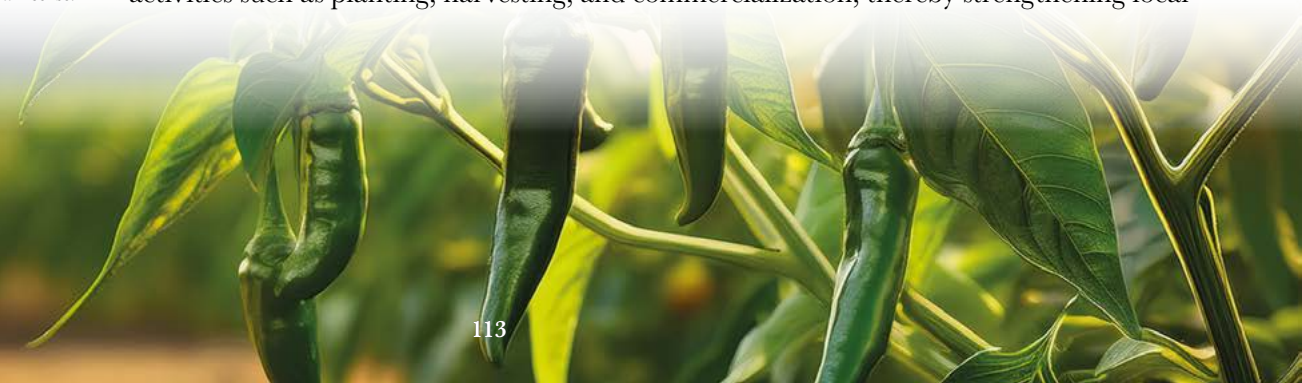
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INTRODUCTION

Tabaquero pepper (*Capsicum annuum* L.) has been a key crop in the agriculture of Tabasco, Mexico, standing out as an economic driver for rural communities. For years, its production generated stable income and promoted both direct and indirect employment in activities such as planting, harvesting, and commercialization, thereby strengthening local



value chains. In 2014, the annual production of dried tabaquero pepper exceeded two tons, representing a value of approximately 19.5 million Mexican pesos, positioning the crop as a strategic element in the regional economy. However, this outlook was severely affected in 2022, when unfavorable environmental conditions led to the outbreak of a devastating disease in the crops. The high sensitivity of tabaquero pepper to humidity variations, as noted by Aceves-Navarro *et al.* (2008), increased the incidence of fungal diseases, severely impacting production. According to an institutional report by Ortiz (2022), the disease was characterized by brown lesions with sunken areas on the outer surface of the fruits, along with circular to ovoid spots. These symptoms were associated with anthracnose caused by the fungus *Colletotrichum*. This situation revealed the vulnerability of the agricultural sector to environmental challenges, the lack of infrastructure, and the absence of accurate diagnostics, all of which limited producers' ability to respond effectively. The consequences were devastating not only for farmers, who faced high phytosanitary control costs and economic losses, but also for the regional economy, as the abandonment of the crop disrupted the productive chains that had previously driven local development. In light of this scenario, there is an urgent need to identify and analyze the causal agent of necrosis in tabaquero pepper, particularly in the pepper-growing region of Macuspana, Tabasco, in order to develop strategies that can mitigate future impacts and help revitalize this high-value crop.

MATERIALS AND METHODS

Study area

The study was conducted in the municipality of Macuspana, Tabasco, in the Pantanos subregion, specifically in the ejido (communal farmland) Nueva Esperanza. Sample processing took place in the Biological Control Laboratory at the Colegio de Postgraduados, Tabasco Campus (17° 58' 34.92" N latitude and 93° 23' 14.10" W longitude).

Koch's Postulates

To confirm the causal agent of fruit and leaf sunken lesions and necrosis in tabaquero pepper, Koch's postulates were applied through the following steps:

Pathogen Collection and Isolation

In 2022, the economic losses derived from the production of tabaquero pepper led to the suspension of its cultivation in the main production areas, making it difficult to collect fruits for research purposes. For this reason, a habanero pepper (*Capsicum chinense* Jacq.) plantation located in the community of Arroyo Hondo Abejónal, municipality of Cárdenas, Tabasco, was selected. This plantation included four cultivated varieties. During field sampling, fruits displaying characteristic sunken lesions were collected, placed in plastic bags, and refrigerated for further analysis at the Biological Control Laboratory of the Colegio de Postgraduados (COLPOS).

The collected fruits were placed under humid chamber conditions at 30 °C for eight days to allow visible symptom development. For pathogen isolation, tissue fragments smaller

than 1 cm² from affected areas were obtained and disinfected sequentially in distilled water, 1% sodium hypochlorite solution, and 70% ethanol. Subsequently, the fragments were plated onto Potato Dextrose Agar (PDA) medium and incubated in Petri dishes at 30 °C for 240 hours.

From the isolates obtained, four strains exhibiting morphological characteristics consistent with the genus *Colletotrichum* were identified. Based on mycelial growth and sporulation parameters, the strain used for the pathogenicity test was molecularly characterized.

Pathogenicity Test

A pathogenicity test was conducted using the microorganism isolated from habanero pepper fruits on a considerable number of healthy tabaquero pepper plants. For this, a conidial suspension was prepared following the methodology of Wang *et al.* (2021) with some modifications. Agar plugs with fungal mycelium were inoculated into Erlenmeyer flasks containing Potato Dextrose Broth (PDB) and shaken for 99 hours. Then, the conidial suspension was filtered, and a concentration of 1×10^8 spores mL⁻¹ was prepared, which was placed into manual sprayers. Spraying was performed at a distance of 20 cm from the plants, while control plants were sprayed with water only. The plants were monitored daily for 30 days, documenting symptom development following the methodology of Lozano *et al.* (2015).

Morphological Identification

To identify the macroscopic characteristics of the isolated pathogen, the following descriptive parameters were considered: presence of mycelium, colony color on the front and reverse sides, texture, shape, growth on the plate, and edge margins (Benítez, 2007). For microscopic identification, descriptive parameters included the structures of hyphae, setae, acervuli, and conidia. Measurements of each structure were taken according to the methodology described by Smith (2004).

Molecular Identification

The selected strain was sent to the Potosino Institute of Scientific and Technological Research (IPICYT), LANBAMA Laboratory, where molecular analyses were performed using the 18S molecular marker (CGACGGGCGGTGTGTACAAGT). The consensus sequence obtained was compared with existing records in the NCBI database using the Basic Local Alignment Search Tool (BLAST) to identify regions of similarity. Sequence assembly was carried out using Chromas Pro software (version 2.6.6), followed by multiple sequence alignment with Clustal X software. Seaview software was used as a graphical editor for multiple sequence alignments (Perdomo, 2011). For the construction of the phylogenetic tree, the MegaAlign tool was employed. Maximum Likelihood analysis was performed using MEGA-X software with 1,000 bootstrap replicates. The model that best fit the sequences was the Tamura-Nei model (Tamura *et al.*, 2021).

RESULTS AND DISCUSSION

Pathogen Isolation

A strain of *Colletotrichum* was obtained from habanero pepper fruits exhibiting dark, sunken circular lesions, designated as “Colle-H”. The colonies presented a circular shape with entire margins and a velvety/cottony texture, showing a dull gray pigmentation at the center and dark gray on the growth rays, along with concentric orange rings. These results are similar to those described by Pérez-Castro *et al.* (2003) in *Discorea* sp., where different morphotypes of the *Colletotrichum* genus exhibited mycelial growth of 1 to 2 cm per day. Likewise, colonies with velvety and cottony textures were observed, with pigmentation ranging from gray, black, to orange. This variability in sporulation and pigmentation among morphotypes is attributed to the different affected hosts and the specific agroclimatic conditions of each region. Microscopically, the strain exhibited typical structures of the genus *Colletotrichum*, such as pointed setae, short conidiophores, conidia, and the presence of acervuli. The conidia were fusiform, hyaline, with curved and pointed ends, measuring 20.2-22.4 μm in length and 3-3.01 μm in width. These results are similar to those reported by Rojo-Báez (2017), who highlighted the presence of acervuli, conidiophores, conidia, and abundant setae formed directly on the hyphae; the setae exhibited brownish coloration. The conidiophores can be hyaline, septate, and the conidia are unicellular, hyaline, smooth-walled, aseptate, with the central part generally slightly curved with parallel walls, measuring between 22.8-23.8 \times 3-3.02 μm . These are typical descriptions of the species *C. truncatum*.

The phylogenetic analysis placed the “Colle-H” strain within the *Colletotrichum truncatum* clade (syn. *C. capsici*). These morphological and molecular results were fundamental for conducting pathogenicity tests on healthy tabaquero pepper plants.

Pathogenicity Test

The analysis conducted on the plants inoculated with the fungus allowed the observation of the sequence of events necessary for the establishment of the infection responsible for anthracnose. The pathogenicity tests confirmed that the fungal isolate (Colle-H) is pathogenic on tabaquero pepper fruits, as evidenced by the appearance of circular and ovoid necrotic lesions with sunken areas displaying light to dark brown tones. However, a relevant aspect of the process was the absence of apparent defense responses or immediate cell death during the initial days of infection, as characteristic symptoms only began to appear approximately eight days after inoculation. During this period, the affected fruits retained a vigorous appearance and showed no visible differences compared to the controls. Auyong (2012) attributes this characteristic to the pathogen’s ability to colonize a wide range of hosts, which may be related to its capacity to adapt to different plant tissues and environmental conditions. As the days progressed after spraying, the symptoms did not appear at the same stage on the fruits as on the leaves, since the fruit cuticle remained intact and apparently undamaged. The infection period became visible during the second week. By day 12, the fruits began to exhibit the characteristic sunken appearance on the superficial epidermis; by day 15, damage caused by the fungus was observed, characterized by prominent, dark, sunken lesions with visible concentric rings. Between days 16 and 18,

the presence of the fungus became more noticeable, with lesion pigmentation progressing to darker tones and a more necrotic appearance. Auyong *et al.* (2015) indicate that the rate of infection by *C. truncatum* may depend on the phenological stage of the host, environmental factors such as humidity and temperature, as well as biochemical changes associated with fruit ripening. In addition, Rojo-Báez (2017), in studies related to the infection process of *C. truncatum*, highlights that this pathogen has the ability to remain in an endophytic state until external conditions favor the manifestation of visible symptoms.

The initial infection events were similar in both green and mature fruit stages; however, on day 20, symptoms were observed in younger fruits, which showed limited development, early maturity, weakened attachment, and the presence of a water-soaked cuticle caused by the gradual degradation of the cell wall. The levels of fungal presence progressively increased and remained nearly constant; likewise, there was no reduction in the infection process, as anthracnose was observed in 90% of the inoculated plants until the end of the monitoring period. Mahasuk *et al.* (2009), in their studies on the pathogenicity of fungi of the genus *Colletotrichum*, specifically *C. truncatum*, during the seedling and fruiting stages of chili pepper, reported that young tissues are more susceptible to the development of anthracnose symptoms. However, the characteristic quiescent phase of the disease allows the fungus to remain latent and infect new host shoots, meaning the persistence of the pathogen can be prolonged in the crops. The symptomatology and appearance of symptoms have been variable between fruits and leaves. Than (2008), in his study on the presence of anthracnose in commercial plants, attributes these variations to the influence of external environmental factors such as temperature, humidity, precipitation, wind, light, among others. However, Rojo-Báez (2017) mentions that the infection process of *C. truncatum* in other hosts mainly involves intracellular and subcuticular colonization as an infection strategy, along with the production of enzymes (cutinase, polygalacturonase, pectate lyase, and pectin lyase) during the necrotrophic phase.

These results open an important space for discussion about the dynamics of fungal establishment in different hosts. The lack of an initial defense response by the infected plants could be related to the fungus's ability to go unnoticed by the plant's defense mechanisms during the early stages of infection.

Morphological Characterization

In this study, the growth rate of the fungus was classified as rapid, as by the tenth day of incubation the colony had already developed a complete circular shape. On the tenth day, the fungus showed invasive growth across the entire plate, reaching a diameter between 72 and 79 mm. This growth exhibited a velvety or cottony texture and was characterized by dark gray tones. In addition, a decrease in color intensity was observed in the radial and concentric rings, resulting in an overall grayish tone of the colony, with a slight white pigmentation at the margins. On the reverse side of the colony, the grayish tone intensified, becoming darker, and the concentric rings were less prominent and paler. Although the gray shades observed in the colony are a common characteristic of the *Colletotrichum* genus, variations in color intensity were noted as the culture developed. Over time, the colors became darker, and the concentric rings became more evident. These findings are

consistent with those reported by Shin *et al.* (2024), who also observed that grayish tones are typical of *Colletotrichum*, although not exclusive to this genus. It is important to note that Adaskaved and Hartin (1997) and Casanova *et al.* (2016) indicated that morphological variations of colonies may be influenced by factors such as the culture medium and temperature fluctuations. According to Rojo-Báez (2017), *Colletotrichum* colonies can exhibit differences even within the same species, which may lead to difficulties in accurate species-level identification (Figure 1).

Microscopically, unicellular conidia measuring 20.2-22.4 μm in length and 3-3.01 μm in width were observed. These conidia had smooth walls, were aseptate, hyaline, and exhibited a slight curvature in the central region, with parallel walls ending abruptly in a rounded and truncated base, containing granular content. Likewise, the presence of acervuli, conidiophores, and abundant setae formed directly on the hyphae was observed. The setae were brown in color, and the conidiophores were hyaline, septate, branched, and densely clustered. The described features are fundamental in the infection process of *C. truncatum*, as they represent key elements for the development and dissemination of the disease in fruits and plant tissues. Rojo-Báez (2016) highlights that the pathogen's life cycle culminates with the production of acervuli loaded with conidia, which serve as the primary source of inoculum for new infections. These acervuli are associated with the appearance of dark, sunken lesions on fruits, characterized by a brown to black coloration.

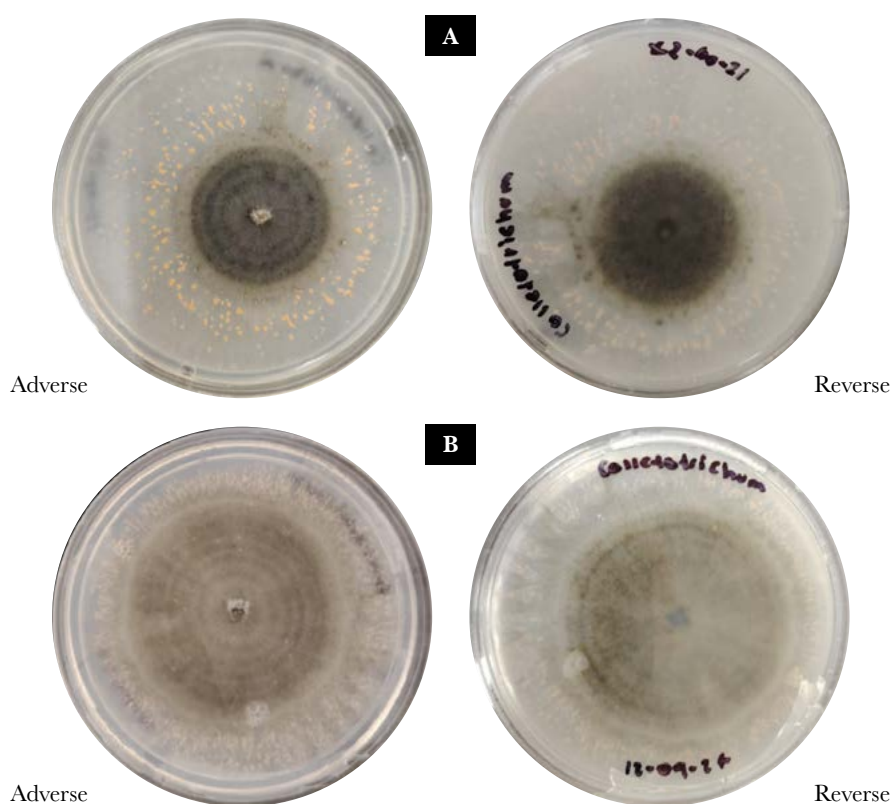


Figure 1. Strain Colle-H grown on potato dextrose agar (PDA) medium, incubated at 30 °C, after five (A) and ten (B) days of growth.

The cellular damage caused during the infection process increases as the acervuli proliferate in the affected areas, creating a favorable environment for the expansion of the fungus. The formation of appressoria —specialized structures that enable the fungus to penetrate the intact fruit cuticle— is crucial for the onset of infection. This mechanism is highly efficient, as it allows for the direct colonization of plant tissue without the need for prior wounding, which explains the pathogen’s ability to infect healthy tissues under favorable environmental conditions (Figure 2).

Molecular Characterization

DNA amplification through PCR of the re-isolated fungal strain Colle-H using the 18S rDNA primer was carried out at the Instituto Potosino de Investigación Científica y Tecnológica (IPICYT), producing a PCR product of approximately 700 bp. The phylogenetic tree (Figure 3), reconstructed using MEGA 5 software, placed the sequence within the taxon of *Colletotrichum truncatum*, with a branch support value of 94%. These molecular results confirm that the isolates, based on the amplification of universal primers targeting the 18S rDNA gene, are considered reliable —since values below 50% are deemed unreliable. Furthermore, the bootstrap value of the Colle-H consensus sequence was greater than 99%, supporting the conclusion that the Colle-H strain corresponds

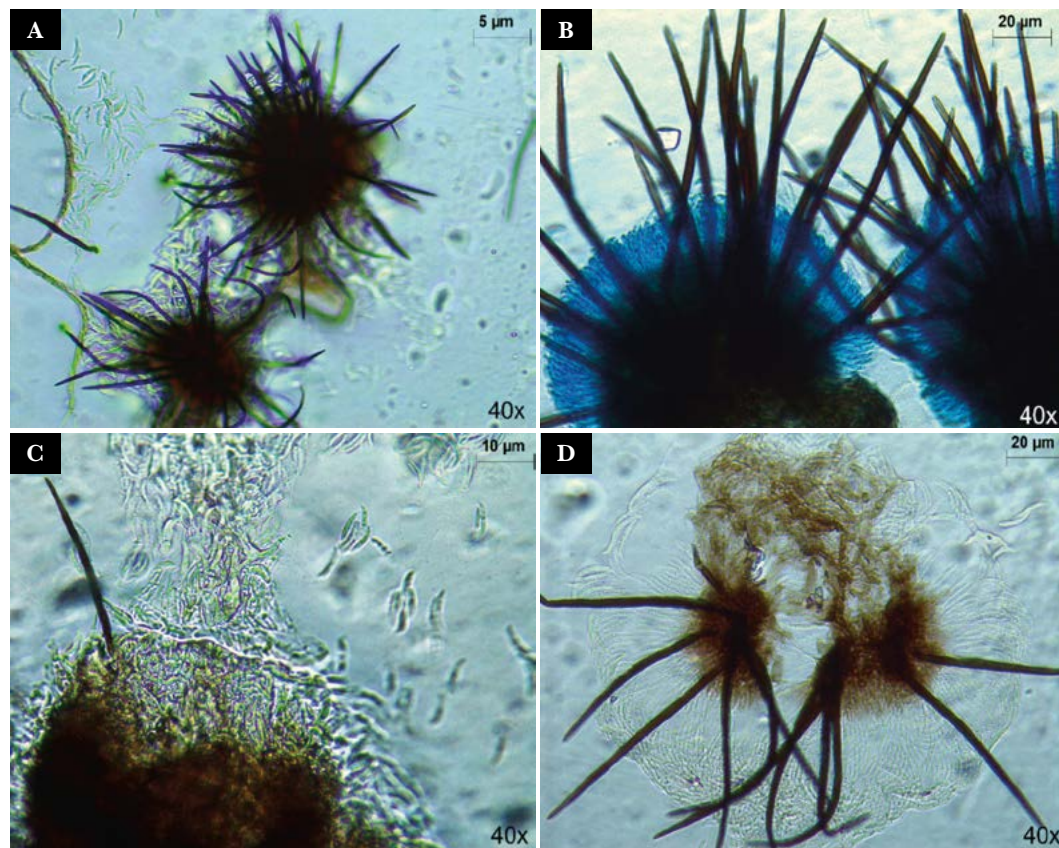


Figure 2. Acervuli (A, B, C, D), conidiophores (B), and setae of the fungus Colle-H (A, B, C), viewed at 40x magnification.

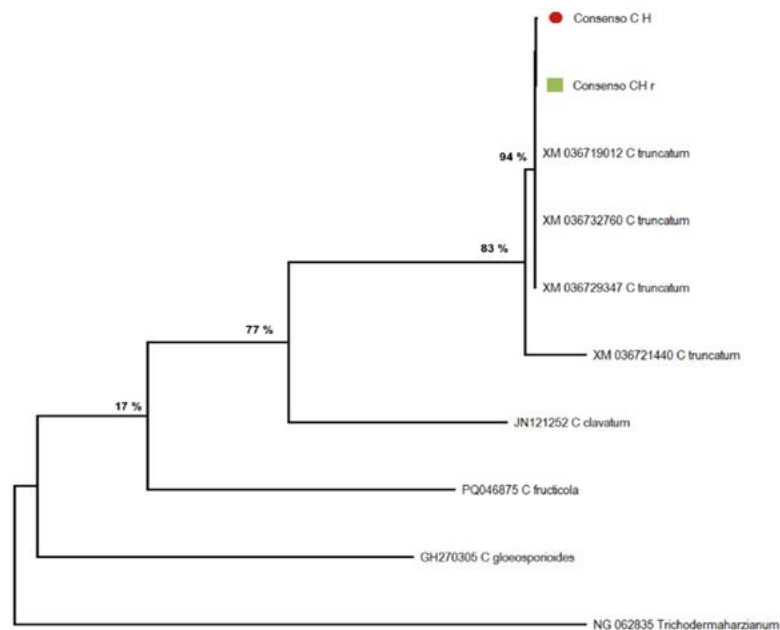


Figure 3. Phylogenetic relationship based on the 18S region. Tree constructed using MEGA 5 software, Maximum Likelihood (ML) method, Kimura 2-parameter model, and 1000 bootstrap replicates. Includes the sequence of the fungus Colle-H (Consensus C H) identified as *Colletotrichum truncatum* and three different species of *Colletotrichum*.

accurately to the species *C. truncatum*. These results are consistent with those reported by Rojo-Báez (2017), who molecularly identified *C. truncatum* isolates in papaya fruits using the 18S rDNA region. The use of this region facilitates amplification and makes it useful for the identification of fungi belonging to the species *C. truncatum*.

This comprehensive approach combining morphological and molecular techniques is essential, especially in the context of plant pathogen diagnosis, where accurate species identification can determine effective phytosanitary management strategies and contribute to the prevention of diseases in economically important crops. Moreover, precise identification not only allows for delimiting the impact of each species but also helps to better understand the ecological and evolutionary dynamics of the genus *Colletotrichum*.

These results open an important discussion regarding the dynamics of fungal establishment in different hosts. The lack of an initial defense response by the infected plants may be related to the pathogen's ability to evade the host's immune system during the early stages of infection. This phenomenon underscores the need to further investigate the interaction mechanisms between the pathogen and the host, taking into account factors such as environmental conditions and possible variations among cultivars. Furthermore, the similarities in symptomatic manifestations across different plant species suggest that certain infection mechanisms may be conserved within the *Colletotrichum* genus, which could provide valuable insights for developing integrated and effective management strategies to control this pathogen.

Finally, these findings highlight the importance of considering the physiological and biochemical characteristics of the host, as well as environmental factors that may influence

symptom expression and disease severity. Further studies could explore the specific interactions between *Colletotrichum truncatum* (syn. *Capsici*) and different chili pepper varieties, aiming to identify potential differences in susceptibility and to develop cultivars more resistant to this type of infection.

CONCLUSIONS

Pathogenicity tests demonstrated that tchihe fungus *Colletotrichum truncatum* can be the causal agent of anthracnose in fruits and leaf spots in tabaquero pepper plantations in the study region. However, further tests and sampling in different tabaquero pepper growing areas where the causal agent is present are necessary to confirm the disease.

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Adoption of robotics and artificial intelligence in agriculture

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ABSTRACT

Objective: to analyze research trends on the adoption of robotics and artificial intelligence in agriculture between 2020 and 2025.

Design/Methodology/Approach: this research is based on a systematic review and bibliometric analysis of articles on robotics and artificial intelligence in agriculture; which were retrieved from the Scopus database. Key players, core topics, and implementation strategies were identified in those articles. Subsequently, the main advantages and challenges, were analyzed in the well-known strategies. Our findings were contextualized with studies on agricultural mechanization and technology transfer to provide a comprehensive perspective looking forward to their adoption.

Results: analysis showed that this technological framework, although emerging, has experienced sustained growth in recent years. India and the United States are notable as the countries with the greatest scientific production. Thematic clusters revealed a shift in dominant terminology, moving from concepts such as “agriculture 4.0” and “agricultural robots” to “smart agriculture” and “digitalization.” Relevant barriers, such as financial and technical limitations, were also identified along with some effective strategies applied in other regions.

Limitations/Implications of the study: this study is limited to Scopus-indexed publications, as well as documents in English and Spanish. However, it offers valuable insights for researchers, policymakers, and other stakeholders in the agriculture sector.

Findings/Conclusions: our research underscores the need to design tailored strategies to overcome adoption barriers, especially among small-sized and medium-sized farmers. The importance of participatory approaches, cooperative models, and the use of multifunctional robots is emphasized as key tools to facilitate effective technology adoption, and to address current challenges.

Keywords: technology transfer, innovation, autonomous systems, precision farming, digitalization, public policy.

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INTRODUCTION

Innovation and technology transfer in Mexico’s agriculture, livestock farming, and fisheries play a key role in increasing productivity and sustainability. Agriculture represented 3.8% of the Gross Domestic Product (GDP) in 2023 according to the World Bank; and



is relevant not only for economic contribution but also for the employment generated, importance in feeding the population, and for the resources utilized. According to the 2022 Agriculture Census (INEGI, 2023), Mexico has 29.8 million hectares dedicated to farming, and the agriculture sector employs more than 27 million people, including permanent and temporary jobs. Globally, these figures rank Mexico in the 11th place in agricultural crop production. These data underscore the importance of studying the adoption of technologies such as robotics and artificial intelligence. Because of their potential to optimize resource use, and to address sustainability challenges, especially in a context where a significant portion of the workforce depends on agricultural activities.

Robotics and artificial intelligence (AI) are technologies, and as such, they are comprised of a set of theories and techniques that enable the practical use of the scientific knowledge, according to the definition by the Royal Spanish Academy of Language (RAE). Therefore, technology transforms the abstract knowledge produced by scientific research into know-how (Pereira, 2018) that involves the use of goal-oriented tools. Technology transfer from one organization to another generally requires a favorable context formed by economics, politics, and cultural aspects. In addition, the management entity that receives innovation technology should have the technical capacity to receive new technology, and the transferred technology needs to be compatible to some degree with the one existing in operation.

In particular, it is suggested that the technology transfer such as robotics or artificial intelligence requires to be studied with care and some specific considerations to reduce the risk of digital exclusion. As well as to avoid increasing inequality, while safeguarding human rights of the population as a whole (Pappa, 2024). Historically, technology shapes the way food is produced; but technology transfer is adopted unevenly, because capital-intensive agriculture is the one that, with its own resources and government support, makes the most use of innovations developed abroad and in national institutions.

On the other hand, small producers in Mexico face challenges such as lack of access to financing, technology, and training appropriate to their needs. In addition to difficulties in adopting sustainable agricultural practices for economic and technical reasons (Amaro-Rosales & De Gortari-Rabiela, 2016). This is how the paradigm of technological agriculture tends to marginalize small-sized and medium-sized farmers (Lule *et al.*, 2016). This has led Mexico, despite being a country open to technology developed around the world, to not adopt technology in all levels of production; and certainly not at the speed it progresses in other countries, especially in the most developed countries.

MATERIALS AND METHODS

In the initial stage of the study, three essential research questions were defined that guided the entire analysis and discussion process. Once defined, a bibliometric analysis and systematic review were conducted on the adoption of artificial intelligence and robotics in agriculture. For this purpose, a search was conducted in the Scopus database on January 6, 2025. A search strategy based on Boolean logic was used with the keywords “digital” AND “agriculture” AND (“robot” OR “AI”) AND (“technology transfer” OR “adoption”). The initial selection of documents by thematic areas was limited to engineering, computer

science, agriculture, environment, earth sciences, social sciences, mathematics, physics, decision sciences, energy, and multidisciplinary studies.

In order to ensure the quality and relevance of the documents, only research articles (ar), conference proceedings (dp), and review papers (re) were considered. After manual data cleaning and homogenization of these results, a bibliometric analysis was performed using the VOSviewer version 1.6.20 tool to create co-citation and keyword co-occurrence maps. In addition, the bibliometrix extension of R version 4.0.5 and the Biblioshiny command were used to obtain metrics on scientific production, collaboration between authors and countries, and analysis of subject evolution. Finally, a manual selection of the documents was done to extract the challenges and solutions in the adoption of AI and robotics in agriculture, as reported by other authors. Also, to compare what was found with other studies related to the adoption of mechanization and overall agricultural technology transfer to contextualize those findings.

The three research questions posed were; What studies exist on the technology transfer of robotics and AI in agriculture, and how has this field of knowledge evolved over time? What are the main challenges in the adoption of AI technologies and robotics in agriculture? What solutions have been proposed or implemented to overcome those challenges?

RESULTS AND DISCUSSION

The delimited search yielded 45 documents, of which 64.4% were research articles and 35.6% were review papers. These articles are from the fields of agriculture and biological sciences, computer science, social sciences, engineering, and others (Figure 1).

Publications on the adoption and technology transfer of AI and robotics in agriculture are on the rise, but still is not a trending topic of study when compared to the number of publications on artificial intelligence applications in agriculture. The latter accounts for 5585 publications in Scopus over the past 5 years (Figure 2).

A map was prepared with the bibliometrix tool in the statistics program R, which shows the countries where the transfer and adoption of robotics and artificial intelligence in agriculture is studied. India and the United States were the countries with the most papers produced; US was the country with the most citations (Figure 3).

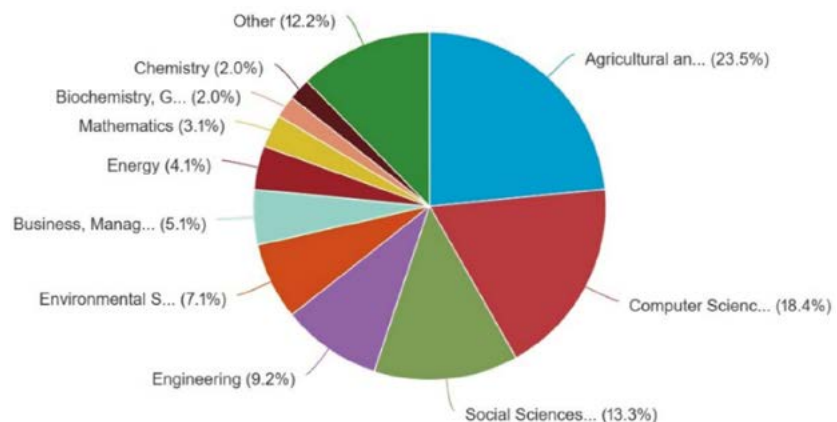


Figure 1. Papers found in the delimited search grouped by subject area.

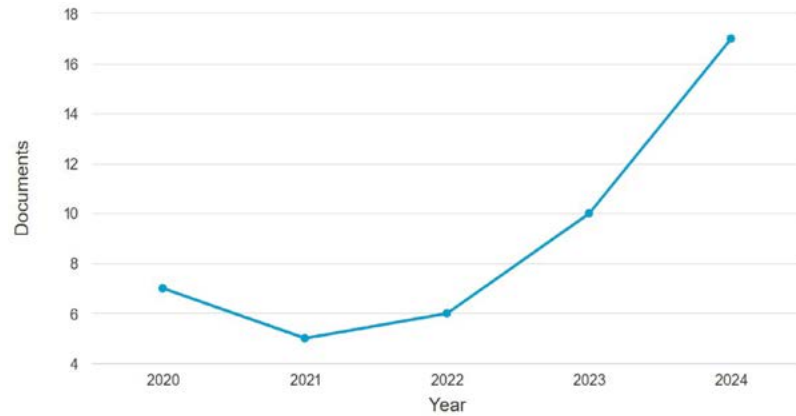


Figure 2. Papers published by year on the adoption of robotics and AI in agriculture that met the searching criteria.

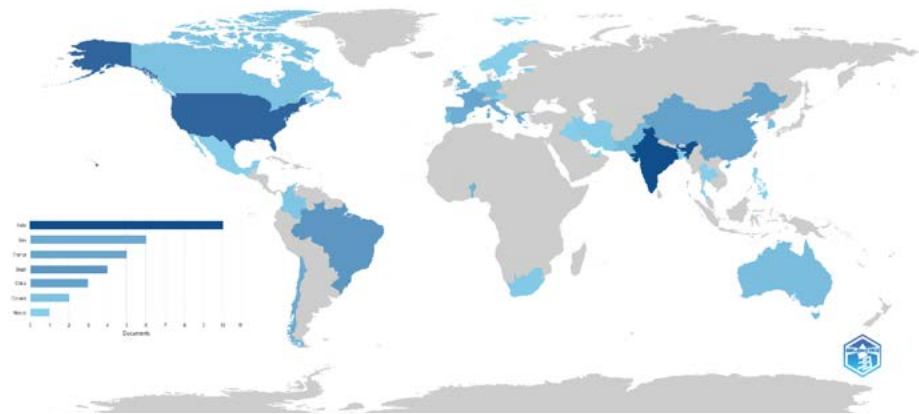


Figure 3. Map of papers published on effective technology transfer to use robotics and artificial intelligence in agriculture by country, generated with R (bibliometrix).

The bibliometric analysis using VOSviewer extracted 469 keywords from the titles and abstracts of the 45 filtered documents. Twenty-two of the most relevant terms that were repeated at least four times were selected. With the keyword co-occurrence map generated, we obtained the main topic clusters of the research (Figure 4).

The analysis results identified four color-coded clusters (red, green, blue, and yellow). Each cluster was manually labeled based on the relationship between the most frequently occurring words that link the terms in the cluster. For example, in the red cluster, the keywords “digitalization” and “agriculture” co-occurred most frequently, leading to labeling that cluster as “agricultural digitalization.” Similarly, the green cluster was labeled “technology adoption for food supply”; the blue cluster “technologies for agricultural sustainability”; and the yellow cluster “artificial intelligence in agriculture”.

The total link strength of two related terms (TLS) is used to predict trends and the main research topic. The yellow cluster in Figure 4 contains the largest number of terms and has the highest total link strength, compared to the blue, green, and red clusters. This indicates that the main research trend over the past five years is focused on the study of artificial

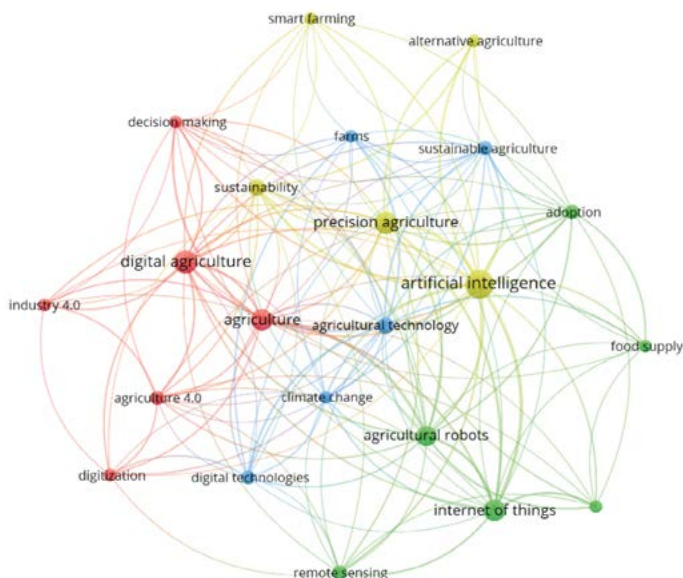


Figure 4. Keywords co-occurrence map of the research on robotics and artificial intelligence used in agriculture, generated in VOSviewer.

intelligence in agriculture, followed by agricultural digitalization, with the adoption of technologies for food supply ranked third, and technologies for agricultural sustainability related to climate change in fourth place (Table 1).

Table 1. Keywords clusters and the total link strength (TLS) value between them.

Cluster	Keyword	total link strength (TLS)
Artificial intelligence in agriculture (yellow dots)	Alternative agriculture Artificial intelligence Precision agriculture Smart agriculture Sustainability	176
Agricultural digitalization (red dots)	Agriculture Agriculture 4.0 Decision making Digital agriculture Digitization Industry 4.0	159
Adoption of technologies for food supply (green dots)	Adoption Agricultural robots Digital storage Food supply Internet of Things Remote sensing	155
Technologies for agricultural sustainability (blue dots)	Agricultural technology Climate change Digital technologies Farms Sustainable agriculture	128

This represents an opportunity, in those places with mobile coverage, for the adoption of robotics in agriculture, especially for the optimization of resources and applications with distributed sensors in lands destined to agriculture. Digital technologies integrated into agriculture promote efficient resource use and waste reduction, aligning with circular economy and environmental sustainability models (Boz & Martin-Ryals, 2023).

Barriers to the adoption of robotics and AI in agriculture

Acquiring advanced technologies, such as robots and AI-based systems, involves high initial costs, which are a significant barrier, especially for small-sized and medium-sized farmers in developing countries (Smidt & Jokonya, 2022). Gil *et al.* (2023) observed that the higher cost of robots compared to other machinery occurs because those AI-based devices have to be prepared to withstand changing environmental conditions; those robots use expensive hardware and sensors, the greater capacity, the higher the cost. Dainelli *et al.* (2021) stated that robustness in the face of environmental conditions is a necessary condition for success in their implementation. Another associated cost is the training of operators in equipment maintenance, which requires some specialization due to the lack of standardization of agricultural robots (Rial-Lovera, 2018). The lack of adequate training programs in rural regions (Bampasidou *et al.*, 2024) is also added as a cost.

On the other hand, cultural and social resistance to change is a phenomenon experienced in farming communities, with a strong reliance on traditional farming methods. This is combined with skepticism toward the usefulness of new technologies, particularly when immediate benefits are not seen (Srivetbodee & Igel, 2021). The lack of local data demonstrating their effectiveness in different climatic and soil conditions hinders their acceptance (Pappa, 2024). And security issues in the use of agricultural data shared with technology providers is also identified as a concern associated with digitalization (Khanna *et al.*, 2022).

A significant barrier to the adoption of technologies such as robotics and artificial intelligence in the agricultural sector comes from the lack of participatory involvement of producers in the technological development and adaptation process. This can result in the design of functionalities that are not perceived as value, because technological solutions offered are disconnected from local problems, thus limiting their acceptance and practical use in farming lands. The lack of specific incentives or legal frameworks that encourage the adoption of these technologies is also a significant barrier (Pappa, 2024). As an example, ecological benefits are not financially rewarded because there is insufficient evidence on those; but incentives exist in other industries, such as electric vehicle mobility.

Strategies for the adoption of agricultural robotics technologies

Strategies to promote the adoption of technology in agriculture are diverse. Europe and the United States, a more mature first-world market, are promoting the use of agricultural robots through a service delivery model (Pappa, 2024). This option helps the end user to avoid the high initial cost of the robots and the training required for their operation. These robotic agricultural services are offered comprehensively, from land preparation to

harvesting. In this way, the contractor extends the period of use of the robotic machines by using them with multiple clients, which allows them to amortize their costs.

Rent-to-own or leasing is an intermediate option that does not require a high initial cost, but does require operator training. This option allows farmers to get used to the technology without experiencing the risk of failing in implementation; also gives them the possibility of constantly renewing the equipment. However, a good advice for a company dedicated to agricultural robotics is, to be successful, they should continue research and development of their products, which may result incompatible with offering low prices (Gil *et al.*, 2023).

On the other hand, the transition to digital agriculture is different in developing countries (Johnson, 2024). To promote adoption among producers, it is suggested that training and practical demonstrations are key to increasing the use of agricultural robotics. The formation of farmer networks can facilitate the exchange of experiences and promote collective adoption (Smidt & Jokonya, 2022). In this regard, various organizations, such as the Autonomous University Chapingo, have proposed encouraging the creation of cooperatives among farmers that can share the use of agricultural robots, which would reduce acquisition cost and operating costs.

Dainelli *et al.* (2021) recommended that successful technological adoption requires user training; machines must be robust enough to adapt to changes in operating conditions, and, in the particular case of forest inventory machines, data must be easy to collect. Emphasis is also placed on education and training to ensure that producers properly understand and adopt technological devices. For large agricultural companies, this is easier; as it can be done by hiring specialized personnel (Milella *et al.*, 2024). For small-sized and medium-sized producers, it is suggested that the development of technological solutions should be accessible and specific to local needs; so considering the type of crops and the climatic or infrastructure limitations in each region (Boz & Martin-Ryals, 2023; da Silveira *et al.*, 2023).

One of the limitations to technology adoption in agriculture is the seasonal nature of production. Agricultural equipment is only used during specific stages of the crop. In contrast, as an example, in the automotive industry that uses the largest number of robotic arms, machinery operates continuously, which facilitates a faster costs amortization. In order to address this challenge, multitasking robots equipped with interchangeable or modular robotic implements could be used (Jensen *et al.*, 2012; Guri *et al.*, 2024). That would allow farmers to acquire technologies in a staggered manner and making them adaptable to different agricultural activities. Another technology adoption strategy is participatory development, with producers bringing together universities and research institutions that provide technical support. In turn, producers contribute ideas to solve their problems, then implementing and testing solutions. This partnership can also reduce the high initial cost of new technologies for producers (Langemeier & Boehlje, 2021).

Governmental role in promoting the adoption of agricultural technologies begins with making the necessary investments in telecommunications in rural areas to facilitate farmers' access to digital and robotic tools, especially in marginalized areas (Parra-López *et al.*, 2024). Furthermore, it is essential to implement public policies that promote digital literacy through training and education programs, allowing farmers to develop the skills

necessary to use these technologies (Smidt & Jokonya, 2022). Financial incentives, such as subsidies, loans, and leasing schemes should also be considered to reduce the initial costs of technological adoption, which are a significant barrier for smallholder farmers (Hasan *et al.*, 2024).

On the other hand, collaboration between the public sector, universities, and technology companies can facilitate research and development of solutions tailored to local needs, fostering innovation and sustainability (Adzenga & Dalap, 2023). Likewise, the government should establish clear regulatory frameworks that guarantee the ethical and responsible use of digital technologies, ensuring data protection and user privacy (Bampasidou *et al.*, 2024). Finally, public policies must be aligned with sustainability goals, promoting the use of robotics and AI technologies that reduce greenhouse gas emissions and improve the efficiency of global resources use such as water (Parra-López *et al.*, 2024), and foster environmentally friendly practices such as integrated pest management or organic farming (Ditzler & Driessen, 2022). All of these actions, combined, can create an enabling environment for the adoption of digital technologies in agriculture.

CONCLUSIONS

India and the United States are leaders in the research evolution on the adoption of robotics and artificial intelligence in agriculture, for effective technology transfer to small producers and communities. In Mexico, the main reasons identified to adopt those technologies are resource optimization and increased productivity that contribute to the economic viability of agricultural activities. The most significant barriers identified are high initial costs, lack of regulated financing, and scarce technical training adequate for small farmers.

In order to face the economic and technical limitations of small-scale farmers, we propose alternatives such as the creation of cooperatives to share costs and the implementation of multitasking robots as viable options. Also, we recommend farmer participation in technological development and targeted training in the use of innovation technology, until the market for robotic technologies and services is consolidated.

The adoption of robotics and artificial intelligence in agriculture in Mexico requires tailored strategies to local needs, combining participatory approaches, cooperative models, and public policies that promote sustainability, digital inclusion, and equitable access to these technologies.

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Theoretical foundations of sustainability and their contribution to innovation in the agricultural sector: a bibliometric analysis

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ABSTRACT

Objective: to gather knowledge of the behavior and trends of publications on the contributions from the dimensions of sustainability to innovation in the agriculture sector, based on their theoretical foundations.

Design/Methodology/Approach: a categorization of scientific papers was obtained through bibliometric analysis. In a first stage, the terms ‘innovation’, ‘sustainability’ and ‘agricultural sector’ were selected as search words. With which 161 papers were selected, all published during the analysis period. Then, information was classified in Excel[®] to generate bibliometric indicators in a second stage.

Results: main agricultural crops, in which innovation research related to sustainability was developed, were maize and beans; followed by rice and coffee. The most studied economic indicators were income, new markets, and production costs. Within the ecological dimension, the most recurrent indicators in research were biofertilization, nitrogen supply, carbon, crop rotation, coatings with organic matter, vermiculture, and climate change.

Limitations/Implications of the study: innovation is continuous, and results can vary if someone takes the articles that will be generated in the coming years. Therefore, our recommendation is to continue with other studies similar to this one, to give continuity and reveals new innovations.

Findings/Conclusions: about 30% of the works were focused on bacterial inoculation, biofertilizers and biostimulants. The search for soil fertility and its prompt recovery form the largest number of research aimed at technological and mainly biotechnological innovation in the agricultural sector worldwide.

Keywords: sustainability, innovation, bibliometric analysis.

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INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) recognizes four dimensions of food security; availability, access, utilization, and stability (Castañeda-Álvarez, 2022). For Ikerd (1990), sustainable agriculture is one capable of maintaining acceptable levels of biological and economic productivity, preserving the environment, and natural resources, satisfying at the same time a requirement of society.

In the second half of 1980s decade, the paradigm of sustainable development emerged as a result of the analysis of the World Commission on the Environment of the United Nations, through the document *Our Common Future*, which defined sustainability as the principle where human beings are at the center of concerns related to sustainable development. Humans have the right to a healthy and productive life in harmony with nature. The primer postulate is the satisfaction of the basic needs of world population for food, clothing, dwelling, and health (Badii, 2004). Sustainability can be understood as a paradigm for thinking about a future in which environmental, social and economic considerations are balanced in the search for development and a better quality of life (Oropesa-Casanova *et al.*, 2021).

The concept of sustainability is related to the principle of the scarcity of resources, the population that exists and the pressure they exert on the territory; through processes as pollution and depletion of natural resources, but also, with clean production strategies in any of the economic fields (Bravo & Bravo, 2016). Sustainable agriculture implies, among other things, long-term conservation of natural systems, optimal production with reduced production costs, adequate income and profit per unit of production. The World Commission on Environment and Development defines sustainable development as one that meets the needs of the present generation without compromising the ability of future generations to meet their own needs. In particular, the satisfaction of basic food needs, and sufficient supply to meet the demands and needs of rural families and communities (Liverman *et al.*, 1988).

On the other hand, bibliometric analyses are responsible for studying scientific activity in an area of study based on statistical and mathematical applications (Aleixandre, 2010). Bibliometrics makes it possible to identify patterns in publications and socialize the information generated on a specific topic (Rubilar-Bernal & Pérez-Gutiérrez, 2018).

The objective of this study was to gather knowledge of the behavior and trends of publications on the contributions from the dimensions of sustainability to innovation in the agriculture sector, based on their theoretical foundations.

MATERIALS AND METHODS

A critical analysis was done of the documents found on the subject 'innovations to agriculture sustainability' in the databases EBSCO and Redalyc in the period from 2019 to 2023. The methodology for bibliometric analysis was followed; in the first stage, 'innovation', 'sustainability' and 'agriculture sector' were used as search words.

That search allowed selecting 161 papers in those databases during the period of analysis. We excluded those that did not report on an innovation related to at least one of the dimensions of sustainability in the agriculture sector. Then, information gathered was classified in Excel[®] to generate bibliometric indicators in a second stage.

Among the participants in innovation, the most representative are transnational private companies with national subsidiaries; followed by agribusiness, brokers or other commercial intermediaries; agricultural producers and public and private research centers. These participants were identified in the published papers on innovation.

RESULTS AND DISCUSSION

One of the important contributions, according to this bibliometric study, on sustainability towards innovation is the technological change in the form of conventional production towards organic production in commercially important crops. The highest costs in organic production are in inputs. This, according to personal interviews with avocado producers in Los Reyes (Michoacan) Mexico, and blackberry fruit producers in Ziracuaretiro, (Michoacan), as well as what was found in several of the articles reviewed in this study.

Innovation in the agriculture sector can be defined as that improvement in production technology that allows, with a certain group of inputs, to obtain more quantity or better quality at the level of production. It was found that biotechnological innovations

predominate in agriculture. Nowadays, the search for sustainability of resources such as soils leads to a large number of research on the inoculation of microorganisms to produce biofertilizers. According to the crop and the specific endogenous requirements of the agricultural production process.

Evolution in the application of science, technology and their innovations in agriculture by agriculture & livestock companies have given rise to major stages of transformation. Types of crops and machinery have made production occurs, in the living conditions of society, with less physical burdens (Zayas, 2018). Companies are mentioned as agriculture & livestock, since it was found that there are innovations centered on forage production, which is part of agriculture, to supply livestock systems.

Adoption of new technology is determined by the size of the farm, farmer experience, education, and industry participation associated with innovation (Zepeda, 1994), and property rights (Perret & Stevens, 2006). Another factor in sustainability that influences the adoption of technological innovations is the efficiency of agricultural production (Cabrini *et al.*, 2013). More than 30% of the papers found are centered on the study of new biofertilizers, mainly based on microorganisms. Another large number of studies focus on the search for efficiency in the use of mineral nutrients and efficiency in the use of water. Few studies deal with credits structure and financing for organic producers.

Another contribution of sustainability to innovation is the development actions associated with social aspects; such as organizing in associations, societies, or agricultural cooperatives that seek collective benefits. One more contribution is based in the creation or improvement of new products or production processes that are friendly to the environment. This can be seen in the studies of new varieties through in vitro production, genetic alterations, hybridization and mutations. All of those were found in a number of documents in this review.

Innovation systems integrate economic, ecological, social, political, cultural and organizational factors that compose, then, the dimensions of sustainability. Although they are generally included in only three, which are environmental, economic and social, this latter grouping the political, cultural and organizational dimensions. Some environmental problems of sustainability that drive innovations in the agriculture sector are climate change, environmental degradation, growing competition for land and water, or high fertilizer prices, due to the depletion of primary sources such as phosphate rock (phosphorite). For this reason, several studies have been directed towards carbon incorporation, nitrite filtration, carbon dioxide generation, and other greenhouse effect gases.

The economic dimension of sustainability is characterized by a production system that allows efficient use of productive factors, generates economies of scale, and increases productivity to levels that improve competitiveness in markets. Within the social dimension, institutions and values are the basis of the development process. While policy creates an environment to promote production and development of human populations. According to García (2018), one of the contributions of sustainability to agricultural innovation is that which refers to the social dimension and its determinants. For example, wages, the rights of rural workers, and other social relationships for production. The innovation he proposed, in terms of the production process, is “network production” where agricultural

capital resorts to service providers towards lowering costs. In such a network, the main participants are producers, also, agricultural service contractors and suppliers of services or inputs.

Innovation profile in the agriculture sector

Agriculture innovation is a process through which farmers improve the production and management practices of their farms. In other words, innovation means the implementation of new crop varieties; the combination of traditional practices with new scientific knowledge; the use of new technologies and machinery; the jointly application of old and new production and post-harvest practices; or participation in markets in a new and more profitable way, among other aspects (Bustamante-Lara & Schwentesius-Rindermann, 2018). However, innovation requires more than just farmer action. Agriculture innovation arises from the interaction between the different stakeholders related to the sector; this is, producers, processors, packers, distributors, consumers, organizations and government (OECD, 2013).

A classification of innovation is shown below, with some examples from agriculture sector:

- **Products innovation:** diversification of new crops for export (fruits, vegetables and grains), new and different varieties of crops, improved and hybrid seeds, seedlings generation, planting of organic and premium-quality crops, reforestation, use of rootstocks, payment of royalties for patents of new varieties, agro-industrial transformation.
- **Production innovations:** intensive-biotechnological production, fertigation; protected and precision agriculture, drones; better management of agrochemicals and fertilizers, organic production, better agricultural practices (safety, health and quality); agroclimatic monitoring; cold rooms and conservation chambers; automated packaging and sorters; greenhouses for seedling germination; fertilization with vermicompost; automation of pumping water from the subsoil; Smart machinery with GPS to make the planting and harvesting processes more efficient, design and construction of harvesting vehicles; computer systems for the management of the production process; use of fumigator planes, new plant growing systems (like pergola system); refrigerated transportation; integrated disease management (genetic, chemical, biological and cultural pest control); agroecological management.
- **Organizational innovation:** financial diversification; installation of computer systems for the management of the administrative and organizational processes the company; rental of agricultural machinery; new forms of flexible hiring of agricultural workers (day laborers), this is, hiring by the day or by the task.
- **Marketing innovations:** contract farming; direct marketing (without intermediaries); search for better market windows (summer and winter); promotional campaigns or agriculture fairs; improvements in presentation and packaging (bags and boxes); world quality certificates, website and management of social networks (Facebook, Twitter, blogs) with company information; search for government support.

To be able to affirm about a change in the degree of sustainability in the agricultural sector due to a technological innovation, it is necessary to compare it with another technology, taking into account time, scale of production and context. One of the main economic incentives to use an innovation that impacts agricultural sustainability is the surplus price for agricultural products in the organic market. Mainly at foreign markets, in the case of Mexico with the United States as the main destination for agricultural exports.

Undoubtedly, the search for soil fertility and soils quick recovery after production, form the largest number of research aimed at technological innovation. This, in order to impact towards greater productivity that results in better profitability, which is part of the ecological and economic dimensions of sustainability. Innovation is mainly biotechnological in the agriculture sector worldwide. There are multiple indicators that can be part of the dimensions in sustainability study that depend on the research objectives and interests to be considered in such studies.

Among the innovations found is conservation agriculture, which is a cultivation system that encourages a minimum of mechanical alteration in soils and the maintenance of permanent soil cover with crop diversification. This system of production enhances biodiversity and natural biological processes that contribute to the use of water and makes the use of nutrients more efficient. It also promotes the sustainability of agriculture production, and impacts profitability by reducing production costs.

In the 161 articles analyzed, 131 main authors were detected. The Cuban Ionel Hernández-Forte was identified as a leader with four articles of innovations in agriculture sustainability, in which he reported research on bacterial inoculation, specializing in rhizobia, in rice crops. He is followed by the also Cuban Carlos José Bécquer with three articles; with studies on inoculation of microorganisms such as *Trichoderma harzianum*, *Azospirillum brasilense* and *Glomus cubense*, to be used as biofertilizers and biostimulants.

According to the search completed in the Redalyc and EBSCO databases during the period 2019-2023, the withstanding countries in the number of research reports on innovations related to several dimensions of agriculture sustainability are Cuba, as the undisputed leader with 61 papers; Mexico with 25; Colombia with 20; Ecuador with 14; Argentina with 10; Brazil and Costa Rica both with 9.

Among the territories of the Mexican Republic that made the most contributions to research on innovations related to agricultural sustainability are the State of Mexico, Chiapas, Coahuila, and Sonora during the 2019-2023 study period. Michoacán is one of the 14 states of the Republic that implemented this type of research of great importance for the biotechnological development in Mexican primary sector.

During the period of analysis, in the 161 articles considered, a total of 32 different species of agricultural crops were studied. The main crops in which research on innovation related to sustainability were maize and beans with 14 and 13 articles during the period 2019-2023, followed by species such as rice, coffee and soybeans with eight documents each, cocoa tree and papaya with six; sugar cane, fodder and tomato with five; lemon and banana with four each. Since livestock production is linked to agriculture, fodder crops were included, and along with tomatoes, lemons and papaya were among the 12 crops with larger number of experiments reported.



Figure 1. Federation Entities (states) in Mexico with innovations related to agricultural sustainability during 2019-2023. Source: original with data from Redalyc and EBSCO.

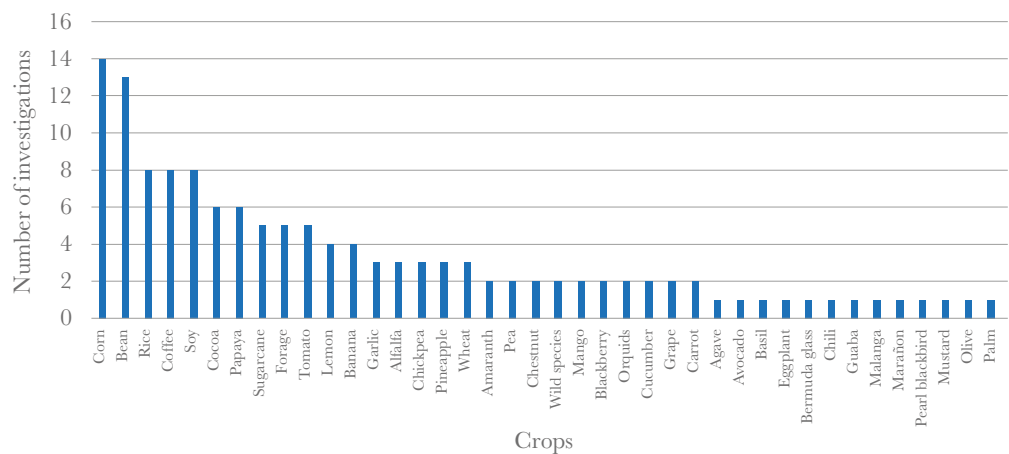


Figure 2. Main crops where sustainability-related innovations were made in the agriculture sector. Source: original based on data from Redalyc and EBSCO (2019-2023) databases.

In regard to the dimensions of sustainability analyzed in this research with their indicators, Economic includes income, new markets, production costs; followed by price, labor costs, input costs, technical assistance, study on clusters, credits, certification and determination of the break-even point. In the Ecological dimension, biofertilization, nitrogen contribution, carbon; crop rotation, organic matter mulch; vermiculture; climate change; protected agriculture; dead and living barriers; crop association (all of which are centered on the recovery of the fertility of agricultural soils and water conservation).

Those main dimensions were followed not so closely by innovations towards other human-related dimensions, cultural, social and political. Here we found education, health, equity, dwelling; recognition of the empowerment of rural women, quality of life for producers, work quality, identification of key participants and production networks.

This means, that mainly, innovations were more technological and biotechnological in nature. In terms of technology, the innovations were mostly in new products, *in vitro* propagation, integrated and post-harvest technology, irrigation technology,

green technologies, labeling systems, multi-scale precision systems, new machinery and equipment, hydroponics, new cultivation systems, mechanized transplantation, thermotherapy, spectroscopic index, use of Single Nucleotide Polymorphism markers with a high degree of precision; nutrient injection, remote sensing, chromatography, weather radar, and molecular markers (Figure 3).

In regard to biotechnological innovations, about 30% of the research papers reported on bacterial inoculation, biofertilizers and biostimulants; 13 articles with focus on the search for new varieties, 5 on new hybrids, 4 on biological control of pests, bioinputs and in vitro cloning, followed by studies of dehydration, plant biomass coverage, organic matter mulch, bacterial DNA extraction and genomics, genetic improvement, nanobiotechnology, mutations, and cross-pollination. Regarding processes, we found few studies; two referring specifically to food sufficiency and one, in the study of rural community-owned enterprises.

The reason behind placing biofertilizers as an innovative bioproduct is they cause a significant impact on each of the dimensions of sustainability. Such innovations are implemented in traditional crops such as basic crops (staplers); maize, beans, rice; also

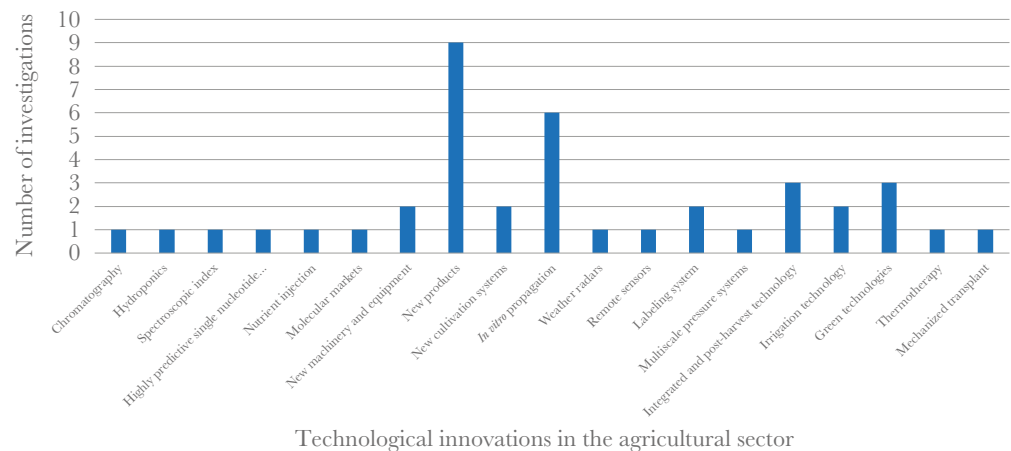


Figure 3. Main types of technological innovations in agriculture during 2019-2023.

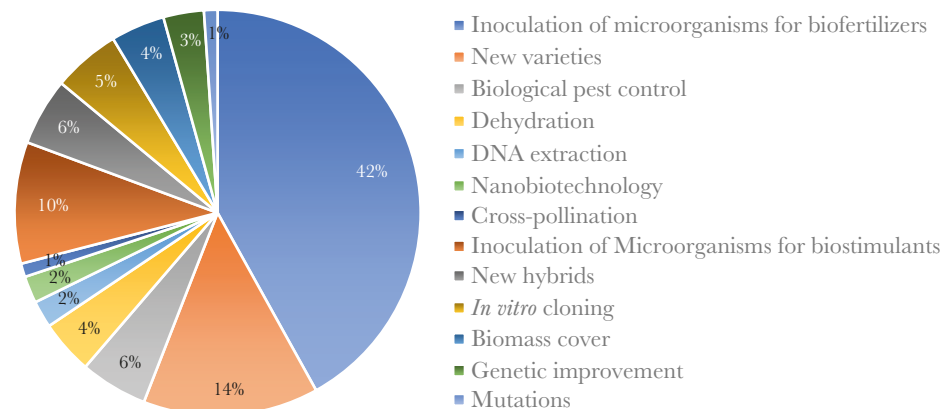


Figure 4. Main biotechnological innovations in Agriculture during 2019-2023.

coffee and cocoa tree generate important innovations in agriculture. As well as other products of great commercial interest such as sugar cane, banana, lemon, as well as berries and avocado that are also part of the findings in this bibliometric study.

Those figures that are taken as indicators of the dimensions of sustainability in agriculture vary according to the subject and the goals pursued by the research. Some additional indicators of the environmental dimension can be erosion risks, productive diversity, management of pests and diseases with biotechnological and conventional methods, health status, waste management, waste reuse, just to mention a few. In the economic sphere are diversification of production, percentage of products destined for sales or for self-consumption, income level, social security, dependence on inputs, production costs, search for the internal rate of return (IR) or break-even point, among many others. Some indicators developed for the political, social and cultural dimensions are type of land ownership, level of education, access to health systems, access to primary services, social integration, training; even others found in this study with focus on the empowerment of rural women, among others.

CONCLUSIONS

There were more than 30 different innovations found, which indicates the great interest in innovation in scientific research in agriculture, mainly for maize, beans, rice, coffee and cocoa tree; also, sugarcane, banana, lemon, berries and avocado. Main sustainability dimensions found were economic and ecological, followed not so closely by social, cultural and political. Withstand economic indicators are revenue, new markets and production costs; also, prices, labor and technical training costs; study on clusters, credits by certification and break-even point determination.

In the ecological dimension, the more recurrent indicators were biofertilizers, conservation agriculture and climate change; the main focus is on the recovery of soils fertility and water conservation. As social indicators, education, health, equity and dwelling. Within political and cultural dimensions, the indicators were quality of life for producers, work quality, recognition of the empowerment of rural women, key participants identification and production networks.

Innovation is continuous; these results may significantly vary in the coming years. A subsequent and similar study is recommended to give continuity and monitor new innovations. Among those, for example, meteorological radars that seek to determine where it may rain and predict rainfall level of rainfall; or those related to genetic mutations and nanobiotechnology, which we found very few mentioned in this period of analysis.

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Nanotechnology, an alternative to prevent and treat bovine mastitis

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ABSTRACT

Objective: to describe the importance of nanoparticles in the prevention, diagnosis and treatment of bovine mastitis.

Design/Methodology/Approach: nanotechnology in the field of veterinary medicine has revolutionized scientific research. As it aims to access a new era in the diagnosis and treatment of dissimilar diseases such as bovine mastitis. In regard to it, this disease was characterized in its most common origin and diagnosis. Then, we examined state-of-the-art papers on the use of nanoparticles as an alternative treatment to verify results and effectiveness.

Results: reviewed reports on the use of nanotechnology as treatment did show favorable, faster and more effective results. Also, it is discussed how nanotechnology has innovated the field of prevention of this disease that can occur in cattle in a clinical or subclinical form, in order to offer innovative solutions to current problems, such as the prevention, diagnosis, and treatment of this common disease in dairy cattle.

Limitations/implications of the study: despite its significant importance in the treatment of bovine mastitis, applied nanotechnology still has certain limitations to be noted. These include the lack of a single, standardized dose; doses used must be tested through clinical studies assessing some aspects such as nanoparticle type, drug load, and infection severity. In addition to other aspects like toxicity, distribution, bioavailability, immunological interactions, and costs.

Findings/Conclusions: the use of nanoparticles in veterinary medicine as treatment of bovine mastitis allows transforming delivery vehicles and assimilation paths for drugs and vaccines, which enhances treatment effectiveness. Therefore, the use of nanomaterials positively impacts animal health and well-being.

Keywords: bovine mastitis, nanotechnology, veterinary medicine.

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INTRODUCTION

Milk is one of the most sought-after products worldwide due to its nutritional properties and the variety of derivatives obtained from it. However, one of the main challenges facing its production is the incidence of diseases such as bovine mastitis.



In the production chain of bovine milk, mastitis is considered the most important economic and productive disease (Alfonso *et al.*, 2020). Because it causes losses related to decreased milk yield, increased clinical treatments for animals, and the culling of affected cows (Bedolla *et al.*, 2020). This disease also produces changes in the protein and lipid composition of milk that affect its quality (Aguilar and Álvarez, 2019). For this reason, it can also threaten the health of consumers through the presence of pathogenic microorganisms in the milk, when is obtained from cows affected by the disease.

In preventing mastitis, proper hygiene and good husbandry practices during milking are essential factors. Antimicrobials are used as the drugs of choice to achieve its control. However, some new, more sustainable and environmentally friendly alternatives have recently emerged, such as the use of nanoparticles.

Based on the above, this paper aims to present the latest advances in this pathology and the new strategies proposed for its prevention and control. The objective was to describe the importance of nanoparticles in the prevention, diagnosis and treatment of bovine mastitis.

Bovine mastitis

Mastitis is the inflammation of the mammary gland (udder) and its secretory tissues. Mastitis incidence decreases milk production, alters the composition and flavor of this product; it also increases the normal bacterial load (Gasque, 2015), in addition to affecting animal well-being (Medrano *et al.*, 2020). It is classified according to its symptoms into clinical and subclinical mastitis (González y Vidal del Río, 2021). Clinical symptoms include increased volume, redness, and pain in the mammary gland. Fever, loss of appetite, and weakness may be seen. Alterations in milk quality such as lumps, changes in color, and an increase in bacterial content are also observed. In the subclinical form, no apparent changes are observed in the udder or in the milk. However, milk production decreases and its composition is altered by the pathogens that may be present (Córdova *et al.*, 2019).

Etiology

The causes of this pathology are related to aspects of cow welfare, health, and hygiene (Córdova *et al.*, 2019). Infectious sources (Benić *et al.*, 2018; Bedolla *et al.*, 2020) and non-infectious sources, such as injuries, high ambient temperatures and stress in animals, are cofactors that cause the incidence of bovine mastitis (Brisuela *et al.*, 2018).

Mastitis is classified according to its causative agents as contagious or environmental. The infected mammary gland is the primary reservoir for contagious agents (García *et al.*, 2018). These infectious source includes bacteria such as staphylococci, streptococci, or coliforms. As well as mycoplasmas, fungi, and yeasts, all of which are pathogens responsible for mastitis (Zoetis, 2023). In studies, contagious and environmental agents have been isolated as causes of the disease. Among those, the following are outstanding, *Staphylococcus aureus* and *Enterobacter* spp. (García *et al.*, 2018); staphylococci coagulase-negative (SCN) and enterobacteria. (Alfonso *et al.*, 2020); *Bacillus* spp., *Streptococcus agalactiae*, *Streptococcus* sp., *Corynebacterium bovis* and *Escherichia coli* (Rodríguez & Espinosa, 2020; Valdivia *et al.*, 2022).

Diagnosis

Different methods are used to detect mastitis, such as the evaluation of physical and chemical changes in milk through conventional bacteriological methods and molecular techniques. Other tools are currently used, ranging from field techniques to sophisticated applications such as biosensors, microchips, and nanotechnology (Aguilar and Álvarez, 2019; Ajose *et al.*, 2022).

Nanotechnology

Nanotechnology (NT) refers to structures whose size is measured in *nm*, which is equivalent to the billionth of a metre (1×10^{-9} m), this is, the millionth of a millimetre (1×10^{-6} mm). Within this scale, nanomaterials and nanostructures are found between 0.1 to 100 nanometers (Cervera-Villaseñor *et al.*, 2023).

Following the contributions from different disciplines, such as biology, chemistry, physics, engineering, and medicine, nanoparticles evolved in recent years and strategies are combined that allow the design, synthesis, and manufacture of nanomaterials (Lira *et al.*, 2018). In fields such as the diagnosis, control, and treatment of diseases, research with these structures has demonstrated great potential (Janiski *et al.*, 2022).

Nanotechnology is a scientific and technological advance that has an important application in different fields of knowledge. In livestock industry, in particular, NT is considered one of the most influential technologies of the 21st century (Feugang, 2019).

Classification of nanoparticles

Nanoparticles are classified according to their origin and the materials they are made of. According to their origin, there are naturally occurring nanoparticles, which are made of organic or mineral materials, and anthropogenic nanoparticles, which are produced by human operations during industrial processes (Frejo *et al.*, 2011).

Regarding the materials that compose them, there are different types; carbon-based nanoparticles, which are mostly made of this material. Metal-based nanoparticles, which are composed of different heavy metals, can in turn be grouped into four categories, metallic nanoparticles (0D), metallic nanowires and rods (1D), metallic sheets and plates (2D), and metallic nanostructures (3D). Dendrimer-based nanoparticles, which are formed by synthetic polymer macromolecules (such as peptioides, lipoids or polysaccharide-modified particles). Finally, composite nanoparticles, which are combinations of similar nanoparticles or nanoparticles mixed in larger materials (Zhang *et al.*, 2016).

Among the most commonly used nanomaterials are metallic nanoparticles such as silver (AgNPs), gold (AuNPs), platinum (PtNPs), copper (CuNPs), selenium (SeNPs); or based on iron oxide (Fe₂O₃NPs), titanium dioxide (TiO₂ NPs), zinc oxide (ZnONPs), among others (Arana *et al.*, 2021).

Nanotechnology in veterinary medicine

Nanotechnology is notable for its potential to revolutionize the veterinary science sector, covering different areas ranging from animal breeding and reproduction, in the field of

animal nutrition, as well as disease diagnosis and the administration of drugs and vaccines (Ali *et al.*, 2021).

For the treatment of infectious diseases of bacterial, fungal (Hassan *et al.*, 2015), parasitic or viral origin (Bogdanchikova *et al.*, 2016) and non-infectious neoplasias (Wójcik *et al.*, 2015), a wide range of metallic nanoparticles are evaluated in veterinary medicine. Research on these nanoparticles is focused on improving anti-inflammatory responses and the wound healing process; As well as in vaccines development, drug delivery, innovation of diagnostic methods for biomolecules detection (DNA, lipids, proteins, metabolites), and for the identification of pathogens and adulterants in food (Kuswandi *et al.*, 2017; Yaqoob *et al.*, 2020).

Nanoparticles in the prevention, diagnosis, and treatment of bovine mastitis

Silver and copper nanoparticles (AgNPs, CuNPs) have shown to have an effect against *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus uberis*, *Candida albicans* and *Candida krusei*, in evaluations with Au, Ag, Cu and Pt nanoparticles, which were tested *in vitro* against microorganisms involved in bovine mastitis (Wernicki *et al.*, 2014). One of the most notable advantages of nanobiotechnology in disease diagnosis is the ability to detect specific biomarkers with high sensitivity and specificity. For the capture and detection of molecules that indicate the presence of diseases in milk samples, nanomaterials (carbon nanotubes, metallic nanowires, and polymeric nanomaterials) can be functionalized with antibodies or other biological recognition molecules (Martínez & Mera, 2024).

Another important advantage is the possibility of developing multiplexed tests, which can reveal multiple mastitis biomarkers in a single sample, providing more specific information about the type of current infection and the severity of the infection. This is beneficial because the precise determination of the causative agent is essential for effective treatment, since there are a variety of bacterial pathogens that can cause mastitis (Singh *et al.*, 2017).

In addition to their high sensitivity and specificity, techniques and devices based on nanobiotechnology provide other significant benefits in the diagnosis of bovine mastitis. For example, some of these methods are fast and portable, which supports a better clinical decision-making and reduces the time required to obtain results (Jensen, 2016; Elsanad, 2020).

Administration of drugs and vaccines

To achieve direct drug delivery to the site of infection, nanotechnology enables encapsulation and controlled release. For the reduction of frequent dosing, antibiotic-loaded nanoparticles easily penetrate the affected tissues, increasing local drug concentration. This minimizes systemic drug exposure, which can reduce bacterial resistance and side effects (Mahendra *et al.*, 2017).

One of the innovative therapies for the treatment of bovine mastitis is nanoparticles loaded with anti-inflammatory agents or immune system modulators that can help reduce inflammation and cause the recovery of the affected mammary gland (Cuca, 2018). An example of this is silver nanoparticles (AgNPs), which penetrate the bacterial cell

membrane, alter its function and cause its death. These are used as delivery vehicles for antibiotics, increasing their efficacy and reducing bacterial resistance. They can be used in concentrations between 10-50 $50 \mu\text{g mL}^{-1}$ (Chen, 2022).

On the other hand, gold nanoparticles (AuNPs) have anti-inflammatory and antioxidant properties. By modulating animal immune response and reducing the production of inflammatory mediators, AuNPs decrease inflammation in the mammary gland, improving the bioavailability and efficacy of drugs by acting as vectors for their delivery (Elsanad, 2020).

Likewise, polymeric nanoparticles (nanohydrogels and polymeric micelles) are used as drug delivery systems. They can encapsulate antibiotics and release them in a controlled manner at the site of infection, extending their therapeutic action and reducing frequent dosing (Sun *et al.*, 2019).

Also, lipid nanoparticles (nanoemulsions and liposomes) are used for drug delivery in the treatment of bovine mastitis, the dose is 1-10 mg of nanoparticles per affected quarter in the udder. This varies according to the type of encapsulated drug and the specific formulation. They can encapsulate hydrophilic and lipophilic drugs, making them versatile in the administration of different types of antibiotics and therapeutic agents. In addition, they improve the solubility and stability of drugs, increasing their bioavailability and efficacy (Chen, 2022).

Finally, magnetic nanoparticles are a promising strategy for the treatment of bovine mastitis. These nanoparticles are functionalized with specific molecules that recognize and bind to bacterial pathogens. Using external magnetic fields, they can be selectively directed to the site of infection, improving local drug concentration and reducing systemic exposure (Buzea *et al.*, 2007; Martínez & Mera, 2024).

CONCLUSIONS

Bovine mastitis is a disease that affects dairy herds, causing significant economic losses to the dairy sector. In addition to adopting good practices and maintaining adequate hygienic conditions during the milking routine, innovative and sustainable alternatives are currently being used to prevent, diagnose, and treat this disease.

The use of nanoparticles in the diagnosis and treatment of bovine mastitis offers an innovative approach. They are an important alternative for the prevention of this disease and also enable the development of a new drug delivery route. Therefore, further research is needed in this area, which has proven to be of great importance in veterinary medicine.

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Validation of the use of unmanned aerial vehicles for population assessment of the bighorn sheep (*Ovis canadensis mexicana* Shaw) in the Sonoran Desert

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ABSTRACT

Objective: To evaluate the use of drones for population monitoring of the desert bighorn sheep in two Wildlife Management Units (WMUs) of the Sonoran Desert.

Design/methodology/approach: Fieldwork was conducted at El Tonuco and Chaparral Ranches, Sonora, during the summer seasons of 2020 and 2021. Direct observations of bighorn sheep were initially carried out, followed by the use of drones to record individual animals. Relative abundance was estimated according to sex and age class. To assess the effectiveness of drone usage, results were compared to those obtained through direct monitoring using the non-parametric Mann-Whitney U test, correction factor, and detectability percentage.

Results: Drone monitoring recorded 118 and 96 bighorn sheep in El Tonuco and Chaparral Ranches, respectively, representing 2.26 and 1.31 times more individuals than those detected through direct observations. Females constituted the most frequently recorded group. No significant differences were found in bighorn sheep abundance between the two monitoring techniques. At El Tonuco Ranch, the correction factor for population estimates based on direct observations, in comparison to drone data, was 62%, while at El Chaparral Ranch it was 24%. This factor varied significantly according to sex and age class ($P \leq 0.05$).

Study limitations/implications: It is recommended to complement the findings with geospatial analyses and to replicate this methodology in other key sites for the conservation of desert bighorn sheep.

Findings/conclusions: The use of drones proved to be a more efficient tool than direct transect observations for detecting bighorn sheep.

Keywords: Detectability, drone, correction factor, xerophilous scrub.



INTRODUCTION

In Mexico, three subspecies of desert bighorn sheep (*Ovis canadensis*, Artiodactyla: Bovidae) are recognized, inhabiting mountainous desert environments: *O. c. cremnobates* and *O. c. weemsi* in the Baja California Peninsula, and *O. c. mexicana* in Sonora, with reintroductions in certain mountain ranges of Chihuahua and Coahuila (Valdez & Krausman, 1999; Medellín *et al.*, 2005; McKinney & Villalobos, 2005; McKinney & Delgadillo, 2006). Due to unregulated hunting, habitat fragmentation, and disease transmission, the species is listed under Special Protection (Pr) according to NOM-059-SEMARNAT-2010 (SEMARNAT, 2019). From an ecological perspective, the desert bighorn sheep plays a key role in the structure and dynamics of plant communities by contributing to vegetation regulation in desert scrublands through grazing, which directly influences plant composition and diversity (Guerrero-Cárdenas *et al.*, 2016; Gastelum-Mendoza *et al.*, 2021, 2024). Economically, it is the most valuable game species in Mexico, with hunting prices exceeding USD 40,000 per specimen, thereby promoting its sustainable use through habitat and population management and conservation (Lee, 2011).

Effective management of desert bighorn sheep populations relies on accurate knowledge of their abundance and structure. In this regard, systematic monitoring is an indispensable tool for decision-making concerning the species' management and sustainable use, as it provides crucial information on population parameters such as abundance, age and sex structure, and harvest rate estimation (Romero-Figueroa *et al.*, 2024). Furthermore, monitoring allows for the identification of demographic trends, the detection of negative anthropogenic impacts, and the assessment of management and habitat restoration efforts (Lee & López-Saavedra, 1994; Lee, 2011; Romero-Figueroa *et al.*, 2024). The most efficient and widely used method for population monitoring of desert bighorn sheep in the Sonoran Desert is aerial helicopter surveys (Remington & Welsh, 1989; Lee & López-Saavedra, 1994). However, this method entails limitations related to high costs, complex logistics, and risks for involved personnel (Miller *et al.*, 1989). Ground-based monitoring, while safer and more cost-effective, only allows for coverage of limited areas, making it difficult to obtain representative population data (Norton-Griffiths, 1978). In this context, the use of unmanned aerial vehicles (UAVs) presents a technological tool that enhances human effort, reduces costs, and facilitates the implementation of standardized protocols for monitoring wildlife in hard-to-reach areas (Hodgson *et al.*, 2013; Martin *et al.*, 2012). This technology offers a systematic and replicable alternative for the continuous evaluation of the abundance and population structure of desert bighorn sheep, particularly in mountainous desert ecosystems where topographic and climatic conditions limit the application of traditional methods. Therefore, the aim of this study was to assess the abundance and structure of a desert bighorn sheep population in the Sonoran Desert using drone-based data, and to compare these findings with those obtained through direct observations. This analysis aims to generate valuable information for the species' management and conservation, and to support informed decision-making toward sustainable management.

MATERIALS AND METHODS

Description of the study area

The study area is located in the municipality of Hermosillo, Sonora, encompassing the ranches El Chaparral and El Tonuco, both registered as Wildlife Management Units (WMUs). El Chaparral Ranch lies 82 km northeast of Hermosillo city and covers an area of 6,515 hectares, while El Tonuco Ranch is situated 37 km in the same direction, spanning 1,560 hectares (Figure 1). According to the Köppen climate classification modified by García, the region corresponds to type BWhw(x'), characterized as very dry and semi-warm, with an average annual temperature ranging from 18 °C to 22 °C. During the coldest month, temperatures drop below 18 °C (García, 1998). The dominant vegetation type is microphyllous and sarcocaulous desert scrub (INEGI, 2016; Figure 1). Precipitation is primarily concentrated in the summer, with a predominantly summer rainfall regime and a winter rainfall proportion ranging from 10.2% to 36% (CONAGUA, 2015). The topography of the sites varies in elevation from 0 to 1,056 meters above sea level. Slopes also differ according to terrain configuration, ranging from 0° to 2.7° in flat lowlands, 9° to 18° on hillsides, and 28° to 58° in the steepest mountainous areas.

Analysis of population abundance and structure

During July 2020 and August 2021, population monitoring of desert bighorn sheep was conducted. This period coincides with the hottest season in the Sonoran Desert, during which the monitoring and identification of the species are more effective due to increased predictability in their activity patterns and location. High temperatures force individuals to concentrate near permanent water sources and in shaded areas or elevated locations with better ventilation, significantly reducing their spatial distribution and facilitating detection (Adame *et al.*, 2017).

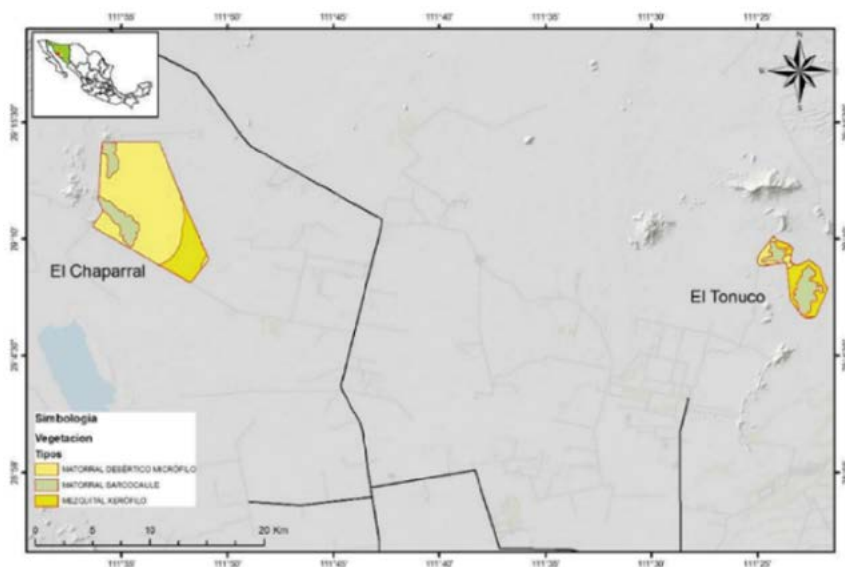


Figure 1. Location and vegetation types of El Chaparral Ranch and El Tonuco Ranch in the state of Sonora, Mexico.

Population monitoring was carried out using a network of roads adjacent to the bighorn sheep's escape terrain, primarily located in lowland foothill areas commonly explored during the legal hunting season. Based on these criteria, permanent observation points were established, spaced 1.5 to 2.0 km apart, to minimize the likelihood of pseudoreplication in individual recordings. Furthermore, due to the presence of permanent water sources in the area, the sheep did not frequently engage in local migrations, thus reducing the risk of overestimation in the observations. Sampling began 30 minutes before sunrise and continued until 11:00 a.m., or until wind speeds exceeded 25 kilometers per hour or drone operating temperatures risked overheating the devices.

At each sampling point, the start time was recorded, and observers searched for bighorn sheep for a period of 10 to 15 minutes, or until the professional guides (certified big-game hunting experts) identified the maximum number of individuals. This ensured accurate identification and detailed knowledge of the study area. At the end of the observation period, once the number of individuals by sex and age class was recorded (Figure 2), a drone flight was conducted over the locations where individuals were detected and surrounding areas to identify any not directly observed. A DJI Mavic 2 Pro drone was used for capturing aerial images and videos, equipped with a Hasselblad camera featuring a one-inch CMOS sensor and an effective resolution of 20 megapixels. The optical system has a 77° field of view and a 28 mm equivalent focal length, with an adjustable aperture ranging from f/2.8 to f/11, allowing optimization based on ambient lighting conditions. ISO sensitivity ranges from 100 to 3200 in automatic mode and up to 12800 in manual mode for photography, while for video, the range is 100-6400 (automatic) and 100-12800 (manual). Images were stored in JPEG and DNG (RAW) formats, and videos in MP4 or MOV using H.264/MPEG-4 AVC and H.265/HEVC codecs. The camera allows for 4K resolution recording (3840×2160 pixels) at 30 frames per second, providing high-quality footage for visual analysis of the observed wildlife. The drone was operated at an altitude between 20 and 40 meters above the mountainous terrain. This methodology yielded both total and relative abundance of the desert bighorn sheep population, where total abundance was defined as the number of individuals observed regardless of sex or age class. To calculate relative abundance, the proportion of individuals was determined for each category: females, young, class I

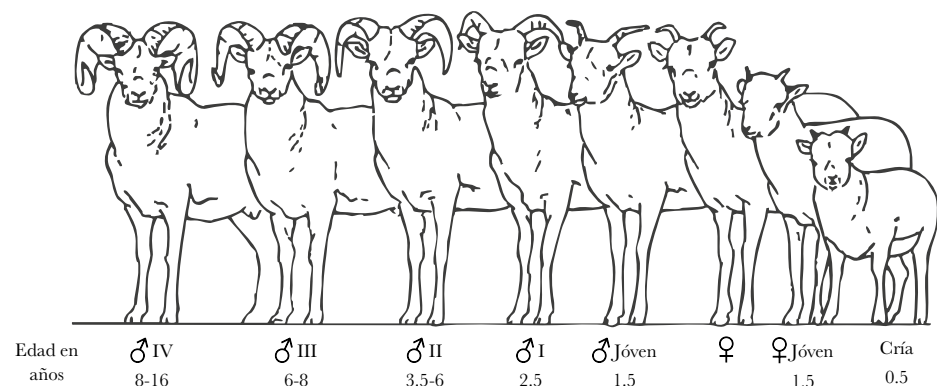


Figure 2. Sex categories and age classes of desert bighorn sheep according to the classification by Geist (1968).

males, class II males, class III males, and class IV males (Geist, 1968), relative to the total population abundance.

At the end of each sampling period at each observation point, the number of individuals detected through direct observation and the number recorded via drone were documented. The population detectability percentage was expressed as the difference between detections obtained through direct observation and those recorded using drones. Additionally, the total population correction factor (regardless of sex or age classes) was calculated using the following formula:

$$\text{Correction factor (\%)} = \left(\frac{\text{Total number of observations with direct observation} \times 100}{\text{Total number of observations using drone}} \right) - 100$$

For relative detectability by sex and age class (females, young, class I, II, III, and IV males), the following formula was used:

$$\text{Correction factor for sex and age class (\%)} = \left(\frac{\text{Number of observations of each sex and age class by direct observation} \times 100}{\text{Number of observations of each sex and age class using drones}} \right) \times 100$$

Finally, the non-parametric Mann-Whitney U test ($\alpha \leq 0.05$) was applied to identify differences in bighorn sheep population abundance based on the two monitoring techniques used.

RESULTS AND DISCUSSION

Although no significant differences were identified in the number of bighorn sheep recorded using the two monitoring techniques, direct observation at El Tonuco Ranch yielded 45 individuals, whereas drone monitoring detected 118, representing a 2.26-fold increase in population abundance compared to direct observation. Similarly, at El Chaparral Ranch, 73 individuals were recorded through direct observation, while drone monitoring identified 96, equating to a 1.31-fold increase in abundance (Table 1).

The use of drones for wildlife and habitat monitoring is still considered emerging, although it is steadily developing as a valuable tool for ecological studies. Several investigations have demonstrated the utility of this methodology in the population assessment of wildlife. Michel *et al.* (2014) validated the use of drones to monitor penguin colonies in the Shetland Islands, Norway. Additionally, drone technology has been successfully applied to monitor other terrestrial mammals, with studies on the abundance and distribution of elk (*Cervus elaphus*) (USGS, 2014), fallow deer (*Dama dama*) (Barasona *et al.*, 2014), and elephants (*Loxodonta africana*) (Vermeulen *et al.*, 2013). In Mexico, Mandujano *et al.* (2017) designed a drone-based methodology for monitoring white-tailed deer (*Odocoileus virginianus*) and their habitats in Actopan, Veracruz, the Tehuacán-Cuicatlán Biosphere Reserve in Puebla, and certain regions of Oaxaca. More recently, Valencia-Maldonado (2021) applied this

Table 1. Comparison of desert bighorn sheep population abundance using direct observation and drone monitoring at El Tonuco and El Chaparral Ranches, Sonora, Mexico.

Sex and age classes	Direct observation		Difference in observation**
	Rancho El Tonuco ($U=11^*$, $P=0.3$)		
Females	16	57	3.56
Offspring	5	25	5
Class I Males	2	3	1.5
Class II Males	4	4	0
Class III Males	6	13	2.16
Class IV Males	12	16	1.33
Total	45	118	2.26
Rancho El Chaparral ($U=15.5^*$, $P=0.75$)			
Females	31	41	1.32
Offspring	22	31	1.4
Class I Males	14	17	1.21
Class II Males	2	2	0
Class III Males	4	5	1.25
Class IV Males	0	0	0
Total	73	96	1.31

* Mann-Whitney U test; number of times greater.

technology to assess the abundance of mule deer (*Odocoileus hemionus eremicus*) in the Sonoran Desert. Contrary to the results of the present study, no differences were reported between direct transect observations and drone-based monitoring.

These findings suggest that the effectiveness of population monitoring techniques drone-based or direct observation depends on habitat characteristics and species ecology (Mandujano *et al.*, 2017). For instance, mule deer are primarily distributed across lowlands and gently rolling hills covered by desert scrub in the Sonoran Desert, where relatively flat terrain and sparse vegetation enhance visual detection and facilitate transect counts (Mackie *et al.*, 2003). In contrast, desert bighorn sheep are associated with rugged mountainous habitats, steep slopes, sparse vegetation, and difficult terrain, which limits the efficiency of direct observation and complicates the application of transect-based sampling (Valdez & Krausman, 1999). Under such conditions, the use of drones offers clear advantages by enabling efficient monitoring in remote areas and facilitating the detection of individuals in complex habitats.

The results of this study suggest that drones are more effective than direct observation in estimating the sex structure of desert bighorn sheep populations (Table 1). Drone-based evaluations more effectively identified the locations of females and young, which are generally more difficult to observe than adult males (Valdez & Krausman, 1999). This is primarily due to the species' sexual segregation behavior. During the non-breeding season, females and young tend to remain in hard-to-reach highland areas of the mountain ranges, using such locations as an anti-predator strategy (Bleich *et al.*, 1997). In contrast, males, after the breeding season, tend to move randomly across the mountain strata in search of

high-quality forage to replenish the energy lost during reproduction (Main *et al.*, 1996), which typically spans from July to December, peaking between August and November.

Adult males, particularly class III and IV, were more frequently recorded via drone in both El Tonuco and El Chaparral Ranches; however, no class IV males were recorded at El Chaparral (Table 1; Figure 3). The low detectability of adult males may be attributed to their low abundance or to the possibility that, during the study period, they were located in the uppermost parts of the mountain ranges or near female groups as they attempted to establish dominance for the upcoming breeding season (Bleich *et al.*, 1997). Moreover, in August one of the hottest months in the Sonoran Desert bighorn sheep tend to reduce activity to conserve energy.

The greater detectability of adult males using drones (Figure 4) reinforces the notion that this technology is effective in identifying individuals dispersed across large areas. These findings align with those of Anderson *et al.* (2018), who concluded that drones overcome the limitations of direct observation, especially in detecting species in open and inaccessible environments. However, while drones increase individual detection rates, the lack of significant differences in the observation of class II males suggests that habitat characteristics or behavior of specific age classes may influence the effectiveness of this technology. Compared to previous studies that reported variability in drone efficacy

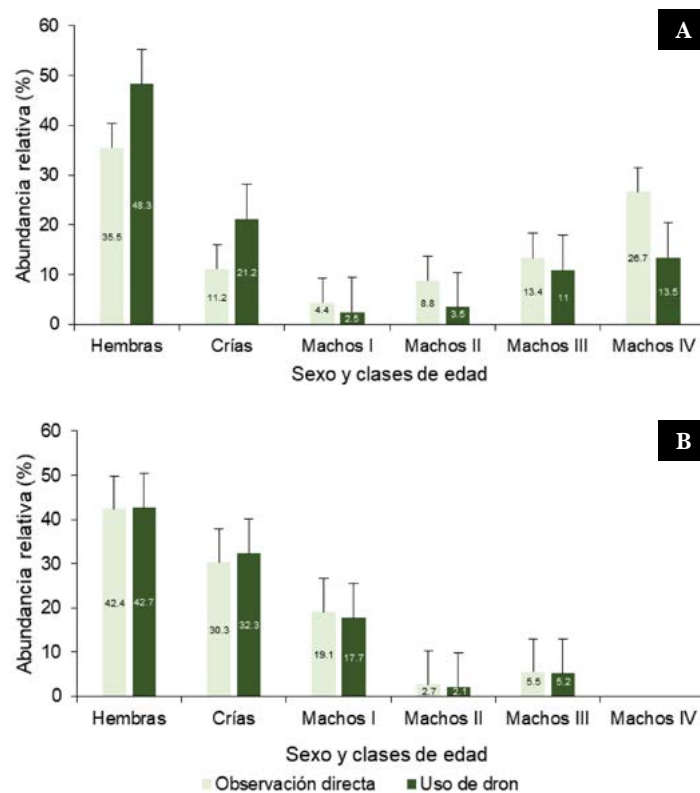


Figure 3. Relative abundance of desert bighorn sheep records by sex and age class, according to the classification proposed by Geist (1968), obtained through direct observation and drone use at El Tonuco Ranch (A) and El Chaparral Ranch (B), located in Hermosillo, Sonora, Mexico (vertical lines on the bars indicate the standard error of the mean).



Figure 4. Desert bighorn sheep specimen classified as a class III male, according to Geist's (1968) criteria, detected using a drone at El Chaparral Ranch at an approximate altitude of 20 meters above ground level (photograph taken by the first author).

depending on species and environment (Wehausen & Bleich, 2007), the present study underscores the importance of integrating multiple monitoring methods to achieve more accurate population estimates.

At El Tonuco Ranch, the overall correction factor for the population estimated through direct observation compared to drone-based monitoring was 62%. This indicates that direct observation underestimates approximately 62% of the population recorded via drones. At El Chaparral Ranch, the correction factor was 24%, suggesting that direct observation underestimates about 24% of the population detected using drones (Figure 5). Additionally, the correction factor was influenced by sex and age classes (Figure 3; $P \leq 0.05$). These results indicate that drone technology enables more efficient detection of bighorn sheep, suggesting that, while useful, direct observation may not be sufficient to obtain accurate population estimates in open and hard-to-access environments.

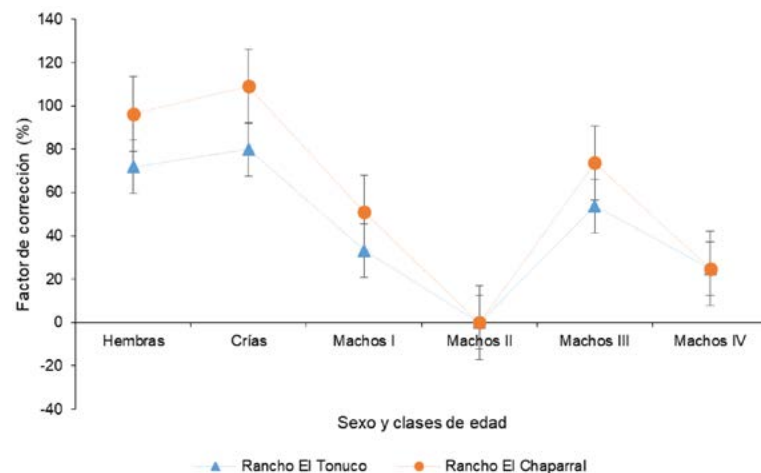


Figure 5. Variation in the correction factor comparing direct observation with drone use, based on sex, age classes, and study site in the Sonoran Desert (vertical lines on the data points indicate the standard error).

The use of drones for regional-scale population assessments of desert bighorn sheep must consider key factors for estimating abundance from aerial surveys, including the number of animals observed, detectability value, the area surveyed, and the total available habitat for the species. Detectability is defined as the probability of observing an animal within the observers' field of view (Steinhors *et al.*, 1989). Unlike drones, this parameter has been calculated for aerial bighorn sheep surveys conducted via helicopter (Romero-Figueroa *et al.*, 2024). For instance, in the Baja California Peninsula, population censuses of bighorn sheep have assumed that only 35% to 60% of the total population is sighted (De Forge *et al.*, 1993; Martínez *et al.*, 2010). This estimation is based on the work of McQuivey (1978) and Hervert *et al.* (1998), who reported that the probability of detecting a group of sheep during a helicopter survey ranged from 0.37 to 0.55. Although drone use in wildlife studies has proven to be an effective tool for obtaining population and behavioral data, its potential limitations are often insufficiently addressed (Ajanic *et al.*, 2020). For example, some animals may alter their behavior or avoid the drone's presence, potentially affecting the representativeness of the observations (Adame *et al.*, 2017). Additionally, the limited battery autonomy restricts flight duration and coverage, especially in extensive or hard-to-reach areas. Further challenges include potential errors in identifying individuals or species from video footage, due to image quality, drone speed, or lighting conditions (Jones *et al.*, 2023). These limitations must be considered when interpreting results and designing more robust methodological protocols. Drone-based wildlife monitoring may present biases in shaded areas or regions with dense vegetation, where visual or thermal detectability of animals may be significantly reduced. For example, in closed-canopy forests, tree cover may conceal animals in both RGB and thermal imagery, thereby lowering count accuracy and increasing the likelihood of missing individuals under foliage (Koh & Wich, 2012; Chrétien *et al.*, 2016). Similarly, Burke *et al.* (2018) noted that thermal interference from the ground and vegetation obstruction may hinder detection with infrared cameras mounted on drones, especially in densely vegetated areas. On the other hand, studies using LiDAR sensors indicate that even advanced systems struggle to penetrate multiple vegetation layers and detect wildlife or understory features without very high point densities (Torresan *et al.*, 2017). Collectively, these findings suggest that shaded or densely vegetated areas may introduce undercounting biases in drone-based projects, thereby affecting data representativeness and validity.

Therefore, it is essential to calibrate drone use to accurately determine the proportion of the bighorn sheep population that can be detected using this technology. Furthermore, drone effectiveness is closely linked to technological aspects. In this regard, integrating thermal and topographic sensors is important to enhance animal detectability and improve habitat assessment efficiency. The findings presented in this study underscore the value and advantages of using drones as a tool for monitoring desert bighorn sheep populations in the Sonoran Desert. However, it is crucial to replicate this research in other regions within the species' natural range in northern Mexico. Additionally, this methodology could significantly contribute to the Conservation, Repopulation, and Sustainable Use Program for Desert Bighorn Sheep in Sonora, by complementing helicopter-based aerial

monitoring through training of authorities, WMU landowners, and wildlife technicians in drone technology applications.

CONCLUSIONS

The use of drones enabled the detection of a greater number of bighorn sheep compared to direct observation, with the most notable difference observed at El Tonuco Ranch. Due to their gregarious habits and ethology, adult females were the most frequently detected individuals. The results demonstrate that drone technology is an effective methodology for monitoring desert bighorn sheep populations in desert scrub habitats. Therefore, it is recommended that this approach be replicated in other areas within the species' natural range in the Sonoran Desert. Lastly, a portion of the photographic and video material obtained during this study is available on the first author's social media platforms (Instagram: @ivanlzns and @wildlifeoldmexico).

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Producer practices, interests, and points of view regarding *Vanilla planifolia* Jacks ex Andrews wilt disease in a Totonac community

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ABSTRACT

Objective: To determine the socioeconomic profile of vanilla (*Vanilla planifolia* Jacks. ex Andrews) producers, their practices, interests, and points of view regarding vanilla wilt disease in the Dimas López community, Olintla, Puebla, Mexico.

Design/Methodology/Approach: A descriptive probabilistic study was conducted through semi-structured interviews with key vanilla producers (n=24). Data analysis was based on descriptive statistics.

Results: All producers were men, 70.8% were between 44 and 68 years old, 95.8% of them spoke Totonac as their native language, and 92% of them were farmers. Acahual was the predominant production system (84%). The maximum planting area was 0.25 ha. The species used as vanilla tutors were piñón (*Jatropha curcas*), pichoco (*Erythrina* sp.), and cocuitle (*Gliricidia sepium*). More than half of producers (62.5%) have found symptoms of wilting in their crops. Producers applied ash, calcium hydroxide, and sodium bicarbonate as a disease management practice. Producers are mainly interested in phytosanitation (58.3%), pollination (29.1%), plant nutrition (8.2%), and curing vanilla (4.1%).

Study Limitations/Implications: The results of this research are only applicable to the study area.

Findings/Conclusions: The Totonac vanilla producers in Dimas López are 44-82-years old. They use the acahual production system. Ash and lime are the main products used for the management of vanilla wilt. The main interest of vanilla producers is phytosanitation.

Keywords: *Vanilla planifolia*, sociodemographic profile, productive profiles, points of view.

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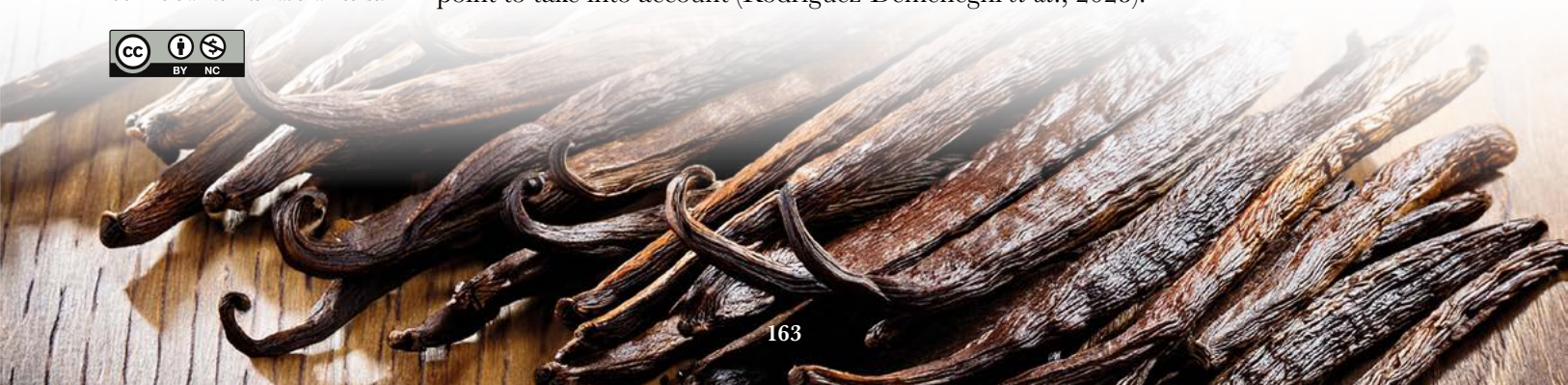
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INTRODUCTION

The genus *Vanilla* comprises more than 110 species distributed in tropical or subtropical regions (Jiménez *et al.*, 2017). Due to its remarkable aromatic properties, the most commercially used species is *Vanilla planifolia* Jacks. ex Andrews (Orchidaceae) (Cervantes-Castillo *et al.*, 2019). Vanilla is native to Mexico and its main habitat is located in the Totonacapan region, which covers over 20 municipalities in the state of Veracruz and 19 in the state of Puebla. This region produces more than 80% of the total domestic vanilla production; therefore, the socioeconomic development of local producers is a major point to take into account (Rodríguez-Deméneghi *et al.*, 2023).



The vanilla grown in the Totonacapan region is widely recognized for its quality, which is the result of the aromatic compounds developed through a combination of natural factors and regional crop management practices (Santillan *et al.*, 2019). However, the vanilla domestic and regional sectors face significant productivity challenges, despite the good reputation of the product (Sánchez-Morales *et al.*, 2024). According to data from SIAP, vanilla production in the municipality of Olintla, Puebla only reached 0.96 t in 2023. This low productivity is mainly the consequence of the abandonment of this crop. The situation is made even worse by management challenges, such as the proliferation of diseases, particularly wilt. The *Fusarium oxysporum* f. sp. *vanillae* fungi causes wilt, rotting both roots and stem (Vargas-Valencia *et al.*, 2019; Kadir *et al.*, 2021).

The municipality of Olintla has specific weather and socioeconomic characteristics. It has a semi-hot sub-humid climate ((A)C(fm) type) with rain all year round (CONABIO, 2011). The area has slopes and low hills, crossed by streams and rivers. The predominant soil type is Lithosol, with a thin, stony, and calcareous texture. The land includes rainforests, agricultural areas, and grasslands, which impacts agricultural dynamics and development of agricultural activities, particularly vanilla cultivation (CONABIO, 2023).

This locality has abundant natural wealth and a diverse vegetation that includes trees, herbs, and shrubs. This agroforestry system is known as acahual and it is where vanilla is grown. Acahuals house both native and introduced species, which enhance the diversity and resilience of the ecosystem (Parada-Molina *et al.*, 2022).

Consequently, understanding the specific challenges faced by vanilla producers is fundamental to determine their priorities. Identifying local needs and practices can improve vanilla productivity and quality, as well as strengthen the resilience of producers to tackle current and future challenges in the sector (Reyes-Hernández *et al.*, 2022). A more prosperous future can be built for those involved in vanilla production, if they work together with local communities and other key participants. Therefore, the objective of this research was to determine the socioeconomic profile of the vanilla producers of the Dimas López community, Olintla, Puebla, as well as their practices, interests, and points of view regarding vanilla wilt disease.

MATERIALS AND METHODS

Study area

Figure 1 shows the study area. The research was conducted in the Dimas López community, municipality of Olintla, located at 516 m.a.s.l., in the Sierra Nororiental, state of Puebla, Mexico (INEGI, 2020).

Data collection

Field data were collected through semi-structured interviews with vanilla producers. The questions included three key aspects: a) sociodemographic profile; b) production profile; and c) points of view on vanilla wilt disease. These interviews analyzed both qualitative (sex, occupation, native language, and points of view and aspects of vanilla production) and quantitative variables (age, years of experience in vanilla cultivation, number of plants, and green vanilla production).

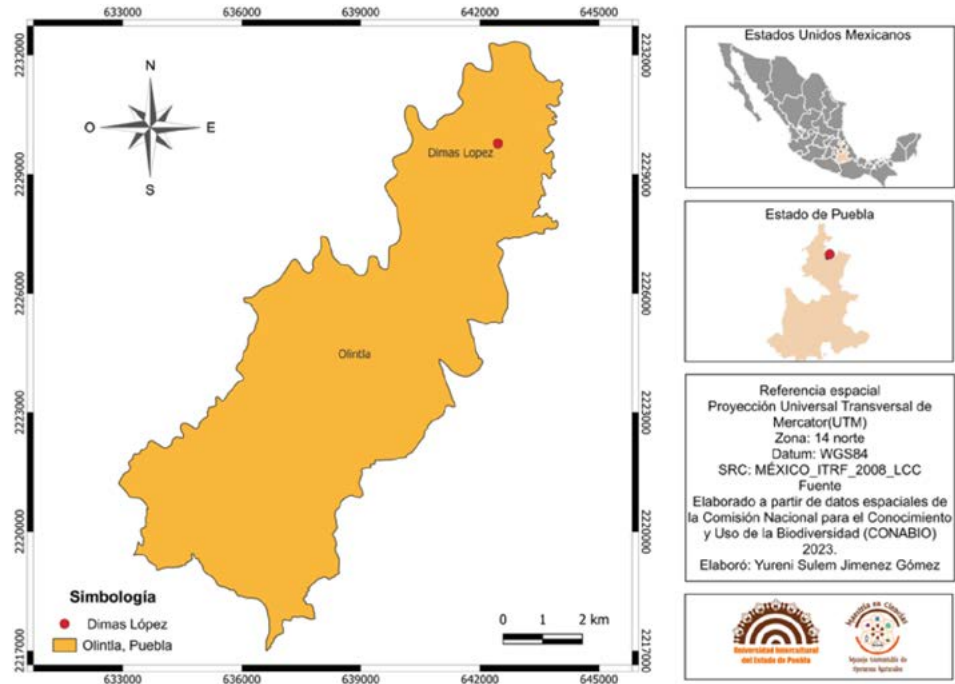


Figure 1. Geographic location of Dimas López, Olintla, Puebla.

In addition, the knowledge of the producer about the control of vanilla wilt, potential management alternatives, and other factors that could influence crop productivity and growth were established. Sixty-five producers participated in the sample (personal communication). However, only 35 out of this total were taken into account, due to their experience in vanilla cultivation (≥ 3 years). The following simple random sampling formula was applied (Castañeda-Guerrero *et al.*, 2020):

$$n = \frac{NZ_{\alpha/2}^2 P_{nq_n}}{Nd^2 + Z_{\alpha/2}^2 P_{nq_n}}$$

Where: N =population size (35 vanilla producers); $Z_{\alpha/2}^2 = 90\%$ reliability; $p=0.5$; $q=0.5$; d =accuracy (0.1).

Therefore, $n=24$ semi-structured interviews were conducted as representative sample of vanilla producers from the Dimas López community. If required, the interviews were conducted in Totonac.

Data analysis

The collected data was organized in a database and analyzed using descriptive statistic methods. Both tables and graphs were used to visualize and better understand the patterns and trends found in the data.

RESULTS AND DISCUSSION

Sociodemographic profile

Table 1 shows the age of producers (44-82 years old). They were a diverse group, with different levels of experience and knowledge.

All interviewees were men, possibly reflecting the traditional role distribution in these communities. Totonac is the mother tongue of most of the participants (95.8%). Santiago-Hernández *et al.* (2023) also reported that a large part of the population in Olintla speaks Totonac. For its part, INEGI (2020) reported that 91.7% of the inhabitants of the municipality speak an indigenous language, either Totonac (99.6%) or Nahuatl (0.2%). Regarding their main occupation, 92% of interviewees were farmers, which highlights the importance of traditional agriculture in the community.

Productive profile

The plots used to grow vanilla were small (≤ 0.25 ha). Despite their size, these plots can be occupied by up to 200 vanilla plants (Table 2).

Although the area had ≤ 0.25 ha, plots can produce up to 20 kg of green vanilla. However, Rodríguez-López *et al.* (2024) reported that 40% of the producers from the Sierra Nororiental in the state of Puebla obtained up to 50 kg of green vanilla per production unit and production cycle, using traditional agriculture systems in $< 1,000$ m² plots. This production results attests to the meticulous care and attention that producers pay to their

Table 1. Sociodemographic profile of vanilla producers in Dimas López, Olintla, Puebla.

Variable		Number	Value (%)
Sex	Man	24	100.0
	Woman	0	0
Age	44 to 56	9	37.5
	57 to 68	8	33.3
	69 to 80	6	25.0
	81 to 92	1	4.2
Occupation	Peasant	22	91.6
	Carpenter	1	4.2
	Muleteer	1	4.2
First language	Totonaco	23	95.8
	Spanish	1	4.2

Table 2. Productive profile of vanilla producers in Dimas López, Olintla, Puebla, Mexico.

Measure	Number of plants it has	Green vanilla production (kg)	Cultivation experience (year)
Maximum value	200	20	15
Minimum value	25	5	3
Mean	67	10	9.7

plants, as well as to the optimal environmental conditions that favor the growth and healthy development of vanilla.

Production systems

Acahual was the predominant (84%) production system. These results match the findings of Herrera-Cabrera *et al.* (2022), who also reported that *acahual* was the predominant system of the Totonacapan region. This system is characterized by intercropping with secondary vegetation, such as timber and fruit trees. Espinoza-Pérez *et al.* (2019) indicated that vanilla cultivation systems in areas with secondary vegetation achieve a greater balance between plant biodiversity and individual plant distribution, resulting in a better ecological balance.

Acahual is a suitable habitat for vanilla plants, since this agroforestry arrangement offers optimal conditions for their cultivation: an adequate level of shade (50-70%) and good ventilation favor a healthy plant development (Ibarra-Cantún *et al.*, 2018). However, 16% of production systems were plantations that used different tutor species, such as piñón (*Jatropha curcas*), pichoco (*Erythrina* sp.), and/or cocuitle (*Gliricidia sepium*). The diversity in production systems may reflect the adaptive strategies and lore applied to crop management. A remarkable example is *Erythrina* sp., which has a significant cultural value thanks to its multiple benefits: it is used in living fences, provides shade, enriches soil by providing organic matter to crops, and potentially restores degraded soils (Parra-Gil *et al.*, 2023). These findings suggest that agricultural practices have a regional coherence. Consequently, this phenomenon can potentially strengthen the importance of sustainability and productive efficiency.

Interests related to vanilla cultivation

Totonac farmers have a keen interest in various vanilla cultivation and production aspects. Their main interest was phytosanitation, which plays a key role, given the susceptibility of vanilla to diseases that damage very part of the plant, from the roots to the fruit (Figure 2).

Wilting and rotting are the most common diseases that impact vanilla production. Hernández-Martínez *et al.* (2020) have reported that pathogens such as *Fusarium* sp. are the main cause of vanilla diseases in the Huasteca Potosina region. This fungus penetrates the plants from the roots, causing the appearance of brown lesions (initial symptoms) in

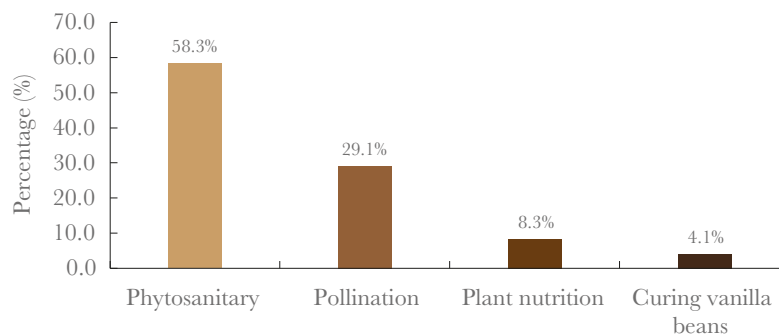


Figure 2. Interests of vanilla producers in Dimas López, Olintla, Puebla.

different parts of the plant. The initial lesions caused by the infection darken and dry the tissue, rotting the base of the stem and impacting the structure of the plant (Koyyappurath *et al.*, 2016). This process does not only weaken the plant, but also interrupts the nutrient and water flow, impacting its health and flowering capacity and, consequently, decreasing the quality and quantity of flowers (Flores-de la Rosa *et al.*, 2018). Pollination was another interest of producers, because the production of vanilla siliques (pods) depends on manual pollination. The rostellum in the flower acts as a barrier that prevents the pollen on the anther from reaching the stigma (Hernández-Apolinar, 2018). Producers invest time and effort in this delicate process, in order to guarantee the proper pod formation and to maintain high quality standards in the final harvest.

Producers were also interested in plant nutrition, since they understand the importance of providing their plants with the necessary nutrients for optimal development and abundant production. Incorporating organic matter into vanilla beds to enrich the soil with essential nutrients and increase its fertility is fundamental. Carrillo-González (2018) reported that organic fertilization is essential for vanilla production. Producers mainly use decomposing plant waste or compost to nourish their crops. However, no regulation currently exists for quality control or the optimal fertilization doses required for the appropriate development of vanilla plants.

Finally, producers were also interested in the curing vanilla. This term refers to the dehydration of the fruit, which also modifies its microstructure and activates enzymatic reactions that induce the characteristic aromas and flavors of vanilla. β -glucosidase is one of the most significant enzymes involved in this process (Vargas-Hernández *et al.*, 2021). Undoubtedly, curing vanilla (drying and dehydration) is fundamental in the production chain, because it transforms the raw material into a value-added product, highly demanded by both domestic and international markets (Jaramillo *et al.*, 2012).

Vanilla wilt disease: points of view and control

Seventy-one percent of the interviewees lacked information about the causative agent of the diseases that impact vanilla plantations. In addition, producers pointed out that they were unaware of the appropriate control practices and of the significant productivity challenge that these diseases pose to their vanilla plantations. Twenty-nine percent of the interviewees are aware about vanilla diseases and implement some type of control management —application of ash, lime (calcium hydroxide), and sodium bicarbonate— that have been somewhat effective for other crops.

According to Arias-Cedeño *et al.* (2021), ash contains not only essential macronutrients but also micronutrients, such as zinc (Zn) and copper (Cu) —metals required for a healthy plant growth. Although a minimal quantity is required, micronutrients are equally vital, because their deficiency can trigger physiological disorders in plants. Therefore, ash can contribute to crop development, functioning as a natural fungicide, since its alkaline nature creates an unfavorable environment for many pathogenic fungi (Romanowska-Duda *et al.*, 2024).

In addition, calcium hydroxide has fungistatic potential, preventing the spread of wilt disease in vanilla plants. Bubici *et al.* (2019) recorded that the application of calcium

hydroxide to the soil played a crucial role for the control of *Fusarium* wilt in banana (*Musa* sp.). This treatment is applied in the base of diseased plants and of nearby healthy plants, in order to raise the pH of the soil and to prevent the spreading of *Fusarium oxysporum* f. sp. *cubense*. In addition, calcium hydroxide is unexpensive, can be easily applied, and it is harmless to the environment and humans. These combination of characteristics make it an alternative for the control of diseases in agricultural crops, particularly when it is used to prevent pathogenic diseases (Cardona and Zapata, 2016).

Likewise, sodium bicarbonate has been effective for the control of postharvest rot. Guédez *et al.* (2010) proved its effectiveness for the control of postharvest fungi in Valencia oranges. These authors reported that sodium bicarbonate healed 80% of the wounds caused by pathogens of the genus *Penicillium* sp., highlighting its potential as a natural and safe treatment to protect crops after the harvest. This compound inhibits the expression and secretion of polygalacturonases, which are the enzymes responsible for the maceration of fruit tissues. Sandoval-Chávez *et al.* (2011) reported that low-pH environments improve the activity of these enzymes.

Vanilla producers also mentioned that wilt disease is more common during the rainy season. These comments match the reports of Reyes *et al.* (2020), who indicated that, while wilt disease impacts vanilla plantations all year-round, its incidence is most severe during the rainy season. In addition, vanilla plantations are extremely vulnerable to this disease before they reach 4+ years.

An encouraging fact is that 8% of interviewees have received technical assistance from government programs. Assistance can help producers to better understand vanilla diseases and to adopt more effective management practices. However, producers are still unaware and untrained regarding the management of diseases in vanilla plantations. Therefore, providing continuous technical support and training activities to producers is fundamental. On their own words, producers want to grow vanilla and receive technical assistance, in order to strengthen their capacity to tackle these challenges and guarantee a long-term crop productivity. During the interviews, producers were asked if they had noticed wilting signs in their vanilla plants and which part of the plant had the highest incidence (Figure 3).

Sixteen-point-six percent of the interviewees admitted they were unsure whether their plants had shown any sign of wilting. In addition, 20.8% stated that their crops had

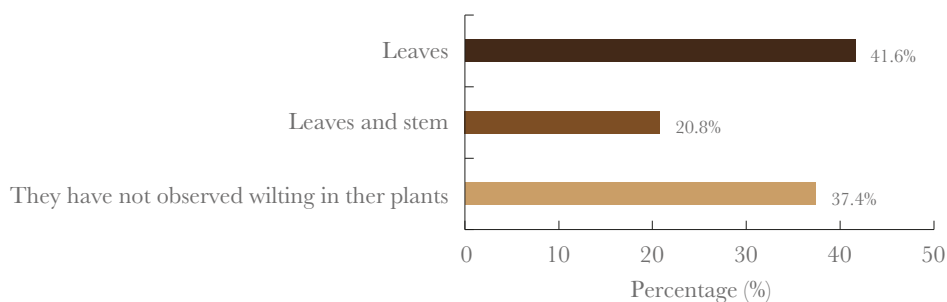


Figure 3. Location of wilt in vanilla plant, based on the experience of producers in Dimas López, Otlintla, Puebla, México.

not shown any wilting symptoms, suggesting a more favorable situation. However, most of producers (62.5%) confirmed signs of wilting in their vanilla plants. This situation is worrying and highlights the seriousness of the problem, especially taking into account that wilting significantly impacts the productivity of vanilla plantations.

CONCLUSIONS

Dimas López producers are members of the Totonac community and grow vanilla in small plots (0.25 ha), using a low-yield production system known as *acahual*. They are deeply interested in the phytosanitation of their plants; therefore, improving disease management strategies and focusing on wilt and rot control in vanilla plantations are fundamental. Furthermore, producers are willing to implement pest and disease control practices to protect their crops. Taking into account the sociocultural context of the community is essential to guarantee that producers effectively apply and accept potential strategies.

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Analysis of chemical composition and heating value of five oak species for their use as biofuels

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ABSTRACT

Objective: To determine the chemical composition and calorific value of biomass from the stump, stem and branches of *Quercus calophylla*, *Q. glaucoides*, *Q. laurina*, *Q. magnoliifolia* and *Q. rugosa*.

Design/methodology/approach: The amount of hemicellulose, cellulose, lignin, and extractable substances was determined in an Ankom fiber analyzer. Pearson's correlation analysis was conducted between the chemical components, and the higher heating value was also calculated.

Results: The highest values obtained were hemicellulose 14.72% in the branches of *Q. laurina*; cellulose 67.19% in the stem of *Q. calophylla*; lignin 21.58% in the stem of *Q. rugosa*; extractable substances 13.00% in the stump of *Q. rugosa*. In particular, the correlation between cellulose and hemicellulose was negative ($r = -0.80$). The results for calorific value varied from 19.32 MJ kg⁻¹ in the stump of *Q. glaucoides* to 20.19 MJ kg⁻¹ in the stem of *Q. rugosa*.

Limitations on study/implications: The shortage of studies about the chemical composition of wood affects the selection of species for their application in the area of biofuels, and a poor selection of raw material translates into inefficient combustion and greater environmental impact.

Findings/conclusions: The species studied are apt for their use as thickened biofuel, given their chemical characteristics. *Q. rugosa* presents a higher percentage of lignin content, extractable substances in the stem and the stump, and high heating value, so it is considered to have greater potential for the elaboration of quality pellets.

Keywords: Dendroenergy, lignin, extractable substances, *Quercus*, higher heating value .

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INTRODUCTION

The *Quercus* genus, with approximately 500 species, belongs to the Fagaceae family (Manos *et al.*, 1999); because of the number of species of this genus, Mexico is considered a diversity center (Valencia, 2004). It is a source of raw material for fuels in timber form and



to produce carbon (Flores and García, 2021); it is also used in permanent constructions where its mechanical resistance is evident. Due to its natural durability, it is used in applications for exteriors, tool handles, fence posts, mine piles, among others (de la Paz-Pérez and Dávalos Sotelo, 2008), as well as for pulp and paper, tannin extraction, adhesive elaboration, thickened biofuel generation, and various additional applications (Honorato and Hernández, 1998).

The applications towards which wood is destined are in function of its chemical composition, because wood is an organic material composed of carbon, hydrogen, oxygen, nitrogen, and other mineral elements; it is heterogeneous because it has cells with different functions; it is hygroscopic from its ability to exchange moisture with the environment; it is porous, and in addition it is an anisotropic material (Suirezs and Berger, 2010). Chemically, it is divided into substances, macromolecular and low molecular weight. In the macromolecular components, there are polysaccharides which in turn are classified into cellulose and hemicellulose, in addition to lignin being present; on the other hand, the substances of low molecular weight are classified into organic and inorganic matter (Fengel and Wegner, 2003).

The parts of a tree such as stem, bark, roots, crown, branches and foliage differ significantly in their chemical composition and amount of water. Consequently, it is important to study the different properties and characteristics of wood to compare them and determine their potential use, as well as their different attributes, to give place to new applications, carrying out a better use, and obtaining added value (Howard, 1973; Suirezs and Berger, 2010).

In the elaboration of thickened biofuels such as pellets and briquettes, the chemical composition of the biomass is an important factor, since it has a close relationship with the amount of energy that these can liberate. Therefore, the chemical composition of the biomass is an important parameter to consider decision making to use some type of additive. For example, lignin present in the biomass acts as an agglutinant at high temperatures, which causes lignin to soften and helps the union of particles (Kaliyan and Vance, 2009; Fearon *et al.*, 2020); therefore, no additional additive is generally used for the thickening process. In communities of the state of Oaxaca, Mexico, wood from species of the *Quercus* genus is widely used as fuel. However, there is a shortage of information related to the chemical composition of wood and its higher heating value, so the objective of this study was to analyze the chemical composition of the stump, stem and branches of *Quercus calophylla*, *Q. glaucoides*, *Q. laurina*, *Q. magnoliifolia* and *Q. rugosa*, with the aim of assessing the wood's potential in its use as biofuel.

MATERIALS AND METHODS

Study area

The study was carried out in the municipality of San Sebastián Coatlán, Miahuatlán, Oaxaca, Mexico, located on the South Sierra of the state (15° 57' and 16° 15' LN, 96° 48' and 96° 58' LW) (Figure 1) with a total of 19,030 ha, at an altitude between 100 and 2300 masl. Regarding the vegetation, 74.73% corresponds to wooded surface, 11.88% is grassland, and 6.47% rain forest (Monjaraz, 2013; INEGI, 2015; INEGI, 2020).

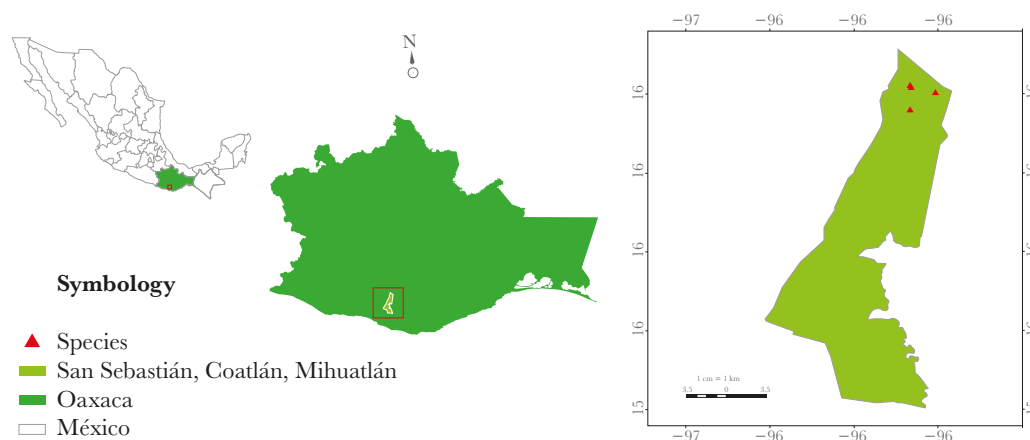


Figure 1. Study area (prepared by the authors).

Tree selection

Samples from five tree species were collected: *Quercus calophylla* Schldl. & Cham (encino de raja), *Q. glaucoides* M. Martens & Galeotti (encino negro), *Q. laurina* Humb & Bonpl (encino blanco), *Q. magnoliifolia* Née (encino yegareche), and *Q. rugosa* Née (encino cucharilla), which, according to Jiménez-Mendoza *et al.* (2023), are the main species that are used for fuel in the study region.

Sample preparation

The trees selected were sectioned into three parts: stump, stem and branches, from which 5 cm-long slices were extracted. The slices were splintered with a manual tool. Then, the splinters were ground in a conventional Micron Mixer[®], K20F mill, and finally sieved (vibratory sieve RO-TAP[®] Model RX-29), collecting samples with particle size 425 μm (Figure 2).

Chemical analysis of the samples

The samples were evaluated in an Ankom[®] Model A200 fiber analyzer (Van Soest *et al.*, 1991). To determine the content of hemicellulose, cellulose, lignin and extractables, wood



Figure 2. Classification and sampling (prepared by the authors).

samples were encapsulated in a filter bag, and the capsules were placed in a suspension tray and put into the fiber analyzer recipient. The fiber analyzer began the process and filtered the samples. When this phase finished, the samples were removed from the analyzer to be dried and weighed, with the purpose of determining the percentage of components of the wood. The content of inorganic substances was determined based on the norm ASTM D 1102-84 (ASTM, 2007).

Higher heating value The higher heating value of the samples was determined according to White (1987), taking as independent variables the content of lignin and the content of extractable substances.

Statistical analysis

Pearson's correlation was conducted with the following variables: hemicellulose, cellulose, lignin, extractable substances, and ashes; for this purpose, the statistical software SAS[®] version 9.0 was used (SAS Institute Inc., 2014).

RESULTS AND DISCUSSION

Hemicellulose content

Quercus laurina presented the highest value of hemicelluloses in its branches (14.72%) and stem (14.27%) (Table 1). These values are lower than what was reported by Herrera-Fernández *et al.* (2017) for this species, with 33.27% for heartwood, 23.51% for sapwood, and 22.72% for bark. Next, the percentage of hemicelluloses in the stem and branches of *Q. rugosa* was 13.84%, similar datum to the value found by Ruiz-Aquino *et al.* (2019) for three broad-leaved species from the forest in Ixtlán de Juárez, Oaxaca, Mexico.

Table 1. Chemical composition of five species of *Quercus* (%).

Species	Component	Hemicellulose	Cellulose	Lignina	Extractable substances	Ash
<i>Q. calophylla</i>	Stump	10.46	63.54	17.60	6.68	1.38
	Stem	9.52	67.19	15.05	6.64	1.28
	Branches	11.34	62.86	15.75	8.38	1.40
<i>Q. glaucooides</i>	Stump	12.26	64.86	10.95	9.15	2.61
	Stem	10.92	62.69	14.02	9.57	2.49
	Branches	10.39	59.39	16.73	10.75	2.41
<i>Q. laurina</i>	Stump	12.90	55.74	18.97	10.29	1.76
	Stem	14.27	56.29	18.66	8.45	1.94
	Branches	14.72	56.52	15.79	10.76	1.79
<i>Q. magnoliifolia</i>	Stump	11.16	64.02	12.52	10.60	1.38
	Stem	12.36	62.89	13.26	9.89	1.33
	Branches	12.54	60.73	14.80	9.46	2.17
<i>Q. rugosa</i>	Stump	13.67	56.39	14.98	13.00	1.59
	Stem	13.84	52.27	21.58	10.31	1.79
	Branches	13.84	55.24	17.05	11.84	1.62

The species that presented the lowest percentage of hemicellulose was *Q. calophylla* in stem, with a value of 9.52%, lower value than what was reported for *Q. candicans* in heartwood with 27.90%, in sapwood with 21.90%, and in bark with 22.04% (Herrera-Fernández *et al.*, 2017; Valencia *et al.*, 2018). *Q. glaucooides* had a hemicellulose content in stump of 12.26%, in stem of 10.92%, and in the branches of 10.39%, lower values for three broad-leaved (34.79-41.67%) reported by Honorato-Salazar *et al.* (2015).

The hemicelluloses of broadleaf species exhibit a different complexity compared to conifers; different proportions of hemicelluloses present in the different compartments of the same tree, performing an intermediate role between the cellulose and the lignin, facilitating the insertion of microfibrils (Ruiz, 2018). Agricultural and lignocellulose forest residues can serve as raw materials to produce bioenergy and chemical products from fermentation (Saha, 2003).

Hemicellulose (also known as polyose) is the second most abundant chemical component in woody and herbaceous biomass which, along with cellulose, is found in nearly all plant cell walls (Li *et al.*, 2013). Hemicellulose has a heteropolymer structure (with molecular weight lower than cellulose) made up of various sugar monomers, such as glucose, galactose, mannose, xylose, arabinose, 4-O-methyl glucuronic acid, and galacturonic acid residues (Figure 3). The exact proportion of each of these monomeric units varies considerably depending on the exact nature or origin of the biomass (Garrote *et al.*, 1999).

Cellulose content

Cellulose was found from 52.27% to 67.19% for stem of *Q. rugosa* and stem of *Q. calophylla*, respectively (Table 1); these values are higher than those obtained for various oak species: *Quercus sebifera* with 45.71%, *Q. tinkhami* with 48.37%, and *Q. rubor* with 41.3% (Bárcenas-Pazos *et al.*, 2008; Laskowska *et al.*, 2018). *Q. laurina* presented a cellulose content in stem and branches of 56.29% and 56.52%, respectively, while for stump it was 55.74%. These values were similar to those recorded by Honorato and Hernández (1998) for stem sapwood and heartwood of *Q. laurina* with 56.20%. For stem of *Q. rugosa*, there was a value of 52.27%, for branch a value of 55.24%, and for stump 56.39%; in these two components of the tree (branches and stump), these values are higher than those mentioned by Bautista

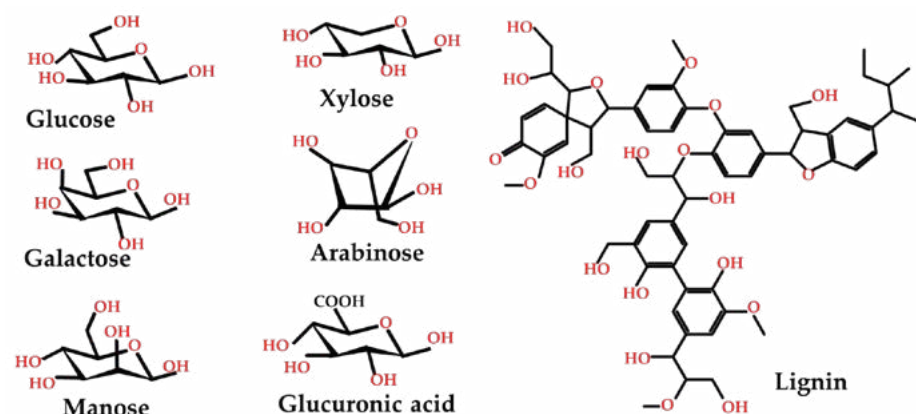


Figure 3. Main monomers of hemicellulose and molecular structure of lignin (Li *et al.*, 2013)

and Honorato (2005) for the mixture of stump heartwood and sapwood of *Q. rugosa* with 52.4%.

The cellulose content in stump of *Q. glaucoides* was 64.86%, followed by *Q. resinosa* with 64.02%, *Q. calophylla* in third place with a value of 63.54%, and *Q. laurina* in fourth place with 55.74%, which were higher values than those for stump of *Quercus oleoides* with 51.68%, *Q. coccolobifolia* with 48.97%, and *Q. durifolia* with 52.82% reported by Bautista and Honorato (2005).

Cellulose is the structural component of the primary cell walls of all the woody and herbaceous biomass and provides resistance and rigidity. Cellulose is of polymeric nature and made up of monomeric units of glucopyranose (Bridgwater, 1994), which at the same time derives from two residues of glucose anhydride united by glycosidic bonds β ($C_1 \rightarrow C_4$) (Figure 4).

Cellulose shows a strong tendency to form intra and inter molecular hydrogen bonds, which induce the formation of microfibrils that are added in highly ordered (crystalline) and less ordered (amorphous) regions. Due to its structure, cellulose is insoluble in most dissolvents and resistant to attack from acids and enzymes, which makes its treatment difficult through non-pyrolytic biomass improvement processes (Sinha *et al.*, 2015).

The variation in results can be related to the use of diverse analytical methods. The values found in this study exceed 50% of cellulose, and therefore, they can be used for paper manufacturing; however, it is necessary to conduct pulping and physical tests of the paper, especially from oak, since its fiber is shorter and could have an adverse effect on some physical and mechanical properties of the paper (Honorato and Hernández, 1998). Cellulose-rich biomass offers many advantages over agricultural raw materials, because of cell elongation, exposure of the secondary cell wall, and programmed cellular death, and with that the large amounts of biomass rich in cellulose can be used to produce bioenergy and biopolymers (Mizrachi *et al.*, 2012).

Lignin content

The lignin content ranged from 10.95% to 21.58% for stump of *Q. glaucoides* and stem of *Q. rugosa*, respectively (Table 1). *Q. laurina* presented 18% of lignin both for stump and stem, the same value (18%) that Gutiérrez-Acosta *et al.* (2021) reported for sawdust from two species of *Quercus* spp., in the state of Durango, and it is also within the range reported by various authors for this species, between 14.67 and 25.5% (Honorato and Hernández, 1998; Ruiz-Aquino *et al.*, 2015; Herrera-Fernández *et al.*, 2017). *Q. calophylla* presented

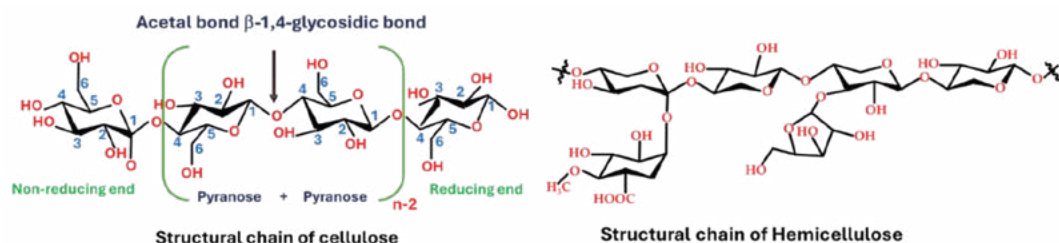


Figure 4. Chemical structure of cellulose and hemicellulose (Bridgwater, 1994).

17.60% of lignin for stump, 15.05% for stem, and 15.75% for branches, values lower than the ones reported by Rutiaga-Quiñones *et al.* (2000) in heartwood of *Q. candicans* with 21.4%. However, Herrera-Fernández *et al.* (2017) reported similar data for *Q. candicans* in heartwood of 16.45%, sapwood of 18.93%, and bark of 29.01%.

The species *Q. rugosa* presented a value of 14.98%, 17.05% and 21.58% for stump, branches and stem, respectively, a similar value to what was found for sapwood of the same species with 14.67%, for heartwood with 16.16% and for bark with 34.67%; it should be highlighted that in bark there is greater concentration of lignin than in wood (Herrera-Fernández *et al.*, 2017; Pintor-Ibarra *et al.*, 2017). *Quercus magnoliifolia* had values of lignin of 12.52% in stump, 13.26% in stem, and 14.80% in branches, lower data for *Q. palustris* with a value of 23.7% (Jaucida *et al.*, 2002).

The lignin content is correlated positively with the calorific value, that is, a high lignin content produces higher calorific value. In addition, this chemical compound has a high resistance to compression, but at the same time it is thermoplastic, which gives wood the ability to change its shape when it is subjected to high temperatures. Therefore, high amounts of lignin in the biomass are an important factor to elaborate quality thickened biofuels; on the other hand, when wood has low amounts of lignin, it can be used to obtain cellulose pulp (Rutiaga-Quiñones *et al.*, 2000; García *et al.*, 2003; Pintor-Ibarra *et al.*, 2017).

Content of extractable substances

The species that presented highest percentage of extractable substances was *Quercus rugosa* in stump with 13.00%, and the species that presented the lowest value was *Quercus calophylla* in stem with 6.64% (Table 1). *Quercus calophylla* was the species that presented the lowest values for the three parts of the tree with values in an interval of 6.64-8.38%; these data are lower than what was obtained by Rutiaga-Quiñones *et al.* (2000), with 10.2 % for stem of *Quercus candicans*.

The stump of *Quercus rugosa* had a value of extractables of 13% (Table 1), which is a similar value to what was reported by Ferreira *et al.* (2018) with 13.20% for bark of *Quercus faginea*. The stump and stem of *Q. glaucooides*, and the stem and branches of *Q. magnoliifolia* presented a value of 9% of extractables, datum higher than what was found by Ruiz-Aquino *et al.* (2020) for *Q. macdougalii*, with total extractables for heartwood of 8.35% and for sapwood of 7.24%. *Q. laurina* had values in stump of 10.29%, in stem of 8.45%, and in branches of 10.76%, similar value to what Jaucida *et al.* (2002) found for *Quercus palustris* with an average of extractables of 7.72%. The content of extractables has an indirect impact on the mechanical properties of wood when influencing their basic density (Ruiz-Aquino *et al.*, 2015). The extracts also have a positive influence on calorific value, since biomass with high content of extractable substances have a higher calorific value (Mauladdini *et al.*, 2022).

Content of inorganic substances

The content of ash varied in an interval of 1.28 to 2.61% for stem of *Q. calophylla* and stump of *Q. glaucooides*, respectively (Table 1). *Q. glaucooides* is the species that showed percentages higher than 2% in the three parts of the tree, that is, stump, stem and branches.

These were similar values to those found by Martínez-Pérez *et al.* (2015) for two broadleaf species with 2.28 and 2.16%. *Q. laurina* presented 1.76% in stump, 1.94% in stem, and 1.79% in branches; these values are lower than the ones reported by Bárcenas-Pazos *et al.* (2008) for *Quercus thinkami* with 2.67%, and for *Q. sebifera* with 3.22%. Vega-Nieva *et al.* (2015) have mentioned that ashes can produce problems during combustion in heaters. In turn, Solla-Gullón *et al.* (2001) argue that depending on the composition of ash, it can function as a nutrient for the soil, decreasing the degree of acidity of some soils, and improving the level of nutrients with ash and nitrogenated fertilization.

Pearson's correlation

Pearson's correlation analysis was conducted between the chemical components, and it was seen that cellulose and hemicelluloses are negatively correlated ($r = -0.80$); that is, as the cellulose decreases, the hemicellulose increases (Table 2). When cellulose is produced in wood, lignin is obtained as byproduct, and it is used mainly as fuel (Ruiz, 2018). For cellulose and lignin, there is a negative correlation ($r = -0.72$), which means that when the cellulose is high, the lignin tends to be low. The presence of lignin has an important role in the response from wood to fluctuations in the moisture content, significantly influencing its behavior in face of dimensional changes (Bárcenas and Dávalos, 1999).

Lignin and extractable substances are not correlated; extractable substances have diverse properties in the biomass such as color, odor, durability, adhesion, drying and discoloration (Álvarez *et al.*, 2012). It should be mentioned that these two variables are essential to select species for their aptitude as biofuels. Cellulose constitutes the fundamental structure, hemicellulose performs a role of connection, lignin confers resistance, and extractable substances influence many specific properties of the wood (Álvarez *et al.*, 2012).

Higher heating value

The higher heating value for stump of *Q. glaucooides* and stem of *Q. rugosa* varied from 19.32 to 20.19 MJ kg⁻¹, respectively. Likewise, the species that presented the highest average was *Q. rugosa* with 20.01 MJ kg⁻¹ (Table 2). The values obtained in this study are

Table 2. Pearson's correlation between the chemical composition of five species of *Quercus*.

		Hemicellulose	Cellulose	Lignin	Extractable substances	Ash
Hemicellulose	Correlation	1.00	-0.80	0.33	0.56	0.06
	Significance		0.00	0.22	0.02	0.81
Cellulose	Correlation	-0.80	1.00	-0.72	-0.61	-0.08
	Significance	0.00		0.00	0.01	0.75
Lignin	Correlation	0.33	-0.72	1.00	-0.00	-0.15
	Significance	0.22	0.00		0.97	0.58
Extractable substances	Correlation	0.56	-0.61	-0.00	1.00	0.15
	Significance	0.02	0.01	0.97		0.58
Ash	Correlation	0.06	-0.08	-0.15	0.15	1.00
	Significance	0.81	0.75	0.58	0.58	

Table 3. Higher heating value of wood from five species of *Quercus* (MJ kg⁻¹).

Species	Stump	Stem	Branches	Average
<i>Q. calophylla</i>	19.65	19.46	19.63	19.58
<i>Q. glaucoides</i>	19.32	19.58	19.86	19.59
<i>Q. laurina</i>	19.99	19.85	19.79	19.88
<i>Q. magnoliifolia</i>	19.53	19.54	19.63	19.57
<i>Q. rugosa</i>	19.88	20.19	19.95	20.01

slightly lower than those calculated for the same species by Ruiz-Aquino *et al.* (2022), using a flat-jacket calorimeter; the authors mention that the species of the genus *Quercus* are among the most frequently used as fuel, due to their calorific capacity and their high basic density, important characteristics in the selection of biomass for bioenergetic uses. Also, the calorific value can vary depending on biomass, climate and soil where the trees are cultivated (Ciolkosz *et al.*, 2010). Based on calorific value, the five species from this study are considered to have aptitude for their bioenergetic use because they exceed 18 MJ kg⁻¹ (Koppejan and Van Loo, 2012; Ngangyo-Heya *et al.*, 2022).

CONCLUSIONS

Quercus rugosa was the species that presented highest value of lignin content and extractable substances in stem and stump, respectively; it also presented the lowest content of cellulose. Four of the species studied have the highest value of hemicellulose in branches, except for *Quercus glaucoides* which has higher content of hemicellulose in stump. Regarding lignin, *Q. calophylla* and *Q. laurina* presented higher content in stump, *Q. glaucoides* and *Q. magnoliifolia* presented higher value in branches, and only *Q. rugosa* showed higher percentage of lignin in stem. As a result of the lignin content of the species, they can have potential for their use as fuels. For the content of extractable substances, *Q. glaucoides*, *Q. laurina* and *Q. rugosa* presented the highest percentage in branches, and *Q. laurina*, *Q. magnoliifolia* and *Q. rugosa* in stump. *Q. glaucoides* is the species that presented highest percentage of ash in the three parts of the tree, in contrast with *Q. calophylla*, which presented lower value of ash in stump, stem and branches. The cellulose and hemicellulose are negatively correlated, as are cellulose and lignin. Lignin and extractable substances were not correlated, and neither was ash with any other chemical component. Based on their chemical composition, the five species studied are apt for their use as biofuels, although *Q. rugosa* is the species that presents higher percentage of lignin content, extractable substances in stem and stump, and high heating value, so it is considered with greater potential for the elaboration of thickened fuels.

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Feminine empowerment and cooperativism: Three study cases of cooperatives in Colombia

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ABSTRACT

Objective: To analyze the perception about challenges and opportunities that women who lead agricultural cooperatives face in Colombia, and their relationship with feminine empowerment.

Design/methodology/approach: A qualitative approach was used by means of the study of three cases of agricultural cooperatives, through structured interviews that addressed three dimensions: 1) sociodemographic data of producers, 2) women's experience in cooperativism, and 3) challenges and limitations faced.

Results: Important challenges were identified in feminine cooperativism, such as limited access to credit, the double workday that women practice within their households, in addition to the complexity of reconciling the demands of agricultural production with domestic responsibilities. There is also a lack of government support, scarce specialized training, and lack of accompaniment and counseling for efficiency in the commercialization of their products. Cooperativism is a platform for collective decisions and social participation, promoting leadership among women in rural zones.

Limitations/implications: Some participants manifested caution when providing key information for the study.

Findings/conclusions: Strategies must be generated to strengthen collective and business capacities of women, with the aim of improving the organizational and economic structure of cooperatives. Policies to promote inclusive governance, financial education, and access to new markets were suggested for a greater impact in gender equity in the sector.

Keywords: Organization, leadership, coffee, women, management.

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INTRODUCTION

Globally, women perform 40% of tasks in the farmland, and a fourth of the world population is constituted by rural women, of which 43% are devoted to agricultural tasks, in addition to household tasks (ONU Mujeres, 2018).

Women have an important role in the rural economy, especially in developing countries, and they represent nearly half of the farmers in the world (Banco Mundial, 2017). They are also drivers of the economy at the global level and even more in the agriculture and livestock



sector, which has particular exogenous and dynamic variables (Bancolombia, 2021), where women carry out a strategic role in economic and social development process. In Colombia, the farming sector generates more than 20% of national employment and represents half of the employment in rural areas. In the case of rural women in Colombia, they are concentrated primarily in the departments of Antioquia, where 11.3% reside, followed by Cauca with 8.1%, Nariño with 7.8%, Córdoba with 7.4%, and Cundinamarca with 6.9% (DANE, 2021).

Despite the advances found in the rural environment, there are still gender inequalities in every dimension of sustainable development, and in many areas progress is slow, so that the Sustainable Development Goals (SDGs) can be attained by the year 2030 (ONU Mujeres, 2018).

Empowerment can be a means for women to show the importance of the meaning of their autonomy, since it leads to improvement of their social conditions and allows them to gain control of the decisions they make (Hernández *et al.*, 2018).

In Colombia, the vulnerability of women is linked to cultural conditions in which patriarchal gender schemes predominate, which restrict their opportunities and establish mechanisms of exclusion and discrimination (Garzón-Segura *et al.*, 2022).

For this study, the empowerment of women through cooperativism is analyzed, which has been understood as a doctrine, a set of principles, a system, a strategy, a movement, and an activity that classifies cooperatives as a model or type of organization directed at the interests of all the members (Dhein *et al.*, 2022). The social importance of cooperatives is relevant because they provide full-time or part-time jobs to a number of local women who do not have other opportunities (Hernández *et al.*, 2018), and the principle of for-profit personalist non-priority and verification of broadened social transferences in kind are elements that are characteristic of every cooperative (Alarcón and Álvarez, 2020).

Cooperatives of rural women are essential to unite and gather women together, so they can make their voice heard, defend their interests and represent them in political and economic spheres, and they also allow women to influence the decisions and institutions that affect their lives (ONU Mujeres, 2018). Ozdemir (2013) points out that the creation of cooperatives implies showing women a way of earning money, generating employment and fighting poverty, which is generally more harrowing for them.

Therefore, women farmers that constitute cooperative societies are women who serve as example for society due to their individual and cultural characteristics (Bezboruah and Pillai, 2015; Bonnan-White *et al.*, 2013; Kamberidou, 2013).

This particular form of social collective undertaking has empowered women members in three different ways: 1) economic security, 2) development of entrepreneurial behavior, and 3) increase of contributions to the family (Datta and Gailey, 2012). Under this context, the objective was to analyze the perception about the challenges and opportunities that women who lead agricultural cooperatives in Colombia face, and their relationship with feminine empowerment.

MATERIALS AND METHODS

The study's approach was qualitative, which allowed identifying patterns and relationships that can complement the qualitative approaches (Yin, 2018). The interview

technique was used and the interview guide as instrument of data collection, with the aim of building cases that are presented through a formulation of questions that had the purpose of gathering information related with variables that help to understand the experience of women as members of cooperatives.

The technique of study case was also applied, which is characterized by being a social process with the aspiration of researching under a methodological purpose (Jiménez-Chaves, 2012), where techniques such as participatory observation and interview were used, in which the researcher interacts with key informants (women farmers, cooperative leaders, among others).

To apply the data collection instrument, the women were contacted for interviews through the Agrarian University Foundation of Colombia; the approach was by means of the women researchers, who were in charge of inviting women who had the following characteristics: a) leaders of agriculture and livestock cooperatives, b) minimum two years of experience in the agricultural market, and c) true willingness of participating in the interview. The study area considered the municipalities of Anolaima and Villa del Rosario de Tena in the Department of Cundinamarca, Colombia, and the study units were three agriculture and livestock cooperatives, which are described in Table 1.

The interviews were applied in the meetings of the Administration Council of the agricultural cooperatives, through a rigid or intentional non-probabilistic sampling, where women were selected in a deliberate manner with the aim of obtaining qualitative data for the research. The interviews were divided into the following themes: 1) data from women farmers; 2) experience of women as members of cooperatives; and 3) challenges and limitations of feminine cooperativism.

RESULTS AND DISCUSSION

Study Case 1: Anolaima Avocado and Other Fruit Producers

Association-APROAFA

APROAFA, founded in 2014 in Anolaima, Cundinamarca, is devoted to the cultivation and transformation of avocado (*Persea americana*). The women farmers' age ranges between 40 and 61 years, and they mention that they have been taught the value of work and

Table 1. Description of the cooperatives.

Case study	Activity carried out by the cooperative
Anolaima Avocado and Other Fruit Producers Association-APROAFA	Promote and develop the cultivation of avocados and other fruit trees in the municipality of Anolaima and the province of Tequendama, thereby achieving the well-being of associated producers, and promote, develop, and research the activity throughout the country.
Association of Women Coffee Growers in Anolaima-MUCCA	A solidarity organization of women coffee growers and entrepreneurs from the municipality of Anolaima, who work throughout the coffee production chain.
ASOFRUGUASIMAL	A rural association that cultivates regional agricultural products, including mangoes, citrus fruits, and avocados, through sustainable production processes using organic ingredients, thereby offering the market a safe, high-quality product.

Source: Prepared by the authors based on fieldwork (2024).

entrepreneurship since they were young, through the sale of products and help with households and farm tasks. Under this idea, Amine and Staub (2009) and Mordi *et al.* (2010) mention that the family characterized by a structure of power is essential, since it carries out a predominant role in women's activities of entrepreneurship and empowering, which drives them to strengthen their self-esteem and vision for work from the family core. There is evidence that cooperative societies empower women since they move beyond being solely entrepreneurial projects; they are capable of generating intangible benefits and promoting women's autonomy (Ferguson and Kepe, 2011).

Women from this organization mention the importance of working under the cooperative approach, because since they joined, the vision has been of being united and committed with the sustainable growth of the association. Under this panorama, Bastida *et al.* (2021) found that some specific characteristics of the cooperatives, particularly their organizational culture and governance model, suggest that this model of collective entrepreneurship is particularly attractive for women entrepreneurs. This is important, since cooperatives have also been viewed as a way of contributing to economic development, especially in developing countries (Guzman *et al.*, 2019; Paudel and Acharya, 2021).

Among the main challenges that the women farmers emphasize, there is lack of access to technology and specialized equipment for transformation; likewise, among the challenges they face in the medium term, they identify creating a processing plant to diversify the offer of products.

APROAFA considers as one of the main factors for success of the cooperative, which has allowed it to remain in the market, the transformation of products and the procurement of a prototype that the women themselves elaborated; this served them to trade their product and also to accelerate processes in the production area.

When the women were asked about who are better, whether men or women, they responded without hesitation that women, because they have the sense of responsibility and dedication, as well as the attentiveness to elaborate manual tasks that guarantee quality products; these factors have helped them to strengthen the loyalty of their clients. In relation to the traditional roles that women fulfill in society, they were asked about whether they contribute or hinder the development of the cooperative, indicating that sometimes traditional roles limit them because they must fulfill domestic activities and care for family members, but also that these challenges make them resilient to face upcoming challenges.

Throughout the interview, women farmers were asked to mention the strengths of the Colombian agricultural sector, emphasizing in their answers the interest for Colombian products from international markets. On the other hand, the main limitations they face is the lack of commitment of people and some women, as well as the limited support from the government.

Study Case 2: Association of Women Coffee Growers in Anolaima-MUCCA

MUCCA, with headquarters in Anolaima, Cundinamarca, gathers women with an average age of 68 years, who are mainly devoted to the production of coffee, mango,

citruses, and other fruit trees. From its start in the year 2016 and with only 10 members, it has sought to improve the living conditions and sustainability of their farms through product transformation. The women farmers highlight their persistence, organization and ability for teamwork, which has helped them to become empowered and to advance towards their objectives. In this regard, Mahmud *et al.* (2012) consider empowerment as a life process and continuous movement of each woman, so women need to reinforce their self-esteem, their position in decision making, freedom of movement, and to have access to cash. Under the same order of ideas, Fundora *et al.* (2017) and Munster and Fleitas (2019) suggest that when a woman attains empowerment and takes the reins of her life, it is possible for her to gain access to various spheres and thus obtain greater representativity, attaining a fairer and more equitable participation.

In relation to leadership, women are described as responsible, with the gift of authority, collaborative, resilient, innovative, persistent, and organized; these qualities have driven them to generate improvements for the cooperative. However, although women's participation in rural development has had important protagonism in recent years, they are not always offered opportunities and conditions for their participation from knowledge and capacities in institutions and cooperatives of their localities, so that their role and personal development is fractioned and minimized (Camejo *et al.*, 2024). On the other hand, Arias *et al.* (2013) mention that women who reside in rural areas experience low investment in human capital training, fewer connections with labor markets, more time devoted to household tasks, low income, fewer connections with the State and greater poverty, which agrees with the limitations of this study case.

MUCCA has achieved milestones such as the development of transformed products, the creation of a local brand, and the strengthening of their transformation abilities, despite women's participation in the sector being limited. As mentioned by Elam *et al.* (2019), Guzman (2019) and Marlow (2019), in spite of the logical variation in the rate of women entrepreneurs between countries, there is wide consensus that women's contribution to entrepreneurship is considerably lower than that of their male counterparts, although the reach of the work that women perform is not just for them, but rather for their families and society.

Cooperative members perceive challenges such as lack of modernization, improvement of communication pathways and transport, as well as the lack of more state backing. Under this scenario, Balachandra *et al.* (2019) and Gupta *et al.* (2014), proposed a gender framework to identify the specific barriers that women face to start a business, including the difficulties to gain access to markets and financial resources (less provision of organizational resources) (Brush *et al.*, 2017), restrictions related to the life sphere, and exclusion from entrepreneurial networks (Bogren *et al.*, 2013; Welsh and Kaciak, 2018), which prevents them from efficiently entering markets and trade channels.

One of the main elements that cooperative members mention to start and coordinate a cooperative is to have management abilities to capture women who are interested in entering the cooperative, since, without an important group of members, cooperativism is projected as limited in its operation. Meanwhile, regarding the success factors that they have achieved in the cooperative, women farmers indicate coffee (*Coffea arabica* L.) exports

as well as the satisfaction of having maintained and driven the cooperative and the creation of a local brand.

Concerning the issue of: Who is better at business, men or women? Half (50%) of the women answered that both men and women are competent to conduct business, and that they only need experience and strengthening of entrepreneurial capacities to reach good agreements and close sales, since men have been naturally devoted to overseeing businesses and in recent years, women have also been included in these activities. The rest mentioned that women are better at conducting business, since they are careful, committed and decided, in addition to having the quality of considering common wellbeing; this statement agrees with what was pointed out by Lee and Huang (2018) describing that women feel rewarded when they center their objectives in collective aims.

Authors like Bastida *et al.* (2020, 2021) point out that women who work under the scheme of cooperativism prioritize mutual help, collaborative work, and non-profit objectives over economic benefit, motivations that are incompatible to a certain extent with the classic individualist behavior attributed to businesspeople (Ahl and Marlow, 2021).

Regarding the traditional roles that women have, they were asked whether they contribute or hinder business development, to which women responded that in certain aspects they are limited to promote the business since there is an unshakable belief that women must oversee domestic responsibilities and family care.

Women identified as the greatest strength of the national agricultural sector the diversity of products that Colombian lands provide because of their geographic and climatic diversity; regarding weaknesses of the sector, they mentioned the lack of modernization and efficient training, as well as the absence of management for commercialization processes.

Study case 3: Fruit-Producing Association of Guasimal-ASOFRUGUASIMAL

Asofruguasimal, located in Tena, Cundinamarca, was established in 2015 with 34 members (men and women), devoted primarily to growing mango (*Mangifera indica*) and citrus (*Citrus* sp.), and they also have hens (*Gallus gallus*) which they received as donation when they formed the association; however, currently only around 15 associates remain. Regarding this, Camejo *et al.* (2024) mention the importance of strengthening cooperatives with a development program that favors the organization of agricultural, commercial and cooperation processes, with the aim of attaining the consolidation of organizations and increasing the number of members.

Authors like Labrador *et al.* (2018, 2020), Marín de León *et al.* (2021), and Núñez Llerena *et al.* (2021) suggest that cooperatives are constituted as economic entities that have a legal personality of entrepreneurial nature and that cooperation between cooperatives is considered as a necessary principle for the full development of the cooperative identity.

The approach of Asofruguasimal is centered on the commercialization and transformation of products, facing obstacles such as the failure to fulfill the calls and the lack of agronomic resources such as fertilizers. The women farmers emphasize the need for financing, the lack of access to networks to broaden the markets, adequate capacities and better support in programming harvests and post-harvests, since they do not have access to updated and efficient training. This agrees with many authors who identify

specific barriers that women face to begin in business or cooperatives, as the difficulties to gain access to markets and financial resources (Balachandra *et al.*, 2019; Gupta *et al.*, 2014); likewise, they have less provision of organizational resources (Brush *et al.*, 2017), restrictions related to the life sphere, and exclusion from business networks (Bogren *et al.*, 2013; Welsh and Kaciak, 2018).

Concerning the traditional roles that women have, they mentioned that the household responsibilities continue to fall on women and this often implies unpaid work and a devotion to family welfare beyond personal or professional aspirations outside the home, which often causes women to have to allocate more time to the family and to not focus completely on improving management of the cooperatives; members of the cooperatives identify this as a weakness to drive the business and commercialization of their products. Under this context, women recognize feminine leadership as a strength, although they face challenges such as incompleteness of key activities, which affects their productivity, as well as machismo. Despite the difficulties, they work to consolidate their limits with clear commitments in transformation, they have a plant where they make fruit pulp, which has contributed to expanding the local market.

A limitation identified was the presence of intermediaries in the entire commercialization chain, which reduces the income of women farmers, since they impose low prices, taking advantage of their power in the market; on the other hand, the excess of intermediaries lengthens the supply chain and causes costs to increase and delivery times to extend.

Therefore, cooperativism in Colombia refers to the participation of women in agricultural cooperatives, which are described as spaces that serve to foster solidarity, mutual help, and strengthening of social networks for support between women, where they provide tools for women farmers to generate income, improve their quality of life, and mainly, reduce their economic dependency. With these elements, women can drive empowerment and thus expand and improve the abilities and talents of the members of feminine cooperatives.

Because of this, the development of rural women in economic activities reinforces their autonomy, gender equity, improves their family context, and expands the opportunities for local and national development (Gasperín-García *et al.*, 2022).

In this sense, Table 2 shows a SWOT analysis, with the purpose of analyzing the strengths, weaknesses, opportunities and threats that feminine cooperatives represent for women.

The SWOT analysis shows that the presence of intermediaries all along the commercialization chain is identified as a limitation for cooperativism in Colombia, as well as the lack of adoption of sustainable and efficient technologies, and the shortage in organizational and entrepreneurial strengthening.

The constant strengthening of the base of women who are members of cooperatives, the promotion of feminine leadership, and the experience women have established in relation to collective work are established; all these elements have driven the active participation of women to strengthen work for equal opportunities, as mentioned by Gasperín-García *et al.* (2023), where the authors indicate that farmers value the collective work and interpersonal relationships that are fostered by cooperativism.

Table 2. SWOT analysis of the 3 case studies of cooperatives in Colombia.

Case study	Strengths	Weaknesses	Threats	Opportunities
Anolaima Avocado and Other Fruit Producers Association-APROAFA	<ul style="list-style-type: none"> Organizational commitment Prototypes of transformed products Strong peasant identity 	<ul style="list-style-type: none"> Limited access to equipment and technology Traditional role still present in women cooperators Lack of administrative organization 	<ul style="list-style-type: none"> Competitive avocado market Price volatility Lack of continuous public policies Weak government support 	<ul style="list-style-type: none"> Potential for commercial expansion Interest in processing plant Future institutional support Growth of the local and national market
Association of Women Coffee Growers in Anolaima-MUCCA	<ul style="list-style-type: none"> Strengthening the women-only organization Solidarity economy Own brand and teamwork 	<ul style="list-style-type: none"> Lack of financing Institutional bureaucracy Lack of continuous technical support and advice 	<ul style="list-style-type: none"> Machismo in rural areas Climatic difficulties Price volatility Unfair competition Lack of efficient road infrastructure 	<ul style="list-style-type: none"> Local brand growth and positioning Participation in rural women's fairs and networks Community support Brand expansion in the national and international market
ASOFRUGUASIMAL	<ul style="list-style-type: none"> Pulp plant installed Female leadership Diversified production (citrus, mango, etc.) Women with a track record and experience in cooperatives (46-76 years old) and practical knowledge in traditional production. 	<ul style="list-style-type: none"> Reduction of active members Internal disorganization Poor technical harvest planning Limitations on the members' education (primary to secondary). Little associative stability 	<ul style="list-style-type: none"> Failure to comply with public calls for proposals Machismo and overload of domestic tasks for women Variability of input prices Climate change Social conflicts and violence Presence of intermediaries 	<ul style="list-style-type: none"> Demand for processed products Generate contacts for linking to improve technical and specialized training. Potential for rural tourism

Source: Prepared by the authors based on fieldwork (2024).

Table 3. Description of the social, productive, economic and cultural aspects of the 3 case studies of cooperatives in Colombia.

Case study	Social aspects	Productive aspects	Economic aspects	Cultural aspects
Anolaima Avocado and Other Fruit Producers Association-APROAFA	Number of members: 6 Age: 40 to 61 years Education: primary to university	Avocado cultivation with a focus on transformation; prototype development; processing plant goal	Need for equipment and technology; limited government support; seeking income diversification	Redefining the traditional female role; integrating peasant identity and entrepreneurship
Association of Women Coffee Growers in Anolaima-MUCCA	Number of members: 10 Age: 48 to 77 years Education: primary to university	Sustainable coffee production; value-added product transformation; active participation throughout the value chain	Brand development; solidarity economy practices; local marketing; limited access to financing; and road infrastructure	Revaluation of the role of women coffee growers; promotion of female coffee identity; cultural transformation from the collective perspective
ASOFRUGUASIMAL	Number of members: 6 Age: 46 to 76 years Schooling: primary to secondary	Mango, citrus, and fruit pulp production; difficulties with harvest and post-harvest scheduling	Lack of agricultural inputs; difficulties in consistent marketing; low external investment	Presence of traditional practices in rural communities; female leadership challenging patriarchal norms

Source: Prepared by the authors based on fieldwork (2024).

CONCLUSIONS

The study shows that agricultural cooperatives are constituted as transformational spaces for rural women, allowing them access to financial resources, strengthening personal and entrepreneurial capacities, as well as increasing the visibility of women to actively participate in leadership positions for decision making. Challenges were identified such as limited access to credit, the double workday of women who participate in the cooperatives and tend to family needs, given the complexity of reconciling the demands of agricultural production with domestic responsibilities, as well as the shortage in government support, the scarce specialized training, and the lack of accompaniment and counseling for efficiency in the commercialization of their products. Feminine cooperativism in Colombia is posed as the access that women have to promoting empowerment and thus improving the quality of life and achieving economic and social growth, as well as personal and entrepreneurial growth of women farmers.

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Adaptive practices to reduce environmental vulnerability due to drought in livestock production

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ABSTRACT

Objective: To analyze the use of different adaptive practices that cattle producers carry out in Concordia, Sinaloa, located on the dry tropics of Mexico, to reduce the environmental vulnerability caused by drought.

Design/methodology/approach: Intentional sampling was used to interview n=40 cattle producers from Concordia, Sinaloa, during the year 2023. Through percentile analysis, four types of producers were characterized. The non-parametric Kruskal-Wallis and Chi-squared tests for qualitative variables were used, in order to determine the differences between groups.

Results: There are adaptive practices for drought, which are poorly understood (22.4% of livestock producers know them) and of low use (under 26%). The producers who use this type of practices more are those who have greater environmental vulnerability to drought (less surface, fewer heads of livestock, and in general insufficient productive resources). The most widely used practice was the availability of shade in paddocks and the use of conservation methods for fodders.

Limitations on study/implications: The results were applied to a sample selected and a region in particular.

Findings/conclusions: Contrary to the hypothesis set out, small-scale producers are the ones that make greater use of adaptive practices and technologies for livestock production. The percentage of livestock producers who use these practices is less than 26%, despite there being local institutions that promote their use.

Keywords: climate change, livestock technologies, dry tropics.

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INTRODUCTION

Climate change is the alteration of climate patterns that change the distribution of key climate variables such as temperature, precipitation, moisture, wind speed, duration of sunlight, and evaporation (Peng *et al.*, 2017). Climate change (CC) and variability have generated great uncertainties in agricultural production, particularly at a small scale



(Zougmore *et al.*, 2018). These uncertainties are characterized by erratic rain patterns and higher temperatures, with prolonged dry periods that cause moisture scarcity in the soil during the growing season of crops. Therefore, cultivation systems that are more resistant to these adverse conditions are necessary (Mhlanga and Thierfelder, 2021).

The increase in temperature, reduction in precipitation, and irregular patterns of rainfall reduce crop yield and livestock production, which expose the impact of climate change on food security of developing countries (Abebaw, 2025). In the presence of effects from CC, strategies are required to strengthen the resilience of agricultural and livestock systems with the aim of reducing the risk of food insecurity both now and in the future (Smith and Frankenberger, 2018).

The impacts of drought, for example, in the rural communities of Mexico can increase by 5% the probability of becoming poor and reduce by 3% female employment and male school attendance (Arceo-Gómez *et al.*, 2020). Climate change in Mexico has led to an increase in the temperature; thus, the mean temperature at national scale has increased by +0.71 °C between 1951 and 2017 (moving average at 10 years, $p < 0.001$), or else by +0.96 °C if it is calculated by ten-year periods ($p < 0.001$); however, the region around the Gulf of California (northwestern states) showed the greatest rise in temperature, increasing up to +2.7 °C, and a decrease of 20% in annual precipitations in the last 67 years (Murray-Tortarolo, 2021).

In Mexico, during the fourth trimester of 2024, livestock production showed a fall in every region, except the south. In the north and center-north of the country, the formation of cattle inventories decreased as consequence of drought, which affected various stages of development in the herd, particularly in previous trimesters, and which, in the case of the northwestern zone of the country, persisted until the beginning of 2025 (Banxico, 2025). In a study conducted about drought in years 2011-2021, Murray-Tortarolo and Jaramillo (2019) found that the existence of cattle and goat livestock decreased around 3% in response to the drought throughout the country. These same authors point out that under the scenario of severe climate change, a higher frequency of extremely dry years (once every three years) would have negative impacts on the livestock production at the regional level in Mexico.

Climate change is the greatest environmental danger intimidating the whole global population; the average global temperature is increasing, the sea level is rising, the glaciers are melting, and the rain patterns are changing in an unpredictable manner. Consequently, strategies for adaptation and confrontation from small-scale producers are essential to mitigate these impacts of climate change (Okolie *et al.*, 2024).

In Mexico, 70% of agriculture is carried out in non-irrigated or rainfed areas (SIAP, 2022), which makes this sector especially vulnerable to climate change. It is important to increase the resilience of small-scale agriculture in developing countries; although studies have focused on the impacts of climate on crops and the decisions for adaptation, obstacles to adopt adaptive measure by small-scale farmers continue to be largely unexplored (Lamichhane *et al.*, 2022).

Although there are many political and economic barriers that hinder the development of adaptation initiatives, detailed and geographically explicit information is still needed

about who and where the most vulnerable small-scale farmers are. This, with the aim of generating recommendations based on evidence about adaptation strategies and practices (or possible new strategies for adaptation), for small-scale farmers who work in different production systems (Donatti *et al.*, 2018). In this sense, the objective of this study was to analyze the use of different adaptive practices that cattle producers perform in the municipality of Concordia, Sinaloa, located in the dry tropics of Mexico, to reduce the environmental vulnerability caused by drought. The research hypothesis was based on the idea that the use of technology and the implementation of adaptive practices for the drought problem has a direct relationship with the scale and availability of resources from the livestock producer; with higher level of productive resources, there will be greater use of adaptive practices.

MATERIALS AND METHODS

Study zone

The municipality of Concordia (23° 04' and 23° 47' LN, 105° 41' and 106° 15' LW), with altitude from 100 to 2300 m, presents subhumid warm climate with summer rains of lower moisture (44.11%), warm subhumid with summer rains of average moisture (23.99%), semiwarm subhumid with summer rains of higher moisture (15.03%), warm subhumid with summer rains of higher moisture (13.20%), and temperate subhumid with summer rains of higher moisture (3.67%) (INEGI, 2010).

Selection of the sample and instrument used

The livestock producers interviewed were selected through non-probabilistic and intentional sampling (Hernández, 2021). The survey was applied in the year 2023 and was obtained this way to decrease the risks from the insecurity prevalent in the study zone. To obtain the information, a structured questionnaire was used, which was made up of four sections: 1) identification of the producer, 2) characterization of the production unit (crops and size of the herd), 3) impact of climate on livestock production, and 4) knowledge and use of adaptive practices and technologies for the drought problem. In total, n=40 livestock producers were interviewed as key agents.

Information analysis

To identify the types of adaptive practices that the livestock producers know and use, the production units were grouped based on the productive scale related to the number of cows that they have. According to Cuevas-Reyes *et al.* (2013), a classification of livestock production units was carried out through the use of percentiles.

The classification obtained was the following: group 1 ($Q2 \leq 11$ cows, small-scale livestock producers - SLP), group 2 ($Q3 >$ than 11 and ≤ 21 cows, medium-scale livestock producers - MLP), and group 3 ($Q4 > 21$ cows, large-scale livestock producers - LLP). A percentile is a value under which a specific proportion of observations are found; percentiles offer an alternative to quotients based on the average (Bornmann *et al.*, 2013). In general, percentiles are not as strongly influenced by extreme values of the distribution as the mean value (Waltman *et al.*, 2011), and they do not depend on the selection of a function of

specific probability density compared with the arithmetic mean, which requires a normal distribution (Bornmann *et al.*, 2013). The analysis of quantitative variables was carried out through the Kruskal-Wallis non-parametric and Chi-squared tests for qualitative variables, to determine the differences ($P < 0.05$) between the groups.

RESULTS AND DISCUSSION

Socioeconomic characterization

Results showed that the three groups of producers have a similar age ($p < 0.05$), from 51 to 59 years of age; they are mature producers that have a median of three children and in terms of education they only have between two and three years of study. The distance between the ranches and the municipal township did not have differences, although the medium-scale producers have their ranches farther from the municipal township (13 kilometers), compared to the medium-scale and small-scale livestock producers (Table 1). The proximity of production units to municipal townships facilitates the economic dynamics through efficient commercialization of products and with cost reduction (Espinoza *et al.*, 2024).

Characterization of the productive unit

The productive resources for fodder production (land and pastureland) had significant differences ($p > 0.05$ and $p < 0.10$), favoring the group of large-scale livestock producers. The total agricultural surface ranged between 19 hectares (MLP) and 80 ha in the LLP; this ratio is kept both for the surface planted and for the total hectares that livestock producers have. That is, the small-scale and medium-scale livestock producers are similar in the variables of agricultural surface and pastureland, but different compared to the LLP in the amount of land for fodder production (Table 2).

Small-scale and medium-scale livestock producers showed a similarity in terms of number of bulls, male calves and female calves produced, but there were differences ($p < 0.05$) compared to the group of large-scale livestock producers. The variable number of adult cows presented statistical differences ($p < 0.05$) between the three groups analyzed. Small-scale and medium-scale livestock producers had one bull compared to those in the LLP which have two, higher number of adult cows, as well as a larger number of male and female calves produced per year (median of seven) (Table 3).

Table 1. Characterization of the producer (median \pm RIC*).

Variables	SLP (n=20)	MLP (n=11)	LLP (n=9)	p**
Age (years)	59 \pm 16.2 ^a	55 \pm 9.0 ^a	51 \pm 15.5 ^a	0.47
Years of schooling	2 \pm 3 ^a	2 \pm 3 ^a	3 \pm 2 ^a	0.40
Number of children	3 \pm 1 ^a	3 \pm 2 ^a	3 \pm 2.5 ^a	0.41
Distance from the ranch (km)	6.5 \pm 23.7 ^a	13 \pm 35 ^a	8.5 \pm 18.7	0.20

Source: Prepared by the authors. *RIC=inter-quartile range, p**=Kruskal-Wallis test.

SLP: small-scale livestock producers; MLP: medium-scale livestock producers; LLP: large-scale livestock producers.

Table 2. Agricultural surface for fodder growing (median \pm RIC*).

Variables	SLP	MLP	LLP	p**
Total area	20 \pm 26 ^a	19 \pm 39 ^a	80 \pm 94.5 ^b	0.01***
Sown area	10 \pm 12 ^a	12 \pm 111.2 ^a	20 \pm 31 ^b	0.04***
Rangeland	9 \pm 22 ^a	6 \pm 35 ^a	24 \pm 63 ^b	0.09****

Source: Prepared by the authors. *RIC=inter-quartile range, p**=Kruskal-Wallis test. ***Significative at 95 %, ****Significative at 90%.

SLP: small-scale livestock producers; MLP: medium-scale livestock producers; LLP: large-scale livestock producers

Table 3. Number of heads of livestock (median \pm RIC*).

Variables	SLP	MLP	LLP	p**
Bulls	1 \pm 1 ^a	1 \pm 1 ^a	2 \pm 2 ^b	0.001***
Adult cows	8 \pm 3.7 ^a	14 \pm 6 ^b	30 \pm 22 ^c	0.001***
Calves	2.5 \pm 3.7 ^a	4 \pm 3 ^a	7 \pm 10 ^b	0.003***
Calves	2 \pm 2 ^a	4 \pm 3 ^a	7 \pm 5 ^b	0.001***

Source: Prepared by the authors. *RIC=inter-quartile range, p**=Kruskal-Wallis test. ***Significative at 95%.

SLP: small-scale livestock producers; MLP: medium-scale livestock producers; LLP: large-scale livestock producers.

Main problems caused by drought

The three groups of producers pointed out that the main problem for livestock production was drought which lasts on average six months in the study zone, so that during this period the lack of food for the livestock (fodder) becomes accentuated. The results agreed with what was described by Perales *et al.* (2000), who point out that in the state of Sinaloa, the scarcity of fodder for livestock feed is the main problem of livestock producers during six months of the year (January to June).

In this regard, the livestock producers agreed ($p>0.05$) in mentioning the existence of four main problems derived from the recurring droughts in recent years: the lack of fodder (84.7%), followed by the lack of water for the livestock (57.7%), and the lack of water for production of fodder crops (33.8%). The problem related to the death of livestock was mentioned in fourth place, although it is more evident in the stratum of large-scale livestock producers (11%), in addition to 5% of small-scale producers mentioning that they have this type of problem (Table 4).

Table 4. Problems related to drought (%).

Variables	SLP	MLP	LLP	Average	X ²
Lack of water for livestock	60	45.5	67	57.7	0.602
Lack of water for forage production	50	18.2	33.3	33.8	0.207
Lack of forage	85	91	78	84.7	0.716
Livestock deaths	5	0	11	5.3	0.526

Source: Prepared by the authors. SLP: small-scale livestock producers; MLP: medium-scale livestock producers; LLP: large-scale livestock producers

Our results converge with what was found by Harvey *et al.* (2018), who revealed that small-scale farmers in Central America have observed climate change, and that most of them are already experiencing the impacts of the increase in temperatures, unpredictable rains, and extreme meteorological phenomena in crop yield and livestock productivity, higher incidence of pests and diseases, as well as the generation of impacts on families' income and, in some cases, food security.

The National Water Commission has identified many years with critical drought periods; in its report from the year 2021, the agency identified that the municipality of Concordia presented conditions of extreme drought (CONAGUA, 2021), which according to the same author, were related to higher losses in crops and grasses.

Adaptive practices and technologies, knowledge and use

In the state of Sinaloa, there have been efforts to solve the problems related to the lack of fodder since 1996, through different strategies for dissemination and transference of livestock innovations carried out by state research centers. Since that period, diverse species of grasses have been generated and validated for the implementation of pasturelands, liberation of fodder sorghum, use of legumes, and methods for fodder ensilage and conservation (Loaiza, 2011; Hernández *et al.*, 2011).

However, despite these efforts, there is still poor knowledge of adaptive practices for the drought problem among the producers interviewed; only 22.4% of the total producers interviewed know the six practices promoted to improve the production of fodders and the wellbeing of livestock. The practices that they least know about are those of adjusting the animal load and the existence of fodder species adapted to the drought conditions (both with 7.5%). The most well-known practices were availability of shade in the paddock and conservation of fodders (37.5 %) (Table 5).

Regarding the application of these adaptive practices in livestock production by the farmers interviewed, no significant differences were found ($p > 0.05$). However, there are indications that small-scale livestock producers are the most prone to use practices that mitigate the vulnerability of the drought problem (on average, this type of producers use 29.2% of the adaptive practices and technologies).

Table 5. Knowledge of adaptive practices and technologies (%).

Practices	SLP	MLP	LLP	Total	X ²
Shade availability in pastures	45	36.4	22.2	37.5	0.501
Adjust stocking rate	5	9.1	11.1	7.5	0.823
Conserve forage (silage, hay)	35	54.5	22.2	37.5	0.315
Implement silvopastoral systems	25	27.3	11.1	22.5	0.642
Forage species adapted to drought	15	0	0	7.5	0.98
Conserve soil and water	25	18.2	22.2	22.5	0.910
Average	25	27.3	14.8	22.4	

Source: Prepared by the authors. SLP: small-scale livestock producers; MLP: medium-scale livestock producers; LLP: large-scale livestock producers.

In general, the adaptive practice of greatest use by the livestock producers interviewed was to provide availability of shade in the paddocks (57.5%). In the case of the tropics, it is known that the effect of thermal stress tends to accentuate during the warmest hours of the day (between 11:00 and 15:00 hrs), and it is higher during the dry season and at the beginning of the rainy season (García, 2010). Tree shade in livestock production systems stands out as a component that improves productivity in the herd. On the one side, it decreases the caloric stress of the livestock and, in addition, shade improves the quality of the pastureland through positive changes in protein contents and a decrease in fiber structures, which consequently improve the digestibility, finally translating into better weight gain of the animals (Obispo *et al.*, 2013).

On the other hand, the practice of fodder conservation through ensilage is recognized as an alternative to obtain good quality feed for the dry season in the tropics, where there is greater scarcity of feed for the livestock (Cuevas, 2019). This is the second practice in importance that livestock producers interviewed have used (55%), which is necessary to decrease the environmental vulnerability related to the lack of feed and quality fodders caused by drought in the study zone (Table 6).

The use of adaptive practices for the problem of lack of fodder caused by long periods of drought in the study zone requires joint efforts from various stakeholders in the sector, in order for more livestock producers to know the alternatives to face the environmental vulnerability in fodder production. However, the adaptation of these technological innovations depends on the producer perceiving the impact of climate change as a risk to be able to implement adaptations, technologies and socially solid policies that mitigate the adverse effects of climate change (Whitmarsh and Capstick 2018; Adger *et al.*, 2009). Viguera *et al.* (2019) described that perceiving the effects of climate change is a necessary condition to implement adaptation strategies by producers and institutions.

Our results showed that it is not enough to have technological alternatives that may be used as adaptive practices, but rather that there are other elements that impact the use or adoption of these alternatives. In this regard, in a study of the agricultural community in Bangladesh, Islam *et al.* (2014) found that there are limitations for the adoption of adaptive measures to climate change in terms of natural, technological, social, economic

Table 6. Use of adaptive practices and technologies (%).

Practices	SLP	MLP	LLP	Total	X ²
Shade availability in pastures	55	54.5	66.7	57.5	0.819
Adjust stocking rate	20	18.2	0	15.0	0.356
Conserve forage (silage, hay)	60	36.4	66.7	55.0	0.326
Implement silvopastoral systems	15	18.2	11.1	15.0	0.908
Forage species adapted to drought	0	18.2	0	5.0	0.068
Conserve soil and water	25	0	0	12.5	0.057
Average	29.2	24.3	24.2	25.9	

Source: Prepared by the authors. SLP: small-scale livestock producers; MLP: medium-scale livestock producers; LLP: large-scale livestock producers.

and institutional processes that are mostly interrelated and collectively influence decisions of adaptation.

The results from this study specify that small-scale livestock producers who have more vulnerability and fewer economic resources are the ones who carry out more adaptive practices, which contrasts to what was found by Schmidt *et al.* (2012), who mentioned that small-scale producers have been the most affected by these changes in climate patterns but do not know how to adapt to these changes to protect their production unit, their family, and guarantee their food security. Harvey *et al.* (2018) mentioned that small-scale farmers are one of the most vulnerable groups to climate change; however, efforts to support the adaptation of farmers are obstructed by the lack of information about how they are experiencing and responding to climate change.

CONCLUSIONS

Problems related to drought in Mexico are increasingly recurrent, so it is necessary to identify, design and implement adaptive practices to reduce the environmental vulnerability caused by this climate phenomenon. The study demonstrates that, contrary to the hypothesis set out, small-scale producers are the ones who use more adaptive practices and technologies for livestock production. However, the percentage of livestock producers who use them is under 26%, even when there are local institutions that promote the use of said technologies. An integral strategy to mitigate the effects of climate change is required, although differentiated according to the characteristics of each group of livestock producers. This would have the aim of including their existing level of adaptation, resources, perceptions, opinions and experiences to strengthen their capacities and tools at the local level.

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DNA presence and viability of *Mycobacterium avium* subsp. *paratuberculosis* in semen of Pelibuey rams during the subclinical stage of paratuberculosis

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ABSTRACT

Objective: To determine DNA presence and viability of *Mycobacterium avium* subsp. *paratuberculosis* in semen of Pelibuey rams in the subclinical stage of paratuberculosis.

Design/Methodology/Approach: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the etiological agent of paratuberculosis (PTB). Reports about the spread of MAP to extraintestinal sites have been confirmed by the presence of MAP-DNA in semen. However, contamination with MAP has not been reported in sheep semen in the subclinical stage of the disease. Such inadvertent contamination would represent a risk factor for reproductive and genetic management, even in the presence of asymptomatic rams. A descriptive, cross-sectional study was carried out with five rams naturally infected with MAP and one PTB-free ram as a negative control. Nested Polymerase Chain Reaction (nested PCR) and MAP culture techniques were used to research the presence of DNA and the viability of *Mycobacterium avium* subsp. *paratuberculosis* in the semen of Pelibuey rams in the subclinical stage of paratuberculosis. The rams came from an infected flock diagnosed with PTB.

Results: The presence of MAP-DNA was detected in the semen of 60% (3 out of 5) of the rams in the subclinical stage. However, MAP did not grow in the culture, suggesting that the MAP from semen samples is not viable.

Study Limitations/Implications: The study was carried out with a small number of naturally infected animals in the subclinical stage, taking into consideration animal welfare and care. However, more animals would result in better measurements.

Findings/Conclusions: The presence of MAP-DNA in the semen of rams in the subclinical stage was confirmed. Apparently, the MAP from the semen is not viable for culture.

Keywords: paratuberculosis, MAP culture, clinical signs, nested PCR, ELISA, semen.



INTRODUCTION

Paratuberculosis (PTB) or Johne's disease (JD) is a chronic infectious disease caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). It mainly impacts domestic and wild ruminants, but it can also affect non-ruminant animals (camels, wild rabbits, pigs, horses, poultry, and carnivores) (Idris *et al.*, 2022). PTB has a 5-30% prevalence, mainly among sheep, goats, and cattle (Guzmán-Ruiz *et al.*, 2016) and its presence impacts the economy, animal welfare, and public health (Whittington *et al.*, 2019). For each animal diagnosed in its clinical stage, 25 more animals may be infected, although they may not show outward signs (Pritchard *et al.*, 2017). Several clinical signs could help to guess the presence of PTB: intermittent scours and weight loss despite a good appetite (Idris *et al.*, 2022). PTB can be detected with several techniques: acid-resistant bacilli through a stool microscopy, a fecal or tissue culture, a serology test, and molecular methods (Idris *et al.*, 2022). MAP mainly spreads through fecal-oral transmission (Whittington and Windsor 2009). MAP can infect the gastrointestinal tract and the mesenteric lymph nodes. However, some studies have proved that it can also spread to extraintestinal sites of cattle, including the uterus, supramammary lymph nodes, udders, testicles, epididymis, and seminal vesicles (Khol *et al.*, 2010). MAP-DNA presence has been reported in the semen of bulls (Ayele *et al.*, 2004; Münster *et al.*, 2013; Sharifzadeh *et al.*, 2010), as well as in the feces, semen, and blood of a subclinically-infected bull. Therefore, semen would be a transmission path without clinical signs (Khol *et al.*, 2010). In conclusion, using infected rams could be a causal agent for the spreading of PTB (Ayele *et al.*, 2004). Eppleston and Whittington (2001) reported the presence of MPA in the ileum, mesenteric lymph nodes, and semen of sheep in the clinical stage. MAP-DNA has also been identified in the epididymis, Cowper's glands, prostate, and semen through a nested PCR (Velázquez-Morales *et al.*, 2019); however, despite the presence of MAP in semen, no alterations have been detected during semen evaluation (Velázquez-Morales *et al.*, 2022). However, the existence and viability of MAP in semen from rams in the subclinical stage of PTB has not been verified; otherwise, new health management strategies should be applied as part of the genetic improvement programs for sheep. Therefore, the aim of this study was to detect the presence of ADN and the viability of *Mycobacterium avium* subsp. *paratuberculosis* in the semen of Pelibuey rams in the paratuberculosis subclinical stage, using the nested PCR and MAP culture techniques.

MATERIALS AND METHODS

Facilities and Animals

The rams were isolated from the herd in pens established in the Colegio de Postgraduados (COLPOS) – Campus Cordoba, a research unit with prior reports of PTB. Campus Cordoba is located in km 384 of the federal highway Córdoba Veracruz, Congregación Manuel León, municipality of Amatlán de los Reyes, Veracruz (18° 51' 20" N, 96° 51' 37" W, 720 m.a.s.l.). The climate is subhumid warm, the average temperature is 18 °C, and the annual precipitation reaches 1,807.3 mm (García, 2004). The study involved six Pelibuey rams: five naturally infected with paratuberculosis

(subclinal stage) and a non-infected specimen as control. The diagnosis was based on an Enzyme-Linked Immunosorbent Assay (ELISA test) and a nested PCR using IS900 primers, which were applied 20 days before the start of the test. Herrold's egg yolk medium with mycobactin was used for the confirmation diagnostic test. The animals had an average weight of 55.9 ± 2.15 kg and were 27 ± 0.6 months-old.

Semen Collection

At the start of the semen sample collection, the prepuce was washed with Dermocleen™ antiseptic soap. The semen samples were collected from each ram with the artificial vagina method, using the methodology proposed by Williams *et al.* (2001).

Serology

An ELISA serology was conducted to detect anti-MAP antibodies, considering that >0.196 optical density values are positive to PTB (Martínez-Covarrubias *et al.*, 2012). Blood samples were collected through a puncture in the jugular vein, using a 20G BD Vacutainer™ needles and BD Vacutainer™ collection tubes without anticoagulant. The whole blood was centrifuged at $1,000 \times g$ for 10 minutes to recover the blood serum, which was kept at -18 °C, until it was processed in the CENID–Microbiología lab of the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP).

PTB Diagnosis through IS900 Nested PCR

To diagnose the PTB infection in Pelibuey rams, DNA was extracted and an IS900 nested PCR was conducted, following the protocol described by Jaimes *et al.* (2008). The test used DNA from fecal and semen samples from each ram. An IS900 nested PCR was conducted to confirm the MAP-DNA presence in feces and semen, using the primers proposed by Erume *et al.* (2001). In the first cycle and the final reaction, 563 and 210 base pairs (bp) were recorded, respectively. The DNA of a MAP strain (ATCC #700535) was used as positive control. The final product of the nested PCR reaction was observed in a 2% agarose gel, stained with GelRed® (Biotium #41003). The gel was visualized with a Gel Doc™ 2000 UV transilluminator system (Bio Rad®).

MAP Culture

The methodology proposed by Martínez-Covarrubias *et al.* (2012) was used to isolate the bacteria. Initially, the semen samples were washed with the acid-alkali method and placed in Herrold's egg yolk medium with mycobactin (2 mg L^{-1} , Allied Monitor Inc.). The samples were incubated at 37 °C for 14 weeks and were subsequently stained with a Ziehl-Neelsen (ZN) stain to search for acid-fast bacilli (AFB).

Body Condition Estimation

Body condition (BC) was scored following the methodology proposed by Russel *et al.* (1969), using the categories proposed by Guerrero-Cárdenas *et al.* (2020): 1) very poor; 2) bad; 3) moderate; 4) good; and 5) excellent.

Statistical Analysis

The output was generated with the SAS[®] 9.4 statistical package and presented as frequencies and percentages.

RESULTS AND DISCUSSION

Table 1 shows the result of the PTB diagnosis with the ELISA and nested PCR tests. All (100%, 5 out of 5) Pelibuey rams tested positive with the IS900 nested PCR diagnostic test. In contrast, ELISA only estimated that 40% (2 out of 5) rams tested positive to MAP; therefore, this serological test recorded a lower percentage of positive diagnosis than the nested PCR. Each test has a different sensitivity and specificity: ELISA has a 79.3% sensitivity and a 82.2% specificity, while IS900 nested PCR has a 67% sensitivity and a 84% specificity (Martínez-Covarrubias *et al.*, 2012). The positive result of the IS900 nested PCR with ram feces was mainly caused by the great volume of bacilli found in the feces of those animals, since an infected animal can excrete between 1.3×10^5 and 5.9×10^9 microorganisms per gram of fecal matter (Manning *et al.*, 2001). Idris *et al.* (2022) have reported the intermittent elimination of MAP in goat feces in the subclinical stage of the infection. For their part, Münster *et al.* (2013) reported the intermittent excretion of MAP through feces in a 18-month old cow—an unusual event for such a young animal. Therefore, they recommended reconsidering the belief that young livestock is not infectious. Meanwhile, Jaimes *et al.* (2008) pointed out that animals who have tested negative to ELISA sometimes tested positive to PCR or bacterial isolation, because, in the early stages of an infection, the prevailing immune response is cellular and the PCR test has a higher inherent sensitivity. During the gestation, the infection expression among females and the seroconversion of animals infected at an early age are boosted, possibly as a consequence of the immune regulation that prevents the rejection of the embryo (Kostoulas *et al.*, 2006). Likewise, Feola *et al.* (1999) studied prolactin and the bovine growth hormone *in vitro* and reported a reduced capacity of macrophages to hinder the growth of intracellular MAP. Therefore, changes in the circulation levels of these hormones during gestation and lactation force phagocytes to allow the intracellular proliferation of *Mycobacterium*.

Table 1. PTB diagnosis in Pelibuey rams in the subclinical stage, using the ELISA test and nested-PCR technique.

Ram Identification	n-PCR IS900: DNA from feces	ELISA
1	+	+
2	+	–
3	+	+
4	+	–
5	+	–
N	–	–

nPCR=Nested Polymerase Chain Reaction. ELISA=Enzyme-Linked Immunosorbent Assay. “+”=positive result. “–”=negative result. N=negative control.

Table 2 summarizes the body condition (BC) score of the rams, recording a 2.5 ± 0.22 average, with a lack of clinical signs (sticky feces and scours). The BC score indicates that sheep have an adequate nutritional status, without weight loss or the presence of clinical signs, possibly because PTB is recorded in 2-4 years old goats, sheep, and cattle (Idris *et al.*, 2022). The weight loss in sheep changes from one specimen to another; in fact, sticky feces or scours only occur in 20% of the cases, during the final stages of the disease (Idris *et al.*, 2022). However, neither sticky feces or scours were reported in this study for 27 ± 0.6 months old rams, which perhaps accounts for the lack of PTB clinical signs among the males. Ayele *et al.* (2001) reported the appearance of clinical signs related to stress-causing factors, such as malnutrition, since these factors can modify paratuberculosis from the subclinical to the clinical stage. However, as has been previously discussed, rams had a moderate BC.

Regarding the search for MAP in semen, the IS900 nested PCR detected the presence of MAP-DNA in goat semen. These results match the findings of Münster *et al.* (2013), who proved that MAP-DNA can be detected intermittently in the semen of bulls in the subclinical stage, with up to 18-week MAP-free intervals. Likewise, they recorded a significant correlation between the presence of MAP in semen and blood. Consequently, they warned against excluding the risk of MAP transmission, despite the lack of the typical signs of clinical paratuberculosis. The presence of MAP outside the intestine is possibly caused by its spread through blood and the lymphatic system (Buergelt *et al.*, 2004). It is even possible that semen samples had been contaminated during the collection (Eppleston and Whittington, 2001; Khol *et al.*, 2010). Unlike the culture, nested PCR can detect viable and non-viable bacteria, because the former has a 100% specificity, but a <50% sensitivity (Jaimes *et al.*, 2008). Table 3 shows the nested PC and culture results; however, no *Mycobacterium* were observed in the semen culture samples.

Figure 1 shows the MAP-DNA presence in semen with the IS900 nested PCR (60%, 3 out of 5).

Figure 1 shows that MAP did not grow in cultures with semen collected from rams in the subclinical stage, possibly as a result of the low number of viable *Mycobacterium* obtained from the sample. Jaimes *et al.* (2008) reported that at least 10^3 bacilli are required

Table 2. Body condition (BC) and clinical signs of Pelibuey rams naturally infected with PTB.

Ram identification	Body condition (BC, score)	Pasty stools	Scours
1	2	–	–
2	2	–	–
3	3	–	–
4	2	–	–
5	3	–	–
N	3	–	–

“+”=positive result. “–”=negative result. Both results were obtained during the recording of the PTB clinical signs.

Table 3. Presence of *Mycobacterium avium* subsp. *paratuberculosis* in the semen of Pelibuey rams in the subclinical stage.

Ram identification	Semen	
	n-PCR IS900	Culture
1	+	-
2	-	-
3	+	-
4	-	-
5	+	-
N	-	-

“+”=positive result. “-”=negative result. Both results were obtained with the nested PCR and MAP culture tests.

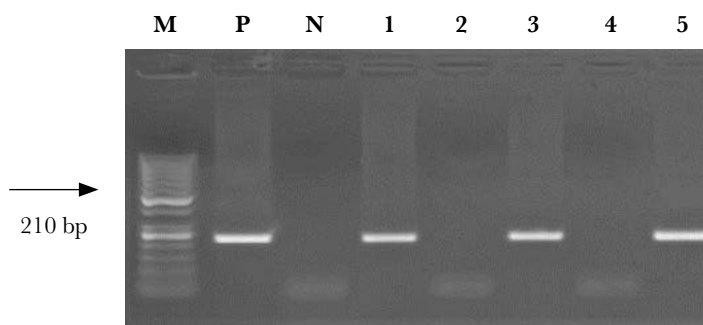


Figure 1. DNA amplification output with IS900 nested PCR in semen samples from Pelibuey rams in the subclinical stage of PTB, using an electrophoresis with 2% agarose gel. Positive fragment of 210 bp. Track M: 50 bp molecular marker. Track P: positive control (MAP-DNA ATCC #700535). Tracks 1, 3, and 5: semen samples with MAP-DNA (positives, 210 bp). Track N: semen samples from the negative control (DNA of a ram without PTB). Tracks 2 and 4: DNA samples with negative response to MAP.

per mL of processed sample to guarantee their growth. For their part, Khol *et al.* (2010) also recorded negative growth results, based on semen samples from PTB-infected cattle, possibly due to the loss of MAP viability during the sample decontamination.

CONCLUSIONS

The presence of *Mycobacterium avium* subsp. *paratuberculosis* DNA was detected with the nested PCR technique in semen from Pelibuey rams in the PTB subclinical stage. Nevertheless, based on the lack of growth of the culture, the MAP from the said samples was shown to be unviable. Therefore, a longitudinal study is recommended to determine if the excretion of MAP through the semen is intermittent and to establish the possibility of its viability.

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Ethical Approval





The experiment followed the technical specifications of the NOM-062-ZOO-1999 Official Mexican Standard (SAGARPA, 1999) for the production, care, and use of laboratory animals, in accordance with guidelines for the use and care of research animals, approved by the General Academic Council of the Colegio de Postgraduados (ColPos, 2016).

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Comparison of sustainability between the dual-purpose system and the silvopastoral system in the Zoque region

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ABSTRACT

Objective: To use the IDEA method (Sustainability of Agricultural Holdings) in the analysis of the dimensions of livestock systems (LS), which allows evaluating sustainability objectively.

Methodology: The evaluation was conducted in n=24 silvopastoral systems (SPS) and n=13 dual-purpose bovine livestock systems (DPBLS) in the Zoque Valley region of Chiapas, Mexico, using the IDEA method.

Design/methodology/approach: The resulting indicators were analyzed with the IBM SPSS statistical package, determining their normality, association and difference in the values obtained.

Results: The sustainability of SPS is defined by the socio-territorial scale (54.26), while in DPBLS the influence of the economic scale predominates (48.92). Statistically, there are no significant differences in the sustainability indicators between both systems.

Limitations on study/implications: In the LS studied, although they have similar levels, sustainability is determined by different factors. This divergence indicates that, while SPS prioritize holistic sustainability through agroecological synergies, DPBLS face the challenge of balancing profitability with responsible environmental practices.

Findings/conclusions: The absence of significant differences in sustainability denotes that management practices are decisive in the management of sustainability in the LS. More studies are required to evaluate the sustainability of the LPUs, as well as implementing public policies that establish rules for the responsible use of natural resources and the transfer of technologies.

Keywords: Agroecological; socio-territorial; economic; sustainability; silvopastoralism.

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INTRODUCTION

Bovine livestock production faces several challenges and key trends; global production of bovine meat continues growing, global consumption shows diverse patterns with increases in developing countries and a tendency towards reduction in some developed regions,



and there is a growing preoccupation over the environmental impact of bovine livestock production (FIRA, 2024), especially regarding greenhouse gas emissions (FAO, 2023). Therefore, this demands increasing productivity without compromising environmental, economic and social sustainability; within this context, dual-purpose bovine livestock systems and silvopastoral systems have been promoted as effective alternatives to balance the production of meat and milk with the conservation of natural resources (Gutiérrez Bermúdez and Mendieta Araica, 2022).

Mexico is an important producer of bovine livestock, both for meat and for milk. Production is divided mainly between meat, dual-purpose, and dairy livestock; the traditional extensive system predominates, especially in tropical and arid zones, although there is a rise in intensive and semi-intensive systems, where small-scale and medium-scale producers represent a significant part of the sector (Pérez Chabela and Lamothe Zavaleta, 2019).

Livestock production in Chiapas is in a phase of growth and modernization, it is an essential economic activity that has shown notable growth in recent years; however, it faces sanitary and environmental challenges, although it has also adopted sustainable practices that seek to ensure its viability and competitiveness in the long term (Vargas-de la Mora, 2018; Orantes-Zebadúa *et al.*, 2014).

The dual-purpose bovine livestock system (DPBLS) maximizes the productive efficiency by obtaining milk and meat simultaneously from the same herd, adapting to various agroecological conditions, and favoring food security; this contributes to the wellbeing of rural communities because it can simultaneously provide security in daily sustenance, conserve ecosystems, generate stable jobs for people in rural areas, and also constitutes the economic basis of millions of livestock and industrial producers (Cuevas-Reyes and Rosales-Nieto, 2018). Dual-purpose livestock production is common in countries with tropical and subtropical climates, where conditions are favorable for grazing and fodder production; it presents great heterogeneity in terms of breeds, levels of production, infrastructure, handling, investment, technology and management, which allows it to adapt to different zones and producers (García-Martínez *et al.*, 2015).

Silvopastoral systems (SPS) integrate tree and fodder species in a productive ecosystem, improving the quality of the soil, animal welfare, and resilience in the presence of climate change, in addition to promoting soil regeneration, carbon sequestration, and biodiversity (Aguilar Pérez *et al.*, 2019; López-Vigoa *et al.*, 2017). SPS seek to improve and modernize livestock production, while they contribute to reducing environmental problems, improving animal welfare, increasing animal productivity, and generating environmental services; they also adapt to the customs and traditions of the regions where they are implemented (López-Vigoa *et al.*, 2017; Alders *et al.*, 2021).

To ensure the long-term viability of these systems, it is crucial to have tools that allow evaluation in an objective and structured manner their level of sustainability. There are various methodologies used to evaluate the sustainability of livestock production systems, through which their environmental, social and economic impact is analyzed. The most frequently used, which include specific metrics (indicators of sustainability) that allow evaluating different aspects of the livestock production system, are: Evaluation Framework

of Natural Resources Management with Sustainability Indicators (*Marco de Evaluación de Sistemas de Manejo de Recursos Naturales con Indicadores de Sustentabilidad*, MESMIS); FAO indicators for the Sustainability Assessment of Food and Agriculture systems (SAFA); Response-Inducing Sustainability Evaluation (RISE); and Sustainability Indicators of Agricultural Holdings (*Indicadores de Sustentabilidad de las Explotaciones Agrícolas*, IDEA) (Berbec *et al.*, 2018).

The IDEA method has been consolidated as an integral model for the evaluation of sustainability thanks to its adaptability to different agriculture and livestock production systems, since it allows analyzing three fundamental dimensions: environmental, economic and social, through gathering and easy measurement of information from the livestock production units (LPUs), providing quantifiable indicators that facilitate decision making and the design of improvement strategies (Prospero Bernal, 2017; Torres-Lemus *et al.*, 2021). Therefore, the objective of this study was to analyze the sustainability of dual-purpose livestock systems compared to silvopastoral systems, using the IDEA method.

MATERIALS AND METHODS

The study was conducted in the Zoque Valley region in Chiapas, Mexico. With a total surface area of 7,393.76 square kilometers, it borders to the north with Region III Mezcalapa and the state of Veracruz, east with Region I Metropolitana, south with Regions VI Frailesca and IX Istmo Costa, and west with the state of Oaxaca; the altitude of the territory ranges from 60 to 2,054 meters above sea level; humid and subhumid warm climate with rainfall regime marked with summer precipitations predominates, and mean annual temperature in the range of 24 °C to 26 °C (CEIEG, 2012).

The information was obtained through intentional or snowball sampling, based on Martín-Crepeo Blanco and Salamanca Castro (2007); surveys and semi-structured interviews were conducted with cooperating producers and workers from 37 livestock production units (LPUs), of which 23 are SPS and 14 are DPBLS located in the municipalities of Cintalapa, Jiquipilas and Ocozocoautla.

The data obtained were organized and analyzed in Microsoft Excel 2019. Then, information analysis was carried out to evaluate the sustainability of the production systems, through the IDEA (Indicators of Sustainability in Agricultural Holdings) methodology, version 3, which was developed in France (Vilain *et al.*, 2008; Zahm *et al.*, 2008; Hamadi *et al.*, 2009).

The resulting indicators of sustainability from the IDEA method for SPS and DPBLS were analyzed using the IBM SPSS statistical package, performing the Shapiro-Wilk normality test to determine if the variables follow a normal distribution. The correlation coefficient between indicators of the systems was also determined, through Spearman's test, to measure the strength and the direction of the association between variables. In addition, the Mann-Whitney U test was carried out to compare the indicators from both systems and to determine if there are significant differences between the samples in terms of their central tendency (Field, 2013).

RESULTS AND DISCUSSION

Silvopastoral systems (SPS) and dual-purpose bovine livestock systems (DPBLS) present structural differences in their priorities of sustainability, as the results from the IDEA methodology show. The sustainability of SPS is determined primarily by the socio-territorial scale (54.26), which evidences a series of limitations: lack of infrastructure for livestock production development; low coverage of social security programs; need to improve productive efficiency; scarce generation of employment; low educational level of producers; and uncertainty regarding the permanence of new generations in the LPUs. All these factors hardly have an average impact on the standard of living. In DPBLS, sustainability is influenced mainly by the economic scale (48.93), which denotes the need to destine resources to animal feeding, as well as to sowing and harvesting of the crops, in addition to a lack of income from alternative activities (Figure 1).

The comparative analysis of the variances did not evidence statistical differences between the values of the indicators corresponding to SPS and DPBLS systems. Consequently, based on the data analyzed, both systems present similar behaviors in the aspects evaluated (Figure 2).

The statistical analysis of the indicators of the agroecological scale (A1-A18) reveals different perceptions. In SPS, strengths with higher diversity of perennial crops (A2: 7.4 *vs.* 6.4) and greater presence of ecological regulation zones (A8: 7.4 *vs.* 5.8) stand out, as well as better management of fertilization (A12: 6.9 *vs.* 2.9), protection of soil (A16: 5.0 *vs.* 3.9), and high energetic autonomy (A18: 8.7 *vs.* 2.7). However, both systems present a shared limitation: the null use of genetic patrimony (A4: 0). For their part, DPBLS present advantages with higher animal diversity (A3: 12.1 *vs.* 8.3), better management of the fodder surface (A11: 2.5 *vs.* 2.3), and lower animal load (A10: 3.3 *vs.* 3.8), which contributes to reducing the ecological pressure.

In the socio-territorial scale (B1-B18), SPS stand out for having greater autonomy in the use of local resources (B7: 7.7 *vs.* 6.7), collective work (B10: 1.5 *vs.* 0.4), and social participation (B5: 3.0 *vs.* 2.3), aspects aligned with the criteria of equity. On the other hand, DPBLS stand out for having greater generation of employment (B9: 4.8 *vs.* 3.0),

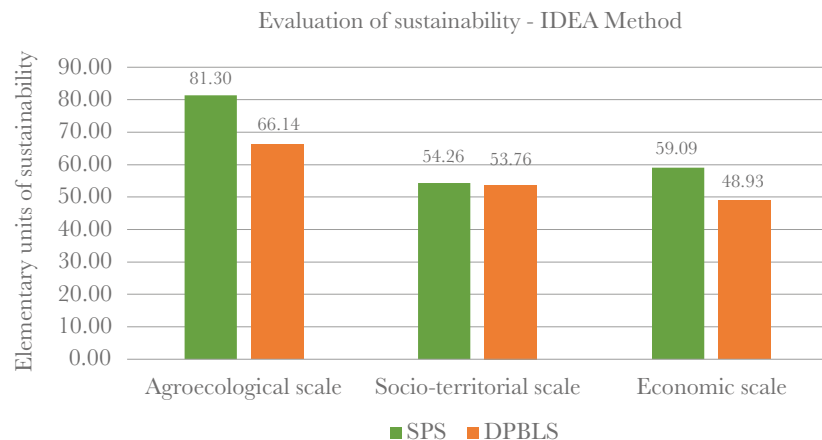


Figure 1. Determination of the sustainability scales using the IDEA Method.

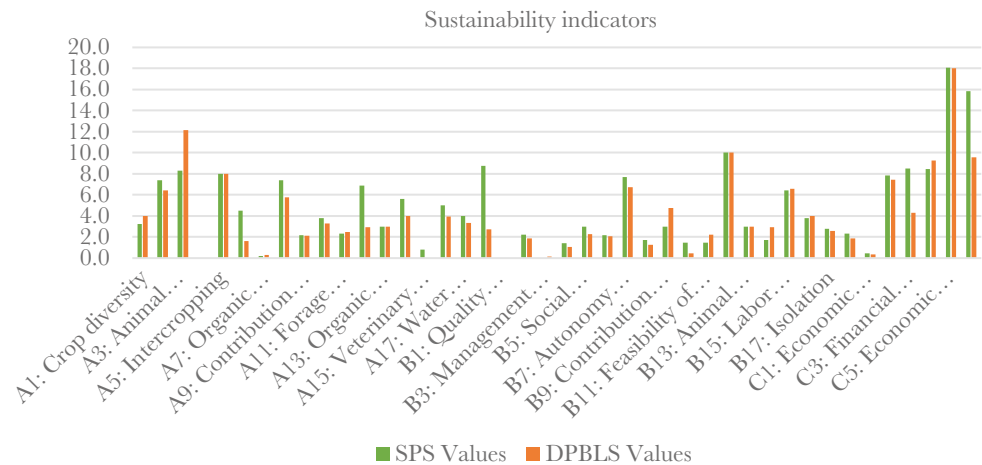


Figure 2. Homogeneity test by comparing variances of SPS and DPBLS.

higher educational level of producers, reflected in the indicator of training (B14: 2.9 *vs.* 1.7), and higher labor intensity (B15: 6.6 *vs.* 6.4), which evidences more active productive dynamics. However, both systems share weaknesses in quality processes (B1: 0) and in the management of non-organic residues (B3: ≤ 0.1).

The indicators that make up the economic scale (C1-C6), show that SPS present greater advantages thanks to their high financial autonomy (C3: 8.5 *vs.* 4.3), higher productive efficiency (C6: 15.8 *vs.* 9.6), and lower dependency on subsidies (C4: 8.4 *vs.* 9.3). For their part, DPBLS show a more marked economic specialization (C2: 7.4 *vs.* 7.8). When it comes to economic transmissibility, the indicators are practically equivalent (C5: 18.0 *vs.* 18.1), and so is the economic viability (C1: 0.4 in both cases), which suggests shared challenges in terms of profitability.

The analysis of relationships and interactions between components of sustainability in both productive systems is presented in Figure 3, which shows that the social, economic and environmental factors are interconnected and contribute in a differentiated manner to the sustainability of each system.

The comparison of components of sustainability indicates a strong relationship and interaction between both systems. However, there are slight trends: SPS have strengths in organizational and ecological aspects, and in the organization of spaces they present better distribution of the territory, making them more efficient and adaptable. In agricultural practices, SPS have higher scoring, revealing the use of more sustainable techniques, which is reflected in a greater efficiency of the system. Meanwhile, DPBLS stand out slightly in the component of diversity, which suggests a greater variety in crops, species and/or activities, as well as in socioeconomic aspects such as employment. Both face important challenges in efficiency, viability and transmissibility of the productive system towards the future, which must be addressed in policies for sustainable rural development.

The Shapiro-Wilk normality test (Table 1) shows that the values of the indicators in both systems do not follow a normal distribution ($p < 0.05$), which justifies the use of non-parametric statistical tests to evaluate differences between them.

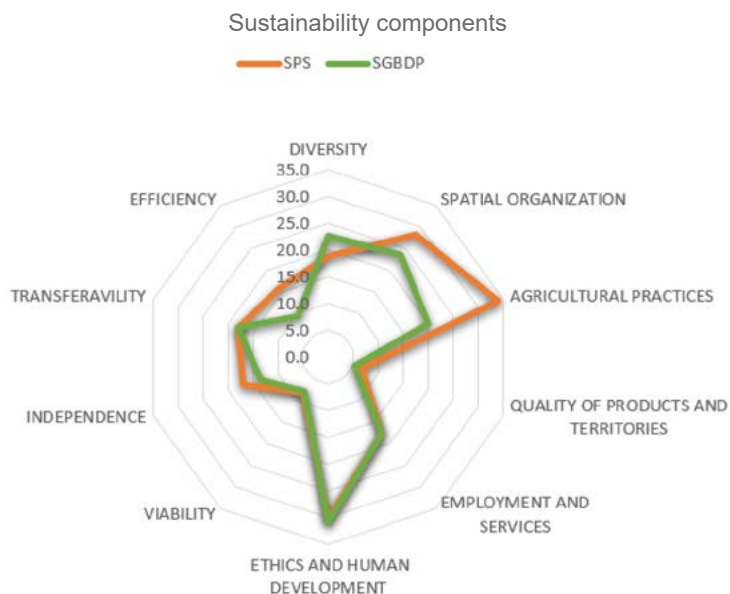


Figure 3. Relationships and interactions of the components that determine the sustainability of SPS and DPBLS.

Spearman’s correlation coefficient (Table 1) indicates a very strong positive relationship between the values of sustainability of SPS and DPBLS ($\rho=0.895$, $p<0.001$). This result confirms that both systems present similar trends in terms of their indicators of sustainability, which suggests that they share key factors that determine their performance.

On the other hand, the Mann-Whitney U test (Table 3) did not show statistically significant differences between the values of the indicators of sustainability of SPS and DPBLS ($p=0.485$). This implies that, although there are differences in the dominating scale of sustainability in each system, their global levels of sustainability are comparable.

Finally, the dispersion graph (Figure 4) shows the strong correlation observed between the sustainability values of both systems, represented by a line of positive tendency, which reinforces the consistency of the statistical findings.

Table 1. Shapiro-Wilk normality test.

	Statistics	Degrees of freedom	P-value
SPS Values	0.86	42	0.00
DPBLS Values	0.84	42	0.00

Table 2. Spearman correlation of SPS and DPBLS sustainability.

	ρ	p	N
SPS-DPBLS	.895**	.000	42

Table 3. Mann-Whitney U test.

	Statistics	p
System value	804	0.485

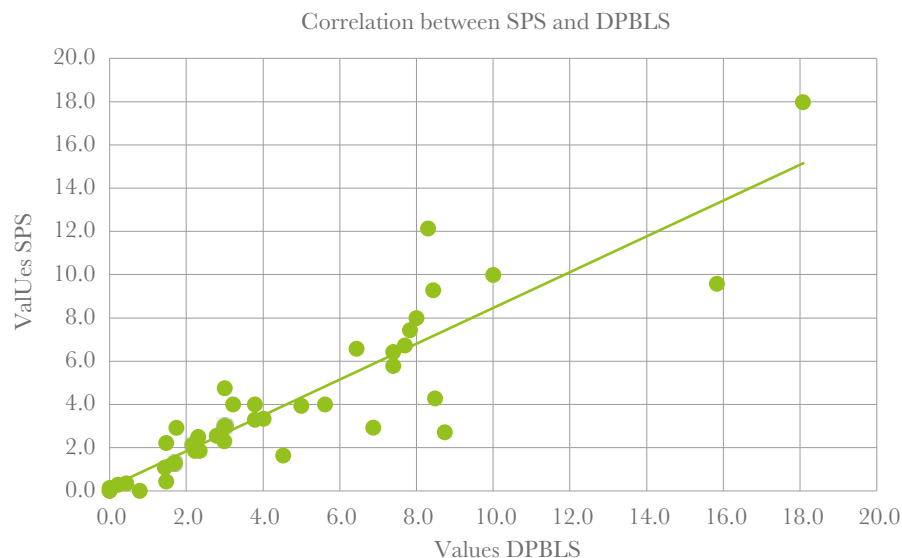


Figure 4. Results of the Correlation Significance Tests.

The results obtained indicate that SPS and DPBLS present a similar behavior in the aspects evaluated. However, the global valuation of these indicators indicates that both systems are still under the thresholds that characterize sustainable management (Otta *et al.*, 2016). This divergence is explained by the operative characteristics and the approaches of each productive system, which agrees with the observations from many authors (Reyes Pontet *et al.*, 2023; Gutiérrez Bermúdez and Medieta Araica, 2022; Haro-Altamirano *et al.*, 2022).

Based on the approach of the IDEA methodology, if the socio-territorial scale is defining for SPS, it reveals the need to strengthen processes such as the improvement in product quality, the conservation of territories, the generation of dignified employment, and the provision of community services. Likewise, human development and ethics emerge as fundamental pillars that must be prioritized in any strategy directed towards sustainability. In line with this perspective, Murgueitio *et al.* (2019) emphasize that sustainable systems should not only be productive, but also capable of generating ecosystemic services that contribute to social and environmental welfare. From this same conceptualization, Rozo *et al.* (2021) and Williams *et al.* (2020) emphasize that these systems contribute to the creation of biological corridors and to strengthening community resilience, essential aspects within the socio-territorial dimension. Chará *et al.* (2017) add that the integration of trees allows producers to diversify their income through the use of wood and non-wood products. These dynamics agree with the findings by Aguilar Pérez *et al.* (2019) and López-Vigoa *et al.* (2017), who highlight the importance of incorporating these systems in rural communities, both because of their contribution to conservation of ecosystems and for their role in sustainable territorial development.

In contrast with SPS, whose sustainability is closely linked to the socio-territorial dimension, in DPBLS the influence of the economic dimension predominates. In DPBLS, sustainability is determined to a greater extent by the economic scale, which evidences a

strong dependency on factors such as profitability and productive efficiency to ensure its viability (Cuevas-Reyes and Rosales-Nieto, 2018; Angón *et al.*, 2016).

While SPS prioritize holistic sustainability through agroecological synergies, DPBLS face the challenge of balancing profitability with environmentally responsible practices. Gallardo *et al.* (2019) and Murgueitio *et al.* (2015) agree that the integration of arboreal components could improve integral sustainability in livestock production systems, in addition contributing to environmental care by reducing the methane emissions by product unit by 20-30%.

SPS showed greater diversity of perennial crops and higher proportion of ecological regulation zones, compared to DPBLS, which reflects significant advantages in terms of biodiversity and environmental conservation. These results agree with what is suggested by Gutiérrez Cedillo *et al.* (2011), who emphasize the need for more effective integration between the agricultural, livestock and forestry subsystems as a pathway to strengthen the sustainability of productive systems.

The high correlation coefficient between both systems suggests the existence of common structural factors that impact their performance in terms of sustainability. This result backs the hypothesis that SPS and DPBLS can share intervention strategies directed at improving management of natural resources, optimizing access to markets, and fostering adoption of sustainable technologies (Zahm *et al.*, 2008). However, the absence of statistically significant differences between both models indicates that neither presents a clear comparative advantage in terms of integral sustainability, which can be related to the variability in management conditions, as well as differences in access to public policies and technical-productive support programs (Prospero Bernal, 2017; Torres-Lemus *et al.*, 2021).

The results obtained highlight the need to design integrated strategies directed at strengthening the different dimensions of sustainability in both productive systems. These strategies must focus on the optimization of the use of natural resources and the adoption of practices that reduce environmental impact without compromising economic profitability (Vilain *et al.*, 2008).

Likewise, it is recommended that future studies approach the evaluation of sustainability in the long term, incorporating emerging variables such as climate change and availability of water resources, since these factors could significantly influence the sustainable performance of the systems analyzed (Hamadi *et al.*, 2009), which will allow identifying territorial patterns of sustainability, as well as designing strategies of differentiated improvement based on specific technical, socioeconomic and ecological indicators. The implementation of systemic and participatory approaches in the formulation of appropriate technologies and support policies could be key to boost the existing synergies and to advance towards a more sustainable and resilient management of both productive systems.

CONCLUSIONS

The findings obtained reveal a high correlation between the SPS and DPBLS systems, which indicates the presence of common structural factors that impact their sustainability. This convergence suggests that both models can benefit from shared

strategies, focused on the efficient management of the resources, access to differentiated markets, and the adoption of sustainable technologies. However, the lack of statistically significant differences between the systems in terms of global sustainability reflects that neither presents a substantial comparative advantage, possibly due to the heterogeneity in the conditions of management, local practices, and availability of policies for productive support.

The high degree of correlation between the systems highlights the importance of implementing integrated strategies that allow improving productive efficiency without compromising environmental and social sustainability. The absence of significant differences in the levels of sustainability between both systems reinforces the idea that management practices can play a crucial role in their sustainable performance.

In this context, the need to study multi-scale and multi-dimensional approaches in more detail, which would allow to identify differentiated territorial dynamics and to direct more contextualized intervention strategies, stands out. The development of specific technical policies, sustained by socioeconomic, ecological and local governance indicators will be essential to promote a sustainable transition in both productive models and to contribute to strengthening their resilience in face of current environmental and economic challenges.

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Research topics and trends on the potential of *Ricinus communis* as biopesticide

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ABSTRACT

Objective: To analyze the scientific production published in journals included in the Science Citation Index Expanded (SCIE) and Social Sciences Citation Index (SSCI) databases of the Core Collection of the Web of Science (WoS), using bibliometric indicators, with the aim of determining the dynamics, quality, structure, and emergent topics on the subject of *Ricinus communis* L., during the 2001-2022 period.

Design/Methodology/Approach: The above-mentioned databases were subjected to an exploratory analysis to extract and evaluate bibliographical records, based on a search equation. The evolution of scientific production and the relationship between documents, authors, and key words were determined and characterized. In addition, relevant research topics and trends were identified. Finally, the VOSviewer software was used to develop one-dimensional (performance) and multidimensional (science mapping) indicators, based on records recovered from the databases.

Results: The results were used to determine the dynamics of scientific production regarding the topic in question. The topic had an oscillatory behavior as time went by, increasing from <5 documents in 2001 to 25 documents in 2022. This increase showed the interest of scientists on the subject. The highest number of publications was recorded in 2014.

Findings/Conclusions: The documents with the highest influence on the generation of new research addressed the use of *R. communis* as biocide, highlighting its harmlessness and environmental preservation. A similar behavior was reported with the use of *R. communis* extracts with larvicide, insecticide, acaricide, and antimicrobial activity. Regarding emerging research trends in *R. communis*, the most notable were *in vitro* studies, its association with nanotechnology; its antimicrobial, antioxidant, and acaricidal activity; and its use in combination with other plant species, as well as its use in metabolic or physiological studies.

Keywords: biopesticides, plant protection, bibliometric analysis, natural products.

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INTRODUCTION

Castor oil plant (*Ricinus communis* L.) is a fast-growing annual or perennial bush, 3 to 5 m tall (Kaur and Bhaskar, 2020; Chouhan *et al.*, 2021). It is a monotypic species from

family Euphorbiaceae. Its oily seeds are not edible. *R. communis* has a polyphyletic origin (Ranjitha *et al.*, 2019): its point of origin has been shrouded by its wide distribution (Muraguri *et al.*, 2020). Four points of origin have been proposed: 1) Eastern Africa (specifically Ethiopia); 2) Northeast Asia, Southeast Asia, and the Arabian Peninsula; 3) the Indian subcontinent; and 4) China (Rukhsar *et al.*, 2018). *R. communis* can thrive under diverse environmental conditions; therefore, it has spread towards temperate, subtropical, and tropical regions of the world (Landoni *et al.*, 2023). It can be found both in the wild and as crop, because it adapts to arid conditions and it can withstand extended droughts (Abel *et al.*, 2022).

The seeds of *R. communis* have a high lipid content, which is extracted to be used by the pharmaceutical and industrial sectors. Despite some harmful effects to the organism, it is widely used in traditional and Western medicine, given the wide impact of its numerous phytochemicals: antifertility, contraception, antinociception, anticarcinogenic, antioxidation, immunomodulation, hepatoprotection, antidiabetic, anti-ulcer, antimicrobial, insecticides, molluscicides, larvicides, bone regeneration, central analgesics, antihistamines, antiasthmatic, cytotoxins, lypolitic, anti-inflammatory, and scar formation (Kumar, 2017; Ergu, 2022). *R. communis* can also be applied for phytoremediation purposes, given its potential to extract metals (*e.g.*, Cd, Pb, Ni, As, and Cu) and pesticide residues. Therefore, it can be a good alternative for the remediation of polluted soils, increasing soil fertility and reducing erosion. It can also be used to produce biofuels, contributing to social development, the generation of local employment, carbon sequestration, and the reduction of greenhouse gases (Bauddh *et al.*, 2015).

Given the numerous harmful effects of synthetic pesticides on the environment and health, the interest on biopesticides as a natural alternative has increased in the last two decades; India and China lead the race to substitute synthetic pesticides with biopesticides (Smith *et al.*, 2021). This segment currently accounts for 5 to 6% of the world pesticide market (Marčić, 2019). Against this background, the use of biopesticides could increase crop production without compromising human health (Sharma *et al.*, 2020). Plant- or microorganism-based pesticides are not toxic for mammals and are biodegradable (Sharma *et al.*, 2020).

Several reviews have determined the state, development, and research trends of biopesticides (Smith *et al.*, 2021; Hernández-Tenorio *et al.*, 2022). *Azadirachta indica* has been the subject of various research works (Vijayakumar and Naqvi, 2002; Singh, 2016). However, as far as this research team was able to determine, no information is available about the scientific production and research trends regarding the use of *R. communis* to control pests and diseases. Therefore, the aim of this research was to identify and characterize the scientific production on the use of *R. communis* as biocide, included in the Science Citation Index Expanded (SCIE) and Social Sciences Citation Index (SSCI) databases of the Core Collection of the Web of Science (WoS) from Clarivate Analytics. Research topics and their trends were emphasized through a bibliometric analysis.

MATERIALS AND METHODS

Information sources and databases

A bibliometric study was carried out to obtain an overview of the scientific production and the research topics and trends regarding the use of *R. communis* to control pests and diseases from 2001 to 2022. Bibliometric indicators (BI) were used to collect bibliographical records of the indexed documents published in mainstream journals (Salager-Zeyer 2015), which can be found in the SCIE and SSCI databases.

Search protocols

The key terms or words were defined *a priori* by the research team and were brought together in the following search equation:

$$(TS = (\text{"ricinus communis*"} \text{ OR } \text{"castor plant"} \text{ OR } \text{"castor oil plant"}) \text{ AND } TS = (\text{pest * OR insect * OR fung * OR acari * OR larv * OR bacter *}))$$

These words and their search expression enabled the recovery of bibliographical records within the title, abstract, and keywords fields from the metadata of the bibliographical records. Additionally, the records were refined based on research articles and data articles. Finally, no language restrictions were considered appropriate for the search criteria. Consequently, 466 bibliographical records were recovered.

Data homogenization and normalization

The extracted records were subsequently subjected to a cleansing, analysis, and selection process to guarantee that the documents did deal with the research subject. Therefore, the records were checked; those who discussed the topic were marked and sent to a marked list within WoS. The final database of this study contained the information of each record consisting of 241 full bibliographical records (including citations). The records were exported to a .txt file, using August 11, 2023, as the final date.

Bibliometric indicators

The file with the extracted records was imported with VOSviewer (van Eck and Waltman, 2007). The said software was used to process the data and obtain the following bibliometric indicators: growth of scientific production, most cited articles, research topics, and research trends. The processing included the normalization of the key words allocated by the authors and the databases. The scientific production growth indicator was developed in Excel, importing the data from WoS, with the result analysis and years of publication options.

Analysis tool

The VOSviewer software, developed by van Eck and Waltman (2010), was used to analyze the records imported from WoS. The research topics and research trends indicators

that make up these networks were developed using the “analysis of co-occurrence” option (van Raan, 1993). The software identified the authors with more citations (globally and during the last five years).

RESULTS AND DISCUSSION

Growth of Scientific Production

Based on the results, the scientific production on the topic did not show a clear linear trend towards a sustained growth throughout time. Instead, interannual oscillations were recorded, although scientific production had a moderate linear growth of $R^2=0.561$ (Figure 1). The annual average of published documents during the study period was 10.95. Figure 1 also shows that the overall number of published documents increased from 5 (1991) to 25 (2021). Likewise, from 2001 to 2009, the average number of documents varied from 3 to 9 per year; however, from 2010 to 2014, the number of publications doubled from 11 to 22 documents, although it subsequently decreased. Finally, scientific production rebounded, reaching 16 and 19 documents in 2021 and 2022, respectively. The increasing trend from 2010 to 2014 was likely related to the worldwide boom of the species as a potential bioenergy source. Likewise, the behavior recorded during the past two years could be associated to the world trend towards green pesticides and the new agri-environmental milieu.

Most Cited Articles

The most cited articles in any area of science are a sign of the interest among academic peers, who believe that their content is important for their research and that the information included in them can back up their work. Table 1 shows the five most cited documents out of the 241 documents from the said period recovered for this study. “Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaeus indicus* juveniles” by Immanuel *et al.* (2004) was the most cited article (139 citations). In second place, “Suitability of different pollen

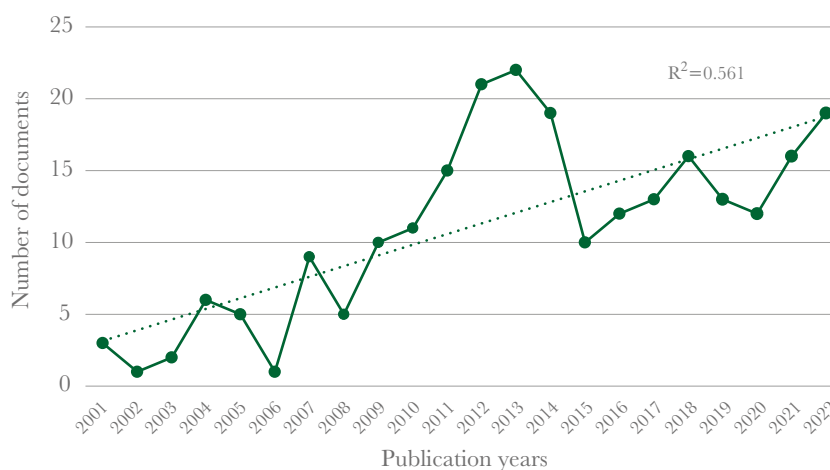


Figure 1. Increase in documents about the use of *R. communis* as a biocide, published in indexed journals included in the Science Citation Index Expanded and Social Sciences Citation Index (2001-2022).

Table 1. Five most cited articles about the use of *Ricinus communis* as biocide, published in indexed journals included in the Science Citation Index Expanded and Social Sciences Citation Index (2001-2022).

Position	Bibliographic reference	Received citations
1	Immanuel, G, Vincybai, VC, Sivaram, V, Palavesam, A, & Marian, MP (2004). "Effect of Butanolic Extracts from Terrestrial Herbs and Seaweeds on the Survival, Growth and Pathogen (<i>Vibrio parahaemolyticus</i>) Load on Shrimp <i>Penaeus indicus</i> Juveniles". <i>Aquaculture</i> , 236(1), 53-65, https://doi.org/10.1016/j.aquaculture.2003.11.033	139
2	Goleva, I, & Zebitz, CPW (2013). "Suitability of Different Pollen as Alternative Food for the Predatory Mite <i>Amblyseius swirskii</i> (Acari, Phytoseiidae)". <i>Experimental and Applied Acarology</i> , 61(3), 259-283 https://doi.org/10.1007/s10493-013-9700-z	99
3	Bigi, MFMA, Torkomian, VL, Groote, ST de, Hebling, MJA, Bueno, OC, Pagnocca, FC, Fernandes, JB, Vieira, PC, & Silva, MFGF da (2004). "Activity of <i>Ricinus communis</i> (Euphorbiaceae) and Ricinine Against the Leaf-cutting Ant <i>Atta sexdens rubropilosa</i> (Hymenoptera : Formicidae) and the Symbiotic Fungus <i>Leucoagaricus gongylophorus</i> ". <i>Pest Management Science</i> , 60(9), 933-938, https://doi.org/10.1002/ps.892	81
4	Luseba, D, Elgorashi, EE, Ntloedibe, DT, & Staden, J Van (2007). "Antibacterial, Anti-inflammatory and Mutagenic Effects of Some Medicinal Plants Used in South Africa for the Treatment of Wounds and Retained Placenta in Livestock". <i>South African Journal Of Botany</i> , 73(3), 378-383, https://doi.org/10.1016/j.sajb.2007.03.003	81
5	Upasani, SM, Kotkar, HM, Mendki, PS, & Maheshwari, VL (2003). "Partial Characterization and Insecticidal Properties of <i>Ricinus communis</i> L. Foliage Flavonoids". <i>Pest Management Science</i> , 59(12), 1349-1354, https://doi.org/10.1002/ps.767	74

as alternative food for the predatory mite *Amblyseius swirskii* (Acari, Phytoseiidae)" by Goleva and Zebitz (2013) recorded 99 citations. The five most cited articles discuss the use of *R. communis* for the management of several phytosanitary and water issues, given its harmlessness to health and the environment. Interestingly, two of the five most cited documents were published in the *Pest Management Science* journal, which likewise was one of the publications with the highest number of cited documents on the topic.

Table 1 also highlights that most of the articles are over 10 years old and that there is a lack of recent articles with high impact or visibility. Therefore, the articles with most citations from 2018 to 2022 were analyzed to identify current research trends on the topic (Table 2). In conclusion, research trends were focused on the combination of *R. communis* with silver, zinc, and magnesium nanoparticles to improve its antimicrobial potential and cytotoxicity. This trend matches the current demand for the reduction of synthesized insecticides and the search for new pesticides with high selectivity and low impact on the environment and agriculture. In this sense, nano-based pesticide formulations have several advantages over conventional pesticide formulations, including a greater environmental stability, controlled release of active ingredients, higher permeability, focused administration, etc. (Manna *et al.*, 2023). Nanotechnology is a new approach that improves the characteristics of insecticides, through the nanomanufacturing of particles. There are several ways in which these

Table 2. Most cited documents about the use of *Ricinus communis* as biocide published from 2018 to 2022 in indexed journals included in Science Citation Index Expanded and Social Sciences Citation Index (2001-2022).

Year	Bibliographic references	Received citations
2022	Ghaffar, N, Javad, S, Farrukh, MA, Shah, AA, Gatashah, MK, Al-Munqedhi, BMA, & Chaudhry, O (2022). "Metal Nanoparticles Assisted Revival of Streptomycin against MDRS <i>Staphylococcus aureus</i> ". <i>Plos One</i> , 17(3), https://doi.org/10.1371/journal.pone.0264588	10
2021	Gul, A, Fozia, Shaheen, A, Ahmad, I, Khattak, B, Ahmad, M, Ullah, R, Bari, A, Ali, SS, Alobaid, A, Asmari, MM, & Mahmood, HM (2021). "Green Synthesis, Characterization, Enzyme Inhibition, Antimicrobial Potential, and Cytotoxic Activity of Plant Mediated Silver Nanoparticle Using <i>Ricinus communis</i> Leaf and Root Extracts". <i>Biomolecules</i> , 11(2), https://doi.org/10.3390/biom11020206	34
2020	Dias, LP, Souza, PFN, Oliveir, JTA, Vasconcelos, IM, Araujo, NMS, Tilburg, MFV, Guedes, MIF, Carneiro, RF, Lopes, JLS, & Sousa, DOB (2020). "RcAlb-PepII, a Synthetic Small Peptide -bioinspired in the 2S Albumin from the Seed Cake of <i>Ricinus communis</i> , is a Potent Antimicrobial Agent against <i>Klebsiella pneumoniae</i> and <i>Candida parapsilosis</i> ". <i>Biochimica et Biophysica Acta-Biomembranes</i> , 1862(2), https://doi.org/10.1016/j.bbamem.2019.183092	31
2019	Panchal, P, Paul, DR, Sharma, A, Hooda, D, Yadav, R, Meena, P, & Nehra, SP (2019). "Phytoextract Mediated ZnO/MgO Nanocomposites for Photocatalytic and Antibacterial Activities". <i>Journal of Photochemistry and Photobiology A-Chemistry</i> , 385, https://doi.org/10.1016/j.jphotochem.2019.112049	78
	Shobha, N, Nanda, N, Giresha, AS, Manjappa, P, Sophiya, P, Dharmappa, KK, & Nagabhushana, BM (2019). "Synthesis and Characterization of Zinc Oxide Nanoparticles Utilizing Seed Source of <i>Ricinus communis</i> and Study of its Antioxidant, Antifungal and Anticancer Activity". <i>Materials Science And Engineering C-Materials for Biological Applications</i> , 97, 842-850, https://doi.org/10.1016/j.msec.2018.12.023	52
2018	Salem, N, Bachrouch, O, Sriti, J, Msaada, K, Khammassi, S, Hammami, M, Selmi, S, Boushah, E, Koorani, S, Abderraba, M, Marzouk, B, Limam, F, & Jemaa, JM Ben (2018). "Fumigant and Repellent Potentials of <i>Ricinus communis</i> and <i>Mentha pulegium</i> Essential Oils against <i>Tribolium castaneum</i> and <i>Lasioderma serricorne</i> ". <i>International Journal of Food Properties</i> , 20:sup3, S2899-S2913, https://doi.org/10.1080/10942912.2017.1382508	26
	Jiang, XY, Xie, Y, Ren, ZF, Ganeteg, U, Lin, F, Zhao, C, & Xu, HH (2018). "Design of a New Glutamine-Fipronil Conjugate with Alpha-Amino Acid Function and Its Uptake by <i>A-thaliana</i> Lysine Histidine Transporter 1 (AtLHT1)". <i>Journal of Agricultural and Food Chemistry</i> , 66(29), 7597-7605, https://doi.org/10.1021/acs.jafc.8b02287	24

particles can be encapsulated, including nanocapsules, nanospheres, nanogels, micelles, liposomes, etc. Overall, nanoinsecticides were more lethal and their residual activity lasted longer than commercial formulations. They can also alter the levels of several enzymes and hormones and delay the development of insects (Graily-Moradi and Asgari-Lajayer, 2021). Despite their advantages, recent research show that several nanoparticles included in the formulation of conventional nanopesticides can be toxic for crops and beneficial organisms, as a consequence of bioaccumulation and trophic transfer. Therefore, Manna *et al.* (2023) do not believe that traditional nanopesticides are advantageous for "green agriculture" (Manna *et al.*, 2023). Consequently, environmentally-friendly green nanotechnology (*e.g.*,

Gul *et al.*, 2021). Meanwhile, Cluster 3 (blue) covered physiological aspects such as: phloem mobility (Yang *et al.*, 2011; Yao *et al.*, 2017), biosynthesis, translocation, and insecticide (Baldin *et al.*, 2007), fungicide (Chollet *et al.*, 2005), and acaricide (Ghosh *et al.*, 2013) activity. Cluster 4 (yellow) accounted for the use of *R. communis* as a biological control agent (*i.e.*, a trap crop that captures phytophagous insects) (Galvao *et al.*, 2008); likewise, it can be used as an outstanding host of predators of pests that attack other crops (Miranda *et al.*, 2021). Finally, Cluster 5 (purple) includes the alternative use of *R. communis* to break the resistance to conventional insecticides, mainly among the greenhouse whitefly (*Trialeurodes vaporariorum*) (Ladys García *et al.*, 2014) and the silverleaf white fly (*Bemisia tabaco*) (Kurra and Pathipati, 2015), and to control fungi (Dikhoba *et al.*, 2019), bacteria (Londoño-Orozco *et al.*, 2010), and nematodes (Tiyagi *et al.*, 2012). According to the in-depth analysis of the scientific production, there is scarce information about the residuality, phytotoxicity, effect on non-target organisms and beneficial fauna of the various presentations (*e.g.*, extracts, essential oils). Such information would be essential to consider its massive use.

Research trends

The trends in the scientific production from 2012 to 2022 about the use of *R. communis* as biocide were addressed from various analytical perspectives. Figure 3 shows the dynamics of the major topic patterns developed by the world research nuclei. The main applications identified were classified, according to their temporality, in three main research areas and are synthesized below:

1. **Purple-blue nodes:** they include studies about its use against insects that are a public health concern—such as *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*—, as well as its use against mites (*Tetranychus urticae*) and nematodes. Emergent studies evaluate their components, toxicity, translocation, and degradation.
2. **Blue-green nodes:** they discuss the combination of *R. communis* with other plant species, such as *Jatropha curcas* and *Azadiracta indica*, to increase its efficiency. Plants and leaves are studied to determine their insecticide and antimicrobial (fungi and bacteria) activity, as well as its use as biocontrol agent.
3. **Green-yellow nodes:** these nodes show the current research trends or the emergent areas of research. Outstanding research work has been made on nanoparticles (Ghaffar *et al.*, 2022) and green synthesis (Gul *et al.*, 2022); *in vitro* studies (Singh *et al.*, 2022); and its antimicrobial (mainly against bacteria) (Gul *et al.*, 2021; Matysiak *et al.*, 2018), antioxidant (El-Sayed *et al.*, 2022), and acaricide (Singh *et al.*, 2022) activity. Other remarkable studies have analyzed its combination with entomopathogenic fungi (Barbosa *et al.*, 2021) and widen their application on the spectrum of insects with economic importance, such as the tobacco cutworm (*Spodoptera litura*) (Vengates *et al.*, 2022). Finally, there has been a permanent and renewed interest in metabolic or physiological studies, mainly aimed at their mobility within the plant and amino acid transport (Li *et al.*, 2021).

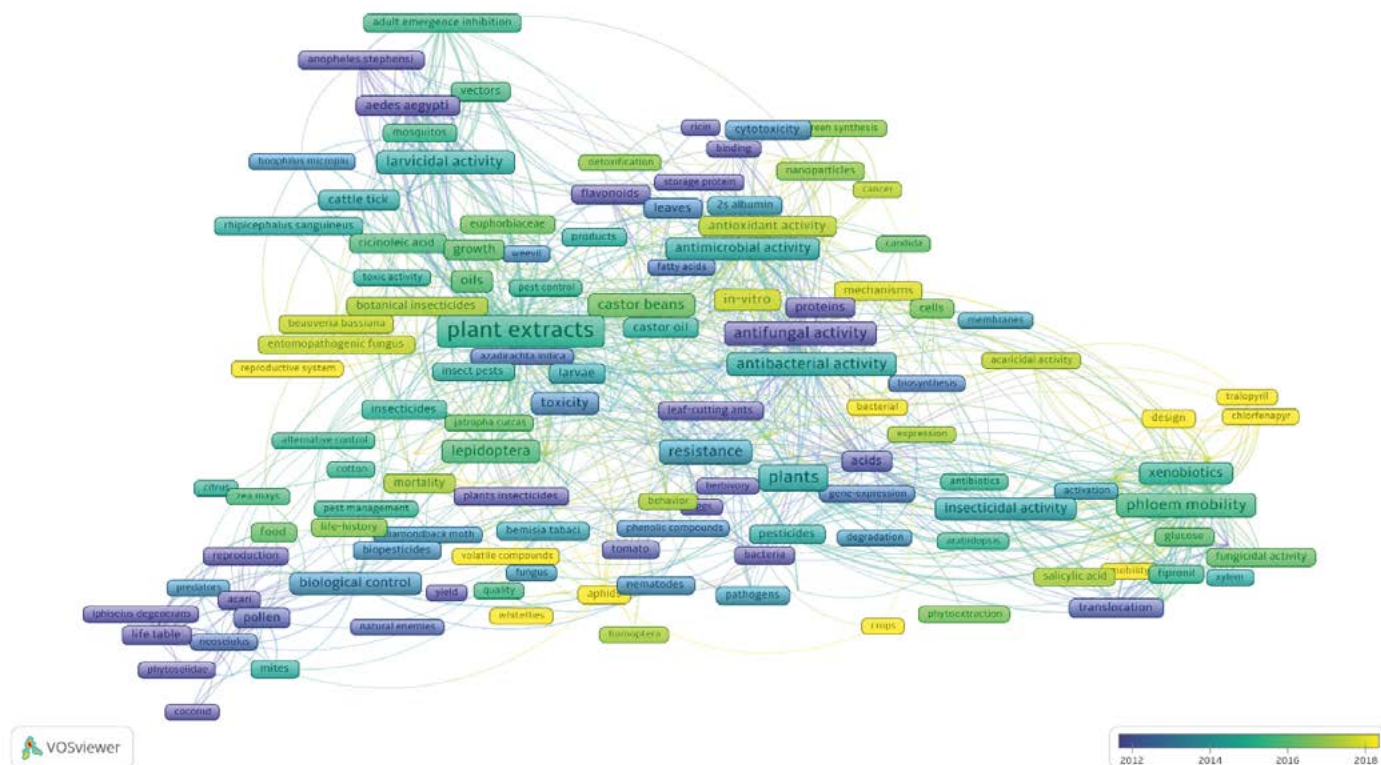


Figure 3. Temporality of research, developed in VOSviewer, of the scientific production about the use of *Ricinus communis* as biocide, published in indexed journals included in Science Citation Index Expanded and Social Sciences Citation Index (2012-2022).

CONCLUSIONS

The scientific production about the use of *Ricinus communis* as biocide is an expanding and emergent study area. Although production is currently low, it has shown an oscillatory yet growing trend during the past two decades.

The most influential documents (per number of citations) discuss the use of *R. communis* in the management of various phytosanitary and water issues, given its harmlessness to health and the environment.

The research topic focused on the use of *R. communis* as a plant extract with larvicide, insecticide, acaricide, and antimicrobial activity. Finally, the following emergent research trends stand out: in vitro studies, its association with nanotechnology, its antimicrobial, antioxidant, and acaricide activity, and its combination with other plant species and entomopathogenic fungi, as well as metabolic or physiological studies about mobility within the plant and amino acid transport.

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Humic substances improve intestinal mucosal integrity in broiler chickens fed two different diets and challenged with a lipopolysaccharide

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ABSTRACT

Objective: To evaluate tibial measurements, microscopic alterations, and goblet cell (GC) counts in the jejunal villi, as well as serum liver biochemical profiles, interleukin responses, and jejunal integrity indices in broilers supplemented with humic substances (HS) derived from vermicompost, subjected to dietary formulation changes and challenged with lipopolysaccharides.

Design/Methodology/Approach: From 7 to 21 days of age, broilers were assigned to four treatments in a factorial arrangement involving two growth promoters AGP: 0.05% bacitracin methylene disalicylate (BMD) and 0.05% nicarbazin; or HS: 0.33% HS and two feeding systems: Continuous diet, consisting of Diet A (corn-soybean meal-based) offered continuously; or Alternating diets, with Diet A alternated every two days with Diet B (sorghum, soybean meal, and canola meal-based). Data were analyzed using analysis of variance.

Results: Broilers receiving HS exhibited significantly higher productivity parameters ($P < 0.05$), greater tibial dimensions, more pronounced microscopic alterations, and higher GC counts in the jejunal villi compared to the AGP group, irrespective of the feeding system. Additionally, HS supplementation upregulated mucin-2 and occludin gene expression in the jejunum, whereas claudin-1 (CLDN-1) and immunoglobulin A (IgA) levels remained unaffected ($P > 0.05$).

Limitations/Implications: Limitations include the small sample size, absence of replication under commercial production conditions, and assessment of only a single HS dose over a short experimental period.

Findings/Conclusions: HS supplementation enhanced the mucoprotective barrier of the jejunal epithelium.

Keywords: Broiler chickens; Humic substances; Goblet cells; Gene expression.



INTRODUCTION

Humic substances (HS) derived from leonardite and lignite have been investigated for decades, with well-documented beneficial effects in the prevention and control of various medical conditions in both animals and humans (Peña-Méndez *et al.*, 2005; Angeles *et al.*, 2022a; EMEA, 1999). HS are primarily composed of humic acids (HA), fulvic acids (FA), and humins, formed through a complex biotransformation of organic matter (Domínguez-Negrete *et al.*, 2021; Maguey-González *et al.*, 2022). The use of HS from vermicompost produced using domestic animal manures as substrate represents a novel, environmentally friendly alternative that is gaining relevance as a potential replacement for antibiotic growth promoters (AGP) in poultry production, particularly in broiler chickens (Angeles *et al.*, 2022a; Maguey-González *et al.*, 2022; Arif *et al.*, 2019). According to the FAO (2024), more than 60 countries have banned or severely restricted AGP use in poultry, and it is estimated that approximately 55% of global poultry production is transitioning toward alternative growth enhancers, such as prebiotics, probiotics, and organic acids. Recent research has demonstrated that HS exert their effects through multiple mechanisms, including metal ion chelation, toxin adsorption, antioxidant activity, and modulation of the gut microbiota (Arif *et al.*, 2019; Xu *et al.*, 2023). These mechanisms contribute to the maintenance of intestinal homeostasis, optimization of nutrient absorption, and regulation of immune balance. Several studies have compared the physiological effects of HS with those of probiotics and prebiotics. For example, Gadde *et al.* (2017) reported that direct-fed microbials (DFM) enhanced the expression of tight junction genes (OCLN, CLDN) and reduced intestinal permeability findings consistent with those observed following HS supplementation (Tang *et al.*, 2023; Mudroňová *et al.*, 2021). Additionally, HS have been shown to modulate gut microbial communities, promoting beneficial bacteria and suppressing pathogenic populations (Wang *et al.*, 2020). It has been proposed that HS form protective layers over the digestive mucosa, limiting the translocation of harmful substances and pathogenic bacteria into systemic circulation (Angeles *et al.*, 2022a; Kühnert *et al.*, 1991). This protective action may involve interactions with epithelial glycoproteins and glycoconjugates, leading to reinforcement of the mucus layer and modulation of signaling pathways such as NF- κ B, which plays a key role in intestinal inflammation (Xu *et al.*, 2023; Salvo-Romero *et al.*, 2015). Moreover, HS have been shown to upregulate the expression of mucin-secreting glycoproteins (*e.g.*, MUC-2) and tight junction-associated genes (*e.g.*, OCLN), both essential for maintaining intestinal structural integrity and functionality (Mudroňová *et al.*, 2021; Tang *et al.*, 2023). In a recent study evaluating goblet cell (GC) counts in the intestinal villi of broilers subjected to abrupt dietary changes, the inclusion of vermicompost-derived HS mitigated the reduction in GC numbers compared to AGP supplementation. Following a second dietary change, GC numbers decreased again, but the decline was less pronounced in HS-fed broilers compared to the initial dietary shift (López-García *et al.*, 2023). These findings suggest a mucoprotective role of HS in the gastrointestinal tract. Under commercial production conditions, alterations in feed ingredient composition are often associated with impaired intestinal function and inflammatory responses of the mucosa (Kuttappan *et al.*, 2015; Tellez *et al.*, 2014), particularly when incorporating ingredients with anti-nutritional factors such as sorghum

and canola meal. The inclusion of these components may negatively impact poultry productivity due to reduced digestibility and nutrient assimilation (Aider & Barbana, 2011; Ambula *et al.*, 2001; Cheng *et al.*, 2022). Consequently, the present study was designed to induce a dietary challenge in order to confirm the mucosal protective effects of HS. This was achieved by alternating a sorghum-canola meal-based diet with a standard corn-soybean meal diet every two days, coupled with an intraperitoneal injection of *Escherichia coli* capsular lipopolysaccharide (LPS) to elicit a more robust immune response both locally in the gut and systemically (Chen *et al.*, 2018). Therefore, the aim of this study was to assess tibial measurements, microscopic alterations, and GC counts in the jejunal villi, alongside serum liver biochemical parameters, interleukin responses, and jejunal integrity indices in broilers supplemented with vermicompost-derived HS, subjected to alternating dietary formulations and challenged with LPS. We hypothesized that dietary inclusion of 0.33% HS would enhance villus length and upregulate tight junction gene expression (OCLN, CLDN-1), while reducing intestinal permeability (measured via FITC-d) under LPS challenge, compared to conventional AGP supplementation.

MATERIALS AND METHODS

Ethical statement

This study was reviewed and approved by the Institutional Subcommittee for the Care and Use of Experimental Animals of the Universidad Nacional Autónoma de México (Protocol No. SICUAE.DC-2021/2-6).

Extraction of humic substances

The HS was extracted from vermicompost produced with sheep manure through alkaline extraction followed by acid precipitation, as described by Domínguez-Negrete *et al.* (2019). In previous studies, its composition and aromaticity were characterized. Briefly, the physicochemical analysis reported by Domínguez-Negrete *et al.* (2021) indicated a pH of 7.8, an ash content of 23.2%, and a humic acid to fulvic acid (HA:FA) ratio of 1.59 (47.1% HA and 29.6% FA). The cation exchange capacity (CEC) was 79.4 cmol(+)/kg. UV-Vis spectra exhibited a λ_{max} at 260 nm and an E4/E6 ratio of 4.3, while FT-IR analysis revealed characteristic absorption bands at 3400, 1715, 1600, and 1230 cm^{-1} (Peña-Méndez *et al.*, 2005; Maguey-González *et al.*, 2023a). The estimated aromaticity was 53.8% (Domínguez-Negrete *et al.*, 2019; Agregado-Palechor *et al.*, 2023).

Experimental design: Animals, treatments and management

The experimental design employed a 2×2 factorial arrangement, incorporating two growth promoters (AGP or HS) and two feeding systems (continuous or alternating). Forty male Ross 308 broiler chickens, seven days of age, with homogeneous initial body weights (mean \pm SD: 130.4 \pm 2.1 g), were randomly assigned to treatments using an initial weight blocking strategy to minimize variability. Each treatment consisted of 10 birds housed individually (n=10). Figure 1 presents a flowchart detailing treatment allocation, experimental phases, and the number of birds at each stage.

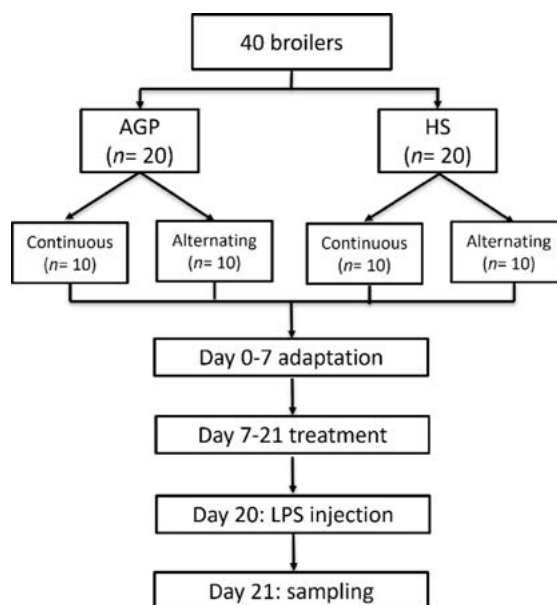


Figure 1. Flowchart of the experimental design and sampling schedule. Forty Ross 308 broiler chickens (7 days old) were assigned to a 2×2 factorial design comprising two growth promoters antibiotic growth promoter (AGP) and humic substances (HS) and two feeding systems (continuous or alternating). All birds underwent an adaptation phase (days 0-7), a treatment phase (days 7-21), a lipopolysaccharide challenge (day 20), and final sampling (day 21). The HS dosage was determined based on results from a previous study (Domínguez-Negrete *et al.*, 2021). In the alternating feeding system, diets A and B were each provided on four separate occasions.

The treatments comprised two types of growth promoters: AGP (0.05% bacitracin methylene disalicylate [BMD] and 0.05% nicarbazin) or HS (0.33% humic substances), and two feeding systems: a continuous diet (Diet A, based on corn and soybean meal, offered without interruption) or alternating diets (Diet A alternated every two days with Diet B, based on sorghum, soybean meal, and canola meal). The composition of the experimental diets is presented in Table 1. Feed and drinking water were provided *ad libitum* throughout the study.

On day 20 of age, all broilers received an intraperitoneal injection of *Escherichia coli* lipopolysaccharide (LPS), serotype O55:B5 (L4005, Sigma-Aldrich Co., St. Louis, MO, USA), at a dose of 3 mg/kg body weight, a route chosen for its effectiveness in eliciting a consistent systemic inflammatory response in poultry (Chen *et al.*, 2018). On day 21, all birds were euthanized by cervical dislocation for sample collection.

Sampling and laboratory determinations

After slaughter, the left leg of each bird was sectioned, and skin, fat, and muscle were removed. Tibias were weighed before and after drying in a forced-air oven at 100 °C for 24 h to determine dry matter (DM) content. Bones were subsequently incinerated in a muffle furnace at 600 °C for 6 h to determine ash content. Both DM and ash values were expressed as percentages and in grams (Angeles *et al.*, 2022).

A 5 cm segment was collected from the mid-jejunum of each bird. Tissues were cut longitudinally, rinsed with saline solution, and fixed onto cork boards by pinning at both

Table 1. Experimental diets.

	Diet A	Diet B
Ground corn	53.56	0.00
Ground sorghum	0.00	54.87
Soybean meal	37.10	31.34
Canola meal	0.00	4.00
Vegetable oil	4.69	5.06
Calcium orthophosphate	1.75	1.73
Calcium carbonate	1.60	1.53
Sodium bicarbonate	0.36	0.43
Salt	0.19	0.11
Methionine	0.23	0.27
Lysine	0.11	0.23
Threonine	0.00	0.04
Choline chloride	0.10	0.10
Vitamin and mineral premix ¹	0.20	0.20
Coccidiostat ²	0.05	0.05
Antibiotic ³	0.05	0.05
Calculated nutrient content		
Metabolizable energy, kcal kg ⁻¹	3.05	3.05
Crude protein, %	22.67	21.81
Digestible Lys, %	1.21	1.21
Digestible Met, %	0.55	0.56
Digestible Thr, %	0.77	0.77
Ca, %	1.00	1.00
Available P, %	0.50	0.50
Na, %	0.21	0.21
Cl, %	0.17	0.17

¹ Each kg provided: 6,500 IU vitamin A; 2,000 IU vitamin D; 15 IU vitamin E; 1.5 mg vitamin K; 1.5 mg thiamine; 5 mg riboflavin; 35 mg niacin; 3.5 mg pyridoxine; 10 mg pantothenic acid; 1,500 mg choline; 0.6 mg folic acid; 0.15 mg biotin; 0.15 mg vitamin B₁₂; 100 mg Mn; 100 mg Zn; 50 mg Fe; 10 mg Cu; and 1.0 mg I.; ² Nicarbazin; ³ Bacitracin methylene disalicylate.

ends, with the serosal surface in contact with the cork and the mucosal surface exposed, preserving its natural shape for subsequent villus morphometric analysis. Samples were fixed for 24 h in 10% buffered formalin, rinsed with distilled water and phosphate-buffered saline, and processed using an automated tissue processor (STP 120, Microm, Tarragona, Spain) with ethanol in ascending concentrations (75%, 90%, and 100%, 6 h each), followed by xylene (6 h), and paraffin embedding at 60 °C for 6 h. Paraffin blocks were sectioned at 5 μm using a rotary microtome (Microm HM 335 E, Germany). Sections were mounted on slides and independently stained as follows: (A) hematoxylin and eosin for villus morphometry (height, thickness) and crypt depth; (B) periodic acid-Schiff for

quantification of neutral goblet cells (GC); (C) Alcian blue at pH 1.0 for strongly sulfated acidic GC; and (D) Alcian blue at pH 2.5 for non-sulfated acidic GC (López-García *et al.*, 2023). The intestinal villus area was calculated using the formula:

$$(2\pi) \times (\text{thickness} / 2) \times (\text{height})$$

The villus height-to-crypt depth ratio was also calculated. Measurements were taken from 10 villi and 10 crypts per bird for each treatment using an inverted microscope (DMi8, Leica, Germany) at 50x magnification, equipped with a digital color camera (MC170 HD, Leica, Germany). Images were analyzed with Leica LAS Interactive Measurement Software. Goblet cell (GC) quantification was performed by longitudinally sectioning each villus into three equidistant regions (basal, medial, and apical). Within each region, the number of cells reactive to the respective stain was recorded. For each staining method, GC counts were obtained for each region and for the total villus. Counts were based on 10 villi per bird, analyzed at 200x magnification using an inverted microscope (DMi8, Leica, Germany) with a digital color camera (MC170 HD, Leica, Germany). Images were processed using Leica LAS Interactive Measurement Software. Blood samples were collected and centrifuged at 4 °C for 15 min at 2,500 rpm, and the resulting serum was stored at –80 °C. Serum biochemical parameters including urea (41043, SPINREACT, S.A./S.A.U., Santa Coloma, Spain), alkaline phosphatase (ALP; 41242, SPINREACT), alanine aminotransferase (ALT; 41283, SPINREACT), and aspartate aminotransferase (AST; 41272, SPINREACT) were quantified using a UV-Vis spectrophotometer (GENESYS 10S UV-Vis, Thermo Scientific, USA) following the protocols of the respective commercial kits. Additional blood samples were collected to determine serum concentrations of proinflammatory cytokines interleukin 1 (IL-1), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF- α) as well as mucin-2 (MUC-2). Quantification was performed by ELISA using commercial kits (IL-1: MBS261118; IL-6: MBS268769; TNF- : MBS2509660; MUC-2: MBS017935; MyBiosource, Inc., San Diego, CA, USA) according to the manufacturers' protocols, and absorbance was read at 450 nm on a microplate reader. On day 21, fluorescein isothiocyanate-dextran (FITC-d, MW 3-5 kDa; 102239527, Sigma-Aldrich Co., St. Louis, MO, USA) was orally administered at 8.32 mg/kg body weight (1 mL/bird). One hour later, blood was collected following the recommendations of Kuttappan *et al.* (2015), centrifuged at 4 °C for 15 min at 2,500 rpm, and serum was stored at –80 °C for subsequent FITC-d analysis. Jejunal samples were collected immediately after slaughter, frozen in liquid nitrogen, and stored at –80 °C. Total RNA was extracted from 20 mg of tissue using the Aurum™ Total RNA Mini Kit (7326820, Bio-Rad, Hercules, CA, USA). RNA concentration was determined using 1 μ L of sample on a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, USA), and RNA integrity was confirmed by 2% agarose gel electrophoresis. cDNA synthesis was performed with the iScript™ Advanced cDNA Synthesis Kit (1725038, Bio-Rad) following the manufacturer's instructions, using reverse transcription at 46 °C for 20 min and enzyme inactivation at 95 °C for 1 min in a thermocycler (CFX96, Bio-Rad, USA). cDNA was quantified and stored at –20 °C. Expression of mature mRNA was measured by quantitative real-time PCR (qRT-PCR)

using EvaGreen Supermix (1864034, Bio-Rad, Hercules, CA, USA). Primer sequences are listed in Table 2. Specificity was verified using the NCBI BLAST tool to ensure no cross-reactivity with unrelated sequences. Primers were synthesized, HPLC-purified, and reconstituted according to manufacturer instructions (Alpha DNA, Canada). Target gene expression was normalized to the expression level of chicken 28S ribosomal RNA (endogenous control).

The qRT-PCR reactions were performed in a total volume of 20 μL , each containing 1,600 ng of cDNA. Amplification was carried out in a thermocycler (CFX96, Bio-Rad, USA) under the following conditions: one cycle of enzyme activation at 95 °C for 5 min; 40 denaturation cycles at 95 °C for 30 s, with annealing and extension temperatures specific to each gene ribosomal RNA gene 28S, occludin (OCLN), and mucin-2 (MUC-2) at 60 °C; claudin-1 (CLDN-1) at 65 °C; and immunoglobulin alpha heavy chain (IgA) at 66 °C followed by one cycle at 4 °C for 5 min for signal stabilization and one cycle at 90 °C for 5 min. Each assay was conducted in duplicate. Absolute mRNA quantification was achieved by generating a linear calibration curve through serial dilutions of the corresponding gene amplicons, ranging from 1×10^{11} to 1×10^3 molecules/ μL . Relative gene expression was calculated using the $2^{-\Delta\text{Cq}}$ method.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) in a completely randomized design with a 2×2 factorial arrangement, considering two growth promoters (AGP or HS) and two feeding systems (continuous or alternating diets), applying the General Linear Model procedure of the SAS software package (SAS, 1990). Each treatment included 10 replicates (one bird per cage). For morphometric measurements (villus height, thickness, and area; crypt depth; and villus height-to-crypt depth ratio) and GC counts (neutral, sulfated, and non-sulfated), 10 complete villi per bird were evaluated. Serum liver biochemical indicators, interleukins, MUC-2, and FITC-d concentrations were determined using five replicates per treatment. For IgA, MUC-2, CLDN-1, and

Table 2. Chicken (*Gallus gallus*) gene-specific primers used for RT-qPCR.

Gene name	Accession number	Oligonucleotide sequence (5 -3)	Direction	Length pb	Author
Mucin 2	NM_001318434	ATTGAAGCCAGCAATGGTGT	F	214	(Holleman <i>et al.</i> , 2020)
		TGACATCAGGGCACACAGAT	R		
Claudin 1	NM_001013611·2	CATACTCCTGGGTCTGGTTGGT	F	100	(Zhang <i>et al.</i> , 2017)
		GACAGCCATCCGCATCTTCT	R		
Occludin	NM-205128·1	ACGGCAGCACCTACCTCAA	F	123	(Zhang <i>et al.</i> , 2017)
		GGCGAAGAAGCAGATGAG	R		
Immunoglobulin alpha heavy chain	S40610	GTCACCGTCACCTGGACTACA	F	192	(Lammers <i>et al.</i> , 2010)
		ACCGATGGTCTCCTTCACATC	R		
28S ribosomal ARN gene	DQ018756	GCGAAGCCAGAGAAACT	F	62	(Lammers <i>et al.</i> , 2010)
		GACGACCGATTTGCACGTC	R		

OCLN gene expression analyses, jejunal samples from two birds were pooled, with five replicates per treatment. Data were tested for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. All variables met parametric assumptions ($P > 0.05$). Significant differences among means were identified using Duncan's multiple range test at $P < 0.05$. Least squares means and standard errors of the mean are presented in the results tables.

RESULTS AND DISCUSSION

Growth performance and tibia measurements

Growth performance from 7 to 21 days of age and tibia measurements at 21 days are summarized in Table 3. Final body weight, weight gain (WG), and feed intake (FI) were significantly higher ($P < 0.01$) in broilers supplemented with HS compared to those receiving AGP. Additionally, FI was greater ($P < 0.05$) in broilers under the alternating feeding system compared to continuous feeding; however, the remaining productive variables did not differ between feeding systems. Table 3 also presents tibia dry matter and ash content at 21 days. No interaction was observed between growth promoter type and feeding system for tibia parameters ($P > 0.05$). Growth promoter supplementation influenced tibia composition, with HS-fed broilers showing higher ($P < 0.01$) tibia dry matter and ash weights compared to AGP-fed birds. The feeding system had no significant effect on tibia variables.

Concentration of biochemical markers in serum

Table 4 presents the serum concentrations of biochemical markers, interleukins, MUC-2, and FITC-d. No significant effects of growth promoter type or feeding system were observed for ALT, AST, ALP, or urea levels, nor for IL-1, IL-6, or TNF- concentrations

Table 3. Productive performance from 7 to 21 days of age and tibia dry matter and ashes content.

Item ^a	Growth promoter				Feeding system			
	AGP	HS	SEM	P <	Continuous	Alternating	SEM	P <
Productive performance								
IBW, kg	0.13	0.13	0.001	0.6843	0.13	0.13	0.001	0.1284
FBW, kg	0.71 ^b	0.79 ^c	0.010	0.0001	0.74	0.76	0.011	0.1926
WG, kg	0.59 ^b	0.66 ^c	0.011	0.0001	0.62	0.63	0.011	0.1311
FI, kg	0.82 ^b	0.93 ^c	0.026	0.0061	0.84 ^d	0.92 ^e	0.026	0.0503
FCR	1.42	1.41	0.049	0.7474	1.38	1.46	0.049	0.3496
Tibia measurements								
DM, %	36.13	36.28	0.225	0.6423	36.36	36.05	0.222	0.3244
DM, g	2.22 ^b	2.60 ^c	0.057	0.0001	2.4	2.42	0.058	0.8063
Ash, %	40.52	41.24	0.287	0.0807	41.16	40.6	0.287	0.1698
Ash, g	0.90 ^b	1.07 ^c	0.028	0.0001	0.99	0.98	0.028	0.8762

Note: WG, weight gain; FI, feed intake; FCR, feed conversion ratio; AGP, antibiotic growth promoter; HS, humic substances; SEM, standard error of the mean; DM, dry matter ^a Least square means with 10 replication per treatment; Values within rows with different superscripts differ significantly, ^{b-c} $P < 0.01$, ^{d-e} $P < 0.05$.

Table 4. Serum concentrations of liver enzymes, urea, proinflammatory cytokines, mucin-secreting glycoprotein 2 (MUC-2), and fluorescein isothiocyanate-dextran (FITC-d).

Item ^a	Growth promoter				Feeding system			
	AGP	HS	SEM	P<	Continuous	Alternating	SEM	P<
ALT, U/L	8.26	8.315	1.21	0.7683	7.95	8.625	1.219	0.7684
AST, U/L	161.68	162.79	37.41	0.9835	135.84	188.63	37.271	0.3335
ALP, U/L	1181.3	773.5	240.60	0.2513	1104.8	850.0	240.974	0.4670
Urea, mmol/L	0.42	0.32	0.05	0.2113	0.39	0.35	0.052	0.5874
IL-1	243.68	304.31	34.67	0.2471	280.68	267.31	35.62	0.7923
IL-6	419.64	286	72.83	0.2152	329.42	376.23	74.83	0.6610
FNT- α	47.55	35.60	13.34	0.5608	28.46	54.69	12.99	0.1736
MUC-2	7.58	6.46	0.40	0.0606	6.88	7.17	0.41	0.6131
FITC-d	55.87 ^b	36.3 ^c	4.68	0.0001	43.47	48.7	4.68	0.4400

Note: AGP, antibiotic growth promoter; HS, humic substances; IL-1, interleukin 1; IL-6, interleukin 6; FNT- α , tumor necrosis factor alpha; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; SEM, standard error of the mean, ^a Least square means with five replication per treatment; ^{b-c} Values within rows with different superscripts differ significantly, $P < 0.01$.

($P > 0.05$). Serum MUC-2 levels in AGP-fed broilers showed a tendency toward higher values compared to those receiving HS ($P < 0.06$). In contrast, serum FITC-d concentrations were significantly higher ($P < 0.01$) in AGP-fed broilers than in those supplemented with HS.

Morphometry of the jejunum villus and number of goblets cells

The morphometry of the jejunal villi is presented in Figures 1-3. Villus height showed no significant differences among treatments ($P > 0.05$; Figure 2A). Villus thickness (Figure 2B) exhibited a significant interaction ($P < 0.01$) between growth promoter and feeding system: under a continuous feeding system with AGP supplementation, villus thickness was reduced, whereas HS supplementation under continuous feeding resulted in wider villi. Under alternating feeding, villus thickness was similar between AGP- and HS-fed broilers. Villus area (Figure 2C) was also significantly affected by the interaction between growth promoter and feeding system ($P < 0.01$). Broilers receiving AGP with continuous feeding had smaller villus areas compared to the other treatments, which showed similar values. Crypt depth (Figure 3A) displayed a significant growth promoter \times feeding system interaction ($P < 0.01$). Birds fed AGP with continuous feeding and those fed HS with alternating feeding had shallower crypts, whereas HS-fed broilers under continuous feeding exhibited the greatest crypt depth. The villus height-to-crypt depth ratio (Figure 3B) was likewise influenced by the interaction between growth promoter and feeding system ($P < 0.01$). Broilers receiving AGP with continuous feeding and those fed HS with alternating feeding had higher ratios. The numbers of neutral, strongly sulfated acidic, and non-sulfated acidic goblet cells (GC) are shown in Figure 3. In HS-supplemented broilers, the number of neutral GC was greater ($P < 0.05$) compared to those receiving AGP (Figure 4A).

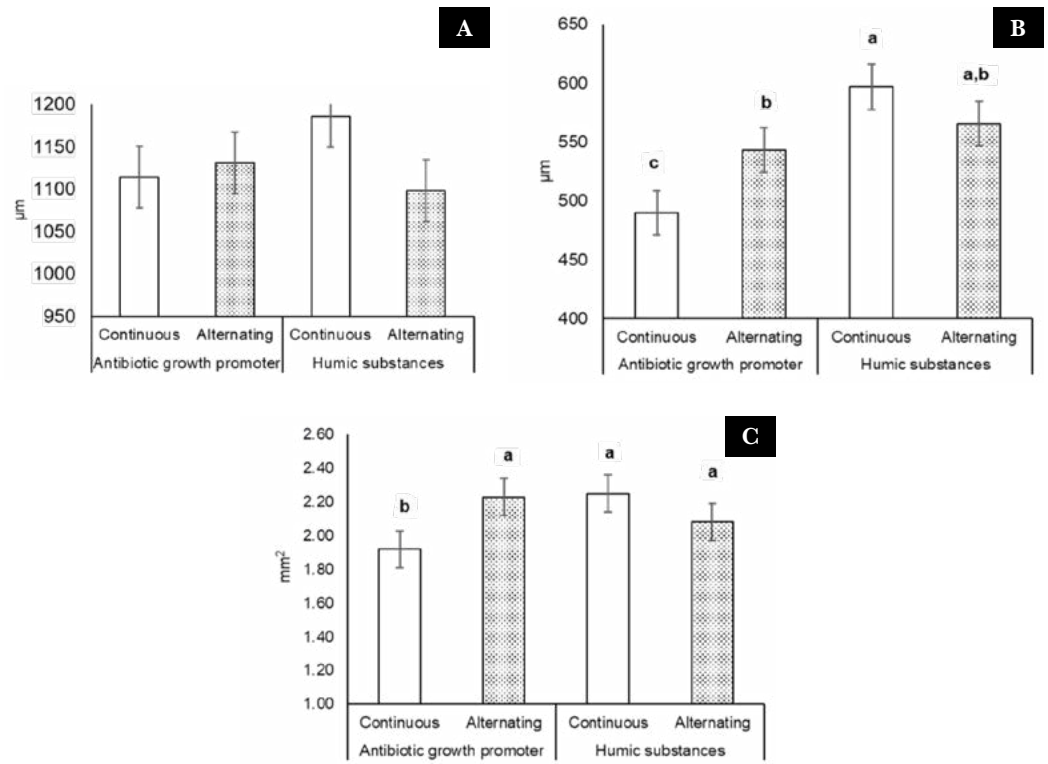


Figure 2. Height of the villi (A, $P > 0.05$; SEM = 36.282); Thickness of the villi (B, interaction of growth promoter and feeding system, $P < 0.01$; SEM = 19.175), and Area of the villi (C, interaction of growth promoter and feeding system $P < 0.05$; SEM = 0.111) in the jejunum. ^{a,b,c} Columns with different superscript are statistically different.

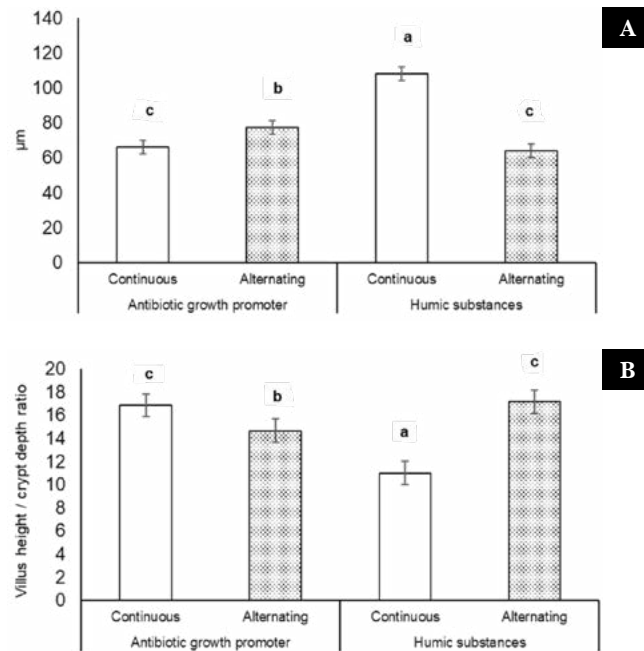


Figure 3. Depth of the crypts (A, interaction of growth promoter and feeding system, $P < 0.01$; SEM = 3.935) and Villus height/crypt depth ratio (B, interaction of growth promoter and feeding system, $P < 0.01$; SEM = 1.000). ^{a,b,c} Columns with different superscript are statistically different.

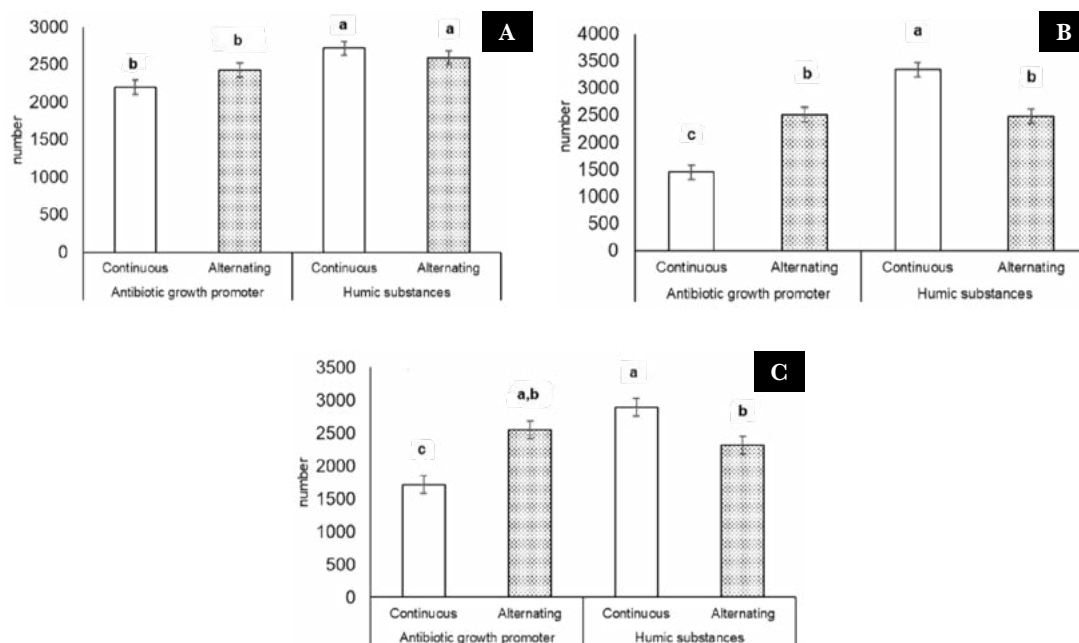


Figure 4. Number of neutral cells (A, effect of growth promoter, $P < 0.05$; SEM=93.846); Number of sulfated acid cells (B, interaction of growth promoter and feeding system, $P < 0.05$; SEM=135.628); and Number of non-sulfated acid cells (C, interaction of growth promoter and feeding system, $P < 0.05$; SEM=133.203) in the jejunum. ^{a,b,c} Columns with different superscript are statistically different.

In the number of sulfated acidic GC (Figure 4B) and non-sulfated acidic GC (Figure 4C), the interaction between growth promoter and feeding system was significant ($P < 0.05$). Under the continuous feeding system, broilers receiving AGP had the lowest GC counts, whereas those supplemented with HS had the highest counts; under the alternating feeding system, birds exhibited intermediate values.

Relative quantification of gene expression

The results of the relative quantification of IgA, OCLN, CLDN-1, and MUC-2 gene expression in the jejunal mucosa, determined by real-time PCR, are presented in Table 5. No significant differences in IgA gene expression were observed between AGP- and HS-supplemented broilers, nor between continuous and alternating feeding systems. Growth promoter type influenced OCLN, CLDN-1, and MUC-2 expression: OCLN and MUC-2 expression levels were higher in HS-supplemented birds compared to those receiving AGP ($P < 0.01$), whereas CLDN-1 expression was higher in AGP-fed broilers than in those supplemented with HS ($P < 0.05$).

Humic substances (HS) derived from vermicompost have been reported to enhance the productive performance of broilers (Maguey-González *et al.*, 2022). In the present study, dietary HS supplementation increased final body weight, weight gain (WG), and feed intake (FI) from 7 to 21 days compared with AGP-fed birds, regardless of feeding system. The absence of differences in feed conversion ratio (FCR) between HS- and AGP-supplemented groups aligns with previous findings, where broilers fed HS from vermicompost showed

Table 5. Relative quantification of immunoglobulin A (IgA), occludin (OCLN), claudine-1 (CLDN-1) and mucin-secreting glycoprotein 2 (MUC-2) genes expression in jejunum ^{*†}.

Genes	Growth promoter				Feeding system			
	AGP	HS	SEM	P<	Continuous	Alternating	SEM	P<
IgA	0.518	0.507	0.008	0.3598	0.518	0.508	0.008	0.4119
Ocludin	0.224 ^b	0.289 ^c	0.015	0.0007	0.241	0.272	0.015	0.1573
Claudin	0.634 ^d	0.544 ^c	0.025	0.0236	0.618	0.560	0.025	0.1263
MUC-2	0.482 ^b	0.569 ^c	0.016	0.0013	0.518	0.533	0.016	0.5195

* Normalized units of the transcript with housekeeping 28S gene; † Data are means of five replicates in pulls of two chicks/treatment.

Note: AGP=Antibiotic growth promoter, HS=Humic substances; SEM=Standard error of the mean; Values within rows with different superscripts differ significantly, ^{b-c} P<0.01, ^{d-e} P<0.05.

similar FCR to control groups (Angeles *et al.*, 2022a; Domínguez-Negrete *et al.*, 2019). The inclusion of 0.33% HS in this study did not significantly improve FCR despite positive trends in FI and WG. This outcome could reflect a suboptimal inclusion level, as recent meta-analyses (Gao *et al.*, 2023) suggest that HS effects on productive parameters may be dose-dependent and influenced by their origin and extraction method. Similarly, in previous work where broilers underwent steady digestive conditions interspersed with two abrupt diet changes, no differences in body weight, WG, FI, or FCR were found between HS- and AGP-fed birds (López-García *et al.*, 2023). To increase digestive and productive challenge compared with the previous study, the current experiment incorporated: (a) diet alternation every two days, (b) inclusion of sorghum and canola meal in diet B, and (c) induction of systemic and digestive disturbances via LPS injection. Remarkably, HS-fed broilers under alternating feeding, despite undergoing eight dietary changes over 15 days and consuming sorghum- and canola-based diets four times, maintained growth performance comparable to those under continuous feeding. This pattern was also observed in AGP-fed birds, although their overall growth rate was lower than that of HS-fed birds.

HS possess one of the highest complexation capacities among natural ligands, allowing strong chelation with various ions and potentially enhancing mineral utilization in both plants and animals (Peña-Méndez *et al.*, 2005). Tibia measurements are reliable indicators of mineral assimilation and mucosal integrity in broilers. Increased tibia ash content, Ca and P percentages at 21 days, and higher dry matter and mineral content at 42 days have been reported in broilers receiving HS from vermicompost in drinking water (Angeles *et al.*, 2022; Gómez-Rosales & Angeles, 2015). In this study, HS supplementation increased tibia dry matter and ash weights by 15.38% and 15.89%, respectively, compared to AGP-fed broilers, irrespective of feeding system. Other studies have also reported higher Ca, P, Fe, and Cu in broiler meat (Ozturk *et al.*, 2010; 2012; 2014) and increased Ca, Mg, Zn, and Fe in breast and thigh tissues (Skalická *et al.*, 2019). Higher serum Ca and P concentrations in HS-fed birds have been linked to improved bone strength and reduced locomotor problems (Disetthe *et al.*, 2017), consistent with the present findings. HS from vermicompost also exerted a trophic effect on the intestinal mucosa, increasing villus thickness (Figure 2B) and villus area (Figure 2C) regardless of feeding system. This enhancement in absorptive surface may contribute to the improved tibia mineralization observed, as nutrient

absorption is maximized in the jejunum. Similar effects greater villus height, thickness, and surface area have been documented in HS- or FA-supplemented broilers (Disethle *et al.*, 2017; Lala *et al.*, 2017; Taklimi *et al.*, 2012; Mao Y, 2019; Tang *et al.*, 2023). In this study, villus architecture in HS-fed birds was maintained across feeding systems, unlike in AGP-fed broilers, which showed structural variation. The protective role of HS has been attributed to their colloidal properties and stimulation of mucin production, forming a barrier over the gastrointestinal mucosa (Angeles *et al.*, 2022a; EMEA, 1999; Kühnert *et al.*, 1991). Previous research has shown that HA supplementation increased intestinal viscosity, reduced bacterial translocation, and lowered permeability (FITC-d) in feed-restricted broilers (Maguey-González *et al.*, 2018a), and upregulated MUC-2 expression in laying hens (Mudroňová *et al.*, 2021). In the present trial, HS supplementation increased neutral GC numbers by 15% (Figure 4A) compared to AGP-fed birds, regardless of feeding system. Under continuous feeding, HS-fed birds had 56.7% and 40.6% more sulfated acidic GC (Figure 4B) and non-sulfated acidic GC (Figure 4C), respectively, than AGP-fed birds. This suggests that HS protect the mucosa by enhancing mucin secretion, thereby limiting pathogen and toxin contact with enterocytes. Despite the LPS challenge and frequent diet changes designed to induce both intestinal and systemic inflammation, liver enzyme and urea levels were unaffected by treatment, consistent with López-García *et al.* (2023). Similarly, no differences in serum IL-1, IL-6, or TNF- α were found, echoing findings in FA-supplemented broilers (Tang *et al.*, 2023). However, reductions in these cytokines have been observed in HS-fed turkey poults challenged with aflatoxin B (Maguey-González *et al.*, 2023b) and in sodium humate-supplemented pigs and calves (Wang *et al.*, 2020; 2022), as well as in HS-fed mice (Xu *et al.*, 2023). A key difference is that those studies compared HS against unsupplemented controls, whereas this trial compared HS to AGP. No significant differences in jejunal IgA gene expression were detected between HS and AGP treatments, regardless of feeding system, paralleling findings in HS-fed broilers (Mudroňová *et al.*, 2020) but contrasting with increased cecal IgA expression in laying hens (Mudroňová *et al.*, 2021). Similar variable effects have been reported for serum IgA in livestock (Wang *et al.*, 2022; Tang *et al.*, 2023; Maguey-González *et al.*, 2018). This study is the first to assess the effects of vermicompost-derived HS on CLDN-1 and OCLN expression in broiler jejunum. HS increased OCLN expression compared to AGP, whereas CLDN-1 expression was higher in AGP-fed birds. Since OCLN and CLDN-1 share localization and function in tight junctions and are co-regulated through phosphorylation (Salvo-Romero *et al.*, 2015), these opposite effects may reflect a compensatory regulatory mechanism. HS supplementation also upregulated MUC-2 expression compared to AGP, consistent with the hypothesis that HS reinforce the mucus layer. Lower serum FITC-d levels in HS-fed birds indicate improved intestinal barrier integrity, aligning with previous HA studies (Maguey-González *et al.*, 2018a) and with other barrier-enhancing additives such as sodium butyrate (Hu *et al.*, 2021) and essential oils (Mohiti-Asli *et al.*, 2020). The concurrent trend toward lower serum MUC-2 in HS-fed birds may indicate reduced leakage of this glycoprotein into circulation, whereas higher FITC-d and MUC-2 levels in AGP-fed birds suggest epithelial barrier disruption. Limitations of this study include the relatively small sample size (n= 10 per group), controlled experimental conditions without

commercial-scale replication, a single HS inclusion level (0.33%), and a short evaluation period (21 days post-challenge). Future research should investigate larger-scale production settings, multiple inclusion rates, and longer trial durations to clarify the long-term potential of HS as AGP alternatives in poultry production.

CONCLUSIONS

Supplementation with 0.33% vermicompost-derived humic substances reduced intestinal permeability by 23% (FITC-d, $P=0.034$), increased villus height by 18% ($P=0.021$), and enhanced tibial bone strength ($P=0.016$) in LPS-challenged broilers. Furthermore, increases in neutral goblet cell counts and the expression of MUC-2 and OCLN genes were observed, suggesting an early epithelial repair response. These findings indicate that HS could serve as a partial alternative to AGPs for preserving intestinal integrity, particularly in sorghum-canola-based diets. Further research should evaluate dose-response effects, microbiota modulation, and economic viability under commercial production conditions.

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