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AGRICULTURA

SECRETARÍA DE AGRICULTURA Y DESARROLLO RURAL

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DIVERSITY AND BURDEN OF GASTROINTESTINAL NEMATODES IN GOAT PRODUCTION SYSTEMS IN THE CENTRAL REGION OF VERACRUZ, MEXICO

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ABSTRACT

Goat herds (*Capra aegagrus hircus* L.) are susceptible to various diseases. One of the most economically damaging in production systems is caused by gastrointestinal nematodes (GINs), which can alter the health, production, and welfare of the animals. The objective of this study was to determine the diversity and burden of gastrointestinal parasites in goat production systems in the central region of Veracruz, Mexico. The municipalities included in the study are located in the mountainous zone (Coatepec and Jalacingo), the highlands (Perote), and the coastal plain (Medellín). A cross-sectional study was conducted, and sampling was carried out during the period from November 2021 to May 2022. Two surveys were applied to farmers. A descriptive analysis was carried out on the characteristics of the production units (PUs). The McMaster technique was used to determine the presence of eggs, and the coproculture, Baermann, and larval identification techniques were used to determine the diversity of GINs. The analysis of the data obtained in the laboratory and the individual survey was carried out with a fixed effects model to analyze the parasite load. The independent variables were the PU, breed, physiological state, and body condition, and the dependent variables were eggs per gram of feces (Epgf) and its natural logarithm ($\ln(\text{Epgf}+1)$). Three types of production systems were found: housed, semi-stabled, and grazing. The genera and species identified were *Haemochus contortus*, *Teladorsagia* spp., *Trichostrongylus* spp., *Chabertia ovina*, *Cooperia* spp., *Oesophagostomum* spp., *Trichuris ovis*, and *Strongyloides papillosus*. The Toggenburg breed had the highest nematode load, and all PUs had high parasite loads with counts greater than 700 Epgf. A great diversity and high loads of GINs were found in the goat PUs of the central zone of Veracruz.

Keywords: gastrointestinal parasites, parasite load, goat farming.

INTRODUCTION

In Mexico, there is a population of around 8 791 894 heads of goat, which are mainly concentrated in Puebla (15.4 %), Oaxaca (12 %), San Luis Potosí (10.5 %), Guerrero (7.9 %), and Zacatecas (6.1 %) (SADER, 2015; SIAP, 2019). Around 162 thousand liters

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of milk and 40 thousand Mg of carcass meat are produced annually. In the state of Veracruz, goat farming has shown gradual growth. In 2010, the estimated goat inventory was 150 306 cattle heads, and by 2021 it increased to 160 625 cattle heads (SIAP, 2022).

Goat production is located in rural communities in arid, semi-arid, mountainous, and highland areas, with little technification and government support and insufficient dissemination of the activity (Ortiz-Morales *et al.*, 2021). It is an activity carried out by small farmers either as a primary productive activity or in combination with other productive species such as cattle and sheep or complementary with agricultural activities (Bedotti *et al.*, 2018; Ortiz-Morales *et al.*, 2021). Farmers lack the economic resources to invest in improving their facilities and accessing technologies that allow adequate zootechnical and sanitary management of their herds (Aréchiga *et al.*, 2008; Ortiz-Morales *et al.*, 2021).

Goats are exposed to gastrointestinal nematode (GIN) infections, which represent a limiting factor for production (Torres-Acosta and Hoste, 2008), mainly in regions with tropical and subtropical climates due to the existence of climatic and environmental conditions that favor the rapid development of the biological cycle of nematodes, especially in grazing production systems (Herrera *et al.*, 2013). Goat production is often done collectively, i.e., they are not separated by age or physiological stages, which causes rapid spread of infective stages (Jiménez *et al.*, 2013). GIN infections can alter animal health and welfare and the productive levels of animals. This is reflected in the economy of farmers (Zapata *et al.*, 2016), showing a decrease in meat and milk production. When the infestation is very high, it is also one of the main causes of mortality in herds (Li *et al.*, 2016).

Parasitosis is a frequent problem in goat production units because of poor sanitary management and reduced goat immunity to parasites, which makes them very susceptible (Mpfungu *et al.*, 2022). The damage caused is related to parasite-specific factors such as gender, degree of infection, and physiological stage, as well as their pathogenicity; this in turn depends on other factors such as weather conditions, vegetation, soil, type of grassland, type of production system, farm management, and characteristics directly related to the animal, such as age, breed, and physiological stage (Mpfungu *et al.*, 2022).

GINs lodge throughout the digestive tract of ruminants to obtain nutrients for survival (Craig, 2018). Within the main gastrointestinal nematode genera identified to affect small ruminants are *Haemochus contortus*, *Trichostrongylus colubriformis*, *T. axei*, *Teladorsagia circumcincta*, *Cooperia* spp., *Oesophagostomum* spp., *Trichuris ovis*, *Strongyloides papillosus*, and *Bunostomum* spp. (Badaso and Addis, 2015).

In the state of Veracruz, there are few studies related to the diversity and impact of gastrointestinal parasitosis in goat herds. Most studies on gastrointestinal nematodes have been carried out in cattle and sheep. Under the hypothesis that gastrointestinal nematodes in the central zone of the state of Veracruz can be caused by different genera, they have an impact on goat production systems. The objective of this research was

to determine the diversity and burden of gastrointestinal parasites in goat production systems in the central region of Veracruz.

MATERIALS AND METHODS

Study area

This research was conducted in four municipalities located in the central zone of the state of Veracruz, Mexico. The municipalities included in the study are located in the mountainous zone (Coatepec and Jalacingo), the highlands (Perote), and the coastal plain (Medellín de Bravo).

Study design

The study was cross-sectional. Sampling of the herds was carried out during the period from November 2021 to May 2022. Herds established in three different physiographic regions of the central zone of the state of Veracruz were considered. The production units (PUs) were selected according to the producer's availability to participate in the study. A total of 123 animals (females) with an average age of five months to eight years were sampled from 14 PUs across four municipalities (Table 1).

Table 1. Locations, number of animals, average age, and breeds of goats (*Capra aegagrus hircus* L.) evaluated in the study areas.

Municipality	Locality	PU	N of animals	Age (years)	Breed
Perote	Xaltepec	1	10	2.00–6.00	Alpine
Perote	Xaltepec	2	10	3.00–7.00	Alpine, Saanen, and Toggenburg
Perote	La Gloria	3	10	0.60–6.00	Saanen
Perote	La Gloria	4	6	0.75–6.00	Saanen
Coatepec	Pacho Viejo	5	10	0.60–7.00	Saanen and Alpine
Coatepec	Coatepec	6	10	2.00–4.00	Alpine, Saanen, Crossbreed, and Toggenburg
Coatepec	Coatepec	7	5	0.50–5.00	Crossbreed and Alpine
Perote	Frijol Colorado	8	10	3.00–4.00	Alpine, Saanen, and Toggenburg
Perote	Frijol Colorado	9	10	1.00–4.00	Saanen and Alpine
Medellín	Mata Ortiz	10	10	1.00–8.00	Crossbreed and Creole
Medellín	Palmira	11	6	1.00–9.00	Creole and Crossbreed
Coatepec	Agrosol	12	6	2.00–4.00	Saanen and Crossbreed
Jalacingo	Melchor Ocampo	13	10	0.41–3.00	Alpine, Saanen, and Toggenburg
Jalacingo	Melchor Ocampo	14	10	4	Alpine, Saanen, and Toggenburg

PU: production unit.

Data collection

To determine the characteristics of the PUs and the conditions of the animals studied, a questionnaire was used to obtain data related to animal identification, age, breed, sex, physiological state (primal, pregnant, lactating, and empty), body condition,

recent diarrhea or diseases, and the last date of deworming. The McMaster test was used to obtain the variable eggs per gram of feces (Epgf), with which the intensity of infection was determined. This metric indirectly represents the intensity of infection of the animals. The breeds found in the PUs studied were Alpine (31.7 %), Saanen (43.9 %), Toggenburg (7.3 %), Crossbreed (13.8 %), and Creole (3.3 %). Body condition (BC) was scored on a scale of 1 to 5, according to Frutos and Ruiz-Mantecón (1994); for better understanding, these scores were associated with qualitative criteria such as 1 (very lean), 2 (lean), 3 (fair), 4 (good), and 5 (fat).

Sampling

Sample collection was carried out by holding the animal by the head through the cleft of the lower jaw with the hands, the trunk of the animal between the hind limbs by an assistant, and extracting the fecal samples directly from the rectum of the goats. A total of 5–10 g of feces was collected and placed in plastic bags properly identified. The samples were coded according to the municipality of origin, PU, and the progressive number of the animal. In addition, in the individual card, the earring or name and number of the animal sampled (from 1 to 10) were recorded. The samples were placed in a cooler with refrigerant for preservation until transferred to the Parasitology Laboratory at the Rancho Torreón del Molino of the Faculty of Veterinary Medicine and Animal Science of the Universidad Veracruzana (UV-FMVZ) for processing.

Diagnostic techniques

The McMaster flotation technique was used to determine fecal counts of gastrointestinal nematodes (Figueroa-Castillo *et al.*, 2015). Fecal egg counts can be classified according to their intensity. Counts of 0 Epgf are considered negative; mild PU infection is up to 200 Epgf, moderate infection is between 200 and 700 Epgf, and high infection is for counts over 700 Epgf (Prada-Sanmiguel and Plazas-Caro, 2010). To identify nematode genera, the coproculture technique was previously used to obtain the third larval stage (L3), providing the environmental conditions that the eggs would have in the grassland and thus achieving the hatching of the eggs to recover the L3 (O'Connor *et al.*, 2006). The Baermann technique was used for the concentration and recovery of the L3, which were differentiated to determine the genera (Figueroa-Castillo *et al.*, 2015). The L3 larvae collected were identified according to dichotomous keys established by Niec (1968), Liébano, (2011), and van Wyk and Mayhew (2013). The main morphological characteristics were size, number, shape of intestinal cells and forelimb, termination of the larval tail, and length and shape of the sheath tail (Niec, 1968).

Statistical analysis

The information from the surveys and laboratory data were integrated in databases elaborated with the Excel program. For the analysis of the laboratory and individual survey data, a fixed effects model was used, where the variables PU, breed, and parasite egg count (Epgf) were included to evaluate the intensity of the infection. This

was analyzed as a generalized linear model using SPSS 23 software (IBM Statistics). The data were transformed with the natural logarithm plus one to guarantee homogeneity of variance of the parasitic load and to improve data distribution (Díaz-Rivera *et al.*, 2000). In the preliminary models, the effects of municipality and locality were not significant. Thus, they were removed from the definitive model and replaced by the PU effect. Additionally, the effect of body condition was included. The effects of age (covariate) and production system, although recorded, were not estimable under the resulting data structure. For the general survey, a descriptive analysis of the characteristics of the PUs was performed.

The following formula used was:

$$\text{Fecal egg count (Epgf and } \ln(\text{Epgf} + 1)) = \mu + PU_i + RZ_j + EF_k + CC_l + \varepsilon_{ijklm}$$

where *Epgf* is the number of eggs per gram of feces and the response variable; μ is the overall mean; PU_i is the effect of the *i*-th production unit; RZ_j is the effect of the *j*-th breed; EF_k is the effect of the *k*-th physiological state; CC_l is the effect of the *l*-th body condition; and ε_{ijklm} is the residual effect on each observation.

RESULTS AND DISCUSSION

Nematode diversity

The following genera, and in certain cases, genus and species of gastrointestinal parasites, were identified from the 123 samples: *Haemochus contortus*, *Teladorsagia* spp., *Trichostrongylus* spp., and *Chabertia ovina* were found in all municipalities; *Cooperia* spp. was found only in the municipality of Jalacingo; *Oesophagostomum* spp. and *Strongyloides papillosus* were found in the municipality of Medellín; and *Trichuris ovis* was found only in the municipality of Coatepec (Table 2). Of the animals sampled,

Table 2. Presence and diversity of gastrointestinal parasites in goats (*Capra aegagrus hircus* L.) in the different municipalities in the central region of Veracruz, Mexico.

Parasite genus	Coatepec	Jalacingo	Medellín	Perote
<i>Haemochus contortus</i>	X	X	X	X
<i>Teladorsagia</i> spp.	X	X	X	X
<i>Trichostrongylus</i> spp.	X	X	X	X
<i>Chabertia ovina</i>	X	X	X	X
<i>Cooperia</i> spp.		X		
<i>Oesophagostomum</i> spp.			X	
<i>Trichuris ovis</i>	X			
<i>Strongyloides papillosus</i>			X	

almost 90 % were found to be highly infected with nematodes. Likewise, all the animals sampled presented nematode associations; that is, they had the presence of more than one species of gastrointestinal nematode.

These results are consistent with Income *et al.* (2021), where samples were collected from nine goat PUs and found *Haemochus* spp. (100 %), *Trichostrongylus* spp. (91.7 %), *Oesophagostomum* spp. (29.2 %), and *Cooperia* spp. (37.5 %). These authors also detected mixed infections with more than one genus of nematodes. In a similar study conducted in sheep and goats by Herrera *et al.* (2013) in Antioquia, Colombia, a similar presence of nematodes was identified in the municipalities studied, with *H. contortus*, *Oesophagostomum* spp., *Trichostrongylus* spp., and *Ostertagia* spp. being the most frequent nematodes. Similarly, in another study in cattle and goats conducted in Quechultenango, Mexico, Figueroa-Antonio *et al.* (2018) determined a positivity of 91.8 % of one or more gastrointestinal nematodes, which is similar to the findings in this study.

Quantification of infection intensity

The model and the intercept (common effect) used were significant ($p \leq 0.01$); the effect of the PU and body condition were also significant ($p \leq 0.01$). The effect of breed was significant ($p \leq 0.05$) only for the transformed variable, and the effect of the physiological state was not important for either of the two response variables (Table 3).

Table 3. Analysis of variance of nematode infection intensity in evaluated in the central region of Veracruz, Mexico.

Variable	Degrees of freedom	Epgf	ln (Epgf+1)
Model	24	25 110 296**	6208**
Intercept	1	117 247 158**	750 032**
Production unit	13	3 671 895**	2755**
Breed	4	3 689 108 ^{NS}	2021 ^{NS}
Physiological state	3	1 691 837 ^{NS}	997 ^{NS}
Body condition	4	70 466 843**	6456**
Error	98	1 774 107	1178

Epgf: eggs per gram of feces; ^{NS} $p > 0.05$ (non-significant); * $p \leq 0.05$; ** $p \leq 0.01$.

Differences between PUs

All PUs were found to have high infection rates, with counts higher than 700 Epgf. The PU with the highest count was AGROSOL, located in the municipality of Coatepec, with an average of 4260 Epgf (Table 4). This could be due to poor animal management, ranging from the indiscriminate use of dewormers to the coexistence of animals from different flocks and other species, like sheep. PUs with loads higher than 2500 Epgf are considered to have serious parasitism problems.

Table 4. Fecal counts of nematode eggs (mean \pm standard error) in the sampled production units in the central region of Veracruz, Mexico.

Production unit	Animals sampled	Epgf	ln (Epgf+1)
4 (La Gloria, Perote)	6	765 \pm 1039 a	4.98 \pm 0.58 a
3 (La Gloria, Perote)	10	898 \pm 906 a	5.75 \pm 0.50 a
13 (Melchor Ocampo, Jalacingo)	10	906 \pm 829 a	6.21 \pm 0.46 a
9 (Frijol Colorado, Perote)	10	1079 \pm 940 a	5.38 \pm 0.52 a
8 (Frijol Colorado, Perote)	10	1090 \pm 1032 a	5.95 \pm 0.57 a
6 (Coatepec, Coatepec)	10	1201 \pm 835 a	6.02 \pm 0.46 a
5 (Pacho Viejo, Coatepec)	10	1369 \pm 796 a	6.83 \pm 0.44 a
11 (Palmira, Medellín)	6	1546 \pm 1048 a	6.68 \pm 0.58 ab
10 (Mata Ortiz, Medellín)	10	2219 \pm 932 a	6.82 \pm 0.52 ab
2 (Xaltepec, Perote)	10	3240 \pm 841 b	7.81 \pm 0.47 b
7 (Coatepec, Coatepec)	5	3351 \pm 945 b	7.87 \pm 0.52 b
1 (Xaltepec, Perote)	10	3356 \pm 1009 b	8.10 \pm 0.56 b
14 (Melchor Ocampo, Jalacingo)	10	3619 \pm 1013 b	8.08 \pm 0.56 b
12 (Agrosol, Coatepec)	6	4260 \pm 994 b	7.96 \pm 0.55 b

Epgf: eggs per gram of feces. Means with different letters within columns indicate significant differences ($p \leq 0.05$).

These results coincide with a study conducted by Income *et al.* (2021) in Kanchanaburi, Thailand, where they studied cattle and goat PUs and found that all goat PUs studied were positive for helminth eggs, presenting 100 % prevalence in the herd, and the positive goats were heavily infected. A study conducted by Cerbo *et al.* (2010) in Lombardy, Italy, showed that almost all the goats sampled were positive in the 110 PUs studied.

Fecal counts of gastrointestinal nematode eggs per breed

Goat production in Veracruz is mainly destined for milk production, which is transformed into fresh cheeses and gourmet cheeses, and therefore, has a preference for specific breeds (SADER, 2021). The results indicate that the Toggenburg breed was the most susceptible to nematode infection (Table 5). This is relevant since it would be necessary to carry out further studies to confirm the presence and susceptibility to nematodes that they maintain. This breed has a small percentage of animals in the herds studied; however, it is present in several PUs.

It is important that producers consider breed susceptibility when acquiring animals and give them special attention. Creole breeds would be thought to have greater resistance because they are animals adapted to their environment; however, they also have high parasite loads, which may be due to the management they receive. Goats, unlike other ruminants, have a weak immunity against GINs (Rossanigo, 2003).

There is scarce literature on parasitism in the Toggenburg breed. Herrera *et al.* (2013)

Table 5. Fecal counts of nematode eggs (mean \pm standard error) by goat breed (*Capra aegagrus hircus* L.).

Breed	Goats sampled	Epgf	ln (Epgf+1)
Crossbreed	17	853 \pm 959 a	6.12 \pm 0.53 a
Saanen	54	1613 \pm 415 a	6.73 \pm 0.23 a
Alpine	39	1856 \pm 514 a	6.19 \pm 0.28 a
Creole	4	2034 \pm 1542 ab	7.27 \pm 0.86 a
Toggenburg	9	3965 \pm 763 b	7.4 \pm 0.42 b

Epgf: eggs per gram of feces. Means with different letters within each column show significant differences ($p \leq 0.05$).

studied goats of the Alpine, Saanen, Crossbreed, Toggenburg, Anglonubian, and La Mancha breeds. The Saanen and Alpine were more susceptible, and the Toggenburg breed was the most resistant. It is worth mentioning that the Toggenburg breed specimens were fewer in number, as well as in this study. In another study conducted by Rossanigo and Colomer (1993) in Argentina, gastrointestinal parasitism had an important effect on the production of Creole goats, which is similar to this study since Creole goats also have high parasite loads.

Susceptibility of animals to infection depends on the breed. The sources of variation depend on natural defense mechanisms. The resistance they exhibit is based on immunological strategies acquired or present in their genetic code and their resilience, which allow the animals to be parasitized, recover, and adapt to the infection, plus the type of management and herd factors (Aguilar-Caballero *et al.* 2008). Some studies describe the susceptibility of high-producing dairy goats to GIN infections (Beasley *et al.*, 2010), and Alpine and Saanen breeds are considered resistant (Baker *et al.*, 2001). However, evidence on the resistance of goat breeds to GINs is limited, which should be considered since the livestock used in the Veracruz are mainly dairy goats.

In relation to the physiological status, it was not possible to detect differences between the statuses analyzed in this study, probably due to the limited number of animals sampled. On the other hand, although there could be an interaction between physiological state and breed, no interactions were included in this study, due to the same limitation as above. The egg counts for each body condition level (Table 6) show that body condition 1 was associated with high gastrointestinal nematode loads, while conditions 2, 3, and 5 had an intermediate load, and the lowest values were observed in animals with body condition 4.

These results suggest that animals with a lower body condition are associated with high loads of gastrointestinal nematodes, while the other conditions seem to have a higher resilience to these parasites. Similarly, Aguilar-Caballero *et al.* (2019) found that goats with poorer body conditions showed the highest loads of GIN.

Table 6. Fecal nematode egg counts (mean \pm standard error) by body condition in goats (*Capra aegagrus hircus* L.) in the central region of Veracruz, Mexico.

Body condition	Number of goats sampled	Epgf	ln (Epgf+1)
1 (Very lean)	11	7533 \pm 508.4 a	8.478 \pm 0.41 a
2 (Lean)	34	1976 \pm 371.7 b	6.784 \pm 0.30 c
3 (Fair)	16	1895 \pm 493.9 b	7.230 \pm 0.40 b
4 (Good)	58	978 \pm 315.4 c	6.480 \pm 0.26 c
5 (Fat)	4	1507 \pm 904.4 b	6.160 \pm 0.74 c

Means with different letters within each column show significant differences ($p \leq 0.05$).

Characterization of the PUs

Fourteen PUs were evaluated, including stabled, semi-stabled, and grazing production systems. Three PUs belonged to the stabled system (17.1 %), four used only grazing (29.3 %) and seven had a semi-stabled system (53.7 %). The age of the animals ranged from 5 months to 8 years, with an average age of 4 years. The effect of age was not analyzed in this study; however, Rojas-Hernández *et al.* (2007) found no effect by age group in a study on the prevalence of GIN in sheep. Stabled PUs should also be considered, since they could be acquiring GINs through the cut grass provided as feed.

Some PUs deworm every 2–3 months, while others do so every 3–6 months or every 6–12 months. The most commonly used products are fenbendazole, ivermectin, closantel, levamisole, and albendazole. The dosage used is the one indicated by the product. The cleaning of the facilities varies from daily to monthly. The feces are used for composting, and some farmers sell them. In almost all PUs, farmers carry out the dosage of the deworming agent, which coincides with what was found by Cáceres *et al.* (2021) in Peru, where producers also dose according to their own criteria, without technical orientation and varying the administration every 3–12 months, and the most used product was albendazole. Inadequate practices lead to resistance to anthelmintics, especially albendazole and ivermectin (Ratanapob *et al.*, 2022).

CONCLUSIONS

The main nematodes found in the study area were *Haemochus contortus*, *Teladorsagia* spp., *Trichostrongylus* spp., and *Chabertia ovina*, since they were found in all municipalities. The intensity of infection found in all production units was high, and most of the animals presented combined infections of different nematodes; therefore, it is important to develop better and integrated strategies for the control of gastrointestinal nematodes. Improved management practices, especially grazing and

parasite control, are recommended to reduce the high infections present in the studied areas. Farmers should take special care due to the high fecal counts of gastrointestinal nematode eggs.

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CHARACTERIZATION OF THE LACTATION CURVE OF RAMBOUILLET EWES IN MEXICO

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ABSTRACT

Sheep (*Ovis aries* L.) in Mexico are mainly used for meat production, especially for the Mexican dish known as 'barbacoa'. There is a growing interest in genetically improving ewe breeds with a focus on milk production for cheese, which is increasing in demand internationally. Among other things, this highlights the importance of understanding the lactation curve (LC) in ewes. The objectives of the present study were: a) to characterize the LC of Rambouillet ewes using Wood's model ($Y_i = at^b e^{-ct} + \epsilon_i$), estimating its three parameters (a , b , c) and other measures of interest: lactation peak (LP), lactation peak day (DLP), and lactation persistency (PER); b) assess the effects of the number of lambs (NL), the type of lambing (TL), and the weaning time of the offspring (WT) on Wood's model parameters and those of the LC; and c) estimate phenotypic correlations between Wood's model and the LC parameters. The results showed that the LC obtained was a 'typical' curve with parameters $a = 901.2$ mL, $b = 0.1206$, $c = -0.01299$, LP = 1045.1 mL, DLP = 9.2 d, and PER = 2.1. There were significant effects ($p \leq 0.05$) of WT on a and of WT and TL on PER; the highest mean of a (957.4) was obtained with weaning at eight weeks. On the other hand, the largest PER averages were found in single lambs and at six of weeks weaning. Phenotypic correlations ($p \leq 0.05$) were estimated between and within Wood's model and the LC parameters. The results of the present study support the recommendation of a milk production system for Rambouillet ewes to make cheese.

Keywords: milk production, mathematical functions, phenotypic correlations, persistence, non-genetic factors.

INTRODUCTION

Sheep are found throughout Mexico, especially in the central-northern region. The 'social sector,' characterized by having fewer than 30 animals, accounts for the greatest percentage of producers (Cuéllar *et al.*, 2012). Currently, sheep farming in the country is focused on the production of meat for the preparation of the traditional dish known

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as 'barbacoa,' using breeds such as Suffolk, Hampshire, Dorset, Charollais, Texel, Pelibuey, Blackbelly, Katahdin, and others. Due to the introduction of specialized breeds for milk production, such as East Friesian, Awassi, Lacaune, and Assaf, ewe milk production systems in Mexico are relatively new (Ángeles-Hernández *et al.*, 2018; Velarde-Guillén *et al.*, 2022). Therefore, information related to production indices and lactation characteristics, such as the lactation curve shape, lactation peak, and lactation persistence, is still limited (Ángeles-Hernández *et al.*, 2013, 2014). However, there is interest in implementing genetic improvement programs for ewes to increase milk production due to a growing market demand, especially for ewe's milk-based cheeses (Haenlein and Wendorff, 2006).

Mexico was a major producer of sheep wool, but because of the low fiber quality and low prices, wool production is no longer significant. In this sense, the Rambouillet breed had a crucial role in the production of wool at the national level; however, this production system was complicated due to the appearance of synthetic fibers, such as polyester, nylon, and linen. Since breeds like the Dorset, Rambouillet, and Suffolk in the USA have demonstrated good potential for milk production (Sakul and Boylan, 1992), Ochoa-Cordero *et al.* (2002) examined the production and composition of milk from Rambouillet ewes in Mexico in order to find a solution for milking ewes and using the milk to make cheese.

Understanding the lactation curve (LC) in ewes is crucial because it serves as a very significant indicator of milk production, in addition to allowing decisions to be made on aspects such as nutrition, health, and flock management. It is also useful for identifying and selecting ewes that are productively superior (Chang *et al.*, 2001). The LC represents the variation of milk production as a function of time and is represented either numerically on a time basis or graphically by postpartum diagrams (Keskin and Dağ, 2006) and is characterized by a mathematical function (Quintero *et al.*, 2007). LC studies have been previously carried out in Mexico, mainly in ewes specialized in milk production (Ángeles-Hernández *et al.*, 2013, 2018).

Ochoa-Cordero *et al.* (2002) concluded that the quality of milk from Rambouillet ewes suggests its use in cheese and yoghurt production in an attempt to obtain additional income from current production systems with this breed. However, they did not characterize the LC, which contains very important information such as lactation peak (LP, date of maximum milk production), lactation peak day (DLP), and lactation persistency (PER), all of which determine the shape of the LC. Therefore, the objectives of the present study were: 1) to characterize the lactation curve in a population of Rambouillet ewes using the Wood model (1967); 2) to evaluate the effect of non-genetic factors affecting the parameters of this model (a , b , c), LP, DLP, and PER; and 3) to calculate phenotypic correlations between Wood's model parameters, LP, DLP, and PER.

MATERIALS AND METHODS

Animals and management

The study was conducted using the milk production records of 45 Rambouillet ewes with one to three lambings, one or two offspring, and an average live weight of 65 ± 7.1 kg. The animals belonged to the sheep flock of the Faculty of Agronomy of the Autonomous University of San Luis Potosí. At an altitude of 1835 m, the climate at the site is cold and dry, with an average temperature of 17.8 °C and annual rainfall of 271 mm (García, 2004).

Ewes were confined and fed daily 3.5 kg of fresh alfalfa (*Medicago sativa* L.) and 1.7 kg of a commercial feed with 14 % crude protein and 2.96 Mcal kg^{-1} of digestible energy. The same diet was offered to the ewes in the morning (8:00) and the afternoon (13:00). Ewes were divided into two groups based on whether their offspring were weaned at six or eight weeks of age. Hand milking was done twice a day (8:00 and 13:00) beginning the first week postpartum, with no stimulus to allow milk let-down. During the lactation period, ewes were milked every seven days, with offspring separated from dams at 16:00 on the day before milking. After milking, the lambs were returned to their mothers. Further details on management are given in Ochoa-Cordero *et al.* (2002).

Statistical analysis

Characterization of the lactation curve

Individual ewe lactation averages were captured in a spreadsheet. Weekly milk production data were adjusted using the Wood model (1967):

$$Y_t = at^b e^{-ct} + \varepsilon_i$$

where Y_t is the milk yield obtained on day t ; a is a scaling factor or milk yield at the start of lactation; b is the slope of the curve in the rising phase; e is the natural logarithm; c is the slope of the curve in the falling phase; and ε_i is the random error. Although it overestimates milk production at the beginning of lactation and underestimates it near and after the LP (Dematawewa *et al.*, 2007; Dijkstra *et al.*, 2010), it is still the most widely used model to model lactation curves in ewes (Pollott and Gootwine, 2000; Nezamidoust *et al.*, 2013; Ángeles-Hernández *et al.*, 2013).

Following a logarithmic transformation, the model was analyzed linearly as follows:

$$Y_t = \ln a + b \ln t - ct$$

Subsequently, the parameters a , b , and c were estimated by multiple linear regression. According to Nezamidoust *et al.* (2013) and taking the averages of all ewes, the lactation peak (LP) was estimated as $a (b/c)^b e^{-b}$, while the lactation peak day (DLP) was calculated as b/c .

Wood's model parameter estimation

In addition, the persistency of lactation (PER), which is a phase of lactation that indicates the ability of an ewe, cow, or goat to continue producing milk after reaching peak production, was included in the statistical analysis (Swalve and Gengler, 1999; Cobuci *et al.*, 2003). The PER was estimated using the Wood method (1968) as follows:

$$PER = - (b + 1) \ln c$$

With this model, PER values are expressed without units of measurement. However, it is understood using a specific regression coefficient (Madsen, 1975), percentages (Sölkner and Fucks, 1987), proportions based on milk production during a specific lactation period in relation to a previous one (Sölkner and Fucks, 1987), random regression genetic coefficients based on a random regression model (Cobuci *et al.*, 2007), or grams per day associated with a rate of decline in milk production during the LC decline (Rojo-Rubio, 2016). In addition, lactation curves were also estimated according to the effects of the number of lambs (NL), the type of lambing (TL), and the weaning time of the offspring (WT).

Analysis of variance for non-genetic effects on total parameters

To evaluate the effect of non-genetic factors on Wood's model parameters (a , b , c) and lactation curve parameters (LP, DLP, PER), the following fixed effects model was used:

$$Y_{ijklmno} = \mu + NL_i + TL_j + WT_k + LP_l + DLP_m + PER_n + \varepsilon_{ijklmno}$$

where $Y_{ijklmno} = a, b, c, LP, DLP, PER$; μ is the overall mean; NL_i is the effect of the i -th number of lambs ($i = 1$ to 3); TL_j is the effect of the j -th type of lambing ($j =$ single, double); WT_k is the effect of the k -th weaning time ($k = 6$ or 8 weeks); LP_l is the effect of the l -th lactation peak ($l = 1$ to 45); DLP_m is the effect of the m -th day of lactation peak ($m = 1$ to 45); PER_n is the effect of n -th persistence ($n = 1$ to 45); and $\varepsilon_{ijklmno} =$ random error \sim NID ($0, \sigma^2$). Double interactions between these variables were initially tested, but none showed statistical significance ($p > 0.05$) and do not appear in the final model. Finally, a Pearson correlation analysis was carried out between the parameters of the Wood's model (a, b, c) and those of the lactation curve (LP, DLP, PER). All analyses were carried out using R-Studio software (R Core Team, 2020).

RESULTS AND DISCUSSION

Characterization of the lactation curve

After fitting the ewe milk production data, the LC was obtained (Figure 1), where the average values of the Wood's model parameters were $a = 901.2$ mL, $b = 0.1206$, and $c = -0.01299$, with $R^2 = 0.66$. On the other hand, LP, DLP, and PER values were 1045.1 mL, 9.2 d, and 2.1, respectively.

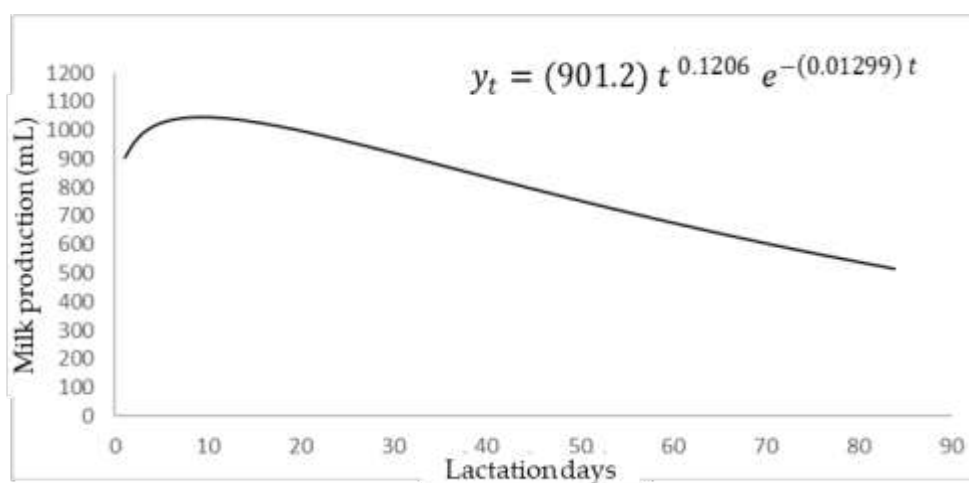


Figure 1. Lactation curve of Rambouillet ewes (*Ovis aries* L.) fitted with the Wood model (1967).

The LC obtained was a typical curve (Ángeles-Hernández *et al.*, 2021) as found in non-dairy ewes, including Merino (Groenewald *et al.*, 1996), Makui (Nezamidoust *et al.*, 2013), Pelibuey (Rodríguez-Álvarez *et al.*, 2021), and St. Croix (Merchant *et al.*, 2021) breeds. Atypical LCs, which are caused by nutrition, animal health, and environmental disturbances, are also reported in the literature (Macciotta *et al.*, 2008). These have been found in F_1 ewes, daughters of Dorset sire and Rambouillet dam (Sakul and Boylan, 1992), and Rambouillet, Suffolk, and Dorset F_1 ewes, daughters of Suffolk sire (Cardellino and Benson, 2002).

In relation to the parameters of Wood's model, it is important to understand the meaning of the combination of signs in parameters b and c , as this provides a clear understanding in relation to the shape of the LC. According to Macciotta *et al.* (2005), when there is a combination of $b > 0$ and $c < 0$, a typical curve is obtained; the combination $b < 0$ and $c > 0$ provides a reverse shape, characterized by an initial phase of decrease to a minimum, followed by an increase; the combination of $b > 0$ and $c > 0$ represents a continuously increasing curve, while the combination $b < 0$ and $c < 0$ represents a continuously decreasing curve.

The literature reports wide variation in mean LP and DLP in ewes. Sakul and Boylan (1992) found LP = 1200 mL and DLP = 14 d in F₁ non-dairy ewes using Wood's model; Groenewald *et al.* (1995) a LP = 1828 mL and DLP = 19 d in Merino ewes using Morant's model; Pollott and Gootwine (2000) a LP = 3.31 L and DLP = 25 d in dairy Awassi ewes using the multiplicative model proposed by Pollot; Dağ *et al.* (2005) a LP = 930 mL and DLP = 60 d in dairy Awassi ewes using the quadratic model; Dağ *et al.* (2005) a LP = 1620 mL and DLP = 10 d in dairy Awassi ewes using the inverse polynomial model; Ángeles-Hernández *et al.* (2013) a LP = 650 mL and DLP = 37 d in F₁ dairy ewes using Wood's model; Miguel *et al.* (2016) a LP = 1337 mL and DLP = 26 d in Ojalada dairy ewes using the Wilmlink model; and Velarde-Guillén *et al.* (2022) a LP = 1.2 kg and DLP = 42 d in F₁ dairy ewes using random regression models with orthogonal polynomials of fifth order.

As for PER, information is limited in ewes. The average PER obtained in the present study was 2.1, which could be interpreted as a moderate persistence. This value is lower than that reported by Torres-Hernández and Hohenboken (1980) in F₁ Hampshire crossbred ewes, which was 5.1 using Wood's model. In lambing, Dairymeade ewes from New Zealand, Marshall *et al.* (2023) estimated PER using a different methodology, where the first lambing ewes had PER = 61.5 %, a value higher ($p \leq 0.05$) than the second (53.7 %), third (54.5 %), and fourth (54.2 %) lambing ewes. Akpa *et al.* (2001), with Red Sokoto goats from Nigeria, found a PER (defined as: $c^{-(b+1)}$) equal to 143.2. In Ecuador, Pesántez *et al.* (2014) found a PER = 69.4 % in F₁ Anglo-Nubian x Criollo goats, a value higher than that obtained by Méndez *et al.* (2019) with Alpine goats from Ecuador, which was 53.6 %. Rojo-Rubio *et al.* (2016) used averages of the rate of decline in milk production during the LC decline phase, obtaining PER averages of 10.7, 8.8, and 14.4 g d⁻¹ in Alpine, Nubian, and Saanen goats, respectively, with the Alpine and Nubian averages being different ($p \leq 0.05$) from the Saanen average.

Differences in LC shape, LP, DLP, and PER values, in addition to ewe genotype, model used to perform the curve fitting, and sample size used, are also subject to the effect of non-genetic factors known to influence milk production, such as milk production time (Carta *et al.*, 1995), milk production level and type of lambing (Cappio-Borlino *et al.*, 1995), number of lambs (Cappio-Borlino *et al.*, 1997a), herd and feeding (Cappio-Borlino *et al.*, 1997b), production year and lactation length (Méndez *et al.*, 2019; Marshall *et al.*, 2023); and climatic factors such as temperature, humidity, wind speed, and radiation (Silanikove, 2000; Naskar *et al.*, 2012).

Non-genetic effects on parameters

The number of lambs (NL), the type of lambing (TL), and the weaning time (WT) showed significant effects ($p \leq 0.05$) on parameters a , b , c , LP, DLP, and PER (Table 1). WT had an impact on a , while TL and WT had an effect on PER. The highest average value for a , according to WT, was at eight weeks of weaning (Table 2). The fact that parameter a had no effect ($p > 0.05$) on single and double lambing ewes could be attributed to the number of offspring suckling that explains milk production, not the

Table 1. Summary of the analysis of variance with mean squares for the test of parameters according to the effects of the number of lambs (NL), the type of lambing (TL), and the weaning time of the offspring (WT) of Rambouillet ewes (*Ovis aries* L.).

	df	a	b	c	LP	DLP	PER
NL	2	54 554.3	0.011	0.000	81 409.5	276.9	0.017
TL	1	24 738.5	0.002	0.003	9820.5	2.04	0.248*
WT	1	203 700.7*	0.000	0.002	138 343.9	669.8	0.334*

df: degrees of freedom; a: milk yield at the start of lactation; b: slope of the curve in the rising phase; c: slope of the curve in the falling phase; LP: lactation peak; DLP: lactation peak day; PER: lactation persistency. * $p \leq 0.05$.

Table 2. Least-squares means of the parameters evaluated according to effects of the number of lambs (NL), the type of lambing (TL), and the weaning time of the offspring (WT) of Rambouillet ewes (*Ovis aries* L.).

	Effect	n	a	b	c	PL	DPL	PER
NL	1	18	897.2±63.6	0.102±0.03	0.011±0.002	1021.2±59.7	17.1±7.9	2.18±0.05
	2	11	955.7±59.2	0.151±0.02	0.016±0.001	1148.8±32.1	9.2±1.2	2.09±0.04
	3	16	847.3±46.9	0.138±0.03	0.026±0.013	1039.5±67.3	9.7±2.2	2.03±0.10
TL	Single	24	888.9±39.1	0.126±0.02	0.013±0.001	1065.2±34.4	13.2±4.2	2.15±0.03a
	Double	21	910.7±67.6	0.129±0.05	0.034±0.021	1043.8±95.7	10.3±1.8	1.94±0.16b
WT	6 weeks	35	838.0±49.2b	0.128±0.02	0.012±0.001	1011.1±56.0	5.9±5.9	2.19±0.04a
	8 weeks	10	957.4±41.7a	0.126±0.02	0.024±0.010	1116.7±34.3	8.6±1.8	2.02±0.07b

n: sample size; a: milk yield at the start of lactation; b: slope of the curve in the rising phase; c: slope of the curve in the falling phase; LP: lactation peak; DLP: lactation peak day; PER: lactation persistency. Different literals between rows and within variables indicate statistical significance ($p \leq 0.05$).

number of offspring born (Peart, 1967; Corbett, 1968). However, milk production in ewes is known to be higher in the case of double lambings (Figure 2) (Abecia and Palacios, 2017; Rosales-Nieto et al., 2018).

In Yankasa ewes, Afolayan et al. (2002) found an effect of the type of lambing ($p \leq 0.05$) on parameters b and c, where the highest averages were found for single lambing ewes. Significant effects ($p \leq 0.05$) of other non-genetic effects on a, b, and c, such as the age at lambing (Carta et al., 1995), milk production level, udder health (Cappio-Borlino et al., 1997a), flock, year, and month of lambing (Ruiz et al., 2000), milking method (Nezamidoust et al., 2013), and lambing time (Ángeles-Hernández et al., 2013), have also been found in other ewe breeds.

For the PER variable, the highest average in TL was obtained for single lambs. This result is consistent with the findings of Ruiz et al. (2000) in Latxa ewes. Furthermore, the highest average in WT was found in the six-week weaning (Table 2). Afolayan et

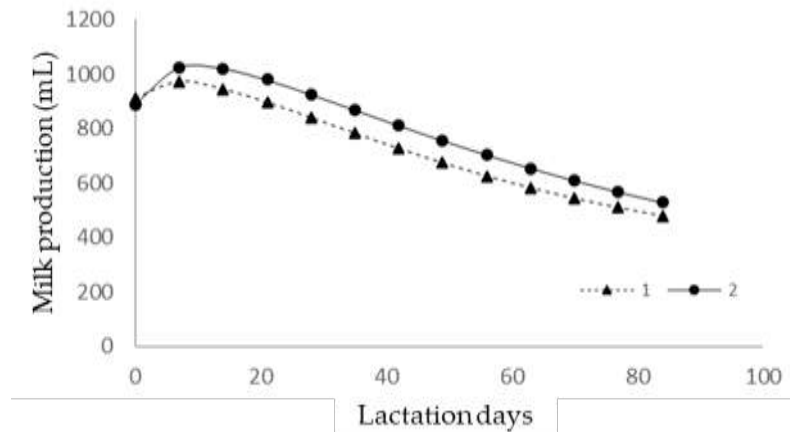


Figure 2. Lactation curve of Rambouillet ewes (*Ovis aries* L.) according to the type of lambing. 1: single lambing; 2: double lambing.

al. (2002) found that parameters a , b , and c , in addition to PER, were affected ($p \leq 0.05$) by the lactation length (84 days of lactation against less than 84 days), finding that the highest averages were in a and PER for the 84-day lactation, while the lowest values were found for b and c . The result for parameter a coincides with the present study but is opposite for PER (Figure 3).

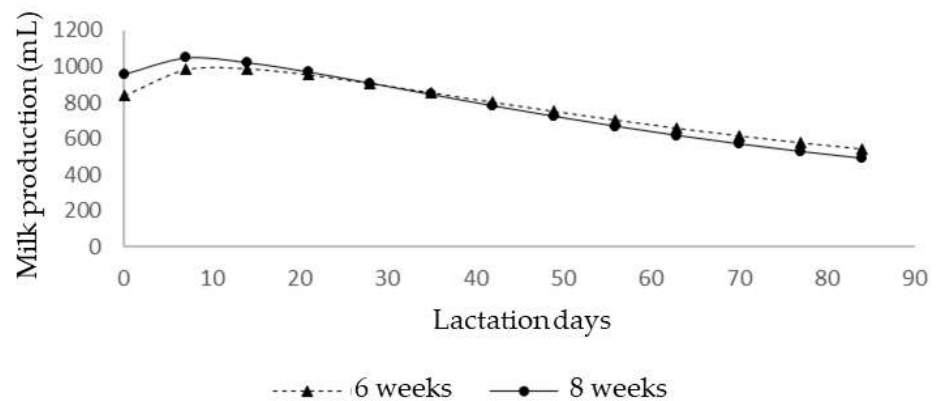


Figure 3. Lactation curve of Rambouillet ewes (*Ovis aries* L.) according to the weaning time of the offspring (six and eight weeks).

Correlation analysis

The correlation $a-b = -0.38$ ($p \leq 0.05$) indicates that curves showing high milk production at the beginning of lactation have a slow growth rate in the slope to reach LP (Table 3). On the other hand, the correlation $b-c = 0.31$ ($p \leq 0.05$) indicates that curves with a

Table 3. Matrix of phenotypic correlations between Wood’s model parameters (*a*, *b*, *c*) and lactation curve parameters (LP, DLP, PER).

Parameter	<i>a</i>	<i>b</i>	<i>c</i>	LP	DLP	PER
<i>a</i>	1.00	-0.38**	0.17 ^{ns}	0.54**	-0.30*	-0.45**
<i>b</i>		1.00	0.31*	0.42**	-0.01 ^{ns}	-0.04 ^{ns}
<i>c</i>			1.00	0.08 ^{ns}	-0.13 ^{ns}	-0.82**
LP				1.00	-0.42**	-0.12 ^{ns}
DLP					1.00	0.33*
PER						1.00

a: milk yield at the start of lactation; *b*: slope of the curve in the rising phase; *c*: slope of the curve in the falling phase; LP: lactation peak; DLP: lactation peak day; PER: lactation persistency. * $p \leq 0.05$, ** $p \leq 0.01$; ns: not significant ($p > 0.05$).

high growth rate in slope during their ascent to the LP have a moderate rate of decline in slope after reaching the LP. The correlation between parameters *a* and *c* was not significant ($p > 0.05$).

The correlations *a-b* and *b-c* of the present study are in agreement with the results of Ángeles-Hernández *et al.* (2013, 2014) using dairy ewes in an organic production system in Mexico. However, the result regarding non-correlation $a-c = 0.17$ ($p > 0.05$) of the present study differs from these same authors, since they obtained values of -0.36 and -0.41 ($p \leq 0.01$), which means that ewes selected for high milk production at the beginning of lactation have a high PER in milk production. However, Ruiz *et al.* (2000) obtained a correlation of 0.46 ($p \leq 0.01$) between *a* and *c* in Latxa dairy ewes, which indicates the opposite of a negative correlation between both parameters, i.e., ewes with a high production at the start of lactation show a low PER in milk production.

It is possible that the lack of statistical significance of the correlation between *a* and *c* in this study is due to the sample size used. Non-significant correlations ($p > 0.05$) between parameters *a* and *c* were also reported by Groenewald *et al.* (1995) in Merino ewes and by Chang *et al.* (2001) in dairy ewe flocks crossed with meat and wool ewes. No results of comparable correlations in ewes between Wood’s model parameters (*a*, *b*, *c*) and LC parameters (LP, DLP, PER) were found in the literature. Waheed and Khan (2013) obtained correlations $a-PL = 0.87$ and $a-DPL = -0.85$ ($p \leq 0.01$) in Beetal goats, indicating that curves with high milk production at early lactation reach a large LP at an early date. These are similar results to those obtained by Shaat (2014) in Zaraibi goats from Egypt, with significant correlations $a-LP = 0.97$ and $a-DLP = -0.56$ ($p \leq 0.05$). In the study with Zaraibi goats (Shaat, 2014), correlations $b-DLP = 0.35$ and $b-PER = -0.33$ ($p \leq 0.05$) were also found, indicating that curves with a high growth rate during the upward slope reach the LP at an early date but have a low PER. In another study with local goats in Tunisia, Atoui *et al.* (2024) also obtained a correlation $b-LP = 0.6$ ($p \leq 0.05$), indicating that curves with a high growth rate in their slope during ascent have a large LP.

Regarding correlations between LC parameters, the LP-DLP = -0.42 ($p \leq 0.01$) correlation means that, on average, curves with a larger LP take less time to reach it. This result is in agreement with Velarde-Guillén *et al.* (2022) in F₁ dairy ewes (mainly ½ Assaf, ½ Awassi, ½ East Friesian, and ½ Lacaune) from Mexico and Shaat (2014) in Zaraibi goats from Egypt, with values of -0.27 and -0.33 ($p \leq 0.05$), respectively. However, it differs from the result obtained by Atoui *et al.* (2024), who reported a correlation of 0.86 ($p \leq 0.01$) between these same parameters. The DLP-PER = 0.33 ($p \leq 0.05$) correlation indicates that curves with a larger PER take longer to reach the LP. This is consistent with the results obtained by Portolano *et al.* (1997) in Comisana ewes from Italy, Shaat (2014) in goats, and Atoui *et al.* (2024) in goats, who found values of 0.88, 0.58, and 0.57 ($p \leq 0.01$), respectively.

CONCLUSIONS

The use of Wood's model allowed the characterization of the lactation curve of Rambouillet ewes, resulting in a typical curve that has an average initial milk production of 901.2 mL and a lactation peak of 1045.1 mL, which is reached 9.2 days into lactation, and a lactation persistency of 2.1, which can be interpreted as moderate. Parameter *a* (milk yield at the start of lactation) of Wood's model was affected ($p \leq 0.01$) by the weaning time of offspring, while the type of lambing and the weaning time of offspring influenced ($p \leq 0.05$) variation in lactation persistency. Phenotypic correlations ($p \leq 0.05$) were estimated within and between the parameters of Wood's model and the lactation curve, providing information that provides insight into peculiar aspects of the lactation curve itself and may be useful for general flock management. Although more research is needed on this subject, and given the high quality of the milk from these ewes, these findings are encouraging enough to recommend a milk production system aimed at cheese production in Rambouillet ewes in Mexico, allowing producers to have another remunerative option, as they cannot economically survive with the current wool production system.

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Agrociencia

COMPUTATIONAL FLUID DYNAMIC MODEL TO SIMULATE THE DISPERSION OF ATMOSPHERIC NH₃ GENERATED BY A COWSTABLE IN LA LAGUNA, MEXICO

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ABSTRACT

Stalls are one of the main causes of ammonia (NH₃) buildup. These harmful gases have increased their concentration in the air in recent years. However, there are few studies on their dispersion to the atmosphere, since they constitute a passive polluting system in livestock areas. Therefore, the objective of this research was to evaluate the distribution of NH₃ pollutants concerning temperature, humidity, and airflow in a stable, through a computational fluid dynamics model and its analysis using the K2 algorithm. The model consisted of simulated 20 production units with similar environmental conditions and took the initial values from data obtained from a nearby weather station in a region characterized by semi-arid climatic conditions. The highest concentration of NH₃ was obtained under a wind velocity of 0 ms⁻¹ due to the stagnation of the pollutant. The results indicated that levels of only 0.48 ms⁻¹ reduce the concentration of NH₃ by 13 %. The results were analyzed using the K2 algorithm to obtain the relationship and inferences between NH₃ emissions, air flow velocity, ambient temperature, and humidity. The analysis of the CFD approach using the K2 algorithm concluded that temperature (85 %) and humidity (98 %) are the main variables that influence the pollution produced by the distribution of NH₃ to the environment derived from livestock activity.

Keywords: Ammonia dispersion, Livestock emissions, Environmental variables, CFD, K2 algorithm, models.

INTRODUCTION

Weather conditions have a decisive influence on the dispersion of pollutants emitted into the atmosphere and affect chemical reactions (Vira *et al.*, 2022). Wind disperses pollutants and transports them away from its point of emission, while its direction relates to the area of affectation (Wang *et al.*, 2022).

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Ammonia (NH_3) at high concentrations, causes throat irritation, lung inflammation, damage to the respiratory tract, and eyes. As the concentration increases, it can produce pulmonary edema. At 50 ppm it causes stress in cattle, inducing low dry matter intake, embryonic deaths due to inhalation poisoning, and low milk or meat production, among others. According to Erisman *et al.* (2008), inefficiencies in the farming system result in large N losses to the environment, including NH_3 emissions to the atmosphere (Houlton *et al.*, 2012). Agricultural activities are the world's largest source of NH_3 in the atmosphere (Bouwman *et al.*, 1997). The stables are one of the main causes that produce accumulation of ammonia and increase in humidity, these harmful gases increase their concentration in the air if the ventilation is deficient. An ammonia level of > 20 ppm is sufficient to cause damage to the respiratory epithelium, the longer the exposure, the greater the damage caused to the mucociliary system.

Atmospheric NH_3 has undesirable consequences. It contributes to the formation of aerosols with implications for air quality (Aneja *et al.*, 2009) and climate change (Adams *et al.*, 2001). Its transport and eventual deposit in non-agricultural ecosystems can affect carbon fixation (Pinder *et al.*, 2012), eutrophication (Grizzetti, 2011), and biodiversity (Bobbink *et al.*, 2010). An annual cost of 18-140 billion USD for NH_3 emissions in the European Union, mainly due to increased mortality associated with aerosols. Without major changes to the current food system, the projected global increase in food demand 70 % by 2050, Conforti (2011) will be accompanied by higher NH_3 emissions (Erisman *et al.*, 2008).

Agricultural NH_3 emissions have proven difficult to estimate over space and time, the resolution required for atmospheric models. Detailed models of nitrogen flux in agricultural systems have been developed for livestock operations (Li *et al.*, 2012) and mineral fertilizers (Cooter *et al.*, 2012). These models require detailed knowledge of local environmental conditions and farming practices that are generally not available. Global NH_3 emission inventories depend on source-specific emission factors, which explain regional practices (Faulkner and Shaw 2008). Recent US National Emissions Inventory (NEI) assessments compiled by the US Environmental Protection Agency (EPA) show good agreement with observations at the national scale (Zhang *et al.*, 2022) but large discrepancies at the regional scale (Fisher *et al.*, 2011). Uncertainties in NH_3 emissions contribute to large errors in simulated ammonium nitrate, which constitutes a significant fraction of the aerosol (Zhang *et al.*, 2020).

NH_3 emissions into the atmosphere affect human health, the climate, and ecosystems, due to the formation of secondary aerosols. Despite many studies, there is inadequate knowledge of agricultural sources (Liu *et al.*, 2022; Luo, 2022; Viatte *et al.*, 2022; Vira *et al.*, 2022; Wang *et al.*, 2022). The increase in world demand for food causes an increase in the concentration of NH_3 produced by dairy farms. Liu *et al.* (2022) established that the punctual emissions of NH_3 from livestock in 2020 ranged between 16.8 and 126.6 kg N ha⁻¹, with an average emission of 42.0 kg N ha⁻¹, with an increasing trend of 5.8 % annually between 2008 - 2020, mainly in spring and summer. From 2008 to 2018, there have been significant increases in global NH_3 emissions in India at 13 %, tropical

Africa at 33 %, and South America at 18 % with the intensification of agricultural activity (Luo *et al.*, 2022).

It is essential to determine the concentration and distribution of gas emissions such as NH₃, which is of increasing importance due to its effect on the health and productivity of animals and workers (Chmielowiec-Korzeniowska *et al.*, 2018; Qin *et al.*, 2020; Tabase *et al.*, 2020; Zhang *et al.*, 2020; Gautam, 2021; Kim *et al.*, 2022; Zhang *et al.*, 2022). NH₃ emissions depend on the design and operation of the barn, as well as manure management (Heidarzadeh-Vazifehkhora *et al.*, 2022). Computer models to simulate the generation and dispersion of gases within the stables constitute an effective strategy for contaminant mitigation.

Computational Fluid Dynamics (CFD) is a versatile technique in those multiple areas of knowledge that have contributed to a comprehensive understanding of the complexity of biological systems and had relevance since the end of the 20th century (Rong, 2016; Ben, 2021). CFD became a tool by combining the fundamentals of physics, chemistry, and biology (Ivanov *et al.*, 2022). Currently, it is possible to couple CFD to systems based on Artificial Intelligence (Bournet and Rojano, 2022; Xin *et al.*, 2022) like the K2 algorithm. The K2 algorithm was developed in the field of artificial intelligence, for approximate reasoning that defines the relationships between multiple variables (De la Torre-Gea, 2014). This work aimed to evaluate the distribution of NH₃ flow generated within a cow stable using a CFD model and its relationship with environmental conditions temperature, humidity, and airflow through probabilistic analysis using the K2 algorithm.

MATERIALS AND METHODS

For this study, it was established the source of contamination as a stable located in the region of La Laguna, Coahuila, Mexico, close to Torreón city, which consists of 20 stables with similar environmental conditions. The results obtained regarding quantity of NH₃ from the approach were considered to establish the source of dispersion pattern of NH₃ to provide an idea of the environmental problem suffered by the region.

By developing a CFD-based methodology, the distribution of atmospheric NH₃ produced by dairy cow barns located in the La Laguna region, which includes the states of Coahuila and Durango, Mexico, was studied; whose geographical coordinates are: 25° 41' 33" North latitude, and 103° 23' West longitude, at an altitude of 1120 m above sea level, during June 15 and 22, 2019, in a region highly characterized by hot weather conditions during the summer that causes environmental pollution.

The stable components were modeled in 3D using the Ansys Geometry version 14.0, commercially available, to obtain the mesh of the modeled objects. The same software tool made it possible to set boundary conditions and initial values of the CFD model (Table 1). Finally, the Ansys Fluent software, simulated ammonia generation. The initial conditions: wind velocity, maximum temperature, and humidity were taken from the corresponding records from June 15 to 22, 2018, 2019, and 2020 from the

Table 1. Boundary conditions and initial values of the CFD model.

Solution	Simulation 3D	Doble precision
Model type	Steady-state	
Mesh type	Automatic Patch	Conforming/Sweeping
Minimum element size	0.20 m	
Number of elements	1,652,901	
Viscosity	K - e with buoyancy	
Energy equation	Active	
	Domain entry	
Velocity inlet	0.15 m s ⁻¹	
Turbulent kinetic energy	1.00 J	
Air temperature	315.00K	
Species	Active	
NH ₃ concentration	100 %	
RH	100 %	
Domain exit	Outlet pressure	1.00 Pa
Kinetic energy	1.00 J	
Solar radiation	900.00 W m ⁻²	
Diffuse solar radiation	400.00 W m ⁻²	
Solar calculator	Active	
Material Physical properties	Air	Soil
Density (kg m ⁻³)	1.22	1,400.00
Specific heat of the air (J kg ⁻¹ K ⁻¹)	1,006.43	1,738.00
Thermal conductivity (W m ⁻¹ k ⁻¹)	24.20 e ⁻³	1.50
Coefficient of thermal expansion (K ⁻¹)	3.39 e ⁻³	
Airborne gases	NH ₃	Water steam
Thermal conductivity (W m ⁻¹ k ⁻¹)	0.04	0.04
Viscosity (kgm ⁻¹ s ⁻¹)	1.72 e ⁻⁰⁵	1.72 e ⁻⁰⁵
Mass diffusivity (kg m ⁻¹ s ⁻¹)	2.88 e ⁻⁵	2.88 e ⁻⁵
Thermal diffusion coefficient (kg m ⁻¹ s ⁻¹)	-8e-06 t + 6 e-05	-2.50 e ⁻³ + 0.13

nearest meteorological station called “El Cuije” in Mexico located at 1,033 m from the stable, whose geographical coordinates are: 25° 41’ 24” North, 103° 20’ 22” West.

1. The proposed methodology to develop the CFD model was in three stages:
2. Continuous flow discretization: Field variables consisted of a number finite of values at points called nodes.
3. Discretization of the equations of motion based on the values of the nodes.
4. System solution of algebraic equations and obtaining the values of the variables in all the nodes.

Each production unit in the stable was 60 m long and 40 m wide, and the height was 10.8 m at the eaves and 12 m at the top (Figure 1). The longitudinal axis of the

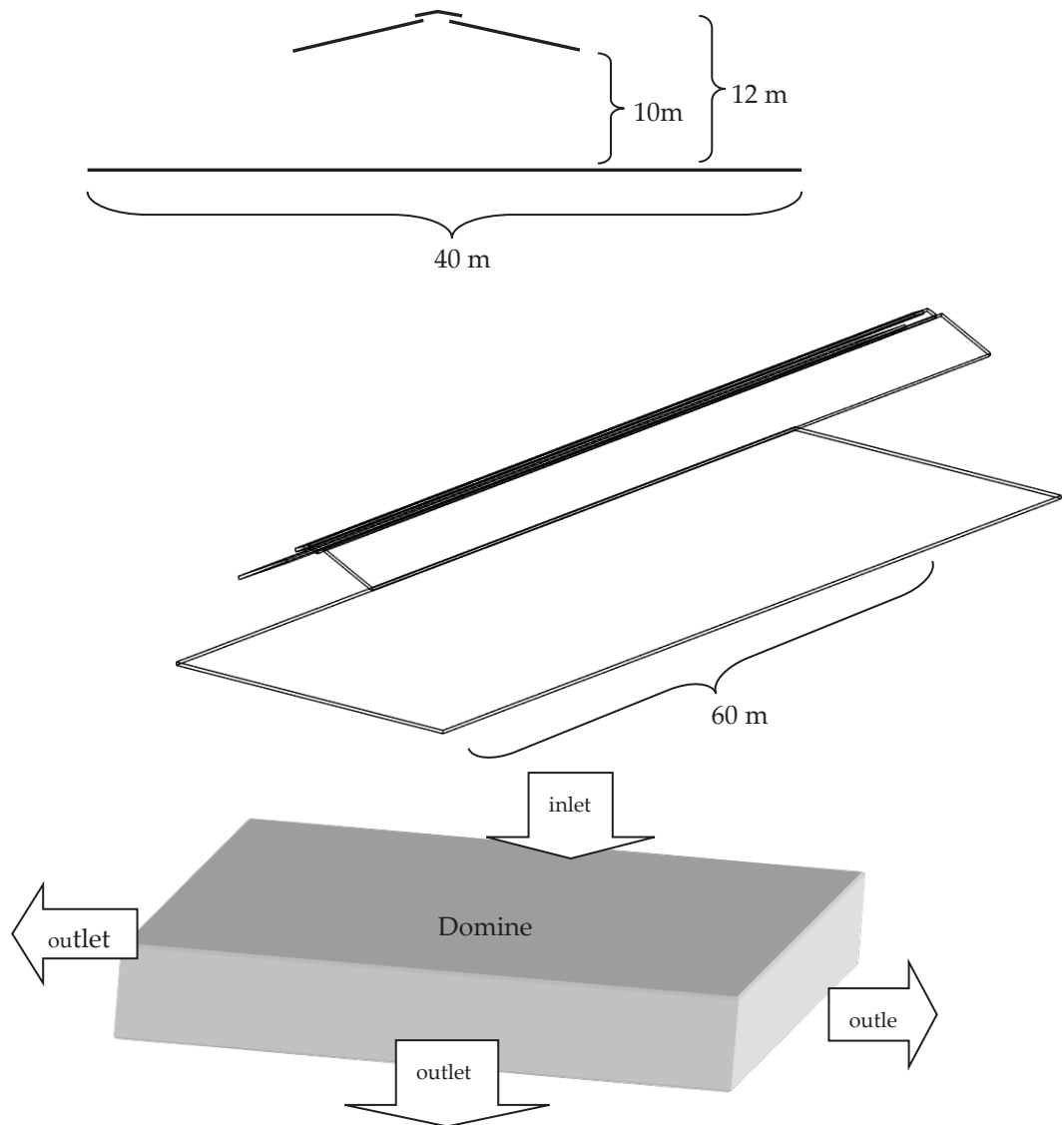


Figure 1. Unit production dimensions and domine.

domain is in the east-west direction, and three sides are completely open, in boundary conditions of 100 m long by 100 m wide and 40 m high.

From the CFD model, a sample of 11,952 records was taken in a vertical plane, at a distance of 40 m from the domain entrance (1/2 the length of the barn), with data on temperature, NH_3 concentration, relative humidity, and height. With them, a database was created and discretized in 5 intervals for each variable, between 300 K and 315 K, 0 to 100% NH_3 and Relative Humidity, and 0 to 40 m of height.

CFD model was validated by analyzing two data sets of temperature; the first from 24 daily measurements from the “El Cuije” weather station: in July 2021 and the second

from data calculated from the CFD model for the same dates (Figure 2). The standard error was computed, finding only 1.49 % of variation between the data set and CFD data, with a standard deviation equal to 1.93 K, which means that the approximation by the CFD model is acceptable (Rico-García *et al.*, 2011).

The variables were defined in a discrete domain, and the functional relationships described the causal inferences expressed in terms of conditional probabilities (Equation 1) using the most common method of Machine learning called the K2 algorithm (De la Torre-Gea *et al.*, 2014).

$$f(i, \pi_i) = \prod_{j=1}^{q_i} \frac{(r_i - 1)!}{(N_{ij} + r_i - 1)!} \prod_{k=1}^{r_i} \alpha_{ijk} \quad (1)$$

Where:

π_i : a data set of NH_3 of node x_i

$q_i = |\varphi_i|$

φ_i : list of all possible instantiations of the variables of x_i in database D. That is, if p_1, \dots, p_s

are the parents of x_i , then φ_i is the Cartesian product $\{v_{p_1}^1, \dots, v_{p_1}^{r_{p_1}}\} \times \dots \times \{v_{p_s}^1, \dots, v_{p_s}^{r_{p_s}}\}$ of all the possible values of attributes p_1 through p_s .

$r_i = |V_i|$

V_i : list of all possible values of the attribute x_i

α_{ijk} : number of cases in D in which the attribute x_i is instantiated with its k^{th} value, and the variables of x_i in π_i with the j^{th} instantiation in φ_i .

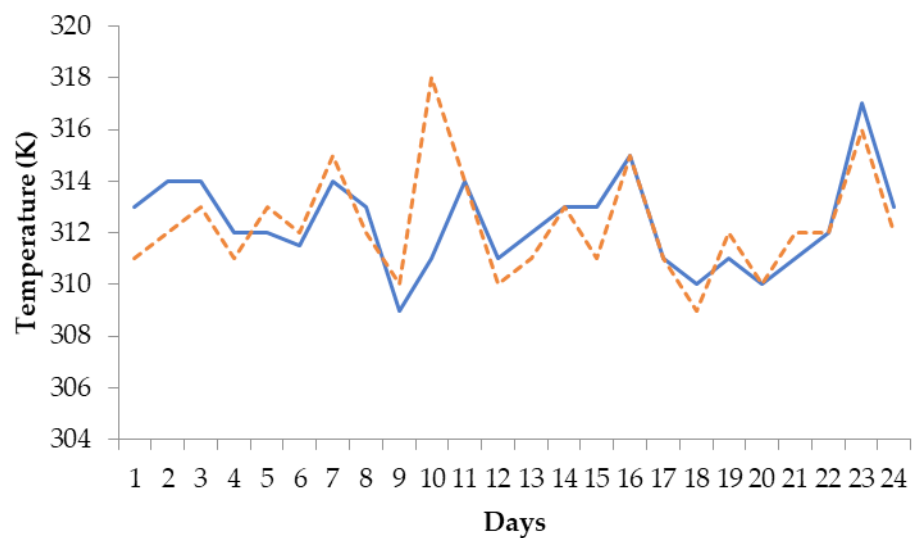


Figure 2. CFD model validation: weather station data solid line, CFD data dotted line.

$N_{ij} = \sum_{k=1}^{\pi_i} \alpha_{ijk}$. That is, the number of instances in the database in which the variables of x_i in π_i is instantiated with the j^{th} instantiation in φ_i .

RESULTS AND DISCUSSION

The results obtained from the CFD simulation show a behavior where the mass of NH_3 (%) depends on the airflow patterns related to the ventilation systems. The highest concentration of NH_3 occurs in the center of the barn at 0.1 m height, where the mass of NH_3 (%) depends on the airflow patterns related to the ventilation systems, these results agree with Rong and Aarnink (2019), Gautam (2021), and He *et al.* (2022) indicate.

According to studies previously cited by Doumbia *et al.* (2021), the highest concentration of NH_3 was found in high-temperature conditions (315 K) and is the main influencing parameter. The maximum temperature of 308 K improves environmental conditions and significantly reduces the dispersion of NH_3 , as indicated by Doumbia *et al.* (2021), Chen *et al.* (2021), and Ahmadi *et al.* (2022).

On the other hand, the highest concentration of NH_3 was obtained under conditions of air velocity of 0 ms^{-1} due to the stagnation of the pollutant. Therefore, levels of only 0.48 ms^{-1} reduce the concentration of NH_3 by 13 %, improving environmental conditions, as previously cited works point out (Doumbia *et al.*, 2021; Chen *et al.*, 2021; Ahmadi *et al.*, 2021; al., 2022).

NH_3 emissions are generated from the 0.1 m boundary layer without airflow. Figures 3 and 4 show the decrease of the contaminant as its dispersion towards the highest layers and wind velocity increases.

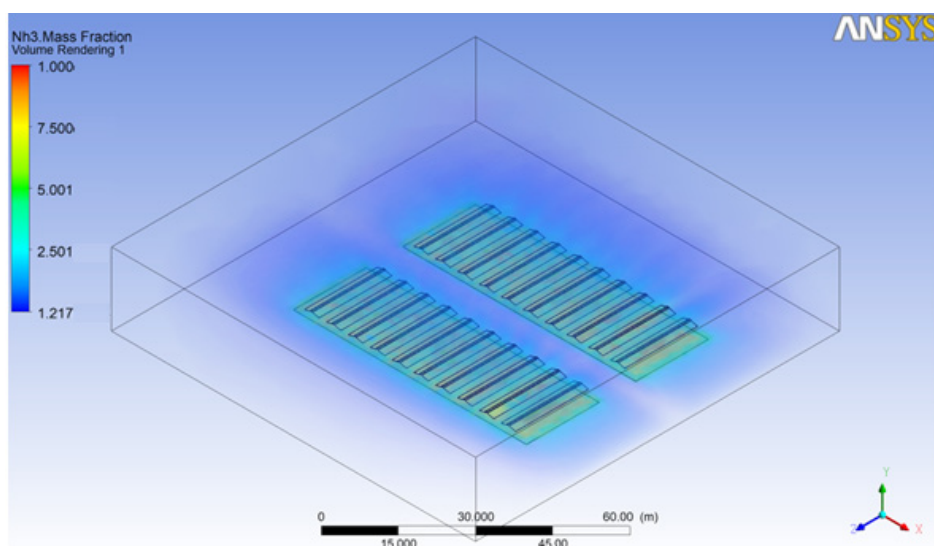


Figure 3. CFD model showing the dispersion of NH_3 concentration.

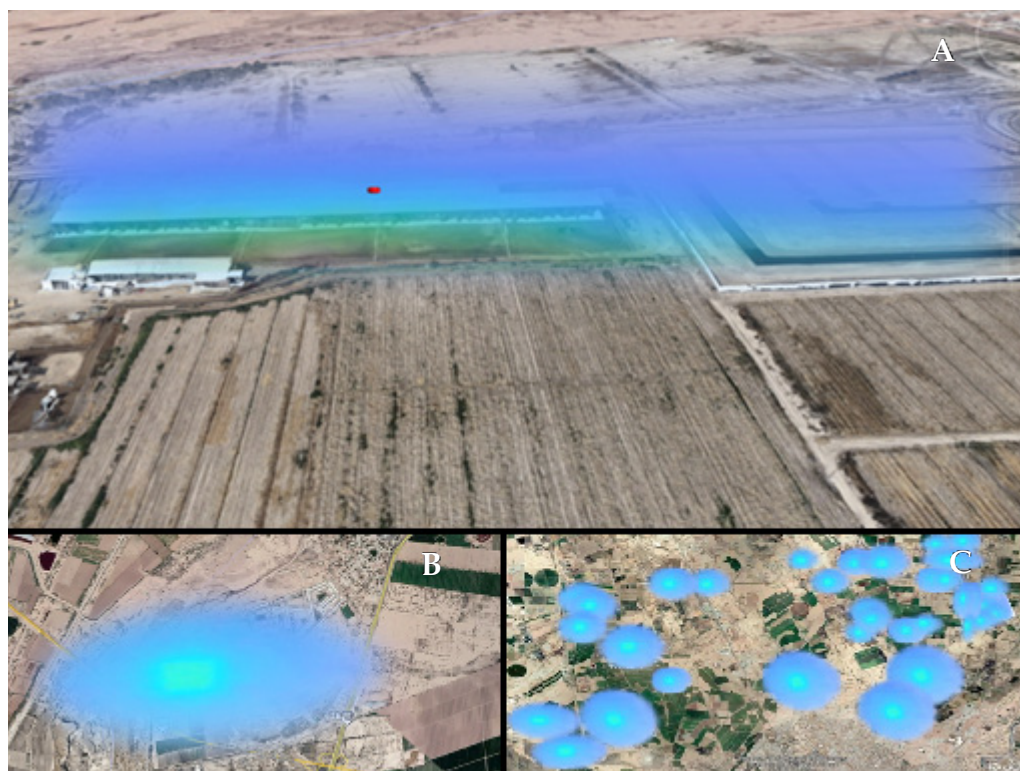


Figure 4. Transposition of the CFD simulation of the dispersion of NH_3 , A: next to a residential community; B: in the location of the stable of La Laguna, Mexico; C: Dispersion of NH_3 from 30 stables conurbation to Torreón city, from 340 registered in La Laguna, Coahuila, Mexico.

Long-term measurements of ammonia concentrations were carried out at three different sites typical of urban, rural, and industrial areas in the La Laguna region of Mexico. NH_3 at the rural site shows a single peak of 14.9 ppm at midday, due to temperature-favored volatilization of agricultural emissions. Showing higher NH_3 levels in summer, ambient temperature was the common determining parameter of atmospheric NH_3 levels, as indicated in Figures 3 A to C.

The NH_3 emissions they generate from the limit layer of 0.1 m without airflow. Figure 5A shows the decrease in the pollutant as its dispersion increases towards the highest layers and wind velocity.

Wind velocity is an important factor in the distribution of NH_3 concentration. Figure 5B shows the effect of the eaves of the barn on the airflow, which causes vertical stagnation zones between 0 to 5 m high and 10 to 12 m below the eave.

Temperature and wind velocity are associated with increasing NH_3 concentrations, in agreement with Viatte *et al.* (2022). However, the highest concentrations of NH_3 are associated with Humidity in the central part of the stable, as shown in Table 2. When only a concentration of 0.5 % NH_3 at 298 K temperature and 0 ms^{-1} air velocity, 90 % of these conditions were found at 0 m height with 0.5 % humidity, *i.e.* on the wet surface.

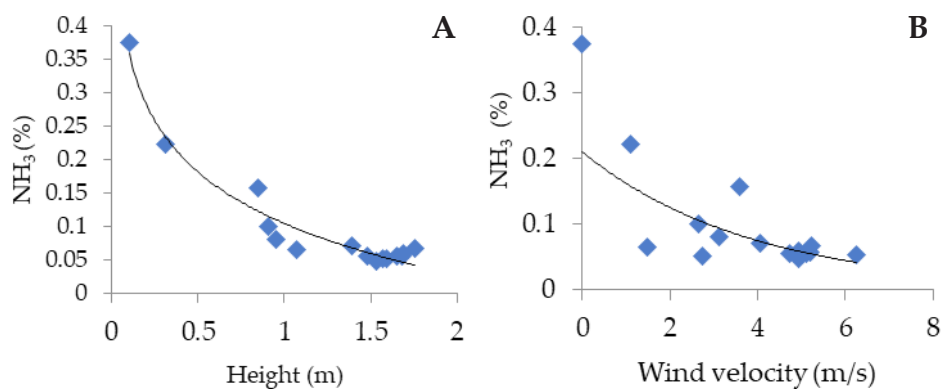


Figure 5. A: NH₃ and height relationship; B: NH₃ and wind velocity relationship.

Table 2. Probabilistic analysis of the CFD domine using the K2 algorithm.

	X (m)	Y (m)	Z (m)	H ₂ O (%)	NH ₃ (%)	T (K)	Wind velocity (ms ⁻¹)
	55.00	0.10	55.00	0.37	0.37	310.70	0.00
<i>A posteriori probability</i>	0.79	0.26	0.77	0.98	1.00	0.85	0.32

The CFD model's approximation analyzed using the K2 algorithm showed that the NH₃ concentration is related to humidity, temperature, and wind velocity, whose gradients depend on height, as shown in Figure 6 like the studies carried out using CAM-chem and Fourier transform infrared spectrometry by Vira and Wang *et al.* (2022), the volatilization of NH₃ is when the highest temperature and humidity occurs on the surface of the stable.

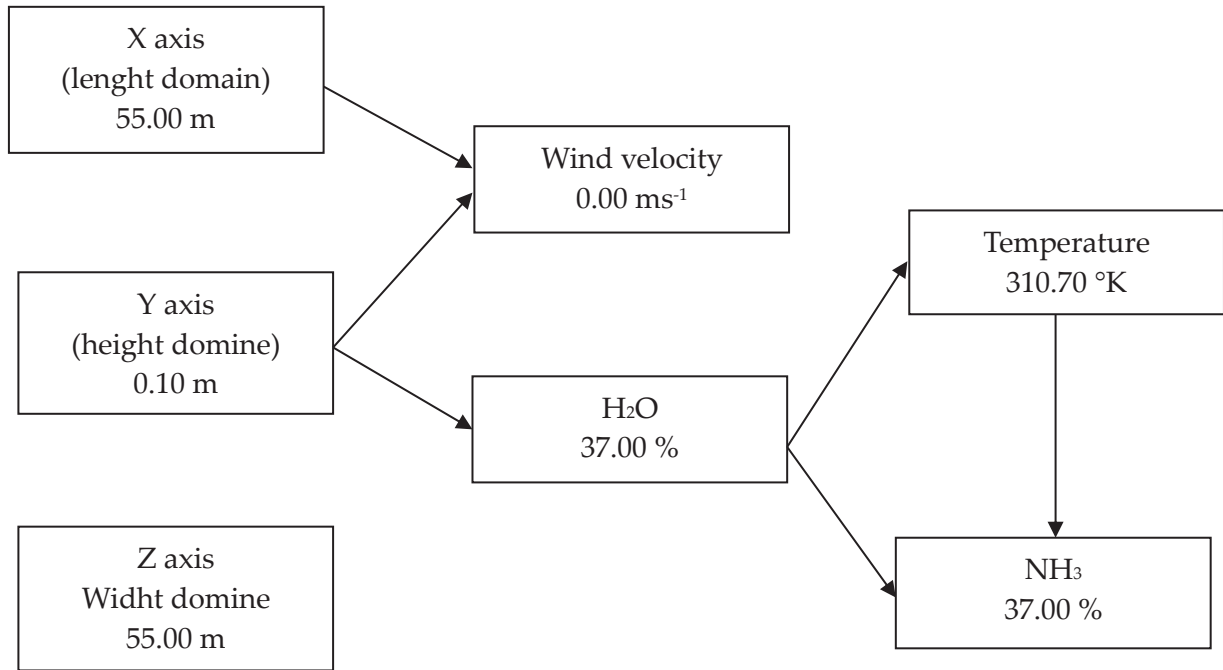


Figure 6. Relationships between the domain dimensions and the variables studied concerning the concentration of atmospheric NH_3 using the K2 algorithm.

CONCLUSIONS

To conclude, the results of this study underscore the significant influence of airflow patterns, temperature, and humidity on the distribution and concentration of NH_3 within cow stable. The CFD simulations demonstrate that NH_3 concentrations are highest near the barn center at lower heights (0.1 m), particularly under low wind velocity and high humidity conditions. This aligns with findings from prior studies, emphasizing that reduced airflow leads to NH_3 stagnation, while even slight increases in wind velocity facilitate dispersion, improving air quality. Temperature was also a critical factor, as increased temperatures enhanced NH_3 volatilization, with peak concentrations observed under high-temperature conditions, similar to observations by Doumbia *et al.* (2021) and Chen *et al.* (2021).

The probabilistic analysis using the K2 algorithm further confirms that NH_3 dispersion is governed by a combination of environmental parameters, with temperature, wind velocity, and humidity gradients varying with height. These results have practical implications for managing NH_3 emissions in agricultural facilities, indicating that optimized ventilation and temperature control could effectively reduce NH_3 concentration and improve environmental conditions.

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SELECTION OF NATIVE AND CONVENTIONAL COTTON (*Gossypium hirsutum* L.) GENOTYPES BASED ON FIBER QUALITY

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) is the main source of natural textile fibers in the world. Properties such as short fiber stickiness, trash content, color, fiber length, and fineness are fiber quality characteristics that determine pricing, profitability, and marketability. Therefore, the description and measurement of all its characters is indispensable to ensure high-quality cotton fiber production. The objective of this work was to evaluate, select, and characterize cotton germplasm (*Gossypium* spp.) with high fiber quality in a randomized complete block experimental design with three replications and 15 plants per replication for the generation of new conventional varieties, based on the hypothesis that genotypes with excellent fiber quality can compete with currently commercialized transgenic varieties. The evaluation was conducted in a greenhouse during the 2019 autumn-winter agricultural cycle at La Laguna Experimental Field of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP-CELALA). Two harvests were carried out, where a random sample of 60 buds was taken from each genotype and the fiber was separated from the seed. A total of 150 g of fiber was taken from each sample and measured for length, strength, fineness, spinnability index, uniformity index, short fiber index, elongation, reflectance, and yellow +b content. Analysis of variance (ANOVA) showed significant differences between genotypes for all variables evaluated, mainly in fiber length, strength, and fineness. The results indicate that TOA-17, TOA-18, GOS-17, GOS-21, and FZ-13 CLON showed outstanding characters of similar quality to the commercial control (DeltaPine®), making these genotypes suitable for the requirements of the textile industry.

Key words: genetic diversity, cotton germplasm, selection.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is the dominant natural textile fiber in the world (Lee and Fang, 2015) and the most widely used raw material in the textile industry (Zheng *et al.*, 2018) because of its high fiber quality for fabric manufacturing. Due to its wide adaptability and excellent performance, it is grown in more than 80 countries (UNIDO, 2023), producing about 92 % of the world's cotton fiber (Zhang *et al.*, 2015) and accounting for 27 % of the fibers consumed in the textile industry (FAO, 2021).

Over the years, breeders have only focused on increasing yield because simultaneously improving complex traits was a major challenge (Li *et al.*, 2016). Currently, genetic improvements in yield and fiber quality are being made to meet the demands of the modern textile industry (Zhao *et al.*, 2022). However, the genetic basis of fiber quality and yield traits in Native American (upland) cotton needs to be discovered for future improvement (Ning *et al.*, 2014).

Around 95 % of the world cotton production corresponds to the *G. hirsutum* (upland) species, as it has excellent yield, good adaptability and fiber quality ("long" category). In some ways, the genetic diversity of these commercial varieties is low, and genetic uniformity poses obstacles to breeders. In addition, it puts the cotton industry at high risk of collapse in the likely event of a disease or pest outbreak or increase (Shim *et al.*, 2018).

Cotton fiber has a wide variation among genotypes, so its description is essential to know all of its properties and maintain a high-quality production. Since the late 1960s, researchers have used the High Volume Instrument (HVI) to select plant populations with high fiber quality, as these parameters are critical in determining cotton profitability (Pinnamaneni *et al.*, 2021).

Fineness (micronaire), length, strength, elongation, moisture index, short fibers, and color are parameters that determine fiber quality (Tarragó *et al.*, 2019) and are mainly controlled by genetic factors (Yehia and El-Hashash, 2022). On the other hand, short fiber stickiness, trash content, color, fiber length, and fineness are fundamental in determining the commercial system of cotton pricing and marketing in the world (Ge *et al.*, 2008).

When fiber quality is low, producers assume that cotton bolls were exposed to adverse events in the field, such as unexpected rains, insect secretions, or dust (Pinnamaneni *et al.*, 2021), resulting in low-quality fibers or fibers with undesirable qualities. However, selection of good cultivars (Orawu *et al.*, 2017), sowing outside optimal dates, fertilization, irrigation (Witt *et al.*, 2020), early crop defoliation, harvesting, and dethatching are factors that can also affect final fiber quality (Hernández-Leal *et al.*, 2022). To some extent, all these factors are affected by the environment, although fiber quality is mainly controlled by genetic factors (Yehia and El-Hashash, 2022).

Selection of genotypes with good genetic traits for yield and fiber quality is critical in the breeding of high-quality cotton (Liu *et al.*, 2020). Yehia and El-Hashash (2022) mention

that most genetic variations in yield components and fiber quality are controlled by non-additive genetic effects. Cotton fiber quality parameters prior to the publication of genomes were studied with linkage analysis methods in biparental segregating populations (Liu *et al.*, 2020). To date, nearly 1000 quantitative trait loci (QTL) are associated with fiber quality and are distributed on all 26 cotton chromosomes (Said *et al.*, 2015).

Therefore, the objective of this study was to evaluate genetic diversity by characterizing the fiber quality of 28 cotton genotypes and a commercial control (DeltaPine®) to select outstanding germplasm. This material can be the basis of a breeding program for the generation of new conventional cotton varieties with excellent fiber quality.

MATERIALS AND METHODS

Area of study, genotypes evaluated, and their characteristics

The work was carried out in a greenhouse at La Laguna Experimental Field of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP-CELALA) in Matamoros, Coahuila, Mexico (25° 32' 1'' N, 103° 14' 79'' W, at an altitude of 1150 m), in the autumn-winter 2019 agricultural cycle. Twenty-eight cotton genotypes and the commercial control DeltaPine® were evaluated (Table 1).

Table 1. Place of collection, origin, and cotton (*Gossypium hirsutum* L.) population type of derived lines and a commercial control evaluated in Matamoros, Coahuila, Mexico.

Genotype	Place of collection	Origin	Description
FZ-10	La Mojonera, Tepecuacuilco, Guerrero	INIFAP Iguala Experimental Field Botanical Garden	Native population
FZ-13 CHAMPION	Cuexcontlán, Tepecuacuilco, Guerrero	INIFAP Iguala Experimental Field Botanical Garden	Native population
FZ-13 CLON	Cuexcontlán, Tepecuacuilco, Guerrero	INIFAP Iguala Experimental Field Botanical Garden	Native subpopulation
FZ-15	Huitzucu, Huitzucu, Guerrero	INIFAP Iguala Experimental Field Botanical Garden	Native population
GOS-17	Ixcateopan, Ixcateopan, Guerrero	INIFAP Iguala Experimental Field Botanical Garden	Native population
GOS-21	El Jordán, Tehuantepec, Oaxaca	INIFAP Iguala Experimental Field Botanical Garden	Native population
GOS-22	Carretera a Mixtequilla, Tehuantepec, Oaxaca	INIFAP Iguala Experimental Field Botanical Garden	Native population
GOS-27	Colonia Centro, Marquelia, Guerrero	INIFAP Iguala Experimental Field Botanical Garden	Native population
US-023	Vicente Guerrero (Barrio el Torque), Venustiano Carranza, Chiapas	INIFAP Iguala Experimental Field Botanical Garden	Native population

Table 1. Continue

Genotype	Place of collection	Origin	Description
TOA-03	Loma de Coyotes, Carretera Iguala- Teloloapan Iguala, Guerrero	INIFAP Iguala Experimental Field Botanical Garden	Native population
TOA-04	Casino de la Unión, Taxco de Alarcón, Guerrero	INIFAP Iguala Experimental Field Botanical Garden	Native population
TOA-06	Teacalco, Amacuzac, Morelos	INIFAP Iguala Experimental Field Botanical Garden	Native population
TOA-11	Cuautlula, Tehuiztingo, Puebla	INIFAP Iguala Experimental Field Botanical Garden	Native population
TOA-12	Sección Primera, Tehuiztingo, Puebla	INIFAP Iguala Experimental Field Botanical Garden	Native population
TOA-17	Ranchería Guadalupe, Chichimá, Comitán de Domínguez, Chiapas	INIFAP Iguala Experimental Field Botanical Garden	Native population
TOA-18	Colonia 28 de agosto, Comitán de Domínguez, Chiapas	INIFAP Iguala Experimental Field Botanical Garden	Native population
TOA-27	Piedras Negras, Pilcaya, Guerrero	INIFAP Iguala Experimental Field Botanical Garden	Native population
PI-589	-	Germplasm bank of the Chapingo Autonomous University	Native population
P1-583 CHAPIS	-	Germplasm bank of the Chapingo Autonomous University	Native population
P1-873	-	Germplasm bank of the Chapingo Autonomous University	Native population
PI-528748	-	Germplasm bank of the Chapingo Autonomous University	Native population
PI-201584	-	Germplasm bank of the Chapingo Autonomous University	Native population
JUÁREZ-91	-	INIFAP Experimental Field La Laguna	Conventional variety
CIAN-95	-	INIFAP Experimental Field La Laguna	Conventional variety
CIAN PRECOZ	-	INIFAP Experimental Field La Laguna	Conventional variety
NAZAS-87A	-	INIFAP Experimental Field La Laguna	Line
NAZA- 87B	-	INIFAP Experimental Field La Laguna	Line
NAZAS SELECTA	-	INIFAP Experimental Field La Laguna	Conventional variety
DELTAPINE®	-	Comercial control	Conventional variety

General crop management and experimental design

The experiment was set up on October 22, 2019, in 200-cavity polystyrene trays using Peat Moss as substrate. Transplanting was done under hydroponic conditions at 35 days after sowing (DDS). The 28 genotypes and the control were established in black polyethylene bags with a capacity of 12 L filled with river sand as substrate. Fertilization was carried out with fertigation using amino acids (300 to 500 mL per 200 L of water), seaweed (2 L ha⁻¹), Bayfolan® (0.4 L ha⁻¹), and Push® (10 to 20 L ha⁻¹). The dose was increased according to the phenological stage of the crop, starting with a concentration of 50 % during the vegetative stage, and from flowering until fruiting, the concentration was increased to 100 %. Two applications were made with fungicides Promyl 50® and Previcur® at a dose of 1 kg ha⁻¹ to control damping off and Captan® to prevent *Rhizoctonia solani*.

Variables evaluated to determine fiber quality

The first harvest took place on May 20 and the second on June 2. A random sample of 60 buds was taken from 15 plants for each genotype, separating the fiber from the seed. Cotton fiber samples were sent to the INIFAP-CELALA fiber quality laboratory for analysis. Fiber length (LON; mm), strength (RES; g tex⁻¹), fineness (FIN; micronaire index), spinnability index (HI; %), uniformity index (UI; %), short fiber index (SFI; %), elongation (ELG; %), reflectance (RD; %), and yellow +b content (PLUS_B) were determined for each sample.

Statistical analysis

A randomized complete block design with three replications and 15 plants per replication was used. An analysis of variance was performed on each variable; when statistical differences between genotypes were detected ($p \leq 0.05$), Tukey's mean comparison test was applied ($p \leq 0.05$). For the analysis of variance, the following model was applied:

$$y_{ij} = \mu + G_i + B_j + E_{ij}$$

where Y_{ij} is the value of the response variable corresponding to genotype i in block j ; μ is the overall mean, G_i is the effect of the genotype i ; β_j is the effect of block j ; and E_{ij} is the experimental error.

A principal component analysis (PCA) was carried out to appreciate the similarities and differences among the 28 genotypes and the commercial control using the statistical package SAS® v.9.3 (SAS Institute, 2011). Furthermore, a cluster analysis was performed by Euclidean distance using the unweighted pair grouping method with arithmetic mean (UPGMA) with the statistical package NTSYS® (Rohlf, 2000). Finally, Pearson's correlation coefficient was calculated with the SAS® v.9.3 statistical package.

RESULTS AND DISCUSSION

Fiber quality performance

The analysis of variance detected significance ($p \leq 0.05$) among genotypes for all nine fiber quality variables (Table 2). The coefficients of variation (CV) ranged from 0.197 to 1.66 %. In this regard, Patel *et al.* (2001) point out that CV varies according to the type of experiment. For cultivar evaluations, acceptable CV values vary between 6 and 8 %, for fertilization between 10 and 12 %, and for pesticide evaluations between 13 and 15 %. The mean CVs in the various tests ranged from 6.1 to 12 %.

Table 2. Mean squares of the analysis of variance for nine fiber quality variables in 28 cotton genotypes (*Gossypium hirsutum* L.) and one control (DeltaPine®) evaluated in Matamoros, Coahuila, Mexico.

Variable	Genotype		Repetitions	Error	CV (%)
Fineness (micronaire index)	1.73	**	0.0002	0.0002	0.286
Length (mm)	0.047	**	0.000005	0.00003	0.437
Resistance (g tex ⁻¹)	33.29	**	0.0538	0.165	1.29
Spinnability index (%)	2330.44	**	1.6	4.12	1.37
Short fiber index (%)	18.42	**	0.0006	0.002	0.533
Elongation (%)	1.41	**	0.0001	0.0093	1.66
Reflectance (%)	40.67	**	0.0125	0.0246	0.197
Uniformity index (%)	11.09	**	0.001	0.0376	0.231
PLUS_B	2.52	**	0.0001	0.0004	0.219
DF	28		2	56	

CV: coefficient of variation; DF: degrees of freedom; PLUS_B: yellow +b content. * $p \leq 0.05$; ** $p \leq 0.01$.

The comparison of means indicates that genotypes TOA-17, FZ-13 CLON, GOS-17, FZ-17, and PI-589 showed a very coarse fineness according to the classification by HVI. In contrast, the NAZAS-87B genotype showed the lowest value, classifying it as fine. Mexican Standard NMX-A-051-SCFI-2000 (DOF, 2000) establishes that the ideal fineness is 3.7 to 4.2 micronaire, with TOA-06, GOS-22, and FZ-13 CHAMPION falling within this parameter (Table 3).

Of the total genotypes evaluated, 21 had very high resistance (> 31 g tex⁻¹). While GOS-22 and FZ-15 showed low resistance, the rest of the genotypes had high resistance. Gourlot *et al.* (2020) note that, for spinners, fineness is crucial in predicting the spinning ability of cotton. Regarding length, GOS-17, GOS-21, GOS-27, TOA-06, TOA-11, TOA-17, TOA-18, NAZAS SELECTA, CIAN PRECOZ, and CIAN-95 had the highest values and fall into the extra-long fiber category (>1.21 mm), so they can be used for the production of very fine yarns. FZ-15 had the lowest value at 0.86 mm. Length and fineness influence yarn strength, while strength and length influence textile processing, affecting yarn quality (Yang *et al.*, 2016).

Table 3. Fiber quality comparison of 28 cotton genotypes (*Gossypium hirsutum* L.) and a commercial control (DeltaPine®) evaluated in Matamoros, Coahuila, Mexico.

Genotype	FIN	LON	RES	HI	SFI	ELG	RD	UI	PLUS_B
TOA-03	4.34 o	1.10 k	31.3 h	138 k	8.69 g	5.2 ijk	79.5 j	83 kl	8 q
TOA-04	4.51 lm	1.06 lm	27.3 j	125 l	8.63 g	6.5 bcd	78.2 l	83.6 jk	8.3 n
TOA-06	4.09 p	1.25 cd	33.4 bg	173 bcd	6.86 q	5.6 h	80 i	85.8 bc	7.4 u
TOA-11	4.70 jk	1.30 j	33.2 cg	139 k	8.1 l	5.1 jk	78.7 k	82.7 l	8.3 n
TOA-12	4.45 n	1.04 n	29 i	122 l	9.57 e	6.4 cde	79.1 jk	82 m	8.2 o
TOA-17	6.51a	1.25 de	34.6 ab	175 bc	6.51 s	6.3 def	76.5 n	85.6 be	10.7a
TOA-18	6.11 c	1.25 cd	32.97 dg	169 cde	7.45 mn	4.97 k	77.7 m	84.6 ghi	10.07 d
TOA-27	4.74 ij	1.09 k	29.7 i	136 k	7.74 k	6.4 cde	77.6 m	84.7 fi	8.5 l
GOS-17	6.13 c	1.40a	32.45 fgh	178 ab	5.81 u	6.8 b	80.6 h	85.3 cf	9.05 i
GOS-21	4.75 i	1.30 b	34.7a	160 ghi	7.26 op	5 k	71.6 q	85.7 bcd	8.2 o
GOS-22	3.87 r	1.05 mn	22.5 m	99 m	14.93 b	5.4 hij	84.2 b	79.1 o	7.9 r
GOS-27	4.78 hi	1.25 cd	33.2 cg	168 def	7.89 j	5.1 jk	83.2 cd	85.3 cf	7.7 s
FZ-10	4.46 n	1.19 gh	32.6 fg	162 fgh	6.59 rs	5.5 hi	80 i	86.6a	7.9 r
FZ-13									
CHAMPION	3.97 q	1.23 e	34.7a	171 cde	7.49 lm	5.5 hi	83.4 c	85.3 cf	8.1 p
FZ13 CLON	6.29 b	1.19 gh	33.3 cg	170 cde	6.61 rs	6.7 bc	78 lm	85.5 be	10.4 c
FZ-15	5.40 d	0.86 p	24.9 l	76 n	16.29a	4.5 l	70.4 r	79.5 o	10.6 b
PI-589	5.31 e	0.91 o	25.7 kl	98 m	10.55 d	6.1 ef	76 o	82.4 lm	8.4 m
P1-583 CHAPIS	4.82 h	0.92 o	26.2 jk	98 m	12.71 c	5.4 hij	73.7 p	81.1 n	9.6 f
PI-201584	4.44 n	1.07 l	33.6 a-f	147 j	7.62 kl	6.5 bcd	84.2 b	83.4 k	7.5 t
P1-873	4.36 o	1.11 k	34.4 abc	154 i	7.33 no	5.7 gh	80.2 hi	84.4 hi	7.9 r
P1-528748	4.90 g	1.1 k	29 i	137 k	7.18 p	6.3 def	82 f	85.1 dg	8.2 o
US-023	4.55 l	1.05 mn	33.3 cg	136 k	8.18 l	6 ef	76.9 n	82.6 lm	8.7 k
NAZAS									
SELECTA	4.48 mn	1.21 f	32.3 hg	157 hi	7.51 lm	5.6 h	82.8 de	85 eh	8.8 j
NAZAS-87A	4.45 n	1.18 h	32.9 df	155 i	8.37 h	5 k	82.5 e	82.4 lm	9 i
NAZAS-87B	3.52 s	1.19 g	33.6 af	158 hi	9.41 f	5.7 gh	84.7a	84.5 ghi	8.5 l
CIAN PRECOZ	4.47 mn	1.21 f	34.1 ae	162 fgh	8.22 i	6 fg	83.4 c	84.8 fi	8.5 l
JUÁREZ	4.47 mn	1.17 i	34.2 ad	157 hi	7.9 j	5.5 hi	82.8 de	84.2 ij	9.8 g
CIAN-95	4.67 k	1.3 b	32.7 fg	182 a	6.73 qr	5.2 ijk	81.5 g	85.9 bc	9.2 h
DELTAPINE®	5.01 f	1.16 i	33.6 af	166 efg	6.26 t	7.2 a	81.1 g	86.1 ab	9.3 g
DSH	0.044	0.016	1.29	6.44	0.141	0.305	0.498	0.615	0.061
RMSE	0.0002	0.00003	0.165	4.12	0.002	0.009	0.025	0.037	0.0004
SE	0.717	0.126	3.33	27.28	2.43	0.659	3.68	1.91	0.914

abc: Mean values per column with different letters are statistically different ($p \leq 0.05$). DSH: honest significant difference; SE: standard error; RMSE: root mean square error; FIN: fineness (micronaire index); LON: fiber length (mm); RES: strength (g tex⁻¹); HI: spinnability index (%); SFI: short fiber index (%); ELG: elongation (%); RD: reflectance (%); UI: uniformity index (%); PLUS_B: yellow content (+b).

For the spinnability index, CIAN-95 reported the highest value with 182 %, surpassing the commercial control (166 %); FZ-15 was the genotype with the lowest value (76 %). FZ-15 had the highest short fiber index (SFI) with 16.29 % and GOS-17 the lowest SFI (5.81 %). The more automated the harvest, the more the fiber is damaged, and

therefore, the shorter the fiber is. Eleven genotypes and the commercial control showed a high uniformity index, with values between 85 and 87 % according to the HVI classification. In contrast, only GOS-22 and FZ-15 had a low uniformity index (with values of 79.1 and 79.5 %). The textile industry seeks longer fibers, as this means more lint production and greater cohesion (Frazier *et al.*, 2023).

Regarding elongation, DeltaPine® had the highest value, followed by GOS-17 with 7.2 and 6.8 %, respectively, while TOA-18 had the lowest value with 4.97 %. If plants lack water in the period immediately after anthesis, fiber growth, elongation, and final fiber length are strongly affected (Zhang *et al.*, 2021).

For the HVI classification system, color is quantified as the degree of reflectance (Rd) and yellowness (+b). The genotypes NAZAS-87B (84.7 %) and PI-201584 (84.2 %) showed higher Rd values, while TOA-17 and FZ-15 had higher yellowness. In contrast, FZ-15 had the lowest value for Rd, with 70.4 %, and TOA-06 for yellowness. Thus, creamy white fibers are considered of higher quality compared to dull, gray, or yellowish fibers, but the application of growth regulators, frost, and drought increase the yellowness (+b) of the fiber due to premature fiber maturation (Zhu *et al.*, 2024).

Relationships between fiber quality variables

Four correlations were positive and greater than 0.6. The spinnability index and uniformity index ($r = 0.939$) had the highest correlation, followed by the length and spinnability index ($r = 0.881$), length and uniformity index ($r = 0.746$), and fineness with yellow +b content ($r = 0.708$) (Table 4). These results have implications for genetic improvement since selection for a higher index of spinnability simultaneously increases the uniformity index and fiber length. In contrast, negative and highly significant ($p \leq 0.01$) correlations were found between the spinnability index and short fiber index ($r =$

Table 4. Correlation between fiber quality traits in 28 cotton genotypes (*Gossypium hirsutum* L.) and a commercial control (DeltaPine®) evaluated in Matamoros, Coahuila, Mexico.

	FIN	LON	RES	HI	UI	SFI	ELG	RD	PLUS_B
FIN	1								
LON	0.042	1							
RES	-0.501 **	-0.155	1						
HI	0.140	0.881 **	-0.095	1					
UI	0.119	0.746 **	-0.026	0.939 **	1				
SFI	-0.185	-0.811 **	0.106	-0.87 **	-0.752 **	1			
ELG	0.256 *	0.081	-0.308 **	0.177	0.048	-0.406 **	1		
RD	-0.470 **	0.296 **	0.369 **	0.432 **	0.419 **	-0.311 **	0.189	1	
PLUS_B	0.708 **	-0.019	-0.277 **	-0.008	-0.035	0.126	-0.005	-0.426 **	1

* $p \leq 0.05$; ** $p \leq 0.01$. FIN: fineness (micronaire index); LON: fiber length (mm); RES: strength (g tex⁻¹); HI: spinnability index (%); SFI: short fiber index (%); ELG: elongation (%); RD: reflectance (%); UI: uniformity index (%); PLUS_B: yellow content +b.

-0.874), length and short fiber index ($r = -0.811$), and fineness with strength ($r = -0.501$). As the units of each variable are different, the principal component analysis (PCA) was performed using the correlation matrix. The first three components explained 81.34 % of the total variation, with 41.99, 69.03, and 81.34 %, respectively (Table 5). López and Hidalgo (1994) indicate that components whose eigenvalues explain 70 % or more of the total variance are acceptable. In PC1, the variables included were length, spinnability, short fiber index, and uniformity index. PC2 was composed of fineness, yellow +b content, reflectance, and strength, while PC3 was only composed of the elongation variable. Yehia and El-Hashash (2022) mention that the PCA analysis is very useful to evaluate the relationship and diversity among cotton collections and to know the relationships between yield, its components, and fiber quality.

Table 5. Vectors and eigenvalues of the principal component analysis (PCA) with variables with the highest descriptive value of the total variance evaluated in 28 cotton genotypes and a commercial control (DeltaPine®).

Variable	PC1	PC2	PC3
Fineness (micronaire index)	0.070	-0.582	-0.002
Length (mm)	0.451	0.000	0.263
Resistance (g tex ⁻¹)	-0.053	0.447	0.273
Spinnability index (%)	0.492	0.000	0.175
Short fiber index (%)	0.473	-0.035	0.105
Uniformity index (%)	-0.496	0.027	0.077
Elongation (%)	0.172	-0.125	-0.833
Reflectance (%)	0.214	0.436	-0.216
Yellow content +b (PLUS_B)	-0.045	-0.503	0.262
Variance explained (%)	41.99	27.03	12.31
Cumulative variance (%)	41.99	69.03	81.34

Genotype clustering according to fiber quality

According to the distribution of the 28 genotypes and the commercial control (DeltaPine®), five groups were identified (Figure 1). Group 1 consists of 14 populations, including all conventional varieties generated by INIFAP, six populations from the botanical garden, and two populations from the Chapingo germplasm bank. This group was characterized by a very strong fiber, average fineness, medium length, acceptable spinnability index, short-low fiber index, and a white fiber (reflectance). The genotypes GOS-27, FZ-13 CLON, TOA-17, TOA-18, and the control formed Group 2 and were characterized by having a very resistant fiber, fine fineness, and average micronaire. These genotypes are good for yarn formation as they are long-fibered and have a good spinnability index. Genotypes from groups 1 and 2 can be used in crosses to obtain new combinations that can perform even better than their parents due to the

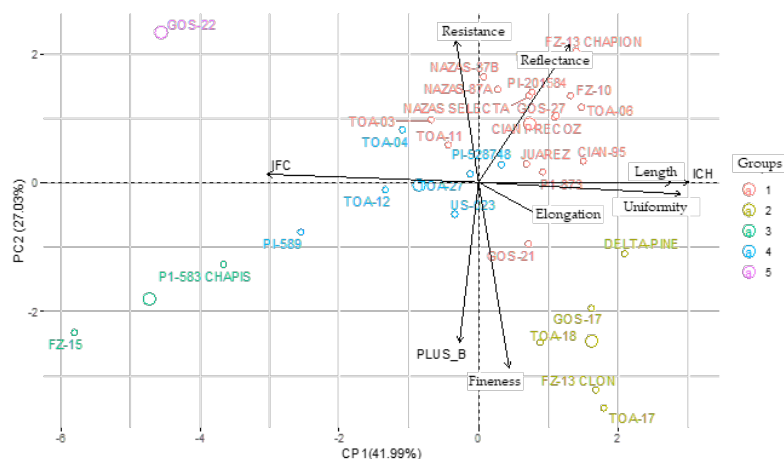


Figure 1. Dispersion of 28 native cotton (*Gossypium hirsutum* L.) and a commercial control (DeltaPine®) on the plane determined by the first two principal components, based on nine variables of the combined analysis. FIN: Fineness (micronaire index); LON: fiber length (mm); RES: strength (g tex-1); ICH: spinnability index (%); UNI: uniformity index (%); IFC: short fiber index (%); ELONG: elongation (%); REF: reflectance (%); PB: yellow content +b (PLUS_B); Rd: reflectance (%).

presence of higher fiber quality in both groups. For example, GOS-27 can be used in regional and/or national trials due to its high fiber quality, which is required by the textile industry.

Meanwhile, Group 3 consisted of genotypes P1-583 CHAPIS and FZ-15, and was characterized by medium fiber length, high strength, and low short fiber index. Genotypes US-023, TOA-04, TOA-12, TOA-27, P1-589, and PI-528748 constituted Group 4 and were characterized by weak and rough fiber strength (micronaire). Finally, Group 5 consisted only of GOS-22. This genotype was characterized by weak fiber strength, average fineness, medium length, high short fiber percentage, weak elongation, and low uniformity index. Alishah (2001) mentions that all this genetic variation can be exploited to obtain varieties with excellent yield and fiber quality.

The cluster analysis produced five groups (Figure 2) that differed in conformation from those obtained by the PCA. This difference results from the PCA only using the variables that explain PC1 and PC2 (69.03 % of the variation), whereas in the cluster analysis, all variables evaluated in the dendrogram are considered. The first two principal components played a key role in describing the main parameters contributing to variability while investigating different traits (Saeed *et al.*, 2014).

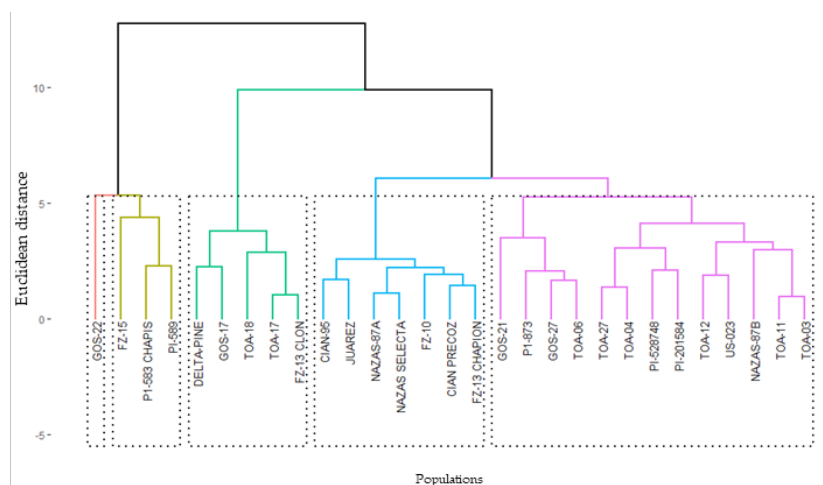


Figure 2. Dendrogram of 28 native cotton genotypes (*Gossypium hirsutum* L.) and a commercial control (DeltaPine®) based on nine variables of the combined analysis.

CONCLUSIONS

Genotypes TOA-17, TOA-18, GOS-17, GOS-27, and FZ-13 CLON showed similar characteristics to the commercial control (DeltaPine®), mainly in the fiber quality traits of fineness, length, resistance, and color (white) required by the textile industry; these genotypes could be used as a source of germplasm for the generation of new commercial varieties and hybrids. Highly significant correlations were found between fineness and strength, spinnability index and short fiber index, and length and short fiber index.

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EFFECTS OF LIGHT AT DIFFERENT DISTANCES AND POWER ON WHEAT (*Triticum aestivum* L.) AND BARLEY (*Hordeum vulgare* L.) GROWTH

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ABSTRACT

Global population growth increases the need for efficient methods of maintaining a stable food supply. Light is one of the most important influences on plant growth and development. Artificial lighting elements used in agricultural applications have different effects on plants. This study analyzed the effects of metal halide (MH) lamps with a wavelength of 450 nm, at distances of 1, 1.5, and 2.5 m, on barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) growth. Growing occurred in light magnification rooms without daylight, using 250, 400, and 600 W MH lamps as the only light source. Plants exposed to light for 13–14 h a day were harvested after 18 days. After harvest, the amount of electrolyte leakage, chlorophyll, carotenoid, and B, Cu, Mn, Zn, K, and Ca intake were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES). The data were analyzed with the SPSS 22 Statistical Package program. Wheat and barley grown at different light distances and intensities showed statistically significant changes in mineral element uptake and plant chlorophyll levels. This research contributes to the optimization of agricultural productivity and the understanding of the interaction between light quality and other environmental parameters.

Keywords: artificial light, chlorophyll, ICP-OES, mineral element uptake.

INTRODUCTION

It is an undeniable fact that light affects every living organism. Plants need a great deal of light as well as different environmental conditions. Light affects plant growth, development, and different metabolic functions while also providing energy for photosynthesis (Yeh and Chung, 2009; Li *et al.*, 2012; Lee *et al.*, 2014). Light could be effective at different levels in all plant developmental stages, such as the germination process. Not only is light necessary for plant development, but the quality of the light is also important (Wu *et al.*, 2007).

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Plants require light at specific wavelengths for optimal growth. Increasing the light intensity to a certain degree improves the photosynthesis rate; however, after a certain value, this becomes adverse (Wu *et al.*, 2007). When plants photosynthesize, they prefer medium-wavelength beams of blue, yellow, and red. These contribute to the formation of chlorophyll, the development of tissues, and blossoming (Taiz and Zeiger, 2008; Hogewoning *et al.*, 2010; Samuolienė *et al.*, 2012). Chlorophyll is the foundation of growth by using inorganic chemicals and generating organic substances (Samuolienė *et al.*, 2012). Light is a critical signal that induces plastid development and carotenoid biosynthesis in plants. During photoreceptors, photomorphogenesis, or de-etiolation, are activated, initiating the production of molecular factors necessary for the biosynthesis of carotenoids and chlorophyll. In fruits, light absorption by chloroplasts during the early stages of ripening facilitates the gradual synthesis of carotenoids in both the peel and pulp, coinciding with the initiation of chromoplast development (Quian-Ulloa and Stange 2021).

Light requirements are directly related to soil fertility. Plants grown in poor soils contain more chlorophyll in their leaves to increase photosynthesis, enhancing both dry matter accumulation and yields (Long and Bernacci, 2003). Moreover, macro- and micronutrients are essential variables influencing plant growth and metabolism in their natural environments (Kumar *et al.*, 2021). A number of studies suggest that nutrient uptake and utilization by plants is modified by light quality, intensity and photoperiod. In particular, light alters the uptake of multiple elements through changes in light quantity and quality. Furthermore, studies reveal links between light perception and nutrient uptake (Chen *et al.*, 2016; Sakuraba and Yanagisawa, 2018; Lin *et al.*, 2020; Xua *et al.*, 2021). Minerals are vital for normal growth, development, fruiting, flowering, and other plant activities (Broadley *et al.*, 2012; Subramanian *et al.*, 2022). Although minerals are essential micronutrients responsible for many regular processes in plants, their excess can have detrimental effects and directly affect plant growth, metabolism, physiology, and aging (Ghori *et al.*, 2019).

Artificial light sources vary depending on wavelengths, colors, and other characteristics. Differences in the light spectrum cause different photosynthetic and morphogenetic responses for each plant species (Urbonavičiūtė *et al.*, 2008). High-pressure sodium steam discharge lamps, incandescent wire lamps, fluorescent lamps, and metal halogen lamps are often used as light sources in plant production, while the usage of LED lamps has lately increased (Lee *et al.*, 2017). Artificial light sources in plant cultivation are used both to support sunlight and as an alternative solution in places with no sunlight.

This study aimed to understand the role of metal halide (MH) lamps in plant growth and to investigate the effects of 250, 400, and 600 W MH lights, set at different distances (1, 1.5, and 2.5 m), on the yields of barley (*H. vulgare* L.) and wheat (*T. aestivum* L.). Given the increasing need for effective strategies to maintain a secure food supply due to issues like the growing global population and the ongoing climate crisis, this study may represent an important contribution to the field of sustainable agriculture.

MATERIALS AND METHODS

In the present study, wheat (Bezostaja), and barley (Kral 97) seeds were planted in soil mixed with animal manure taken from agricultural fields. The soil was prepared for 750 g and placed in plastic pots. The seeds were weighted, and 6 g of wheat seeds and 5 g of barley seeds were planted in each pot. Three pots of barley and three pots of wheat seeds were prepared for each light source application. Then, the planted seeds were covered with soil. After germination, the wheat and barley plants were exposed to light at different distances (1 m, 1.5 m and 2.5 m) under 250, 400, and 600 W metal halide (MH) lamps with a wavelength of 450 nm for approximately 13–14 hours a day. Plants were irrigated during the growing process, taking into account field capacity and irrigation methods, and harvested at the end of 18 days.

Electrolyte leakage in the cell

After harvest, 0.1 g of fresh leaf samples were taken and placed in test tubes separately. Each tube was filled with 4 ml of pure water and stored at 4 °C for 24 h. Additionally, to determine cell damage, the amount of ions transferred from the samples to the water was examined using an electric conductometer (Griffith *et al.*, 1992; Duman and Koca, 2014).

Chlorophyll and carotenoid concentrations determination

To evaluate the amount of chlorophyll, 0.5 g of the harvested leaves were placed in a porcelain mortar with 20 mL of 80 % acetone. The mixture was homogenized using a pestle, filtered using filter paper, transferred to a 10 mL centrifuge tube, and centrifuged for 10 min. The absorbance values at 663, 646, and 440 nm were determined individually using a spectrophotometer. The amount of chlorophyll (Chl) and carotenoids (Car) was calculated using the equations below. Results were reported in mg g⁻¹ FW (fresh biomass weight) (Porra *et al.*, 1989; Osma *et al.*, 2014; Duman and Koca, 2014).

$$\text{Chl } a = 12.25 A_{663} - 2.55 A_{646}$$

$$\text{Chl } b = 20.31 A_{646} - 4.91 A_{663}$$

$$\text{Chl } a + b = 17.76 A_{646} - 7.34 A_{663}$$

$$\text{Car} = 4.69 A_{440} - 0.267 \text{ Chl } a + b$$

Mineral elements determination

Harvested wheat and barley samples were dried in an oven at 80 °C for 24 h, ground into a powder in a mortar, and placed in separate plastic bags for storage. To avoid mixing the powders of the samples during the powdering process, ethyl alcohol was added after each sample was powdered. Following the addition of 10 mL of 65 %

HNO₃. 0.5 g of each plant were weighed into Teflon cells and burned for 20 min at 180 °C using a microwave device at 280 Psi = 1930.5 kPa. The cells were allowed to cool, and the samples inside were adjusted to 50 mL with distilled water. The samples were then filtered using filter paper, and the element concentrate on was determined at the appropriate wavelengths using an inductively coupled plasma optical emission spectroscopy (ICP-OES) instrument (Duman *et al.*, 2015; Osma *et al.*, 2017; Elveren and Osma, 2022). Results were reported in mg kg⁻¹ DW (dry weight).

Statistical Analysis

The data were analyzed with the SPSS 22 Package Statistical program (IBM Statistics) using an ANOVA test with a 95 % confidence interval. The differences between samples in multiple comparisons were determined by S-N-K and Tukey's B test (Elveren and Osma, 2022).

RESULTS AND DISCUSSION

Chlorophyll-*a* concentration ranged from 8.16 to 13.56 mg g⁻¹ FW in barley, with the maximum value observed in the group that received light from a 250 W lamp from a 1.5 m distance. In wheat, the amount of chlorophyll-*a* ranged from 9.58 to 17.75 mg g⁻¹ FW, and the highest value was found in the group that received light from a 400 W lamp from a 2.5 m distance (Figure 1).

Chlorophyll-*b* concentration ranged between 3.24 and 5.48 mg g⁻¹ FW in barley, with the best results obtained using a 600 W lamp from a 1 m distance, whereas in wheat, it ranged between 4.45 and 5.90 mg g⁻¹ FW, with the best results obtained using a 600 W lamp from a 1 m distance (Figure 2).

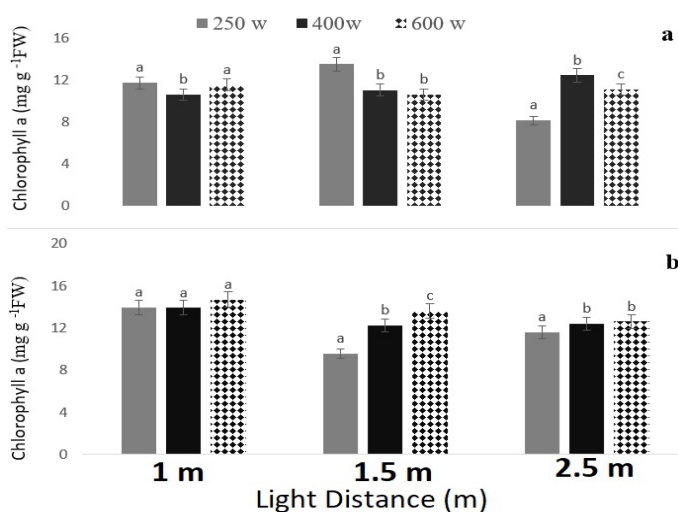


Figure 1. Chlorophyll *a* in leaves of plants cultivated with light at different distances and radiant power values. a: barley; b: wheat.

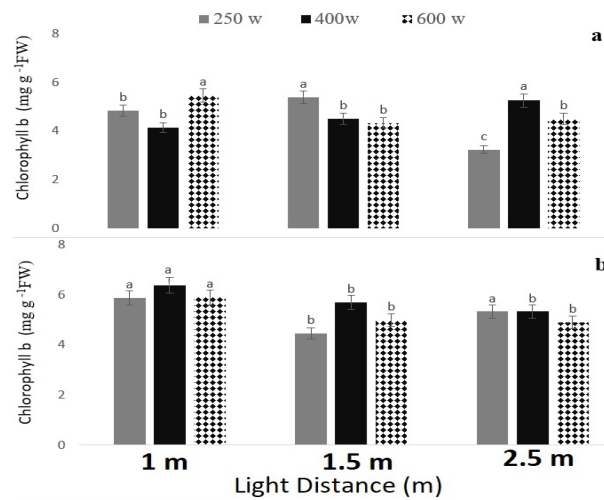


Figure 2. Chlorophyll *b* in leaves of plants cultivated with light at different distances and radiant power values. a: barley; b: wheat.

The total amount of chlorophyll in barley ranged between 11.4 and 18.95 mg g⁻¹ FW, with the highest values obtained using a 250 W lamp from a 1.5 m distance. In wheat, it ranged between 14.04 and 20.61 mg g⁻¹ FW with a 600 W lamp from a 1 m distance (Figure 3).

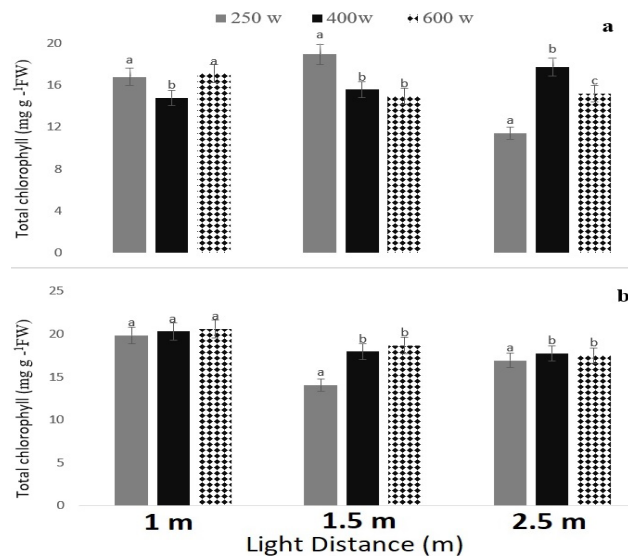


Figure 3. Total chlorophyll in leaves of plants cultivated with light at different distances and radiant power values. a: barley; b: wheat.

The results indicated that there were 1.56–2.70 mg g⁻¹ FW of carotenoids in barley, with the highest concentrations observed using a 250 W lamp from a distance of 1.5 m. The optimal results were obtained using a 600 W from a 1 m distance in wheat, with carotenoid levels of 1.81–2.84 mg g⁻¹ FW (Figure 4).

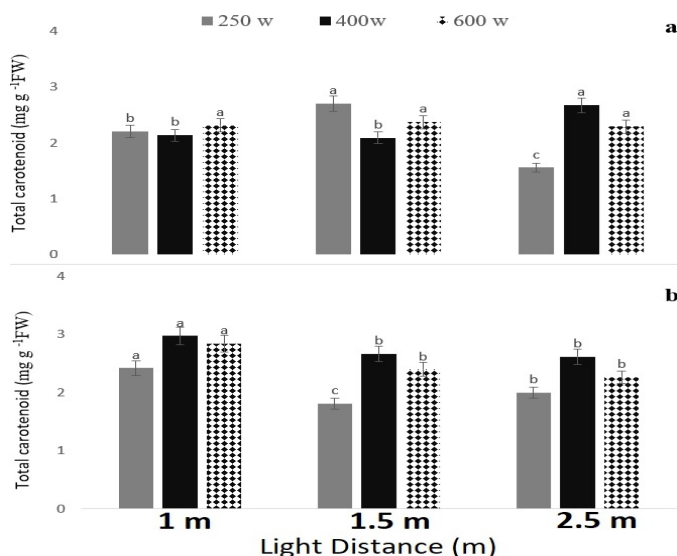


Figure 4. Total carotenoids in leaves of plants cultivated with light at different distances and radiant power values. a: barley; b: wheat.

In this study, it was found that the concentration of electrolyte leakage in wheat and barley plants varied depending on the distance and power of the applied light source. This finding reveals the effects of light intensity and distance on the cellular membrane permeability of plants and provides important clues about plant physiology. In particular, new information has been provided on how different light conditions affect the stress tolerance mechanisms of plants, thus contributing to the development of important strategies for increasing agricultural productivity and protecting plant health (Figure 5).

Statistically significant differences were observed when comparing the chlorophyll levels of plants grown at different light intensities and distances. These results indicate that growing conditions have a determining effect on chlorophyll production. In this study, the amount of chlorophyll in barley was higher in samples grown at 400 W and a distance of 1.5 m. In wheat, in general, the amount of chlorophyll was found to be higher in samples grown with a 400 W light source and at a distance of 1 m.

Given the element concentration data obtained in the present study, in barley, it was found that the values obtained for B (5.11–15.48 mg g⁻¹ DW), Mn (35.79–58.06 mg kg⁻¹

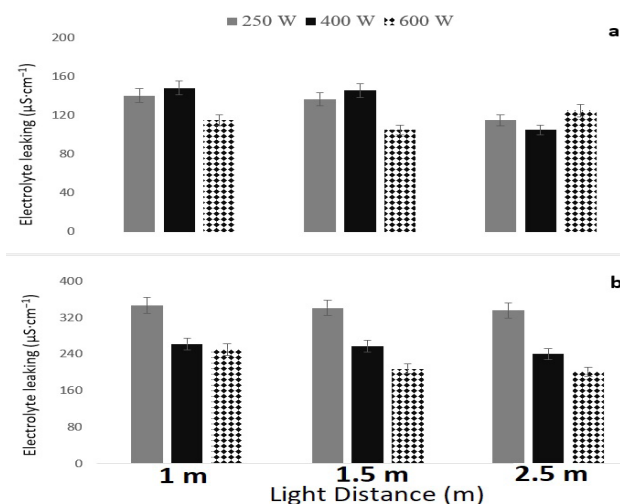


Figure 5. Electrolyte leaking in leaves of plants cultivated with light at different distances and radiant power values. a: barley; b: wheat.

DW), Zn (42.38–61.55 mg kg⁻¹ DW) and K (55 399.22–63 472.64 mg kg⁻¹ DW), were the highest when using a 250 W light source at a distance of 2.5 m; for Cu, concentration was higher (11.47–14.82.5 mg kg⁻¹ DW) with a 600 W lamp at a 2.5 m distance; and for Ca (2 069.43–2 954.47 mg kg⁻¹ DW), the highest values were found using a 400 W lamp at a distance of 2.5 m (Table 1).

Table 1. Mineral element concentrations in barley leaves grown with light at different distances and radiant power values (mg kg⁻¹ dry weight).

Element	Distance (m)	250 W	400 W	600 W
K	1.0	63 620.09 ± 638.50	57 697.99 ± 790.39	57 353.81 ± 700.23
	1.5	56 474.17 ± 948.92	61 662.83 ± 872.96	55 399.22 ± 880.10
	2.5	63 338.64 ± 795.55	58 022.29 ± 942.19	60 791.34 ± 757.24
Ca	1.0	2 166.38 ± 34.99	2 295.42 ± 48.11	2 085.18 ± 23.61
	1.5	2 069.43 ± 33.88	2 169.52 ± 96.43	2 901.06 ± 17.33
	2.5	2 477.25 ± 25.00	2 954.47 ± 38.33	2 556.94 ± 20.77
B	1.0	7.64 ± 0.04	11.96 ± 0.22	7.41 ± 0.10
	1.5	5.11 ± 0.08	10.96 ± 0.16	11.57 ± 0.12
	2.5	15.48 ± 0.38	15.30 ± 0.11	11.50 ± 0.09
Mn	1.0	51.72 ± 0.34	45.68 ± 0.70	50.36 ± 0.69
	1.5	51.61 ± 0.67	37.72 ± 0.44	55.74 ± 0.92
	2.5	58.06 ± 0.21	35.79 ± 0.78	46.75 ± 0.86
Zn	1.0	51.02 ± 0.43	45.34 ± 1.21	47.10 ± 0.99
	1.5	51.19 ± 0.26	45.08 ± 0.66	42.38 ± 0.30
	2.5	61.55 ± 0.28	46.90 ± 0.62	43.96 ± 0.14
Cu	1.0	13.60 ± 0.48	13.44 ± 0.46	14.81 ± 0.43
	1.5	13.44 ± 0.25	12.79 ± 0.37	13.77 ± 0.25
	2.5	14.10 ± 0.22	11.47 ± 0.47	14.82 ± 0.21

In wheat, it was determined that the concentration of B (10.14–17.46 mg kg⁻¹ DW), Cu (14.02–19.22.5 mg kg⁻¹ DW), Mn (26.02–79.47 mg kg⁻¹ DW), Zn (54.53–95.74 mg kg⁻¹ DW), and Ca (2 118.20–4 527.11 mg kg⁻¹ DW) was higher in the samples grown using a 250 W light source at a distance of 2.5 m, while and the concentration of K (55 804.74–77 735.07 mg kg⁻¹ DW) was higher in the samples grown at a distance of 2.5 m using a 600 W light source (Table 2).

Table 2. Mineral element concentration in wheat leaves grown with light at different distances and radiant power values (mg kg⁻¹ dry weight).

Element	Distance(m)	250 W		400 W		600 W	
K	1.0	55 804.74 ±	766.65	72 275.60 ±	677.35	66 225.41 ±	1 469.05
	1.5	64 262.84 ±	1 243.47	60 815.29 ±	789.35	66 496.31 ±	590.98
	2.5	62 666.88 ±	762.25	68 985.34 ±	594.97	77 735.07 ±	1 370.60
Ca	1.0	3 237.02 ±	51.52	2 795.42 ±	19.11	2 138.25 ±	21.39
	1.5	3 183.73 ±	26.04	2 501.06 ±	63.81	2 118.20 ±	16.70
	2.5	4 527.11 ±	43.20	2 510.24 ±	18.55	2 482.91 ±	37.63
B	1.0	16.08 ±	0.46	11.44 ±	0.09	12.41 ±	0.25
	1.5	17.46 ±	0.51	10.14 ±	0.43	12.38 ±	0.16
	2.5	14.67 ±	0.12	11.65 ±	0.43	10.55 ±	0.05
Mn	1.0	40.03 ±	0.87	32.42 ±	0.35	26.02 ±	0.57
	1.5	51.81 ±	0.52	34.19 ±	1.30	28.49 ±	0.43
	2.5	79.47 ±	2.56	35.50 ±	0.72	45.89 ±	0.34
Zn	1.0	71.96 ±	0.45	59.94 ±	1.08	62.18 ±	0.66
	1.5	82.88 ±	0.88	54.53 ±	1.72	75.76 ±	1.00
	2.5	95.74 ±	0.73	63.57 ±	0.96	76.37 ±	0.81
Cu	1.0	14.57 ±	0.20	17.17 ±	0.40	14.02 ±	0.24
	1.5	17.32 ±	0.25	15.69 ±	0.46	16.14 ±	0.21
	2.5	19.22 ±	0.42	17.44 ±	0.32	19.14 ±	0.56

It was determined that the concentrations of K, B, Mn, and Zn were generally higher in wheat samples grown at a distance of 2.5 m with a 250 W light source. On the other hand, for barley, it was observed that the concentration of Ca, Mn, Zn, and Cu increased in samples grown at a distance of 2.5 m with a 250 W light source. These results indicate that plants can uptake nutrients in different ways according to environmental conditions and that appropriate cultivation strategies are important in optimizing plant nutrient uptake. Therefore, it is necessary to carefully evaluate the effects of lighting intensity and distance on plant nutrition and growing practices. Previous studies found that there were considerable changes in element concentrations at different light intensities and distances. In a study on pea seeds (*Pisum sativum* L.), Wu *et al.* (2007) found no difference in stem diameter between seedlings produced in the dark and those grown under LED lights, but light quality had a significant impact on stem length, leaf area, and seedling weight. Islam *et al.* (2012) examined LED and

classic high-pressure sodium lamps to grow the poinsettia plant (*Euphorbia pulcherrima* Willd. ex Klotzsch), and those growing under LED were found to be 20–34% shorter. Furthermore, Yamaguchi *et al.* (2019) studied the relationship between LED lighting system power usage and lettuce (*Lactuca sativa* L.) leaf development in a hydroponic plant-growing system. The development of lettuce in a growing system with minimum electrical power and a long illumination time was found to be outstanding. Limprasitwong and Thongchaisuratkrul (2018) evaluated shallot plants (*Allium ascalonicum* L.) under daylight, LED light, and in nursery conditions under a grow lamp. In the first seven days, shallots grown under LED light and the grow lamp had a greater germination rate, and the grow lamp performed better than the LED in the first three days. In another study, Hamamoto and Yamazaki (2009) supplemented greenhouse tomatoes (*Solanum lycopersicum* L.) with a 40 W fluorescent lamp for 2 h at sunrise and found that tomato productivity and fruit quality increased by 10 % when conducting trials in the spring-summer and autumn-winter months.

Lin *et al.* (2013) used red-blue (RB) LED, red, blue, white (RBW) LED, and fluorescent lamp (FL) as a control group in their study in Taiwan and evaluated the responses of chlorophyll accumulation, plant biomass, carotenoids, soluble protein, sugar, and nitrate to three different light sources in lettuce leaves. Plants cultivated under RBW LED applications had high soluble sugar and low protein content compared to RB LED combinations. Accordingly, there were no significant differences in chlorophyll, carotenoid, or soluble proteins in lettuce leaves.

It was found that LED lamps can affect the production of phenolic compounds in buckwheat sprouts (*Fagopyrum esculentum* Moench), and certain light intensities and LED burning time could be important in the growth chamber to increase phenolic compounds (Lee *et al.*, 2014). It has been observed that external lighting increases seedling growth and quality in vegetable-growing greenhouses. A new approach in greenhouse lighting systems is the strong illumination of tomatoes, peppers, and cucumbers throughout the entire growth cycle (Olle and Viršilė, 2013).

Experiments have been devised to clarify the impact of monochromatic LED light or different combinations on plant morphogenesis and physiology. Significant differences in several parameters were observed when comparing plants under fluorescent light. In a study on *in vitro* banana (*Musa acuminata* Colla) plantlets, a significant increase in chlorophyll-*a*, chlorophyll-*b*, total chlorophyll levels, and a number of stomata was observed under LED illumination compared to fluorescent lamp illumination (Vieira *et al.*, 2015).

Both the color temperature and wavelength of commonly used white LED fixtures have significant effects on plants. As the color temperature increased, there were corresponding increases in plant height and weight values, chlorophyll and carotene levels, and mineral absorption capacity (Şahin *et al.*, 2022a). In their study, Şahin *et al.* (2022b) analyzed the effects of three commonly used light sources on wheat and barley and found that chlorophyll levels (*a*, *b*, and *a+b*) in plants with stress factors differed according to the light sources. Furthermore, in the study, El Haddaji *et al.*

(2023) concluded that producing micro-greens under artificial LED lighting is a very important model to solve the problem of decreasing arable land stocks per capita due to problems such as increasing population, decreasing water resources, urbanization, and climate change.

Miao *et al.* (2023) found that increasing the light intensity had a positive effect on plant height and leaf number in two lettuce varieties (*Lactuca sativa* L.) and one spinach variety (*Spinacia oleracea* L.), which is consistent with previous studies. While Crunchy lettuce plant width was not affected by changing light intensities, Deangelia lettuce width decreased as light intensity increased. However, Shawen spinach width was positively correlated with light intensity. These results highlight the influence of species-specific traits, such as morphological and physiological traits, that make certain varieties more or less suitable for certain environments.

Compared to previous studies investigating the effects of light on plants, this study shows that different applications of light have significant effects on plants. In particular, in parallel with previous studies investigating the effects of different wavelengths and intensities of light on plant growth, development and productivity, this study found that specific light spectra and application methods caused significant differences in photosynthetic activity, biomass production and morphological characteristics of plants. Artificial lighting has an impact on plants in all of the aforementioned investigations. In the present study, unlike the previous studies, it was observed that light affects mineral element uptake and the amount of chlorophyll in plants by considering the light intensity and distance together. Artificial light sources are expected to become more essential in plant growth in the future as the world population increases. The developments in artificial light sources, especially with developing technology today, warrant further research studies, which may lead to innovations in indoor farming, vertical farming, and greenhouse production, where artificial lighting plays a crucial role in providing the necessary light energy for photosynthesis. Additionally, understanding how different light spectra affect plant growth can lead to more efficient and sustainable agricultural practices, potentially reducing the need for chemical inputs and optimizing resource use.

CONCLUSION

This study demonstrates the importance of artificial lighting for agricultural productivity by addressing the effects of light intensity and distance on elemental uptake and chlorophyll content in plants. This approach is important as artificial light sources are becoming increasingly important in indoor plant cultivation and greenhouse production. From this study, researchers and agricultural practitioners can develop more efficient and sustainable practices, potentially reducing the need for chemical inputs and optimizing resource use. This knowledge will contribute to meeting the growing global demand for food and addressing challenges such as limited arable land and climate change.

With the use of artificial light methods in plant cultivation, appropriate lighting should be provided for the plants. In addition to sunshine in agricultural production, the primary goal of using artificial lighting is to boost agricultural productivity by delivering adequate light under economic conditions. Benefiting from light that creates perfect conditions for the plant may be more efficient. The results obtained in this study emphasize that plant species respond differently to various lighting conditions and that cultivation methods should be carefully determined to ensure optimum chlorophyll production. Therefore, careful adjustment of lighting conditions in plant breeding practices can be a decisive factor in plant productivity and quality.

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Agrociencia

THE EFFECT OF STORAGE TEMPERATURE ON SOME PHYSICOCHEMICAL PROPERTIES OF FLOWER HONEY

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ABSTRACT

The aim of the study was to determine the pH, Brix, moisture, free acidity, hydroxymethylfurfural (HMF) level, and diastase activity that are effective on the quality of unpasteurized and pasteurized flower honey produced in the South-eastern Anatolia Region of Türkiye after storage. A total of 40 samples brought for analysis were divided into two groups: raw and pasteurized at 70 °C. The honey samples were stored at different temperatures (4–6, 21–24, and 35–37 °C) and time periods (3, 6, and 9 months) for analysis. The data were statistically analyzed in a three-factor experimental design with repeated measurements at the levels of one of the factors. Considering the storage periods, it was found that HMF levels were 2.79 ± 3.08 , 4.36 ± 3.08 , and 184.17 ± 3.08 mg kg⁻¹, while the diastase values were 9.48 ± 0.39 , 8.51 ± 0.39 , and 1.46 ± 0.39 , respectively, and statistically significant ($p \leq 0.01$). Considering temperatures, it was determined that HMF and diastase values at 4–6 and 20–22 °C and 3-month storage periods were in accordance with the Turkish Food Codex Honey Communiqué, while the storage periods of the samples at 35–37 °C were above the legal limits. As a result, it can be recommended that flower honeys should be stored between 4–6 and 20–22 °C since the shelf life and honey quality will be negatively affected when the storage temperature exceeds 35–37 °C.

Key Words: honey quality, hydroxymethylfurfural, diastase activity, storage temperature.

INTRODUCTION

Bees produce honey from various floral nectars and insect secretions (Erbakan *et al.*, 2020). In general, honey is composed of sugar (glucose and fructose) (80 %), water (17 %), and proteins, enzymes, mineral substances, organic acids, and vitamins (3 %) (Khan *et al.*, 2015). Treatments, storage conditions, and the composition of honey have a significant effect on its quality. Some technological processes can be used to maintain the quality criteria of honey during the storage period. One of these processes is the application of heat to improve the its microbiological and physicochemical properties. Heat treatments are performed in double-walled tanks or using technological applications including microwaves and infrared. These processes

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result in both positive impacts and undesirable effects such as an increased amount of hydroxymethylfurfural (HMF) and heat-induced alterations of the diastase enzyme on the quality of honey (Erbakan *et al.*, 2020).

HMF is formed by the reaction of sugars and amino acids during heat treatments. It can be produced by either the breakdown of hexose in an acidic environment or the Maillard reaction (Güzel and Bahçeci, 2020). It is formed by the dehydration of monosaccharides, i.e., boiling in highly acidic environments, and the formation of furfural from pentoses and 5-hydroxymethyl furfural from hexoses as a result of monosaccharide molecules losing three molecules of water (Das *et al.*, 2022). HMF can be produced in honey over time as a result of heating, long-term storage, or other processes such as filtering, transporting, and packaging. While HMF is naturally present in very low concentrations even in freshly harvested honey, its formation is dependent on pH, ambient temperature, heat treatment time, and sugar concentration, which is regarded as one of the most important quality criteria for honey. Since the amount of HMF in honey is known to have a negative impact on health, HMF is a critical public health criterion.

The dominant climate group in South-eastern Anatolia, where the study was conducted, is continental, with very hot summers and rarely cold winters. The long-term average temperature of the region in August was 30.7 °C, and it was 32.1 °C in August 2024. The lowest temperature recorded in the region was 16.4 °C, while the highest was 46.1 °C (MGM, 2024). If the storage temperatures of the honeys are not taken into consideration due to the high temperature of the region, it may have negative effects on the quality of the honeys obtained in the region. While the amount of HMF increases at high temperatures, the diastase activity decreases. In other words, there is a negative correlation between HMF and diastase activity (Güzel and Bahçeci, 2020).

The International Agency for Research on Cancer classifies HMF as a probable carcinogen to human health. Related studies have shown that HMF has a highly toxic effect, and its intake at high doses may cause irritation of the upper respiratory tract, eyes, skin, and mucous membranes, as well as genotoxicity and tumor formation. Furthermore, the reaction of HMF with reactive oxygen disrupts antioxidant structures, causing oxidative stress or pathological disorders. Oxidative stress has a negative impact on DNA and damages cells and tissues by forming lesions (Batu *et al.*, 2014). The structure of HMF includes aromatic alcohol, aromatic aldehyde, and furan rings. In Türkiye, the maximum authorized concentration in honey is 40 mg kg⁻¹. Although heating processes and their duration affect the formation of HMF, its value can also rise under appropriate conditions when storage time and temperatures are not taken into consideration (Das *et al.*, 2022).

The diastase enzyme is another important factor in honey quality. Enzymes in the structure of honey are considered to be its most valuable components. It has been reported that the bee adds enzymes from its own glands while the rest come from the nectar source (Güzel and Bahçeci, 2020). If natural and freshly harvested honey has

not been subjected to any other process (such as heating, filtration, pasteurization, or packaging), it is considered to have a high diastase value (enzyme content) and to be of high quality.

The diastase enzyme is associated with honey maturation and has a direct effect on its quality. The diastase value is the volume of a 1 % starch solution that can be completely hydrolyzed by the diastase enzyme found in 1 g of honey in 1 h at 40 °C. Therefore, the amount of this enzyme is determined by the diastase value, and a count of more than eight indicates high quality. This is because the diastase enzyme degrades at high temperatures, poor storage conditions, and long storage times. As a result, the minimum diastase value in honey offered for consumption is eight according to legal regulations (Bell and Grainger, 2023).

The Turkish Food Codex, Honey Communiqué No. 2012/58 stipulates the parameters related to the consumption and quality of honey (TFC, 2020). Its chemical composition and quality vary based on the current conditions inside and outside the hive (after harvest), packaging, and storage conditions, as well as geographical and botanical origin. It has become necessary to carry out a series of chemical analyses to determine the changes in the chemical composition and dynamic structure of honey during the processing, packaging, and preservation processes (Amariei, 2020). Therefore, the aim of this study was to determine the pH, Brix, moisture, free acidity, HMF levels, and diastase activity that are effective on the quality of unpasteurized and pasteurized flower honey produced in the South-eastern Anatolia region of Türkiye after storage at different temperatures and time periods.

MATERIALS AND METHODS

Raw honey samples were delivered to the facility in March, numbered, and separated into two groups: unpasteurized and pasteurized at 70 °C for 5 min. The samples were stored at different temperatures (4–6, 20–22, and 35–37 °C) for nine months and then analyzed every three months (June, September, and December). The analysis included pH, moisture, Brix value, free acidity, hydroxymethylfurfural (HMF), and diastase activity.

Moisture analyses were carried out according to the TS 13365 method (Martin, 1979). The pH and free acidity of honey were determined using the TS 1728 method (ISO, 1970). Brix values were determined by placing 5 g of honey between the prisms of a refractometer (ATC model BX-90, China). The device was properly closed, and the dry matter content of the sample was determined using the indicator (AOAC, 2006).

The diastase activity was measured with a T80+ UV/VIS spectrometer according to the TS 13364 method (Schade *et al.*, 1958). The absorbance values of solutions prepared for HMF analysis were measured using a spectrometer (T80+ UV/VIS, United Kingdom) at 550 nm. The wavelength and absorbance values from the control group (HMF content, the solution in the tube with water was considered as the control group) were subtracted, multiplied by 192 (A factor found by taking into account the extinction

coefficient of the colored substance formed in the experiment and the concentration unit chosen for the result) coefficients, and recorded as HMF (AOAC, 1995).

Statistical Analysis

Statistical analyses of changes in each parameter were carried out using a three-factor experimental design with repeated measures at one of the factor levels, followed by Tukey's test for multiple comparisons, using SPSS Statistics 21.0 (IBM Statistics). The following model was used:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \pi m(ij) + \gamma k + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\pi\gamma)_{km(ij)} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl}$$

where μ is the general mean; α_i represents the effect of honey group i (1 and 2, unpasteurized and pasteurized); and β_j represents the effect of temperature j (4–6, 20–22, and 35–37 °C). $(\alpha\beta)_{ij}$ represents the interaction between honey group and temperature; $\pi m(ij)$ represents the effect of the experimental unit (at honey group i and temperature level j); and γk represents the effect of moisture level k . $(\alpha\gamma)_{ik}$ is the effect of the honey group x moisture interaction; $(\beta\gamma)_{jk}$ is the effect of the moisture x temperature interaction; $(\pi\gamma)_{km(ij)}$ is the effect of the experimental unit x moisture interaction; $(\alpha\beta\gamma)_{ijk}$ is the effect of the honey group x temperature x moisture interaction; and ε_{ijkl} is the error.

RESULTS AND DISCUSSION

From the descriptive values of the properties analyzed in the study (Table 1), it was observed that the HMF value increased in both honey groups as the storage temperatures increased.

While the highest HMF value was determined at 35–37 °C, the lowest diastase value was recorded at the same temperatures during the 3-month storage period among all replicates (Table 1).

The diastase values in the samples decreased as the storage temperature increased, as opposed to the HMF values. The diastase value was zero when the storage temperature rose to 36 °C in the second and third replicates.

No significant differences were found between the values of the other properties (pH, Brix values, moisture content, and free acidity). In all replicates, the pH ranged between 3.89 and 3.92, the brix values ranged between 81.24 and 81.42 %, the moisture content ranged between 16.89 and 17.01 %, and the free acidity values ranged between 17.7 and 18 meq kg⁻¹ at the different storage temperatures evaluated for both honey groups (Table 2). These results are similar to the required values in the literature (Czipa *et al.*, 2019; Güzel and Bahçeci, 2020; Balos *et al.*, 2023).

The HMF and diastase values were statistically significant ($p \leq 0.05$) at the storage temperatures evaluated (Table 2). There was no statistically significant difference between the means for both groups of honey. The difference between the means of

Table 1. Descriptive values of the physio-chemical properties evaluated in unpasteurized and pasteurized honey at different temperatures and storage time periods.

Replicate	Temperature	N	Unpasteurized		Pasteurized	
			HMF $\bar{x} \pm S_x$	DV $\bar{x} \pm S_x$	HMF $\bar{x} \pm S_x$	DV $\bar{x} \pm S_x$
June	4–6 °C	20	1.65 ± 0.62b	10.10 ± 3.50a	2.43 ± 0.83b	9.83 ± 3.53a
	20–22 °C		2.33 ± 2.07b	9.37 ± 3.14a	2.77 ± 1.23b	9.05 ± 3.10a
	35–37 °C		94.87 ± 28.34a	4.60 ± 1.45b	122.38 ± 31.62a	4.19 ± 1.24b
September	4–6 °C	20	2.06 ± 0.64b	9.37 ± 2.92	2.79 ± 0.85b	9.29 ± 2.61
	20–22 °C		4.50 ± 1.91b	8.78 ± 3.04	5.33 ± 1.70b	8.59 ± 3.02
	35–37 °C		150.38 ± 31.61a	0	175.31 ± 32.89a	0
December	4–6 °C	20	3.52 ± 1.31b	9.21 ± 2.83	4.31 ± 1.37b	9.11 ± 2.76
	20–22 °C		5.38 ± 1.62b	7.77 ± 3.03	5.89 ± 1.68b	7.52 ± 3.01
	35–37 °C		269.56 ± 54.89a	0	292.56 ± 55.89a	0

N: numbers of observation; \bar{x} : mean; S_x : standard error; HMF: hydroxymethylfurfural; DV: diastase value. Means with different letters are statistically significant ($p \leq 0.01$).

other properties measured in the study (pH value, Brix values, moisture content, and free acidity) was statistically insignificant at different storage temperatures for both honey groups.

Sajid *et al.* (2019) stored fresh honey samples from *Acacia modesta* (Wall.) P.J.H. Hurter, *Justicia adhatoda* L., *Brassica rapa* L., *Ziziphus jojoba* Mill., *Citrus x sinensis* (L.) Osbeck, *Trifolium repens* L., and *Dalbergia sissoo* Roxb. at room temperature (29 °C) and reported HMF values of 33.53 ± 3.46 , 25.24 ± 4.32 , 31.67 ± 4.32 , 24.45 ± 2.82 , 31.83 ± 3.75 , $40.68 \pm$

Table 2. Physicochemical properties evaluated in unpasteurized and pasteurized honey at different temperatures and storage time periods.

Factors	N	pH	BV	FA	DV	MC	HMF	
Temperature	4–6 °C	40	3.91 ± 0.02	81.33 ± 0.16	17.79 ± 0.90	9.48 ± 0.39a	16.95 ± 0.15	2.79 ± 3.08b
	20–22 °C	40	3.90 ± 0.02	81.33 ± 0.16	17.84 ± 0.90	8.51 ± 0.39a	16.95 ± 0.15	4.36 ± 3.08b
	35–37 °C	40	3.91 ± 0.02	81.33 ± 0.16	17.80 ± 0.90	1.46 ± 0.39b	16.95 ± 0.15	184.17 ± 3.08a
Honey group	Unpasteurized	40	3.91 ± 0.01	81.23 ± 0.13	17.75 ± 0.73	6.57 ± 0.31	17.01 ± 0.12	59.36 ± 2.51
	Pasteurized	40	3.91 ± 0.01	81.42 ± 0.13	17.87 ± 0.73	6.39 ± 0.31	16.89 ± 0.12	68.19 ± 2.51

BV: Brix value; FA: free acidity; DV: diastase value; MC: moisture content; HMF: hydroxymethylfurfural. Means with different letters are statistically significant ($p \leq 0.01$).

4.24 and 28.55 ± 3.6 mg kg⁻¹, respectively. They also reported HMF values in packaged honeys (Quersh Honey, Marhaba Honey, Sulman Honey, Saudi Honey, Young Honey, and Alshifa Honey) of 389.18 ± 296.95 , 316.86 ± 205.54 , 319.63 ± 268.43 , 331.26 ± 263.38 , 318.78 ± 214.42 , and 516.26 ± 273.2 mg kg⁻¹, respectively.

Balos *et al.* (2023) reported that the HMF content in honey stored at 22 °C was 2.08 ± 0.91 mg kg⁻¹. Kedziersk-Matysek *et al.* (2016) stated in their study on rapeseed honey that the HMF content of newly harvested honey samples was 3.07 ± 1.77 %, which significantly increased (19.74 ± 2.84) while storing the honey at room temperature (20–26 °C). Diafat *et al.* (2017) also reported that the HMF value of honey stored at 4 °C was 11.4 mg kg⁻¹, while Moloudian *et al.* (2018) reported HMF values between 30.7 ± 15.22 and 97.24 ± 129.1 in honey samples of different origins stored at room temperature (20 °C). The values obtained from these studies are higher than the values obtained in the present study. The HMF values obtained in Czipa *et al.* (2019) and Seraglio *et al.* (2021) showed similar values with the results obtained in the present study.

The freshness and quality of foods containing carbohydrates are strongly correlated with the HMF content, which is a crucial criterion used to assess the quality of honey and assess the processing and storage temperatures and conditions. HMF is formed when sugar in honey is broken down due to heat treatment and longer storage periods (Balos *et al.*, 2023). In general terms, the HMF value of honey increases to a certain extent even at temperatures such as 30–35 °C. Consequently, honey from areas with high temperatures has higher HMF values.

Karabournioti and Zervalaki (2001) evaluated honey from five different botanical origins at temperatures of 35, 45, 55, 65, and 75 °C for 24 h, and reported that the initial HMF levels in pine, citrus, sunflower, cotton, and thyme honey were 1.20, 2.25, 26.80, 9.70, and 8.78, respectively, whereas these values rose to 1.95, 3.45, 29.20, 9.90, and 10.78 at 35 °C and 43.40, 63.30, 226.35, 173.4, and 191.35 at 75 °C, respectively. Given that the honey was stored for 24 h in their study and a year in the current study, it can be argued that the lower HMF rates of the honey may be the result of different storage times.

It was also observed that the levels of HMF in honey samples varied depending on the source from which they were harvested, but they changed significantly with temperature and time.

Diastase loss in honey is one of the undesirable quality criteria, but the presence of very high levels of diastase is also not desirable. A high level of diastase increases fermentation (Korkmaz and Küplülü, 2017). In another study, Şahinler *et al.* (2007) stored honey samples at 55, 65, and 75 °C for 15, 30, 45, and 60 min, then stored them at room temperature for one year and examined the diastase activity. At room temperature at the end of the ninth month was 13.6. In this study, diastase activity was lower.

In their study, Khan *et al.* (2015) heat treated flower honey samples at 40, 50, 60, 70, and 80 °C for 5, 10, 15, 20, and 25 min each and found that the diastase values were 13.6, 13.9, 14.0, 14.1, and 14.0 at 40 °C (5, 10, 15, 20, and 25 min); 14.0, 14.3, 14.7, 15.7,

and 15.2 at 50 °C; 10.2, 9.8, 8.5, 5.3, and 5.6 at 60 °C; 8.2, 7.1, 5.7, 4.9, and 3.2 at 70 °C; and 6.2, 5.1, 3.1, 2.9, and 2.1 at 80 °C, respectively. These values were higher than those obtained in the present study despite the higher temperature of the heat treatments, and this may be attributed to the considerably shorter storage periods.

Korkmaz and Küplülü (2017) stored the extracted flower honey samples collected from different facilities at certain temperatures (10, 22, and 35 °C) in three-month periods (3, 6, 9, and 12 months) and examined the diastase activity in honey. The study found that the mean diastase values at 10 and 22 °C did not fall below 8 (the threshold value established by the Turkish Food Codex Honey Communiqué), but fell below 8 for honey stored at 35 °C after the sixth month. The results obtained are similar to those obtained herein.

Balos *et al.* (2023) determined the diastase activity of honey samples stored at 22 °C as 17.35 ± 3.97 . Moloudian *et al.* (2018) stated that diastase activity values ranged between 11.88 ± 0.05 and 28.68 ± 17.75 in honeys of different origins at room temperature (20 °C). Diafat *et al.* (2017) reported that the diastase activity of honeys stored at 4 °C was 129.49 ± 43.67 , which is considerably higher than the diastase value found in this study. In their study on raw rapeseed honey, Kedziersk-Matysek *et al.* (2016) reported that diastase activity in freshly harvested honey was 28.37 ± 6.53 , while its value for honey stored at room temperature (20–26 °C) decreased to 21.44 ± 4.37 .

Sajid *et al.* (2019) reported that the diastase activity of fresh honey samples *Acacia modesta* (Wall.) P.J.H. Hurter, *Justicia adhatoda* L., *Brassica rapa* L., *Ziziphus jojoba* Mill., *Citrus x sinensis* (L.) Osbeck, *Trifolium repens* L., and *Dalbergia sissoo* Roxb. stored at room temperature (29 °C) were 41.47 ± 5.70 , 29.57 ± 14.52 , 29.27 ± 7.75 , 26.97 ± 22.23 , 43.46 ± 10.46 , 35.55 ± 12.64 , and 34.5 ± 14.02 , respectively, which is higher than the values in the present study. Sajid *et al.* (2019) reported the diastase activity of packaged honeys (Quersh Honey, Marhaba Honey, Sulman Honey, Saudi Honey, Young Honey, and Alshifa Honey) stored at room temperature (29 °C) as 9.45 ± 1.48 , 5.95 ± 1.20 , 8.61 ± 1.99 , 6.9 ± 0.55 , 10.35 ± 1.48 , and 6.15 ± 0.72 . The diastase activity values obtained by Boussaid *et al.* (2018), Erbakan *et al.* (2020), and Bell and Grainger (2023) were similar to those obtained in this study.

Yılmaz and Eskici (2017) determined the diastase activities of 45 honey samples by using the spectrophotometric method. They stored the honey samples at 20 °C for one year and found that the mean diastase activity dropped from 14.6 to 10.7. The diastase activities found in their study were similar to those herein. The differences observed between previous studies and the current study were due to the values resulting from long-term storage in inappropriate storage conditions (high storage temperature) following honey harvesting as a result of the high temperatures to which the honey is exposed during factory processing.

Brix value and sugar rates in adulterated honey differ from those in natural honey. It is reported that the Brix value in natural honey ranges between 78.8 and 84 %. The moisture content of honey correlates with its sugar content (Kanbur *et al.*, 2021). The brix values obtained here ranged between 81.2 and 81.42 %, which were comparable

to those found in natural honey and other studies (Geana *et al.*, 2020). Natural honey is an acidic product, and pH is one of the most important properties used to determine honey quality. The concentration of amino acids, peptides, carbohydrates, organic acids, and minerals is important in determining pH values and, thus, acidity (Mohammed Hassan *et al.* 2021). Escuredo and Seijo (2019) found that the enzymes in honey had a significant impact on acid formation, with honey with high enzyme levels being more acidic. Natural honey should have a pH of 3.7 to 6.4 (Mutlu *et al.*, 2017). The pH of the honey obtained herein was found to be within the range (3.89–3.92) expected in natural honey.

The pH values detected in numerous studies are lower than those obtained in the present study (Aydın *et al.*, 2008; Yılmaz and Küfrevioğlu, 1999). Batu *et al.* (2013) found that Sivrice and Erzurum region honey had similar pH levels as this study, while Malatya, Ovacık, Muş, Artvin, Rize, and Bayburt region honey had higher pH levels than this study. The pH values obtained by Akyüz *et al.* (1995) and El Sohaimy *et al.* (2015) were higher than the present study. The variations in results can be attributed to differences in honey sources, regions, and climatic and ecological conditions.

The moisture content of honey is important for granulation, and it has been reported that an increase in moisture content may not only lead to microbial degradation but also to the crystallization of honey (Chen, 2019). The moisture content of honey should be approximately 17 % in order to attain harvest maturity (TFC, 2020). The moisture content obtained in the present study ranged between 16.89 and 18 %, falling within the values of the Turkish Food Codex Honey Communiqué. While the moisture values recorded in previous studies (Batu *et al.*, 2013) were lower than those recorded here, the moisture rates recorded in the studies of Akyüz *et al.* (1995), Serin *et al.* (2018), and Amariei *et al.* (2020) were similar to those detected in the current study. The differences between the studies can be attributed to the honey's different sources and regions of harvest.

CONCLUSION

The two most important criteria for determining the quality and freshness of honey are the hydroxymethylfurfural (HMF) content and diastase activity. The recommended limit values for honey storage conditions are a maximum of 40 mg HMF and a minimum of 8 for the diastase value. The HMF and diastase values of honey stored in June, September, and December deviated from the specified limit values when the temperature exceeded 21 °C. As a result, honey is best stored at room temperature.

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QUALITY OF STRAWBERRY (*Fragaria x ananassa* Duch.) FRUITS FROM PLANTS CULTIVATED UNDER COLORED SHADE NETS

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ABSTRACT

Strawberry (*Fragaria x ananassa* Duch.) fruit has a high demand worldwide for its organoleptic and nutraceutical properties that are beneficial to human health. Colored shade nets alter the light quality reaching plants, influencing specific physiological reactions and the phytochemical composition of fruits. The present study aimed to investigate how different photosensitive shade nets (black, 288 photosynthetically active radiation (PAR); blue, 429 PAR; green, 271 PAR; and beige, 519 PAR) affect the physical and biochemical quality of strawberry fruits cv. Albion. The study was conducted in a glass greenhouse in the spring-winter season of 2022. Strawberry seedlings were placed in 5-L containers in an open soilless system. A completely randomized experimental design was used, with four treatments and a control (973 PAR), with nine replicates; the experimental unit consisted of one plant per pot. The concentration of total soluble sugars, total soluble solids (TSS), phenolic compounds, anthocyanins, flavonoids, ascorbic acid, and antioxidant capacity were evaluated; as well as titratable acidity (TA), pH, and TSS/TA ratio. The results showed that the fruit's response was influenced by the color of the net. Fruits from plants grown under the blue and beige nets had 42 % higher total soluble sugars, while those under blue nets had 86 % more anthocyanins compared to the control plants. TSS values were similar in the control fruits and blue and beige nets. The fruits harvested from the beige net showed an increase of 24 % in TA and 4 % more phenolic compounds than the control. The blue and beige nets could serve as an alternate production strategy to enhance the physical and biochemical parameters of strawberry fruits.

Keywords: photosensitive net, anthocyanins, antioxidant capacity, phenolic compounds.

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INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch.) is a fruit characterized by its delicate flavor and high vitamin content (Zoratti *et al.*, 2015). The plant is cultivated in several regions from the Arctic to the tropics (Hancock, 2020), and the fruit contains a diversity of organoleptic and nutraceutical properties, including ascorbic acid, anthocyanins, and phenolic compounds, which possess a strong antioxidant capacity that is beneficial for human health (Stevenson and Scalzo, 2012). Strawberries are considered a functional food (Giampieri *et al.*, 2014) and are a rich source of phenolic compounds.

Shade nets in agriculture offer protection to crops from extreme radiation, winds, or pests (Shahak, 2008), birds, hail, and viral diseases transmitted by insects (Tezcan *et al.*, 2023). Black shade nets alter the amount of radiation plants receive, while color or photo-selective nets can modify light quality and enhance the transmission of specific wavelengths, potentially affecting physiological responses like photosynthesis and photomorphogenesis. The color of the nets can boost photosynthetically active radiation (PAR) or photosynthetic photon flux density (PPFD) within the range of 400–700 nm (Arthurs *et al.*, 2013) and increase the phytochemical and pigment content of crops.

In coriander (*Coriandrum sativum* L.), the use of black nets increased the content of total phenols, flavonoids, antioxidant activity, and ascorbic acid in the leaves (Buthelezi *et al.*, 2016). Miao *et al.* (2016) covered strawberry 'Yueli' fruits with transparent polyethylene colored filter light films (red, yellow, green, blue, and white); the fruits of the red and yellow covers had a higher content of total anthocyanins than those of the white (control), and the opposite effect was observed with the green and blue covers. With the use of red, green, and black nets with 40 % shading in strawberry plants cv. Kabarla, it was observed that all the nets increased the leaf anthocyanin content compared to the control, but with black, the content was the highest (Aras and Eşitken, 2019).

Retamales *et al.* (2008) found that utilizing colored nets altered the reproductive and vegetative patterns of *Vaccinium corymbosum* L., resulting in a 44 % increase in yield with white nets, 25 % with gray nets, and 32 % with red nets. Wang and Wang (2014) discovered that as the shading percentage increased, there was a 20 % longer duration in the flowering stage and a 13 % greater maturation of strawberry fruits; however, there were no significant effects in firmness, total soluble solids, ascorbic acid, and titratable acidity. The variability in research results suggests that the physiological responses of plants to light quality and intensity depend on the type, color, and spectral composition of the net (Kotilainen *et al.* 2018).

This study aimed to examine the impact of various colored shade nets (black, blue, green, and beige) on the physical, biochemical, and nutraceutical quality of strawberry fruits cv. Albion, and evaluate if this technology could be a feasible production method to improve these fruit characteristics.

MATERIALS AND METHODS

Plant material and growing conditions

The study was conducted in a glass greenhouse during the spring-winter season of 2022. Strawberry seedlings (cv. Albion) were placed in black polyethylene containers of 5-L capacity in an open soilless system. The substrate consisted of a 70:30 v/v mixture of sphagnum peat and perlite. Plants were set at a density of 11 plants per square meter. Three times a week, plants were hand-watered with 800 mL of a nutrient solution (Steiner, 1984) (pH 6.0, electrical conductivity 1.7 dS m⁻¹). Tap water was used to prepare the nutrient solution, considering its ion composition; the nutrient solution contained 9 mEq L⁻¹ NO₃⁻, 0.39 mEq L⁻¹ H₂PO₄⁻, 0.26 mEq L⁻¹ SO₄⁻², 4.43 mEq L⁻¹ K⁺, 5.83 mEq L⁻¹ Ca⁺², 1.94 mEq L⁻¹ Mg⁺², 3.24 ppm Fe-EDTA, 0.14 ppm Zn-EDTA, 0.05 ppm Cu-EDTA, 0.73 ppm Mn-EDTA, 0.05 ppm Mo-EDTA, and 0.13 ppm B-EDTA.

Temperature, relative humidity, and spectral irradiance underneath the nets

On the 25th day after transplant (dat), the plants were covered with shade nets (80 % shade) of various colors (black, green, blue, and beige), while a control group without a shade net was also set up. Nets were positioned at a height of 1.5 m above the plants. Temperature and relative humidity were recorded underneath each net using a datalogger (RC-51H, Elitech Technology, CA, USA). Spectral irradiance was recorded at 70 dat in each net at noon on a clear sky day using a multispectral radiometer (PS-300, Apogee Instruments Inc., UT, USA) (Table 1). An experimental design with four treatments and a control was implemented, utilizing 15 replications per treatment; a one-plant container was considered the experimental unit.

Table 1. Temperature, relative humidity, and spectral irradiance (μmol m⁻² s⁻¹) measured under the colored shade nets assed.

Variable	Control (no net)	Black	Blue	Green	Beige
Air temperature (°C) [†]	36.53±0.7	33.20±0.5	35.45±0.6	33.10±0.5	34.95±0.4
Relative humidity (%) [†]	28.55±1.5	41.21±1.9	38.21±1.7	38.46±2.2	38.50±1.8
PAR (400–700 nm)	973	288	429	271	519
Ultraviolet-B (280–320 nm)	0.71	0.15	0.11	0.04	0.09
Ultraviolet-A (320–400 nm)	43.44	14.51	16.38	12.61	21.05
Blue light (400–500 nm)	293.80	90.25	71.78	82.75	150.06
Green light (500–600 nm)	343.91	01.98	151.53	97.16	185.41
Red light (600–700 nm)	335.19	96.32	105.66	90.61	183.88
Far red light (700–800 nm)	297.24	82.89	120.99	81.98	163.77

[†]Data are expressed as mean ± standard error of 18 replications (18 weeks). PAR: Photosynthetically active radiation.

Fruit physical characterization

Five plants were selected randomly for each treatment. All fruits were harvested when they reached a bright red color covering three-quarters of the fruit. The length and width of the fruit were measured using a digital vernier caliper (Steren®, Mexico City, Mexico). The roundness index was calculated by dividing the length by the diameter of each fruit (Martínez-Bolaños *et al.*, 2008). Firmness was measured at the equatorial diameter (Martínez-Bolaños *et al.*, 2008) using a penetrometer equipped with a 1.9 mm wide tip (model FT O2, Qa Supplies®).

Fruit biochemical characterization

The total soluble sugar concentration was measured using the anthrone technique (Witham *et al.*, 1971) in 15 fruits (three-quarters covered in red) per treatment. A sample of 5 g of fresh fruit was prepared for each treatment replicate. The measurements were taken using a spectrophotometer (model Thermo Spectronic Genesys 10 UV, NY, USA) at a wavelength of 600 nm. The sugar concentration was calculated using a standard curve from 0 to 250 µg of glucose mL⁻¹. The total soluble solids (TSS) were measured using a digital refractometer (PAL-1 Atago®, Saitama, Japan) on 20 harvested fruits per treatment (also when three-quarters covered in red). The pH and titratable acidity (TA) of the fruit were measured from 10 fruits (in the same state of maturation mentioned above) per treatment. A sample of 10 g of fresh fruit was blended in 50 mL of distilled water for each treatment replicate. The pH was measured using a CONDUCTRONIC potentiometer (model PC45, Puebla, Mexico), and titratable acidity was evaluated using the AOAC (1980) method with acidity percentage calculated based on citric acid (mEq = 0.064). The equation used was:

$$\% \text{ citric acid} = \frac{\text{mL NaOH} \times N \text{ NaOH} \times \text{mEq citric acid} \times V \times 100}{\text{sample weight} \times \text{aliquot}}$$

The TSS/TA ratio was also calculated.

Fruit nutraceutical quantification

A methanolic extract was prepared from a sample of 15 fruits per treatment. Strawberry pulp (1 g) was crushed in 10 mL of aqueous methanol solution at 80 % v/v. The mixture was homogenized and sonicated (BRANSON 2510 sonicator, Danbury, CT, USA) for 15 min, allowed to rest for 24 h, and then centrifuged at 1400 x g for 10 min (Chang *et al.*, 2002).

Phenolics compounds

An aliquot of the methanolic extract (250 µL) was mixed with 250 µL of Folin-Ciocalteu reagent (0.2 N) and 2 mL of Na₂CO₃ (0.7 M). The mixture was left to incubate for 2 h at room temperature in the absence of light. The absorbance was determined at 765 nm (Thermo Spectronic Genesys 10 UV spectrophotometer, NY, USA). The phenol concentration was determined using a gallic acid standard curve ($y = 0.0060x + 0.0389$;

$R^2 = 0.991$) and expressed as mg gallic acid equivalents per 100 g fresh weight (mg GAE 100 g⁻¹ FW) following the Waterman and Mole (1994) technique.

Flavonoids

A 500 µL aliquot of the methanolic extract was mixed with 1.5 mL of 95 % ethanol, 100 µL of 10 % AlCl₃, 100 µL of CH₃COOK (1 M), and 2.8 mL of distilled water. The mixture was incubated for 30 min at room temperature, and the absorbance was measured at 415 nm. Flavonoid content was quantified using a standard curve of quercetin ($y = 0.006x - 0.0026$; $R^2 = 0.996$) as reported by Chang *et al.* (2002). The results were quantified as milligrams of quercetin equivalents per 100 grams of fresh weight (mg QE 100 g⁻¹ FW).

Anthocyanins

Two test tubes were prepared with 0.1 mL of the methanolic extract. A 0.025 N KCl solution (5 mL) at pH 1 was added to one test tube, and a 0.4 M CH₃COONa 3H₂O solution (5 mL) at pH 4.5 was added to the second test tube. The reaction was allowed to stabilize for 15 min, and then the absorbance was measured at 510 and 700 nm in both mixtures. The concentration of anthocyanins was measured in mg of cyanidin-3-glucoside equivalents per 100 g of fresh weight (mg CGE 100 g⁻¹ FW) (Giusti and Wrolstad, 2001).

Ascorbic acid

The method 967.21 from AOAC (1995) was utilized. A sample of 0.5 g of fresh fruit was prepared for each treatment replicate. The concentration of ascorbic acid was calculated using a standard curve equation ($y = -0.0038x + 0.2205$; $R^2 = 0.991$) at a wavelength of 515 nm. The results were quantified in milligrams of ascorbic acid equivalents per 100 grams of fresh weight (mg ASCE 100 g⁻¹ FW).

Antioxidant capacity

The antioxidant capacity was determined with the 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method (Re *et al.*, 1999). A Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) standard curve ($y = -0.2274x + 0.6746$; $R^2 = 0.993$) was utilized at a wavelength of 734 nm. The results were expressed as µM Trolox equivalents per 100 g of fresh weight (µM TE 100 g⁻¹ FW).

Statistical analysis

A completely randomized experimental design was used with four treatments and a control, with nine replicates for the physical, chemical, and nutraceutical characteristics of the fruit. Analysis of variance (ANOVA) and mean comparisons were performed using Tukey's test ($p \leq 0.05$) with SAS[®] version 9 software.

RESULTS AND DISCUSSION

Fruit physical characterization

Fruits from plants covered with colored nets exhibited significant variations in diameter, roundness index, and firmness compared to the fruits from the control plants ($p \leq 0.05$) (Table 2). Fruits harvested from colored nets had a diameter approximately 39 % smaller than those from the control group, with no significant impact on length. The decreased diameter caused a rise in the roundness index, leading to the production of elongated fruits. Fruits collected from plants under the blue and green nets had a 12 % drop in firmness compared to the control. The findings of this study do not coincide with those of Retamal-Salgado *et al.* (2017), who observed a decrease in firmness of 30 to 90 % in fruits of *Vaccinium corymbosum* L. grown in the field compared to the control group when the shade was increased using a black net, suggesting that using nets to reduce light exposure can lead to a shorter postharvest life.

Table 2. Physical characteristics in strawberry (*Fragaria x ananassa* Duch.) fruit cv. Albion cultivated under colored shade nets.

Color net	Equatorial diameter (mm)	Length (mm)	Roundness index	Firmness (N)
Control	39.23 ± 2.59 a	32.03 ± 0.55 a	0.88 ± 0.06 b	1.42 ± 0.03 a
Black	24.19 ± 0.55 b	32.42 ± 0.93 a	1.34 ± 0.03 a	1.34 ± 0.01 ab
Blue	24.36 ± 0.25 b	31.35 ± 0.56 a	1.28 ± 0.02 a	1.24 ± 0.03 b
Green	23.35 ± 0.58 b	30.87 ± 0.99 a	1.32 ± 0.03 a	1.26 ± 0.04 b
Beige	24.33 ± 0.39 b	31.23 ± 0.53 a	1.28 ± 0.01 a	1.35 ± 0.04 ab
HSD	4.96	2.98	0.14	0.12
CV (%)	13.60	7.01	8.50	6.80

Data are expressed as mean ± standard error of nine replications. Different letters in the same row indicate significant statistical differences (Tukey, $p \leq 0.05$). HSD: honest significant difference; CV: coefficient of variation.

Fruit biochemical characterization

Fruits from plants grown beneath blue, beige, and green nets exhibited an increase of 42, 28, and 22 % in total soluble sugars compared to those of the control ($p \leq 0.05$) (Table 3). There was no significant effect in sugar content between fruits cultivated under the black net and those grown in the control. The total soluble solids in fruits harvested from blue and beige nets was similar to that of the control group ($p \leq 0.05$) and 23 % higher than in fruits harvested with black and green nets. All fruits harvested from the nets, regardless of color, had a greater titratable acidity in comparison to fruits from the control treatment. The titratable acidity of fruits obtained from the beige net was 24 % higher than that from the other nets ($p \leq 0.05$). The pH of fruits from the control and blue nets was similar, while the fruits under the black and green nets were 5 and

Table 3. Chemical characteristics in strawberry (*Fragaria x ananassa* Duch.) fruits cv. Albion cultivated under colored shade nets.

Color net	Total soluble sugars (g 100 g ⁻¹ FW)	TSS (%)	TA (%)	pH	TSS/TA ratio
Control	2.63±0.16c	7.00±0.09a	1.00±0.03c	2.58±0.04a	7.07±0.31a
Black	2.62±0.12c	5.63±0.07b	1.11±0.01b	2.44±0.03bc	5.06±0.06c
Blue	3.73±0.13a	6.94±0.13a	1.10±0.01b	2.50±0.02ab	6.25±0.15b
Green	3.21±0.12b	5.60±0.07b	1.08±0.02b	2.35±0.02c	5.15±0.06c
Beige	3.36±0.07ab	6.78±0.06a	1.24±0.01a	2.48±0.01b	5.47±0.06c
HSD	0.49	0.34	0.07	0.09	0.64
CV (%)	11.76	3.99	4.89	2.83	8.31

Data are expressed as mean ± standard error of nine replications. Different letters in the same column indicate significant statistical differences (Tukey, $p \leq 0.05$). FW: fresh weight; TSS: total soluble solids; TA: titratable acidity; TSS/TA: TSS/TA ratio; HSD: honest significant difference; CV: coefficient of variation.

9 % more acidic than the control, respectively. Fruits from beige, green, and black nets had, on average, a TSS/TA ratio 26 % lower than control fruits, whereas fruits from blue nets had a ratio 11 % lower ($p \leq 0.05$).

In 'Hayward' kiwifruit vines under blue, white, red, and grey nets, it was observed that the red and white nets increased the size of the fruit and concentration of total soluble solids during cold storage, while the blue and grey nets decreased fruit size and concentration of total soluble solids compared with the control (Basile *et al.*, 2012). Zoratti *et al.* (2015) found a 12 % decrease in the yield of *Vaccinium corymbosum* L. fruits when grown under a black net (90 % shade) compared to the control group. Fruits collected from green and black nets had the lowest total soluble solids levels in this study, possibly due to the lower photosynthetic rate caused by the decrease in the radiation intensity reaching the plants.

This study demonstrates that fruits produced under nets exhibited more titratable acidity compared to the control plants (Table 3). The readings fall within the range specified by Hancock (2020) of 0.29 to 1.24 % citric acid in strawberry. Fruits grown under black, green, and beige nets exhibited a lower pH compared to the control and blue net fruits; in general, the use of nets yielded acidic fruits, due to the state of ripeness of the fruit in which it was harvested (when they reached a bright red color covering three-quarters of the fruit). The pH measured in this study was lower than the range of 3.4 to 3.7 reported by Nunes *et al.* (2021) for various strawberry fruit cultivars. Gunness *et al.* (2009) stated that pH plays a crucial role in the sensory characteristics of strawberry fruit by influencing the impression of sweetness, especially when the pH is less acidic. Simkova *et al.* (2024) found that the total soluble solids, titratable acidity, and the sugars/acid ratio are highly correlated with the ripening of strawberry as sugars increase and titratable acidity decreases as the fruit matures.

The data indicates a correlation between the far-red light passing through colored nets (Table 1) and the levels of total soluble sugars, total soluble solids, and pH in strawberry fruit. These parameters showed an increase with greater exposure to far red light, reaching a peak near 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ before decreasing (Figures 1A–1E). However, titratable acidity (Figure 1C) and the TSS/TA ratio (Figure 1E) consistently increased even when far red light exceeded 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Kim *et al.* (2020) found in tomato fruits (*Solanum lycopersicum* L.) that the combination of far-red wavelength and red lighting enhanced total soluble solids and titratable acidity, as opposed to red light alone; the authors suggested that this may be due to alterations in the phytochrome system and the functioning of the enzyme sucrose phosphate synthase as the fruit

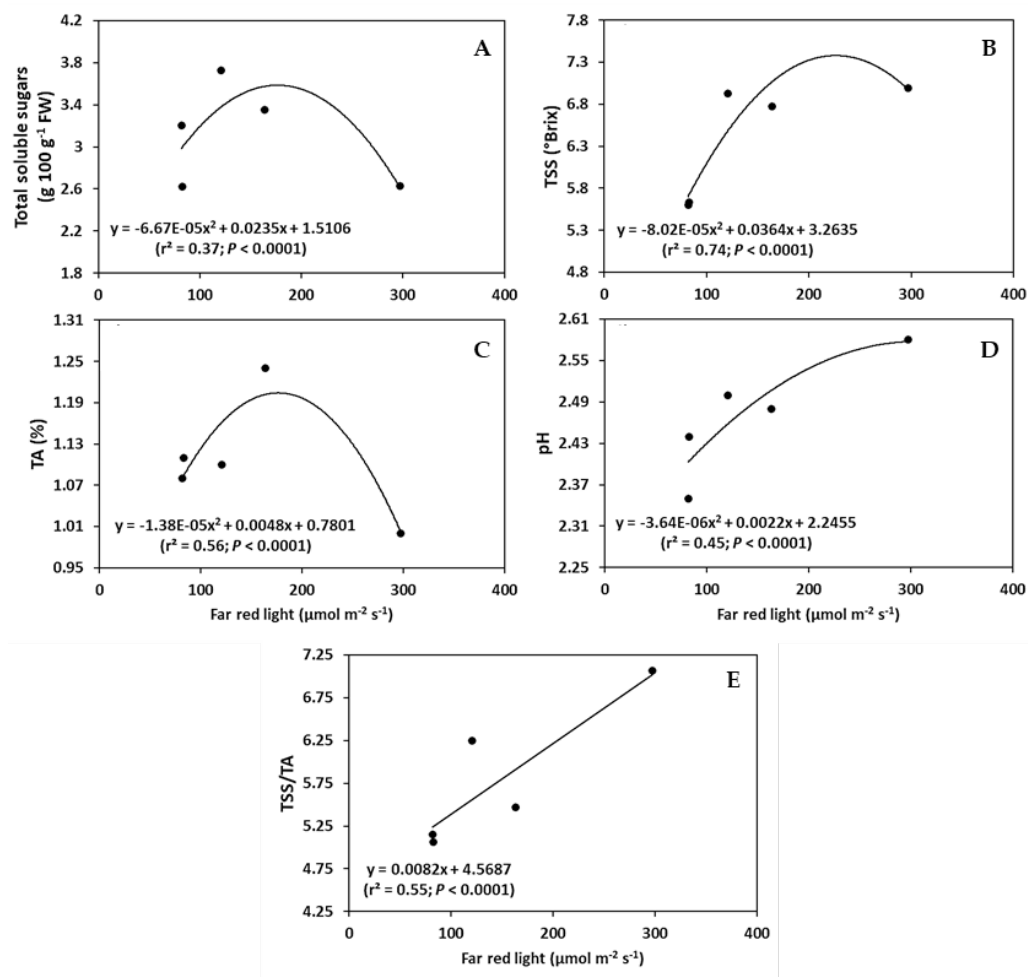


Figure 1. Chemical characterization of strawberry (*Fragaria x ananassa* Duch.) fruit cv. Albion cultivated under colored shade nets as influenced by far red light (700–800 nm). A: total soluble sugars; B: total soluble solids (TSS); C: titratable acidity (TA); D: pH; E: TSS/TA ratio. FW: fresh weight.

develops. Zhen *et al.* (2022) noted a synergy between far-red light photons and short-wavelength photons.

Studies have shown that far-red photons from LED light can enhance the photochemical efficiency and photosynthetic rate when combined with red/blue or white light (Zhen and van Iersel, 2017). In this study, a synergistic effect probably occurred in the control, beige, and blue net fruits that were exposed to a higher level of far-red wavelength light (Table 1). This exposure may have resulted in increased carbohydrate production and translocation to the fruits, leading to elevated total soluble solids, as suggested by Kim *et al.* (2020) in tomato.

Fruit nutraceutical quantification

Differences in nutraceutical concentrations in strawberry fruits were observed between treatments (Figure 2). The total content of phenolic compounds in fruits grown with beige nets was 4 % higher than that of the control and 5 % higher than that of fruits grown with black and blue nets ($p \leq 0.05$) (Figure 2A). The total phenols measured in this study were within the range reported by Nunes *et al.* (2006) for different strawberry cultivars grown in the field (110, 70, and 90 mg de GAE 100⁻¹ g for cv. Chandler, Oso Grande, and Sweet Charlie, respectively). However, it was lower than the concentration observed by Ornelas-Paz *et al.* (2013) for organically cultivated fruits of cv. Albion (209 mg de GAE 100⁻¹ g). In both studies, the fruits were harvested when three-quarters of the fruit had become red.

There were no significant differences in flavonoid concentrations between the control and net fruits; however, fruits from the beige and green nets were around 41 % higher in flavonoids than the blue net (Figure 2B). These results suggest that the strawberry plants cultivated under beige and green nets may have experienced stress conditions due to the quantity and ratio of UV-A and blue light that they received. Although the plants grown under the blue net received a high intensity and quantity of short wavelength light (UV-A and blue), the fruits did not demonstrate a high flavonoid content but a lower concentration, implying that these compounds were in the leaves. Zoratti *et al.* (2014) found that intense solar radiation, particularly in the blue spectrum (400–500 nm), alters flavonoid production in fruits. However, this response may vary depending on the interaction between light conditions and environmental factors. Additionally, Falcone *et al.* (2012) stated that exposure to UV-B radiation (280–320 nm) not only affects plant growth and development but also results in the production of reactive oxygen species. In response to this UV-B radiation, plants synthesize flavonoids to absorb it and to counteract the reactive oxygen species.

The anthocyanin concentration in fruits produced with the blue net was 86 % bigger than the control, 54 % higher than the beige net, and 95 % higher than the green and black nets. ($p \leq 0.05$) (Figure 2C). This may be due to the excessively high blue radiation may have caused photo-oxidative stress in the plants, resulting in increased production of antioxidants for protection, as indicated by Alrifai *et al.* (2019), and the increase in anthocyanin levels in the fruits. Qian *et al.* (2016) observed that the

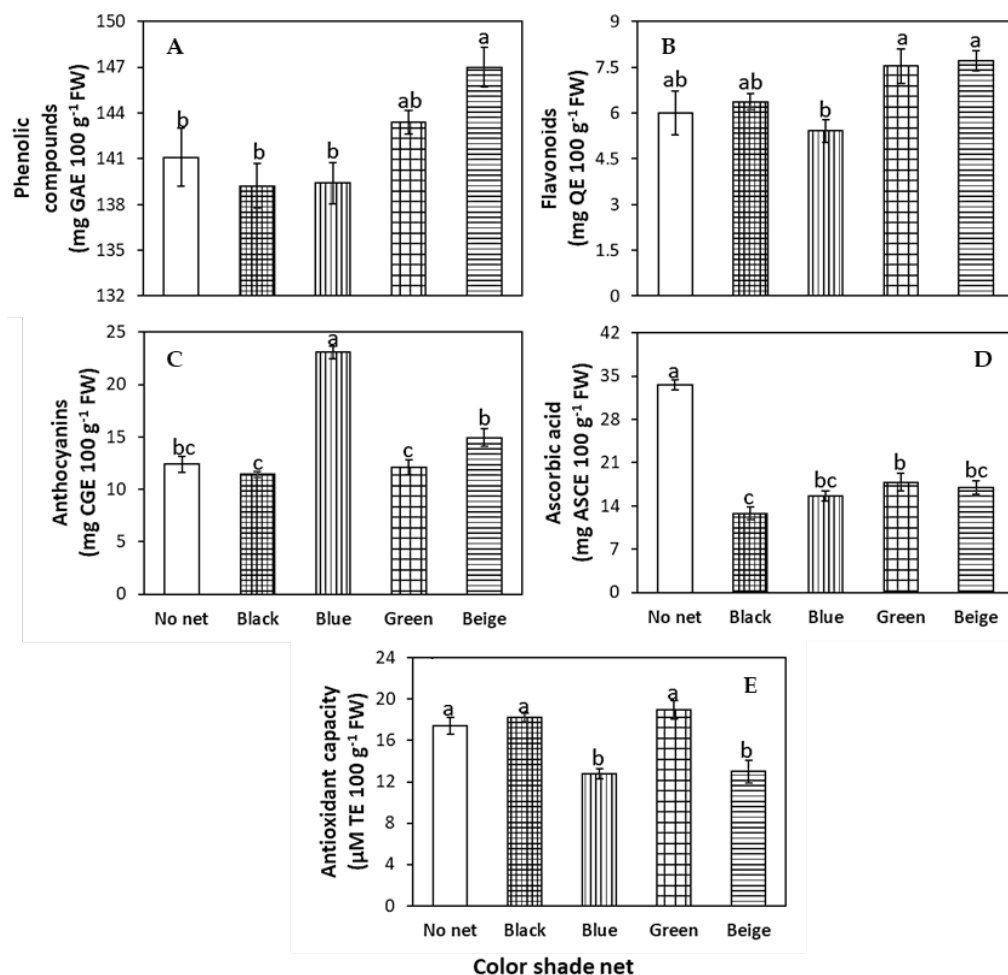


Figure 2. Antioxidant concentration in strawberry (*Fragaria x ananassa* Duch.) fruit cv. Albion cultivated under varying colored shade nets. A: phenolic compounds; B: flavonoids; C: anthocyanins; D: ascorbic acid; E: antioxidant capacity. Data are expressed as mean \pm standard error of nine replications. Different letters on the bars indicate significant statistical differences (Tukey, $p \leq 0.05$). FW: fresh weight; GAE: gallic acid equivalents; QE: quercetin equivalents; CGE: cyanidin-3-glucoside equivalents; ASCE: ascorbic acid equivalents; TE: Trolox equivalents.

accumulation of anthocyanin in fruits is influenced by light as it is required for their synthesis, and that light spectral quality can affect the process. Short wavelengths in the blue and UV spectrum are reported to enhance anthocyanin concentration when fruits are ripening (Zoratti *et al.*, 2015).

The color of the nets did not promote the buildup of ascorbic acid in the fruits; these levels were half of those found in the control fruits (Figure 2D). According to Flieger *et al.* (2021), stressful conditions lead to the production of reactive oxygen species in

plants, which are responsible for damaging biomolecules such as membrane lipids, proteins, and nucleic acids. Plants have developed a defensive mechanism against these reactive oxygen species by creating antioxidant substances like ascorbic acid to neutralize the negative impact of free radicals. The present study showed that the control fruits contained the highest level of ascorbic acid, indicating that these plants were experiencing stressful conditions. However, the use of colored shade nets alleviated such stressful conditions caused by high solar intensity, temperature, and low relative humidity, as their use was associated with a 3 °C drop in temperature and a 37 % increase in relative humidity when compared to the control. Temperature and relative humidity are environmental conditions that significantly impact the growth of strawberries (Hancock, 2020).

The antioxidant capacity of fruits from plants grown under black and green nets was similar to that of the control and 29 % higher than those under blue and beige nets (Figure 2E). Fruits' antioxidant activity refers to the capacity they have to counteract or decrease oxidation produced by atoms or groups of atoms with unpaired electrons that steal electrons from stable molecules, known as free radicals (Niki, 2011). The results obtained suggest that the plants from the control, black net, and green net produced fruits with greater capacity to neutralize free radicals because they contained higher levels of certain antioxidants.

The antioxidant capability observed in the control may be linked primarily to the stress induced by the intense light and high temperature that the plants were subjected to in comparison to plants under the colored nets, since the control exhibited a high quantity of ascorbic acid. The fruits collected from plants grown under blue and beige nets exhibited a lower antioxidant capacity compared to those collected using black and green nets; however, phenolic compounds and anthocyanins were the main contributing chemicals in the beige (Figure 2A) and blue (Figure 2C) nets, respectively. Warner *et al.* (2021) comment that the secondary metabolites that are predominant in strawberry fruit and influence antioxidant capacity are phenolic compounds. This activity varies depending on the cultivar and maturation stage of the fruit (Hwang *et al.*, 2019). The present study suggests that the antioxidant capacity of fruits obtained using colored nets may not be solely due to the concentration of a specific compound, and additional determinations like the FRAP and DPPH methods were necessary.

CONCLUSIONS

Fruit quality was affected by the color of the shade nets. Fruits harvested from plants grown under the blue and beige nets showed higher total soluble sugars, and with the blue net, the highest concentration of anthocyanin was presented, whereas those harvested with the beige net had increased concentrations of titratable acidity and phenolic compounds. The study suggests that using blue and beige nets could serve as an alternate production strategy to enhance the quality and nutritional properties of 'Albion' strawberry fruit. Using other methods is recommended to accurately assess the antioxidant capacity.

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TOWARDS BIOCULTURAL CONSERVATION OF STINGLESS BEES IN CÓRDOBA, VERACRUZ, MEXICO

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ABSTRACT

Based on biological and ethnobiological data from 2020 to 2022, this study provides a base line for the biocultural conservation of stingless bees. These bees in Mexico, like many other pollinators, are facing population declines and this biological loss impacts traditional human knowledge and its uses. This paper aims to record the traditional ecological knowledge about these bees to establish sustainable meliponiculture. Cultural values were obtained through participant observation, interviews, free listing, and structured questionnaires, and a bee inventory was conducted. Data were analyzed using Smith's Prominence Index and descriptive statistics using SPSS v. 25 software. Inhabitants recognized 21 floral visitors, including invertebrates, mammals and birds. 'Tencales' (local term for *Scaptotrigona mexicana*), hummingbirds and butterflies are the most culturally prominent floral visitors. The meliponini belonging to *Scaptotrigona mexicana* were recognized as 'tenchal', although different common names were obtained for other meliponini, denoting morphological and behavioral differences among the following species: *Partamona bilineata*, *Plebeia pulchra*, *Scaptotrigona pectoralis*, *Scaura argyrea*, *Trigona corvina*, *Trigona fulviventris*, *Trigona fuscipennis* and *Trigona nigerrima*. Although the research area is located near a large city, there is substantial diversity of melliferous flora, floral visitors, and traditional ecological knowledge used by the inhabitants in their daily activities. Stingless bees are perceived as the primary pollinators that help maintain their forests and thus help sustain their lives. This paper promotes better awareness of bioculturally effective community conservation strategies.

Keywords: Meliponini, native bees, floral visitors, pollinators, biocultural richness.

INTRODUCTION

Human cognition implies adaptation and coevolution with the environment in which humans develop the knowledge that allows them to survive and ensure success for their descendants in future generations. This cultural process has been

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named variously as: 'native', 'traditional', 'empirical', 'ancient', 'indigenous', 'folk', 'rural', 'local' or 'traditional ecological' (Ellen, 2009). Also, Ellen and Harris (2000) refer to it as 'indigenous environmental knowledge' (IEK) and list some of its more commonly asserted characteristics. This reservoir of experience and biological and ecological information about species richness at the local level is transmitted between generations orally through cultural transmission, i.e., the emergence, acquisition, storage and communication of ideas and practices. This can be transmitted from parents to children (vertical transmission), between two individuals of the same generation (horizontal transmission), and/or from non-parental individuals of the parental generation to members of the filial generation (oblique transmission) based on Ohmagari and Berkes (1997).

Mexican ethnobiological studies regarding stingless bees (i.e. Simms and Porter-Bolland, 2021; Vásquez-García *et al.*, 2021) describe traditional knowledge of bee biology, ecological relationships, morphology, behavior, nesting, handling and harvesting honey, wax, propolis, symbolic aspects and taxonomic determination of species. However, in the preceding studies the information obtained was based exclusively on the testimony of adult producers or beekeepers.

This paper arises from the interest of Colegio de Postgraduados' researchers in using this knowledge as a basis for a strategy for developing and conserving these bees and other pollinators in Cervantes y Lozada. It could be hypothesized that a base line of traditional-local knowledge rooted in this community will ensure acceptance and success in establishing colonies that can be used in a sustainable way.

It is recognized that insects in general and bees in particular have been gradually declining worldwide, mainly due to changes in land use, large-scale agriculture, deforestation, excessive use of agrochemicals, climate change, impacts of genetically modified organisms, pathogens, pests and exotic species, which have affected plant reproduction and crop yields (Hristov *et al.*, 2020; Nava-Bolaños *et al.*, 2022). From the ecological point of view, bees have importance as efficient pollinators of wild and cultivated plants, thus contributing to the maintenance of ecosystems and the conservation of biodiversity.

MATERIALS AND METHODS

Study area description

Cervantes y Lozada (CyL) is located at 18° 57' 14.4" N and 96° 55' 35.04" W with an average elevation of 1214 m (Figure 1), in the region called 'Sierra del Gallego', 12 km northeast of the nearby city of Córdoba, Veracruz, Mexico. The local climate is semi-warm and humid, with abundant rainfall in summer (1900-2100 mm annually) and a temperature range of 18-24 °C. The area has the following types of vegetation and land use: a) montane cloud forest in the highest parts of its territory; b) tropical evergreen forest in the lower parts, c) a transition strip or ecotone between the two, at the same elevation of the CyL community, d) secondary vegetation derived from these types

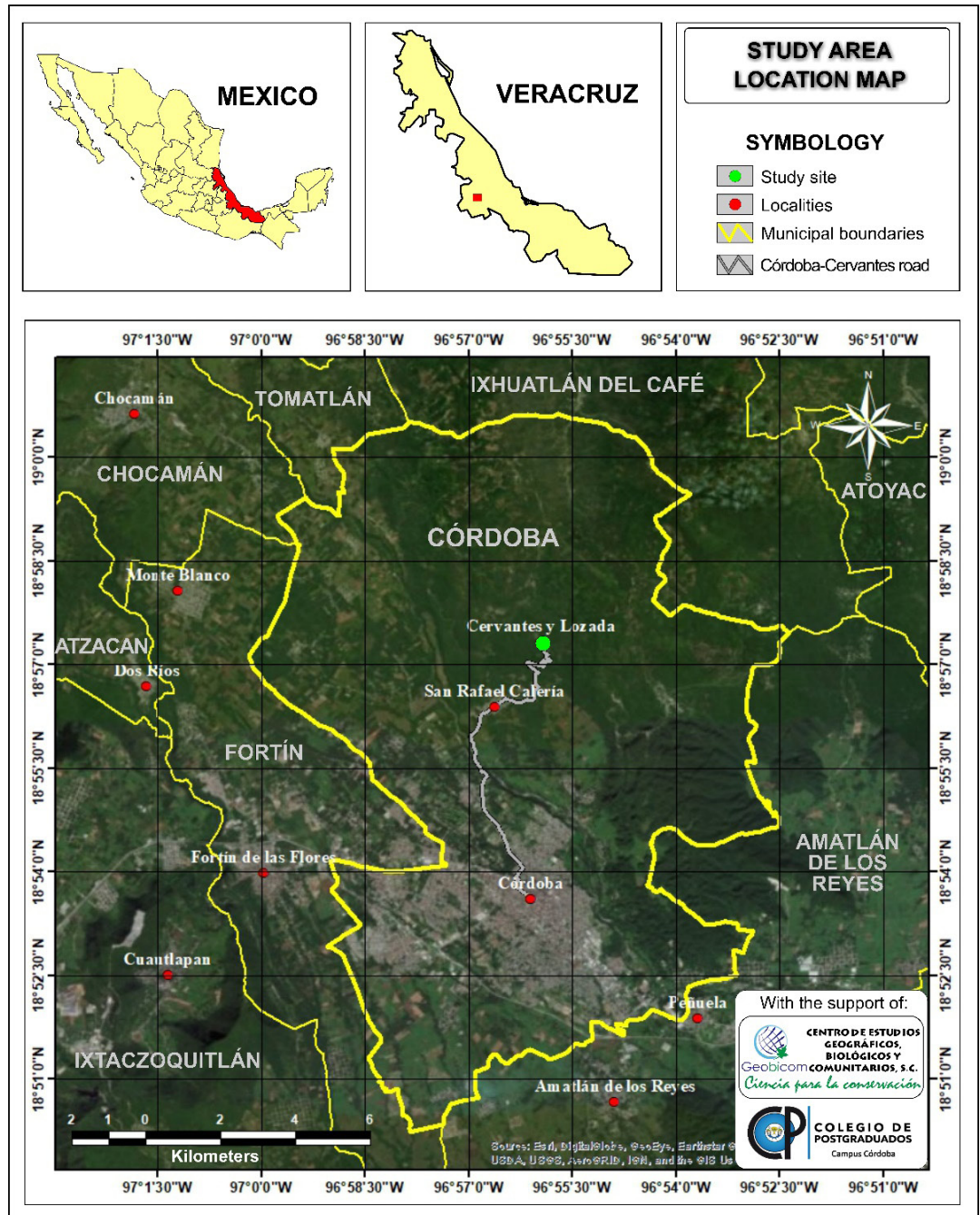


Figure 1. Location of Cervantes y Lozada, Córdoba, Veracruz, Mexico. Source: prepared by authors using Google Earth images.

of vegetation, and e) areas of different crops (Real-Luna *et al.*, 2021; 2024; Alcántara-Salinas *et al.*, 2020).

Sociocultural context of Cervantes y Lozada

The community has a population of 173 inhabitants in 54 households. The basic dwelling usually consists of two main spaces, one that serves as a kitchen-dining room-living room and the other as a bedroom. These homes are constructed with cement materials with windows without a pitched roof, reflecting the kind of dwelling found in Córdoba city. Behind or around the dwelling are orchards with fruit-bearing trees, medicinal plants and spaces where the people raise domestic animals, mainly ducks, turkeys, chickens and, in a few cases, pigs. There is electricity and tap water. Some use gas for cooking (most dwellings use firewood) and most dwellings have a mobile phone.

The principal subsistence activity is agriculture, mainly carried out by older people with the participation of all family members. They harvest the main crops such as: 'coffee' (*Coffea arabica* L.), some maize, beans, chili and squash. Coffee farms are intercropped with different varieties of banana (*Musa x paradisiaca* L.) and ornamental species planted mainly for their foliage, such as: 'palma camedor' (*Chamaedorea elegans* Mart. and *Ch. metallica* O.F. Cook ex H.E. Moore), 'maicera' (*Dracaena fragrans* (L.) Ker Gawl.) and 'dracena' (*Cordyline fruticosa* (L.) A. Chev.). Some people earn a wage income by being hired at harvest time and as workers in Córdoba City; some migrate to the U.S.A. and Mexico City where wages are higher. Coffee is the main agricultural product traded outside CyL. Although this town is considered highly marginalized with a significant degree of illiteracy (CONAPO, 2021), members of the community have their own dwelling as private property and share rights and duties as citizens, such as the 'faena' (meaning 'work' or 'tribute'), wherein they perform communal labor on collective resources such as the maintenance of schools, fencing and roads. Formal education is available for children, which consists of preschool and grades 1 to 6 in a primary school with one teacher. The primary school has two classrooms with a playground area. If a family should decide to educate their children further, San Rafael Calería, 4.3 km from CyL, is the nearest town with a secondary school. For undergraduate studies youths must leave CyL.

On the main street there is a Catholic church, an evangelical temple, and a community house which serves as a place for meetings and training programs. As in other communities of the region, CyL faces changes in land use for agriculture (mainly coffee and banana) and deforestation for firewood. To raise agricultural production, some people use herbicides for weed control or other chemical products affecting wildlife. There is also the problem of poaching of game by people outside the community.

In 2017 researchers from the Colegio de Postgraduados initiated biological studies and training of local participants for community bird monitoring in CyL (Alcántara-Salinas *et al.*, 2020). The Colegio de Postgraduados also established meliponini nests for research proposes and some for reintegration. Given the community relationship

fostered by the doctoral thesis developed by Natalia Real Luna in 2020, this site was considered ideal for promoting the establishment of bee nests as a biocultural conservation strategy. In addition, the Colegio de Postgraduados joined The National Strategy for Conservation of Pollinators in 2021 (Secretaría de Agricultura y Desarrollo Rural-SADER and Secretaría de Medio Ambiente y Recursos Naturales-SEMARNAT, 2021) to provide experience on patented box construction (Salazar-Vargas *et al.*, 2019) and sustainable management of meliponini.

Data collection

This research was conducted by using a combination of biological and ethnobiological techniques. Researchers adopted a broadly participatory approach and, as far as possible, joined in local subsistence and cultural activities.

Information was obtained in two kinds of data: 1. Data on pollinators (floral visitors) emphasizing meliponini, collected and analyzed from the perspective of scientific entomology and biology; and 2. Ethnographic data to address the research questions concerning differences and similarities regarding traditional knowledge of pollinators (floral visitors) within the CyL population, especially meliponini, where individual respondents engaged with a combination of approaches and elicitation techniques, such as interviews, free listing, questionnaires and participatory workshops, the last mainly at the primary school. The research work began in March 2020, paused due to the Covid-19 pandemic from April to November 2020, then continued from December 2020 until March 2022, with weekly visits to the community.

During the first incursion into the field, researchers established contact with local authorities and citizens to request approval to conduct workshops with children. Overall, the norms established in the Code of Ethics of the International Society of Ethnobiology (ISE, 2020) were adopted.

Data on stingless bee inventory

Stingless bees were collected *in situ* from February 2021 to March 2022. When species determination was problematic, the specimens were collected with an entomological net on roadsides and in the community gardens from 10:00 am to 4:00 pm. For bee collection the usual preservation process was followed, with specimens being individually labelled and mounted in special boxes. Taxonomic determination was made using published keys for stingless bees developed by Arnold *et al.* (2018), Ayala (1999) and Bonet and Vergara (2016). The collected specimens are kept in the wildlife laboratory of the Colegio de Postgraduados Campus Córdoba.

Traditional knowledge

Participant observation was used as the default qualitative research strategy, as well as different kinds of interviews such as 1) free interviews, 2) *ad hoc* or informal interviews, and 3) structured interviews (see Alcántara-Salinas *et al.*, 2013 for details), in addition to workshops with children at the school where interviews were conducted. All these techniques were carried out in different contexts while people were doing

daily activities. Quantitative methods were used to analyze free listing and structured questionnaire data.

Free listing was employed to generate data about the cultural prominence of floral visitors organized at the same level of contrast. Free lists were solicited from 25 randomly chosen people from the community. The frequency, average rank and the value of Smith's Prominence Index of items listed were calculated (Alcántara-Salinas, 2021; Puri, 2011; Quinlan, 2017).

The local knowledge regarding floral visitors was determined for different age and gender groups to differentiate traditional-local ecological knowledge between age groups. Then stingless bees were used for formal questionnaires, as these were the highest-ranking type of floral visitors elicited.

The questionnaire was formulated with the following topics: 1. Common name, 2. Biology, 3. Colony products, 4. Uses of colony products, and 5. Oral tradition. These topics were obtained through a pilot test carried out at the beginning of the fieldwork. Questionnaires were conducted at each individual's home to avoid crowding and distractions. For children, questionnaires were applied individually after a workshop held in the classroom.

The analysis divided age categories into three main groups, following the protocol of Alcántara-Salinas (2013): children (6 to 13 years), youths (14 to 39 years), and adults (40 to 96 years).

All responses were systematized and coded in a Microsoft Office 365 Excel worksheet, then the information was analyzed. We also analyzed by percentages, by gender and by age to relate traditional ecological knowledge of meliponini with the questionnaire responses by constructing a 9 x 75 matrix (nine questions by 75 individuals) using SPSS v. 25 software SPSS (IBM, 2017).

The questionnaire was applied to a total of 75 people, another 65 individuals were interviewed to obtain information about pollinators and stingless bees, and 13 primary school children were included for the ethnobiological approach.

RESULTS AND DISCUSSION

Stingless bee inventory

Mexico has 46 species of stingless bees; 25 of them have been recorded in the state of Veracruz and 11 species have been recorded in the municipality of Córdoba (Ayala, 1999; Bonet and Vergara, 2016). Nine species were recorded in Cervantes y Lozada: *Partamona bilineata* (Say, 1837), *Plebeia pulchra* Ayala, 1999, *Scaptotrigona mexicana* (Guérin, 1845), *Scaptotrigona pectoralis* (Dalla Torre, 1896), *Scaura argyrea* (Cockerell, 1912), *Trigona corvina* Cockerell, 1913, *Trigona fulviventris* Guérin, 1845, *Trigona fuscipennis* Friese, 1900 and *Trigona nigerrima* Cresson, 1878 (Figure 2).

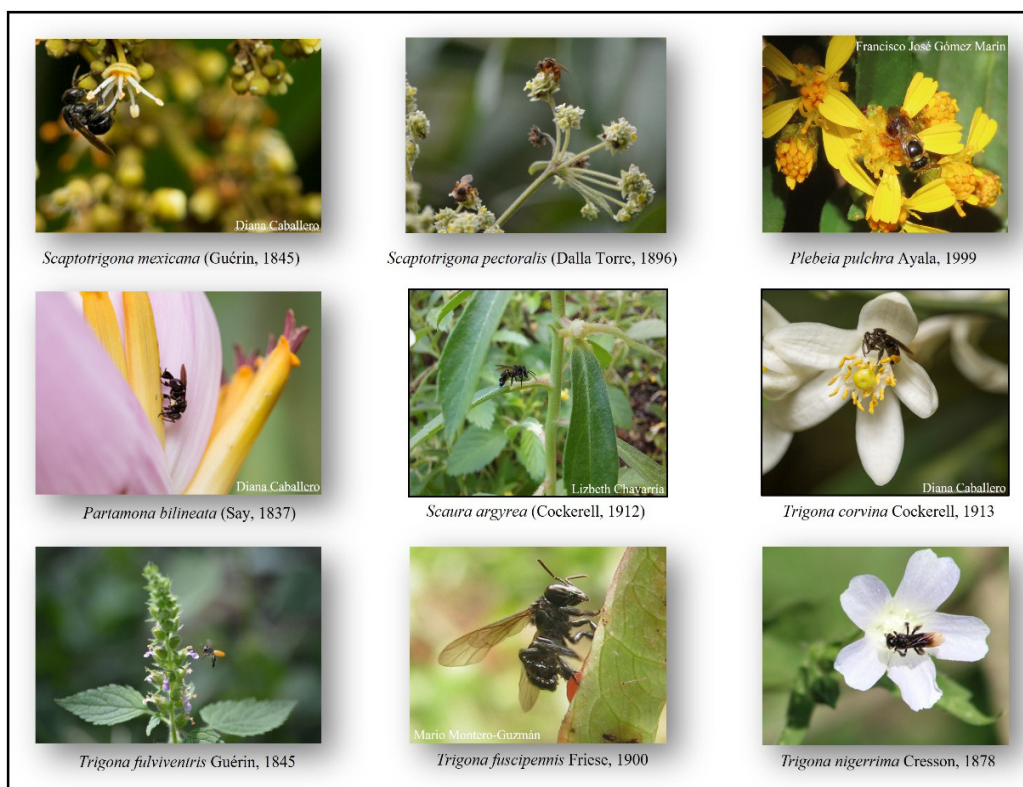


Figure 2. Species of stingless bees found in Cervantes y Lozada, Córdoba, Veracruz, Mexico.

Traditional knowledge

Free listing was conducted with 25 individuals (12 men and 13 women) representing 15 % of the population, which generated 155 answers. Twenty-one species were mentioned as feeding on nectar or otherwise related to flowers: arthropods (86 %), birds (9 %) and mammals (5 %). Figure 3 shows the Smith's Prominence Index results: bee (*Apis mellifera*), butterfly (various species), hummingbird (various species), 'tenchal', 'jicote' (bumble bee), and wasp were the most frequently mentioned floral visitors. Free listing results emphasize the perception of insects as floral visitors, but also recognize other flower visitants, whether for feeding purposes or some other form of interaction, such as for predation (birds, ants, spiders, small beetles, dragonflies) or herbivory (crickets, worms, stick insects) similar to the food networks reported in Crespo-Pérez *et al.* (2020). In general, people recognized the value and function of pollinators to maintain the surrounding forests by providing environmental services, such as air pollution reduction, biological control, plant regeneration, etc. Perception of floral visitors among individuals by gender showed that women mentioned 17 of the 21 animals listed, while men mentioned 16. The highest Smith's Prominence Index values (Figure 4) by gender indicated that women and men

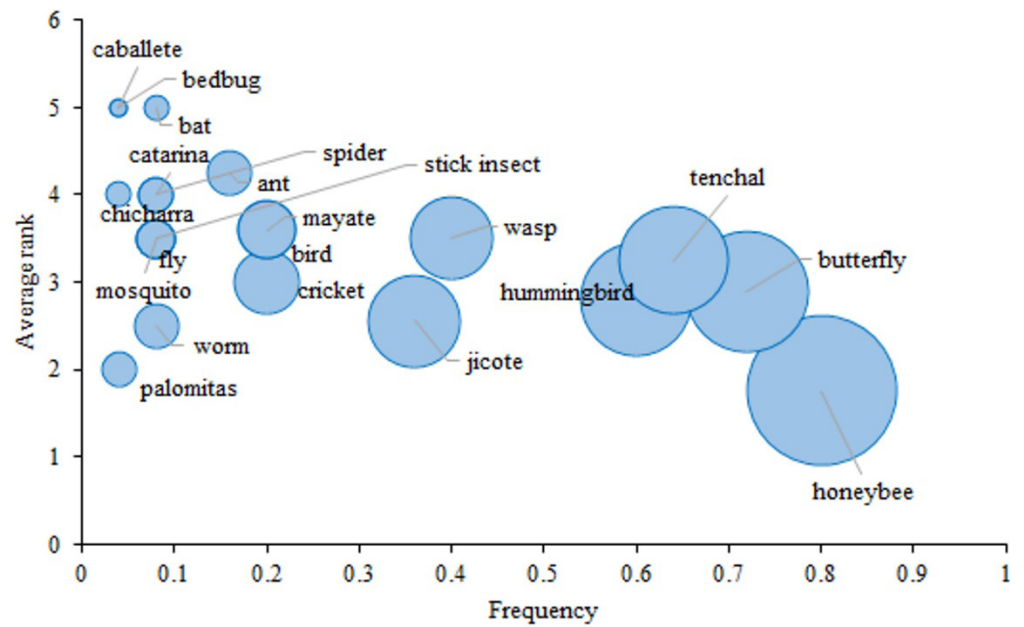


Figure 3. Plot of average rank versus frequency; the size of the sphere shows the value of Smith's Prominence Index with respect to the floral visitors mentioned in free listing data.

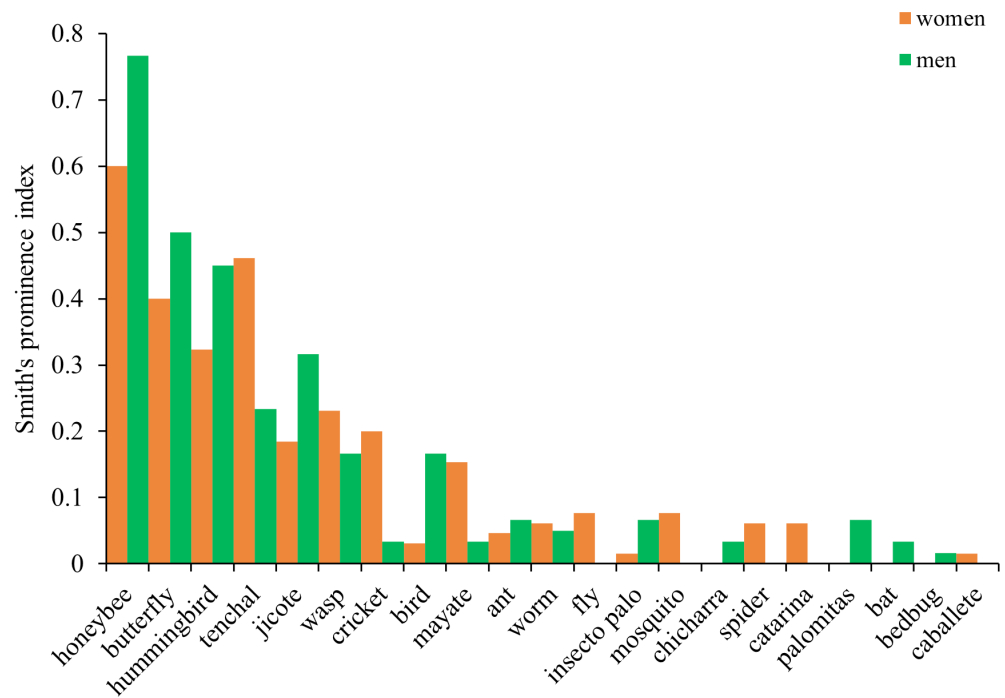


Figure 4. Smith's Prominence Index for floral visitors mentioned in the free listing by women and men.

mentioned bee (*Apis mellifera*), butterfly (several species), hummingbird (several species), 'tenchal', 'jicote' (bumble bee), and wasp as the most frequent floral visitors. Since stingless bees appeared as one of the most represented in free listing, the questionnaire was focused on them.

Questionnaires focused on stingless bees as the highest ranked floral visitor species and were completed by 75 people: 52 % by men and 48 % by women, 17 % by children, 39 % by youths, and 44 % by adults.

Common names of stingless bees

Most of the people can clearly differentiate similar insects. For instance, European bees (*Apis mellifera*) are never confused with native bees, and it is recognized that their swarms or hives are different from those of stingless bees by their morphological and behavioral characteristics. In fact, most of the people in CyL recognize the term 'tenchal' for the species *Scaptotrigona mexicana* (Guérin, 1845); this term has an important cultural prominence. 'Tenchal' was the response of 58 % of adults against 38.5 % of children in the questionnaire applied.

Although the term 'tenchal' is used for *S. mexicana*, some people preferred other terms, such as 'comejé', 'abeja negra', 'mosquito', 'abeja real' and 'melipona', to distinguish it from other meliponini species like: *Partamona bilineata* (Say, 1837), *Plebeia pulchra* Ayala, 1999, *S. pectoralis* (Dalla Torre, 1896), *Scaura argyrea* (Cockerell, 1912), *Trigona corvina* Cockerell, 1913, *T. fulvoventris* Guérin, 1845, *T. fuscipennis* Friese, 1900 and *T. nigerrima* Cresson, 1878.

All the terms and meliponini species mentioned above were corroborated *in situ*, while doing interviews, collecting bees and performing participative observation. The fact that people recognize species by different terms stresses some multidimensional principles of classification (Alcántara-Salinas *et al.*, 2013), where some aspects like habitat, morphology, color, or the relationship with humans are involved in naming meliponini.

Also, the fact that some species are given different descriptive common names can be explained, as Ellen (1999) noted: 'that knowledge of names ('lexical' knowledge) is unevenly distributed within the community (presumably mainly among older people); or that knowledge of names in themselves does not always reflect 'substantive knowledge' or a combination of both'. The task of obtaining local names of species of interest has great importance for maintaining a clear dialogue with local people, as it allows participants to manage the same cultural codes, not only in the use of the language, but also in cultural perception, and to have direct access to people's psyches for their conceptual words (Alcántara-Salinas, 2021). Common names are evidence of traditional knowledge transmission (Alcántara-Salinas *et al.*, 2022; Geng *et al.*, 2016) and are needed to repatriate traditional-local knowledge to the community to facilitate projects or elements to promote biocultural conservation.

Stingless bee biology

Common names not only encode useful recognition of biological species of bees, but also encode features such as color, size, and behavior. In this regard, CyL inhabitants distinguish bees 50.7 % by color and 30.7 % by size. In addition, people encode more detailed aspects of bee size; for example, 'they are like an ant' or 'smaller than bees or wasps'. They also stress their color: 'some are black' as is *Scaptotrigona Mexicana*; 'brown, yellow or orange' as is *Scaptotrigona pectoralis*; 'some are small, glittery, have brown heads and legs', 'some have white wing tips', a characteristic of *Trigona nigerrima*; 'there is a little bee with a yellow tail', referring to how the abdomen appears in *Trigona fulviventris*. Some of the above affirmations are also mentioned in Zamudio and Hilgert (2012). There is no doubt that morphological characteristics such as size and color are predominant features used by people to identify stingless bees (Figure 5).

Defensive behavior is another characteristic of these bees to protect their nest, described by 64 % of the individuals surveyed. When people find these bees in the field while doing their activities, they noted they 'are not dangerous', 'bees do not sting', although 'they bite' and 'get tangled in the hair', 'they leave like wax on the skin', 'they buzz', and 'they bother the eyes and try to enter the nose and ears', which is also described by Michener (2007) and Shanahan and Spivak (2021).

People know these bees are attracted to sweet smells: 'when we use perfume, [they] come closer', 'they like people's sweat'; bees are even known for some habits considered unhygienic, such as: 'they stand in dog excrement', 'they walk on dead animals' and some 'smell bad'.

Other characteristics recognized for these bees among local people is their gregarious behavior, also reported by Dos Santos *et al.* (2008) and Zamudio and Hilgert (2015): 'when they go to the nest [they] gather with their group', 'they do not live alone', 'they live in family'. Stingless bees are also recognized as restless and very active: 'When one arrives, more come after', 'went back to the nest, immediately came out again, doing that the whole day'.

Regarding their habitat, people mentioned that these bees can be found in different locations in their territory, such as: 'mainly in the field', 'in the bush', 'in the forest', 'in the coffee plantations', 'in areas where there is vegetation', 'where there are flowers and trees', 'in the village', 'in the gardens of houses', that is, bees interact with people constantly and in most of the places where people carry out their daily activities.

People in CyL recognize the nests of meliponini, with 70.7 % knowing that meliponini complete their life cycle in them. People know that these nests may be inside tree cavities, between tree branches, or just lying exposed on the ground. People distinguish species by the form of their nests, as can be seen in Figure 6. Their nest is described as a ball shape, 'with the color of the wood', and made of organic matter and soil; in addition, these nests have an entrance in a very peculiar shape, described as 'a little bugle or trumpet'. People also described not only the microhabitat, but also the characteristic materials of which the nest is made. Nests can be established on 'the ground', 'in rock crevices', 'in walls', 'in electric light poles', 'in active termite mounds' and 'in anthills'. All this has also been described by Shanahan and Spivak (2021).



Figure 5. Species of stingless bees that the inhabitants of Cervantes y Lozada recognize due to their morphological characteristics and nesting sites.

While doing fieldwork, the presence of several bee colonies accompanying inhabitants was confirmed. For example, a 52-year-old man had a ‘nest within his house’ for more than 20 years; another 45-year-old man also showed us a nest ready to be sold, as people from the city or surrounding localities do business with local residents, earning around \$500.00 pesos (\$25 USD) per colony. Another 40-year-old man mentioned that he gives the nests he finds to a person from another community.

Traditional knowledge indicates an important relationship recognized by age and gender between the habitat and behavior of meliponini. That may be explained by the fact that the local natural environment offers the same possibilities for learning and accumulation of traditional ecological knowledge to both men and women, as

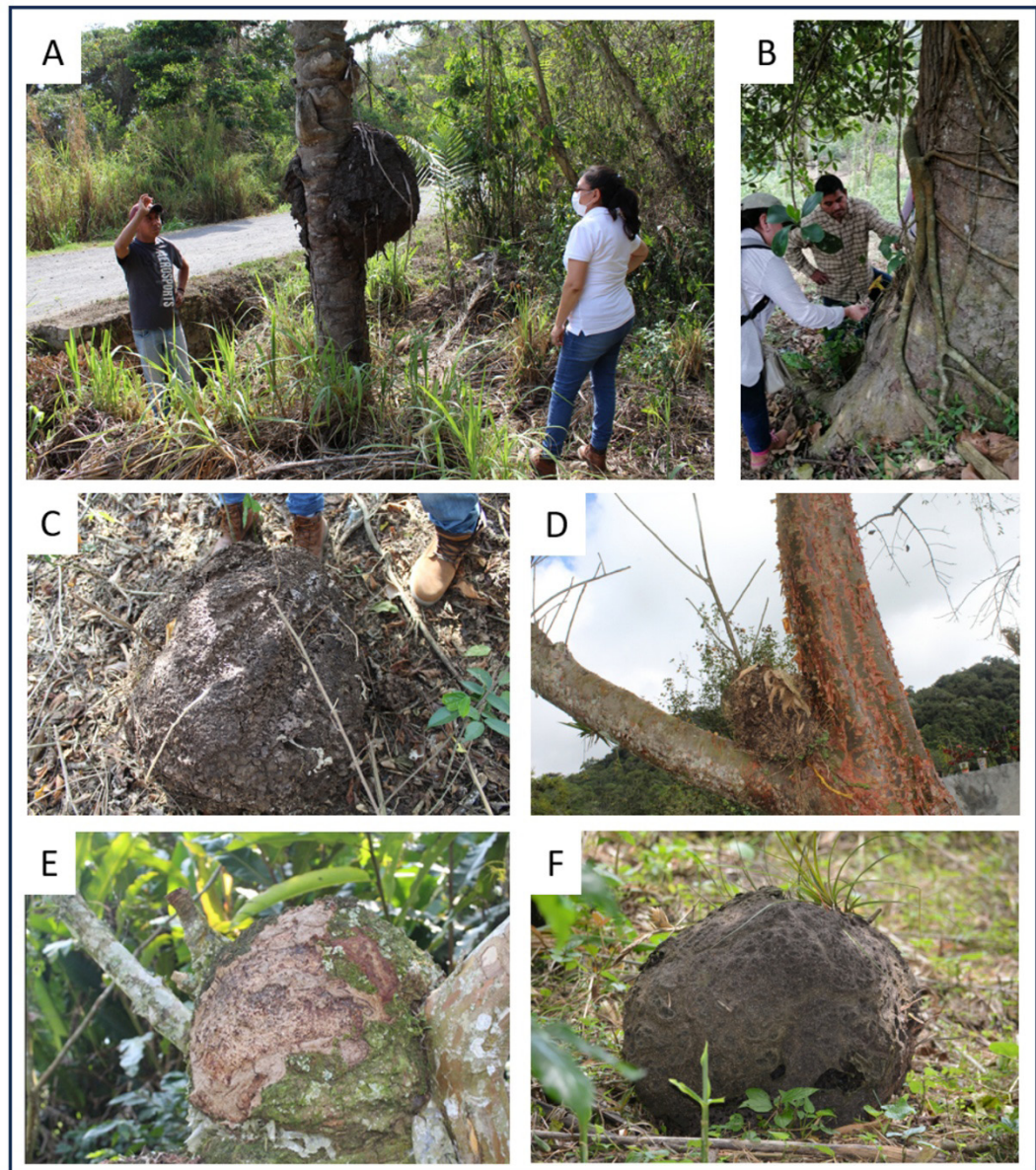


Figure 6. Stingless bee nests in Cervantes y Lozada. A) *Scaura argyrea*; B) *Scaptotrigona mexicana*; C) *Scaura argyrea*; D) *Trigona fuscipennis*; E) *Trigona nigerrima*; and F) *Trigona corvina*.

was likewise reported by Doyle *et al.* (2017) and Geng *et al.* (2016). Therefore, there is homogeneity of this knowledge by gender, which is mainly acquired in the conduct of their daily activities within the forest in the company of family.

Even though men and women have different roles in society, bees are present in most of the spaces where they carry out their daily activities.

In relation to age groups (children, youths, and adults), the results show that the children differ from youths and adults in the traditional knowledge of meliponini by making fewer distinctions among names and species; this is likely because they are building up their abilities and are in a learning process, reflecting limited experience and contact with the stingless bees found in the locality.

In addition, some children already have a mobile phone within their dwelling, spending more time on it or watching television instead of exploring their surroundings, limiting themselves to playing on the soccer field only on weekends or some afternoons; however, it could not be said that traditional knowledge of meliponini is being lost among children, since it tends to be transmitted gradually, as mentioned by Lima *et al.* (2021). It is important to mention that children who completed the questionnaire do not represent the community as a whole.

We noticed that cultural transmission of knowledge regarding meliponini and other floral visitors takes place when people cultivate coffee or when they harvest it.

Colony products

When people responded about the uses of the colony products, 16 % mentioned honey and 18.7 % wax; most of the people who answered the questionnaire showed ignorance of any product obtained from the nest of these bees. However, when we were doing fieldwork, we noticed that many of the people recognized honey, wax, pollen and larvae as principal products obtained from the nests of these bees.

Uses of colony products

Extracting honey from bee nests was referred to as 'castrar nidos' ('to castrate nests'), 'ordeñar nidos' ('to milk honey') or 'cosecha de miel' ('honey harvesting'). This activity is mainly performed by men, also mentioned by Athayde *et al.* (2016). Occasionally women help men with this activity. Both women and men use the same techniques in beekeeping. One person mentioned that he 'castrates' nests' at night, in order to avoid the bees' active defenses and to cause less disturbance; other people mentioned that they use smoke to make the bees disperse, while some people cut the tree to extract the nests.

Extracting nests by cutting down trees brings partial or total destruction of the nests; once it is exposed to the sun and air, bees abandon it or are prey to other animals, such as ants. Therefore, it is important to provide training for the sustainable management of these organisms and to ensure that the colonies remain in their original habitats, as well as to guarantee the honey harvest is carried out in a safe manner. It is also important to train women in this activity, in which all members of the family can participate, from children to the elderly.

Honey harvesting is mainly carried out during the spring, as this is the period in which most honey is produced and when it has the best flavor. This activity is pursued once a year to procure the honey that may be required for self-consumption. People also recognize stingless bee honey by its taste, texture, or color. It may be described

as having an acid or bittersweet taste and a liquid texture, but in CyL people more often mentioned the color of the honey, which may be dark yellow or bright yellow, depending on the species of meliponini.

The commercialization of honey is limited, as it is known for low annual productivity, e.g., one liter per year. When people extract honey for sale, they may earn \$1,000.00 Mexican pesos (\$50 USD) per liter. However, it is better appreciated by local people for their health by virtue of its medicinal properties, such as for treating respiratory tract diseases like the flu and coughing, for skin problems such as for healing wounds or as an anti-inflammatory for scrapes and bumps. The pure form of this honey is used for eye injuries known as cataracts (cloud vision). Other uses include oral infusions mixed with lemon, medicinal plants or garlic, as also reported by Zamudio and Hilgert (2011).

The medicinal use of meliponini honey may save money for medical consultation, also described by Carvalho *et al.* (2018). The use of honey in CyL contributes to maintaining traditional remedies for specific diseases and for maintaining familial and social ties, due to the fact that it is primarily shared with good wishes for good health to those who receive it (relatives, neighbors or friends).

Beeswax is known as “cera de Campeche” (Campeche wax). “Campeche” does not refer to the Mexican state of Campeche, but it is the most widely used term by which beeswax is recognized.

People said that “cera de Campeche” was used as glue and sealant for leaks in pipes or holes in the roof, to make candles, even as glue for small pieces of ceramic. Some of those uses are also reported by Dos Santos and Antonini (2008). In CyL bee nests can also be used as ornaments inside the dwellings, as can wasp nests (Figure 7).

Pollen and larvae are consumed as a source of protein in CyL. Bee larvae could be an ideal food due to their nutritional composition of proteins, fatty acids and minerals, so they could improve food security. It seems that children acquire traditional knowledge gradually over the time, for which reason they are separated from youths and adults. We consider that stingless bees are among the most salient pollinator species. The analysis helps us to see adults as the principal social group maintaining significant traditional knowledge concerning colony products, particularly honey use and harvesting. This traditional ecological knowledge seems to be transmitted either directly from parents to children or youths (vertically) or between individuals not necessarily belonging to the same descendent group (horizontally or obliquely), following Ohmagari and Berkes (1997). Horizontal transmission is increasingly limited as there is less time available for organized games and leisure time in the field, as young people may prefer interacting on social networks or watching television. However, some children lacking those technologies acquire skills they may use later, especially when involved in a wide range of activities in the field during the year.

Oral tradition and importance of stingless bees

Stingless bees are represented in local oral traditions; however, only a few individuals (8 %) mentioned that these bees may be beneficial when they build their nests in



Figure 7. Wasp and stingless bee nests used as decorative artefacts in dwellings

the forest or near houses where people are working, as bees may warn of snakes approaching. Some believe that bees serve as omens, announcing weather changes, such as thinking that a strong wind will come if the bee guardians do not appear to defend their nest.

Most local people appreciate stingless bees as suppliers of honey and wax, but mainly as an elemental protagonist as pollinators, given that they maintain the ecological functions of the forest. Some described them as 'part of nature', valued 'because God created them', or asserted that 'every little animal has its function', and 'they produce medicinal honey for us'.

Finally, when including all responses of all individuals, there is a strong relationship overall between the questionnaire responses of all the individuals. However, when looking in detail, we can see three main groups: 1) women show more agreement on ecological importance, bee morphology, and cultural aspects of stingless bees, while 2) men are correlated with biology and uses of colony products as they are the ones who most often extract nests and their products, and 3) children are more correlated with the answer "do not know", but, as we mentioned before, children who participated

in the questionnaire do not represent most of the children of CyL, and they may also simply be in the process of acquiring traditional knowledge. We are not able to attest that they have lost traditional knowledge. Youths fall in the middle of adult men and women, but not on the side of the children, which suggests they have acquired some but not all aspects of traditional knowledge.

Further actions for biocultural conservation of stingless bees in Cervantes y Lozada

When it was mentioned during workshops and interviews in CyL that the intention of the Colegio de Postgraduados as an institution is to provide the community with stingless bees colonies along with the technical advice for their sustainable harvesting, some people manifested interest by saying: 'I like it', 'I find it interesting', 'I want to know more', 'to help nature', 'I want to learn', 'to know what we have in the community', or 'to produce honey'.

We believe that by providing colonies of these bees to a certain number of people in CyL, we may help establish biocultural strategies for their conservation, such as: to avoid the extraction of wild colonies, to promote traditional uses of the products of the colonies, to motivate children to learn about harvesting bee products and their uses, and to increase family income by selling honey and other products.

The Colegio de Postgraduados may also provide people with technical information for commercialization of honey, pollen or wax in their natural form, dehydrated or as a supplement for meals.

Meliponiculture would be useful to promote awareness of biodiversity conservation consistent with local needs, avoiding the loss of traditional knowledge due to social changes, such as out-migration that disrupts the line of knowledge transmission and contributes to an increasing detachment from the communities' own traditional rural activities. Extinction of species and reduction of biodiversity also contribute to a 'cascade effect', not only in terms of natural resources depletion, but in terms of social and cultural indices. Therefore, there is a need for pollinator and bee conservation to safeguard local knowledge and to promote sustainable subsistence strategies that are essential for local agriculture, local ecosystems, and traditional knowledge (Alcántara-Salinas, 2021)

CONCLUSIONS

The perception of bees' value and diversity among the inhabitants of Cervantes y Lozada highlights the fact that each species is culturally salient by virtue of its size, color, habitat and behavior, among other distinctive aspects. People consider the main pollinators to be stingless bees, bees, butterflies, hummingbirds, tenchales, jicotes and wasps.

Adults maintain traditional knowledge of stingless bees, such as their common names and the morphological, behavioral, ecological and utilitarian characteristics of the

colony's products; it is important to continue transmitting this traditional knowledge to new generations so that it is preserved and may endure. There was no clear division of responsibilities between women and men; it is gender neutral, as women and men work together in agriculture, leading to a sharing of knowledge between men and women for all participants.

Ethnobiological studies on stingless bees are important for projects that promote the sustainable use of natural resources and the conservation of cultural aspects. Based on this research, the people of CyL have enough traditional knowledge to sustain a progressive program of technical training to establish a communitarian meliponary. The training and the Meliponini colonies may be provided in the near future by the Colegio de Postgraduados to promote their biocultural conservation.

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Agrociencia

FUNGICIDAL ACTIVITY OF CHITOSAN IN SYNERGY WITH *Trichoderma harzianum* AND *Bacillus subtilis* FOR THE CONTROL OF CHILI WILT DISEASE

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ABSTRACT

Chili (*Capsicum annuum* L.) wilt disease is considered one of the main diseases of this crop. It is caused by a complex of phytopathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotium rolfsii*. These fungi have developed resistance to fungicides, resulting in environmental contamination that harms human health. These factors motivate the search for environmentally safe and efficient products to control this disease without causing adverse effects to ecosystems. Chitosan, which is innocuous and has antagonistic activity, favors the growth of some beneficial microorganisms. In this study, the compatibility of chitosan at eight concentrations (75, 150, 300, 600, 1200, 2400, 4800, and 9600 ppm) with *Trichoderma harzianum* and *Bacillus subtilis* was evaluated, as well as its combined inhibitory effect against the causal agents of chili wilt disease (*F. oxysporum*, *R. solani*, and *S. rolfsii*). *T. harzianum* and *B. subtilis* were found to be compatible with chitosan. However, at concentrations of 2400 ppm and above, their growth and sporulation were affected. In the inhibition tests, low concentrations (75–600 ppm) stimulated greater mycelial growth of *Trichoderma* and had greater inhibition of mycelial growth of the phytopathogens, while concentrations of 600–1200 ppm stimulated their parasitism against *F. oxysporum* and *R. solani*. Similarly, the presence of chitosan in the culture medium favored greater growth of *B. subtilis* and higher inhibition percentages (91.65, 71.04, and 68.55 %) for *S. rolfsii*, *F. oxysporum*, and *R. solani*, compared to the treatment with *B. subtilis* without chitosan. Therefore, the combination of these microorganisms with chitosan could be considered as an alternative for the control of this disease.

Keywords: Biopolymer, compatibility, phytopathogens, harmless, inhibition, beneficial microorganisms.

INTRODUCTION

In Mexico, chili (*Capsicum annuum* L.) is of cultural, gastronomic, and economic importance (SIAP, 2023). It is affected by several diseases, which considerably reduce

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its yield. Chili wilt is one of the most important diseases (Andrade-Hoyos *et al.*, 2019; Reyes-Tena *et al.*, 2019; Villar-Luna *et al.*, 2022), affecting several varieties of this crop (Moctezuma-Bautista *et al.*, 2021) due to root rot, causing loss of turgor, yellowing, flower abortion, and plant death (Espinoza-Ahumada *et al.*, 2019; Villar-Luna *et al.*, 2022), and resulting in losses of up to 100 % (Mojica-Marín *et al.*, 2009; Villar-Luna *et al.*, 2022). This disease is caused by a complex of fungi such as *Fusarium* spp., *Rhizoctonia solani* (Andrade-Hoyos *et al.*, 2019; Espinoza-Ahumada *et al.*, 2019; Vallejo-Gutiérrez *et al.*, 2019), *Sclerotium rolfsii*, and *Verticillium* spp., among others, and oomycetes such as *Pythium* spp. and *Phytophthora capsici* (Pérez-Acevedo *et al.*, 2017; Leos-Escobedo *et al.*, 2022).

To control these phytopathogens, farmers apply chemically synthesized fungicides such as 2-thiocyanomethyl (TCMTB), Azoxystrobin, Carbendazim, Thiabendazole, and Tebuconazole (Espinoza-Ahumada *et al.*, 2019), which do not solve the problem and are harmful to people's health and the environment (Andrade-Hoyos *et al.*, 2019; Villar-Luna *et al.*, 2022). In addition, they cause phytopathogens to acquire resistance to chemical compounds, thus raising production costs (Andrade-Hoyos *et al.*, 2019; Espinoza-Ahumada *et al.*, 2019). However, there are other control alternatives, such as the use of antagonistic microorganisms (Espinoza-Ahumada *et al.*, 2019; Ley-López *et al.*, 2022).

Rhizobacteria of the genus *Bacillus* have antagonistic effects against phytopathogens (Leos-Escobedo *et al.*, 2022) through various mechanisms of action, such as competition for nutrients, parasitism, and antibiosis by the production of enzymes (chitinases) and antibiotics (bacillomycin, fungimycin, mycosubtilin, and zwittermycin) (Mojica-Marín *et al.*, 2009). Similarly, some rhizospheric fungi, such as *Trichoderma* spp., have been shown to be effective for the control of phytopathogens through various mechanisms of action, such as mycoparasitism, competition for space, antibiosis by enzyme production, plant defense mechanism activation, and promotion of plant growth (Espinoza-Ahumada *et al.*, 2019; Gallegos-Morales *et al.*, 2022).

In addition to the application of antagonistic microorganisms, there are other control alternatives, such as the use of chitosan, which is a harmless biopolymer (Escudero *et al.*, 2017; Pérez-Madruga *et al.*, 2019) consisting of glucosamine units and, to a lesser extent, N-acetyl-D-glucosamine, linked together by β 1-4 type bonds. It is extracted by deacetylation of arthropod chitin, mainly from the exoskeleton of crustaceans. This polymer exhibits inhibitory activity against a wide variety of phytopathogens such as bacteria, oomycetes (Pérez-Madruga *et al.*, 2019), and fungi. Also, it possesses other qualities of agronomic importance, such as improving seed germination, favoring mineral absorption, promoting plant growth, activating plant defense mechanisms (López-Corona *et al.*, 2019; Pérez-Madruga *et al.*, 2019), and stimulating the growth of some beneficial microorganisms such as *Trichoderma asperellum* (Rautela *et al.*, 2019) and *Bradyrhizobium japonicum* (Costales *et al.*, 2017).

Since reports that demonstrate the compatibility of chitosan with beneficial microorganisms are scarce, the objectives of this work were to demonstrate the

compatibility of chitosan with *Trichoderma harzianum* and *Bacillus subtilis*, as well as to prove their synergism in combination for the control of the complex of phytopathogens causing chili wilt.

MATERIALS AND METHODS

Experiment location

The experiment was carried out at the Microbiology Laboratory of the Department of Agricultural Parasitology of the Antonio Narro Autonomous Agrarian University (UAAAN) in Saltillo, Coahuila, Mexico (25° 21' 13" N, 101° 1' 56" W), at an altitude of 1742 m.

Biological material

Chitosan with a 100 % deacetylation grade extracted from shrimp exoskeletons by bacterial fermentation and 50 % sodium hydroxide (NaOH) was used. The strains of antagonistic microorganisms (*Trichoderma harzianum* and *Bacillus subtilis*) and phytopathogens causing chili wilt disease (*Fusarium oxysporum*, *Sclerotium rolfsii*, and *Rhizoctonia solani*) were taken from the microbial collection of the microbiology laboratory. All fungi were reactivated on potato dextrose-agar (PDA) culture medium (DIBICO, Mexico), and the bacteria on nutrient agar medium (DIBICO, Mexico).

Compatibility of beneficial microorganisms with chitosan

Mycelial growth and sporulation of *T. harzianum* were measured *in vitro* in Petri dishes with PDA culture medium supplemented with chitosan at eight concentrations (Q-75, Q-150, Q-300, Q-600, Q-1200, Q-2400, Q-4800, and Q-9600 ppm) and a control treatment without chitosan, with pH adjusted to 6.5 and with four replicates per treatment. Once the culture medium was solidified, a 5 mm diameter explant of culture medium with 72 h mycelial growth of *T. harzianum* was placed in the center of each Petri dish. They were then incubated at 28±2 °C with a photoperiod of 12 h light and 12 h dark.

Every 24 h, with the help of a digital Vernier (Truper, Mexico), mycelial growth was measured (in millimeters) until one of the control treatments completely filled the Petri dish. Subsequently, at 96 h, the percentage of sporulation was measured, for which 10 mL of sterile distilled water was added to each Petri dish, and scraping was performed with the help of a sterile glass rod. The contents with spores were placed in a 50 mL Falcon tube. Spore counting was performed with the aid of a Neubauer chamber and a compound microscope at 40 X, and the number of spores per milliliter was determined.

The compatibility of *B. subtilis* with chitosan was evaluated in Erlenmeyer flasks of 250 mL capacity, with 100 mL of nutrient broth culture medium supplemented with chitosan at the same concentrations mentioned above, plus a control treatment without chitosan, for a total of nine treatments with three replicates. The treatments

were adjusted to 6.5 pH and incubated at 28 ± 2 °C with a photoperiod of 12 h light and 12 h dark, under constant agitation at 150 rpm for 72 h. Colony-forming units (CFUs) were then counted in the same way as for *T. harzianum*.

Synergism of beneficial microorganisms in combination with chitosan as an inhibitor of *in vitro* mycelial growth of phytopathogens

The combination of *T. harzianum* and chitosan in dual confrontation against phytopathogens was performed in Petri dishes with PDA culture medium supplemented with chitosan at six concentrations, medium without chitosan, and a control (the phytopathogen without confrontation), adjusted to pH 6.5, for a total of eight treatments (Q-75 ppm + *Trichoderma*, Q-150 ppm + *Trichoderma*, Q-300 ppm + *Trichoderma*, Q-600 ppm + *Trichoderma*, Q-1200 ppm + *Trichoderma*, Q-2400 ppm + *Trichoderma*, *Trichoderma*, and control) for each phytopathogen, with four replicates per treatment. Once the culture medium had solidified, a 5 mm diameter portion of culture medium with mycelial growth of *T. harzianum* was placed at one end of the Petri dish, and at the other end of the dish, the phytopathogen with 96 h of growth. The dishes were incubated at 28 ± 2 °C with a photoperiod of 12 h light and 12 h dark until the control treatment completely full the Petri dish.

Inhibition was assessed using a five-level visual scale, where 1 = *Trichoderma* completely covers the phytopathogen on the Petri dish; 2 = *Trichoderma* covers at least two-thirds of the Petri dish; 3 = *Trichoderma* and the phytopathogen colonize half of the Petri dish; 4 = the phytopathogen covers at least two-thirds of the Petri dish and appears to resist invasion by *Trichoderma*; and 5 = the phytopathogen completely covers *Trichoderma* and the Petri dish. *Trichoderma* was considered an antagonist of the phytopathogen when level 3 was not exceeded, this level being the lowest antagonism level and level 1 the highest. It was considered to have no antagonism capacity when the phytopathogen reached levels 4 and 5 (Bell *et al.*, 1982).

The combination of *B. subtilis* and chitosan was carried out in a similar way, using the same concentrations and treatments, substituting *Trichoderma* for *B. subtilis*. Once the culture medium was solidified, *B. subtilis* was inoculated with a bacteriological loop in each of the four cardinal points of the Petri dishes and a control only with the phytopathogen, with four replicates per treatment. Then, a 5 mm diameter portion of culture medium with mycelial growth of one of the phytopathogens was placed in the center of each box and incubated at 28 ± 2 °C with a photoperiod of 12 h light and 12 h dark.

Every 24 h, mycelial growth was measured in millimeters with a digital Vernier (Truper, Mexico) until the control treatment completely filled the Petri dish. With the data obtained, the percentages of inhibition (% *in*) of each phytopathogen were obtained using the following formula:

$$\% \textit{ inhibition} = \frac{C - T}{C} * 100$$

where C is the diameter of phytopathogen growth in the control treatments and T the diameter of phytopathogen growth in the treatments with *B. subtilis*, with and without chitosan (Castillo-Reyes *et al.*, 2015).

Experimental design

Each experiment was established in a completely randomized experimental design. The data obtained were subjected to an analysis of variance (ANOVA), and a Tukey's test for comparison of means ($p \leq 0.05$) was performed using the statistical program InfoStat version 2019.1.2.0.

RESULTS AND DISCUSSION

Compatibility of *Trichoderma harzianum* and *Bacillus subtilis* with chitosan

Mycelial growth of *T. harzianum* at different concentrations of chitosan in PDA culture medium showed that concentrations from 75 to 1200 ppm (Figure 1A–E) did not affect fungal growth because at these concentrations there were no significant differences with the control (Figure 1I) (Table 1). The fungi in these treatments filled the Petri dishes at 72 h. However, when the concentration increased to 2400 ppm, mycelial growth of *T. harzianum* was impaired (Figure 1F). In the sporulation evaluation, concentrations of 75 and 150 ppm favored fungal sporulation with 1.5×10^8 and 5.2×10^8 spores per mL, respectively, which were statistically ($p > 0.05$) different from the control (1.2×10^8) and the rest of the treatments (Table 1).

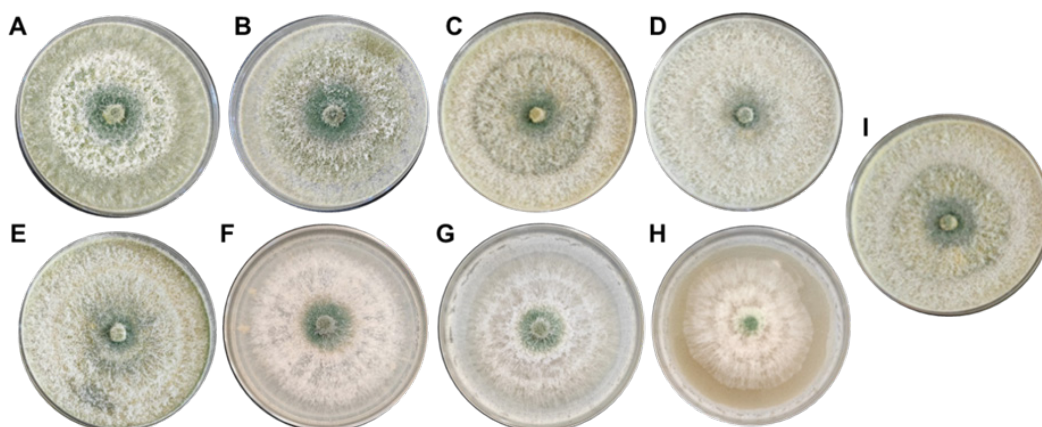


Figure 1. Growth of *Trichoderma harzianum* in potato dextrose-agar (PDA) culture medium supplemented with chitosan at different concentrations. A: 75 ppm; B: 150 ppm; C: 300 ppm; D: 600 ppm; E: 1200 ppm; F: 2400 ppm; G: 4800 ppm; H: 9600 ppm; I: PDA without chitosan.

Table 1. Growth of *Trichoderma harzianum* and *Bacillus subtilis* in culture medium supplemented with chitosan at different concentrations.

Treatment	<i>Trichoderma harzianum</i>		<i>Bacillus subtilis</i>
	Growth (mm) Mean ± EE	Spores per mL Mean	UFC per mL Mean
Q-75 ppm	85 ± 0.00 A	1.5 × 10 ⁸ B	1.7 × 10 ⁹ BC
Q-150 ppm	85 ± 0.00 A	5.2 × 10 ⁸ A	1.9 × 10 ⁹ BC
Q-300 ppm	85 ± 0.00 A	1.1 × 10 ⁸ C	2.8 × 10 ⁹ A
Q-600 ppm	85 ± 0.00 A	6.8 × 10 ⁷ E	3.1 × 10 ⁹ A
Q-1200 ppm	85 ± 0.00 A	7.9 × 10 ⁷ D	1.95 × 10 ⁹ B
Q-2400 ppm	59.02 ± 1.15 B	4 × 10 ² F	9.3 × 10 ⁸ D
Q-4800 ppm	58.11 ± 0.94 B	3.3 × 10 ² F	8.6 × 10 ⁶ E
Q-9600 ppm	48.49 ± 0.61 C	2.8 × 10 ² F	1.3 × 10 ⁵ E
Control	85 ± 0.00 A	1.2 × 10 ⁸ C	1.5 × 10 ⁹ C

*Means with the same letter are not significantly different ($p > 0.05$). UFC: colony forming units; EE: standard error.

These results coincide with those reported by other authors, who mention that *Trichoderma* is tolerant to chitosan at certain concentrations. Hernández-Domínguez *et al.* (2021), when using chitosan (Sigma-Aldrich at 85 % distillation) at different concentrations (1, 4, 6, 8, 10, and 12 mg mL⁻¹), showed that *Trichoderma parareesei* did not present significant differences with the control; however, there was a decrease in growth at concentrations of 6, 10, and 12 mg mL⁻¹.

Chittenden and Singh (2009) reported that mycelial growth of *T. harzianum* was not affected by low molecular weight chitosan (50–190 kDa) at concentrations of 0.5 and 0.75 % (5000 and 7500 ppm). In contrast to this study, *T. harzianum* was more sensitive, since concentrations from 2400 to 9600 ppm (2.4 to 9.6 mg mL⁻¹) did affect its growth, as they used chitosan with different properties, such as deacetylation percentage (the higher this value is, the more active chitosan is). Zavala-González *et al.* (2016) suggest that the tolerance of *Trichoderma* species to this biopolymer is related to a low linoleic acid content and low fluidity of their cell membrane. Escudero *et al.* (2017) mention that some fungi, such as *Pochonia chlamydosporia*, use chitosan as a source of nutrients for their growth and that low concentrations of this biopolymer increase their mycelial growth.

In the case of compatibility of *B. subtilis* with chitosan, at 72 h of incubation, the bacteria presented the highest number of UFC at concentrations of 300 and 600 ppm, with values of 2.8 × 10⁹ and 3.1 × 10⁹, respectively, which was statistically ($p > 0.05$) higher than the other treatments and the control (Table 1). However, at concentrations higher than 2400 ppm, the UFC of the bacteria decreased.

These results are similar to those reported by Costales *et al.* (2017), who tested the *in vitro* compatibility of *Bradyrhizobium japonicum* with chitosan. At a concentration

of 10 mg mL⁻¹ in the culture medium, the bacteria increased their number of UFC compared to the control, while at concentrations of 500 mg mL⁻¹ they decreased. In this experiment, low concentrations (75–600 ppm) of chitosan increased the UFC of *B. subtilis*, and decreased at high concentrations (1200–9600 ppm). These results are of great relevance because the information related to the compatibility of beneficial bacteria with chitosan is very scarce, especially the combination of bacteria antagonistic to phytopathogens with the biopolymer.

Combination of chitosan and *Trichoderma harzianum* against phytopathogens

In the combination of chitosan with *T. harzianum* against *S. rolfsii* (Figure 2), as the concentration of chitosan increased, the growth of the phytopathogenic fungus decreased; however, the growth of *Trichoderma* was not affected, since it presented inhibition levels of 2 (Figure 2, Aa–Ac) and 1 (Figure 2, Ad–Af). The latter had the highest inhibition compared to the *T. harzianum* treatment without chitosan (Figure 2, Ag), which presented a level 2 on the evaluation scale.

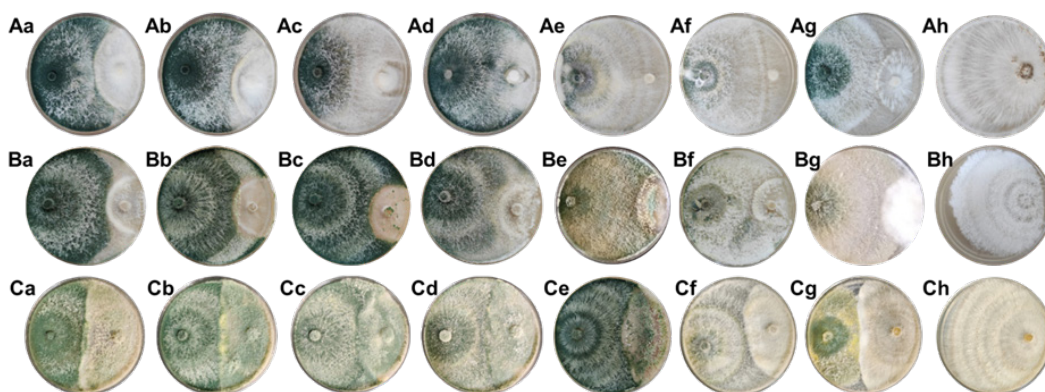


Figure 2. *In vitro* inhibitory effect of the combination of chitosan and *Trichoderma harzianum* against *Sclerotium rolfsii* (A), *Fusarium oxysporum* (B), and *Rhizoctonia solani* (C). Combinations of *T. harzianum* with chitosan at concentrations of 75 (a), 150 (b), 300 (c), 600 (d), 1200 (e), 2400 ppm (f), *T. harzianum* without chitosan (g), and controls (h).

In the assay against *F. oxysporum* (Figure 2, Ba–Bh), the presence of chitosan in the culture medium stimulated greater growth of *Trichoderma* (Figure 2, Ba–Bf). At chitosan concentrations of 75 to 300 ppm (Figure 2, Ba–Bc), *Trichoderma* ranked at level 2 inhibition of *Fusarium*; however, at concentrations of 600 to 2400 ppm (Figure 2, Bd–Bf), it ranked at level 1 inhibition, as it favored parasitism of *T. harzianum* against the phytopathogen, which was not present in the rest of the treatments or in the *Trichoderma* treatment without chitosan (Figure 2, Bg), which was located at level 2 of the scale.

In the bioassay of *R. solani* (Figure 2, Ca–Ch), it was observed that the presence of chitosan in the culture medium favored a greater growth of *Trichoderma* (Figure 2, Ca–Cf) in the treatments with concentrations of 150 to 1200 ppm (Figure 2, Cb–Ce), since they increased the parasitism of *T. harzianum* against the phytopathogen, which grew on *Rhizoctonia* and managed to fill the Petri dish. This placed these treatments at level 1 on the scale, while the *Trichoderma* treatment without chitosan presented the lowest level of inhibition (level 3), as both microorganisms filled half of the Petri dish, indicating no dominance of one over the other (Figure 2, Cg).

These results coincide with those reported by some authors; for example, Chittenden and Singh (2009) demonstrated the synergistic effect of the *in vitro* combination of chitosan at 0.05 and 0.075 % plus *T. harzianum*, which completely inhibited the mycelial growth of *Leptographium procerum* and *Sphaeropsis sapinea*. This was better than the treatment with chitosan alone, where *L. procerum* presented growth of approximately 45 and 25 mm, and *S. sapinae* was not affected in its growth. Hernández-Domínguez *et al.* (2021) refer to the fact that chitosan induces a greater production of metabolites such as chitinases in *Trichoderma* and that these showed greater control against *F. oxysporum*.

In turn, Rautela *et al.* (2019) demonstrated that the presence of chitosan (500 ppm) improved the enzymatic activity (cellulases and chitosanases) and the production of secondary metabolites (tetradecanoic acid and 1,2-benzenedicarboxylic acid) of *Trichoderma* compared to the treatment of *Trichoderma* without chitosan. This could be beneficial to increase its antagonistic activity against phytopathogens. An increase in the antagonistic capacity of *T. harzianum* against the three phytopathogens was noted with the presence of chitosan, behaving differently according to its concentration. At low concentrations (75–300 ppm), it stimulated a greater growth of *Trichoderma*, and at intermediate concentrations (600–1200 ppm), it stimulated its parasitism against *F. oxysporum* and *R. solani*, with which a greater control of the phytopathogens was obtained compared to the treatment without chitosan.

Combination of chitosan and *Bacillus subtilis* against phytopathogens

The combination of chitosan with *B. subtilis*, as with *Trichoderma*, favored the growth of the bacteria, which was visualized in a higher percentage of inhibition of the phytopathogens. In the experiment against *S. rolfsii* (Figure 3, Aa–Ah), the presence of chitosan in the culture medium inhibited the mycelial growth of the fungus, while it stimulated the growth of the bacteria. It presented an inhibition of 91.35 % at a concentration of 2400 ppm (Figure 3, Af); furthermore, the presence of 75 ppm of chitosan in the culture medium with *Bacillus* obtained 75.26 % inhibition of the phytopathogen (Figure 3, Aa), presenting an increase of 15.64 % compared to the treatment of *B. subtilis* without chitosan (Figure 3, Ag), which was the treatment that showed the lowest inhibition of the fungus with 59.62 % (Table 2).

In the assay against *F. oxysporum* (Figure 3, Ba–Bh), the presence of chitosan stimulated a higher percentage of inhibition by *B. subtilis* against the phytopathogen, where it

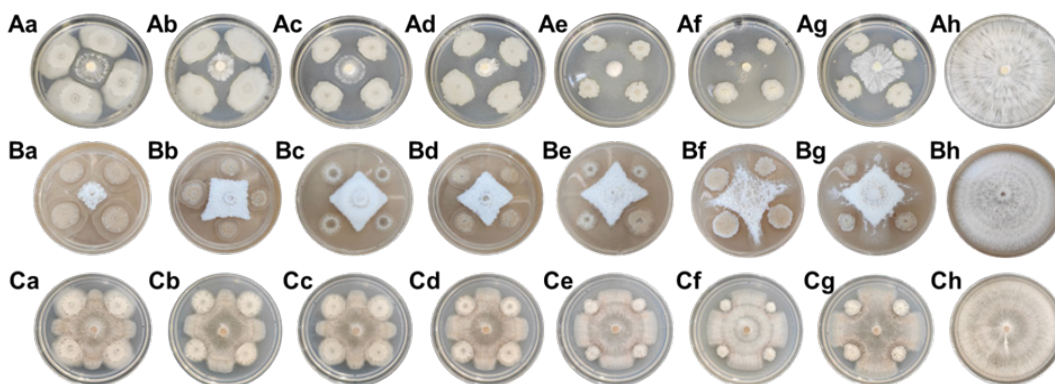


Figure 3. *In vitro* inhibitory effect of the combination of chitosan and *Bacillus subtilis* against *Sclerotium rolfisii* (A), *Fusarium oxysporum* (B), and *Rhizoctonia solani* (C). Combinations of *B. subtilis* with chitosan at concentrations of 75 (a), 150 (b), 300 (c), 600 (d), 1200 (e), 2400 ppm (f), *B. subtilis* without chitosan (g), and controls (h).

Table 2. *In vitro* inhibition of phytopathogens by the combination of *Bacillus subtilis* and chitosan at different concentrations.

Treatment	<i>Sclerotium rolfisii</i> Mean ± EE	<i>Fusarium oxysporum</i> Mean ± EE	<i>Rhizoctonia solani</i> Mean ± EE
Q-75 ppm + <i>Bacillus</i>	75.26 ± 0.72 C	71.04 ± 0.53 A	63.84 ± 0.94 AB
Q-150 ppm + <i>Bacillus</i>	72.10 ± 1.04 C	64.58 ± 0.19 B	68.55 ± 2.81 A
Q-300 ppm + <i>Bacillus</i>	74.30 ± 0.50 C	65.82 ± 0.30 BC	59.16 ± 0.79 BC
Q-600 ppm + <i>Bacillus</i>	81.77 ± 0.36 B	63.70 ± 0.53 BC	58.20 ± 0.28 CD
Q-1200 ppm + <i>Bacillus</i>	84.72 ± 0.16 B	63.15 ± 0.50 C	53.90 ± 0.16 DE
Q-2400 ppm + <i>Bacillus</i>	91.35 ± 0.52 A	60.63 ± 0.50 D	52.43 ± 0.23 E
<i>Bacillus</i>	59.62 ± 1.17 D	56.85 ± 0.85 E	52.51 ± 0.63 E
Control	0.00 ± 0.00 E	0.00 ± 0.00 F	0.00 ± 0.00 F

*Means with the same letter are not significantly different ($p > 0.05$). EE: standard error.

obtained 71.04 % inhibition with the concentration of 75 ppm chitosan (Figure 3, Ba), a value statistically superior to the other treatments.

In the combination of chitosan and *B. subtilis* against *R. solani* (Figure 3, Ca–Ch), the bacteria did not present inhibition of the fungus by antibiosis, but by competition. Greater bacterial growth was observed in the treatments with chitosan (Figure 3, Ca–Cf), with a higher percentage of inhibition (68.55 %) in the treatment of chitosan at 150 ppm plus *Bacillus*. On the other hand, the *B. subtilis* treatment without chitosan (Figure 3, Cg) only reached 52.51 % inhibition (Table 2).

It is worth mentioning that there are few studies that demonstrate the compatibility of chitosan with antagonistic bacteria used against phytopathogens. However, there

are some studies related to the combination of this biopolymer with plant growth-promoting rhizobacteria. Ortega-García *et al.* (2021) demonstrated that by combining *Bacillus amyloliquefasciens* with chitosan (65 % deacetylation) at a concentration of 50 and 100 %, they obtained higher germination, growth, and production of *Asparagus officinalis* compared to the application of these treatments individually. Holguin-Peña *et al.* (2020) promoted sorghum growth by the combined application of chitosan, *B. subtilis*, *B. polymyxa*, and *B. amyloliquefasciens*. Similarly, Agbodjato *et al.* (2016) demonstrated this in maize by combining chitosan with *Azospirillum lipoferum*, *Pseudomonas fluorescens*, and *Pseudomonas putida*.

Costales-Menéndez *et al.* (2021) demonstrated that chitosan favors the survival of *Bradyrhizobium* in soybean seeds, in addition to nodulation in the plant, which causes greater growth. Álvarez-Sánchez *et al.* (2021), through the combined application of chitosan with *B. japonicum*, promoted seed germination, growth, and turnip production when these treatments were applied individually; in addition, there was a lower incidence of *Peronospora brassicae*. These results are like those obtained in this research, since the mixtures of chitosan with *B. subtilis* achieved greater inhibition of phytopathogens compared to the treatment where only the bacteria were applied.

Sawaguchi *et al.* (2015) demonstrated that the application of chitosan to silty soil increased the bacterial population up to 20 times, where chitinolytic microorganisms such as *Kitasatospora* and *Streptomyces*, which are capable of degrading chitosan, predominated. Therefore, the inclusion of microorganisms such as *Bacillus*, *Streptomyces*, and *Trichoderma* sp. could be considered since they are capable of producing chitinases and chitosanases that are compatible with this biopolymer (Castañeda-Ramírez *et al.*, 2011). This is considered a possible reason why the treatments of *B. subtilis* with chitosan achieved a greater inhibition of phytopathogens; however, the characterization of the compounds produced by these microorganisms in culture medium with and without chitosan will be considered for future studies to understand the effect produced by chitosan on these bacteria.

CONCLUSIONS

The compatibility of *Trichoderma harzianum* and *Bacillus subtilis* with chitosan was demonstrated. The presence of this biopolymer at low concentrations favored the growth of these microorganisms. Similarly, growth and inhibition by *Trichoderma* were stimulated at chitosan concentrations of 75–300 ppm, while at 600–1200 ppm, parasitism against *Fusarium oxysporum* and *Rhizoctonia solani* was promoted. The presence of chitosan in the culture medium caused greater growth of *B. subtilis*, with higher inhibition percentages for *Sclerotium rolfsii*, *F. oxysporum*, and *R. solani* compared to the treatment without chitosan. *T. harzianum* and *B. subtilis*, mixed with chitosan, exhibited a synergistic effect resulting in a greater inhibition of phytopathogens, so these combinations can be considered as bioalternatives for the control of phytopathogens causing chili wilt disease.

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Agrociencia

INTEGRATION OF GENOTYPES OF MAIZE (*Zea mays* L.) AND PLANT POWDERS AGAINST *Sitophilus zeamais* Motschulsky (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

The objective of this study was to determine the combined effect of maize (*Zea mays* L.) genotypes with the application of epazote (*Dysphania ambrosioides* L.), castor oil plant (*Ricinus communis* L.), Mexican pepperleaf (*Piper auritum* Kunth), moringa (*Moringa oleifera* Lam.), neem (*Azadirachta indica* A. Juss.), and Pacific Coast mahogany (*Swietenia humilis* Zucc.) as an alternative to control the maize weevil (*Sitophilus zeamais* Motschulsky). It was hypothesized that combining certain maize genotypes with specific plant powders would significantly reduce *S. zeamais* damage without affecting germination. Three bioassays were conducted. In the first, the biological activity of six plant powders to control weevil populations and their impact on seed germination was evaluated; in the second, the effect of the hardness of three maize hybrids and the “Ancho Pozolero” variety on weevil was determined; and in the third, the interaction between genotypes and the most effective powders was explored. With five replicates per treatment and a control without application, 100 g of maize and 1 % plant powder were placed in 250 mL glass containers using a fully randomized design. Mortality, emergence, insect damage, and maize germination were assessed. In the evaluation of plant powders, *D. ambrosioides* and *S. humilis* stood out for their insecticidal and insectistatic effects and protection of maize, although they affected germination by 6.5 and 7.2 %, respectively. *P. auritum* showed good insecticidal and insectistatic effects without affecting germination. The hybrid “Tlaoli Puma,” classified as hard, reduced the damage caused by *S. zeamais* by one third. The combination of the hybrid “Tlaoli Puma” with 1 % plant powder of *D. ambrosioides* or *S. humilis* reduced the chances of attack and damage by *S. zeamais*, contributing to reducing post-harvest losses of stored maize and thus validating the hypothesis.

Key words: maize weevil, plant insecticides, pest management in storage.

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INTRODUCTION

Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae), known as the maize weevil, is one of the most economically important insect pests of stored grains in the world. In storage, it directly consumes the grain, reducing its quality and contaminating it (Matías-Oregán *et al.*, 2022). In addition, the damage promotes susceptibility to plant pathogens and leads to market rejection (Nwosu *et al.*, 2015). This insect is a pest of wheat, barley, sorghum, rice, and millet in storage, but its main host is maize (*Zea mays* L.). If the grain is 10 % infested at the time of harvesting and is not controlled, it can result in total losses after six months. Post-harvest losses caused by *S. zeamais* in subsistence maize producers can reach up to 80 % if no control measures are implemented (Rangel-Fajardo *et al.*, 2020a).

Synthetic fumigants such as aluminum phosphide and insecticides such as deodorized malathion are used to combat *S. zeamais*, which pose a risk to human health (Silveira-Gramont *et al.*, 2018), promote the selection of resistance genes in target populations, and contaminate the environment while accumulating residues in grains and leading to technological dependence. In view of this situation, agro-ecological and sustainable strategies are being sought to help combat this pest.

Improved maize varieties with resistance to grain-attacking insects are one of the most desirable factors in preventing pest damage (López-Castillo *et al.*, 2018). The physical, chemical, and biochemical characteristics of the grain are closely related to resistance, which is based on the hardness, amount of protein, moisture, and phenols in the grain (Nwosu *et al.*, 2015). For example, hardness is a type of mechanical-structural resistance considered to be antigenic and is negatively correlated with damage and susceptibility to insects. Another type of resistance is antibiosis, with the performance of compounds such as phenolic acids concentrated in the pericarp of the grain (Cabrera-Soto *et al.*, 2009). These compounds can act as an anti-feed and anti-nutritional factor, causing the insect to reduce its ability to digest and absorb nutrients from the maize grains.

Another viable alternative for the control of *S. zeamais* is the use of botanical insecticides in the form of powders, extracts, or oils, which are generally biodegradable and have a low impact on the ecosystem (Ileke *et al.*, 2020). These powders have been used especially by small producers where maize is produced for self-consumption, and are a renewable, accessible, and inexpensive resource compared to synthetic insecticides (Quiñones-Dena *et al.*, 2017). Their secondary metabolites with insecticidal and insectistatic effects can protect grain in storage (García-García *et al.*, 2019). Some experiments conducted on maize grains treated with plant powders were effective against *S. zeamais*, such as *Piper nigrum* L. and *Ruta graveolens* L. powder, which caused 68.8 and 60.4 % mortality, respectively (Quiñones-Dena *et al.*, 2017).

Although there is previous work on maize grain resistance or the use of plant powders to combat storage pests, few combine the two technologies. The objective of this work was to determine, under laboratory conditions, the effect on the grain of four maize genotypes in combination with different plant powders for the control of *S. zeamais*.

MATERIALS AND METHODS

Location of the experiment

The study was conducted in the insectary of the Entomology and Acarology Program of the Postgraduate College, Campus Montecillo, in Texcoco, State of Mexico, Mexico, during the summer-autumn period of 2023.

Maize genotypes tested

Maize grains of three hybrids were used: H-50 (MAZ-427-030904), one of the most commercially used in the High Valleys of Mexico; Tlaoli Puma (MAZ-1844-231117) and Tsiri Puma (MAZ-1571-290514), which have prospects for commercial use (Tadeo-Robledo *et al.*, 2016, 2021; SNICS, 2023); and the open-pollinated variety of broad maize “Ancho Pozolero.” The hybrids were provided by Dr. Alejandro Espinosa Calderón and Dr. Margarita Tadeo Robledo from the National Institute of Forestry, Agriculture, and Livestock Research (INIFAP-CEVAMEX) and the National Autonomous University of Mexico (UNAM, Faculty of Higher Education (FES) Cuautitlán Campus), respectively. The Ancho pozolero variety was obtained from Catalina Armendáriz Guzmán, a producer in the municipality of Ozumba, State of Mexico, Mexico.

In order to have sufficient material for testing, hybrids were grown in the spring-summer 2021 cycle in experimental plots at Rancho Almaraz, UNAM FES-Cuautitlán. Harvesting was done by hand when the maize cobs reached physiological maturity. The grain was sieved to separate impurities. It was then exposed to -4 °C for 48 h to kill pathogens and insects. Grain hardness was determined indirectly with its volumetric weight (kg hL^{-1}) using the GAC 2100 grain analysis equipment (Dickey-John®, USA).

Insect breeding

Mass reproduction of *S. zeamais* started with around 2000 individuals from an infested storage. Insects were kept in quarantine and without selection pressure to insecticides for four generations. Adults (150) were placed in 1 L glass containers with 750 g of Ancho Pozolero maize, previously washed and disinfected. The lid of each container was perforated and covered with organza cloth to allow ventilation. Adults were kept on the grain for 15 d to oviposit and then removed with an aspirator. The infested jars were kept in a rearing chamber at 26 ± 2 °C, relative humidity (RH) of 37 ± 2 % and a 12 h photoperiod. This process was carried out periodically in order to have biological material during the experiment.

Plant powders

Plant species were selected based on history of previous use as insecticides, their abundance in regions of Mexico, and history of use in stored grains (Table 1). For each plant species, 10 kg of fresh plants were collected, and five specimens were mounted in presses for taxonomic identification, which was carried out by Dr. Monserrat Vázquez Sánchez, head of the Botanical Herbarium-Hortory of the Postgraduate College.

Table 1. Species, family, organ used and collection place of plants tested against the maize weevil (*Sitophilus zeamais* Motschulsky) under laboratory conditions.

Scientific name	Common name	Family	Organ used	Place of collection
<i>Azadirachta indica</i> A. Juss.	Neem	Meliaceae	Leaf	Veracruz, Ver.
<i>Dysphania ambrosioides</i> L.	Epazote	Amaranthaceae	Leaf	Texcoco, Mexico
<i>Moringa oleifera</i> Lam.	Moringa	Moringaceae	Seed	Veracruz, Ver.
<i>Piper auritum</i> Kunth	Mexican pepperleaf	Piperaceae	Leaf	Veracruz, Ver.
<i>Ricinus communis</i> L.	Castor oil plant	Euphorbiaceae	Leaf	Texcoco, Mexico
<i>Swietenia humilis</i> Zucc.	Pacific Coast mahogany	Meliaceae	Seed	Culiacán, Sin.

The collected plant material was subjected to dehydration in the shade for 20 d. It was then pulverized by structures in an electric mill (Dakota®, model 1500) and sieved on a 0.25 mm mesh, 24 h before setting up the bioassays. The powder obtained was stored in properly labelled plastic containers.

Bioassays

Three bioassays were conducted to evaluate the effects of plant powders, maize genotypes, and their interaction against *S. zeamais*. In the first, the biological activity of the six plant powders to control *S. zeamais* populations and their impact on seed germination was evaluated. The second bioassay was to determine the effect of the hardness characteristics of the genotypes (three maize hybrids and the variety Ancho Pozolero) on the weevil. In the third bioassay, the interaction between genotypes and the most effective powders was explored.

Effect of plant powders on *S. zeamais*

In this bioassay, each treatment was evaluated at a dose of 1 % (1 g of plant powder per 100 g of grain). For these tests, 250 mL glass containers were used, in which 100 g of Ancho Pozolero maize with a moisture content of 13 % were placed and the corresponding plant powder was added. It was mixed uniformly by oscillatory and vertical movements for 1 min and infested with 10 pairs (females and males) of adults *S. zeamais* from 1 to 10 d of age. Treatments were kept in a chamber under brood-like conditions for 15 d to allow adults to copulate and oviposit. At the end of this time, the adults were extracted to quantify mortality, considering as dead the one that, after being touched with a brush, did not respond to the stimulus.

To assess the effect of powders on emergence and level of damage, the same procedure was followed as for the mortality tests, except that first-generation adults were counted 55 d after infestation. Adult emergence and grain damage in the control group were considered to be 100 %.

Finally, treatments that showed promise ($\geq 40\%$ mortality and/or $\geq 50\%$ reduction in emergence) were selected to determine the effect of powders on germination. For this purpose, 500 seeds per treatment were randomly selected to establish five replicates of 100 seeds each. The seeds were arranged in groups of 20 and wrapped in folded paper towels, moistened with distilled water, and placed in plastic bags. Treatments were randomized and placed in a germination chamber at 25 °C and a 12 h photoperiod for 7 d. After this time, the germination percentage was determined according to ISTA criteria (ISTA, 2024).

Effect of genotypes on *S. zeamais*

The objective of the second bioassay was to determine the effect of the hardness characteristics of the maize hybrids (H-50, Tlaoli Puma, and Tsiri Puma, and the variety Ancho Pozolero) on the weevil. The same procedure was followed as in the previous trial to maintain the experimental unit, and the same variables (mortality, emergence, damage, and germination) were determined without using plant powder impregnation. Five replicates were carried out for each genotype to ensure consistency of results and minimize random variability.

Powder-genotype interaction on *S. zeamais*

In the third bioassay, the interaction between the best plant powders and genotypes was analyzed using similar experimental units to the second bioassay. A total of 1 g of powder from the best treatments of the first bioassay was added, and a control group without powder was included, with five replicates each. This configuration allowed a specific evaluation of the effect of the interaction between powder and genotype under controlled conditions.

Experimental design and statistical analysis

The design was completely randomized. The data obtained were tested for assumptions of normality and homogeneity of variances ($p \leq 0.05$); when these were met, analysis of variance (ANOVA) was performed. Means were compared using Tukey's test ($p \leq 0.05$). When the assumptions were not met, the data were analyzed with the Kruskal-Wallis test, and a comparison of means and ranks was performed with the InfoStat software version 2020 (InfoStat, 2020).

RESULTS AND DISCUSSION

Effect of plant powders against *S. zeamais*

The evaluated plant powders caused an insecticidal and insectistatic effect on *S. zeamais* ($p \leq 0.05$) (Table 2). The best results were obtained with the impregnation of plant powders of *D. ambrosioides* (leaf), *S. humilis* (seed), and *P. auritum* (leaf). These caused more than 90 % mortality and reduced adult emergence and grain damage by

Table 2. Results of the application of 1 % plant powders against *Sitophilus zeamais* Motschulsky adults in maize stored in Montecillo, Texcoco, State of Mexico, Mexico.

Plant powder	Mortality (%)	Emergence (%)	Damage (%)	Germination (%)
<i>Dysphania ambrosioides</i> (leaf)	100 ± 0.0 ⁺ a*	0.0 ± 0.0 c	0.0 ± 0.0 c	93.5 ± 8.0 bc
<i>Moringa oleifera</i> (seed)	2.2 ± 0.8 c	81.2 ± 18.1 b	88.9 ± 4.1 b	88.4 ± 10.2 c
<i>Swietenia humilis</i> (seed)	95.5 ± 1.0 a	1.3 ± 1.7 c	1.4 ± 0.6 c	92.8 ± 9.4 bc
<i>Piper auritum</i> (leaf)	90.0 ± 3.3 a	5.8 ± 6.7 c	4.8 ± 1.9 c	98.2 ± 2.6 a
<i>Ricinus communis</i> (leaf)	28.0 ± 4.4 b	99.0 ± 31.8 ab	104.8 ± 8.9 ab	94.0 ± 4.6 c
<i>Azadirachta indica</i> (leaf)	6.0 ± 1.6 c	156.7 ± 38.9 a	152.4 ± 9.5 a	98.6 ± 4.0 a
Control	3.5 ± 0.7 c	100 ± 22.7 ab	100 ± 8.7 b	100 ± 6.5 a

⁺Means ± standard error. *Same letters in the columns indicate no significant difference among means of the ranges (Kruskal-Wallis, $p \leq 0.05$).

94.2 %. In contrast, the application of *M. oleifera* seed powder, *A. indica* leaf, and *R. communis* leaf did not affect *S. zeamais* and did not protect the grain; on the contrary, *A. indica* leaf favored emergence and damage. In addition, the impregnation of the latter three powders affected germination (Table 2).

The insecticidal effect caused by *D. ambrosioides*, *S. humilis*, and *P. auritum* was reported by several authors on various pests due to the action of secondary metabolites (Ortega-Arenas *et al.*, 2014; Rangel-Fajardo *et al.*, 2020a). These species are characterized by alkaloids, steroids, flavonoids, phenols, and saponins, distributed in different organs of the plant, mainly in roots, leaves, and seeds. These compounds can interfere with the metabolism of organisms, causing variable effects, such as phagorepellency, sterilization, blockage of metabolism, interference with metamorphosis, and death.

In this research, *D. ambrosioides* leaf caused total mortality in *S. zeamais*; however, there are reports indicating that the effect may be minor and/or variable, depending on the plant part evaluated, phenological stage, and prevailing environmental conditions where the plant developed (Rangel-Fajardo *et al.*, 2020b). The leaf and stem powder mixture of *D. ambrosioides*, for example, caused only 10.5 % mortality in *S. zeamais*, while 1 % *P. auritum* powder caused 6.7 % mortality (Gómez-Herrera *et al.*, 2018).

The null biological activity of *M. oleifera* seed powder at 1 %, as found in this study, was also evidenced previously, even at concentrations ranging from 10 to 100 % (Emeka *et al.*, 2020; Asare *et al.*, 2023). The results found with *P. auritum* leaf powder coincide with those reported by Agüero-Cabrera *et al.* (2020); however, these authors point out that greater effectiveness can be obtained when the plants are harvested in cold weather because they contain a higher concentration of secondary compounds. Effectiveness can decrease by up to 6.7 % (Gómez-Herrera *et al.*, 2018).

In this research, it was evident that the treatments with the highest mortality also significantly inhibited emergence ($p \leq 0.05$) (Table 2). *D. ambrosioides* leaf (0 %), followed

by *S. humilis* seed (1.3 %) and *P. auritum* leaf (5.8 %). This is in agreement with the work of Aros *et al.* (2019), who found a reduction of 82.8 and 38.6 %, respectively, in weevil emergence with 1 % leaf and stem powder and 1 % essential oil of *D. ambrosioides*.

The decrease in emergence in this work was probably due to the fact that females were removed before they laid their normal load of eggs, which had a direct effect on female mortality. It is also possible that secondary metabolites in these structures caused a rapid toxic effect in the adult, which prevented copulation from occurring and thus decreased oviposition. Another aspect that may have influenced reproduction was the possible sterility of females or the death of insects in the immature stages, which made insect emergence impossible (Gómez-Herrera *et al.*, 2018).

Contrary to what was found with most plant powders, the 1 % *A. indica* leaf powder did not cause a toxic effect on *S. zeamais*, which is in line with what is reported in the literature (Erenso and Berhe, 2016), but had an unusually increased emergence of individuals in the first generation (and thus the damage to the grain). This can be attributed to the chemical composition of the leaf, which stimulates the insects' reproductive capacity and development. This finding is in contrast to that reported by Erenso and Berhe (2016), who found significant weevil mortality when applying *A. indica* powder at doses of 1 to 3 %. However, it is likely that the differences in activity compared to what was found in this study are due to the fact that they used seed powder, which concentrates the highest amount of azadirachtin, the compound to which the insecticidal properties are attributed. The least grain damage was also recorded with *D. ambrosioides* (0 %), followed by *S. humilis* (1.4 %) and *P. auritum* (4.8 %). This reduction could be a consequence of the insecticidal effect on the adult or the growth-regulating or antifeedant effect of the metabolites present in the plant powders studied (Shiberu and Negeri, 2017).

Regarding the effect of powders on seed germination, differences between treatments were found ($p \leq 0.05$) (Table 2). The most evident reduction was recorded with 1 % *M. oleifera* seed powder (11.6 %) and *R. communis* leaf (4 %). In the rest of the treatments, no adverse effect was observed, which suggests that the compounds in the powders of the different organs of the species studied exert biological activity against weevils but have no effect on the germination of the grains studied. This is in line with other studies on species of the same family, which is important as it represents a good alternative for use in seed storage (Yuya, 2017; Aros *et al.*, 2019).

For the assessment of the 1 % plant powders, *D. ambrosioides* and *S. humilis* showed insecticidal, insectistatic, and protective effects on maize, although they affected germination. *P. auritum* is noted for its insecticidal, insectistatic, and grain-protecting effect without reducing germination. In contrast, *R. communis* showed a slight toxic effect against *S. zeamais*, affecting germination. *M. oleifera* does not impact *S. zeamais* or protect the grain and also affects germination. *A. indica* does not kill the weevil but increases emergence and damage without influencing germination. Among these treatments, *D. ambrosioides* leaf achieves total mortality in *S. zeamais* and shows the best performance compared to other treatments and previous studies.

Effect of genotypes on *S. zeamais*

The results of *S. zeamais* development on the four maize genotypes (Table 3) show that they were suitable substrates for feeding and reproduction of the insect since they did not cause significant mortality ($p \leq 0.05$), and that, on the contrary, the hybrids Tsiri Puma and H-50 favored the increase of the adult population in the first generation. Out of the three hybrids evaluated, only Tlaoli Puma reduced damage to maize (29.5 %) ($p \leq 0.05$). All three hybrids showed higher germination percentages compared to the control treatment (Ancho Pozolero) (Table 3).

Table 3. Effect of maize genotype on *Sitophilus zeamais* Motschulsky adults and its outcome on damage and germination in bioassays evaluated in Montecillo, Texcoco, State of Mexico, Mexico.

Genotype	Mortality (%)	Emergence (%)	Damage (%)	Germination (%)
Tlaoli Puma	3.0 ± 1.0 [†] a [‡]	150.5 ± 11.6 a	70.5 ± 8.4 b	118.4 ± 5.5 a
Tsiri Puma	0.0 ± 0.0 a	179.4 ± 8.3 a	92.0 ± 7.5 a	111.8 ± 1.3 b
H-50	4.0 ± 2.0 a	160.8 ± 11.5 a	104.5 ± 10.2 a	[†] 118.7 ± 0.5 a
Ancho Pozolero	2.0 ± 1.0 a	100.0 ± 5.3 b	100.0 ± 10.1 a	100.0 ± 0.9 c

[†]Means ± standard error. [‡]Different letters in columns indicate significant differences between means (Tukey, $p \leq 0.05$).

When comparing all treatments, the resistance of the hybrid Tlaoli Puma stands out for reducing *S. zeamais* damage by almost a third without affecting the pest; however, the increase in germination, which is presumably related to the hardness (76.6 kg hL⁻¹) presented by this material (Palacios-Rojas, 2018). This differs from the hybrids Tsiri Puma and H-50, with “intermediate” hardness (75 and 73.9 kg hL⁻¹), and from the variety Ancho Pozolero, with “soft” hardness (63.8 kg hL⁻¹). This coincides with the work of several authors, who found that hard maize inhibited 47 % (López-López *et al.*, 2023), 75.8 % (López-Castillo *et al.*, 2018), and 78.3 % (Rodríguez-Cobos and Iannacone, 2012) of the insect damage.

Hardness is negatively correlated with damage and susceptibility to insects (López-Castillo *et al.*, 2018) and can be mechanical-structural or due to the action of secondary metabolites such as phenolic acids that are concentrated in the grain pericarp (Cabrera-Soto *et al.*, 2009). These compounds can act as an anti-feed and anti-nutritional factor, causing the insect to reduce its ability to digest and absorb nutrients from the maize grains.

Effect of genotype-plant-powder interaction-*S. zeamais*

In the interaction of *D. ambrosioides* (leaf), *P. auritum* (leaf), and *S. humilis* (seed) powders with the hybrid Tlaoli Puma (Table 4), it is observed that *D. ambrosioides* caused total mortality and complete inhibition of emergence and damage. This could

Table 4. Effect of genotype-powder interaction against *Sitophilus zeamais* Motschulsky adults and its result on damage and germination in bioassays evaluated in Montecillo, Texcoco, State of Mexico, Mexico.

Treatment	Mortality (%)	Emergence (%)	Damage (%)	Germination (%)
Tlaoli Puma- <i>Dysphania ambrosioides</i> (leaf)	100 ± 0.0 [†] a*	0.0 ± 0.0 b	0.0 ± 0.0 b	97.3 ± 2.3 a
Tlaoli Puma- <i>Piper auritum</i> (leaf)	70.0 ± 4.6 a	20.8 ± 9.5 b	21.4 ± 2.8 b	97.0 ± 1.6 a
Tlaoli Puma- <i>Swietenia humilis</i> (seed)	100 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b	97.0 ± 2.7 a
Tlaoli Puma-Control	3.0 ± 0.5 b	100 ± 21.0 a	100 ± 4.2 a	100 ± 2.5 b
Tsiri Puma- <i>Dysphania ambrosioides</i> (leaf)	100 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b	81.3 ± 4.3 bc
Tsiri Puma- <i>Piper auritum</i> (leaf)	94.0 ± 1.6 a	2.3 ± 3.0 b	2.8 ± 0.9 b	86.6 ± 1.6 ab
Tsiri Puma- <i>Swietenia humilis</i> (seed)	96.0 ± 0.8 a	3.5 ± 2.7 b	5.8 ± 0.8 b	76.6 ± 2.0 c
Tsiri Puma-Control	0.0 ± 0.0 b	100 ± 15.0 a	100 ± 3.8 a	100 ± 1.9 a
H-50- <i>Dysphania ambrosioides</i> (leaf)	100 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b	97.5 ± 1.3 b
H-50- <i>Piper auritum</i> (leaf)	95.0 ± 0.8 a	1.0 ± 1.8 b	1.1 ± 0.9 b	97.5 ± 1.5 b
H-50- <i>Swietenia humilis</i> (seed)	93.0 ± 1.1 a	0.0 ± 0.0 b	0.0 ± 0.0 b	97.1 ± 1.3 b
H-50-Control	4.0 ± 0.8 b	100 ± 20.8 a	100 ± 5.0 a	100 ± 1.1 a
Ancho Pozolero- <i>Dysphania ambrosioides</i> (leaf)	100 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b	92.7 ± 1.0 c
Ancho Pozolero- <i>Piper auritum</i> (leaf)	100 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b	99.1 ± 3.4 a
Ancho Pozolero- <i>Swietenia humilis</i> (seed)	93.0 ± 1.1 a	1.4 ± 0.5 b	1.8 ± 0.5 b	94.3 ± 2.6 bc
Ancho Pozolero-Control	2.0 ± 0.5 b	100 ± 9.7 a	100 ± 4.6 a	100 ± 2.5 a

[†]Means ± standard error. *Same letters in the columns indicate no significant difference between the means of the ranges (Kruskal-Wallis, $p \leq 0.05$).

be attributed to the effectiveness of the plant powder, which has no effect on the hybrid, supporting the inappropriateness of this interaction, particularly the reduction in germination, in contrast to *P. auritum* (leaf), where the insecticidal effect is reduced and the insectistatic effect of the powder dominates the interaction, and the protective effects of both strategies on maize do not add up. On the other hand, antagonism is evident in the sum of both measures, even with a reduction in germination.

Treatment of the hybrid Tlaoli Puma with *S. humilis* seed achieved total elimination of the infesting adult population and totally inhibited emergence and damage. This interaction represents an excellent alternative as an enhancement with a slight reduction in germination caused by the plant. The other treatments (Table 4) showed no significant effects, as there is no natural resistance in Tsiri Puma, H-50, and Ancho Pozolero, so the insect is not affected and the maize is not protected. There is no contribution of these materials to the interaction, manifesting the full activity of the plant powders.

Impregnation of 1 % *D. ambrosioides* leaf powder on any maize hybrid or variety eliminates infesting adults within 15 d and prevents their offspring and damage to the grain before death; however, germination is affected by 2.5 to 18.7 %. In the treatment with Tlaoli Puma and *S. humilis*, the resistance effect of the maize is combined with the

effect of the plant powder; however, *S. humilis* does not cause total mortality in other non-resistant maize; even germination is always affected. The interaction of Ancho Pozolero with *P. auritum* eliminates adults, prevents emergence and damage, and does not affect germination; however, *P. auritum* at 1 % does not always cause total mortality.

In view of the incipient interaction of plant powders and resistant maize, 1 % *D. ambrosioides* leaf powder impregnated into any maize genotype should be used to cause total elimination of adults, prevent the development of the first generation, and fully protect the maize at the risk of a 2.5 to 18.7 % decrease in germination. These findings support the notion that by combining the right maize genotype with an effective plant powder, it is possible to achieve satisfactory results in controlling *S. zeamais*, as corroborated by Rangel-Fajardo *et al.* (2020b), who mention that some maize varieties are less affected by the presence of *S. zeamais* and show less damage when treated with specific doses of plant powders.

It is important to note that there are several strategies for the control of *S. zeamais*. In cultural control, emphasis is placed on preventive measures aimed at avoiding damage in the field, such as early harvesting, given that delayed harvesting significantly increases the percentage of maize cobs affected by *S. zeamais* and early infestations in storage (Rangel-Fajardo *et al.*, 2020a). Another effective strategy is biological control, which focuses on the introduction of natural enemies to combat pests. Although this method has shown positive results in laboratory settings, it has not yet achieved widespread implementation in silos and storage rooms. Finally, physical control relies on proper moisture management, as most damaging insects do not thrive in grains with balanced moisture levels, especially below 40 %. Each of these activities contributes to the reduction of maize postharvest losses, and together, it is estimated that they could reduce losses by up to 60 % (López-Castillo *et al.*, 2018).

CONCLUSIONS

In the evaluation of six 1 % plant powders, *Dysphania ambrosioides* and *Swietenia humilis* stand out for their excellent insecticidal, insectistatic, and protective effects on maize, although they slightly affect germination. *Piper auritum* shows good insecticidal and insectistatic effects, protecting the grain without affecting germination. The hybrid Tlaoli Puma, classified as hardy, proves to be tolerant to *Sitophilus zeamais* attack, as it reduced one third of the normal damage caused by this pest. The combination of the hybrid Tlaoli Puma impregnated with 1 % plant powder of *D. ambrosioides* or *S. humilis* reduces the chances of attack and damage by *S. zeamais* and contributes to reducing post-harvest losses of stored maize.

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