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Synchronization of the estrous during a short period, using a low dose of equine chorionic gonadotropin (eCG) in primiparous and multiparous ewes

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ABSTRACT

Objective: To evaluate the reduction effect of the synchronized luteal phase and the eCG application in the reproductive variables and hormone profile response of primiparous and multiparous ewes.

Design/Methodology/Approach: The experimental design was completely random, with a 2×2×2 factorial arrangement. Based on their reproductive activity, the 81 specimens were divided into primiparous (n=38) and multiparous (n=43) ewes. The estrous and pregnancy stages were analyzed using the χ^2 test. An analysis of variance and the Tukey's mean comparison test were used to determine the start and the duration of the estrous. P_4 concentration was measured using the PROC MIXED which influenced the fixed effects of the treatment and the day, as well as their interaction.

Results: There was no difference between treatments, regarding the occurrence of the estrous; however, its start and duration were not impacted by the physiological state and the synchronized luteal phase. P_4 concentrations in plasm were higher in primiparous ewes than in multiparous ewes. The main effects did not impact the pregnancy and the prolificity rates.

Study Limitations/Implications: The variation in the start and the duration of the estrous was caused by the physiological reproductive state and the duration of the synchronized luteal phase (6 and 12 days). Therefore, these effects should be taken into account when the artificial insemination takes place at a fixed period.

Finding/Conclusions: The combination of the cronolone sponges with 100 UI of eCG during short periods (6 days) effectively synchronizes the estrous. P_4 concentrations in serum were higher in primiparous ewes, although these concentrations were not a determining factor in the increase of pregnancy and prolificity.

Keywords: Cronolone, estrous, sheep, progesterone, synchronization.

Image by Manfred Richter at Pixabay

INTRODUCTION

Several hormone methods have been developed to synchronize the estrous of ewes. They are mainly based on natural and synthetic progesterone, gonadotropins, prostaglandin $F_{2\alpha}$, and the male effect (Ungerfeld and Rubianes, 2002). Intravaginal sponges and CIDR devices are inserted for 12-14 days. The equine chorionic gonadotropin (eCG) is used before, in the moment, or after the sponge or device is removed. Their combination is the most common hormone treatment used to simulate the luteal phase for the synchronization of the estrous (Ataman *et al.*, 2006). In this context, eCG application has been proven to have positive effects on the fertility and productivity of ewes (Ince and Karaca, 2009). The aim of using eCG in progesterone synchronization programs is to increase the ovulation rate and, consequently, to increase the multiple birth rate (Macías *et al.*, 2013). Nevertheless, several factors can modify the reproductive efficiency of ewes, including: breed, seasonality, age, environment, nutrition, diseases, semen quality, reproductive state, hormone treatment, progesterone concentration in plasm, and vaginal microbiota (Mustafa *et al.*, 2007).

Currently, a demand for clean products has arisen and, consequently, the use of chemical and hormone treatments for domestic animals has been reduced or completely avoided (Martin *et al.*, 2004; Eisler *et al.*, 2014). Therefore, researches have focused on developing alternative reproductive methods, which interact both with animal welfare and the environment. Their objective is to improve the reproductive efficiency of ewes (Fierro *et al.*, 2017). Consequently, protocols for the synchronization of the estrous during short periods have been recently established, in order to determine an optimal biological response to a hormone treatment. The aim is to obtain similar results to the conventional treatments regarding the luteal phase simulation, improving pregnancy rates, even during the seasonal anestrous (Ustuner *et al.*, 2007; Özyurtlu *et al.*, 2011; Nasser *et al.*, 2012). Therefore, the objective of this study was to evaluate the effect of the synchronized luteal phase reduction and the application of a low eCG dose on the reproductive efficiency of primiparous and multiparous ewes, during their reproductive season.

MATERIALS AND METHODS

The study was carried out in the sheep unit of the experimental farm of the Colegio de Postgraduados, Campus Montecillo, Texcoco, State of Mexico (19° 48' 23" N and 98° 48' 27" W, at 2,241 m.a.s.l). The climate is subhumid warm, with a mean annual precipitation of 632.5 mm and a temperature range of 12 to 18 °C (García, 1988). The animals were handled according to the ethic and biosecurity standards of the Council for International Organizations of Medical Sciences (CIOMS, 1986) and in compliance with the NOM-062-ZOO-1999 Mexican law, regarding the use of animals in experiments (DOF, 2001).

Animals and treatments

Eighty-one Dorset ewes, in their reproductive season, with a 54±4.2 kg average weight, and a body condition score of 3 (scale: 1-5), were used for the experiment. The sheep were previously dewormed with ivermectin; additionally, they were injected with Bayer® ADE vitamin. Additionally, a Sonovet 600 ultrasound scanner (Medison, Inc., Cypress, California, USA) was used to carry out a transrectal ultrasound, in order to determine if the ewes were pregnant. All the ewes were fed *ad libitum* with oat (*Avena sativa*) hay and 600 g of commercial forage, which included 14% raw protein (RP) and 2.4 Mcal kg^{-1} of metabolizable energy (ME), in compliance with the sheep nutritional requirements (NRC, 2007). They also had *ad libitum* access to water. Based on their reproductive activity, ewes were divided into: primiparous (n=38) and multiparous (n=43). Subsequently, they were randomly further divided into groups for their hormone treatments. Before the sponges were inserted, ewes were pre-synchronized using two doses of prostaglandin $F_{2\alpha}$ (65 mg cloprostenol, Celosil®, Schering-Plough), at 8-day intervals. The first primiparous group (P) was synchronized with cronolone sponges (20 mg, Chronogest®, Intervet) in two periods: 12 days —with (P12+eCG, n=8) and without (P12+0, n=9) the application of 100 UI equine chorionic gonadotropin (eCG; Folligon®, Intervet)— and 6 days —with (P6+eCG, n=9) and without (P6+0, n=12) the application of 100 UI equine chorionic gonadotropin (eCG; Folligon®, Intervet). Both eCG applications were carried out when the sponge was removed. The same 12- and 6-day synchronization program was used for the multiparous ewes (M): M12+0, n=11; M12+eCG, n=12; M6+0, n=11; M6+eCG, n=9.

The detection of the estrous started 24 h after the removal of the sponge, using males with antimating aprons; subsequently, ewes were monitored every 4 h, during 72 h, in order to determine the duration and end of the estrous. Ewes mated at least twice with males of proven fertility, at 12 h intervals. The return of the estrous was detected twice a day (morning and evening), between 15 and 18 days after the mating. The pregnancy was confirmed transrectally 30 days after the mating, using a Sonovet 600 ultrasound scanner and a 7.5 MHz transducer.

Sampling and lab analysis

The 5 mL blood samples were gathered through a puncture in the jugular vein at 08:00 h (from fasting ewes). In order to determine the P_4 concentration in serum, the samples were collected two days before inserting the sponges and, subsequently, every 48 h during the estrous synchronization (12 days). All the samples were centrifuged at 1,500 g at 4 °C for 15 minutes in an IEC Centra 8R (International Equipment Company, USA). The blood serum was separated and stored in polypropylene tubes; subsequently, they were preserved in a freezer at −20 °C, awaiting the hormone analysis. The P_4 analysis was carried out using an enzyme-linked immunosorbent assay (Immunometrics, UK Ltd., 280 Muster Road, London SW6 6BQ). The analytical sensitivity was 0.13 ng mL^{-1}, with an inter- and intra-Assay Coefficients of Variability of 9.59 and 13.7%, respectively.

Statistical Analysis

The experimental design was completely random, with a 2×2×2 factorial arrangement; each ewe was an experimental unit. The main factors were the reproductive physiological state (primiparous and multiparous), the period of protocol of the synchronization of the estrous (6-12 days), and the application of a low eCG dose (0-100 UI). The percentages of the occurrence of the estrous and pregnancy were analyzed using the χ^2 test, through the PROC FREQ. An analysis of variance was used for the start and duration of the estrous, using the PROC GLM and the Tukey's mean comparison test. P_4 concentration was subjected to an analysis of variance, with repeated measurements throughout time, using the PROC MIXED, which included fixed effects of the treatment and day, as well as their interaction. For this procedure, the covariance structure was modeled using the effect of the sheep inside the group. For this variable, the first-order autoregressive model (AR 1) was used to determine the correlation between sequential measurements within the same animal. The mean values were compared using the least squares method. All the procedures were carried out using the statistical analysis system software suite (SAS, 2009).

RESULTS AND DISCUSSIONS

Occurrence, start, and duration of the estrous

Whether the period of the synchronization of the luteal phase (Table 1) was short or long or regardless of the primiparous (P: 97.9%) or multiparous (M: 95.6%) physical state of the ewe, the estrous was not different ($P>0.05$) between hormone treatments. The response to the start of the estrous was not different ($P>0.05$) between hormone treatments during the synchronization program. However, there were differences caused by the reproductive physiological state of the ewe ($P<0.05$) (Table 1). Differences were found between treatments for the duration of the estrous variable ($P<0.05$) (Table 1). These differences were caused by the effects of the reproductive physiological state (P: 37.2±1.5 h *vs.* M: 41.3±1.4 h) and the duration of the synchronized luteal phase (6 days: 42.2±1.4 h *vs.* 12 days: 36.3±1.5 h of synchronization).

The differences detected on the occurrence of the estrous after a hormone treatment are the consequence of several factors, including breed, season, location, nutrition, weather, and the presence of a male after the removal of the intravaginal sponge (Khalilavi *et al.*, 2016). Nevertheless, the reproductive physiological state of the ewe, the synchronization time, the concentration or dose must be also taken into account before using the different devices or hormone treatments. Other researchers obtained similar results regarding the presence of the estrous, even after the reproductive season. The following synchronization protocols were reported: 6-14 days (Ungerfel and Rubianes, 2002), 6-12 days (Ustuner *et al.*, 2007), and 7 days (Özyurtlu *et al.*, 2011). For their part, Alves *et al.* (2016) reported lower results than those obtained in this experiment: 72-80% estrous responses, using synchronization periods of 6, 9, and 12 days. However, regarding the simulation of the luteal phase, these results only prove that the short period treatment (6 days) with progesterone is as effective as the conventional treatment (12 days). This treatment has an excellent induction to the estrous and, therefore, can be used as an alternative for the synchronization of ewes during their reproductive stage (Özyurtlu *et al.*, 2011).

Several researches report that approximately 90% of the estrous start within a 48-72 interval, after the removal of the sponge or hormone device, combined with low eCG doses (Koyuncu and Altcekic, 2010). Nevertheless, the start of the estrous in this study was caused by the effect of the hormone treatment during the synchronization. In average, the estrous started 35.9±2.3 h for the groups treated for the short period (6 days) and 37.5±1.4 h for the usual period (12 days). However, the estrous occurred earlier than the results reported by Khalilavi *et al.* (2016), who recorded an average of 44.73±4.4 h (6 days) and 45.62±3.76 h (12 days) of synchronization using MAP. The estrous was longer in the group of multiparous ewes than in the primiparous ewes. There are few comparative studies about the occurrence of the estrous in ewes with different reproductive physiological states. On the one hand, the results of the duration of the estrous obtained with multiparous ewes are higher than the 17.38-27.27 h range reported by Khalilavi *et al.* (2016). On the other hand, the results obtained by the primiparous ewes are similar to the 39-42 h period reported by Alves *et al.* (2016). The duration of the estrous was also influenced by the synchronization period and was longer for the 6 days group than for the 12 days group. These results are similar to those reported by Ustuner *et al.* (2007) and Nasser *et al.* (2012).

Hormone profile of progesterone (P_4)

P_4 concentrations in plasm were different ($P<0.05$) during the synchronized luteal phase and after the estrous. The results obtained in this experiment reflected a higher concentration in primiparous ewes (1.8 ± 0.1 ng mL^{-1}) than in multiparous ewes (0.9 ± 0.1 ng mL^{-1}), showing a quick increase in the P_4 concentrations, 2 days after the removal of the sponge of the new estrous cycle (Figure 1). Bartlewski *et al.* (1999) established that P_4 concentrations higher than 1 ng mL^{-1} are the result of a functional corpus luteum at the moment that the sponge is inserted. Additionally, the insertion of the sponge soaked with progesterone does not affect the P_4 production by the corpus luteum. Only when the sponge is inserted at the beginning of the luteal phase, the production and its average life could be affected, because the corpus luteum does not secrete enough P_4 during this stage for a normal average life. However, it makes it susceptible to an $PGF_{2\alpha}\alpha$early secretion injury (Ainsworth, 1985). Nevertheless, the primiparous ewes' group had a higher P_4 concentration in plasm. These results match the findings of Husein and Kridli (2002), Husein and Haddad (2006), and Khalilavi *et al.* (2016), who reported that the P_4 levels increased 2 days after the insertion of the sponge and diminished when the sponge was removed, driving the estrous behavior. However, the P_4 concentrations in the primiparous ewes again had a sudden increase during the next estrous cycle (Figure 1). These results

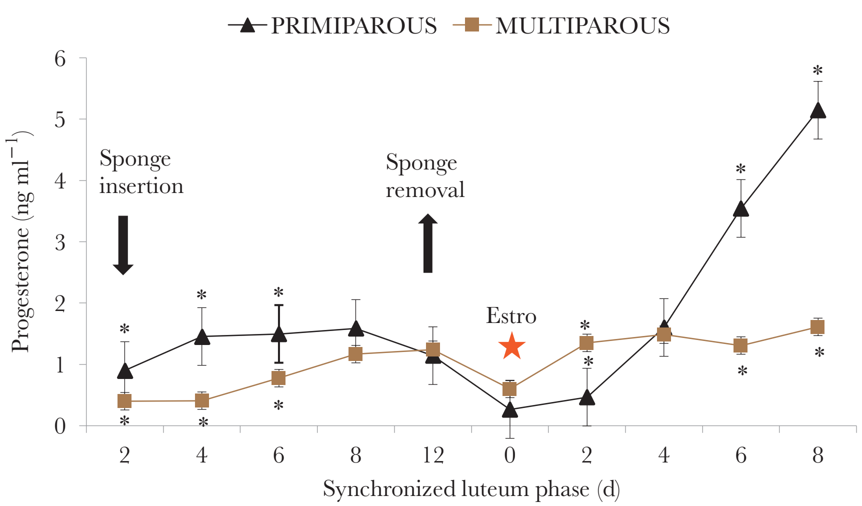

Figure 1. Concentration of progesterone in plasm (mean ± standard error) during synchronized luteum phase in primiparous and multiparous ewes. * Indicates statistical difference ($P<0.05$) between experimental groups.

suggest that these ewes can continue to develop bigger corpus luteum or that their metabolism is not capable of diminishing its concentration in blood plasm, unlike the multiparous ewes.

Pregnancy and prolificity rates

Neither the synchronization period and the eCG application, nor the reproductive physiological state of the ewe, resulted in differences ($P>0.05$) in the pregnancy and prolificity rates. The pregnancy rate of the ewes at 30 days after the mating was 95.3% and 97.3% for the multiparous and the primiparous groups, respectively. Likewise, the multiparous group recorded a 1.26 index, while the primiparous group had a 1.29 index (Table 1).

The pregnancy percentages obtained in this study are similar to those reported by Mustafa *et al.* (2007), who recorded a 91.6% pregnancy among ewes. However, our results are higher than those obtained by Ali (2007), who reported 91.6% pregnancies, using 40 mg of FGA for 8 days and 500 UI of eCG for two periods (48 h before and at the moment of the removal of the sponge). Ali concluded that the use of eCG improves the ovulation rates, but not the pregnancy percentage.

In this regard, Alves *et al.* (2016) reported 45.5, 36.4, and 20% pregnancy rates for 6-, 9-, and 12-days synchronization protocols, respectively. These synchronizations were carried out with ewes inseminated by laparoscopy. In contrast, Özyurtlu *et al.* (2011) reported 58.3-66.7% pregnancy rates during an anestrous period, applying 500 UI of eCG to ewes with 7-14 days synchronization. Likewise, Khalilavi *et al.* (2016) reported 66.6-80% pregnancy rates, as a result of the application of 300 UI of eCG to ewes with 6-12 days synchronization. In both studies, the ewes were directly mated with studs of proven fertility. There was no difference in the prolificity index between treatments. Nevertheless, our results are similar to those reported by Mustafa *et al.* (2007), who used 500 UI of eCG and obtained 1.18 and 1.11 prolificity with hormone treatments of 12 and 14 synchronization days, respectively. The use of eCG in the estrous synchronization protocols has an important effect on the ovulation rate of the ewe and, therefore, in its prolificity. According to Koyuncu and Ozis (2010), the application of 500 UI of eCG should take place 24 h before or at the moment of the removal of the sponge. Additionally, the subcutaneous application of eCG has better results for multiple lambings and fecundity than the intramuscular application of eCG. In this research, the application of 100 UI of eCG sought to support the follicular growth, in order to induce the estrous, mainly in the primiparous group. Quintero *et al.* (2011) obtained similar results in hair sheep: 1.8, 1.9, and 2.2 prolificity, with the application of 100, 200, and 400 UI of eCG, respectively. Likewise, Macias *et al.* (2013) pointed out that low doses of eCG (140-280 UI) can induce the estrous in hair sheep, even under heat stress. They obtained 1.8 (140 UI) and 2.2 (280 UI) prolificity rates.

Overall, the pregnancy percentage and the prolificity index obtained in this study are acceptable, regarding the hormone treatments used. These results suggest a development of the corpus luteum and an increase of the secreted progesterone, which was required to provide the endometrium with the appropriate conditions for the implantation of the embryo and the preservation of the pregnancy.

Table 1. Reproductive response variables in primiparous (P) and multiparous (M) ewes during short (6 d) and long (12 d) periods of synchronization with low dose of equine chorionic gonadotropin (eCG).

Reproductive Variables	Multiparous				Primiparous			
	M12+eCG (n=12)	**M12+0 (n=11)**	**M6+eCG (n=9)**	**M6+0 (n=11)**	**P12+eCG (n=8)**	**P12+0 (n=9)**	**P6+eCG (n=9)**	**P6+0 (n=12)**
Estrus response (%)	100 (12/12)	100 (11/11)	100 (9/9)	100 (11/11)	100 (8/8)	100 (9/9)	100 (9/9)	91.6 (11/12)
Estrus onset (h) [†1]	33 ± 4.5^a	35.2 ± 3.2^a	30.6 ± 4.6^a	37.4 ± 2.4^a	40.5 ± 1.8^b	41.3 ± 2.4^b	37.3 ± 1.5^a	38.3 ± 1.4^a
Estrus duration (h)	34 ± 1.4^b	40.3 ± 1.4^{ab}	48 ± 1.5^a	42 ± 1.4^{ab}	34 ± 1.5^b	36 ± 1.5^{ab}	40 ± 1.5^{ab}	38 ± 1.4^{ab}
Gestation (%)[2]	91.6 (11/12)	100 (11/11)	100 (9/9)	90.9 (10/11)	100 (8/8)	100 (9/9)	100 (9/9)	91.6 (11/12)
Prolificity index	1.27±0.1	1.27±0.1	1.30±0.1	1.20±0.1	1.25±0.1	1.33±0.1	1.44±0.1	1.18±0.1

1 Time after sponge removal.
2 Based on P_4 profiles in serum and ultrasound on day 30.
3 Number of lambs born per ewe.
[a, b] Values with different letters in columns are different ($P<0.05$).
[†] Means±standard error.

CONCLUSIONS

The combination of intravaginal cronolone sponges with 100 UI of eCG in short (6 days) and long (12 days) synchronization periods favors the occurrence of the estrous in primiparous and multiparous ewes, during the mating season. However, the variations of the start and duration of the estrous are caused by the reproductive physiological state of the ewe (primiparous and multiparous) and by the duration of the synchronized luteal phase (6 and 12 days). Consequently, these results must be taken into account when artificial insemination takes place in a fixed time. Finally, the P_4 concentrations in serum were higher in primiparous ewes, although this result did not determine a pregnancy and prolificity increase in the treated ewes.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Luteolytic failure as the cause of low efficiency in synchronization with prostaglandins in cows under tropical grazing

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ABSTRACT

Objective: To determine the reason for the low response to the exclusive use of prostaglandin in synchronization programs in cows under tropical grazing compared with the use of progesterone (CIDR).

Design/Methodology: Thirty-five cows with CL were randomly distributed in two groups. The first group (GPG; n=23) was synchronized using two doses of PGF2αα(25 mg of Dinoprost®) with a 12-day interval. The second group (GCIDR; n=12) was synchronized with an intravaginal device (1.9 g of progesterone, 2 mg of estradiol benzoate, and 50 mg of progesterone); a PGF2α (pm) dose was applied on day 7, before removing the CIDR (am) on day 8. CL regression, luteolytic failure, progesterone concentration, and CL size were determined. Data was subjected to a normality test, followed by the Mann-Whitney U test or independent Student's t-test and chi-square test.

Results: Only 82.6% (19 out of 23) of the GPG cows that received the second dose of prostaglandins have a functional CL (<1 ng mL^{-1} of progesterone). The CL recorded a regression only in 43.5% of the cows in GPG *vs.* 91.7% in GCIDR (P=0.0001). In addition, GPG cows showed a luteolytic failure of 39.1% and an asynchrony of 17.4%.

Conclusions: The low effectiveness of prostaglandin on the synchrony and regression of the CL (luteolytic failure) in cows fed under tropical grazing can be attributed to the low efficiency of the synchronization programs.

Keywords: regression of the corpus luteum , luteolytic pattern, luteolytic efficiency.

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INTRODUCTION

For decades, as a result of its luteolytic action, prostaglandin was used in estrous and ovulation synchronization programs (Córdova-izquierdo *et al.*, 2011; Colazo *et al.*, 2017).

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However, the results reported in sheep show variability: Arroyo-Ledesma *et al.* (2015) reported a 100% estrous response, while Meilán and Ungerfield (2014) and Ungerfield (2011) reported a 90% response. Synchronization is reported in only 70% of the female bovines (Giordano *et al.*, 2013; Liu *et al.*, 2018), as well as an estrous response that ranges from 87% (Mérola *et al.*, 2012; Bover *et al.*, 2019) to 64% (Gioso *et al.*, 2005). However; a failure in the effectiveness of prostaglandin on the CL regression is reported in 62.7% of sheeps (Hernández-Cerón *et al.*, 2001). CL regression is attributed to the action of prostaglandins, ending diestrous and triggering proestrous (3 and 2 days for cattle and sheep, respectively) (Atuesta and Diaza, 2011). This results would enable an estrous response the following day, basing its action within 72 h in sheep (Thimonier 1981; Ávila-Castillo *et al.*, 2019) and 96 h in cattle (Córdova *et al.*, 1983; Liu *et al.*, 2017, 2018). In published reports, the period considered is greater than the effect attributed to the action of prostaglandin. There are few exclusive studies about the effectiveness of prostaglandin in CL regression in cattle; a mere 51.6% efficiency has been reported in embryo transfer programs —*i.e.*, a synchrony failure in 48.4% of recipient cows (Baruselli *et al.*, 2000). This phenomemon can be attributed to inefficiency on the lysis of the corpus luteum (Hernández-Cerón *et al.*, 2001), which would allow females with luteolytic failure to have a natural/normal estrous cycle which may coincide with the assessment periods. The lack of evidence about the luteolytic failure of prostaglandin in synchronization protocols in cattle merits further research about the efficiency of the prostaglandin application to lyse the corpus luteum in a period no longer than 4 days (response period). The effectiveness of prostaglandin on the CL regression in cows synchronized with 2 single doses was assesed for that purpose.

MATERIAL AND METHODS

The study was performed in Villahermosa, Tabasco, in the tropical region of southeastern Mexico (18° 20' N, 17° 78' S, 92° 95' E, and 93° 15' W), which has a warm-humid-dry climate with a maximum temperature of 43.5 °C and a minimum temperature of 10.5 °C (SMN, 2010).

Animals used for the experiment

Thirty-five cows (3-4 years old) fed with humidicola grass (*Brachiaria brizantha*) and an average body condition of 3.2±0.49 points (scale: 0 to 5) (Edmonson *et al.*, 1989) with at least one corpus luteum (CL) were randomly distributed as follows: the first group (GPG; n=23) was subjected to a synchronization protocol with two luteolytic doses of PGF2α (25 mg of Dinoprost, Lutalyse® by Zoetis), with a 12-day interval between doses (Selk *et al.*, 1988); and the second group (GCIDR; n=12) was subjected to a synchronization protocol consisting of an intravaginal device inserted on day 0 (1.9 g of progesterone, CIDR® by Zoetis), plus 2 mg of estradiol benzoate (IM; Sincrodiol® by Ourofino), and 50 mg of IM progesterone (Progesvit A-E® by Brovel), followed by a 25-mg dose of PGF2α (Lutalyse® by Zoetis) administered on day 7, and the removal of the CIDR on day 8 (Baruselli *et al*, 2011). Both groups were provided with clean, fresh water on a daily basis.

Variables

Luteal dynamics: A Mindray DP-10 Vet ultrasound with a 7.5-MHz intracavitary real-time, linear array transducer was used to perform an ultrasonography, in order to determine the diameter of CL from the second PGF2α dose on day 0, 2, 4, and 7. The diameter of CL was determined with the equation described by Sartori *et al.* (2004):

$$D = \frac{L + A}{2}$$

D=Diameter of CL (mm), L=lenght of CL (mm), A=width of CL (mm).

Blood progesterone concentration (P4)

Progesterone was determined 0, 1, 2, 5 and 7 days after the second PGF2α dose or the removal of the CIDR using blood samples obtained by venipuncture, using BD Vacutainer® tubes and needles with 6 ml of anticoagulant (80-100 IU of heparin) which were then refrigerated at 4 °C. Subsequently, they were centrifuged (3000 x g for 10 min at room temperature) and stored at −20° C before they were analyzed. The solid-phase enzyme-linked immunosorbent assay (ELISA) technique was used for this determination, based on the principle of competitive binding described by Siregar *et al.* (2017), using a DGR® EIA 1561 commercial kit (GmBH, Germany) and a 450±10 nm calibrated microplate reader.

Data analysis

Data was analyzed in 2 phases. The first compared the efficiency of two synchronization protocols (prostaglandins, GPG; n=23 *vs*. Progesterone, GCIDR, n=12) in cows fed with grazing. The second determined if the cause of the low efficiency of prostaglandin (GPG) on CL regression in bovines is similar to the cause reported in sheep by Hernández-Cerón *et al.* (2001). The diameter of CL (according to the equation described by Sartori *et al.*, 2004) was determined based on the luteal dynamics of GPG, while the functionality of the corpus luteum was determined according to the blood progesterone concentration. Less than 1.0 ng mL^{-1} was considered non-functional CL and a higher concentration was considered functional CL (Ribeiro *et al.*, 2012). Therefore, a luteolytic failure was determined, when the CL remained functional throughout the assessed process (Callejas *et al.*, 2003; Ribeiro *et al.*, 2012; Liu *et al.*, 2017). Structural lysis was determined based on a statistical decrease in diameter of the CL of up to 9 mm (Balaro *et al.*, 2017).

The resulting data were subjected to a Kolmogorov-Smirnov normality test, followed by a statistical test ($P \leq 0.012$). Subsequently, they were analyzed using non-parametric tests (Mann-Whitney U Test) or, when appropriate, they were subjected to a Student's t-test for independent groups. The proportion of cows showing lysis of the corpus luteum was submitted to a chi-square (χ^2) statistical test. Data were described as the arithmetic mean ± standard error of the arithmetic mean, using the SYSTAT statistical package version 13 (Chicago, Illinois, USA).

RESULTS AND DISCUSSION

Efficiency of synchronization programs on CL lysis

In both groups, the assessment began at the end of the synchronization program (GPG, n=23 and GCIDR, n=12); all the cows showed at least one CL. The percentage of cows showing CL regression from day 1 to day 7 is higher in cows with GCIDR than the efficiency of GPG cows ($p \leq 0.04$, Table 1). However, blood progesterone concentration did not differ between the two groups on day 0, 1, and 2 ($p \geq 0.09$; Table 1). Meanwhile, on day 4 and 7, the progesterone concentration was lower in cows with GCIDR *vs*. GPG ($p \leq 0.005$). Furthermore, the size of CL was similar in cows with GCIDR *vs*. GPG on day 0, 2, and 4 ($p \geq 0.21$), while on day 7 the size of CL was greater in cows with GPG ($p=0.006$). This demonstrates the low efficiency of a prostaglandin dose on day 4 in 56.5% and on day 7 in 43.5% of the specimens.

Effectiveness of prostaglandin on the regression of corpus luteum

The presence of at least one CL was observed in 100% of the cows, following the application of the second prostaglandin dose; however, only 82.6% (19 out of 23) recorded a functional CL (>1 ng mL^{-1} progesterone; P=0.002) after the second dose, while 17.4% of the cows recorded the presence of a non-functional CL. Nevertheless, they had a functional CL (>1 ng mL^{-1} progesterone) on day 4 (Table 2).

On day 4, a low efficiency in prostaglandin synchronization was observed in 56.5% of the cows with GPG (CL regression in 43.5%), reaching 43.5% on day 7. This inefficiency is attributed to two factors: the failure to synchronize the second prostaglandin dose in 17.4% of the cows that had a CL regression of the CL on day 0; and a luteolytic failure in 39.1% and 26.1% of the cows subjected to prostaglandin synchronization on day 4 and day 7, respectively. Therefore, in the following GPG analysis, cows with a functional CL

Table 1. Efficacy of synchronization on luteolysis in cows fed under tropical grazing (GCIDR=with CIDR and GPG=synchronization with two doses of prostaglandins).

	Days later of the 2^{nd} dose of prostaglandins.				
	0	**1**	**2**	**4**	**7**
Proportion of cows with luteolysis, %					
GCIDR, n=12	0	66.7	75	91.7	91.7
GPG, n=23	0	13	39.1	43.5*	56.5
P^1	---	0.002	0.039	0.008	0.04
Progesterone concentration, ng/ml					
GCIDR	4.5±5.5	2.2±2.4	1.0±0.9	0.57±0.4	0.6±0.3
GPG	3.8±3.6	2.2±1.8	2.2±2.2	2.2±2.2	4.0±6.6
P^1	0.27	0.39	0.088	0.004	0.005
Corpus luteum size, mm					
	0		2	4	7
GCIDR	20.9±3.4		11.2±3.3	9.3±4.2	7.9±2.0
GPG	19.9±4.9		12.8±4.5	14.5±5.1	13.3±5.6
P^1	0.47		0.21	0.31	0.006

Table 2. Size of CL and proportion of cows with functional CL (with luteolytic failure or CL regression) or with non-functional CL.

		Effect of prostaglandins			
Second dose, day 0		**1**	**2**	**4**	**7***
CL- Functional, % 82.6	Failure	69.5	43.5	39.1	26.1
	luteolysis	13	39.1	43.5	56.5
	P[1]	0.000	0.732	0.732	0.016
CL- Non-Functional, % 17.4	Functional	13	4.4	0	0
	Non-Functional	4.4	13.0	17.4	17.4
Corpus luteum size, mm					
CL-Functional	Failure	18.3±4.3	17.1±3.5	17.9±3.8	15.8±6.0
	luteolysis	23.6±2.8	8.9±1.7	6.8±5.2	6.8±5.5
	P[1]	0.005	0.000	0.000	0.03
CL- Non-Functional		11.1±1.4	13.0±0.4	15.5±4.3	15.9±5.5

after the application of the second prostaglandin dose (cows that underwent a regression of the CL *vs.* failure of CL regression) were compared with the evolution of cows with a non-functional CL after the application of the second prostaglandin dose. The proportion of cows with CL regression was similar to those showing a luteolytic failure on days 2 and 4 ($p=0.7$); meanwhile, a failure decrease was recorded on day 7 ($p=0.016$) (Table 2).

The progesterone concentration at day 0 was statistically similar between cows with CL lysis and cows with luteolytic failure ($p=0.5$) (Table 3), while the progesterone concentration at day 1, 2, 4, and 7 was higher in cows with luteolytic failure ($p\leq0.03$) (Table 3). However, the size of the CL was lower in cows with luteolytic failure throughout the assessed period ($p=0.005$) (Table 2). The CL size was greater in cows that manifested luteolytic failure than in cows that suffered CL regression ($p\leq0.03$) (Table 2). Therefore, the blood progesterone concentration did not differ in cows with luteal failure or lysis ($p=0.51$) (Table 3), while the progesterone concentration was higher in cows with luteal failure from day 1 to day 7 than in with cows that suffered CL regression ($p\leq0.03$). Likewise, the size of the CL and the progesterone concentration in cows with non-functional CL increased from day 1 to day 7 after the application of the second prostaglandin dose (Table 3).

According to these results, 100% of the cows have at least one corpus luteum at the first and second luteolytic dose of prostaglandin. This potential synchronization is supported

Table 3. Blood progesterone concentration in GPG cows with functional CL (with luteolytic failure or lysis of CL) or with non-functional CL.

		Effect of prostaglandins				
Second dose, day 0		**0**	**1**	**2**	**4**	**7***
CL-Functional	Failure	4.2±4.2	3.4±2.1	4.2±2.9	3.9±2.6	5.6±6.9
	luteolysis	4.8±3.5	1.5±1.2	0.7±0.2	0.7±0.5	0.9±0.8
	P[1]	0.51	0.03	0.001	0.001	0.027
CL- Non-Functional		0.6±0.1	1.2±0.9	1.3±0.5	2.1±1.0	8.3±11.2

by several studies (25 mg, Moreno *et al.*, 1986; Bó *et al.*, 2004; Montiel-Palacios *et al.*, 2011). However, progesterone profiles determine that 74.1% of the cows are synchronized (functional CL at the second dose), which matches the results of Liu *et al.* (2018), who reported that only 69.6% of the cows become synchronized with the use of prostaglandins; meanwhile, Giordano *et al.* (2013) mentions that 70 to 80% of the cows become synchronized. In contrast, our results indicate that only 35.7% of the subjected cows showed luteolysis within the 4-day period attributed to the action of exogenous prostaglandin (Córdova *et al.*, 1983; Liu *et al.*, 2017; 2018); however, 7.1% showed lysis before the second prostaglandin dose, with a functional CL after the application, as indicated by Olivera (2007) and 14.3% showed a CL regression after the action period of the exogenous prostaglandin (day 7).Our results differ from some studies in bovines, including Liu *et al.* (2017) and Ribeiro *et al.* (2012), who show that a standard prostaglandin dose causes CL lysis in 60% of the cows. However, a 35.7% (Hernández-Cerón *et al.* 2001) to 50% (Granados-Villareal *et al.* 2017) and 42.8% (Álvarez-Reyna 1994) effectiveness of prostaglandin is reported in sheep. This phenomenon is attributed to the high percentage of luteolytic failures recorded in females. Therefore, these studies do not report the percentage of females which had a luteolytic failure since the first prostaglandin dose. Based on our results, 28.6% of the cows do not have functional CL at the second dose, perhaps as a consequence of a possible luteolytic failure of prostaglandin since the first application.

These results —obtained from a high luteolytic failure— are attributed to various causes, such as age, functionality, and size of the CL at the time of prostaglandin action (Stevenson *et al.*, 1984; Moreno *et al.*, 1986; Berroa-Pinzón 1988). For example, Oliveira *et al.* (2007) mention that the sensitivity of the CL to the action of prostaglandin in cattle starts at day 5 of maturation, while Menchaca and Rubianes (2004) report a sensitivity at day 3 after ovulation in sheep. In our study, synchronization with the first prostaglandin dose suggests that the CL had a minimum age of 7 days (3 to 4 days after the start of the estrous and ovulation of the first dose, plus 8 to 9 days following the development of the CL). However, we observed that 28.6% of the cows did not have CL functionality at the time of the second dose, which could indicate CL lysis before this dose; therefore, luteolytic failure in some cows from the first dose of prostaglandin can be inferred, as has been shown in some studies on luteolytic failure in the second application of prostaglandin.

Prostaglandin sensitivity is related to size and progesterone concentrations (Spell 2001; Sartori *et al.*, 2002). In our study, cows that suffered luteolysis showed a larger CL (23.6 mm *vs.* 17.9 mm in cows with luteolytic failure); however, no differences in progesterone concentration (3.8 mL^{-1} and 2.6 mL^{-1}) were recorded between cows that suffered lysis and those that had luteolytic failure. This result is different from the findings of Granados-Villarreal *et al.* (2017), who pointed out that a high progesterone concentration prior to prostaglandins results in a higher proportion of females with luteolytic failure.

In this study, 91.9% of the cows subjected to synchronization with a vaginal device and prostaglandins synchronized at the onset of the estrous and ovulation in a 4-day period, while in the protocol with two prostaglandin doses only 71.4% of the cows became synchronized with the first dose of prostaglandin and only 50% are synchronized with the second dose after a 7-day period. These results can be attributed to an effect in progesterone release by CIDR, which improves the sincronization response as indicated by Beard and Lamming (1994). In a long progesterone period, it increases estrogen receptors; consequently, they increase the oxytocin receptors that, along with exogenous prostaglandin, induce endogenous prostaglandin on day 7 after CIDR insertion, which improves the efficiency on CL regression in GCIDR synchronized cows. These results demonstrate that the low pregnancy rate observed in synchronization protocols with two prostaglandin doses is caused by the luteolytic failure of prostaglandin in the application of the first and second doses. For example, Riveiro *et al.* (2012) report a 28% pregnancy rate using prostaglandin and artificial insemination; in contrast, the CIDR and prostaglandin protocols have been shown to achieve a pregnancy rate up to 81% (Hernández *et al.*, 2008).

CONCLUSIONS

The low efficiency of prostaglandin in the synchronization programs can be attributed to the high proportion of cows that do not synchronize and the high proportion of cows with luteolytic failure. This factor may be the cause of the low pregnancy rate in prostaglandin synchronization programs reported by some studies. Likewise, synchronization with progesterone is more effective than the use of two prostaglandin doses applied with a difference of 12 days.

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Taro (*Colacasia esculenta* (L.) SCHOTT) value chain in Veracruz, Mexico

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ABSTRACT

Objective: To identify and analyze the links of the taro (*Colocasia esculenta*) value chain in Actopan, Veracruz, Mexico, focusing on its deficiencies and opportunity areas, and to simulate the yields generated by this crop.
Design/Methodology/Approach: The research was carried out in the town of Santa Rosa, in Actopan, Veracruz, Mexico, with local producers and manufacturers, using both an exploratory and a random descriptive method and a qualitative and quantitative approach. Direct observation, interviews with key informants, and reflection and analysis workshops were carried out to find out which are the links of the value chain and how they operate within the taro agroecosystem in the study region.
Results: The links between inputs supply, production, harvest and post-harvest, collection and distribution, transformation-packing-crating, and commercialization were determined. The main limitation was the area cultivated by half of the producers (0.5 to 3.0 hectares) who obtain an average annual yield of 50 t ha^{-1}. The cost of agricultural inputs is high, the market prices for the product are low, and pest organisms impact the production. Regional middlemen (both retailers and wholesalers) are in charge of commercialization and most of the agreements are informal. The main market for taro and its destination is international (90% for the American market). The best organized packing plant has a 126 to 169% monthly yield.
Study Limitations/Implications: Performance simulations must be carried out in medium and small packing plants to obtain an overall comparison.
Findings/Conclusions: The production and commercialization of taro in the study area generate economic income and local employment throughout the year. Taro also has lower production costs than other crops, as well as a high return on investment. Overall, it benefits the economic agents of the value chain. However, the lack of organization of the participants does not allow them to use economies of scale or to access preferential markets. Consequently, the economic benefits are not distributed equally among all the links.

Keywords: Taro, value chains, performance.

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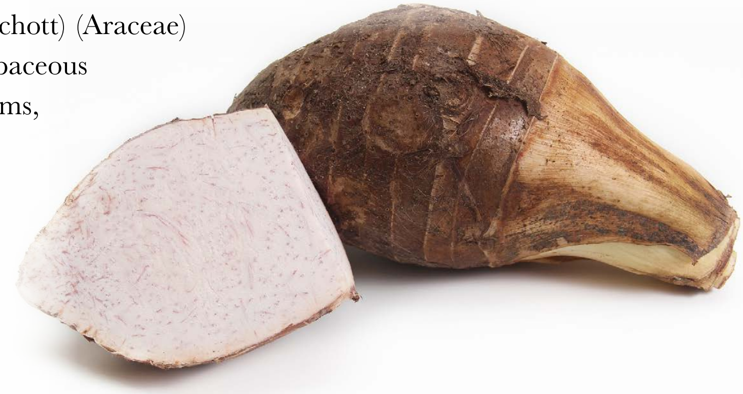
INTRODUCTION

Taro (*Colacasia esculenta* (L.) Schott) (Araceae) is a tropical and subtropical, herbaceous perennial plant. It lacks aerial stems, but it has large leaves that come from a primary underground corm —its commercial product. It is classified as a



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non-traditional exotic product and its global consumption has experienced a significant boom, taking advantage of the interest shown by growing consumer sectors. Its importance as a nutritious and highly digestible food comes from the microgranular structure of its starch, as well as from its minerals and vitamins (magnesium, iron, phosphorus, potassium, sodium, copper, and manganese; and vitamin C, vitamin E, and vitamin B6). It is grown in humid and tropical regions of southeastern Mexico, Central, and South America. Its easy cultivation makes it highly viable for its commercialization as fresh and processed products, providing it with great potential in the domestic and foreign markets. Taro develops well in humid areas and only its underground fraction —a modified stem known in the field of botany as corm— can produce more than 50 t ha^{-1}. One fifth of the total biomass stored in it constitutes dry matter; 80% of it is made up of high digestive quality starches (Olguín-Palacios, 2018).

In Mexico, the main producing states are Oaxaca, Tabasco, Nayarit, Sinaloa, and mainly Veracruz. The last state accounts for 84.80% of the national total (577 ha). In Veracruz, the main producing municipalities are Paso de Ovejas, La Antigua, Puente Nacional, Úrsulo Galván, and Actopan. In this last municipality, the average annual growth rate of taro has been higher than other crops in the same period, including sugarcane (*Saccharum* spp.), chayote (*Sechium edule*), and manila mango (*Mangifera indica*) (Lezama, 2020).

Currently, value chain analysis is one of the main tools that companies have available to carry out their strategic planning and to generate competitive advantage —a topic of vital importance nowadays, given the number of competitors whom they have to face, mainly as a consequence of globalization, e-business, and the opening of markets, among other factors (Zamora, 2016).

A value chain (Porter, 1985) is a basic business tool to analyze the sources of competitive advantage, because it is a systematic way to examine all the activities that are carried out, as well as their interactions. Consequently, the processes can be divided in their strategically relevant activities, in order to understand the cost behavior, as well as the current and potential sources of differentiation. A value chain is surrounded by economic agents; the agent can also be a participant in all the activities carried out in the said chain (Riemann, 2013). Jan van Roekel (1995), mentioned that "In the future, agri-food producers, processors, logistics service providers, and distributors will no longer compete as individual entities; rather, they will collaborate in a strategic "Value Chain", competing against other value chains in the market."

In this scenario, the proper management of sustainable agri-food value chains plays an important role, since they provided a clearer reflection of the complex reality of agriculture and the relationships that develop between the various participants (Sayers, 2003). In this sense, a value chain must be evaluated based on its profitability (as a relative measure of profits). It is determined by the comparison of the net profits of the company through its sales (profitability or net profit margin on sales) with the investment made (economic or business profitability) and the funds contributed by its owners (financial or owner profitability) (Morillo, 2001). More specifically, financial profitability indicates the company's ability to obtain profits from the investment made by shareholders, including the undistributed profits of which they have been deprived (Urias, 1991). Consequently, the promotion of the economic and social development of the producing region requires a clear identification of the members of the taro value chain in the study region and their functions, as well as an economic measurement of their production process.

MATERIALS AND METHODS

Location of the study area

The research was carried out in the town of Santa Rosa Actopan, Veracruz, Mexico (19° 23' and 19° 44' N and 96° 20' and 96° 48' W) with local producers and manufacturers.

Data collection and analysis

The research was exploratory and descriptive, using a qualitative and quantitative approach; it had the participation of key actors. To determine the links of the value chain and how they operate within the taro agroecosystem in the study region during 2018, 2020, and 2021, the following research instruments were used: direct observation, interviews and questionnaires answered by key informants, and reflection and analysis workshops.
The first approach consisted of a meeting with the manager of the largest packing plant in the area, as well as with a group of producers. The objectives of the research work were explained to both the businessman and the producers and they were asked their opinions about the taro value chain. Their answers laid the foundations for the development and arrangement of the data collection instruments, specifically semi-structured interviews and questionnaires that covered the following topics: information about the members of the links (name, age, sex, etc.), knowledge of value chains, participation in the value chain (link to which they belong), types of interaction with the different links, and contribution to the value chain.

Subsequently, field visits were carried out in the area to learn about the operation of the value chain. The producers and the workers answered guiding questions with their point of view and opinion. In addition, periodic semi-structured interviews were conducted with the manager of the packing plant and with some producers who work with him. Consequently, the resulting data were used to identify the components and the function of each link of the taro value chain in the study area.

The data was subjected to a three-stage analysis. In the first stage, the framework data was subjected to a qualitative analysis, considering the stages of separation of units according to functional and process criteria, as well as the identification and classification of units. The value chain model proposed by Michael Porter (1985) was taken as a basis to identify which stages add value for clients and interested parties of the taro agroecosystems. In the second stage, a cost and component analysis of the taro production process was carried out using the Vensim software. And in the third stage, the same software was used to develop a predictive model to project the profitability generated by the productive process of the taro value chain.

RESULTS AND DISCUSSION

The state of Veracruz has mostly extensive and monoculture production systems (INIFAP, 2019) and, for the most part, taro is grown under this modality. Taro is grown in

679 ha of the Mexican territory (SIAP, 2018), out of which 577 ha (84.80 %) are located in the Veracruz.

Identification of the participants of the value chain

Six links of the taro value chain were identified in the Actopan municipality (Figure 1): inputs supply; production, harvest, and post-harvest; collection and distribution; transformation; packing and crating; and commercialization.

Agricultural input suppliers are the first link in the value chain. These are considered relevant participants or agents of the chains and are represented by agrochemical stores that supply agricultural inputs to producers in various parts of the Actopan municipality. These stores advice the producers during the initial stage of planting and development of the crop, regarding the use, dosage, and application of fertilizers. However, not all producers receive this service, since it depends on their type and their economic capacity. Regarding the value chain approach, there is no special link between the first and other links and the input purchase and production activities lack any kind of organization. All the respondents indicated that they did not belong to any organization, which limits their lobbying power, hindering their access to government financial support and preferential wholesale prices, and limiting their preferential access to the markets, among other advantages that they would obtain if they belonged to a group of that kind.

The production includes the producers as main participants. Most of them (57.1%) have a small productive unit ranging from 0.5 to 3.0 ha; producers with plots between 4.0 and 6.0 ha account for 14.3%, while those with plots of 7.0 to 9.0 ha are 21.4% of the total; finally, producers with more than 10 ha and whose average yields reach 50 t ha^{-1} account for 7.1%. Most of the producers (75%) grow a local or native variety identified as coconut taro and obtain a 75.0% profitability. The rest of the producers (25%) indicates that they produce it to increase the profitability of the planted area. This result matches the findings of a study carried out by Arce (2018), in which most of the producers explained that the production of taro enhances their income. The main problems or limitations that producers have faced are high prices of agricultural inputs (32%), low market prices for the corm (28.5%), pest and disease control (25%), high production costs (14.2%), and commercialization problems (3.5%). To calculate the total production sowing costs, the following elements were added: labor costs of sowing per month, total water costs per hectare and per month, and total land treatment costs per year and per month, as well as the total and monthly number of hectares (Figure 2).

The next link is divided into harvest and post-harvest activities. The harvest is carried out 9 to 12 months after planting. In order to keep the corm fresh during the

Figure 1. Taro value chain in the municipality of Actopan, Veracruz.

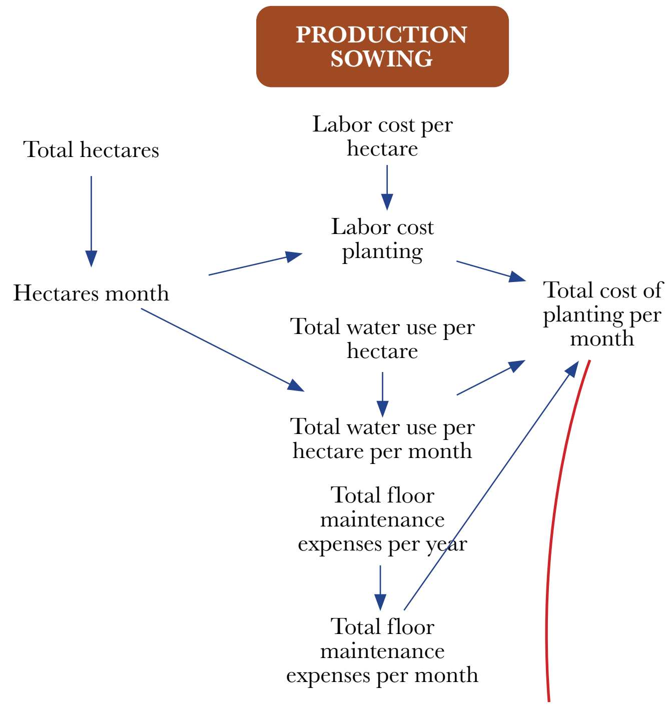

Figure 2. Elements of the production link.

transportation from the plantation to the packing facilities, it must include part of the stem where leaf growth begins (Zelendon, 2010). The intermediaries hire personnel to visit the production unit to schedule the purchase with the producers; the said personnel are aware of the quality standards of the exported produce, the main destination of this crop. Once the corms have arrived at the various packing plants, a process of selection, washing, drying, and packing in 18-kg net sacks (arpillas) begins. The total labor per crop and the daily labor payment were used to calculate the total expenses per crop (harvest) (Figure 3).

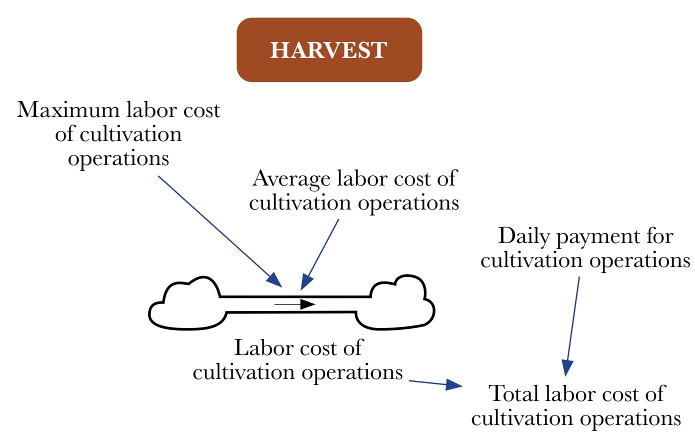

Figure 3. Elements of the harvest and post-harvest link.

The activities of the collection and distribution link (Figure 4) are carried out by regional rural collectors, retailers, and wholesalers. The wholesalers are the dominant force in the region: they go directly to the production unit where the harvest is carried out. This type of negotiation means that the producers avoid the inconvenience of transporting the product. The product is paid according to the regional price (López *et al.*, 2018).

Transformation, packing, and crating are the penultimate link. The seven regional packing plants were the sole participants of this link. Empacadora Santa Rosa is the biggest packing plant in the region. Only 10% of its total production is transformed into fried food which are sold by its subsidiary, Empresa de Frituras Doña Leo. The rest (90%) is packed fresh for its exportation. It is important to emphasize that, following the selection process, the percentage of the corms used to produce fried food (10%) do not comply with the exportation requirements. Other local packing plants follow the same process: they also pack close to 100% of their production for exportation as fresh product. Unlike Empacadora Santa Rosa, low-quality corms (20%) that do not qualify for exportation are sold in the domestic market (20%), which means that they have an 80% loss. Finally, in the last link (commercialization), 90% of taro production is destined for export to the United States by land —five 20-ton shipments per week, along with three weekly shipments by sea. The packing plants are the sole participants in this link. They have their own clients, along with well-defined distribution channels. Figure 5 shows the elements used to calculate the total distribution expenses; the data comes exclusively from Empacadora Santa Rosa.

Empacadora Santa Rosa has total annual expenses of approximately $14,297,396 —calculated adding the monthly expenses of the production process (Table 1)— and a total income of approximately $33,344,230 —the total annual income, which has an approximate 165 % annual return regarding the initial investment (Table 2).

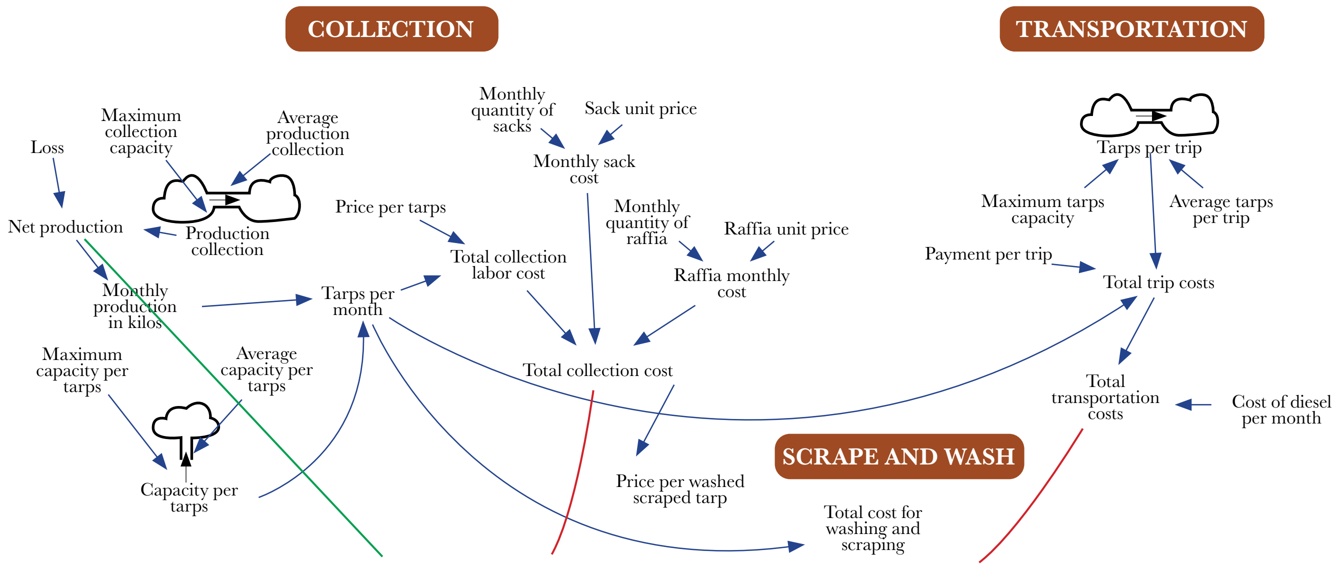

Figure 4. Elements of the transportation and distribution link.

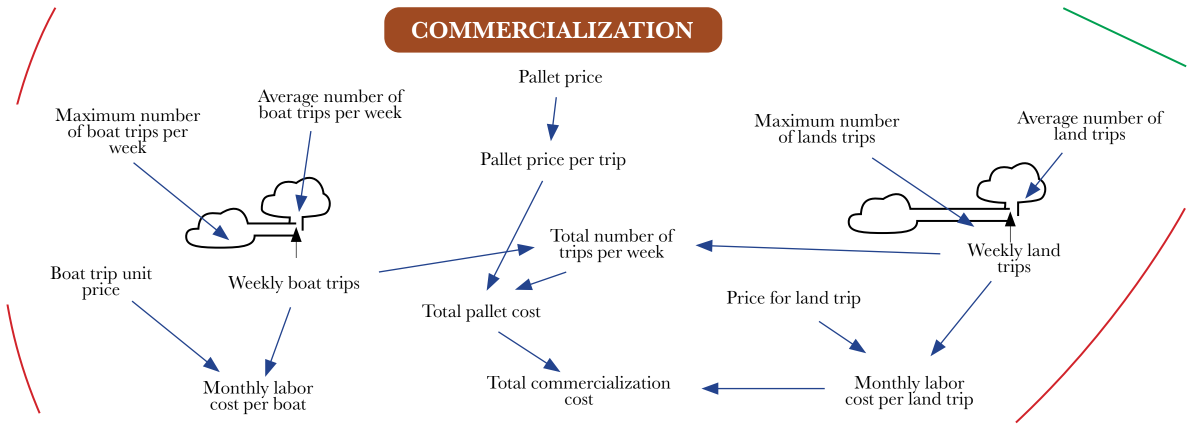

Figure 5. Elements of the commercialization link.

Table 1. Annual expenses for the cultivation and commercialization of taro (*Colocasia esculenta*).

Month	Labor Crop	Harvest	Transp.	Wash. & Scrap.	Packaging	Total Cost
0	181186.3	160917	11922.4	176026	239257	997,642
1	173314.2	160611	12226.3	175679.1	243302.8	993,466.70
2	187087.9	199127.6	14056.9	219331.3	317086.3	1,165,023.40
3	159784.3	181685.2	13417.8	199563.2	373657.6	1,156,441.50
4	182173.9	184746.8	12515.9	203033	314577.4	1,125,380.40
5	184221.6	186570.8	12679.4	205100.3	311926.8	1,128,832.40
6	151263.5	172439.8	13402.6	189085.2	288063.3	1,042,587.70
7	168012.2	198306.1	13381	218400.2	259984.5	1,086,417.30
8	204778.6	199204.1	13926.9	219417.9	363511.8	1,229,172.60
9	181053.2	188410.8	13139.4	207185.6	219901.2	1,038,023.60
10	140510.9	184342.7	13596	202575.1	299256.2	1,068,614.30
11	152196.5	199417.1	12957.9	219659.4	360699	1,173,263.30
12	192343.1	182941.8	12360	200987.3	275565.6	1,092,531.00
TOTAL EXPENSES						$14,297,396

Table 2. Total annual earnings.

Months	Total earnings ($)
1	2,754,200.00
2	2,816,190.00
3	2,763,010.00
4	2,788,030.00
5	2,782,250.00
6	2,787,980.00
7	2,783,710.00
8	2,779,390.00
9	2,762,360.00
10	2,814,400.00
11	2,752,880.00
12	2,759,830.00
Total	33,344,230.00

CONCLUSIONS

Six links were identified within the taro value chain (inputs suppliers, production, harvest and post-harvest, collection and distribution, transformation, packing and crating, and commercialization). All of them have very precise and independent participants, characteristics, and functions. They have scarce interaction and cooperation, unless a single person or company (*e.g.*, packing plants) is involved in more than one link. Otherwise, there are only informal relationships between links and the communication and exchange of information is non-existent. Except for a few participants, there is generally no organization and they do have a specific weight to negotiate with suppliers or customers or to influence the creation of specific government support and development programs. Regardless of the abovementioned problems, the regional packing plant with the best organization has very attractive profitability indexes, which shows that taro is a profitable crop and that, if the other links in this value chain manage to organize themselves, they can obtain better profits and contribute to the socioeconomic development of the region.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of the sowing ratios on the yield and quality of seed from maize genotypes

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ABSTRACT

Objective: To identify optimal sowing ratios, validation of production technology and seed quality in maize hybrids.

Design/methodology/approach: Sowing ratios of 4:2 and 6:2 were studied to observe their effect on the genotypes, as well as their quality and response in seed production; this was done by evaluating the following variables: days to male and female flowering, plant and ear height, percentage of ear and seed in cobs, commercial seed yield, thousand seed weight, number of seeds per kilogram, standard germination and hectoliter weight of the seed. An analysis of variance was carried out using a completely randomized design with factorial arrangement and four replications, the experimental unit being furrows of 20 linear meters.

Results: Significant differences were detected in the hybrids with respect to the variables and sowing ratios, which specifically influences the weight, size and number of seeds; the hybrid H-386A had the best response to the environment and ratios, exceeding the rest of the hybrids evaluated by 72.92%.

Limitations on study/implications: The interaction of genotype and environment are factors that limit seed production; however, performing evaluations in different environments allows finding stable hybrids with high yields.

Findings/conclusions: The generation of knowledge allows us to make decisions regarding crop establishment and quality in seed production lots, being a recommended environment in CIRPAC's area of influence.

Keywords: *Zea mays* L., INIFAP hybrids, production, flower synchronization.

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INTRODUCTION

The National Institute for Forest, Agriculture and Livestock Research (*Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias*, INIFAP), generates and liberates hybrids for different agroecological zones of the country, and the acceptance of these hybrids by

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farmers is possible because parents are supplied in category registered to different national companies devoted to seed production. In addition to this, it is necessary to develop technologies to identify the environments that are favorable and the seed quality (physical, physiological, sanitary and genetic) for sowing. Through the Centro Altos Experimental Field from Jalisco, which depends on the *Centro de Investigación Regional Pacífico Centro* (CIRPAC), activities in genetic improvement of maize are developed, to obtain new hybrids of white and yellow grain, which can be used in the subtropical zone of the Central Pacific of Mexico. The adoption of these new materials will depend on the yield potential and seed quality from the parent lines, as well as the yield potential of the grain from hybrids at the commercial level.

In the generation of improved varieties and the production of maize seed, agricultural research and teaching institutions have contributed to the offer of quality seed (Larque *et al.*, 2013; Domínguez and Donnet, 2014). Likewise, García *et al.* (2018) mention that the use of quality seeds constitutes one of the most profitable investments in the economy of every farmer, and widely justifies the research in adequate techniques to produce the maximum amount of high quality hybrid maize (*Zea mays* L.) seed. The seed yield and quality can be increased with changes in agricultural practices, such as the sowing pattern, referring to the number of furrows with female plants in relation to the furrows with male plants.

During the production process of the seed, its quality is controlled; however, there is scarce information about the technology of seed production of registered and certified categories. In hybrids, the potential yield and seed quality of parents (lines and simple crosses) have been evaluated, and seed production sites have been identified (Virgen *et al.*, 2010; Arellano *et al.*, 2011).

It has been found that the maize seed yield of the compact sowing pattern is equal (García *et al.*, 2014) or superior (Beck, 2004) to the conventional system; however, there is no information about its effect on the physical and physiological quality of the seeds obtained, which is why it is necessary to evaluate the quantity and the quality of the hybrid seeds produced based on attributes that favor the establishment and development of the plants in the field.

However, it is still essential to understand the behavior of the parents, lines and simple crosses, in production localities, and their interaction with the environment to obtain a quality hybrid seed (Virgen *et al.*, 2016). Therefore, in order to determine whether the materials fulfill the requirements mentioned before, this research study was established in one of the main producing zones of certified maize seed and influence area of national companies linked to INIFAP-CIRPAC, with the objective of identifying the optimal sowing ratios, validating the production technology and seed quality of the hybrids H-384A, H-385A and H-386A.

MATERIALS AND METHODS

The research study presented here was conducted during the Fall-Winter 2012/2013 cycle, in Coquimatlan, Colima. This is an important zone in the production of maize seed, with regards to the area of influence (Table 1), whose characteristics are presented next.

Table 1. Location of the agroclimate conditions of the locality of study.

Locality	Municipality and State	Altitude msnm	Temperature mean °C	Weather	Location	
					Latitude N	Longitude W
Coquimatlán	Coquimatlán, Colima.	400	26.1	Warm Subhumid	19° 11' 52"	103° 48' 41"

In this study, three hybrids generated by INIFAP in the Pacific-Center region were evaluated. In this locality, seed production lots of the yellow grain hybrids H-384A (G1), H-385A (G2), H-386A (G3) were established, whose parents are: simple cross of lines B-4A×B-6A (female), B-7A (male), and B-4A×B-6A (female), B-7A×B-8A (male) and B-3A×B-6A (female), B-9A (male), respectively. The ratios of females and males were: 4:2 (R1) and 6:2 (R2) (Table 2).

Establishment and management. For the study of the three hybrids, they were established with a female:male furrow ratio of 4:2 (R1) and 6:2 (R2); in furrows by 0.8 meters of width, with a depth of 5 cm. Sowing was done simultaneously for G1 and G2, while for the hybrid H-386A, the male was established first and five days later the female, with 7 to 8 seeds being deposited per linear meter.

The response variables considered were: days until male flowering (MF), days until female flowering (FF), plant height (PH), ear height (EH), percentage of ear in female cobs (PE), percentage of seed in female ears (SFE), commercial seed yield (CSY), thousand seed weight (TSW), number of seeds per kilogram (S/kg), standard germination (G), and hectoliter weight of the seed (HW), which were taken based on the procedure described by ISTA (2004).

Statistical analysis. The information obtained from each of the variables studied in this study was analyzed with the SAS 9.4 software. A completely randomized design was used, and the treatments had a factorial arrangement with four repetitions, where each experimental unit consisted in furrows of 20 linear meters. Analysis of variance was carried out, and in the response variables where a significant statistical difference was observed a means comparison was carried out (Tukey P≤0.05) (SAS Institute, 2002).

Table 2. Relation of hybrids, parents, Split and sowing rate or female:male (F:M) furrow proportion.

Experimental hybrid	Parent		Split (days)	Row ratio Female:Male
H-384A (G1)	B-4A×B-6A	(Female)	Simultaneous	4:2 (P1) y 6:2 (P2)
	B-7A	(Male)		
H-385A (G2) H-386A (G3)	B-4A×B-6A	(Female)	Simultaneous more 5 days AT	4:2 (P1) y 6:2 (P2) 4:2 (P1) y 6:2 (P2)
	B-7A×B-8A	(Male)		
	B-3A×B-6A	(Female)		
	B-9A	(Male)		

(G1) genotype one, (G2) genotype two, (G3) genotype three, (R1) proportion one, (R2) proportion two and AT; on time, that is, the first sowing establishment when there is Split in days between female and male.

RESULTS AND DISCUSSION

In this study, the genotypes and proportions were mainly analyzed with regards to the variables studied. Tukey's means comparison test (Table 3) established differences in the variables studied, regarding the variation factors, although there are numerical differences between them. Concerning the effect in the yield (t ha^{-1}), genotype three (G3) exceeds G2 and G1, in 36.01 and 50.99 %, respectively, which compared to other variables that can be moved by several factors corresponding to the crop's management (fertilization, irrigation, etc.) and their statistical and numerical difference indicates specific decision making for the locality and the genotypes in study.

For the physical characteristics of the grain, it was observed (Table 3) that the 1000 seed weight TSW (g) for genotype three (G3) was 357.38 g, exceeding G1 and G2, obtaining the highest weight with ratio two (R2); in addition, in this locality the number of seeds per kilogram resulted in the G3 presenting the lowest number of seeds, which means that the seeds of this material are larger than the rest of the genotypes in study, and this did not affect an increase in female:male ratio to 6:2, that is, six furrows of female by two of male. In the analysis of variance, significant statistical differences were observed due to the ratio of male *vs*. female plants, the means test ($P \leq 0.05$) only showed difference in plant height and ear height, but did not present statistical difference in male and female flowering. Regarding the ratios, the contrary case in the genotypes where more precociousness is seen is G3, followed by G2, and the slowest in flowering being G1. This could probably be attributed to the genetic characteristics of the parents, standing out or presenting this in parent three (G3). There are key factors in the success of seed production, among them the ratio of female furrows to male furrows. In this regard, MacRobert *et al.* (2014) point out that the most common ratios are: 2:1, 3:1 and 6:2. According to this, we could reconcile

Table 3. Means comparison of the variables evaluated for genotypes and sowing ratios.

Variables	Genotypes				Proportions		
	G1	G2	G3	DMS	P1	P2	DMS
FM (Days)	69.50 a	69.00 b	63.50 c	0.00	67.33 aa	67.33 a	0.00
FF (Days)	70.00 a	69.00 b	68.00 c	0.00	69.00 aa	69.00 a	0.00
AP (m)	2.40 abb	2.28 b	2.42 aa	0.11	2.32 b	2.41 a	0.07
AM (m)	1.25 c	1.43 b	1.64 a	0.10	1.50 a	1.38 b	0.07
OLO (%)	26.03 a	18.30 b	21.08 bb	4.51	22.65 aa	20.96 a	3.03
SEM (%)	73.96 b	81.69 aa	78.91 a	4.51	77.34 a	79.03 aa	3.03
PH(Kg hL^{-1})	78.27 abb	81.20 aa	76.21 b	2.98	78.10 a	79.02 aa	2.00
PMS (g)	300.38 b	317.00 bb	357.38 a	26.86	313.33 b	336.50 a	18.05
Sem kg^{-1} (No.)	3338.13 aa	3174.38 a	2813.88 b	254.42	3207.08 a	3010.50 b	171.00
G (%)	98.87 a	98.87 aa	96.50 b	1.76	97.66 a	98.50 aa	1.18
REND (t ha^{-1})	4.45 b	5.81 bb	9.08 a	1.86	6.95 aa	5.93 a	1.25

MF: days until male flowering, FF: days until female flowering, PH: plant height, EH: ear height, PE: percentage of ear, PS: percentage of seed, CSY: commercial seed yield, TSW: thousand seed weight, S/kg: number of seeds per kilogram, G: standard germination and WS: hectoliter weight of seed. MSD: Minimum Significant Difference. Values in the same line for each source of variation, followed by the same letter, are not statistically different (Tukey, $P \leq 0.05$).

that the ratio is a determinant factor in seed production, and this is because when it is increased the quality and the seed yield is not sacrificed.

Table 4 shows the effect of the variables with regards to the genotypes and ratios. The response variables showed significant differences (P≤0.01) compared to the genotype (PE, PSE, PH, TSW, S/Kg, G, and REND), while in the ratios they only showed significance in variables TSW and S/Kg, which means that they influence the size and quality of the seed. Regarding the genotype × ratio interaction, there is significance in the percentage of the ear and the seed, as well as the seed yield, and with this combination there is a positive response. This response was similar to what was exposed by Virgen *et al.* (2014), Espinosa *et al.* (2003), Arellano *et al.* (2011). In this study, highly significant differences were observed in the parameters of seed yield and quality, the results shown agree with those reported by García *et al.* (2018), when revealing highly significant differences in physical and physiological quality.

The physiological quality can be evaluated with laboratory seed germination tests (Copeland and Mcdonald, 2001). In addition, the physical quality of the seed can be evaluated considering criteria such as moisture content, physical purity, mechanical damage, appearance, thousand seed weight, and volumetric weight, among other attributes (Tillmann *et al.*, 2003). When observing Table 5, corresponding to the means of the variables studied and their genotype × ratio interaction, as well as its effect on the seed quality and yield, it shows that there is difference between genotypes regarding the means of

Table 4. Mean squares and statistical significance of the variables analyzed.

S.V.	D.F..	Cobs (%)	Seed (%)	PH (kg hL^{-1})	TWS (g)	Seeds per kg (number)	SG (%)	Yield (t ha^{-1})
Genotype	2	122.65*	122.65*	50.16*	6874.04**	575483.16**	15.04*	45.30**
Proportion	1	17.10ns	17.10ns	5.15ns	3220.16*	231870.04*	4.16ns	6.20 ns
G*P	2	105.95*	105.95*	9.14ns	906.54ns	51800.66ns	6.54ns	25.07**
EE	18	12.50	12.50	5.46	443.36	39750.34	1.91	2.14
Mean		21.80	78.19	78.56	324.91	3108.79	98.08	6.44
CV		16.21	4.52	2.97	6.48	6.41	1.41	22.72

SV: source of variation; ns: not significant; * and ** significant values with P≤0.05 and P≤0.01, respectively.; DF: degrees of freedom; EE: Experimental error; CV: coefficient of variation; FM: days to male flowering, FF: days to female flowering, PH: plant height, TWS: thousand weight seeds, SG: standard germination.

Table 5. Means of the variables studied, corresponding to the genotype x ratio interaction, regarding the seed quality and yield.

Hybrid	Ratio Level	Cobs (%)	Seed (%)	PH (Kg hL^{-1})	TWS (g)	Seeds per kg (number)	SG (%)	Yield (t ha^{-1})
H-384A (G1)	4:2	31.07	68.92	77.09	295.0	3402	99.5	3.00
	6:2	20.98	79.01	79.46	305.7	3273	98.2	5.89
H-385A (G2)	4:2	17.14	82.85	81.96	311.5	3214	98.0	6.77
	6:2	19.46	80.54	80.43	322.5	3134	99.7	4.84
H-386A (G3)	4:2	19.73	80.26	75.24	333.5	3004	95.5	11.08
	6:2	22.44	77.56	77.18	381.2	2623	97.5	7.07

PH: plant height, TWS: thousand weight seeds, SG: standard germination.

the variables studied, although hybrid H-386A exceeds H-384A and H385A, with relation to the yield with 72.92% and 38.89%, respectively, in what corresponds to ratio 4:2, and with 16.69% and 31.54%, respectively in ratio 6:2; however, it stands out that statistically in the variables of seed quality (PE, PS, SHW, S/Kg and G), there are differences regarding the three hybrids (G1, G2 and G3), to which the numerical difference is not representative, with regards to the variables.

To cover the demand for seed requested from any seed company, it will always be necessary to determine the optimal conditions to achieve the maximum benefit. Virgen *et al.* (2016) pointed out that the loss in seed production can be attributed to the vigor of the lines, the variation of the environments, and the genotype x environment interaction, among others.

Likewise, the results observed indicate that the relationship there is between hybrid H-384A and H-385A, in relation to H-386A, can have an influence because the female parent defines or participates in both materials (B-4A×B-6A), as mentioned by Tadeo *et al.* (2004), in the variables and hybrids studied.

According to Ramírez *et al.* (2010), the environmental factors influence the growth and development of the plant. In this regard, the environment studied for seed production, Coquimatlan, Colima, shows good conditions to establish the three hybrids; however, the ratios 4:2 and 6:2 have a significant response between the materials and the variables, obtaining a better response from hybrid G3 in ratio 4:2. This is attributed to the low number of lateral primary branches (4-5), and with it the ramification of the spike from the male is lower than in the male parents of G1 and G2; however, through the mechanical dispersal of pollen the ratio 6:2 can be established to increase the yield in grain production without losing surface to be established, which is what happens when establishing four female furrows by two male furrows. Regarding this, García *et al.* (2014) mention that in their results they found that in the production of hybrid seed H-135 it is possible to use compact sowing patterns 4:1 and 8:1, and they found that the seed quality of the sowing patterns 4:1 and 8:1 is the same as that obtained by the conventional pattern 6:2, which is why in addition to taking advantage of the entire surface in the multiplication of the hybrid seed, they obtained good physical and physiological quality of the seed.

CONCLUSIONS

A large part of the success in seed depends on the production technology implemented. For this purpose, it is important to generate it, validate it, and make it reach the national seed-production companies and organizations; in this study, the behavior of the parents that participated in the yellow grain maize hybrids H-384A, H-385A and H-386A, with regards to the locality of Coquimatlan, Colima, covered the expectations of production and quality. However, when contemplating the topological arrangements or sowing ratios 4:2 and 6:2, the best ratio for seed production is R1 or 4:2; that is, four female furrows of and two male furrows, with hybrid H-386A being the one that obtained the best response. This allows making decisions regarding the establishment of the crop and quality in seed production lots, with it being a recommended environment in the influence area of the CIRPAC.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Somatic indices and nutritional composition of the roe of the native fish *Dormitator latifrons*

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ABSTRACT
Objective: To evaluate some reproductive aspects of *Dormitator latifrons* and the nutritional quality of its eggs.
Design/methods/approach: Eighty-two fish were randomly collected (August 2021), and their sex, length, weight, somatic indices, Fulton condition index (K), proximate composition, and amino acid and lipid composition (fatty acids) of the gonads were determined.
Results: Of the total specimens collected, 62% were females; length and weight values were higher in males, but their gonadosomatic index (GSI) was lower than in females. Somatic indices did not show differences between different weight ranges. In the roe of *D. latifrons*, the average proximate composition was 24.3% protein and 8.5% lipids. The most abundant essential amino acids were leucine and lysine. Linoleic acid (C18:2n6) was the fatty acid with the highest concentration.
Limitations/implications: It is necessary to complement the analysis of the amino acid and fatty acid profile of the roe in wild organisms to relate the changes caused by balanced feed.
Findings/conclusions: This study shows that the *D. latifrons* roe is a good source of amino acids and PUFA.

Keywords: proximate composition, fatty acids, essential amino acids.

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INTRODUCTION

Fish by-products have become increasingly important worldwide due to their nutritional, economic, and environmental benefits (Chakraborty *et al.*, 2020). Mexico is home to species with great economic potential that have not yet been exploited, including the species *Dormitator latifrons*, which is only consumed in some locations in the southeast of the country, despite its delicious flavor and excellent nutritional properties. (López-Huerta *et al.*, 2018). Food products with high nutritional value and acceptability could be produced from this species (Montoya-Martínez *et al.*, 2022). Moreover, the characteristics of this fish make it ideal for farming, even though there is still little information on this species (Vega-Villasante *et al.*, 2021).

Although roe is considered a high-quality food, due to its nutritional value and importance in the international market, little information is available on its chemical composition, quality, and safety (Vasconi *et al.*, 2020), especially for freshwater species.

The present study aimed to determine the somatic indices, proximate composition, and amino acids and fatty acids profile of the roe of *D. latifrons* to expand our knowledge about the reproductive performance of the species and the nutritional quality of its roe.

METHODS

Sample preparation

Adult *D. latifrons* fish were collected in August 2021 from the ponds of a shrimp farm (*D. latifrons* is considered a pest in shrimp farming) in the municipality of San Blas, in the state of Nayarit. The collected fish were killed by immersion in ice water and then frozen at −20 °C. The fish were transported in coolers to the Laboratorio de Calidad de Agua y Acuicultura Experimental of the Universidad de Guadalajara in Puerto Vallarta, Jalisco, where they were kept at −20 °C for further processing.

The specimens were thawed in a refrigerator at 5 °C for 17 h. Once thawed, the length and weight of each specimen were determined, and the Fulton condition index (K)=(total weight/length3)×100 (Ricker, 1975) was calculated. The fish were then dissected and eviscerated. The liver and gonads were separated and weighed to determine the gonadosomatic index (GSI)=(gonad weight/total weight)×100, the hepatosomatic index (HSI)=(liver weight/total weight)×100 and the viscerosomatic index (VSI)=(viscera weight/total weight)×100. The 82 collected specimens were grouped into three weight ranges (200-299, 300-399, 400-499).

The female gonads (roe) were cleaned of foreign material such as scales, blood, etc. Their surface was manually washed with tap water and rinsed with drinking water, and they were then frozen at −20 °C for later lyophilization.

Proximate analysis

The roes of 6 fish were used for the proximate analysis. The roe was first lyophilized; once dry, it was ground, mixed, and divided into two groups of 3 gonads each. Moisture was determined from weight loss during lyophilization. All analyzes were performed using the AOAC (1995) method. Crude protein was determined by the micro Kjeldahl method, using a protein conversion factor of 6.25. Lipids were determined by the Soxhlet method, using hexane as solvent. The ashes were determined by the calcination method at 550 °C for 4 h until reaching constant weight. All analyzes were performed in triplicate.

Determination of amino acids and fatty acids

For the analysis of amino acids and fatty acids, the lyophilized samples were sent to the Laboratorio de Nutrición Acuícola of the Universidad Autónoma de Baja California. The content of amino acids was determined on 100 mg of material taken from previously defatted and dried samples. The samples were hydrolyzed with a mixture of 6 N HCl and phenol (0.06%) and refrigerated at −30 °C until further processing. Derivatization was performed on an Agilent HPLC (1200 infinity series). The calibration curve was generated using a standard amino acid solution (P.N. 061-3330). The area under the curve was estimated using the "OpenLAB" program (Agilent Technologies 2000) to determine the proportion of amino acids to the protein content in the samples.

The content of fatty acids (FA) was determined using the technique of Folch *et al.* (1957) with modifications. Butylhydroxytoluene 0.01% ($C_{15}H_{24}O$) was added as the antioxidant solution, using the lowest possible temperature for lipid extraction. The FA were separated, identified, and quantified on an AGILENT GC 7820A gas chromatograph, equipped with a Split/Splitless injector, a flame ionization detector, and an AGILENT 122-2361 capillary column (DB-23, 60 m × 0.25 mm with an internal diameter of 15 mm). Nitrogen (N_2) was used as carrier gas. Fatty acids were identified by comparison with the retention times of the following standards: 37 Component FAME Mix (Supelco/Sigma-Aldrich®), GLC 87, GLC 96 (Nu-Chek Prep®), RM-2, RM-6, and GLC 90 (Supelco/Sigma-Aldrich®). In addition, polyunsaturated fatty acids (PUFAs) from marine oils (PUFA1 and 3, Supelco/Sigma-Aldrich®) were used as an identification pattern. Nonadecanoic acid (C19:0) was used as an internal standard. The composition of each FA was estimated based on the corresponding area in the respective chromatogram. The calculations were made using the GC Chemstation Data Analysis software and expressed as the weight percentage of the total lipids in the roe of *D. latifrons*. All analyzes were performed in duplicate.

The protein quality of the gonads was evaluated using the chemical score method (CS) (Block & Mitchell, 1946) by dividing the EAA content of the evaluated protein by the amino acid content of a reference protein (mg/g protein) defined according to the amino acid requirements for children (2-3 years) established by the U.S. Food and Nutrition Council (FNB/USA, 2002 in Hernández-Triana, 2004).

Statistical analysis

The Shapiro-Wilk normality test and Bartlett's homogeneity of variance test (α=0.05) were applied to the results of weight, length, and somatic index. Compared were made with the one-way analysis of variance (ANOVA) (weight, length, and k index in sexes) or the Kruskal-Wallis (somatic indexes, weight, and length in different weight intervals). Statistically significant differences ($P<0.05$) between treatments were determined using Tukey's test. All statistical analyzes were performed using SigmaPlot 11.

RESULTS AND DISCUSSION

The sex distribution of the 82 collected specimens was 62% (51) females and 38% (31) males. The males were significantly larger in both length and weight, but their GSI was lower than that of females ($P<0.05$) (Table 1). In fish, the Fulton condition index (K) is used as an indirect estimate of the state of robustness (Urquidez *et al.*, 2016) or physiological and reproductive condition (Arellano-Martínez *et al.*, 2001). For this species, Chang & Navas (1984) report K variations during the year, finding the maximum peaks (approx. 1.8) during the rainy season. The average K value (Table 2) was 2.3±0.3, which means that

D. latifrons has high K values since this index, although it varies according to the species, is close to 1.0 in marine fish (Urquidez *et al*., 2016). Cifuentes *et al*. (2012) reported the condition indices of 12 species of native fish from Chile during two annual cycles, with values ranging from 0.1 to 1.3. They found the most significant variability (from 0.1 to 2.6) in *Percilia gillissi*, probably associated with the reproductive seasons. In most fish, the highest condition index values coincide with the time of sexual maturity, and the lowest values with weight loss after spawning. This difference is the reason for the high value of K found in the present study in *D. latifrons* since most of the organisms analyzed were between stage V (gravid) and VI (spawning) of the sexual maturity scale based on empirical data on gonadal development (Vega-Villasante *et al*., 2021). This result coincides with the reports from Mexico that the reproductive phase of this species lasts between June and November (Rojas-Herrera *et al*., 2009).

The somatic indices did not show significant statistical differences (P>0.05) between different weight intervals (Table 2). Chang & Navas (1984) report for *D. latifrons* the maximum GSI values between March and April (approx. 17.5%) during the rainy season in Ecuador because fish present gonadal development in this period. Despite the variability observed in the K index, the highest values were found in the maturation period, which shows a certain tendency to coincide with the GSI. Rojas-Herrera *et al*. (2009) reported a seasonal variation in the GSI of *D. latifrons*, with a maximum value in June (approx. 8.2%) and 4.8% in August. The GSI values obtained in the present study were higher than 9% in August. These high GSI values correspond to the state of maturity in which the fish gonads were found since it has been reported that this index tends to increase with increasing gonad size as the gametogenesis process progresses, which is why this index is considered an indicator of sexual maturity in fish (Re-Vega *et al*., 2020). The HSI is considered a good indicator of the physiological condition since it is associated with the energy storage in the

Table 1. Total length, body weight, and gonadosomatic index (GSI) and Fulton condition index (K) of females and males of *D. latifrons* were used in this research.

Organisms	Number of fish	Length (cm)	Weight (g)	GSI	K
Total	82	24.6±1.6	346.2±68.0	10.61±4.9	2.3±0.3
Females	51	24.1±1.4[a]	331.1±58.8[a]	13.59±3.5a	2.3±0.3
Males	31	25.4±1.6[b]	371.1±75.6[b]	5.72±2.3b	2.3±0.4

Table 2. Weight, length, and somatic index values of *D. latifrons* according to weight range.

Variables	Weight range (g)		
	200-299 (n=27)	300-399 (n=34)	400-499 (n=21)
Weight (g)	272.7±24.0 [a]	349.5±32.2 [b]	435.4±25.0 [c]
Length (cm)	23.1±0.9 [a]	25.0±1.3 [b]	26.0±1.0 [c]
Gonadosomatic index	12.3±4.9	10.3±4.7	9.0±5.1
Hepatosomatic index	4.2±1.5	4.4±1.1	4.1±1.1
Viscerosomatic index	22.5±6.2	21.5±4.2	19.5±5.2

liver, which decreases during vitellogenesis and spawning (Arellano *et al*., 2001); however, these values may vary with the species. The HIS values found in the present study for *D. latifrons* are probably low, but no published data was used as a reference. Higher GSI values were found in the present study than those reported in other species. This GSI value is probably associated with the morphology of this fish, which has a wide abdominal cavity since the females produce a large amount of roe (they are very fertile). This roe has excellent commercial potential and an enjoyable flavor and texture.

Table 3 shows the proximate composition of the roe of *D. latifrons*, with an average protein content of 24.3% and an average lipid content of 8.5%, higher values than what has been reported for this fish meat (Montoya-Martínez *et al*., 2022). Balaswamy *et al*. (2009) evaluated the proximate composition of four freshwater species (*Catla catla* (catla), *Cyprinus carpi* (carp), *Labeo rohita* (rohu), and *Channa striatus* (murrel). They found that the roe protein content of these species ranged from 16.6 to 28.2%, while the lipid content ranged from 3.2 to 9.5%. Iwasaki & Harada (1985) analyzed the proximate content of the gonads of 14 species of marine fish, finding that the protein content ranged from 11.5 to 28.7% and the lipid content ranged from 3.0 to 19.8%.

Table 3 shows the amino acid composition of the roe of *D. latifrons*. The most abundant non-essential amino acid was GLU, followed by ASP and the essential amino acids LYS and LEU. The least abundant amino acid was the essential amino acid MET. The ratio between essential and non-essential amino acids (E/NE) in the roe protein was 0.9. These results coincided with what Iwasaki and Harada (1985) reported for gonads of 14 marine species. They found that GLU was the most abundant amino acid, followed by LEU or ASP, while the least abundant amino acids were MET and HIS, with an average E/NE that ranged from 0.71 to 0.75. As these authors indicated, a favorable and balanced E/NE ratio suggests that the roe of *D. latifrons* can be considered a food source of high-quality protein.

The fatty acid analysis of the roe of *D. latifrons* (Table 3) showed that 34.2% of the total fatty acids (FA) were polyunsaturated (PUFA), and 34.0% were saturated (SFA), and 30.7% were monounsaturated (MUFA). Linoleic acid (LA, C18:2n6) was the fatty acid with the highest concentration (23.6%), while eicosatetraenoic acid (ETA, C20:4n3) was the least abundant (0.6%), and the n-3/n-6 ratio was 0.4. This coincided with what López-Huerta *et al*. (2018) reported for the muscle of farmed *D. latifrons*, where LA was the most abundant fatty acid (20.5%), but the n3/n6 ratio was 0.85. These authors found differences between the concentrations of FA in wild and farmed fish, which may be related to the type of oil used in the formulation of the balanced feed since increasing the content of vegetable oils in the feed decreases the content of long-chain n-3 fatty acids in the cultivated fish, thereby reducing the nutritional quality of the fish meat.

In the roe of *D. latifrons* (Table 3), the most abundant SFA was palmitic acid (C16:0, 18.0%). In contrast, the most abundant MUFAs was oleic acid (OA, C18:1n9, 21.1%), similar to what has been reported for the roe of other freshwater species such as *Coregonus albula*, *Rutilus frisii kutum*, and *Silurus glanis* (Kaitaranta, 1980; Ghomi & Nikoo, 2010; Saliu *et al*., 2017), and in some marine species (Rincón-Cervera *et al*., 2009; Garaffo *et al*., 2011). The PUFA/SFA ratio was higher in the roe of *D. latifrons* than the minimum value (>0.45)

Table 3. Proximate composition and amino acid and fatty acid profile of *D. latifrons*.

Proximate analysis (%)		**Fatty acids (%)**	
Moisture	61.9±2.5	C13:0	8.4±0.7
Proteins	24.3±1.1	C14:0	1.4±0.2
Lipids	8.5±0.3	C16:0	18.0±0.9
Ash	3.5±0.4	C18:0	5.8±0.1
Amino acids (g/100g protein)		C24:0	0.8±0.2
Essential amino acids		**∑SFA**	**34.0±0.8**
HIS	2.2±0.1	C16:1n7	5.1±0.2
ILE	3.7±0.1	C18:1n9	21.1±1.2
LEU	5.8±0.1	C18:1n7	3.2±0.4
LYS	5.8±0.3	C22:1n9	1.5±0.4
MET	1.4±0.7	**∑MUFAS**	**30.7±1.3**
THR	3.5±0.1	C18:2n6	23.6±0.4
VAL	4.3±0.1	C18:3n3	1.7±0.0
PHE	3.0±0.1	C20:2	0.7±0.1
Non-essential amino acids		C20:4n3	0.6±0.0
ALA	4.4±0.2	C20:5n3	1.0±0.0
ARG	4.8±0.1	C22:5n3	2.4±0.2
ASP	5.8±0.1	C22:6n3	4.2±0.5
GLU	8.9±0.1	**∑PUFAS**	**34.2±1.2**
GLY	2.5±0.1		
SER	4.4±0.1	**n3/n6**	0.4±0.0
TYR	3.0±0.1	**DHA/EPA**	4.1±0.4
E/NE	0.9	**PUFA/SFA**	1.0±0.0

recommended for human consumption (Saliu *et al.*, 2017). The content of FA n-3 in the roe of *D. latifrons* was lower than the content of n-6 because LA (C18:2n6) was the most abundant FA (23.6 %) in the roe of *D. latifrons* (Table 3). This is like what was reported for *Gadus morhua* (Rincón-Cervera *et al.*, 2009), in which LA was the most abundant PUFA. However, these results differ from most of the results reported for other species, in which FA n-3 was the most abundant. The content of docosahexaenoic acid (DHA, C22:6n3) in the roe of *D. latifrons* was higher than that of the content of eicosapentaenoic acid (EPA, C20:5n3). The content values of these fatty acids were lower than those reported for other species (Kaitaranta, 1980; Rincón-Cervera *et al.*, 2009; Ghomi & Nikoo, 2010; Garaffo *et al.*, 2011; Saliu *et al.*, 2017). Lipid levels and FA composition vary according to species, sex, age, the season of the year, food availability, salinity, and water temperature (Vlieg & Body, 1988). The content values of the amino acid MET and FA in the roe of *D. latifrons* are mainly the result of the feed used in the shrimp farms where the fish were found since the content of oils of vegetable origin in the feed used for cultivation can lead to an increase in FA n-6, mainly of LA (Vasconi *et al.*, 2020).

Bastos-Rosales *et al.* (2020) and López-Huerta *et al.* (2018) reported the content of amino acids and fatty acids in the meat of *D. latifrons* in the wild and farmed specimens fed with a commercial feed for tilapia. They found that the only limiting amino acid was lysine, but only in wild fish. When comparing the FA content, they found a higher concentration of OA and LA and a lower proportion of EPA and DHA in farmed fish compared to wild fish, so they recommended paying attention to the quality of the oil used in the feed of farmed fish.

The consumption of fish products and by-products, of all kinds without exception, is widely recommended because they contain less SFA and cholesterol than other meat products (Acuña-Reyes, 2013). Furthermore, fish products are rich sources of amino acids and essential fatty acids that are beneficial for human health (Garaffo *et al.*, 2011), such as PUFAs of the n-3 family. Even when farmed lean and semi-lean fish, as well as their by-products, do not have sufficient amounts of n-3, they have a high content of OA and LA considered of high nutritional value (Garaffo *et al.*, 2011).

Even though MET is the limiting amino acid in the roe of *D. latifrons*, the roe was not deficient in the other analyzed amino acids (Table 4). The amino acid content of the roe of *D. latifrons* meets the essential amino acid requirements for children, according to the FNB/USA (2002).

Table 4. Chemical score (CS) of *D. latifrons* roe.

Essential amino acids	Children 1-3 years old	CS
Histidine	18	1.22
Isoleucine	25	1.48
Leucine	51	1.14
Lysine	55	1.05
Methionine/Cysteine	**25**	**0.56***
Phenylalanine/Tyrosine	47	1.28
Threonine	27	1.30
Valine	32	1.34

Data from the US Food and Nutrition Board (FNB/USA, 2002). *Limiting amino acids.

CONCLUSIONS

Mexico has a long tradition of eating fish roe, especially in coastal and riverside areas, where roe is considered a delicacy, as is the case of the fish roe of the same species, *D. maculatus*, which is widely consumed in the states of Veracruz and Tabasco, reaching a price of $140 per kilo of fresh roe. This study presents the first report on the composition of *D. latifrons* roe, showing that they are a good source of amino acids and PUFAs so that they could be used as food supplements. Having ample sources of cheap, fresh *D. latifrons* roe, which can be processed and used as raw material in the production of various food products to improve its protein value and help combat malnutrition, should be considered an important objective. Moreover, using the roe would improve the commercial potential of the native fish *D. latifrons*.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Characterization of the understory associated with a pine-oak forest in the Sierra Madre de Chiapas region

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ABSTRACT

Objective: To characterize the understory species associated with a pine-oak forest in the Sierra Mariscal region, Chiapas.

Design/methodology/approach: Six 40 m^2 subplots were established to evaluate the cover, abundance and identity of shrub, herbaceous and sapling components of the understory. Floristic diversity was determined. The level of disturbance was evaluated using the observation method. An analysis of variance and comparison of means by Tukey ($p<0.05$) between structural variables was applied. The relationship between disturbance levels and study sites was performed by correspondence analysis.

Results: The understory is composed of five tree species, three herbaceous and two shrub species. The largest diameter ($p \leq 0.0001$ and $F=27.6$) corresponds to herbaceous *Cortaderia selloana* (5.38 ± 0.94 cm) and *Pteridium aquilinum* (4.5 ± 0.96 cm). The greatest height corresponds to *Quercus* sp. saplings (126.3 ± 75.9 cm) and the herbaceous *Cenchrus* sp. (110.2 ± 54.1 cm) and *Pteridium aquilinum* (91.7 ± 40.9 cm), the latter having the highest density (4050 ind. ha^{-1}) and cover (16.2%). Floristic diversity was low in all six sites. Site six was the most diverse and site three the most disturbed.

Study limitations/implications: The study comprised a limited and insufficient area to generalize the conditions of pine-oak forests in the Chiapas highlands. It is suggested to expand the study universe and increase the number of replications.

Findings/conclusions: Variability in understory structure and composition was found that corroborates the relationship between forest diversity and disturbance.

Keywords: understory, saplings, floristic diversity, disturbance.

Image by Gaby Stein at Pixabay

INTRODUCTION

Forests make up a wide range of terrestrial ecosystems, where trees are the dominant structural element (Chazdon *et al.*, 2016). The state or condition of the forest can be described by three characteristics: 1) spatial position or distribution, 2) species diversity and mix, and 3) the arrangement of vertical and horizontal differentiation (Castellanos-Bolaños *et al.*, 2008). The role played by the understory, made up of shrubs, herbaceous, grasses, climber plants, lianas and other low vegetation, also favors the dynamics and stability of forest ecosystems (Nakhoul *et al.*, 2020).

The understory is spatially distributed in a stratum below the canopy and subcanopy, being a key component in the functioning of forests with effects on wildlife (Echiverri and Mcdonald, 2019). This vegetative stratum provides habitat for wildlife and contributes to nutrient cycling, intervening in the maintenance and productive capacity of forests under management (Ampoorter *et al.*, 2014; Davis & Puettmann, 2009), which favors forest productivity and tree regeneration (Landyut *et al.*, 2019). In addition, the understory acts as an important buffer in reducing carbon emissions and mitigating climate change (Jin *et al.*, 2022).

Unfortunately, traditional forest management practices continue to encourage the removal of understory species to minimize competition for resources between upper canopy trees and the understory (Giuggiola *et al.*, 2018). Indeed, soil biochemical processes such as microbial activity, community composition, carbon sequestration and nutrient turnover rate have been affected (Trentini *et al.*, 2018; Fang *et al.*, 2021). Significant alterations in the understory have also been brought about by human disturbances and global changes, such as warming, nitrogen deposition, and changes in precipitation (Chen *et al.*, 2023). All this has an impact on the loss of productivity and stability of the forests (Zhang *et al.*, 2022). Given the importance of the understory as an essential component of forests, it is necessary to implement diagnoses that integrate the structural elements, composition, diversity and abundance of species that make up this vegetative stratum. This allows an approximation of the current state and condition of the forest and the possible variability between zones that show some type of disturbance. Based on the above, the objective of this study was to characterize the understory species (shrubs, herbaceous and regeneration) associated in a pine-oak forest in the Sierra Mariscal region, Chiapas, taking into account elements such as density, cover, diversity and the influence of the levels of disturbance present in the ecosystem studied.

MATERIALS AND METHODS

The present study was conducted in a pine-oak forest in the municipality of El Porvenir, in the Sierra Mariscal de Chiapas, Mexico. The study area is located between the coordinates 15° 45’ 11” LN and 92° 24’ 75” LO, at an average altitude of 2840 m above sea level and a total area of 4 ha (Figure 1).

Sampling and site distribution

A random site sampling design was established in which six circular sites were established with an area of 400 m^2 for each site in which the tree, canopy and subcanopy

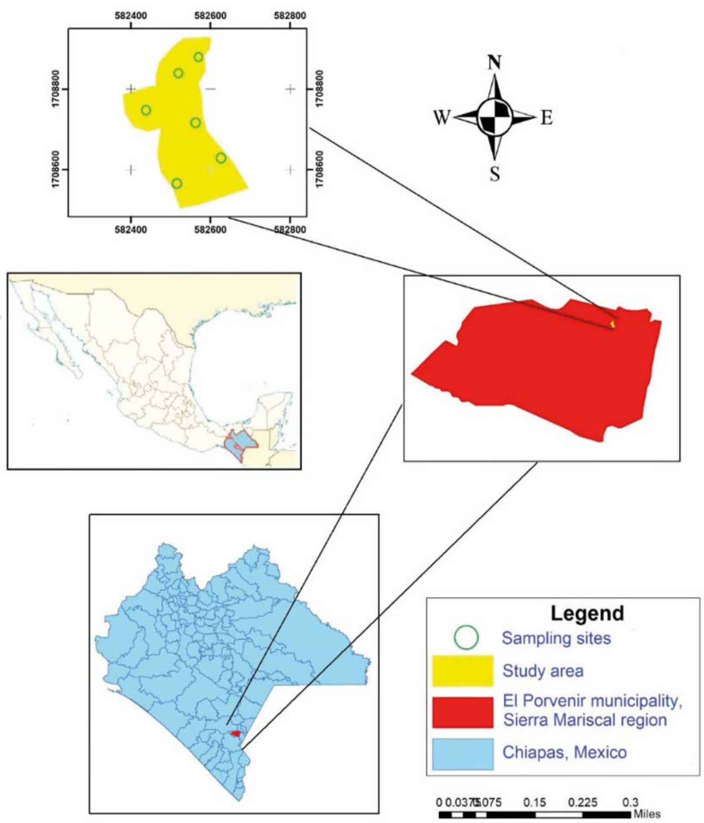

Figure 1. Geographic location of the study area and distribution of sites.

were evaluated, the results of which correspond to another work. For the present study, six concentric circular sub-sites were defined (within each site). In each circular subsite a central point was taken, and from this, four ropes with a radius of 3.57 m were established to obtain the desired surface of 40 m^2 (0.004 ha).

Characterization of the understory

The characterization of the understory includes shrubs, herbaceous and saplings (saplings with DN $<$2.5 cm and height $\leq$1.3 m), the latter according to the criteria used by López-Toledo *et al.* (2012). An inventory of the understory species was conducted, evaluating structural parameters such as density (ind. ha^{-1}), normal diameter (DN) and average height by species. In each subsite, four 1 m^2 squares were established to average the percentage of cover per species, according to the indicators of Westhoff and Van der Maarel (1978) as shown in Table 1.

Floristic diversity by sites was determined using the Shannon, Simpson and Hill Equity indices (Moreno, 2001) with the help of Past software version 3.2 (Hammer *et al.*, 2009).

The level of disturbance was evaluated using the observation method, based on the criteria established by García *et al.* (2016). For this, three criteria were established: i) not disturbed, ii) semi-disturbed and iii) disturbed, considering as undisturbed that area that resulted in little or almost no alteration (less than 5%), semi-disturbed when the area presents

Table 1. Scale used to calculate percent cover in herbaceous and shrub vegetation (adapted from Westhoff and Van der Maarel, 1978).

Value	Percentage of coverage
9	More than 75% of the total
8	Between 50 and 75% (62.5%)
7	Between 25 and 50% (37.5%)
6	Between 12.5 and 25% (18.75%)
5	Between 5 and 12.5% (8.75%)
4	Less than 5%, but more than 10 individuals, too many to count
3	Less than 5%, more than 10 individuals, and can be counted
2	Less than 5%, between 3 and 10 individuals
1	Less than 5%, 1 to 3 individuals

close to a 50% of affectations produced by man and disturbed when high anthropogenic influence is appreciated (more than 80%).

Statistical analyses were performed with jmp pro 14 software (Statistical Analysis System [SAS], 2019). The results obtained were analyzed by analysis of variance (ANOVA) and comparison of means by Tukey ($p \leq 0.05$) between the structural variables (normal diameter and height) evaluated in the study sites. A correspondence analysis was performed to determine the relationship between the nominal variables (degree of anthropization) and the sites. The dimensionality of the solution was determined from the minimum ([rows, columns] -1). For this, the statistical package SPSS version 23.0 was used.

RESULTS AND DISCUSSION

The understory vegetation consists mainly of five tree species, three herbaceous and two shrub species. The herbaceous stratum is composed of the species *Pteridium aquilinum* with a density of 4050 ind ha^{-1}, *Cenchrus* sp. with 2500 ind ha−1 and *Cortaderia selloana* with an average of 400 ind ha^{-1}. The shrubs found were *Baccharis* with a density of 3650 ind ha^{-1} and *Rubus ulmifolius* with an average of 250 ind ha^{-1}. The regeneration is made up of saplings with a diameter $<$2.5 cm of the species *Pinus ayacahuite* (550 ind ha^{-1}), *Pinus maximinoi* (300 ind. ha^{-1}), *Quercus* sp. (250 ind ha^{-1}), *Prunus serotina* (100 ind ha^{-1}) and *Cupressus lindley* (100 ind. ha^{-1}).

The diameter of these species shows significant differences ($p \leq 0.0001$ and $F=27.6$), whose highest mean value corresponds to the herbaceous *Cortaderia selloana* (5.38±0.94 cm) and *Pteridium aquilinum* (4.5±0.96 cm) and the lowest mean value to *Cenchurus* sp. (1.06±0.22 cm) (Table 2).

With respect to height, the regeneration of *Quercus* sp. (126.3±75.9 cm) and the herbaceous *Cenchrus* sp. (110.2±54.1 cm) and *Pteridium aquilinum* (91.7±40.9 cm) show the highest average values. The density of understory species is 8350 ind ha^{-1}; the highest number of individuals belongs to *Pteridium aquilinum* (4050 ind ha^{-1}) with a coverage of 16.2%. This density of understory species determines the dynamics of the

Table 2. Main tree, herbaceous and shrub species forming the understory in the study area.

Species	Common name	Biological form	ND (cm±S.D.)	H (cm±D.E.)	D (ind ha^{-1})	Co (%)
Quercus sp.	Tulán, roble	tree	1.26±0.65bc	126.3±75.9a	150	0.6%
Pinus ayacahuite	Pino tabla	tree	1.62±0.33bc	64.3±36.9ab	550	2.2%
Pinus maximinoi	Pino ocote	tree	1.18±0.94bc	95.8±91.88ab	300	1.2%
Prunus serotina	Capulín blanco	tree	0.5±0.14bc	22±5.6b	100	0.4%
Cupressus lindley	Ciprés nuculpat	tree	0.45±0.07bc	18.5±4.9b	100	0.4%
Pteridium aquilinum	helecho, chipe	herbaceous	4.5±0.96a	91.7±40.9a	4050	16.2%
Cortaderia selloana	paja	herbaceous	5.38±0.94a	87.2±31.1ab	400	1.6%
Baccharis sp.	escobillo	bush	2.43±0.27b	78.3±39.5ab	3650	15%
Cenchrus sp.	mozote	herbaceous	1.06±0.22c	110.2±54.1a	2500	10%
Rubus ulmifolius	mora	bush	0.68±0.22bc	41.2±21.1ab	250	1.2%
Total					8350	48.8%

ND: normal diameter, H: height, D: density, Co: cover. Different letters show significant statistical differences ($p \leq 0.05$).

overstory (Nakhoul *et al.*, 2020) and can be influenced by the structural arrangement of the dominant canopy, which controls the availability of light, water and soil nutrients (Barbier *et al.*, 2008) in the study area. In the understory of the evaluated community, the predominant life form is herbaceous, followed by shrubs and trees, a result that supports the record of Mejía *et al.* (2018) for the understory in dense pine and oyamel forest in Nevado de Toluca, Mexico, where the shrub stratum was the richest. The authors refer that it is possible that species richness in the herbaceous stratum is determined by multiple factors, such as slope or orientation, temperature, precipitation, solar radiation, among others.

Floristic diversity was low for all study sites (Table 3), due to anthropogenic actions. Site six was presented as the most diverse area, expressed through the Shannon index, with higher equitability (Equity) and lower dominance (Simpson). These results correspond with what was reported by Valdés *et al.* (2014) and García *et al.* (2016) for pine forests, attributing the low diversity to the influence of anthropization and ecosystem type. According to Alvis Gordo (2009), natural tropical forests are heterogeneous and are made up of a high diversity of species, with different successional ages: sapling, latizal and fustal. In the study areas this does not correspond, first, to the nature of the pine forest where few species are abundant and, second, to the disturbances that limit and interrupt the successional stages of the forest.

Table 3. Values of floristic diversity in the understory sites in the study area.

Indexes	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Shannon (H')	1.00	1.42	1.39	1.47	1.46	1.59
Equity (J')	0.72	0.79	0.71	0.79	0.79	0.82
Simpson (D)	0.43	0.29	0.31	0.34	0.34	0.28

Disturbance levels

Correspondence analysis showed a significant correlation ($p \leq 0.05$) between study sites and disturbance levels (Table 4) with total inertia values of 1.746. The solution indicated that only the first axis is significant with an inertia ratio that explained 53.6% of the total variance. This demonstrates the degree of deterioration of the understory where inappropriate management practices are carried out.

The permuted correspondence analysis allowed the formation of three groups, depending on the disturbance levels (Figure 2). Sites 1, 2 and 6 were in the undisturbed category, sites 4 and 5 semi-disturbed and site 3 disturbed, considered the most vulnerable to disturbances, which demonstrates the effect of disturbances associated with clear-cutting without management criteria and grazing in this study site.

Although three of the six sites evaluated are associated with the conserved level, inadequate management practices such as grazing, logging and opening of trails were observed in these sites, which can change the condition of the pine-oak understory in

Table 4. Correspondence analysis between disturbance levels and study sites.

Dimension	Own value	Inertia	Chi-square	Sig.	Inertia ratio		Confidence	
					Explained	Accumulated	Standard deviation	Correlation
1	0.97	0.94			0.54	0.54	0.02	0.17
	0.90	0.81			0.46	1.00	0.03	
Total		1.75	420.89	0.00[a]	1.00	1.00		

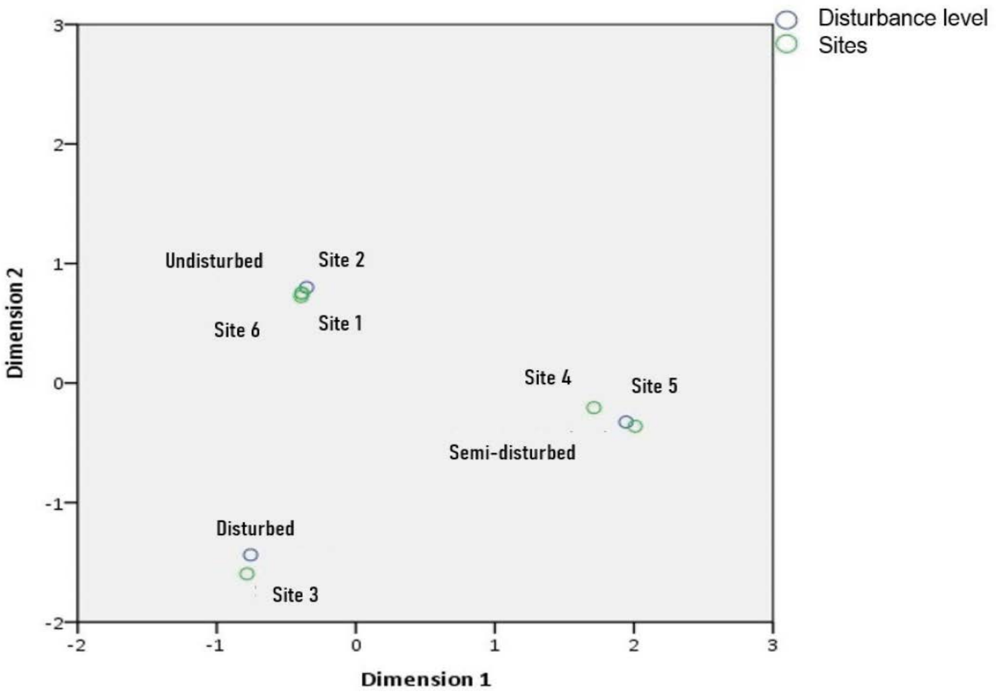

Figure 2. Two-dimensional representation of the relationship between disturbance levels and sites.

a short period of time. Disturbance of the vegetation caused by anthropogenic factors significantly alters the communities (De la O-Toris *et al.*, 2012). In this regard, Ruiz *et al.* (2022) assert that any change in the historical disturbance regime of an ecosystem can alter species composition by decreasing the importance of native species, creating opportunities for the entry of exotic species, or both.

CONCLUSIONS

Given the lack of works oriented to evaluate the structure, composition and diversity of understory species in the Pine-Oak forests of the Sierra de Chiapas, the analysis of the state and condition of the forests allowed us to identify the configuration of structural parameters and species dominance. Statistical differences were found in the height and normal diameter of understory species, with variation in the mean of the data obtained. Variability was found in the structure and composition of the understory, which demonstrates the relationship between diversity and forest disturbance, linked to logging without management criteria and grazing in the most disturbed sites. The results suggest management, conservation and restoration tasks in the evaluated forest ecosystem.

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AGRO PRODUCTIVIDAD

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Coverage changes of pine trees at risk in collection sites in northern Mexico

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ABSTRACT

Objective: To analyze the changes in land-use and vegetation (LUV), from 1985 to 2014, in the sites in northern Mexico where 16 pine trees listed in the NOM-059-SEMARNAT-2010 are collected.

Design/Methodology/Approach: Based on consultations in botanical collections, a georeferenced database of the species under study was developed. A point coverage was created to which LUV vector information from Series I (1985) and VI (2014) was added. Subsequently, LUV class changes were identified during the evaluation period through spatial analysis and geoprocessing.

Results: A total of 2,242 georeferenced records of pine species were obtained. Fifty percent of the pine records consulted in collections with Series I information belong to other non-primary forest categories of LUV, while with Series VI information with this condition rose to 58.7%. Consequently, in the study period major changes occurred in the conversion of primary forests to agricultural lands, pasture, secondary vegetation in coniferous and oak forests, and mainly urban areas.

Limitations/Implications: The availability of larger-scale cartographic material limited the generation of a risk analysis study about the changes and threats to forest conservation.

Findings/Conclusions: The number of collection sites registered in coniferous forest, scrubland, and primary oak forest in Series I —which are now agricultural lands, water bodies, pastures, and urban areas— was significantly lower than in Series VI.

Keywords: Temperate forests, botanical collection, forest ecosystems, threatened species, *Pinus* spp.

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INTRODUCTION

Forest ecosystems cover 70.5% of the national territory; 10.7% of the said territory is made up of pine and mixed forests (CONAFOR, 2019). The forests found within the Mexican territory are home to 94 species that belong to the six conifer families recognized worldwide; 43 of these species are endemic and 18 can only be found in three or fewer states (Gernandt and Pérez, 2014).

Image by Zdeněk Chalupský at Pixabay

Mexico is one of the three countries considered as a secondary diversification center of the genus *Pinus*, since 49 out of the 111 species currently existing on the planet can be found in its territory (CONABIO, 2020; Gernandt and Pérez, 2014; Sánchez, 2008). According to NOM-056-SEMARNAT-2010, some of these pine species are classified within a level of risk (special concern, threatened, or endangered), because their distribution and habitat quality are considered to be limited. Given its biological condition, its population is highly fragile in the face of anthropic activities and impacts (Aguirre and Duivenvoorden, 2010; SEMARNAT, 2010). According to data from CONAFOR (2020), the deforestation of the national territory reached 166,337 ha in 2018, which suggests the need to identify the areas where the species at risk are distributed, in order to determine their vulnerability to vegetation changes. In practice, the evolution of the natural geographic distribution of the pine species at risk in Mexico has not received any attention (if any at all). The objective of this study is to determine the changes in land-use and vegetation (LUV), from 1985 to 2014, in the sites in northern Mexico where 16 pine trees listed in the NOM-059-SEMARNAT-2010 are collected. The research hypothesis is that the geographic distribution of the species has at least remained constant during the evaluation period.

MATERIALS AND METHODS

Description of the study area

The study area corresponds to the north of Mexico. It is made up of the states of Baja California, Baja California Sur, Sonora, Chihuahua, Coahuila, Nuevo León, Tamaulipas, San Luis Potosí, Zacatecas, Aguascalientes, Durango, Sinaloa, and Nayarit (Figure 1). From a geographical point of view, the scope of the work included more than the northern half of the Mexican territory.

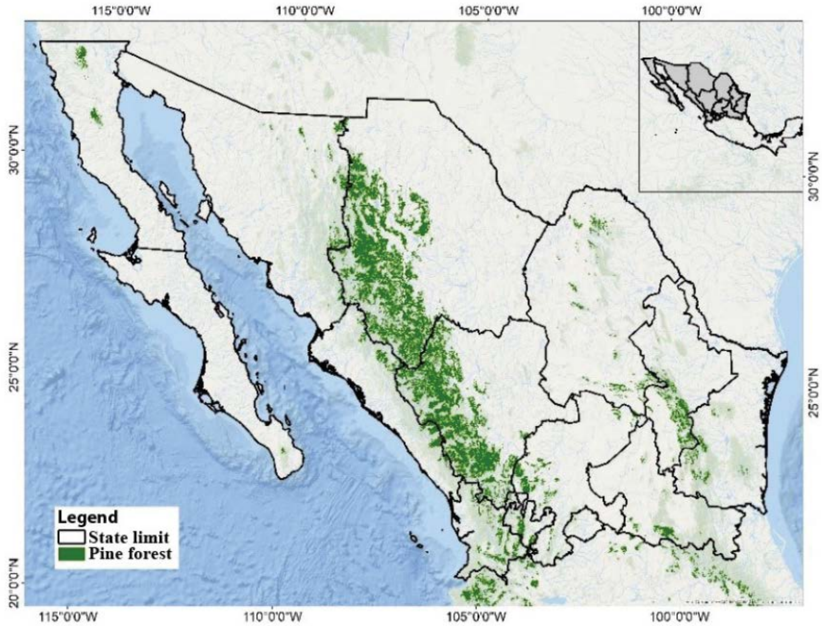


Figure 1. Location of the study area (Source: INEGI, 2016a).

Record of pines at risk

This research focuses on the pines that grow naturally in northern Mexico and are in a level of risk status, according to the Official Mexican Standard NOM-059-Semarnat-2010 (SEMARNAT, 2010). The study focused on the following 16 species: *Pinus Attenuate* Lemmon, *P. contorta* Douglas ex Loudon var. *murrayana* (Balf.) Engelm, *P. coulteri* D. Don, *P. culminicola* Andresen et Beaman, *P. jeffreyi* Greville & Balf., *P. johannis* M. F. Rob.-Pass., *P. lagunae* (Rob.-Pass.) Passini., *P. martinezii* E. Larsen, *P. maximartinezii* Rzed., *P. monophylla* Torr. & Frém., *P. muricata* D. Don., *P. nelsonii* Shaw., *P. pinceana* Gordon, *P. quadrifolia* Parl. Ex Sudw., *P. remote* (E. Little) D. K. Bailey *et* Hawksw., and *P. strobiformis* Engelm.

The methodology used in the research was based on the suggestions of Ruiz *et al.* (2018), who mainly recommended obtaining botanical-geographic records of pines in a level of risk from different herbaria whose specimens were collected from 1836 to 2018. However, the analysis considered only the years between the creation of Series I and Series VI of INEGI. For this purpose, the types of vegetation were assumed to be constant and coincide with those reported in the collection dates before the base year of Series I of INEGI. Both physical and virtual botanical-geographical records were obtained from national and international sources. The database was complemented with information queries from the Inventario Nacional Forestal y Suelos (CONAFOR, 2014) and corroborated with information available in another bibliographic source (Pérez *et al.*, 2019).

Integration and geoprocessing of geographic data

A database was developed based on the following variables: identifier, registration date, scientific name of the species, geographic coordinates in decimal degrees, state, municipality, and locality. A cleansing was carried out in order to eliminate repeated observations and incomplete records. The .xls database was exported to ArcMap™ 10.2.1 in order to create a points shapefile using the *XY add data* module. Subsequently, the georeferenced database of the point layer was analyzed and validated with information about the coverage of states and municipalities (INEGI, 2021), as well as land-use and vegetation from Series VI (INEGI, 2016a).

The modifications to the landscape of the pines collection sites were analyzed based on the layers of land-use and vegetation (LUV) Series I (INE-INEGI, 1997) and Series VI (INEGI, 2016a), at a 1:250,000 scale. The former contains 88 LUV types and the latter, 76. Based on the differences between the classes defined for LUV in Series I and VI, a homogenization process was carried out using documents from INEGI (1998), INEGI (2016b), Meave *et al.* (2016), and Velázquez *et al.* (2002). The first step was to list all the vegetation types in each series to identify those that had elements in common, in order to bring down the classes to a manageable number. Based on INEGI's own documentation and other authors (Meave *et al.*, 2016; Velázquez *et al.* 2002), 15 large classes were obtained that grouped the respective categories of Series I and VI. The main large classes were: agriculture, coniferous forest, oak forest, water bodies, scrubland, pasture, jungle, without vegetation, hydrophytic vegetation, secondary vegetation (coniferous forest), secondary vegetation (oak forest), secondary vegetation (scrubland), secondary vegetation (pasture), secondary vegetation (jungle), and urban area.

Based on this information, the ArcMap™ 10.2 Intersect geoprocessing tool was used, with the point layers of the records of pines at risk and Series I and VI of INEGI as inputs. This method was used to obtain the point layer (shapefile) and its attribute for the LUV categories of each Series. As a final step, the table of attributes of the coverage resulting from the previous operation was exported to Excel™ 2016 and Rstudio™ 3.5, to analyze the descriptive statistics and graphs, in order to compare the LUV changes between series of every record of pines at risk.

RESULTS AND DISCUSSION

Number of consulted records

A database with 2,242 records of the 16 pine species in the risk category from the year 1836 to 2018 was developed. *Pinus attenuate* and *P. strobiformis* were the species with the lowest and highest percentages in the records, with values of 0.5 and 34.28%, respectively. The rest of the species had percentages that did not exceed 11% (Table 2). The states that make up the study area are characterized by low coniferous forests (Sierra Madre Oriental and Occidental of Mexico). The main states included in this area are Chihuahua, Durango, Coahuila, and the northern end of Baja California (Figure 2).

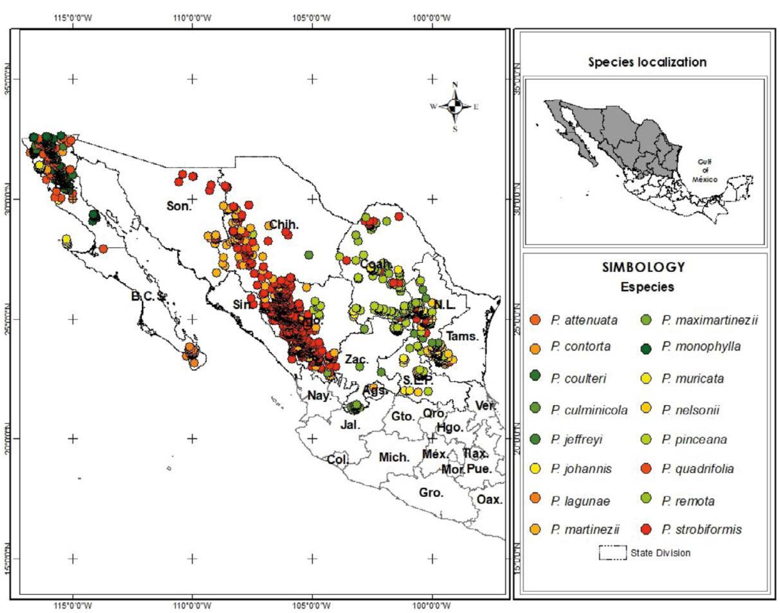


Figure 2. Geographic location of the records of pine species at risk per state in northern Mexico.

Table 2. Percentages of the 16 pine species at risk recorded in northern Mexico, consulted in herbaria and data collections.

Number	Especie	%	Number	Especie	%	Number	Especie	%	Number	Especie	%
1	Patt	0.50	5	Pjef	4.50	9	Pmax	3.12	13	Ppin	7.85
2	Pcon	2.50	6	Pjoh	2.90	10	Pmon	3.93	14	Pqua	8.15
3	Pcou	4.60	7	Plag	1.87	11	Pmur	2.27	15	Prem	1.56
4	Pcul	6.50	8	Pmar	5.00	12	Pnel	10.47	16	Pstf	34.28

P. attenuata: Patt; *P. contorta*: Pcon; *P. coulteri*: Pcou; *P. culminicola*: Pcul; *P. jeffreyi*: Pjef; *P. johannis*: Pjoh; *P. lagunae*: Plag; *P. martinezii*: Pmar; *P. maximartinezii*: Pmax; *P. monophylla*: Pmon; *P. muricata*: Pmur; *P. nelsonii*: Pnel; *P. pincena*: Ppin; *P. quadrifolia*: Pqua; *P. remota*: Prem; *P. strobiformis*: Pstrf.

Records by states

The states that record the highest number of pine species were Durango, Baja California, Chihuahua, Coahuila, Nuevo León, Tamaulipas, and Zacatecas. Those Figures amounted to 93.68% of the national total. In particular, given their relative frequency, *P. strobiformis* stands out in Durango, *P. nelsoni* in Tamaulipas, and *P. quadrifolia* in Baja California (Figure 3). Although the pine species under analysis can be found in most of the states of the study area, their representativeness in Aguascalientes is practically null (0.04%).

Distribution of pine trees per land-use and vegetation

Figure 4 shows the distribution of the pine records under study, according to the different LUV categories of Series I and VI. Globally, Series I had proportions (percentages) of up to 0.8, while in Series VI the maximum value was 0.6.

In coniferous forests, scrublands, oak forests, and secondary vegetation (scrubland), the number of records decreased in the passage from Series I (Figure 4a) to Series VI (Figure 4b). In fact, the reduction percentage in the coniferous forest exceeded 5%. Meanwhile, for agriculture, pasture, secondary vegetation (coniferous forest), secondary vegetation (oak forest), and urban area, the number of records increased during the same evaluation period. The secondary vegetation (coniferous forests) category obtained the best benefits: its gain percentage rose by almost 10 %.

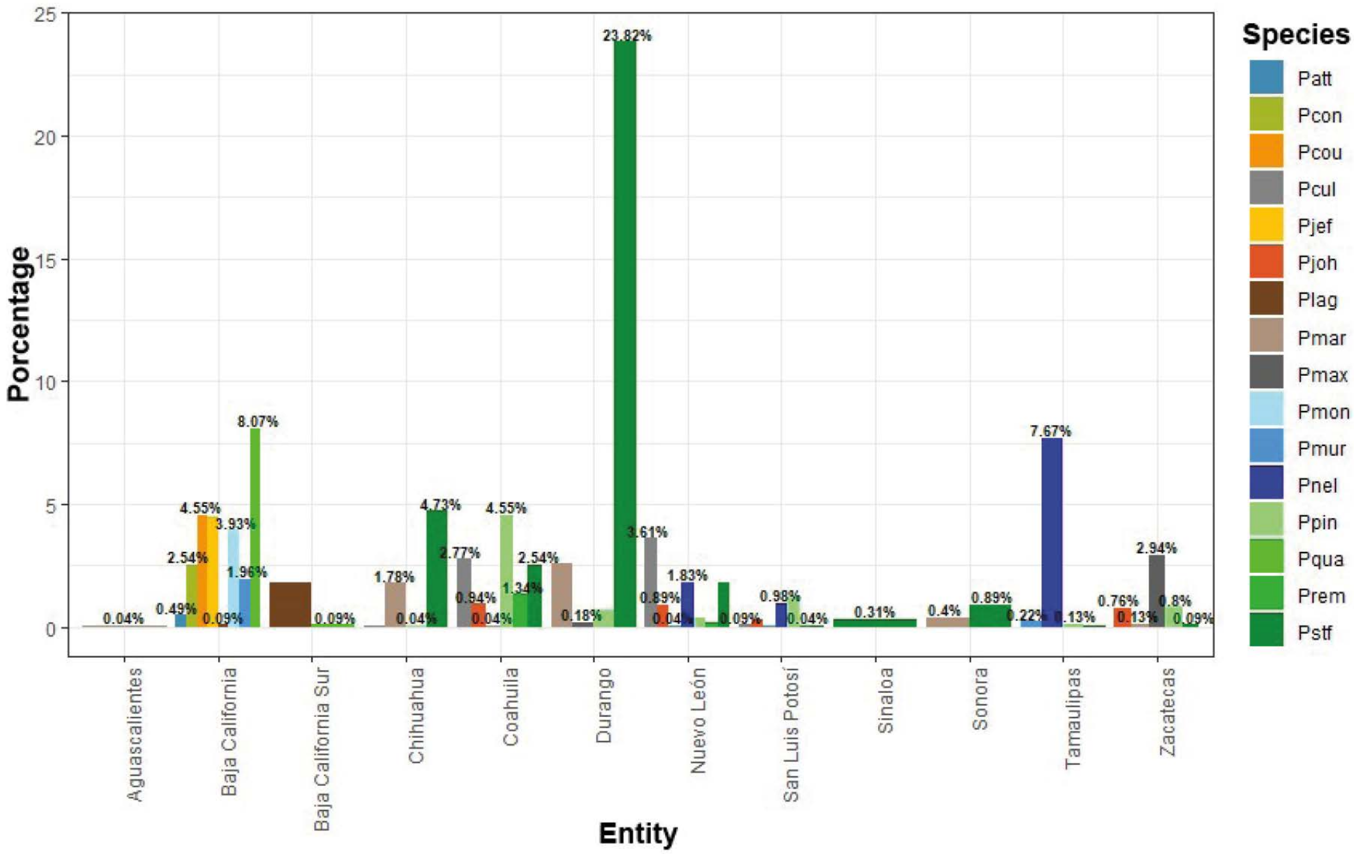
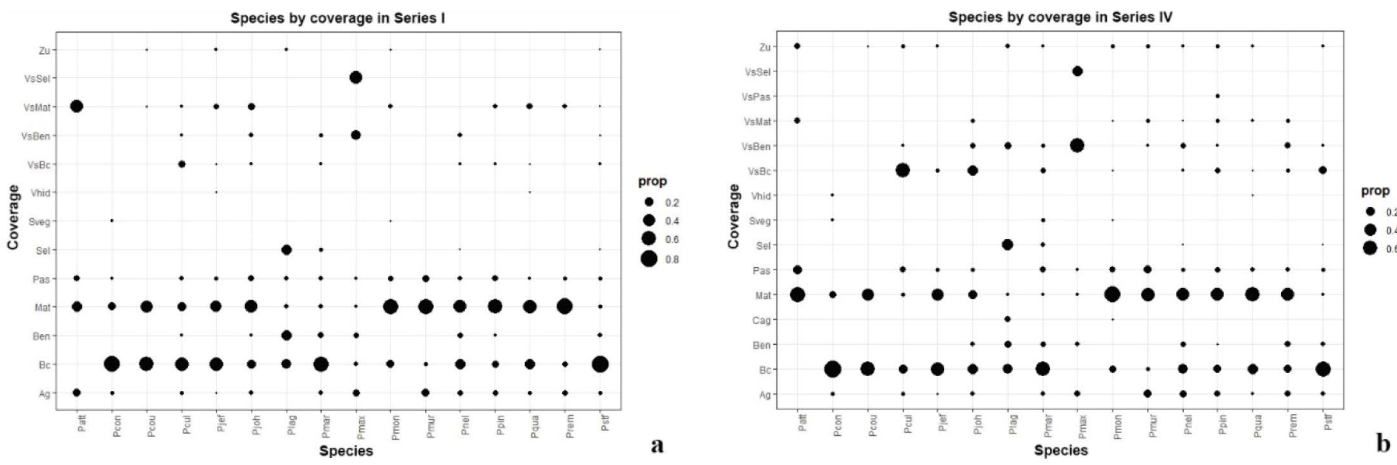


Figure 3. Percentage of the records of pine species at risk in the northern states of the Mexican Republic.



Agriculture: Ag; Coniferous Forest: Bc; Oak Forest: Ben; Scrubland: mat; Pasture: Pas; Jungle: Sel; Without vegetation: Sveg; Hydrophytic vegetation: Vhid; Secondary vegetations: Vs; Urban area: Zu.

Figure 4. Percentage distribution of the records of the pine species at risk, according to the coverage of Series I (a) and VI (b) per states in the north of the Mexican Republic.

In particular, the area of the oak forest and scrubland categories was reduced and, consequently, the number of records was also negatively altered, while the area of agriculture, pasture, secondary vegetation (coniferous forest), secondary vegetation (oak forest), and urban zones increased in the period analyzed (Table 2).

The comparison of Series I with Series VI (Table 2) shows that the points on the diagonal that represent the LUV remain stable in the same category in the two Series and represent 72.26%. The points outside of the diagonal indicate movements between categories. Changes from scrubland records to coniferous forest (equivalent to 1.83% of Series I), oak forests (0.31%), secondary vegetation (coniferous forests, 1.83%), and secondary vegetation (oak forest) were identified. There were changes in the pine records, from coniferous forest to secondary vegetation (coniferous forest, 7.40%), to secondary vegetation (oak forest, 0.67%), and to pasture, agricultural, and urban areas (2.63% in total). There were also records of pines in oak forests that changed to secondary vegetation (oak forest, 0.58%).

The changes in the number of records in the categories from Series I to Series VI are mainly attributed to anthropogenic factors (García *et al.*, 2012; Pineda *et al.*, 2009). These authors highlight that population growth and density contribute to the transformation of the natural landscape, the increase in land used for agriculture, and the reduction of forest area.

Fires have negative impacts on forests and consequently alter the ecosystem goods and services they provide. Despite the planning, coordination, and execution efforts carried out by the Comisión Nacional Forestal to timely detect and fight such catastrophic events, the annual loss of forest resources in Mexico is inevitable. From 1998 to 2021, an average of 340,466 ha/year was reported in the whole nation; in 2015, a total of 88,538 ha was impacted, 32.11% of which is located in the study area (CONAFOR, 2022; FAO, 2020). This situation is reflected in the results of our research: a reduction of 12.4% was observed in the records of pine species that fell initially in the category of coniferous and oak forests

Table 2. LUV change matrix of the number of collection sites for pines at risk from Series I (S1) to Series VI (S6).

S1 \ S6	Ag	Bc	Ben	Cag	Mat	Pas	Sel	Sveg	Vhid	VsBc	VsBen	VsMat	VsPas	VsSel	Zu	Total
Ag	63	6			6	4				1				3	19	**102**
Bc	29	855	10	2	14	27				166	15				3	**1121**
Ben		8	62			1	1	2		5	13		3			**95**
Mat	15	41	7		460	13	1			41	14	13			6	**611**
Pas	7	9	1	3	5	54		1	1	5	1	3	3	1	6	**100**
Sel							20									**20**
Sveg								2								**2**
Vhid					1				2							**3**
VsBc	2	2				2				43						**49**
VsBen		2				4					37					**43**
VsMat	2	3			41	4				4		2			2	**58**
VsSel						1					16			15		**32**
Zu															6	**6**
Total	**118**	**926**	**80**	**5**	**527**	**110**	**22**	**5**	**3**	**265**	**96**	**18**	**6**	**19**	**42**	**2242**

Agriculture: Ag; Coniferous Forest: Bc; Oak Forest: Ben; Water bodies: Cag; Scrubland: mat; Pasture: Pas; Jungle: Sel; Without vegetation: Sveg; Hydrophytic vegetation: Vhid; Secondary vegetation (coniferous forest): VsBc; Secondary vegetation (oak forest): VsBen; Secondary vegetation (scrubland): VsMat; Secondary vegetation (pasture): VsPas; Secondary vegetation (jungle): VsSel; Urban area: Zu.

and were subsequently included in another type of LUV. In a study carried out from 2013 to 2017, in an area of 1,260.73 ha in southwestern Chihuahua identified as burned areas using the Normalized Burn Ratio (NBR) and field verification, Valdez *et al.* (2019) recorded a change of coverage and a transition from forest coverage to secondary vegetation in 82% of the area.

The findings of this research —which in themselves reflect the dynamics of forest ecosystems— are similar to the results of other studies about land-use change in Mexico. In this sense, increases in spaces transformed by humans were observed, including agricultural, induced pasture, and urban areas. In addition, a loss in forest area was recorded, as a result of conversions to agricultural areas and pasture (Pérez *et al.*, 2011). Challenger and Dirzo (2009) affirm that by 1976 the original vegetation coverage of the country had suffered a 38% reduction; around 1993, it only covered 54% and, by 2002, it occupied only 50% of its original surface. Sanchez *et al.* (2009) refer that the growth of human settlements and urban areas have had a significant increase since the 1970s. Currently, this is the most important land-use change in some regions of the country (SEMARNAT, 2010).

Finally, as a result of the specific characteristics of cartographic studies about the regional or national LUV changes, the scale of work is a highly relevant factor, since the availability, temporality, and spatial resolution of the databases directly influence the accuracy of the results (Franco *et al.*, 2006; Peralta *et al.*, 2019). In this sense, the findings of the present research depend on the information contained in Series I and VI of INEGI, and, therefore, may change if more accurate scales are used in specific study areas.

CONCLUSIONS

The records of the 16 pine species at risk in northern Mexico showed changes in the land-use and vegetation categories during the analysis period. From 1985 to 2014, a decrease in the number of matches in the records of the pine species at risk in coniferous forests, scrublands, and oak forests was observed. This phenomenon is part of the land-use changes that took place between the two series. In addition, an increase in records was identified in categories deeply altered by humans, such as urban areas and agricultural lands. Consequently, it is uncertain whether or not these recorded species can currently be found in the initial collection areas, highlighting the importance of further studies on a smaller scale to evaluate national programs for the conservation of the genus *Pinus* in the risk level and the ecosystem services they provide.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Synthetic fertilizers and vermicompost in juvenile Persian lime (*Citrus×latifolia* Tanaka ex Q. Jiménez) trees

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ABSTRACT

Objective: To evaluate fertilization with vermicompost and NPK mineral fertilizer in young Persian lime (*Citrus×latifolia* Tanaka ex Q. Jiménez) trees.

Design/Methodology/Approach: A randomized block design was established with eight treatments and four repetitions: T1, 0 kg tree^{-1}; T2, 90-22.5-22.5 N-P-K kg ha^{-1}; T3, 2 kg tree^{-1} of vermicompost; T4, 3 kg tree^{-1} of vermicompost; T5, 4 kg tree^{-1} of vermicompost; T6, 90-22.5-22.5 N-P-K kg ha^{-1} + 2 kg tree^{-1} of vermicompost; T7, 90-22.5-22.5 N-P-K kg ha^{-1} + 3 kg tree^{-1} of vermicompost; and T8, 90-22.5-22.5 N-P-K kg ha^{-1} + 4 kg tree^{-1} of vermicompost. The study variables were tree height, stem thickness, crown diameter from North to South (N-S) and East to West (E-W), soil pH, and soil moisture (%). The data were statistically analyzed applying the MINITAB V.17 statistic through an ANOVA ($P \leq 0.05$), while a multivariate analysis was used for the means comparison.

Results: The tree height and crown diameter variables had different results in the Persian lime trees treated with 4 kg tree^{-1} of vermicompost (T5). The tree canopy had a similar development than T5 with vermicompost treatments combined with NPK mineral fertilizer.

Study Limitations/Implications: Conventional lime production indiscriminately uses synthetic fertilizers, polluting natural resources. Organic fertilizers are a nutritional alternative for the trees.

Findings/Conclusions: The vermicompost treatment efficiently maintains adequate soil moisture during the dry season, improving the growth and development of Persian lime trees.

Keywords: Nutrition, citrus, vermicompost.

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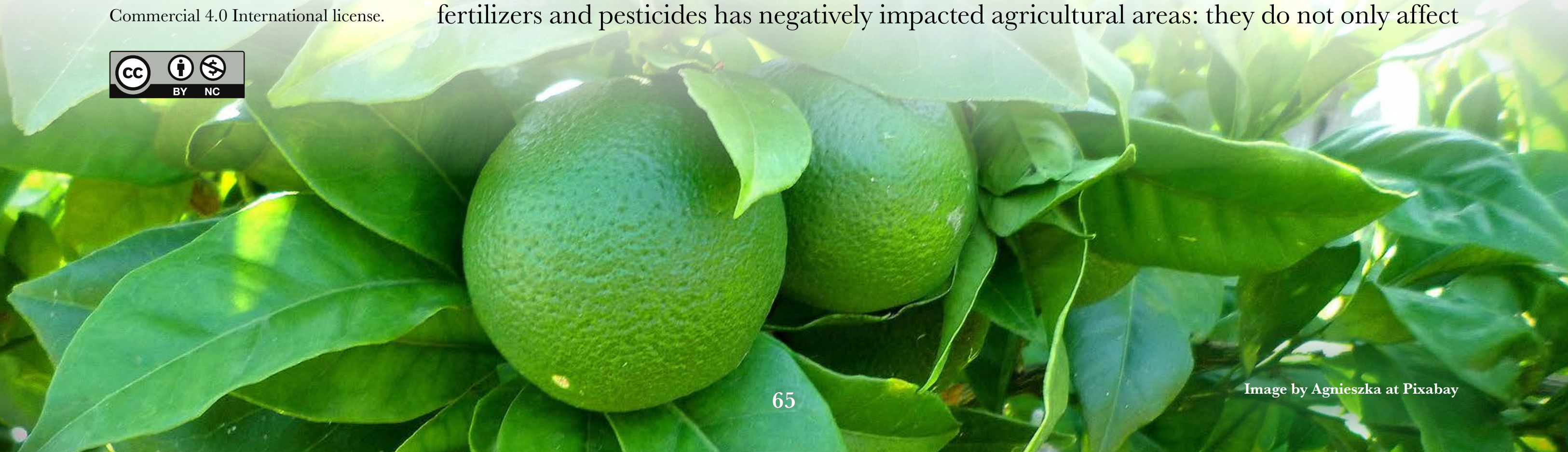
INTRODUCTION

The environmental awareness about pollution, environmental impacts, food safety, and food security has continuously increased in recent years. The intensive use of synthetic fertilizers and pesticides has negatively impacted agricultural areas: they do not only affect



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plant growth, but they also decrease the biological control of soil-borne diseases and reduce biodiversity, since they destroy the ecosystem, as well as the beneficial insects and the species from which birds and other animals feed (Andrade and Ayaviri, 2017; Castillo *et al.*, 2020; Castañeda *et al.*, 2021). As a consequence of the use of chemical fertilizers, nitrates run off or infiltrate into groundwater. The large amounts of fertilizers that are applied to crops are carried by water and wind before being absorbed by the plants and retained by soil particles. In developed countries, the use of synthetic fertilizers has been restricted, in favor of other forms of fertilization that are more sustainable and have less impact on the environment and public health (Eugencios *et al.*, 2017; FAO, 2020). The vermicomposting technique is a rational environmental tool, focused on the organic waste processed by earthworms. They carry out the composting process and produce a final output of high economic value called "vermicompost". Additionally, vermicompost is a clean, safe, and low-cost process for the producer (Ersahin *et al.*, 2009; Villegas and Laines, 2017).

Some earthworm species can consume organic material residues very quickly and fragment them into very small particles. This process is known as "grinder gizzard" and it is carried out by an organ of earthworms that promotes greater microbial activity. Consequently, the material (nitrogen, potassium, phosphorous, and calcium) is released and transformed into forms that are much more soluble and available to plants (Olivares *et al.*, 2012; Silveira *et al.*, 2018; Ramos *et al.*, 2019; Acosta *et al.*, 2021).

Organic fertilization in citrus trees has already been applied in Veracruz, Mexico. For example, Berdeja *et al.* (2018) evaluated the effect of two types of fertilizers (inorganic and organic) on Persian lime (*Citrus*×*latifolia*) fruits, proving that there are no significant differences in the values of the fruit parameters (weight, peel thickness, nutritional content, etc.). According to the results, the growth and development of the fruit achieved with organic fertilization was very similar to the fruits fertilized using an inorganic (chemical) fertilizer. Therefore, the objective of this research was to evaluate the fertilization with vermicompost and synthetic fertilizer formula in Persian lime trees recently planted in Jesús Carranza, Veracruz, Mexico.

MATERIALS AND METHODS

The experiment was carried out at the facilities of the Tecnológico Nacional de México/ Instituto Tecnológico Superior de Jesús Carraza, located in the municipality of Jesús Carranza, Veracruz, Mexico.

Figure 1. Location of the municipality of Jesús Carranza, Veracruz.

Treatments

The tree plantation was sown in December 2020. The tree management partially consisted of formative pruning and pruning of suckers and branches resulting from the pattern of the trees. Two types of foliar applications were made: 1) abamectin and humic and fulvic acids; and 2) liquid inorganic fertilizer (5.9% nitrogen+20.9% phosphorus). Additionally, hormonal applications were carried out with the Agromil V product (cytokinins, gibberellins, auxins, and folic acid).

The lime trees were nurtured at 127 days, applying the different fertilization treatments at 50 cm from the trunk of the trees (Table 1).

In the tree plantation, the different doses of fertilization based on vermicompost, and chemical formulas were evaluated. The fertilization treatments were applied per grafted Persian lime tree; some treatments were mixed with mineral fertilization based on N-P-K.

The mineral fertilization dose was determined per hectare and the dose per tree was calculated based on the planting density (357 trees per hectare). Only one full-dose application formula nutrition (N-P-K) was made per tree (Table 1).

The experimental unit consisted of two Persian lime trees, with an edge tree on each side. Each treatment has four replications, and the fertilization dose treatments are distributed in a randomized complete block design (Table 1).

The fertilizer sources corresponded to urea (46N-0-0), triple 17 (17N-17P-17K), and vermicompost. The composition is C: 18.57%, total N: 2.24%, C/N ratio: 8.13, N-NO_3: 583 mg kg^{-1}, P: 0.12%, K: 0.79%, Ca: 1.33%, Mg: 1.21%, Na: 0.12%, Fe: 357 mg/kg^{-1}g, Zn: 91 mg kg^{-1}g, Mn: 196 mg kg^{-1}g, Cu: 38 mg kg^{-1}g, and pH: 6 (Olivares *et al*., 2012). The variables measured were tree height, stem thickness, crown diameter from North to South (N-S) and East to West (E-W), soil pH, and soil moisture (%). The last variable was measured with a VT-05q humidity monitor hygrometer. The variables were measured at 136 days and at 164 days.

The statistical analysis of the growth and development variables of the fertilized trees was carried out with the MINITAD version 17 software, performing an analysis of variance (95% reliability), a three-dimensional relationship analysis, and a multivariate analysis.

Table 1. Fertilization treatments with vermicompost and mineral in Persian lime trees.

Treatment	Source	Soil fertilization dose
1	0	0
2	Mineral	90-22.5-22.5 NPK/tree
3	vermicompost	2 kg/tree
4	vermicompost	3 kg/tree
5	vermicompost	4 kg/tree
6	Mineral + vermicompost	90-22.5-22.5 NPK/tree+2 kg/tree
7	Mineral + vermicompost	90-22.5-22.5 NPK/tree+3 kg/tree
8	Mineral + vermicompost	90-22.5-22.5 NPK/tree+4 kg/tree

RESULTS AND DISCUSSION

The results regarding the tree height variable indicate that the T3 treatment had statistically significant differences with respect to the other treatments, although they were like the T5 treatment on the first date of sampling (136 days) (Table 2). In the second and third samplings (carried out at 164 and 244 days, respectively), there were statistically significant differences in the behavior of the T3 treatment trees than in the other treatments, but the behavior was statistically like T5.

At 288 and 356 days, the Persian lime tree with T5 was statistically higher regarding fertilization. This result indicates that the lime trees' response to the application of fertilization doses of 4 kg/tree with vermicompost. The next treatment used a dose of 2 kg tree^{-1} of vermicompost. Seven-year-old Persian lime trees have responded favorably to the use of organic fertilizers, which provide them with good nutrition for their development and growth. The recommended management dose is 15 kg tree^{-1} of kudzu applied to the soil in the months of January, April, and August. The organic fertilizer provides the following nutritional percentages: 3.73 (N), 0.189 (P), 1.96 (K), 0.78 (Ca), 0.32 (Mg), etc. (Berdeja *et al.*, 2018).

For the stem diameter variable of the lime trees (Table 3), no statistically significant difference was found between the different treatments evaluated. However, at 356 days, the stem thickness variable of the T5 treatment was higher than the control without fertilizer

Table 2. Tree height with different fertilization treatments using vermicompost and formula in 356-days old Persian lime.

Treatment	136 days	164 days	244 days	288 days	356 days
T1	62.88 A B	68.63 AB	71.8 E	73.6 E	76.7 E
T2	53.88 B	57.38 B	60.0 H	58.7 H	64.3 H
T3	73.75 A	78 A	83.5 A	73.7 D	82.5 B
T4	59.63 AB	62.38 AB	68.4 F	68.7 G	68.0 G
T5	70.50 AB	72.88 AB	77.4 B	82.0 A	86.5 A
T6	61.25 AB	66.0 AB	73.0 D	70.5 F	76.4 F
T7	60.38 AB	66.0 AB	76.5 C	78.5 C	76.8 D
T8	59.75 AB	62.25 AB	65.6 G	79.7 B	82.5 C

Means that do not share a letter are significantly different. Tukey test at 95%.

Table 3. Stem diameter in Persian lime trees.

Treatment	136 days	164 days	244 days	288 days	356 days
T1	7.0 A	7.3 A	9.2 A	12.3 A	17.5 A
T2	7.0 A	7.5 A	9.0 A	10.3 A	13.2 A
T3	6.2 A	7.8 A	9.4 A	11.8 A	14.7 A
T4	7.2 A	7.4 A	9.5 A	10.5 A	15.0 A
T5	7.3 A	8.0 A	11 A	12.8 A	18.6 A
T6	6.3 A	7.9 A	9.8 A	10.3 A	17.0 A
T7	7.7 A	8.3 A	11 A	11.8 A	16.9 A
T8	7.4 A	8.3 A	9.6 A	10.3 A	14.4 A

Means that do not share a letter are significantly different. Tukey test at 95%.

application. In an experiment with lime associated with coconut (*Cocos nucifera*) palms, the development and growth of the trees had a positive response to the application of organic sugarcane amendments and corn harvest waste. The incorporation of corn dry matter into the soil between the alleys improved the productivity of lime trees and the development in stem diameter and tree height (Rebolledo *et al.*, 2019).

The treatments showed statistically significant differences regarding the crown diameter variable, measured in the last sampling dates in the North to South position (Table 4). The T5 fertilization dose was higher than all treatments at 288 and 356 days. The slow release of nutrients by vermicompost in lime trees caused a response at 288 and 356 days, since only a fraction of the total nitrogen content is mineralized. However, vermicompost is a good substitute for chemical fertilization and can obtain similar results to synthetic fertilizers, given the high concentration of nitrogen in the organic matter particles. It is important to consider that 40% of phosphorus is available to trees (Olivares *et al.*, 2012).

In the crown diameter variable with orientation from East to West (Table 5), statistically significant differences were observed at 136 days with T4, T6, and T7. These three treatments had better results than other treatments; however, T8 treatment recorded higher values at 164 days. At 288 and 356 days, the T6 treatment had statistically significant differences, which were higher than the results obtained by other treatments regarding the crown development variable.

Table 4. North-South canopy diameter of lemon trees.

Treatment	136 days	164 days	244 days	288 days	356 days
T1	33.3 A	36.6 A	47.3 A	54.8 C	49.1 D
T2	33.7 A	41.4 A	54.2 A	46 G	40.1 H
T3	34.6 A	36.7 A	47.8 A	48.3 F	46.5 F
T4	32.2 A	36.8 A	47.5 A	51.6 E	47 E
T5	38.6 A	43.0 A	52.3 A	61.7 A	68.5 A
T6	35 A	37.0 A	43.1 A	60.5 B	58.5 B
T7	48 A	44.5 A	44.7 A	53.6 D	50.3 C
T8	37.2 A	41.3 A	41 A	44.9 H	43.2 G

Means that do not share a letter are significantly different. Tukey test at 95%.

Table 5. East-West canopy diameter of lemon trees.

Treatment	136 days	164 days	244 days	288 days	356 days
T1	27.2 C	34.2 B	49.8 A	58.8 B	52.3 C
T2	31.7 BC	34.2 B	45 A	39.3 G	33.5 G
T3	30.5 BC	33.7 B	37.4 A	37.8 H	30.0 H
T4	34.6 ABC	40.6 AB	47 A	49.3 F	38.4 F
T5	32.6 BC	40.6 AB	48.2 A	54.7 D	55.3 B
T6	38.6 ABC	41 AB	40.7 A	59.0 A	61.2 A
T7	42.1 AB	43.2 AB	48 A	57.3 C	43.5 E
T8	47.0 A	50.1 A	37.2 A	50.6 E	47.8 D

Means that do not share a letter are significantly different. Tukey test at 95%.

The soil pH at 136 days showed that there are statistically significant differences between treatments T1, T2, T3, T4, T5, and T7. The pH ranged from 5.8 to 7.0. The treatments with high fertilization doses had acid pH: T6 and T8 had a pH of 5.4 and 5.5, respectively. The pH in the soil at 164 days for all the treatments was neutral (7) compared to the control. At 244, 288, and 356 days, variations in soil pH were observed, without significant differences (Table 6). Regarding other research about Persian lime grafted on sour orange (*Citrus aurantium* L.), lime developed at an age of 9 and 12 years, in moderately acid soils (5.66 and 6.16 pH), at a depth of 0-30 cm in the soil profile. Consequently, pH close to neutral favor the absorption of nutrients and water. The Persian lime had more roots at a depth of 0 to 30 cm than at other depths (30 to 60 cm and 60 to 100 cm). This condition is the result of soil aeration at shallower depths (Contreras *et al.*, 2008). pH values of 6.2 were recorded in Persian lime trees managed with 1.0 kg of dry matter at the bottom of the stump and dry matter during crop development. The dry matter applied in lime trees favored growth in height and stem diameter, reinforcing the concept that "the contribution of organic fertilizers meet the nutrient requirements and demands of the crops, perhaps in a different way to the action generated by the chemical formulas" (Rebolledo *et al.*, 2019).

When measuring the soil moisture (%), no statistically significant differences between the treatments were recorded. In the last dates, the highest and more homogeneous soil moisture was recorded in treatments T4 and T7 regarding the absolute control, although the treatments fertilized with vermicompost had a better response to moisture retention (Table 7). In irrigated Persian lime plantations in Nayarit, moisture did not affect nutrient concentration in the slices of the lime fruits, but it did intervene in lime flowering. Zn mainly impacts the tree with the management of irrigation water. In non-irrigated plantations, phosphorus and potassium concentrations in lime slices are lower than those found in irrigated plantations; these concentrations are also related to soil characteristics. The behavior of the nutrients of calcium, sulfur, iron, copper, and boron was the same between rainfed and irrigated lime (Mellano *et al.*, 2017).

Figure 2 shows the relationship between tree height and soil pH and moisture, where 60-80 cm tall lime trees had a 6-6.2 soil pH and 70-80% moisture. Meanwhile, 100-cm tall Persian lime trees recorded a 6.5 soil pH and 70-80% moisture.

Table 6. Soil pH in Persian lime trees.

Treatment	136 days	164 days	244 days	288 days	356 days
T1	6.6 AB	6.9 A	6.7 A	5.6 A	6.6 A
T2	6.5 AB	7.0 A	6.1 A	6.1 A	6.6 A
T3	7.0 A	7.0 A	6.3 A	5.9 A	6.7 A
T4	6.9 A	7.0 A	7.0 A	6.3 A	7.0 A
T5	6.5 AB	7.0 A	6.8 A	6.5 A	6.8 A
T6	5.4 C	7.0 A	6.6 A	5.4 A	7.0 A
T7	5.8 AB	7.0 A	6.7 A	5.8 A	7.0 A
T8	5.5 BC	7.0 A	7.0 A	5.8 A	6.8 A

Means that do not share a letter are significantly different. Tukey test at 95%.

Table 7. Soil moisture in Persian lemon trees.

Treatment	136 days	164 days	244 days	288 days	356 days
T1	31.3 A	78.8 A	66.8 A	92.5 A	100 A
T2	50 A	82.5 A	80 A	100 A	90 A
T3	27.5 A	85 A	80 A	100 A	97.5 A
T4	40 A	86.3 A	78.7 A	100 A	100 A
T5	49.4 A	81.3 A	80 A	93.1 A	100 A
T6	57.5 A	83.7 A	80 A	92.5 A	100 A
T7	33.8 A	85 A	80 A	100 A	100 A
T8	62.5 A	87.3 A	75 A	100 A	95 A

Means that do not share a letter are significantly different. Tukey test at 95%.

Regarding the relationship of the crown diameter variables measured from North to South with the soil pH and soil moisture variables, the results of the pH and soil moisture variations were more significant for lime trees with a 20-cm crown diameter, a 6.4-6.6 pH, and a 60-80 % soil moisture (Figure 3).

No variability was recorded regarding crown diameter measured from East to West, when it was correlated with the soil moisture and soil pH variables and when the crown

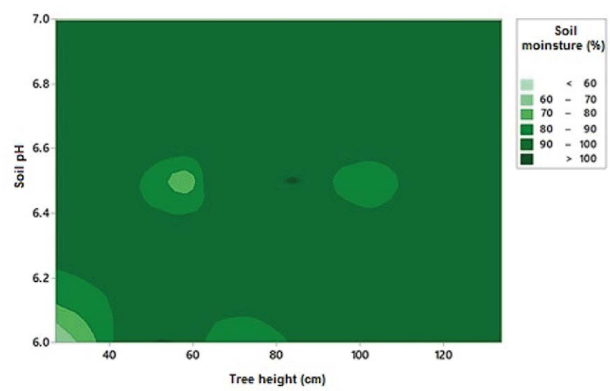

Figure 2. Relationship of lime tree height with soil pH and moisture at 356 days.

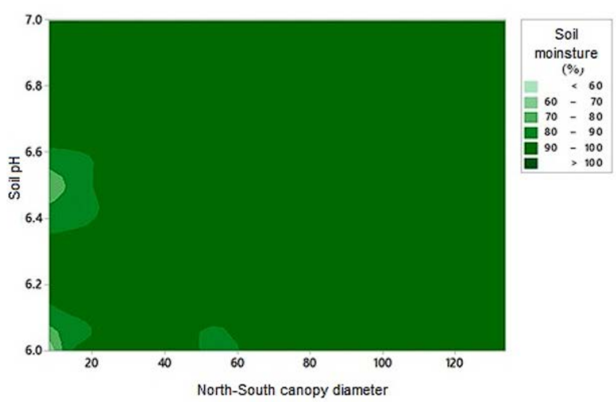

Figure 3. Relationship between the canopy diameter of the North-South tree, with the pH and moisture of the soil at 356 days of age.

diameter was greater than 20 cm. The soil moisture and soil pH variables are closely correlated with the development of the canopy. This phenomenon can have a positive or negative influence —*i.e.*, water deficit can induce flowering in lime trees (Vélez *et al.*, 2012) (Figure 4). When studying the processes that intervene in the growth and development of trees and the characteristics of the soil, the response variables that intervene in the process of chemical and organic fertilization were unified. Table 8 shows the resulting means. Therefore, the fertilization doses based on mineral and vermicompost (T5 and T7) have the best response, enhancing the growth and development of Persian lime trees. The fertilization dose recommended for commercial purposes in organic and agroecological plantations belong to T5. The lime growth variables can be influenced by soil moisture since it participates in stomatal conductance and citrus transpiration processes. That is to say, in the drought stage the trees that were subjected to mineral fertilization could present water stress, which would impact the physiological processes, consequently stopping the development of the leaves and causing wilting and curling (Vélez *et al.*, 2012).

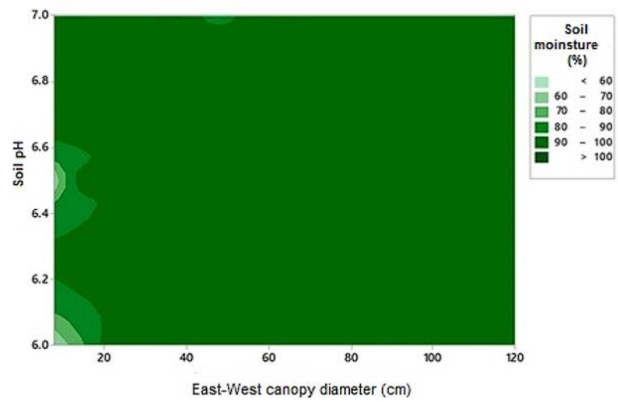

Figure 4. Relationship of canopy diameter of the East-West tree with the pH and soil moisture at 356 days of age.

Table 8. Means and standard deviations of the multivariate analysis of the different vermicompost and mineral treatments.

Treatment	Mean	Standard deviation
T1	41.39	28.89
T2	38.97	27.60
T3	41.29	31.31
T4	41.12	29.03
T5	45.74	30.82
T6	43.75	29.81
T7	43.8	30.02
T8	42.83	29.69

CONCLUSIONS

The treated Persian lime trees had a greater growth and development than the trees to which no nutrients were applied. Applying 4 kg tree^{-1} of vermicompost increased tree height and crown development. The applications of a mixture of vermicompost and synthetic formula led to a better tree canopy development; however, tree height was similar to the height of trees fertilized with vermicompost. A slightly acidic pH in the soil and treatments managed with vermicompost-based fertilizers had a neutral tendency which improves nutrient assimilation. In the dry season, the rainfed lime trees fertilized with vermicompost had a better response to moisture retention in the soil.

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AGRO PRODUCTIVIDAD

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Photodegradation Diuron herbicide with TiO_2-Al_2O_3 catalysts supported on graphene nanoplatelets

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ABSTRACT

Objective: To photodegrade Diuron with TiO_2-Al_2O_3 nanomaterials supported on graphene nanoplatelets (GnPs)

Design/methodology/approach: The synthesis of the materials was carried out by the sol-gel method under mild conditions. Subsequently, the obtained materials were subjected to thermal processing for structural stabilization and pulverized. Synthesized nanomaterials were then characterized by nitrogen adsorption/desorption, X-ray diffraction, scanning electron microscopy, and Uv-Vis spectroscopy.

Results: The adsorption/desorption results indicated that the ternary TiO_2-Al_2O_3/GnPs nanomaterials were found to have complex porosity, which suggested that TiO_2-Al_2O_3 was formed on agglomerated GnPs. X-ray diffraction data revealed that the anatase phase of TiO_2 and the αAl_2O_3 phase coexist with the crystalline phase of graphene. The morphology of the materials indicates that the nanoplatelets were randomly dispersed in a continuous mixed oxide phase. About the UV analysis, the presence of GnPs at 1 wt % concentration reduces the band gap by 6%.

Limitations on study/implications: The physical and chemical properties of GnPs make the material an excellent candidate for the degradation of pollutants by photocatalysis.

Findings/conclusions: The addition of GnPs improved the Diuron degradation, probably by forming a nanostructured interface or heterojunction.

Keywords: Diuron, GnPs, TiO_2, Al_2O_3, Photocatalysis.

INTRODUCTION

Pesticides and herbicides are continuously used for pest control. Herbicides are classified: 1) according to their interaction with plants, which can be by direct contact and translocation through the soil, 2) by their total or partial effect on vegetation, and 3) their chemical composition. Diuron (1,1-dimethyl, 3-(3', 4'-dichlorophenyl) urea) is a broad-spectrum herbicide and algaecide used in agriculture for weed and algae control especially in and around water bodies (Malato *et al.*, 2003) it inhibits photosynthesis by preventing oxygen production and blocks electron transfer at the plant photosystem level (Wessels, J.S.C. & Van der Veen, R., 1956). This chemical compound has been used to control the growth of a wide variety of seasonal weeds (Giacomazzi & Cochet, 2004). However, compound agriculture has resulted in the contamination of soil and aquatic environments by leaching (Thurman *et al.*, 2000). Furthermore, synthetic herbicides in agriculture in the last ten years have caused significant damage to the ecosystem due to specific physicochemical properties (resistance to biodegradation, toxicity, and solubility, among others) (Fenoll *et al.*, 2013) . For this reason, there is an earnest effort to optimize removal techniques, either by chemical or physical degradation. Among the known techniques, advanced oxidation processes and photodegradation via solar energy are famous (Bamba *et al.*, 2008). On the other hand, semiconductor compounds are used as catalysts for the photodegradation of pollutants. In this area, TiO_2 is widely studied mainly because of its low cost and availability in addition to its physicochemical properties and a favorable redox capacity. Three crystalline forms of TiO_2 have been reported, however, the anatase phase is more active when compared to rutile and brookite (Escobar *et al.*, 2000) (Navarrete *et al.*, 1996) (Araña *et al.*, 2008). Despite its excellent qualities, titanium oxide has a disadvantage, electron-hole recombination. The adverse effects of titanium oxide are inhibited by combining it with other metal oxides and even doping it. Among the examples reported is $[Ru(bipy)_3]Cl_2$-doped with TiO_2/SiO_4, used for wastewater degradation, obtaining 70% efficiency. The materials performed well during photodegradation, and their activity was four times higher than known commercial materials (Bernardes *et al.*, 2016). Graphene oxide (GO) based photocatalyst GO-TiO_2, was investigated under UV-Vis in the photodegradation of a mixture of four pollutants, including Diuron and the results showed a reasonable rate of photodegradation of pesticides in both natural and ultrapure water (M. Cruz *et al.*, 2017). For nanostructured TiO_2/ZnO/rGO (TZR) composites, 5% rGO gave the highest photocatalytic activation (99%) concerning the pure individual components; the rGO load on the TZR composite has attributed an effect on its photocatalytic activation (Nguyen *et al.*, 2020). Boron-doped TiO_2 did not produce a significant degradation of different pesticides studied; even so, a slight decrease in the concentration was observed for the solutions studied (Rey *et al.*, 2015) . Tungsten-doped titanium oxide (W) was tested in the degradation of Diuron under simulated solar irradiation. The best nanophotocatalyst $W_{0.03}Ti_{0.9}7O_2$ showed 70% degradation w.r.t. pure TiO_2 (Publishing & Science, 2017). In another study, TiO_2 doped with 0.5 wt% Sm^{3+} had mesmerizing photocatalytic activity performance for Diuron degradation by sunlight but it was low (42%), and 0.3 wt% Sm^{3+} showed the best performance in photodegradation with Diuron (80%) (D. De Cruz *et al.*, 2011). The work of multiple researchers to discover processes that are more efficient in photodegradation has led them to combine Al_2O_3 and TiO_2 and achieve a mixed oxide that increases the photocatalytic activity by increasing the contact surface with the molecule to be degraded, so it is considered an excellent binary system for efficient degradation of organic molecules (Anderson & Bard, 1997) (Choi *et al.*, 2006). This research aimed to synthesize and characterize nanostructured ternary materials based on TiO_2, Al_2O_3, and GnPs and correlate the resulting physicochemical properties with the photodegradation efficiency of Diuron.

MATERIALS AND METHODS

Reagents

XG Sciences Inc.'s GnPs, (xGnP®) are nanoparticles consisting piles of a few graphene sheets, single graphene sheets, and nanostructured graphite; the average surface area is 750 m^2 g^{-1}. This material is Grade C, typically consisting of platelets with a particle diameter of fewer than 2 m and a few nm thicknesses

Catalyst synthesis

The synthesis method for the materials was sol-gel, which consists of placing graphene nanoplatelets (GnPs) with titanium and/or aluminium precursors in a three-hole flask. With an addition funnel, a 50:50 water-ethanol mixture was added drop by drop, and then, the mixture reaction was heated at 60 °C, maintaining agitation and with constant reflux for 24 h. Later, the reaction product was dried in the rotary evaporator and then placed in an oven at 80 °C for 12 h. Finally, the material was calcined at 500 °C for 4 h with airflow and a heating ramp of 2 °/min^{-1}.

Catalytic Evaluation

A 30 mg kg^{-1} solution was prepared, and 250 mL was poured into the reactor. The catalyst was added to achieve a concentration of 0.4 g L^{-1} and left in agitation for 30 min in the absence of light to reach equilibrium. Subsequently, a first sample was taken before turning on the lamp. It was left in visible irradiation, and 6 mL were taken at 5, 15, 30, 30, 45, 60, 120, 180, 240, and 300 min. From the corresponding calibration curve, UV-Vis readings of the samples were taken. For determining degradation percentages using the following equation: $x(\%) = ([A°] - [A]) / [A°]$. Where (mg kg^{-1}) is the concentration when the light is turned on to start the photodegradation process and (mg kg^{-1}) is the concentration after five hours under irradiation (Richard Felder, 2004).

Characterization

Nitrogen physisorption: This characterization was carried out in the Micrometrics equipment model Tristar II. First, degassing treatment with nitrogen flow was given to each sample, and then, a liquid nitrogen cooling at −196 °C was supplied.

SEM: This characterization was performed in a Field Emission Scanning Electron Microscope FE SEM FEI Tecnai F30 (300 keV) vacuum of 80 Pa.

X-Ray diffraction: Diffractograms were obtained with a BRUKER diffractometer model D-8, with a Cu Kα radiation $\lambda = 1.54059$ Å, 300 W.

Solid UV-Vis: Readings were carried out in an UV-Vis Cary 300 equipment in a beam range from 200 to 800 nm wavelength at room temperature.

RESULTS AND DISCUSSION

Isotherms represent the volume of nitrogen physisorbed in the solid w.r.t. the relative pressure of nitrogen, because of the adsorption and desorption process of the gas in the solid (Figure 1). The adsorption-desorption isotherms for TiO_2, Al_2O_3, and GnPs materials presented V-type, indicating weak interactions between the adsorbate and adsorbent. The hysteresis loop for GnPs was of H3 type characteristic of plate-like materials. Meanwhile for TiO_2 and Al_2O_3 samples, the hysteresis loop was H5, characteristic of partially blocked mesoporous materials, according to the IUPAC classification (Cychosz *et al.*, 2017).

The pore diameter distribution in samples was calculated from the BJH method. GnPs have a distribution between 400 to 50 nm, with a maximum of 150 nm. TiO_2 and Al_2O_3 samples presented bimodal distribution with maximal diameters centered at 65 and 88 nm, respectively (Figure 1B). Specific surface area was obtained with the BET method; the results are shown in Table 1. The area of GnPs and Al_2O_3 samples are in the typically published ranges (Daş *et al.*, 2019), (Tang *et al.*, 2020). For TiO_2, the surface area depends on the sol-gel synthesis conditions; areas between 50-90 $m^2\ g^{-1}$ have been reported in the literature (Tahir *et al.*, 2020).

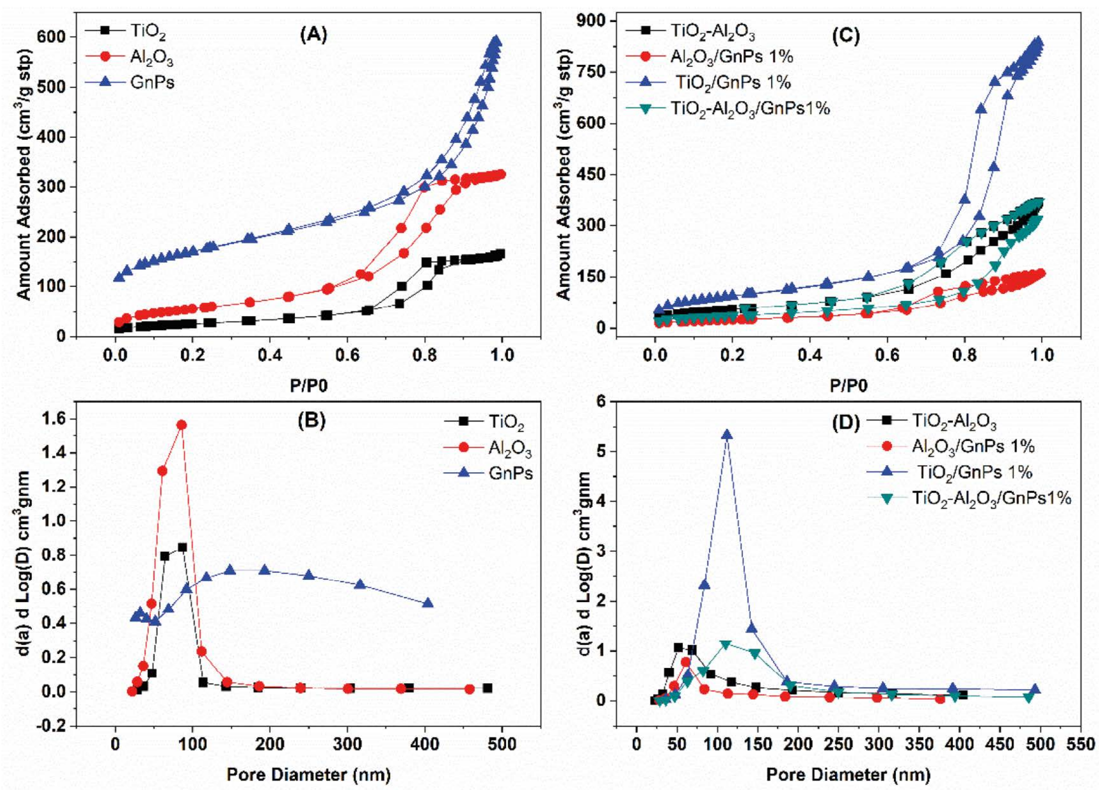

Figure 1. Nitrogen adsorption/dissociation as a function of relative pressure (P/P0) nitrogen for reference materials (A), incorporation of GnPs (C) and pore size distribution of reference (B) and with GnPs (D).

Type (IV) isotherm for TiO_2-Al_2O_3 composite was identified, the pore shape was modified compared to pure composites (TiO_2 and Al_2O_3), and the hysteresis loop was H3. The incorporation of 1% GnPS (TiO_2/GnPs 1%) showed the same isotherm and hysteresis loop as the TiO_2 sample. There was no change in the isotherm type in the case of the Al_2O_3/GnPs 1% sample, but the hysteresis type changed from H3 to H4. This loop is caused by the presence of micro and mesoporosity which is typically observed in carbon-based materials. The combination of the three species resulted in an isotherm type (IV) and a hysteresis loop H2b which represents materials with complex porosity and pore networks connected with bottle-shaped pores (Figure 1C).

Pore diameter distribution was unimodal for Al_2O_3/GnPs 1% and TiO_2/GnPs 1%, with an average diameter of 61 and 112 nm, respectively. TiO_2-Al_2O_3 and TiO_2-Al_2O_3/GnPs 1% materials presented bimodal distribution with diameters 53-70 and 110-148 nm, respectively (Figure 1D). The addition of GnPs 1% to TiO_2 decreased the surface area by 3.2%; however, this same addition to Al_2O_3 was favored and increased by 68%. The TiO_2-Al_2O_3 composite presented a higher surface area (199 m^2 g^{-1}) than the pure TiO_2. However, the surface area for the TiO_2-Al_2O_3/GnPs 1% composite (131 m2 g-1) is lower to that of TiO2-Al2O3 material since TiO2-Al2O3 was formed and grown on agglomerated GnPs (Table 1).

Results of the X-ray diffraction analysis of the TiO_2, Al_2O_3, and GnPs samples are shown in Figure 2A. GnPs diffraction pattern corroborated that they are grade C graphene nanoplatelets (See annexes). According to the diffraction signal at 2θ=25.5°, corresponds to a crystalline graphitic material with few layers. Aluminum oxide showed diffraction signals at 2θ=37.38 and 45.98°, characteristic of the γ-Al_2O_3 phase (Campos *et al.*, 2013). Titanium oxide presented diffractions at 2θ=25.39, 38.03, and 48.19°, characteristic of the anatase phase according to JCODS Chart No. 21-1272 (Urbano *et al.*, 2011) .

Results of the binary samples (Al_2O_3/GnPs 1wt%, TiO_2/GnPs 1 wt %) obtained by the sol-gel method are shown in Figure 2B. Characteristic diffraction signals of GnPs and γ-Al_2O_3 were identified for the Al_2O_3/GnPs 1% sample. In case of TiO_2/GnPs 1wt% sample, anatase diffraction signals were superimposed with the signals of GnPs at 2θ=25°. A weak signal at 2θ=30.88° corresponding to the brookite structure was also observed.

Table 1. Specific Brunauer-Emmett-Teller (BET) area, volume, and pore size of the reference samples and obtained composite materials.

Sample	Surface area (m^2 g^{-1})	Pore volume (cm^3 g^{-1})	Pore size (nm)
TiO_2	93	0.25	7
Al_2O_3	203	0.50	6
GnPs	598	0.72	8
TiO_2-Al_2O_3	199	0.55	8
TiO_2/GnPs 1%	90	0.24	6
Al_2O_3/GnPs 1%	341	1.25	10
TiO_2-Al_2O_3/GnPs 1%	131	0.48	10

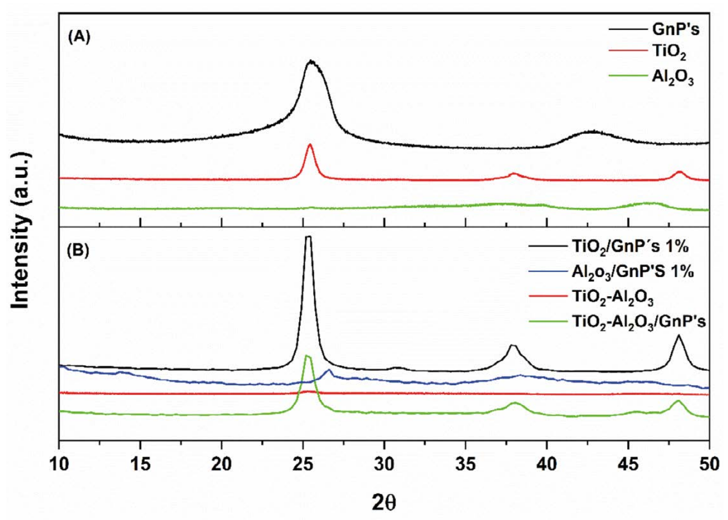

Figure 2. Diffractograms for reference samples (A) and samples with 1% GnPs (B).

Figure 2B shows diffractograms of the ternary and binary mixture of the materials. Both mixtures showed characteristic patterns of anatase phase of TiO_2 at 2θ=25.35, 38.13, and 48.19°, a slight diffraction signal at 2θ=45.33° corresponding to γ-Al_2O_3. In addition, overlap with the signal corresponding to the 002 planes of GnPs at 2θ=25.35° was also identified. The intensity of the diffraction signals corresponding anatase phase of the ternary mixture was much higher than that of the binary sample because of the more highly ordered phases. Table 1 shows the crystal size of the materials obtained from the Scherrer equation (Scherrer & Hillberry, 1978).

According to the data in Table 2, the addition of GnPs to TiO_2 stabilizes the brookite structural phase and decreases the crystal size. The Al_2O_3/GnPs 1% sample showed no significant change in the gamma-alumina structure, except the increase in the crystal size. Finally, in the TiO_2-Al_2O_3/GnPs 1% sample, despite with the high concentrations of aluminum oxide and simultaneous presence of the graphene crystalline phase, the anatase structure prevails without altering the interplanar distance of the graphitic layers. Therefore, to incorporate GnPs into the TiO_2-Al_2O_3 mixture, serves as stabilizing support.

The field emission scanning electron microscopy technique was used and provided highly focused images, through which morphology and constituents' arrangement of the TiO_2/GnPs, Al_2O_3/GnPs, and TiO_2-Al_2O_3/GnPs materials, were studied.

Figure 3A shows a 4.4 μm cluster, which is constituted by graphitic piles of length between 300 and 44 nm (Figure 3B).

Figure 4A shows TiO_2 dispersed clusters of between 1 and 11 μm, formed from semispherical conglomerates of titanium oxide. Figure 4B shows these conglomerates with semisphere sizes between 1.7 and 1.8 μm.

Micrographs of the Al_2O_3/GnPs 1% sample suggest a morphology characterized by irregular particles of different sizes, see Figure 5A. The observation and arrangement of the GnPs in Figure 5B are difficult to determine; Al_2O_3 probably covers them.

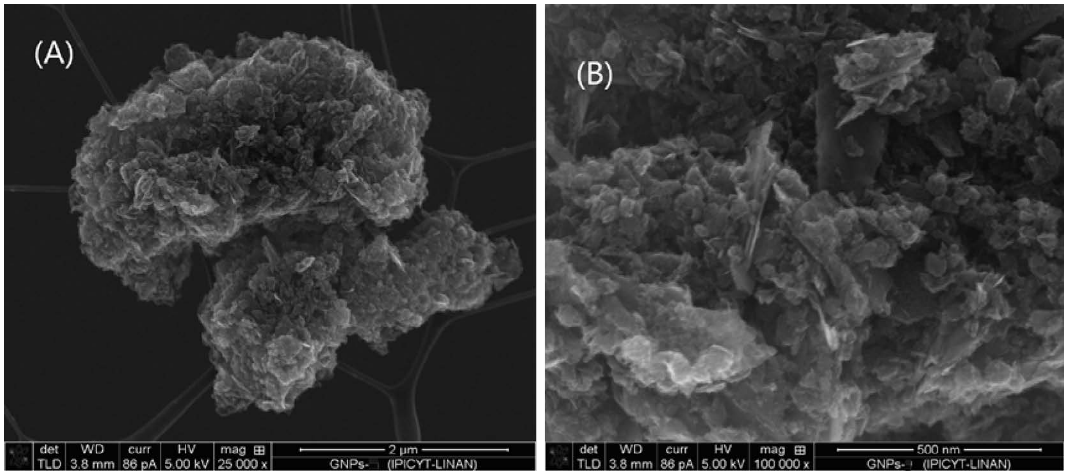

Figure 3. SEM micrographs of graphene nanoplatelets (GnPs) at 25 000X and 100 000X magnification.

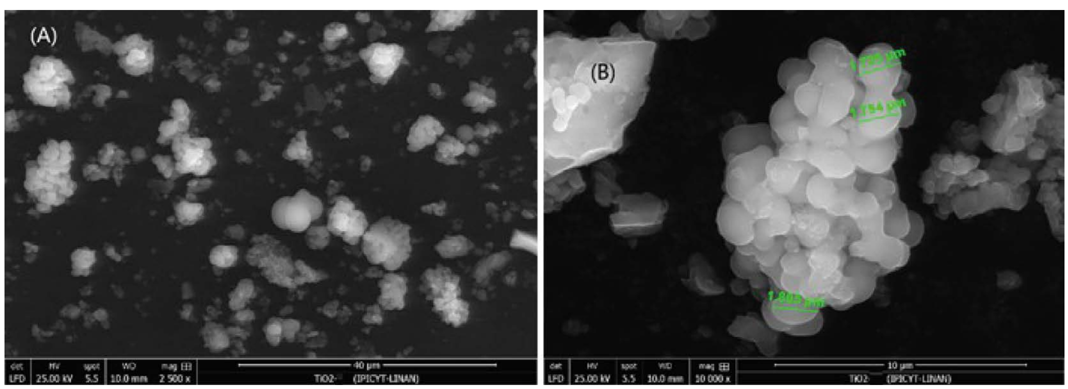

Figure 4. SEM micrographs of TiO_2 at 2500X magnification in A and 20 000X in B.

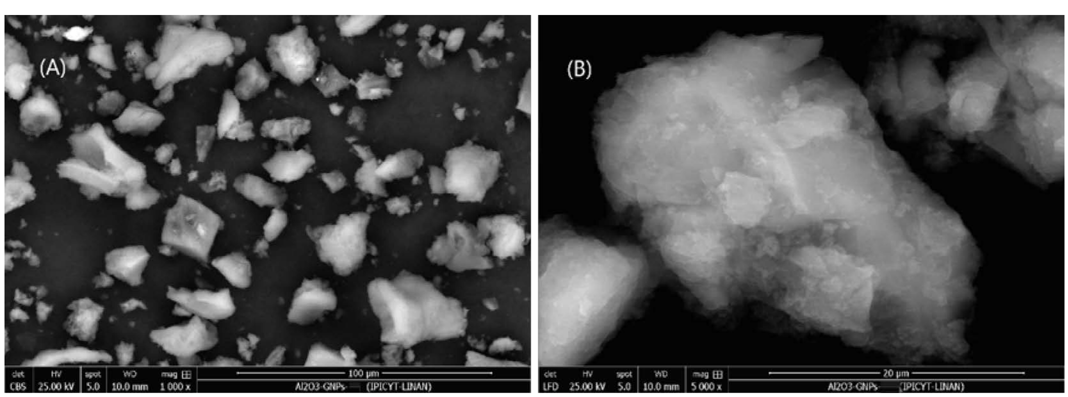

Figure 5. SEM Micrograph of Al_2O_3/GnPs 1% at 2000X magnification in A and 5000X in B.

As in Figure 6A is shown, titanium oxide was grown on the surface of GnPs. The morphology of the accumulated TiO_2 particles is semispherical, with sizes between 501 and 286 nm (Figure 6B).

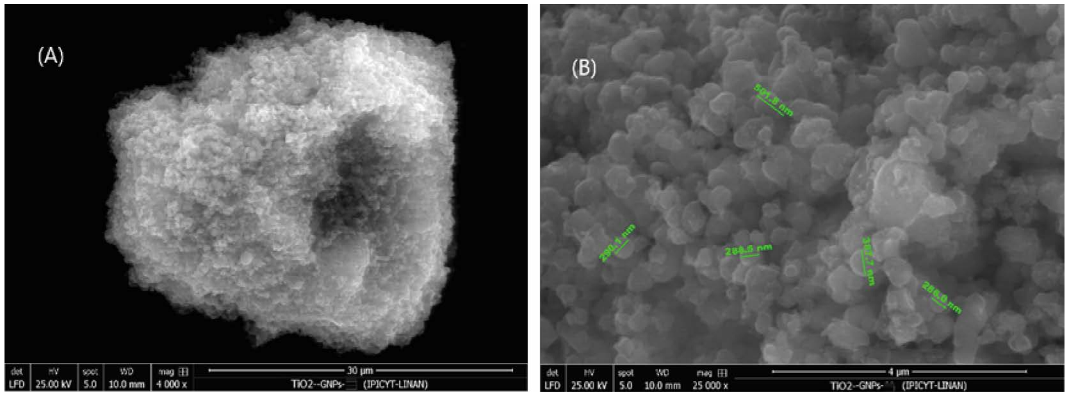

Figure 6. SEM micrograph of TiO_2/GnPs 1% at 4000X magnification in A and 25000X in B.

Figure 7A shows SEM micrograph of TiO_2-Al_2O_3/GnPs 1%, it can be observed that part of the graphene nanoplatelets was well dispersed in the TiO_2-Al_2O_3 binary mixture. However, no preferential arrangement of the nanoplatelets in the binary matrix was observed in both vertical and horizontal directions (Figure 7B).

Results of Uv-Vis analysis are shown in Figure 8. The Al_2O_3 UV-Vis spectrum corresponds to typically insulating material. The TiO_2 spectrum is like that reported in the literature, with an absorption maximum at 400 nm wavelength (Raja *et al.*, 2006). The corresponding GnPs films, in turn, showed a linear trend in absorbance.

Incorporation of GnPs into TiO_2 resulted in a higher absorption capacity than that of the pure GnPs. However, the GnPs have a different effect on alumina, *i.e.*, the absorption capacity was favored, which is a function of the GnPs concentration in sample.

On the other hand, the titanium-aluminum mixture showed a shift to lower wavelengths (370 nm), according to the absorption of the titanium oxide reference. The sample TiO_2-Al_2O_3/GnPs 1wt% presented an absorbance near 420 nm, slightly higher than TiO_2. Based on the spectra, the band gap of all materials was calculated using Tauc's method (Tauc, 1987); the results are shown in Table 2.

TiO_2 band gap resulted to be close to that reported in the literature (Kim *et al.*, 2011), and decreased when GnPs were incorporated. In the case of Al_2O_3-GnPs, calculated band gap was like that obtained for titanium oxide. There is no change in the band gap when

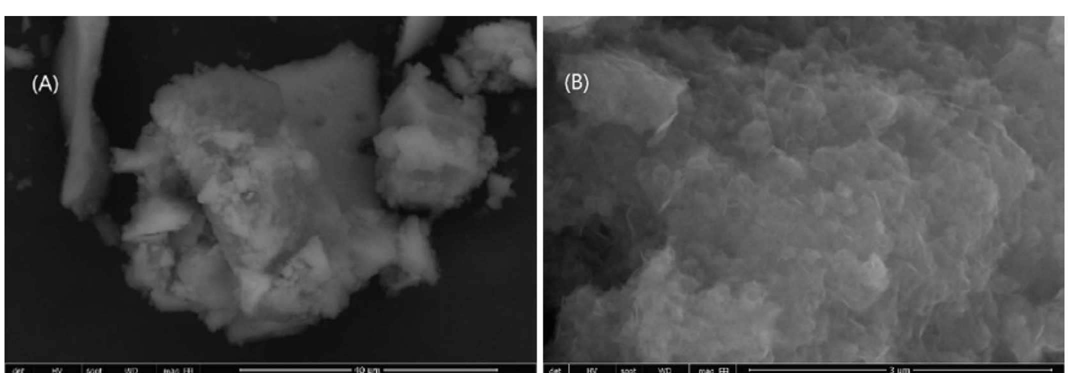

Figure 7: SEM micrograph of TiO_2-Al_2O_3/GnPs 1% at 2500X magnification in A and 40 000X in B.

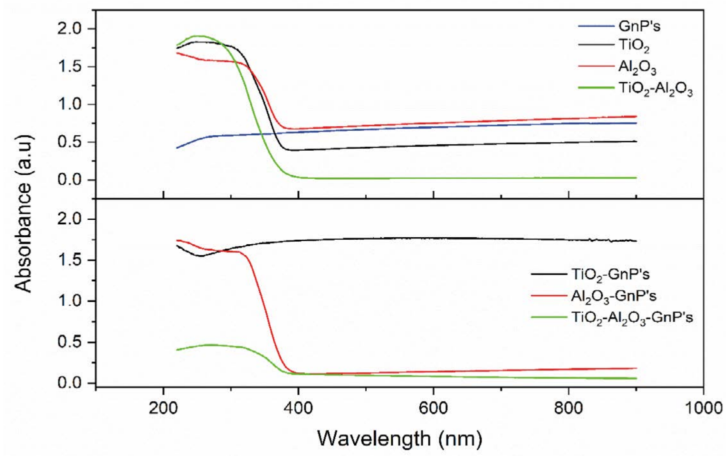

Figure 8. UV-Vis spectra for powder samples.

integrating aluminum and titanium oxide together. However, 1% GnfPs in the binary mixture reduced the gap by 6%.

A solution was prepared at 30 ppm of Diuron, from which 250 mL was taken and poured into the photoreactor, and then 0.01mg of the catalyst were added as well. The equilibrium stage consisted of keeping the catalyst in contact with the Diuron solution during 1 h. Subsequently, a 5 mL aliquot was taken as a reference (concentration at zero time). Once this stage was completed, the simulator was turned on; aliquots were taken at 5, 15, 30, 30, 45, 60, 60, 120, 180, 240, and 300 min of reaction time, UV-Vis reading followed the contaminant. A calibration curve was previously performed.

Figure 9 shows results of the normalized Diuron concentration in the photodegradation process. Upon probing the reference materials, considerable decrease in concentration due to the photolysis was observed, but alumina presented the least decrease. Binary mixtures presented slow concentration decrease w.r.t. TiO_2-Al_2O_3/GnPs 1%, also showed the smallest decrease in concentration.

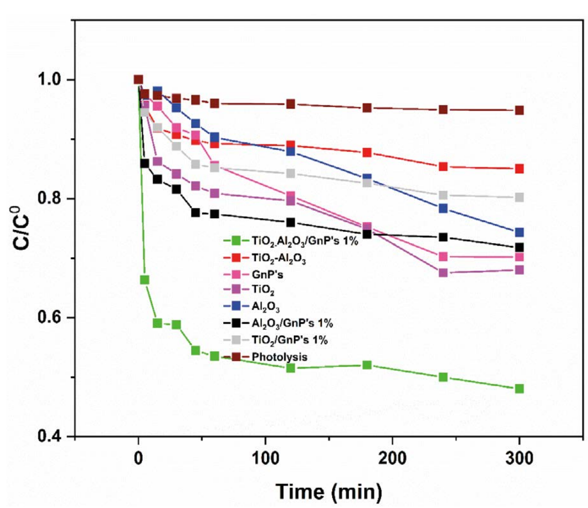

Figure 9. Normalized Diuron concentration *vs.* reaction time for reference materials, binary, and ternary samples.

TiO_2-Al_2O_3/GnPs 1% showed the highest conversion, while the lowest when TiO_2-Al_2O_3 was probed. The kinetic constant (k) was calculated to compare the photocatalytic activity of all materials as well. The results are shown in Table 2.

Finally, the TiO_2-Al_2O_3/GnPs 1% sample presented a fast and constant drop out of convention throughout the reaction. According to the values of the constant kinetic k, the activity showed the following trend: TiO_2-Al_2O_3 /GnPs 1%.>Al_2O_3/GnPs 1% >TiO_2> TiO_2/GnPs 1%>TiO_2-Al_2O_3>GnPs>Al_2O_3.

If it's assumed that due to the interaction among the three species in ternary material (TiO_2-Al_2O_3/GnPs) band gap was narrowed, and that the recombination of electron-hole pairs was apparently mitigated by Al_2O_3 presence, then the photocatalytic activity of ternary mixture was favored by a type II heterojunction.

Such heterojunction consists of interactions between two semiconductors with very similar band gaps. Consequently, photo-excited electrons are transferred from the conduction band of component A (GnPs) to the conduction band of component B (TiO_2), and the holes can travel in the opposite direction of the electrons. In addition, the insulator (Al_2O_3) helps to avoid recombination by enhancing electron trapping. This type of interface reduces the band gap and generates an efficient charge separation by decreasing the recombination rate, and then improving the photocatalytic activity in the materials. Figure 10 schematizes the heterojunction propitiated for the ternary material.

Table 2. Crystal size and band gap of materials, and herbicide conversion rates.

Sample	Crystal size (nm)	Band gap (eV)	k (h^{-1})[a]	x (%)[b]
TiO_2	3.19	3.0	0.55	32
Al_2O_3	11.08	---	0.06	26
GnPs	-	1.7	0.17	30
TiO_2-Al_2O_3	7.03	2.5	0.30	15
TiO_2/GnPs 1%	10.76	3.0	0.31	20
Al_2O_3/GnPs 1%	10.36	3.0	0.60	28
TiO_2-Al_2O_3/GnPs 1%	9.05	2.8	1.81	52

[a] Apparent kinetic constant
[b] Conversion (x) to 300 min of reaction time

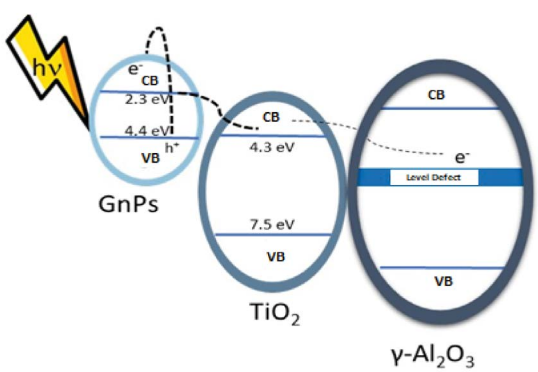

Figure 10. Scheme of photoactivity and heterojunction of ternary material.

CONCLUSIONS

The sol-gel method allowed the synthesis of the mixed oxide (titanium oxide-aluminum oxide). The presence the GnPs crystal structure was reflected in the texture of the resulting material. The interactions between the species had a positive effect on the electronic properties of the mixed oxide. The incorporation of GnPs into the mixed oxide influenced the fine structure of the product. However, the textural properties were modified by increasing the surface area and incorporating new porosity. The distribution of GnPs in the mixed oxide was random. Electronic properties such as band gap were slightly reduced. The properties modified by GnPs presence were of great relevance in the photoactivity because a heterojunction regales the electron-hole pairs and, consequently, the higher activity yield in the degradation of Diuron.

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ZnO nanoparticles synthesized by chemical precipitation to increase germination and bioactive compounds in sprouts of *Raphanus sativus* L.

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ABSTRACT

Objective: To examine how priming radish (*Raphanus sativus* L.) sprouts with zinc oxide nanoparticles (NPs-ZnO) affects their germination, photosynthetic pigments, phenolic compounds, and zinc content.

Design/methodology/approach: We evaluated five NPs-ZnO treatments and a control sample with four replications under a completely randomized design.

Results: Sprouts treated with NPs-ZnO showed increased germination variables, photosynthetic pigments, phenolic compounds, and zinc content as compared to untreated radish sprouts.

Study limitations/implications: It is hard to establish a response model for the effects of NPs since their shape, size, surface charge, chemical composition, and concentration may have a differentiated impact on seed germination.

Findings/conclusions: Using NPs-ZnO could be an effective way to enrich crops, since the passage of Zn through plant tissues will cause an accumulation of this micronutrient.

Keywords: *Raphanus sativus*, Nanofertilizer, Zinc oxide.

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INTRODUCTION

Due to the growing demand for agricultural products, boosting crop production and productivity is a great challenge worldwide. To increase sustainability in modern agriculture, we must introduce innovative technologies (Rai-Kalal & Jajoo, 2021). Using and applying nanoparticles to stimulate plant growth and modulate plant physiological responses is a relatively recent practice. Nanopriming can improve seed germination and reduce aging (Mahakham *et al.*, 2017) by triggering metabolic processes usually activated during the early phase of germination, which in turn improves germination and emergence

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rates, thus promoting the sustenance of seeds under different abiotic stresses (Singh *et al.*, 2020). Moreover, zinc oxide nanoparticles (NPs-ZnO) have shown positive effects on the growth of mung beans, chickpeas, cucumber, alfalfa, tomato, and other crops (Mahajan *et al.*, 2011; De la Rosa *et al.*, 2013). Radish (*Raphanus sativus*) (Brassicaceae) is an annual horticultural crop consumed worldwide because of its nutritional value (Xie *et al.*, 2018), and has proven to be a healthy food for human diet and health. It is a widely studied species that has been incorporated as a scientific biological model to describe the toxic effects of some chemical substances on germination (Taladrid & Espinosa, 2021). Our objective was to use NPs-ZnO, synthesized through a conventional method of controlled chemical precipitation, to improve germination, growth, bioactive compounds, and zinc concentration in radish sprouts.

MATERIALS AND METHODS

Zinc oxide nanoparticles

We used zinc oxide nanoparticles (NPs-ZnO), measuring between 67 and 71 nm, with a purity of 99%, white color, and structurally hemispherical and polygonal shapes. We synthesized the NPs-ZnO through a controlled chemical precipitation method following Galindo-Guzmán *et al.* (2022), and using zinc acetate $Zn(CH_3COO)_2$ as a precursor.

NPs-ZnO applied treatments

In order to test how different concentrations of NPs-Zno affect seed viability, we applied six treatments, divided into one control sample (deionized water), and 25, 50, 75, 100, and 125 mg L^{-1} NPs-ZnO doses.

Seed germination

We used radish seeds of the Champion variety (Hortaflor®). The seeds were disinfected with 75% ethanol and washed with deionized water. We performed germination tests by placing ten seeds per treatment in 90 mm Petri dishes containing Whatman filter paper. The treatments were applied only once during imbibition, for which we added 5 mL of NPs-ZnO suspension per treatment with a pipette. Four replications per sample were prepared. The Petri dishes were sealed with Parafilm paper and placed in a Novatech CA-550 artificial growth chamber at 22 ± 2 °C for seven days (Don *et al.*, 2013).

Parameters evaluated during the experiment

The trial was completed according to the International Seed Testing Association (ISTA) guidelines, which consider the following parameters: percentage of vigor and germination (%), radicle length (cm), and biomass accumulation in the plumule and radicle in milligrams per sprout.

Determining chlorophyll and carotenoid content

Chlorophyll and carotenoids were determined following the Lichtenthaler & Wellburn (1983) method. We recorded absorbance readings at 665, 649, and 470 nm using a Jenway 7305 UV-visible spectrophotometer.

Total phenol content

The total phenolic compounds were quantified using the Folin-Ciocalteu method (Singleton *et al.*, 1999). Results were reported in gallic acid equivalent mg on a 100 g fresh weight basis (mg equiv AG 100 g^{-1} FW).

Zinc

Zn was quantified in a hydride generation atomic absorption (AA) spectrophotometer (Lab Wrench Varian SpectrAA®, 220Fast model). We presented the results in μg kg^{-1} of dry weight.

Statistical analysis

The experiment used a completely randomized design with six treatments and four replications. We verified the data normality and homogeneity of variances for each response variable with the Bartlett and Kolmogorov-Smirnov test (Bartlett, 1937; Steinskog *et al.*, 2007). Results were evaluated by analysis of variance and comparison of means with Tukey's test ($p \leq 0.05$), using the statistical package (SAS) version 9.4. We normalized the variables reported as percentages (vigor and germination) by applying the arcsine square root transformation (Steel & Torrie, 1960).

RESULTS AND DISCUSSION

Effects of NPs-ZnO on radish seed vigor and germination

Our results show that the NPs-ZnO did not significantly affect ($P \leq 0.05$) the seeds' vigor, which remained similar to the control sample (Table 1). The best dose for the germination variable was 100 mg L^{-1}, with a germination average of 90%, 29.42% higher than the control sample. Meanwhile, no significant statistical differences ($P \leq 0.05$) were obtained with the 0, 25, 50, 75, and 125 mg L^{-1} doses. It is hard to establish a response model of the effects of NPs because their shape, size, surface charge, chemical composition, and concentration can have a differentiated impact on seed germination and seedling growth (Ahmad *et al.*, 2021). Moreover, molecular factors such as kinases acting as DNA "checkpoints" and links between genotoxic stress and seed aging also affect germination (Taladrid & Espinosa, 2021).

Fresh weight of plumule and radicle

An increase in the fresh weight of the plumule and radicle was observed in the sprouts prepared with NPs-ZnO. The maximum increase of fresh weight occurred in the 100 mg L^{-1} dose, which surpassed the control sample by 15.49% for the plumule and 20.57% for the radicle. This behavior relates to the increase in germination percentage, which ultimately increases biomass production. The increase in biomass could be a result of the Zn ions present in the seeds treated with NPs-ZnO. Said ions play a vital role in the biosynthesis of natural auxin (indole-3-acetic acid) in plants, which consequently activates cell division and enlargement (Ali & Mahmoud, 2013). These results concur with previous research by Rai-Kalal & Jajoo (2021), who reported that Zn plays an effective role in increasing biomass in wheat seedlings.

Radicle length

The NPs-ZnO significantly affected ($P \leq 0.05$) the radicle length (Table 1). In the 25 mg L^{-1} dose, the length was longer than in the control sample (7.10 cm), with a numerical difference of 14.51%. For the 50 mg L^{-1} dose, the length obtained was 6.22 cm, statistically equivalent to the control sample (6.20 cm), with a numerical difference of 0.32%. The 75 and 125 mg L^{-1}

treatments were statistically equivalent. An increase also occurred with the 100 mg L-1 dose, which reached a root length of 9.62 cm. Several studies have shown that root elongation improved in wheat, sweet sorghum, and soybean when exposed to NPs-ZnO as compared to control samples (López-Moreno et al., 2010; Elhaj Baddar & Unrine, 2018; Naseeruddin et al., 2018). Nanoparticles enter the seed coat through its pores, causing a greater penetration of water molecules and inducing the enzymatic activity that generates reactive oxygen species (ROS), and that degrades starch to physiologically improve seed germination (Mahakham et al., 2017).

Photosynthetic pigments

Chlorophyll content has a positive relation to photosynthetic rate. Therefore, a change in total chlorophyll content can be considered an indicator of plant health. As observed in Figure 1a, the sprouts treated with NPs-ZnO presented a significant difference ($P \leq 0.05$). When applying the 25 mg L^{-1} dose, there was a chlorophyll increase of 4.74% in comparison with the control sample. The maximum increase was observed in the 50 mg L^{-1} dose, with an average difference of 32.03% as compared to the control sample. The 75 and 100 mg L^{-1} doses were statistically equivalent to each other. However, we registered a decrease of 13.96% in the 125 mg L^{-1} dose as compared to the control sample. Zn could explain the positive effects of NPs-ZnO on chlorophyll content because it plays a vital role in chlorophyll biosynthesis by protecting the sulfhydryl group of the chlorophyll molecule (Cakmak, 2008). Zn also plays a role in the development of chloroplasts and takes part in the repair process of photosystem II by recycling the damaged D1 protein (Hänsch & Mendel, 2009). However, Acharya *et al.* (2020) argue that using nanoparticles at high

Table 1. Means comparison in germination, vigor, plumule and radicle fresh weight, and radicle length of radish sprouts when treated with NPs-ZnO. *Values with different letters within the same column indicate a significant difference according to Tukey's test ($p \leq 0.05$). (±) standard deviation.

Treatment NPs-ZnO (mg L^{-1})	Vigor (%)	Germination (%)	Fresh weight of plumule (mg)	Fresh weight of radicle (mg)	Radicle length (cm)
0	69.53 ± 4.06^{a}	69.53 ± 4.06^{b}	55.90 ± 2.41^{bc}	26.30 ± 0.57^{b}	6.20 ± 0.31^{c}
25	63.80 ± 6.04^{a}	61.77 ± 3.32^{b}	60.98 ± 6.34^{b}	22.86 ± 1.16^{cd}	7.10 ± 0.43^{bc}
50	69.53 ± 4.06^{a}	69.53 ± 4.06^{b}	47.66 ± 1.53^{d}	23.85 ± 0.57^{bc}	6.22 ± 0.22^{c}
75	69.53 ± 4.06^{a}	69.53 ± 4.06^{b}	45.03 ± 1.41^{d}	21.25 ± 0.55^{d}	8.05 ± 0.12^{b}
100	67.50 ± 4.69^{a}	89.99 ± 0.00^{a}	69.18 ± 4.17^{a}	31.71 ± 1.26^{a}	9.65 ± 0.35^{a}
125	67.50 ± 4.69^{a}	69.53 ± 4.06^{b}	48.28 ± 3.21^{cd}	20.39 ± 1.91^{d}	7.27 ± 0.83^{b}

concentrations in seed priming will cause toxicity in the sprouts, which can be observed in a reduction of photosynthetic pigments.

Carotenoids play a vital role in the photoprotective mechanism of plants. Figure 1b shows the effect of exposure to NPs-ZnO on total carotenoid content in radish sprouts. For the 0, 25, 50, 75, and 100 mg L^{-1} doses, no statistically significant differences ($P \leq 0.05$) were obtained, but the 50 mg L^{-1} dose registered the highest carotenoid content (7.95 mg g^{-1} PF). Meanwhile, the 125 mg L^{-1} dose presented a decrease in comparison with all treatments.

Total phenol content

High doses of NPs-ZnO had a significant effect on the synthesis of total phenolic compounds in the sprouts (Figure 2). The concentration of total phenolic compounds in radish extracts ranged from 95.88 to 168.37 mg AG 100 g^{-1}. The maximum value (168.37 mg AG 100 g^{-1}) was recorded after applying a 50 mg L^{-1} NPs-ZnO dose. The main compounds responsible for the defense mechanism in plants are secondary metabolites of a phenolic nature, which also improve the nutritional value of crops due to their known antioxidant activity.

Zinc

As the NPs-ZnO doses increased, the Zn content in the radish sprouts also increased (Figure 3). In the control sample, the amount of Zn was 45.95 μg kg^{-1} DW. The maximum

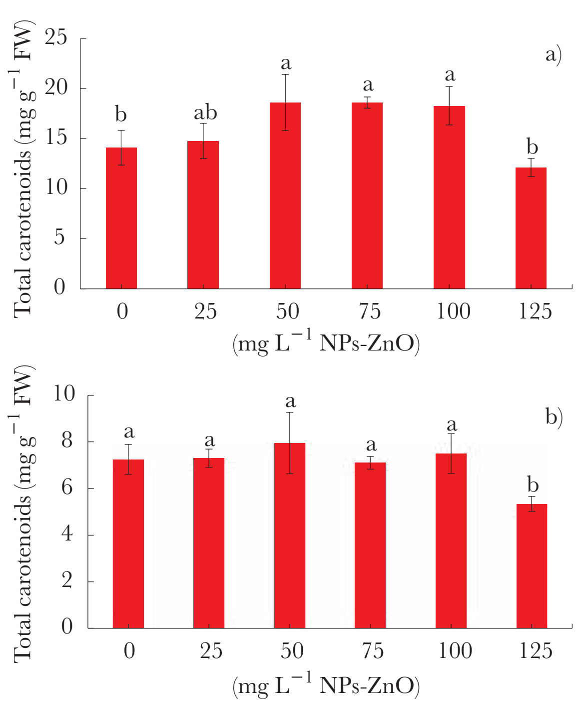

Figure 1. Chlorophyll content (a) and carotene content (b) in radish sprouts treated with NPs-ZnO. The vertical bars indicate the standard deviation of the mean. Different letters indicate a significant difference according to Tukey's test ($P \leq 0.05$).

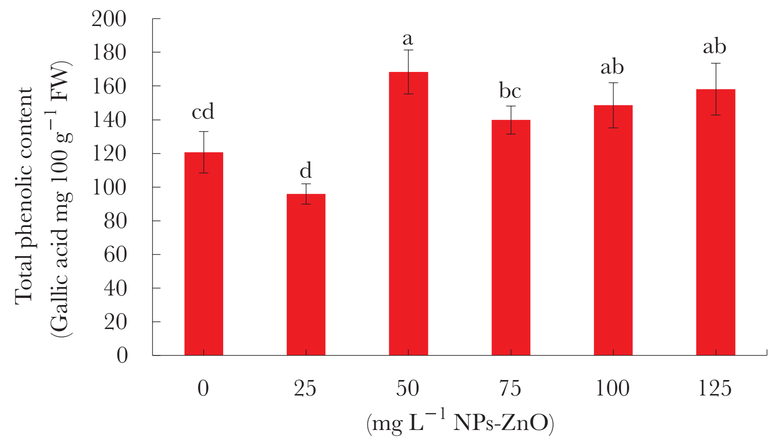

Figure 2. Total phenol content in radish sprouts when treated with NPs-ZnO. The vertical bars indicate the standard deviation of the mean. Different letters indicate a significant difference according to Tukey's test ($P \leq 0.05$).

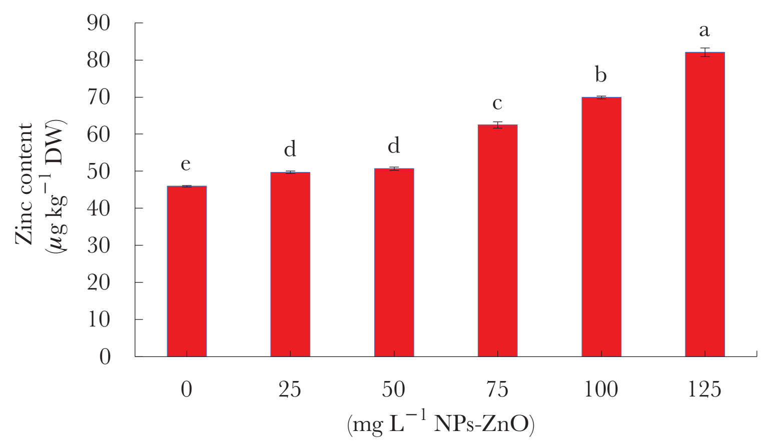

Figure 3. Zinc content in radish sprouts after applying NPs-ZnO. The vertical bars indicate the standard deviation of the mean. Different letters indicate a significant difference according to Tukey's test ($P \leq 0.05$).

zinc content occurred in the 125 mg L^{-1} dose (82.07 μg kg^{-1}). Although this concentration is favorable, we must point out that photosynthetic pigments decrease. Therefore, using higher concentrations can be harmful. According to the Instituto de Medicina (2001), the recommended intake of Zn for adults is 8 mg day^{-1} for women and 11 mg day^{-1} for men. The accumulation of Zn in radish sprouts could complement the daily intake of this micronutrient.

CONCLUSIONS

Radish seeds responded positively to NPs-ZnO treatments, which showed different efficacy depending on the parameters tested. In this regard, using NPs-ZnO could be an effective way to enrich crops, since the passage of Zn through plant tissues will cause its accumulation.

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AP AGRO PRODUCTIVIDAD

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Evaluation of the efficiency of rhizobial biofertilizers in guava crop (*Psidium guajava* L.) using statistical quality control

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ABSTRACT

Objective: Apply statistical quality control to evaluate the efficiency of biofertilizers for the sustainable development of crops of guava (*Psidium guajava*) in Chiapas, Mexico.

Design/methodology/approach: Physicochemical parameters were analyzed to determine soil fertility in guava crops. The structure and diversity of the bacterial community was studied by structural metagenomics. A quality control statistical analysis was applied to determine the effect of biofertilization on the growth and production of the guava plant crop.

Results: The soils were silty clay and had variations in pH and cation exchange capacity. Guava plants inoculated with PGPB rhizobial bacteria had higher growth and number of fruits. The cause-effect analysis determined that soil nutrients, plot phytotechnical management and bacterial diversity significantly influence the effectiveness of biofertilizers.

Limitations on study/implications: Atypical climatic variations in the region, deficient pest control and high genetic variability in plants influence the productivity of guava crops. It is important to explore a larger area of crops to detect more cause-effect elements.

Findings/conclusions: Experimental statistical analyzes and quality control are effective tools to determine the efficiency of biofertilizers in fruit crops.

Keywords: Guava crop, biofertilizers, quality control, rhizobial bacteria.

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INTRODUCTION

Conventional agricultural systems in Mexico demand the use of large amounts of fertilizers and a wide variety of agricultural inputs in order to maintain crop yields (Arriaga *et al.*, 2017). The constant and systematic use of these agricultural inputs causes alterations in the soil fertility and unfortunately severe damage to human health and

Image by Rajesh Balouria at Pixabay

the environment (Mozumder and Berrens, 2007). Biofertilizers emerge as a sustainable biotechnological alternative to reduce the use of fertilizers and improve the yield of crops (Itelima *et al.*, 2018). Biofertilizers are beneficial microorganisms that promote plant growth and improve soil health. Bacteria known as plant growth promoters (PGPB) are commonly used as biofertilizers (Vessey, 2003). Among the PGP bacteria, the *Rhizobium* genus stands out for its ability to fix nitrogen, solubilize phosphate, among other biological qualities (Chen *et al.*, 2003). Several authors have reported the positive effect of biofertilization on different types of agricultural crops. However, information on the application of biofertilizers formulated with *Rhizobium* bacteria in fruit crops is very scarce (Rincón-Molina *et al.*, 2022). In southern Mexico, fruit growing is one of the most important agricultural activities since it strengthens food security and the economy at a regional and national level. The cultivation of the guava in the metropolitan region of Chiapas, Mexico, has increased in recent years, becoming a strategic crop with high agricultural and socioeconomic potential (Rincón-Molina *et al.*, 2022). Guava (*Psidium guajava* L.) belongs to the family Myrtaceae, which includes more than 70 genera and approximately 2,800 species (Vitti *et al.*, 2019). Guava fruit is rich in antioxidant activity, maybe due to its high vitamin C content. This crop requires important conditions to achieve good yields, mainly that the soils have a good nitrogen and potassium content (Montes *et al.*, 2016). Local guava farmers apply large amounts of chemical fertilizers to increase crop yields. However, despite this agricultural practice, the desired results have not been achieved and, on the contrary, the deterioration of the soil and the contamination of the environment have increased. The application of biofertilizers in guava crops emerges as an important agrobiotechnology for the improvement and sustainable development of this type of fruit crops (Mosa *et al.*, 2021). In agricultural production systems, the modern use of quality control tools makes it possible to identify, correct and improve those factors that negatively influence crop yields (Miroslav *et al.*, 2016). In this way, it is possible to apply the continuous improvement of the processes. In this work, statistical designs and cause-effect quality control (Ishikawa diagram) were applied to evaluate the efficiency of rhizobial biofertilizers applied in a guava crop

MATERIALES Y MÉTODOS

Experimental site

The biofertilization trials were carried out in an agricultural field of guava cultivation called “Rio Grande” located in the Ribera de Monte Rico in Chiapas, Mexico (16° 71’ 04” N and 93° 03’ 12” W), at an average height of 400 m.a.s.l (Figure 1). The inoculation tests and the different samplings were carried out in an area of 2100 m^2.

Soil characterization

Rhizospheric soil samples were collected from five randomly located points in the guava experimental plot 20 cm deep from the top layer. The samples collected from each point were mixed to obtain a single representative sample that was used for a chemical fertility analysis. The pH and electric conductivity (EC) were measured by using a digital pH meter. Cation exchange capacity (CEC) was determined according to the Official Mexican

Figure 1. Location of the experimental plot of guava cultivation.

Standard NOM-021-SEMARNAT-2000. The determination of total nitrogen and total carbon was carried out by using a FLASH 2000® auto-analyzer. Total phosphorus was determined with the solubilization method of $HNO_3/HClO_4$ (Rincón *et al.*, 2020). Additionally, soil samples were obtained to study the structure and diversity of the bacterial community through a metagenomic analysis (Rincón-Molina *et al.*, 2022).

Bacterial strain used in biofertilization trials

The bacterial strain *Sinorhizobium mexicanum* ITTG-R7 (DQ411930) was used in biofertilization trials. The strain was isolated from the legume *Acaciella angustissima* and is characterized by its high nitrogen fixation and plant growth promoting capacity (Rincón-Rosales *et al.*, 2021).

Experimental design for guava crop biofertilization

Inoculation trials were conducted at the experimental plot on 4-year-old guava plants, which were uniform in size and vigor, and spaced 7.0 m between the rows and 7.0 m between plants. The experimental unit consisted of one guava tree. A completely randomized design was used for the experiment with six replications of each treatment. In this experiment, the effects of five treatments on the growth of guava plants were evaluated. Treatments consisted of: T_1: (*S. mexicanum* ITTG-R7), T_2: (ITTG-R7 + Fertilizer Triple 17), T_3: (*Azospirillum brasilense* CD), T_4: (Triple 17), and T_5: [non-inoculated and non-chemically fertilized plants]. The strain *A. brasilense* CD which is commercially available, was applied as a PGPB reference. The guava plants were inoculated with *S. mexicanum* ITTG-R7 and *A. brasilense* CD strains to a concentration of 10^6 CFU mL^{-1}. Plants were inoculated with 100 mL of bacterial suspension, which was applied directly to the plant base. Every 3 months, the plants were inoculated over an experimental period of 9 months. In the same way, triple 17 fertilizers, was applied to the plants using a fertigation system. At the end of the experiment, the variables: total plant height, foliar cover, basal diameter, flowers number, fruits number and total chlorophyll were determined. Data obtained from the inoculation test were analyzed by ANOVA at a significance level of alpha=0.05 by using the statistical software Statgraphics Centurion XV.2. The comparison of means was carried out by the Tukey test ($p<0.05$).

Quality control analysis applied to the biofertilization process

Statistical analysis was applied to determine the Quality Control Limits (QCL) to the morphometric variables (plant height, foliar cover, basal diameter) and number of fruits in the guava cultivation system to know if the production is adequate or requires some improvement (Gutiérrez and De La Vara, 2013). Also, a cause-effect analysis (Ishikawa diagrams) was applied to detect the causes that affect crop yield in the guava production system and thus establish countermeasures to improve the efficiency of biofertilization (Acosta *et al.*, 2019).

RESULTADOS Y DISCUSIÓN

The soil sample obtained from the guava plot was silty clay loam in texture, shallow and with good water drainage. The pH was slightly acidic [6.1±(0.022)] and the EC was 0.82±(0.021) dSm^{-1}. Guava grows well in a wide range of soil types, but prefers well-draining soil with a pH between 5 and 7 (Shukla *et al.*, 2014). The cation exchange capacity (CEC) value was 26.32±(0.011) Cmol kg^{-1}. In relation to the fertility parameters N, C, and P. The chemical analysis indicated low levels for the content of total N [0.18%±(0.017)] and for C content [0.91%±(0.012)]. In the case of available P, high value [52.06 mg kg^{-1}±(0.328)] was determined. The C:N ratio is considered as an important parameter related to soil fertility. In this study, the soil of the guava crop had a low value of the C:N ratio (5.0±0.18) according to the Official Mexican Standard NOM-021-SEMARNAT-2000, indicating a rapid mineralization and release of N, which is available for plant uptake.

Regarding the study of the bacterial community of the rhizosphere soil of the guava crop. 16S rRNA gene sequences showed a wide diversity of bacterial species in the soil of the guava crop. Actinobacteria was the most abundant phylum (>20% relative abundance) followed by Proteobacteria, Acidobacteria, and Firmicutes (Figure 2).

Bacillaceae family dominated in soil samples. Gaiellaceae and Vicinamibacteraceae, which were also found, are related to carbon transformation in soil. Among the identified bacterial genera, *Bacillus* is characterized by its high potential as PGPB. These bacteria have the ability to produce auxins and other plant growth promoters (Rincón-Molina *et al.*, 2022).

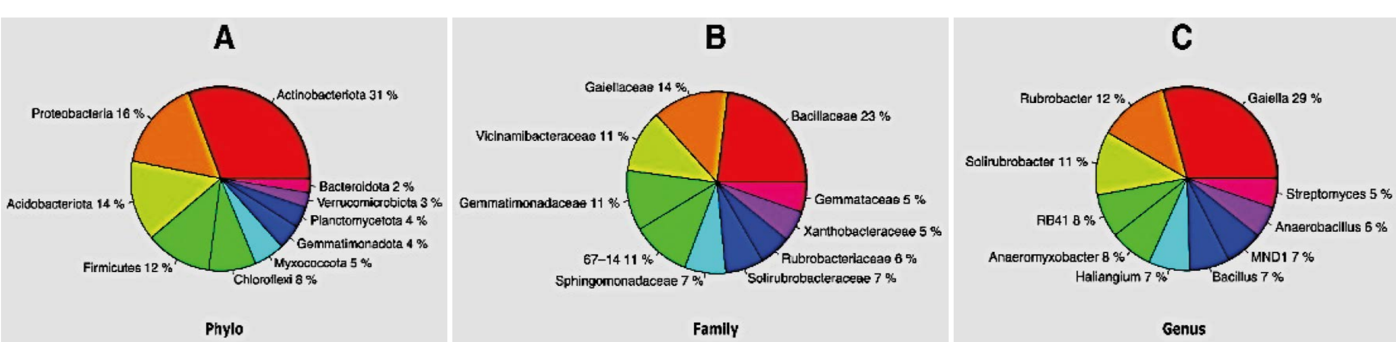

Figure 2. The bacterial community structure of the rhizospheric soil of the guava crop.

Biofertilization with diazotrophic bacteria in guava cultivation positively influenced the growth of guava plants (Table 1).

The plants inoculated with ITTG-R7 strain had a higher height compared to the other treatments. The application of the ITTG-R7 strain alone or mixed with the fertilizer (Triple 17) had a significant effect ($p<0.05$) on the foliar cover of plants. The basal diameter of the stem increased in those plants that were treated with both the fertilizer as well as with the biofertilizer. A significant effect ($p<0.05$) was observed in the plants treated with the ITTG-R7 strain in relation to the number of flowers. Trees inoculated with *S. mexicanum* ITTG-R7 strain as well as those chemically fertilized registered a significant increase in the number of fruits compared to the control plants (without fertilization/uninoculated). The amount of total chlorophyll was higher in the plants inoculated with *S. mexicanum*. The positive effect on growth in guava was observed with the inoculation of ITTG-R7 mixed with chemical nutrients. Similar effects have been found in fruit crops inoculated with *A. brasilense*. For fruit crops, it has previously been reported that combinations of biofertilizers with nitrogen and other nutrients provide the best effects on plant development, and thus, on yields (Dwivedi *et al.*, 2012). Bacteria as biofertilizers with plant growth potential when combined with inorganic and organic inputs help with crop nutrient uptake; thus the results suggest that ITTG-R7 plays a key role in nutritional improvement.

In relation to the application of quality control analysis in an agricultural production system. In the first instance, we determined the quality control limits for different growth and production parameters in guava crop plants that were biofertilized with the *S. mexicanum* ITTG R7 (Figure 3).

For the total plant height, it was determined that 82% of the plants were within the central limit (CL). For leaf cover, the CL value was 85%. Basal diameter was above 84%. For the number of fruits, a CL value of 86% was obtained and for the chlorophyll

Table 1. Effect of biofertilization on growth parameters in guava plants.

Treatment	Plant height (cm)	Foliar cover (cm)	Basal diameter	Flowers number	Fruits number	Total Chlorophyll (mg mL^{-1})
T1 (Strain ITTG R7)	369 a*	553 a	98 ab	36 a	63 a	2.8 a
T2 (ITTG R7 + Triple 17)	327 b	516 ab	107 a	29 b	53 ab	3.7 b
T3 (*A. brasilense* + Triple 17)	312 b	462 b	64 c	22 c	35 bc	2.6 b
T4 (Fertilizer Triple 17)	336 b	475 b	86 b	23 bc	50 ab	2.7 b
T5 (Negative control)	267 c	375 c	57 c	19 c	25 c	2.0 c
p-value	0.000	0.005	0.004	0.003	0.001	0.001
HSD ($p\leq0.05$)	24.7	58.5	12.1	6.73	20.0	0.31

*Mean values of six replicates. Means followed by the same letter are non-significant (HSD Tukey test, $p\leq0.05$).

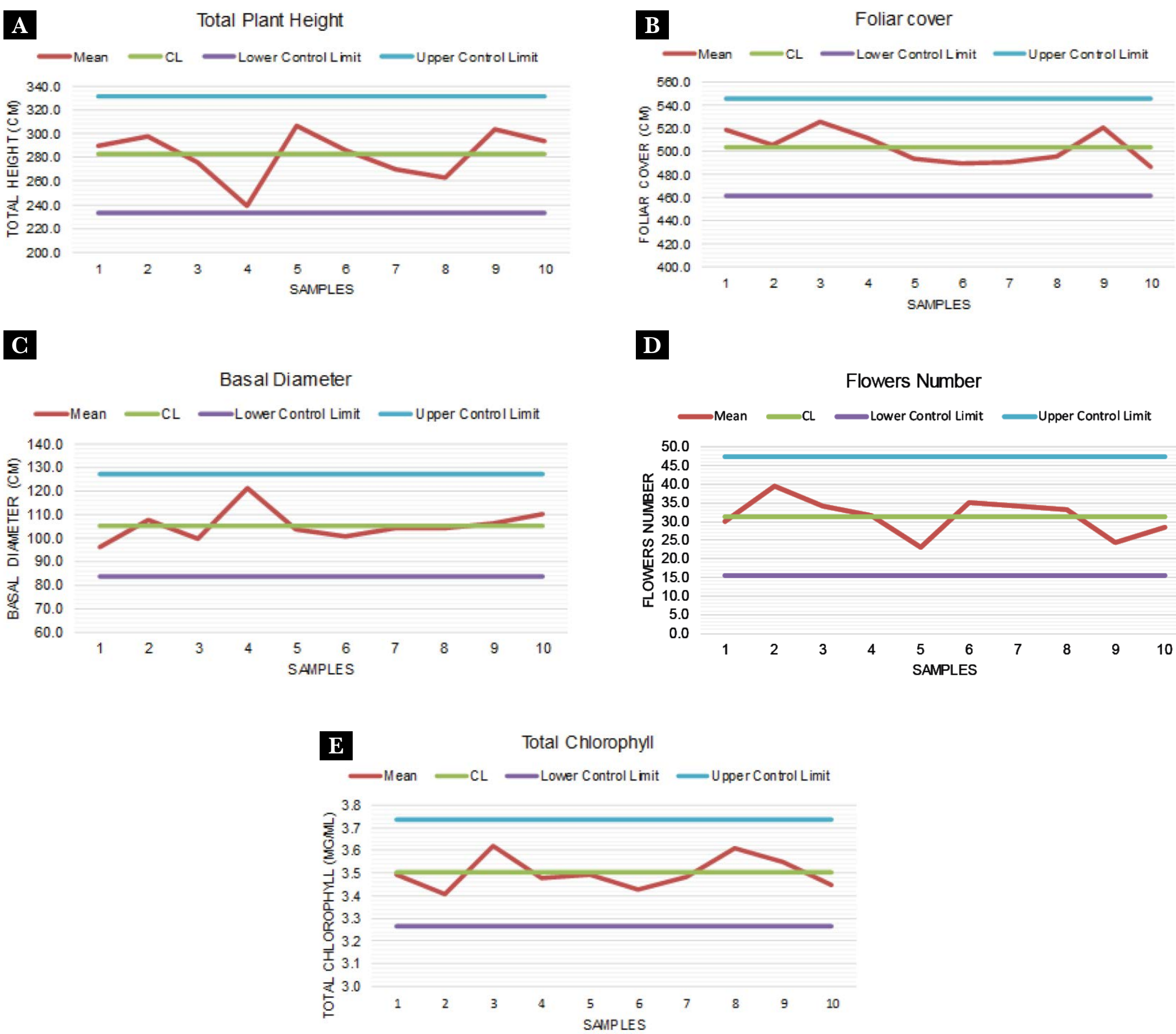

Figure 3. Quality control charts for development and production parameters in a biofertilized guava crop.

content the CL value was 84% (Figure 3). In general, in statistical quality control analyzes it is important to maintain a confidence level (1-alpha) greater than 95%, in this case we consider that biofertilized guava plants showed a positive effect on growth and production. However, it is necessary to implement some additional measures to improve the production process.

On the other hand, cause-effect analysis allowed us to determine that phytotechnical management, agricultural inputs, soil nature, labor, raw material were the main causes that are related to yield in guava cultivation. In Figure 4, an Ishikawa diagram is shown, with the effects main causes and the sublevels in each of these. In this work it was possible to apply countermeasures especially for the phytotechnical management of plantations and programs to improve crop growth and yield through the detection and elimination of pests, weeds and control of birds and insects that damage plants and fruits. Likewise, it was observed that the application of biofertilizers positively influence plant growth, flowering, and fruit production.

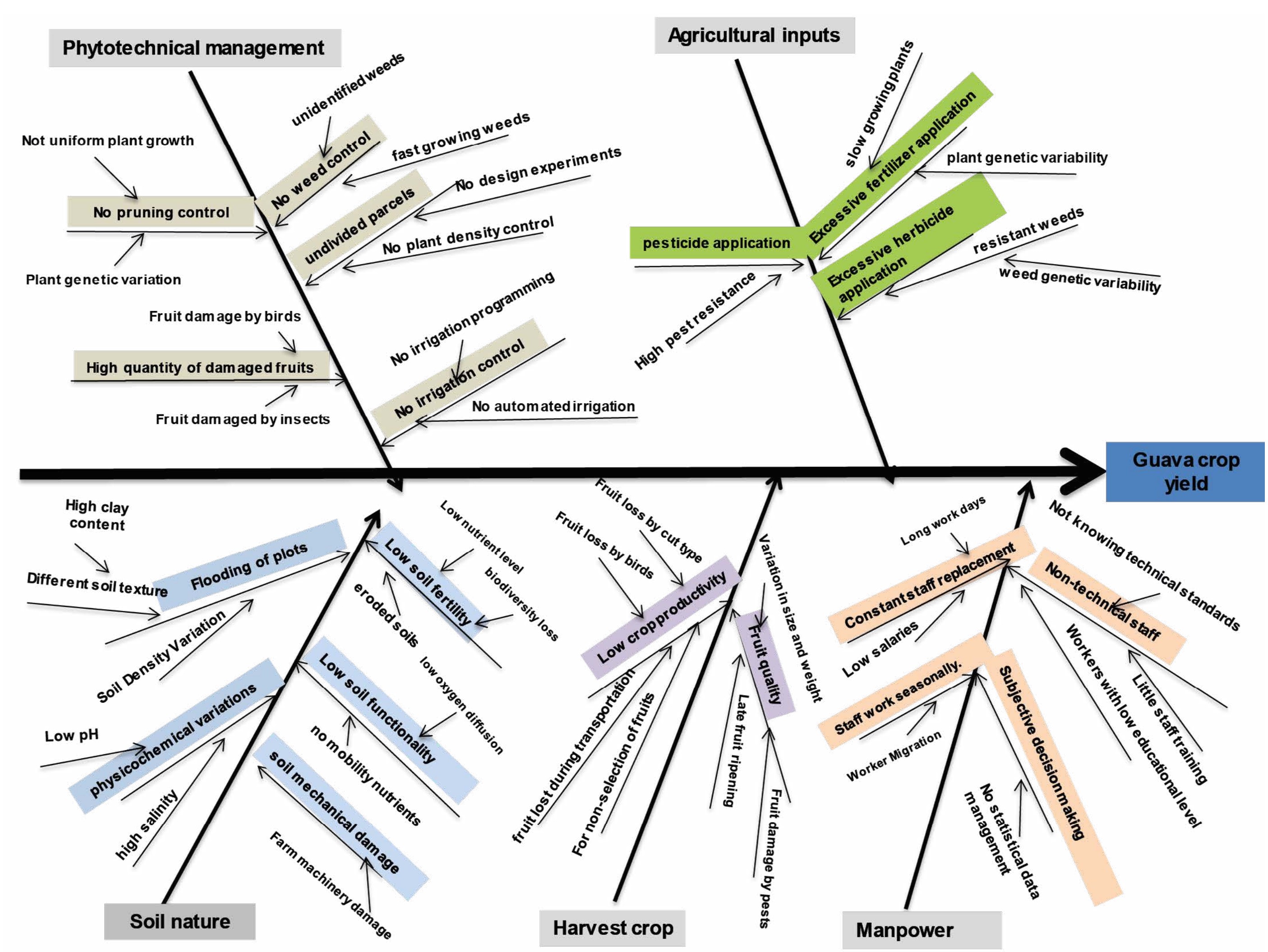

Figure 4. Cause-effect diagram (Ishikawa) on guava crop production process.

CONCLUSIONS

Rhizobial biofertilization significantly improved the growth and production of the guava crop. *S. mexicanum* ITTG-R7 stood out as a PGPB species that can contribute to the growth of guava plants and also improve the quality and functionality of the soil. Phyto technical management, agricultural inputs, soil nature, labor, raw material were the main causes that are related to low yield in guava cultivation. Experimental statistical analyzes and quality control are effective tools to determine the efficiency of biofertilizers in fruit crops.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Fec, *CA5A* and *CLSTN2* genes and their function during sheep ovulation: a review

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ABSTRACT

Objective: To describe the function of *Fec*, *CA5A* and *CLSTN2* genes during ovulation in ewes.

Design/methodology/approach: A search and analysis of scientific information related to *Fec*, *CA5A* and *CLSTN2* genes in sheep was performed.

Results: *Fec*, *CA5A* and *CLSTN2* genes are involved at the ovarian level; ewes carrying the first gene were found to have increased ovulation rate, folliculogenesis and granulosa cell differentiation. *CA5A* stimulates an increased follicular rate and plays an important role in pre-implantation. While *CLSTN2* has activity in ovarian development and growth; it also can interact with other genes involved in follicular maturation, granulosa cell differentiation and development of the ovarian follicle.

Limitations on study/implications: Ewes carrying these genes increase the prolificacy rate in the flock.

Findings/conclusions: The expression of these genes acts synergistically in the ovulatory process, enhancing the ovulatory response by contributing to endocrine, paracrine, and molecular synchronization, so that the maturation of the oocyte occurs, leading to ovulation.

Keywords: genes, *Fec*, *CA5A*, *CLSTN2*, sheep.

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INTRODUCTION

Sheep are one of the most important livestock species in the world due to their productive and reproductive potential. For this reason, one of the main objectives of selection and genetic improvement programmes is to identify animals with the best productive characteristics (Cardona-Tobar *et al.*, 2020). Reproductive efficiency is a variable that allows the productivity of the flock to be evaluated; it is determined by ovulation rate, embryo development, fertility rate; among others, which help to determine the profitability

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of sheep production systems. The development of an oocyte depends on a combination of paracrine and autocrine factors acquired during folliculogenesis in synchrony with oocyte growth and differentiation, giving it the ability to restart meiosis and support the early stages of embryonic development (Torres-Osorio *et al*., 2019; Figure 1). Ovulation rate is genetically regulated by the combined action of a group of genes with small effects and, in some cases, by the action of a gene with a larger effect. Genetic variants related to fertility in sheep are referred to as *Fec*, and the different alleles are assigned a letter or the initials of the breed in which the variant was discovered (Luna and Alonso, 2014).

***Gen BMPR1B* (*FecB*)**. In 1990, Piper and Bindon observed an increase in the heritability of the number of lambs born per ewe per lamb (prolificacy) in a flock of Booroola Merino sheep and concluded that the increase could be due to the action of a gene affecting ovulation rate. It was the first time that the existence of a prolificacy gene was demonstrated, and it was later identified as a mutation in the gene encoding the Booroola or *FecB* gene, located in the Bone Morphogenetic Protein Receptor 1B (*BMPR1B*) fragment of sheep chromosome 6, which has a mutation at A746G position 249 of the protein, which changes from glutamine to arginine in animals carrying this mutation. Catalogued as an important gene with additive effect on ovulation rate, associated with high prophilicity in Booroola Merino ewes (Mulsant *et al*., 2001; Souza *et al*., 2001; Wilson *et al*., 2001). In sheep, *FecB* is the main biomarker of fertility and is associated with an increase of 1.5 ovulations per estrus, which translates into an increase of 0.4 to 0.7 lambs per ewe. Bethancourt and Valerio-Mena (2022) reported a fertility of 1.17 in sheep carrying this mutation. Guo *et al*. (2018) proposed that the *FecB* gene not only affects the rate of oocytes and prolificacy, but also favours the uterine environment for correct implantation and subsequent pregnancy, concluding that ewes with this mutation (homozygotes and heterozygotes), compared to those without it, suffer changes in the sequence of amino acids

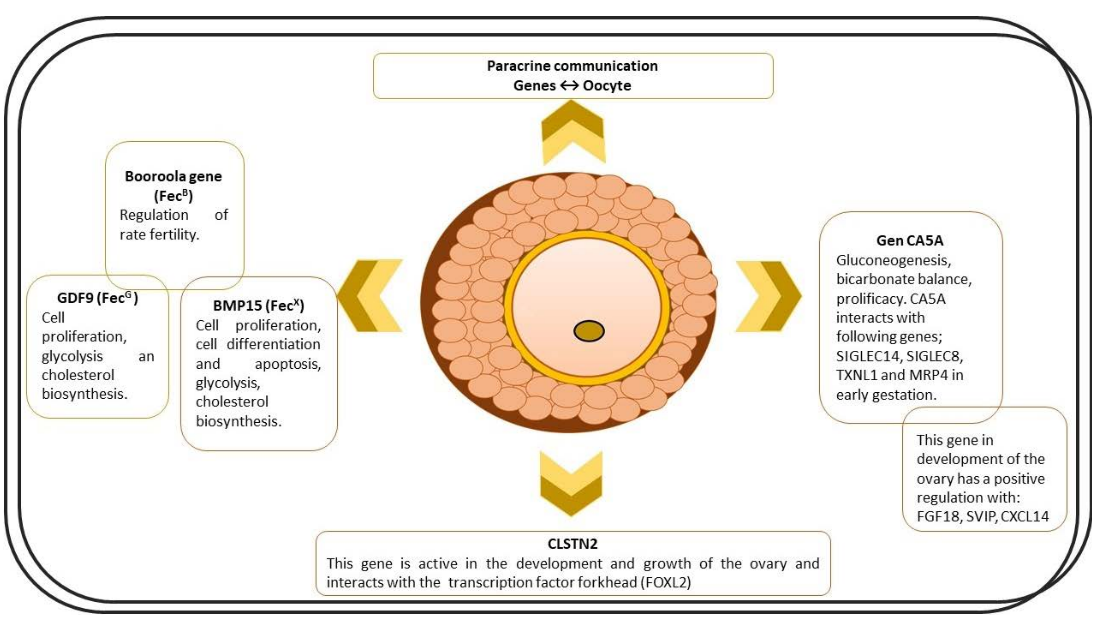

Figure 1. Autocrine, paracrine and genetic factors involved in the regulation of oocyte development.

that make up the oviductal fluid, which favours the prooxidant response in favour of the antioxidant capacity. However, Chen *et al.* (2021) point out that further research is needed on the involvement of the *FecB* gene during embryonic development and interaction with the uterus, as well as the interaction between mRNAs and long non-coding RNAs (lncRNAs) at the oviduct level in sheep. Li *et al.* (2021), identified five new mutations in the *FecB* gene (DD4, DD23, DD17, II12 and II10) in the homozygous genotype of the Chinese white ewe, and that the heterozygous genotype increases litter size at third lambing.

Gen *GDF9* (*FecG*). In sheep, it is located on chromosome five, has a length of 1365 base pairs (bp; Bodensteiner *et al.*, 1999) this gene is expressed in the oocyte from follicular development until ovulation (Hanrahan *et al.*, 2004; Juengel *et al.*, 2013) it is involved in the proliferation and organisation of theca cells surrounding the ovarian follicle, it also promotes granulosa cell proliferation and differentiation through paracrine signalling, as well as steroidogenesis by increasing estradiol secretion (Miyoshi *et al.*, 2012; Strauss and Williams, 2019). The *GDF9* protein is secreted by the growing oocyte and acts as a paracrine factor as it is recognised by specific receptors on the cells of the oocyte cumulus complex, stimulating bidirectional communication and promoting growth and development of the ovarian follicle (McNatty *et al.*, 2005). Eleven mutations have been identified in this gene: $FecG^{T}$ (Nicol *et al.*, 2009), $FecG^{E}$ (Silva *et al.*, 2010) and $FecG^{WNS}$ (Våge *et al.*, 2013), from G1 to G8, of which the G1, G4, G6, G7 and G8 polymorphisms generate changes in the amino acid sequence of the protein they encode, G8 presenting sterility in homozygous females and increased prolificacy rate in heterozygous females (Hanrahan *et al.*, 2004). For the $FecG^{E}$ (Embrapa) variant mentioned above, homozygous ewes carrying the $FecG^{E}$ allele show increased ovulation rate (82%) and prolificacy (58%; Silva *et al.*, 2010). In Pelibuey ewes, Muñoz-García *et al.* (2021) agreed that the $FecG^{E}$ gene mutation in homozygous ewes increased ovulation rate, prolificacy rate and fecundity.

Gen *BMP15* (*FecX*). The bone morphogenic protein gene (*BMP15* or *GDF9B*), located on the X chromosome in sheep, is 1179 bp in length. The protein encoded by the *BMP15* gene is a member of the *TGFβ* family of bone morphogenic proteins (Hanrahan *et al.*, 2004), which acts through a series of signalling proteins (SMAD pathway) responsible for a wide range of physiological behaviours at the cellular level, including oocyte development and maturation, as *BMP15* is expressed in secondary ovarian follicles, stimulating the growth and differentiation of granulosa cells through homotypic connexin-37 junctions, promoting the exchange of nutrients and signals with the oocyte (Luna and Alonso, 2014).

Expression of the *BMP15* gene is a key factor in determining the rate of ovulation and fertility in mammals. In sheep, nucleotide changes in the *BMP15* gene increase ovulation rate by at least 1.0 and litter size by 0.6 (Luna and Alonso, 2014). The following mutations have been reported Fec^{XI}, $FecX^{H}$, $FecX^{G}$, $FecX^{B}$, $FecX^{L}$ and $FecX^{R}$, $FecX^{O}$, $FecX^{Gr}$, $FecX^{Bar}$ and a 17 base pair deletion in $FecX^{R}$, as well as *BMPR1B* and $FecX^{2W}$ gene, which increases the number of antral follicles (Feary *et al.*, 2007; Albarella *et al.*, 2015; Pineda *et al.*, 2018; Hernández-Montiel *et al.*, 2020). Of these, six mutations have been found in this gene that affect prolificacy: $FecX^{G}$ and $FecX^{B}$ (Hanrahan *et al.*, 2004), $FecX^{H}$ and $FecX^{I}$ (Galloway *et al.*, 2000), $FecX^{L}$ (Bodin *et al.*, 2007) and $FecX^{R}$ (Monteagudo *et al.*, 2009) $FecX^{H}$. Argüello-Hernández *et al.* (2014) found the presence of $FecX^{G}$ and $FecX^{L}$ polymorphisms in the *BMP15* gene in Pelibuey ewes and reported an increase in double lambing, as this mutation increases ovarian sensitivity to FSH and increases the ovulation rate in ewes with this mutation. Demmers *et al.* (2011) and Lahoz *et al.* (2011) mentioned that it is a great advantage to use ewes with these mutations for lamb production. However, it has also been reported that the presence of homozygotes ($FecX^R$ /$FecX^R$) of this variation causes sterility due to ovarian failure, which causes morphological abnormalities such as hypoplasia, leading to infertility (Lahoz *et al.*, 2011; Alabart, 2016).

The *BMP15* gene has a similar expression to the *GDF9* gene, so they are often related, as it is expressed in the oocyte as well as in the follicle and acts through a series of signalling proteins (via SMAD; Luna and Alonso, 2014; Figure 2).

Gen *CA5A* (Carbonic anhydras 5a). Among the candidate genes associated with prolificacy in sheep is the *CA5A* gene, a gene of the carbonic anhydrase (CA) family of zinc-containing metalloenzymes, whose main function is to catalyse the reversible conversion of carbon dioxide to bicarbonate $\left(HCO_{3-}\right)$ and is also involved in gluconeogenesis. The *CA5A* gene plays an important role in the bicarbonate supply of many mitochondrial enzymes and is involved in biological and metabolic processes (Pokharel *et al.*, 2020). Hernandez-Montiel *et al.* (2019) showed that *CA5A* is positively expressed in the ovaries of Pelibuey ewes; they observed that the presence of the gene triggered double births compared to non-carriers who had single births. Pokharel *et al.* (2020) studied the regulation of the *CA5A* gene in the ovaries of Texel ewes and found that it was expressed in F1 crosses, leading to the hypothesis that this gene has an important function at least up to the pre-

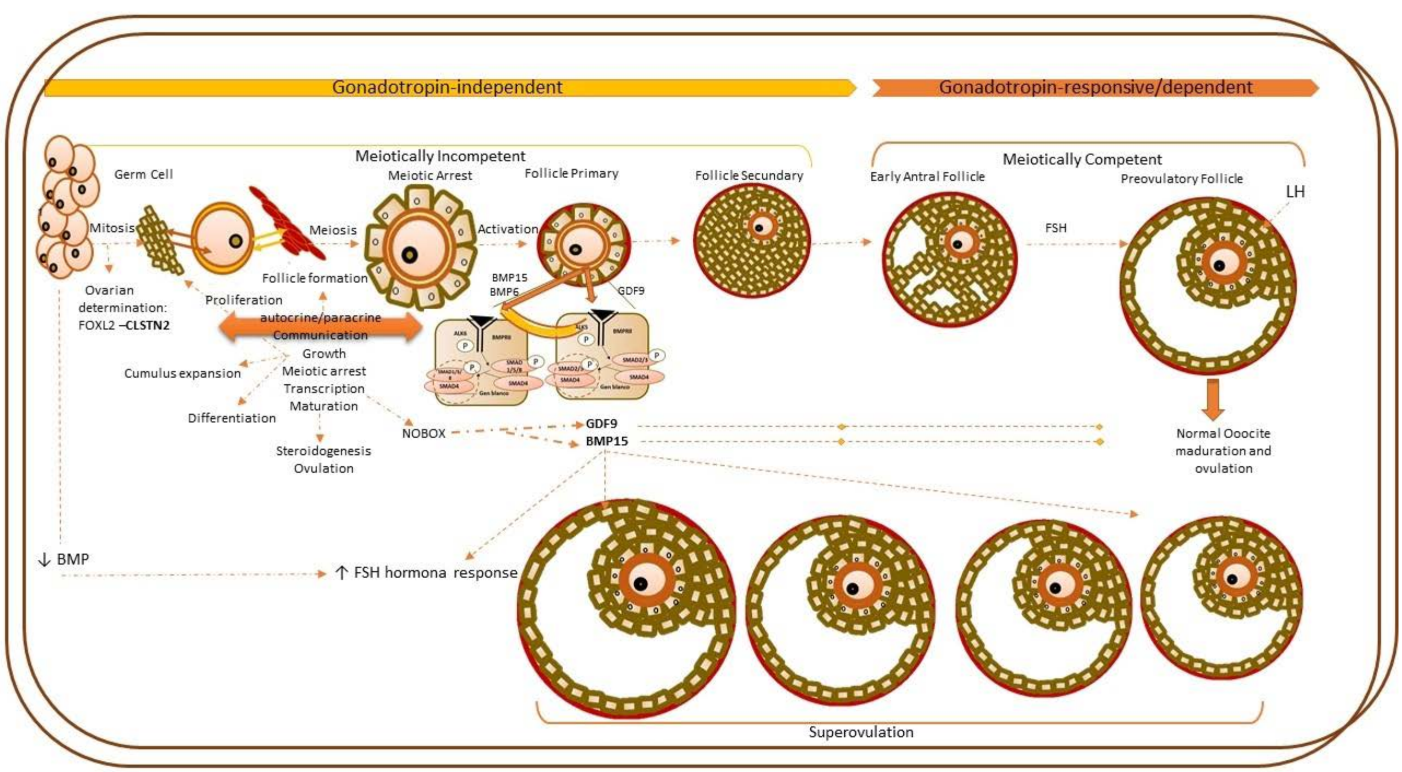

Figure 2. Folliculogenesis and oocyte development through gene expression, paracrine and autocrine (Adapted from Sanchez and Smitz, 2012).

implantation stage, since it was observed that the expression in F1 crosses of the corpus luteum showed a similar behaviour to that of the dams. This gene is associated with several genes during early pregnancy, also participates together whith other genes in the process of ovarian development in ewes (Pokharel *et al.*, 2018; Hernández-Montiel *et al.*, 2019).

Gen *CLSTN2* (Calsyntenin 2), this gene is involved in several processes of vital importance such as: lipid metabolism (Ugi *et al.*, 2014) calcium regulation, metabolic disorders such as glucose and insulin. In addition, this gene is involved in cell proliferation, differentiation, cell death, tumorogenesis, ovarian development and growth together with the transcription factor forkhead (FOXL2), follicular expression: growth and maturation of ovarian follicles, sex determination, and also participates in granulosa cell differentiation with subsequent follicle development (Pisarska *et al.*, 2011).

In Brahman heifers, it was found to be involved in the regulation of insulin and glucose levels, which are very important for cell metabolism and proper communication of the endocrine axis, resulting in carrier females achieving oocyte development and quality leading to first ovulation and puberty faster than non-carriers (Amstalden and Williams, 2014). In sheep and goats, CLSTN2 has been identified as a potential candidate gene for fertility and can be used for marker-assisted selection (Wijayanti *et al.*, 2022).

CONCLUSIONS

This review describes the genetic control of ovulatory development, the mechanisms involved in the control of ovulation rate in mammals and the interaction with other genes. These findings provide useful DNA markers for the selection of sheep with genes associated with increased prolificacy, which is a valuable genetic resource for livestock production when establishing a breeding programme to increase the profitability of the flock.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

The relationship between the fishermen and the American crocodile (*Crocodylus acutus*) in the Mexican central western Pacific: a narrative analysis

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ABSTRACT

Objective: To analyze the relationship between the fishermen of central western Mexico and the river crocodiles (*Crocodylus acutus*), emphasizing the resulting conflict.

Design/Methodology/Approach: The work was carried out with 22 fishermen from the Cuyutlán and Alcuzahue lagoons in Colima, Mexico. Four participative workshops were organized per community, in order to explore the positive and negative aspects of the relationship, the perception and knowledge about the ecosystem functions of the crocodile, and the management practices and actions associated with this relationship, as well as to determine the natural participants and factors that influence the said relationship. The workshops were recorded and the resulting data was subjected to a narrative analysis and a summary.

Results: Harmonious relationships are recorded, including the creation of bonds between humans and crocodiles and recognizing the benefits to fishing and the ecosystem function of the crocodile. The conflictive relationships identified were related to fishing, the reduction of fishing resources, and accidents. The strategies used to prevent conflicts are related to the dangerous zones, the behavior of the crocodiles, and the killing of the animals to reduce their presence. Additionally, compensation schemes, a collective management of fishing resources, and the replacement of fishing by the exploitation and capture of the crocodile were taken into consideration.

Study Limitations: Besides the fishermen's, the point of view of other participants should be determined.

Conclusions: The relationship between fishermen and crocodiles is both harmonious and conflictive. On the one hand, both receive benefits; on the other hand, fishermen suffer economic, operative, and life style impacts, which create inauspicious scenarios for the conservation of the reptile.

Keywords: animal damage, conflict, crocodile, relationship, fisheries.

INTRODUCTION

Fishing has shaped several life styles around the world for at least 92,000 years (Dahlet, Himes-Cornell, and Metzner, 2021; Lackey, 2005) and, even in the early 19th century, fishing resources were considered inexhaustible (Lackey, 2005). Afterwards, the scarcity of the resources led to their initial management and farming. By the mid-twentieth century, the management of fishing was based on the maximum possible yield (Andrew *et al.*, 2007; Lackey, 2005).

The studies about fisheries management have been focused on biological and ecological indicators, paying scarce attention to the social mechanisms and dynamics that are part of the activity (Andrew *et al.*, 2007). These type of studies include works that seek to understand the social conflicts involved in fishing (Dahlet *et al.*, 2021). Some researches focus on the fishermen-wildlife conflict, in which both parties cause and suffer damage (Szteren and Páez, 2002; Tixier *et al.*, 2021).

These conflictive relationships are usually caused by the loss of fishing, the risk of facing wildlife, the damage caused to the work equipment, and the association of wildlife with the reduction of fishing resources. All these elements threaten food and economic security, making the life style of fishermen even harder (Guerra, 2019; Tixier *et al.*, 2021). Wildlife dies as a result of the reprisal of the fishermen or trapped in their nets; consequently, their populations and conservation are endangered (Guerra, 2019; Wickens *et al.*, 1992).

Researches about this subject have analyzed this phenomenon from a quantitative point of view, calculating economic losses (Guerra, 2019; Tixier *et al.*, 2021). For example, Szteren and Páez (2002) determined that the damage caused to the fishermen's equipment by wildlife amount up to 71% of their operative cost.

Nevertheless, the relationship between fishermen and wildlife is not well known and there is a gap of qualitative variables, including experiences, beliefs, myths, discourses, values, and perspectives (Serfass, Bohrman, Stevens, and Bruskotter, 2014; Tixier *et al.*, 2021). Additionally, the studies have been focused on the interaction between mammals or birds and fishermen. There is less information about the fishermen-crocodile relationship, although some studies have determined that it is conflictive in nature (Aguilar-Olguín, Rivera-Rodríguez, Hernández-Hurtado, and Ramírez-Martínez, 2021; Aranda-Coello *et al.*, 2016; Das and Jana, 2018). However, the analysis has been almost exclusively focused on the general aspects of the fishermen-crocodile conflict or in their relationship (Brackhane *et al.*, 2019; Kpéra *et al.*, 2014; Peña-Mondragón *et al.*, 2013). Few studies analyze the specific aspects of that relationship. Most of the studies are focused on the quantitative and economic aspects of the fishermen-crocodile relationship (Aust, Boyle, Fergusson, and Coulson, 2009). Therefore, the objective of this study was to analyze that relationship, focusing on the resulting conflict, but also seeking to reflect the phenomenon from a coexistence point of view (Pooley, Bhatia, and Vasava, 2021).

MATERIALS AND METHODS

Study sites

The study was carried out with fishermen that work in two water bodies inhabited by *Crocodylus acutus*, in Colima, in western Mexico. The first is the Alcuzahue lagoon (18° 54'

42.50" N and 103° 46' 32.33" W), located in the municipality of Tecomán. The second is the Cuyutlán lagoon, a massive body of water which —as a result of its hydrological characteristics— is usually divided into four sections. This lagoon extends into the territory of various municipalities. This research was carried out in the Vaso IV (18° 54' 05" N, 104° 01' 53" W), in the sites popularly known as Estero Palo Verde and La Laguna. Both sites are located in the municipality of Armería. In both localities, the fishermen have formed cooperatives, which have an exclusive permit for the exploitation of the fishing resources. However, some fishermen carry out this activity without being members of the said cooperatives or without a permit. Both groups use fishing methods such as cast netting or trammel net. Their catch is used for self-consumption and/or sold at the local market. Consequently, their activity is considered artisanal or small-scale fishing.

Data generation

Data generation was carried out through participative workshops (Geilfus, 2002; Gerritsen, 2016) that took place from January to February 2021. The participants were members of the Sociedad Cooperativa de Producción Pesquera Laguna de Alcuzahue and non-associated fisherman from both Alcuzahue and Cuyutlán. The fishermen were 36-64 years old and all of them had at least 5-year experience as fishermen in the said sites. However, most of them had been fishing for 20-30 years in coastal areas. Twenty-two fishermen attended the four participative workshops that were organized in each community, following the methodology proposed by Geilfus (2002) and Gerritsen (2016). Overall, the workshops sought to understand the fishermen-crocodile relationship. The first workshop explored the positive and negative aspects of the relationship, while the second confirmed these aspects, emphasizing perceptions and knowledge about the ecosystem functions of the crocodile (Somaweera *et al.*, 2020). The third workshop focused on management practices and actions associated with these relationships. Finally, the fourth workshop sought to understand the natural participants and factors that influence the phenomenon, especially management practices and actions.

Data analysis

The workshops were recorded with the prior authorization of the participants. Subsequently, the dialogues were transcribed (Elliott, 2005; Poland, 1995) and the transcriptions were subjected to a narrative analysis. Categories based on common and contradictory elements of the different narratives of the participants were developed for the narrative analysis. After reading and re-reading the workshop transcriptions, these patterns

were grouped , in order to create a synthesis (Elliott, 2005; Lieblich, Tuval-Mashiach, and Zilber, 1998; Riessman, 2008; Wertz *et al.*, 2011). Finally, the results are expressed as an unified text, where some categories are depicted as prototype fragments of the fishermen's words (Riessman, 2008). The fishermen are identified in the results section with their code plus an ordinal number (*i.e.*, Fisherman 1, Fisherman 2).

RESULTS AND DISCUSSION

In average, the fishermen were 40 years old. Five of the fishermen were not members of a fishing cooperative and only three were women, who occasionally fish along with their partners. Additionally, six fishermen were directly or indirectly related to crocodile conservation projects. Regarding the fishermen-crocodile relationship, two types of relationships were established: harmonious and conflictive.

Harmonious relationships with crocodiles

The fishermen mentioned that they create bonds with some of the crocodiles and that they acknowledge the benefits that crocodiles bring to fishing. According to Himes and Muraca (2018), these aspects influence their lifestyles.

The harmonious relationships that were found have already been reported in other researches. However, they were no more than short comments in extensive articles that mainly focused on the conflict. This is a typical characteristic of the studies about the overall human-wildlife relationship (Pooley, Bhatia *et al.*, 2021). Consequently, this research aimed to find the highest possible number of positive fishermen-crocodile relationships.

Regarding the bonds, those fishermen said that they are created because they spend a lot of time in the fishing spots and coexist with certain specimens. They like and name these animals. Additionally, they feed them and are aware of their temperament. Fishermen consider that this is a reciprocal behavior because the reptile also recognizes them. For example, Fisherman 1 told us:

> *"Bonds are created between humans and animals. [...] The crocodile comes closer, and I feed her. [...] Some of them have names. [...] Some of them have a different temperament. The one I always talk to in the canal (Muñeca) doesn't come closer. [...] She has her babies and I pass near them and the animal doesn't attempt to attack me."*

Nevertheless, some researchers argue that feeding crocodiles is a potentially dangerous situation that can result in attacks (Pooley *et al.*, 2021). About this situation, Fisherman 1 said:

> *"Of course, it's dangerous, [...] we know how dangerous crocodiles are. [...] Fishermen feed crocodiles because they enjoy watching them eat."* (Fisherman 1).

Crocodiles provide ecosystem benefits to fishing. Four of these benefits can be included as part of their ecosystem functions (Somaweera et al., 2020) they are top predators; they favor the nutrient flux; they are an ecosystem indicator and engineers; and they are the "guardians" of fishes. Since this last function is not included in the literature, we will attempt to explain it.

The crocodile cause fear among some people. Fear drives fishermen away and, consequently, more fishes can be found in the water bodies. Fisherman 3 explains:

> *"Crocodiles protect the fishes and there are still enough fishes. [Otherwise] people would go as far as they could and fish as much as they could."* Fisherman 3

Regarding their ecosystem functions, crocodiles are predators that reduce the populations of certain species that also feed on fishes. Additionally, crocodile constructions such as "caves", "canals", "pools", and "*echaderos*" (places where the crocodile sunbathes) create ideal conditions for fish abundance and diversity.

Meanwhile, some fishermen say that they use these "*echaderos*" as indicators that "*there are fishes here*" and, consequently, good fishing. Camacho (2015) reported similar results in other places. Other fishermen affirm that fishes eat the nutrients "*freed*" by the movements of the reptile.

These benefits for fishing and the bonds created with the crocodiles have been scarcely explored in scientific researches, which mainly focus on the crocodile-fishermen conflicts (Pooley, Bhatia *et al.*, 2021). However, understanding these conflicts provide useful information to manage conflictive situations, because fishermen that had harmonious relationships with the reptiles were the most involved in or receptive to crocodile conservation.

Conflicts between fishermen and crocodiles

The conflict between fishermen and crocodiles has three aspects: the first is the "*theft*" of the catch and the damage caused to the nets; the second relates to accidents involving crocodiles; and the third is the association of the crocodile with the reduction of fishing resources. These situations are separated for analytical purposes; however, they all can happen simultaneously.

The "*theft*" and the damage to the equipment take place when a fish is caught in the net and the crocodile "*comes and takes it*" (Fisherman 7) to eat it, ruining the fishing equipment. This phenomenon has been reported in other researches about the coexistence of fishermen and crocodiles (Aust *et al.*, 2009; Kpéra *et al.*, 2014). Similar conflicts have been recorded between humans and aquatic mammals (Guerra, 2019; Szteren and Páez, 2002).

The damage caused to the nets depends on the particularities of each fishing method. Passive fishing methods (such as the trammel net method) require that the nets stay longer in the water, which results in greater damage (Szteren and Páez, 2002), while cast netting is a more active and maneuverable method which results in longer-lasting nets. Fisherman 8 told us:

> *"I've got cast nettings from last year. They last longer, because they occupy a small area. If you see [the crocodile], you just quickly pull them out."*

In short, this conflict causes economic damages, which is the main operative expenditure of the fishermen (Aust *et al.*, 2009; Tixier *et al.*, 2021). If the damage can be fixed, it takes time and money (Aust *et al.*, 2009; Guerra, 2019). Additionally, since most of the interviewees does not know how to fix their nets, they can spend at least US$15.00 in repairs, tantamount to what they normally earn in a day. Besides, while the nets are being repaired, the fishermen cannot fish (Guerra, 2019), unless they own more than one piece of such equipment. Fisherman 7 says that "*you need to own at least four*" to mitigate the crocodile effect.

Therefore, each time their equipment is completely damaged, fishermen have to invest about US$45.00-US$100.00 in new equipment. These situations vary in frequency: some of the fishermen told us that these damages take place every time they fish, while others told us it happens two or three times a month. The frequency of the attacks mentioned by the fishermen matched the findings of other studies (Aust *et al.*, 2009). Finally, in addition to these expenditures, fishing is reduced because of damaged equipment, as Fisherman 6 told us:

"*Right now, I catch 5 kg of fish; if there are no crocodiles, I catch 20 kg.*"

The conflict with the crocodiles causes great operative difficulties for the fishermen. This situation is similar with other wildlife species (Szteren and Páez, 2002). However, accidents are a very important characteristic of the relationship with crocodiles. These accidents can take place when the fishermen are moving throughout the water body, while they operate their equipment or they "clean" the catch, which can attract crocodiles. All the fishermen that participated in the workshops recounted accidents involving those repitles. Fishing is the human activity that is impacted by more crocodile attacks in Latin America (Pooley, Siroski *et al.*, 2021). However, although the frequency of the attacks is high, in some cases they are "*just a fright*", while in other cases, the consequences are wounds that leave permanent scars and, in a few cases, lead to amputations. Additionally, some fishermen mentioned that, when they "*fight*" with a crocodile to retrieve the net, the risk of falling into the water poses a deadly risk, especially where the mud has turned "*rubbery*". Understandably, many fishermen fear for their lives. Therefore, fishing is a high risk activity, just like in other continents (Das and Jana, 2018; Wallace, Leslie, & Coulson, 2011). Some fishermen have doubts about pursuing this activity, while others are angry:

"*I would like to pull out a gun and just kill it.*" (Fisherman 12).

The last reason for the conflict is the "*excess of crocodiles*" (Fisherman 9) related to the reduction (both in quantity and diversity) of animals of commercial interest. For example, Fisherman 6 argues that "*one of the reasons why sometimes we do not find fishes*" is because crocodiles eat them. This situation was also reported in other studies (Aranda-Coello *et al.*, 2016) and it is one of the most common elements in the conflicts between fishermen and wildlife in general (Recharte, Bowler, and Bodmer, 2008; Serfass *et al.*, 2014). However, from a scientific point of view, these affirmation cannot be proved, because quantitative data about the populations of commercial interest and the trophic relationship of the crocodile are required (Hilborn, Oscar, Anderson, Baum, and Branch, 2020; Maunder and Punt, 2013).

Overall data is scarce (Nifong *et al.*, 2014; Somaweera *et al.*, 2020) or non-existent, particularly for the sampling sites; however, this perception is not based on scientific facts and, along with abovementioned impacts, justifies the negative behavior and perspectives about the conservation of the crocodile (Guerra, 2019; Vaclavikova, Vaclavik, and Kostkan, 2011). Additionally, the alleged reduction (regardless of the cause) can increase the conflicts (Szteren and Páez, 2002). Nevertheless, fishermen also mentioned that the reduction of resources could be caused by fishermen who do not comply with the "*close season*", their chosen fishing method, and how long they keep the trammel nets in the water. Consequently, there is an overexploitation of the sites, which has a direct impact on the resources. African fishermen have already mentioned that crocodiles are not the only cause of the reduction of the fishing resources (Kpéra *et al.*, 2014); meanwhile, from a scientific point of view, overexploitation is the main cause of the reduction of fishing resources (Hilborn *et al.*, 2020).

Strategies to avoid conflict

There are only few scientific reports about the strategies used by fishermen to avoid the impacts of the crocodiles (Hayman *et al.*, 2014). The information usually focuses on the imprudent activities of the fishermen that could lead to an incident (Pooley, Siroski *et al.*, 2021). However, the fishermen interviewed in this research are aware of the dangerous areas, they understand the behavior of the crocodiles, they refrain from attracting them, and they also kill them to reduce their presence in the area.

Fishermen told us that crocodiles can be found all over the fishing sites but that in certain, more dangerous areas they are "*more aggressive*". These areas match with places of biological importance for the crocodiles: their caves, their nests, and their "*echaderos*". Crocodiles usually defend these sites, because they are "*territorial*" or "*they are nesting*" (Fisherman 7). These findings match the findings of Aranda-Coello *et al.* (2016) and González-Desales *et al.* (2021) who reported that the reproductive season of the crocodiles is the most dangerous period.

This zoning indicates danger; nevertheless, it does not imply that fishermen avoid those sites, because they are known as places "*where more fish can be found*" (Fisherman 9). Additionally, this spatial acknowledgment of the danger is unknown by fishermen that do not usually fish in the area. They do not notice the temporal changes associated with the biological, social, or climatic variables, such as the reproductive cycle of the reptile, feeding the crocodiles, or the rainy season (which modifies the water level). The third variable is a factor associated with accidents suffered by fishermen in other sites (Das and Jana, 2018).

In order to avoid crocodiles, some fishermen told us that they carry out their activities at 11:00 am, when crocodiles "*are not [in the water], most of them are out [of the water], sunbathing on the banks, as if they are just waking up.*" (Fisherman 1). Additionally, some fishermen have a wide knowledge about the biological and ethological aspects of the crocodile. However, they have had accidents involving crocodiles. This situation contradicts the findings of other authors, who mention that accidents involving crocodiles are caused by the ignorance of people (Pooley, Siroski *et al.*, 2021).

Nevertheless, this knowledge allows fishermen to understand certain "*signs*" that the crocodile sends, showing that it is angry or at ease. Fisherman 1 told us that when crocodiles are angry, they "*keep still and splash, their trunk curves, and their tail bends*". Fisherman 1 also told us that it is better to keep away from these specimens. However, when they approach him, he follows this strategy:

> "*This is what I do: I come closer in a much more aggressive way, so instead of feeling fear, I try to scare them. I swear [yelling] to make them afraid. They feel the aggression in my voice and may be [this is how] you can make them feel fear*" (Fisherman 1).

Additionally, a fisherman who fishes on foot told us that he avoids killing the fishes, because their blood can attract crocodiles. He has already observed this behavior when he eviscerates his catch on the shore. Therefore, he prefers to keep the catch alive in a burlap bag that he ties loosely to his body, keeping it in the water all the time. This is how he avoids the risky behavior followed by other fishermen who keep dead fishes close to their chest (Pooley, Siroski *et al.*, 2021; Wallace *et al.*, 2011).

Nevertheless, fishing would be less dangerous on a boat (Pooley, Siroski *et al.*, 2021) domesticated animals and humans. Large‐scale transformations of crocodilian habitats across the Latin America and Caribbean region, alongside significant use of crocodilians as a resource, and retaliatory killing of crocodilians following (or to prevent but, "*not anybody can fish on a motorboat. You need to be a member of a cooperative. If you are not, they don't let you. And right now, a share costs 60 thousand pesos. Then you have to add the boat, the engine [...]*" (Fisherman 1). It is not that the fishermen are unwilling to belong to a cooperative; rather, the lack of social structures causes a conflict between cooperative members and "free" fishermen, giving rise to accidents involving crocodiles. This conflict between humans for fishing rights (Peterson, Birckhead, Leong, Peterson, and Peterson, 2010) is very common in this sector, whose management is partially the responsibility of the government institutions (Dahlet *et al.*, 2021).

Finally, some fishermen attempt to get rid of these negative impacts by killing the crocodiles. This situation is less frequent in Cuyutlán than in Alcuzahue, where some fishermen declared that they kill the reptiles, while others do not disapprove this activity, although they do not kill the crocodiles themselves. Meanwhile a third, small group opposes the killings. Their stance also has led to risky situations, as Fisherman 6 told us:

> "*There were two young men fishing, they both carried rifles. 'If it [the crocodile] shows up now, I will kill it.' Hey! [we told them], you are shooting at the crocodiles. You can't do that. And I heard one of them say: we will even kill you!*"

However, the fishermen that do kill crocodiles say that they eat them. In fact, the consumption of crocodile meat is widely known (Klemens and Thorbjarnarson, 1995) and it has also been reported in other sites (Camacho, 2015). Fisherman 12 told us:

"*When I kill one, I eat it. The meat is very good, my friend!*"

Conflict solution and management proposals

Meanwhile, four potential solutions or strategies to manage the conflict were proposed. These proposals were classified as activities that fishermen could carry out themselves and those that require external participants, especially government institutions.

The fishermen believe that at least four aspects require "*more control*" (Fisherman 3): gun ownership, poaching, accident prevention and solution, and the payment or replacement of equipment damaged by the crocodiles. Fisherman 9 told us:

"*[The government] should come and say that crocodiles are here to stay and that they will provide a compensation every two months, so we don't have to risk being eaten by a crocodile. That will help us to survive.*"

Compensation schemes to deal with conflicts involving other animal species have already been used with a certain level of success (de Klemm, 1996; Guerra, 2019). However, some operative difficulties should be taken into account for the implementation of the compensation scheme, including obtaining a source of funds (Maclennan, Groom, Macdonald, and Frank, 2009) and reducing the bureaucracy and the waste of time of the mechanism (Vaclavikova *et al.*, 2011). These are instrumental schemes and they do not necessarily resolve the relationship conflicts (Himes and Muraca, 2018). Some fishermen proposed quitting fishing, which implies searching for a job that involves less risks and involves earning enough money to continue their life style (Than, Zaw, and Hughes, 2020). The fishermen themselves proposed exploiting the crocodiles' meat and skin, which would allow them to change from fishermen to "*businessmen*". This alternative has been economically and environmentally successful (Larriera and Imhof, 2006; Webb, 2020). Nevertheless, there is scarce scientific proof about its impact on life styles; therefore, its efficiency should be consider instrumental (Himes and Muraca, 2018).

Finally, the discussion turned towards the need to comply with fishing regulations, in order to avoid overexploitation. This proposal should include the management of the conflict and the protection of the crocodiles within an ecosystem conservation perspective, changing the competitive view that prevails in the area where the crocodile-human conflict exists (Balaguera-Reina and González-Maya, 2010) for a vision in which crocodiles carry out useful ecosystem functions (Somaweera *et al.*, 2020) guaranteeing fishing resources. This objective could be achieved using strategies based on the collective management of the fishing resources (Domondon, Tirona, Box, and Pomeroy, 2021; Marriott *et al.*, 2021) and the abovementioned perceptions and knowledge about the environmental role of the reptile and the bonds with specific crocodiles.

CONCLUSIONS

The relationship between fishermen and crocodiles is both harmonious and conflictive. On the one hand, both receive benefits; on the other hand, fishermen suffer economic, operative, and life style impacts, which create inauspicious scenarios for the conservation of the reptile. Economic and operative damages can be covered with compensatory schemes; however, their planification and implementation should take into account operative deficiencies and challenges.

The fishermen of the study sites have developed a set of strategies to reduce the conflicts. They have a great knowledge about the ecology, biology, and even ethology of the crocodiles. Nevertheless, the impacts caused by crocodiles remain the same. The substitution of economic activity can be a tool to modify the crocodile-human relationship. However, further research must be carried out to evaluate the feasibility of this type of projects and the effects that they will have on the life style of the fishermen. The reduction of fishing resources is a priority for the fishermen. Generating projects for the collective management of ecosystems will require technical data and social work. The said project will allow the integration or the promotion of a positive view about the crocodile and its environmental functions. Finally, the results of this research mirror the fishermen's point of view. This is the first research to analyze this specific phenomenon. Nevertheless, government institutions and the academia also play an important role. Therefore, further research should be carried out, in order to analyze other social sectors involved in the fishing industry.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Bean rust resistance and yield of black bean genotypes under field conditions

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ABSTRACT

Objective: To identify black beans lines resistant to bean rust under field conditions, which have similar or higher yield than three commercial varieties sown in Veracruz, Mexico.

Design/Methodology/Approach: The genotypes were evaluated using a completely randomized block design. The reaction of the genotypes to bean rust was qualified using a 1-9 incidence rate, while grain yield was estimated in kg ha^{-1}. An analysis of variance was carried out and the resulting means were divided using a 0.05 Least Significant Difference (LSD). Additionally, a correlation analysis between the incidence values of bean rust and the yield of the genotypes was carried out in each evaluation site.

Results: Nine lines were resistant to bean rust (incidence average: 1.78-3.33). Out of these lines, Jamapa Plus/XRAV-187-3-4-4 was the most productive line, with a 2,183.4 kg ha^{-1} average yield. This result is statistically similar to the results of the Jamapa Plus/XRAV-187-3-4-1 and Jamapa Plus/XRAV-187-3-1-2 lines and the Verdín variety. In addition, the results of Jamapa Plus/XRAV-187-3-4-4 are slightly higher than the results obtained by the rest of the genotypes.

Study Limitations/Implications: Although bean rust was recorded in three of the localities of the study area, the incidence degree and the stage when it was found were different. However, in all the cases, the infection degree was enough to evaluate the reactions of the genotypes to this disease.

Findings/Conclusions: Three bean rust-resistant lines were identified under field conditions. They had a significantly higher average yield than the Negro Jamapa and Negro Medellín varieties.

Keywords: *Phaseolus vulgaris* L., *Uromyces appendiculatus*, productivity.

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INTRODUCTION

There is a great physiological variation among the races of *Uromyces appendiculatus* var. *appendiculatus* (Pers.) in Mexico; this pathogen causes bean rust, which is one of the main fungal diseases of bean (*Phaseolus vulgaris* L.) crops [1]. All the bean production areas of the state of Veracruz have been impacted by this fungus, although it has a higher frequency incidence in the areas located at an altitude >800 m, with environmental temperature between 17 and 27 °C and a 90% relative humidity [2]. The yield of the Negro Jamapa and other native varieties can be reduced by more than 40% when the bean rust appears before the flowering [3]. Mancozeb® and cooper oxychloride® are the main fungicides used to control bean rust [2]; they are usually applied in more than one occasion, increasing the production cost of the crop [4]. A more economical and environmentally friendly alternative is the use of improved bean rust-resistant varieties, which can be successfully adapted to the bean cultivation areas in Veracruz [5]. The Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) has developed several tropical black bean varieties, which have proven to be naturally resistant or tolerant to some *U. appendiculatus* physiological races, under field conditions [6,7]. However, in order to guarantee the identification of bean lines and to develop varieties which are resistant or tolerant to the pathogens, they must be inoculated with spores of the main *U. appendiculatus* races that impact commercial bean crops [5]. Therefore, in 2019, the reaction of 53 advanced bean lines and three opaque black beans varieties (the preferred crop in Veracruz) were evaluated under greenhouse conditions. These lines and varieties were subjected to an artificial inoculation with spores obtained from different bean production areas of the states of Veracruz and Chiapas. Eleven lines with high resistance [5] and good adaptation to the bean production areas of Veracruz [8] were selected for this study. The evaluations took place under field conditions, in three environments of Veracruz, in which bean rust is frequently reported. The objective of this study was to identify the lines with higher resistance to bean rust and which have a similar or higher yield than the three control varieties. Additionally, it should be possible to release these lines as commercial varieties.

MATERIALS AND METHODS

The genotypes were evaluated in Rincón Grande (18° 51' N and 97° 06' W, 1,248 m.a.s.l.), during the 2019-2020 autumn-winter (A-W) cycle and the 2020 winter-spring (W-S) cycle and in Rincón Chico (18° 50' N and 97° 05' W, 1,191 m.a.s.l.), during the 2019-2020 autumn-winter cycle. Both localities are part of the Orizaba Municipality, located in central Veracruz. Out of the 11 evaluated lines, 5 were a cross of the Negro Papaloapan/SEN 46, 3 were a cross of the Negro Citlali/XRAV-187-3, and 3 were a cross of the Jamapa Plus/XRAV-187-3. These crosses were developed by the Programa Nacional de Frijol y Garbanzo (Bean and Chickpea National Program) of the INIFAP, at the Campo Experimental Bajío in Celaya, Guanajuato. The Negro Medellín, Verdín, and Negro Jamapa varieties were used as control crops. The genotypes were sown in a density equivalent to 250,000 plants ha^{-1}, in a completely randomized block design, with three repetitions. Each experimental unit consisted of three 5-m long furrows, with a 0.80 m separation between them. In the A-W cycle, the experiment was conducted under residual humidity conditions, while six gravity irrigations were applied in the W-S cycle, at 10-15 days intervals.

During the bean pod filling stage, the reaction of some genotypes to the bean rust incidence was visually evaluated, using the 1-9 general scale proposed by the International Center for Tropical Agriculture [9]. This scale has different levels of reaction: 1-3 means that the plant is resistant (non-visible or very minor signs); 4-6 means that the plant has an intermediate reaction (visible symptoms that cause a limited economic damage); and 7-9 means that the plant is susceptible (severe to very severe symptoms that cause significant yield loses or the death of the plant). During the ripening stage, yield grain was calculated in kg ha^{-1}, with 14% humidity. The bean rust incidence and yield grain data were subjected to an individual and joint analysis of variance for the three evaluated environments. In order to divide the means, a test based on the Least Significant Difference was applied, with a 5% error probability (LSD, α=0.05). Additionally, correlation analyses were carried out to determine in which evaluated environments bean rust caused a significant grain yield damage.

RESULTS AND DISCUSSION

The bean rust incidence was significantly different (P≤0.05) between the evaluated environments. Only in Rincón Grande, a significant grain yield reduction was recorded during the 2020 W-S cycle; this reduction was caused by a greater and earlier incidence (Table 1) of this disease (from the beginning of the pre-flowering stage). During this stage the disease can cause a greater damage [1]. Fifteen days after the sowing (das), relative high humidity (>80%) and temperature (15.4-28 °C) conditions prevailed in the area for a 20-d period which resulted in a greater incidence [10]. Meanwhile, during the 2019-2020 A-W cycle, no significant grain yield reduction was recorded in Rincón Chico (Table 1), although bean rust had a statistical similar average than in the previous environment, because this disease only appeared during the bean pod filling stage. During the same agricultural cycle, in Rincón Grande, although bean rust appeared during the pre-flowering stage, the minor incidence of the disease did not cause a significant damage to bean yield (Table 1).

Bean rust incidence also had a significant variation (P≤0.01) between genotypes as well as in the three evaluated areas. Table 2 shows that most of the lines had very mild symptoms of the disease in all the environments; only Negro Citlali/XRAV-187-3-1-8 had an intermediate reaction, with significative bean rust damage, during the 2020 W-S cycle in Rincón Grande. Meanwhile, nine lines had a 1.78-3.33 bean rust incidence average, which means that they are resistant to this disease [9]. In previous studies carried out under greenhouse conditions, these lines had already shown a hypersensitive reaction to the inoculation with bean rust (*i.e.*, they only showed small chlorotic spots without sporulation [5]); consequently, they are highly resistant to this disease [11]. Meanwhile, the control varieties shown significant damages in two out of the three studied environments;

Table 1. Bean rust mean values per evaluated environment and its impact on bean yield.

Environment	Incidence (scale 1 - 9)	Correlation coefficient rust *vs.* yield
Rincón Grande, I-P 2020	3.81 a	r=−0.608 *
Rincón Chico, O-I 2019-20	3.31 ab	r=−0.289 ns
Rincón Grande, O-I 2019-20	2.88 b	r=−0.200 ns
DMS (0.05)	0.579	

Bean rust incidence mean values with the same letter in the environment (ambiente) column are not statistically different, according to the LSD (DMS) (α=0.05). *=Significant Correlation; ns=Non-Significant Correlation.

Table 2. Bean rust incidence mean values of 14 black beans genotypes, in three environments of the state of Veracruz (2019-2020 Autumn-Winter cycle and 2020 Winter-Spring cycle).

Genotype	Rincón Grande O-I 2019-20	Rincón Chico O-I 2019-20	Rincón Grande I-P 2020	Incidence average
Papaloapan/SEN 46-2-6	3.33	2.00	3.67	3.00 def
Papaloapan/SEN 46-3-2	3.33	4.67	4.00	4.00 bcd
Papaloapan/SEN 46-7-7	2.33	2.67	4.00	3.00 def
Papaloapan/SEN 46-7-10	2.33	3.67	3.67	3.22 cde
Papaloapan/SEN 46-7-12	3.00	2.33	3.67	3.00 def
Negro Citlali/XRAV-187-3-1-5	2.00	1.00	2.33	1.78 f
Negro Citlali/XRAV-187-3-1-6	1.00	1.33	3.00	1.78 f
Negro Citlali/XRAV-187-3-1-8	2.33	4.33	4.67 *	3.78 cd
Jamapa Plus/XRAV-187-3-1-2	2.67	3.00	2.67	2.78 def
Jamapa Plus/XRAV-187-3-4-1	2.00	1.67	2.33	2.00 ef
Jamapa Plus/XRAV-187-3-4-4	3.00	3.67	3.33	3.33 cd
Negro Medellín (TR)	3.33	7.00 *	5.67 *	5.33 a
Negro Jamapa (TR)	4.33 *	4.00	5.00 *	4.44 abc
Verdín (TR)	5.33 *	5.00	5.33 *	5.22 ab
DMS (0.05)	1.118	1.275	1.521	1.252

RC (TR)=Regional Control. A-W (O-I)=Autumn-Winter. W-S (I-P)=Winter-Spring. *=Bean rust incidence values were statistically higher in each evaluated environment, according to the LSD (DMS) (α=0.05). Bean rust incidence mean values with the same letter in the genotype column are not statistically different, according to the LSD (DMS) (α=0.05).

additionally, they also recorded the highest bean rust incidence mean values (Table 2). The susceptibility to bean rust of the Negro Jamapa, Negro Medellín, and Verdín varieties had been previously recorded [3,1,12].

Additionally, a grain yield statistical significance was detected ($P \leq 0.01$) between environments and genotypes. During the 2019-2020 A-W cycle, the highest mean yield was obtained in Rincón Chico (Table 3), because the crop developed under the appropriate humidity and temperature conditions [13] and bean rust appeared belatedly. Meanwhile, during the 2020 W-S cycle, the early bean rust incidence in Rincón Grande had a significant impact on grain production. An excessive rainfall (802.1 mm) in Rincón Grande resulted in the lowest grain yield during the 2019-2020 A-W cycle; 60.5% of the total rainfall was recorded during the first 30 das [9], causing a deficient development of the plants [13].

Jamapa Plus/XRAV-187-3-4-4 was the most productive line among the bean rust-resistant lines in the three evaluated environments (Table 3). The grain yield average of Jamapa Plus/XRAV-187-3-4-4 was statistically similar to the results obtained by Jamapa Plus/XRAV-187-3-4-1 and Jamapa Plus/XRAV-187-3-1-2, as well as by the Verdín variety; it was significatively higher than the rest of the genotypes (Table 3). Tosquy *et al.* (2019) reported that the Jamapa Plus/XRAV-187-3-4-4 and Jamapa Plus/XRAV-187-3-1-2 had a remarkably high productivity. Negro Jamapa and Negro Medellín recorded the lowest yield averages mainly as a consequence of the damages caused by bean rust.

Table 3. Bean rust incidence mean values of 14 black beans genotypes, in three environments of the state of Veracruz (2019-2020 Autumn-Winter cycle and 2020 Winter-Spring cycle).

Genotype	Rincón Grande O-I 2019-20 (kg ha^{-1})	Rincón Chico O-I 2019-20 (kg ha^{-1})	Rincón Grande I-P 2020 (kg ha^{-1})	Average (kg ha^{-1})
Papaloapan/SEN 46-2-6	1166.67	2718.67	1640.00*	1841.7 abcd
Papaloapan/SEN 46-3-2	1233.33	2933.33	1213.33	1793.3 bcd
Papaloapan/SEN 46-7-7	1222.00	2654.67	1266.67	1714.4 bcde
Papaloapan/SEN 46-7-10	1117.67	2503.33	1464.33*	1695.1 bcde
Papaloapan/SEN 46-7-12	1326.67*	2662.00	1246.33	1745.0 bcd
Negro Citlali/XRAV-187-3-1-5	789.00	2531.00	1433.33*	1584.4 de
Negro Citlali/XRAV-187-3-1-6	715.33	3227.67*	1206.67	1716.5 bcde
Negro Citlali/XRAV-187-3-1-8	1131.00	2528.33	1184.67	1614.6 cde
Jamapa Plus/XRAV-187-3-1-2	1388.67*	2827.00	1671.33*	1962.3 abc
Jamapa Plus/XRAV-187-3-4-1	1462.33*	3260.33*	1409.00*	2043.8 ab
Jamapa Plus/XRAV-187-3-4-4	1497.67*	3315.00*	1737.67*	2183.4 a
Negro Medellín (TR)	1069.00	2364.33	1122.33	1518.5 de
Negro Jamapa (TR)	849.00	2021.00	1180.00	1350.0 e
Verdín (TR)	1244.33*	3093.67*	1204.33	1847.44abcd
Promedio de ambiente	1158.05 c	2760.02 a	1355.71 b	1757.93
DMS (0.05)	261.65	367.38	378.78	375.78

RC (TR)=Regional Control. A-W (O-I)=Autumn-Winter. W-S (I-P)=Winter-Spring. *=Bean rust incidence values were statistically higher in each evaluated environment, according to the LSD (DMS) (α=0.05). Bean rust incidence mean values with the same letter in the genotype column are not statistically different, according to the LSD (DMS) (α=0.05).

CONCLUSIONS

The Jamapa Plus/XRAV-187-3-4-4, Jamapa Plus/XRAV-187-3-4-1, and Jamapa Plus/XRAV-187-3-1-2 lines showed resistance to the spore inoculation of different *U. appendiculatus* physiological races, found in the commercial crops of Veracruz and Chiapas, México. They also recorded a significantly higher grain yield average among these varieties than in the Negro Jamapa and Negro Medellín control varieties. Therefore, these three varieties can be released as commercial varieties in the short term.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Elements to improve the management and commercialization of dragon fruit (*Hylocereus undatus* (Haworth) D.R. Hunt)

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ABSTRACT
The objective of this study is to identify production and commercialization problems and potential of dragon fruit (*Hylocereus undatus*) in the socioeconomic context of San Luis Atolotitlan, Caltepec, Puebla, Mexico. A questionnaire was applied between March and May 2020 to n=20 producers-marketers of the Asociación de Producción Rural de San Luis Atolotitlan, Caltepec. The cultivation of dragon fruit is part of a crop diversification system within a social context characterized by migration, low education, and extensive dependence on local buyers. In addition, its maintenance practices, use of organic fertilizers, and average yields are minimal. However, if management is improved and transformation and marketing channels are identified and diversified, the system will be able to boost the economy of the region. Young producers, with a higher educational level and with an innovative attitude, could improve those measures.

Keywords: Marketing, production costs, production technology.

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INTRODUCTION

Dragon fruit (*Hylocereus undatus*) is native to southeastern Mexico and Central America (Zee *et al.*, 2004; Legaria *et al.*, 2005), although currently its commercial cultivation has been expanded to Southeast Asia, Europe, and the United States, where it is consumed more than in Mexico. From 2009 to 2019, the production of dragon fruit (*Hylocereus undatus*) in Mexico was carried out in the states of Yucatán, Quintana Roo, Puebla, Nayarit,

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Tabasco, Guerrero, Sinaloa, and Nuevo León (SIAP, 2020). As a whole, Quintana Roo and Yucatán accounted for 95% of that production. Dragon fruit production has increased from 1,493 t to 9,029 t: an average annual growth rate (AAGR) of 22.14%. Quintana Roo and Yucatan had an average harvested area of 356 and 272 ha, respectively —94% of the total area destined for cultivation. From 2009 to 2019, the state of Puebla accounted for 4.76% of the average domestic production (*i.e.*, 174 t and average yields of 4.1 t ha^{-1}). The cultivation has been carried out in the Distritos de Riego (Irrigation Districts) of Izúcar de Matamoros, Tecamachalco, and Tehuacán, with an average participation of 28.2%, 21% and 26.2%, respectively (SIAP, 2020). The objective of this research was to describe the production of dragon fruit (*Hylocereus undatus*) in San Luis Atolotitlan, Caltepec, Puebla and to identify its technological needs and existing commercialization channels.

MATERIALS AND METHODS

The study was carried out in the municipality of Caltepec (Figure 1) in southwestern Puebla. It limits to the northwest with the municipality of Zapotitlán and to the northeast with the municipality of San José Miahuatlán, both in the state of Puebla. To the south it limits with the state of Oaxaca.

It is part of economic region VII, whose municipal seat is located in Tehuacán. It has an area of 391.74 square kilometers that makes it the 11th biggest municipality in Puebla. It has five auxiliary governing boards: Acatepec, Acotolototlan, Coatepec, Acatitlan, and Tlalocuitepec (INEGI, 2017). For this research, the information was obtained through the application of a survey. This technique can be used when there is not enough information about certain topics or when the information cannot be obtained through other techniques (Rojas, 2002). The survey was applied to 20 dragon fruit producers-marketers who belong to the Asociación de Producción Rural de la comunidad de San Luis Atolotitlan, Caltepec, from March to May 2020. To calculate profitability, the average production costs and productivity of the surveyed producers were obtained. The following algebraic expressions based on economic theory were used for that purpose (Krugman and Wells, 2006; Samuelson and Nordhaus, 2010):

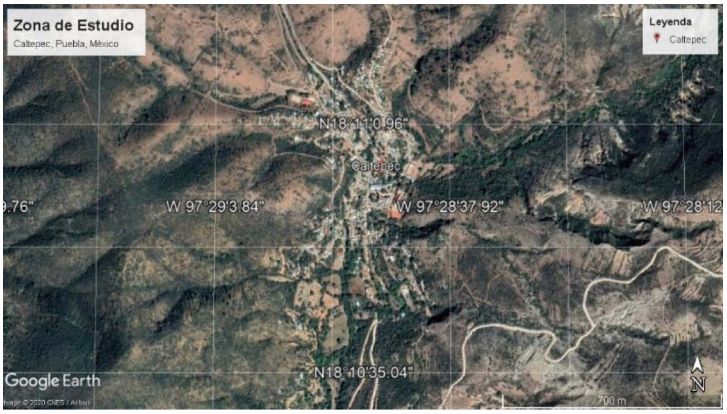

Figure 1. Dragon fruit (*Hylocereus undatus*) study area. Caltepec, Puebla, Mexico.

$$CT = PxX$$

Where: CT = Total cost; Px = Price of input or activity X; X = Activity or input.

$$IT = PyY$$

Where: IT = Total income (\$ ha^{-1}); Py = Market price of crop Y (\$ t^{-1}); Y = Crop yield (t ha^{-1}).

$$Profitability = IT - CT$$

RESULTS AND DISCUSSION

Producers' characteristics

Dragon fruit producers from San Luis Atolotitlan are in average 62-years old and their age range goes from 39 to 69 years. Seventy-eight percent of the producers are over 50; therefore, the population of the rural primary sector faces an aging problem. According to SAGARPA-FAO (2014), the aging of the population has been more pronounced in rural areas, mainly as a consequence of the national and international migration of the working age young population. The age of the producer is decisive for the adoption of new practices and technologies. In this study, only 22% of the producers were 50-years old or younger, have attended secondary school and are interested in knowledge, innovation, and the adoption of the new technologies. The rest of the producers showed no interest in this regard. None of the producers have studied beyond the secondary level: 78% had completed primary school and 22% secondary school. According to INEGI (2020), the 15+ population in the state of Puebla has attended a 9.2 average schooling level (little more than the third year of secondary school). This figure is lower than the national level (9.7 levels, a little more than completed secondary school). According to SAGARPA-FAO (2014), analyzing the schooling level of agricultural producers has a major implication in their capacity to implement productive technologies and to manage the production units.

Seventy-eight percent of the interviewees had relatives or they themselves had had to leave their homes looking for other places of work where they could improve their income. The USA was the destination of 43% of them. According to INEGI (2020), Puebla ranks 19th among Mexican states, regarding the migration of its population. Meanwhile, producers mention that remittances are an important source of income. Another major characteristic is that the producers fail to take advantage of their membership in the Asociación de Producción Rural de la comunidad de San Luis Atolotitlan, Caltepec. They neither make massive purchases, nor sell their production as a group. According to Ayala *et al.* (2013), the lack of productive organization prevents them from generating economies of scale; consequently, they must deal with intermediaries, who establish the price of the product.

Production characteristics

The producers are smallholders with an average of 4 ha, who raise backyard cattle species. The crops they grow along with dragon fruit are papaya, corn, and avocado. On average, producers use one hectare for dragon fruit, which is considered a rainfed crop; however, in some cases it has support irrigation. Producers are unaware about technological packages endorsed by agricultural or academic research institutions. The technology developed by INIFAP allows producers to handle up to 2,500 plants ha^{-1}. Applying the recommended technological components, as well as high-yield cultivars, returns of $\geq$18 t ha^{-1} can be achieved (Del Ángel *et al.*, 2012).

Dragon fruit propagation and cultivation

The crops are planted in May, transplanting cuttings into the ground with the help of posts (mostly made of concrete). Previously, the propagation of the cuttings is carried out during the new moon of April, according to the beliefs of the producers. The cuttings are planted in plastic bottles where they are allowed to grow until the end of April and, after rooting, they are planted directly in the ground. Before the dragon fruit trees are planted, the land is weeded and fertilized with a combination of donkey manure and organic matter.

Plant maintenance

A fertilizer is applied in May, while Malathión® (a pesticide used to combat the fruit fly and other dragon fruit pests) is applied in May, July, and August. The amount varies according to the condition of the crop: when a persistent pest is identified, producers tend to apply a higher dose. Dragon fruit plants are mainly attacked by parasitic pests, particularly the worm known as capichi or owlet moth (*Noctuidae* sp.) and the fruit fly (*Dasiops* sp.). Producers use support irrigation, although the crop is practically rainfed. Plant pruning is also carried out in May, before flowering begins. Together with the pruning, the bushes are subjected to a *rameada*, an activity that consists clearing the plant to guarantee that the buds about to bloom receive enough sunlight to grow without problems. This activity should be a sanitation pruning.

Harvest

Plants bear fruit until the second year. To satisfy the demand, producers plant around 150 cuttings per year. They record an average mortality of 15% —*i.e.*, for every 10 cuttings planted, at least two are lost in the process. This situation takes place when the roots of the plants have been fully developed before they are planted. Dragon fruit plants bloom three to five times a year. For each cut, 800 to 1,000 fruits ha^{-1} are obtained, as a result of the low planting density. The harvest is carried out in three cuts; however, five floral emissions are common and they normally overlap.

Production costs

At the beginning of the dragon fruit plantation, concrete posts are set up to serve as tutors for the plants' development. This investment was calculated based on 2020 prices, estimating a cost of $9,700 Mexican pesos. The posts receive an annual maintenance and are replaced after five years. Table 1 shows the total production costs for dragon fruit.

The fruit is harvested in three cutting periods, which take place at the end of July, August, and October, with an average production of 10 fruits per plant and an average of 100 plants per hectare. Table 2 shows the profitability calculations for the producer, breaking down cuts and average price paid per fruit.

The crop is profitable, as long as it finds a buyer (Table 2). The collectors usually visit the dragon fruit plantations located in the municipality; however, if collectors fail to visit the plantation, the harvest of that cut would likely be lost. The fruit is sold per piece and not per weight (kg), because some producers consider that they can obtain a higher profit margin that way. The dragon fruit is a promising crop for the producer, because it is profitable and does not require a great deal of investment, once the plant has started to

Table 1. Costs per hectare (2020 prices) of dragon fruit maintenance and production in San Luis Atolotitlan, Caltepec, Puebla.

Actividad	Unidad	Cantidad	Costo unitario ($)	Subtotal ($)
Tratamiento del tallo				
Obtención del esqueje	Pieza	150	5	750
Cortador de esqueje	jornal	2	120	240
Abono	kg	730	1.4	1,022
Aplicación de abono	jornal	1	120	120
Labores para el transplante				
Transplante	jornal	3	120	360
Aplicación de abono	kg	1,180	1.4	1,647
Labores culturales de mantenimiento				
Composta	kg	1,650	1.0	1,650
Aplicación de composta	jornal	1	120	120
Abono	kg	1,680	1.4	2,352
Aplicación de abono	jornal	1	120	120
Plaguicida	l	1	415	415
Aplicación de plaguicida	jornal	1	120	120
Insecticida	l	1	251	251
Aplicación de insecticida	jornal	1	120	120
Riego	l	2,000		1,200
Poda de formación (rameado)	jornal	2	120	240
Poda de limpieza	jornal	2	120	240
Deshierbe	jornal	2	120	240
Cosecha				
Corte 1	jornal	2	120	240
Corte 2	jornal	2	120	240
Corte 3	jornal	2	120	240
Total				11,927

Source: developed by the authors based on the information collected in the field.

Table 2. Fruit production, production costs, and profit per hectare (2020 prices) for the dragon fruit producer in San Luis Atolotitlan Caltepec, Puebla.

Concepto	Primer corte	Segundo corte	Tercer corte	Subtotal
Frutos por planta	10	10	10	30
Plantas por ha^{-1}	100	100	100	300
Producción de fruta por ha^{-1}	1000	1000	1000	3000
Precio pagado por fruta	7	7	7	
Ingreso por corte	7000	7000	7000	21,000
Costo de producción				11,927
Utilidad por ha^{-1}				9,073

Source: developed by the authors.

bear fruits; nevertheless, the main issue is having a market that constantly demands the product. The fruit is sold right outside the plantation. The price is established by the buyer, depending on its size and appearance. Producers mention that market saturation hinders the sale of fresh dragon fruit in times of maximum production. Ideally, producers should start transformation processes to add value to the fruit. This measure could generate local employment and improve the profitability and quality of life of the producers. According to Castillo (2006), this exotic fruit is accepted and reaches good prices in domestic and foreign markets. Although some of its characteristics still constitute a problem, the plants are ideally suited for their exploitation. Dragon fruit is a product with scarce diffusion in the domestic market; therefore, the promotion of non-traditional products with development potential becomes a fundamental task for the sustainable development of the agricultural sector (García and Quirós, 2010). The production and commercialization of this product is limited to the local market, since there are no land plots used exclusively for this crop and most of the production is collected from backyards. As a result of its nutritional value, the dragon fruit may have various opportunities in the market. The properties of the fruit are auxiliary in the treatment of some diseases (Lezama *et al.*, 2007).

CONCLUSIONS

The production is carried out without a technological package endorsed by a public institution and the producers use their empirical knowledge for the maintenance of the crop. The management of cultural practices carried out by the producer only takes into consideration weeding and pesticides. The producers lack any connection with experts in the field. Regarding the commercialization, it is necessary to establish a transformation process for the use of pulp or by-products. As an alternative to commercialization, we propose working under a rural cooperative arrangement that allows regional producers to collect and transform the fruit, in order to position the dragon fruit in competitive markets and boost demand. Positioning the dragon fruit in the domestic market requires campaigns that raise awareness about its benefits and promote its consumption. The dragon fruit is a promising crop for the smallholding producers, if they establish an adequate agricultural management and if there is a growing demand.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Analysis of tobacco productivity in Mexico between 1980 and 2020 through the KLEMS methodology

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ABSTRACT
Objective: Tobacco production experienced a gradual growth during the first half of the 20th century, but its increase has been truncated in the last four decades. The objective of the study is to analyze the productivity of tobacco in Mexico in order to certify its importance in Mexican society and to measure its productivity.
Design/methodology/approach: The article presents an analysis of tobacco's productivity in Mexico in the 1980-2020 period, through the KLEMS methodology. Analysis of census samples from the period studied has confirmed the decrease experienced by this agricultural production.
Results: The importance of production has been visibly decreased within macroeconomic factors such as the Gross Domestic Product (GDP), which leads to a loss of participation of this agricultural product both in the national and international context. This has resulted in the contraction, in addition, ostensibly, of the number of jobs that are directly related to this agricultural activity and the ulterior consequences that come up for the family economy.
Limitations on study/implications: The statistical records from the period 1980 to 2020 were taken into account, since there were no records available before 1980.
Findings/conclusions: Through the research results, the decrease experienced by the tobacco industry from 1980 to 2020 was confirmed, and also that production will disappear at the industrial level.

Keywords: Total productivity of factors, tobacco, KLEMS.

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Introduction

The tobacco industry in Mexico sets out social goals directed fundamentally at creating and preserving sources of employment in rural communities as a way to increase workers' income in this field, and of the families that depend on it. It has a quality that it shares internationally which identifies processes of concentration and monopolization of goods, and the tobacco industry has followed a pattern of business concentration and, consequently, of capital (Meneses, 2002).

Image by John Lambeth at Pixabay

The state of Nayarit, as has been mentioned, occupies the first place in tobacco production, aspect that stands out since the first decades of the past century when it became a strategic place in the distribution of this production for Latin America (Mackinlay, 2008). An important aspect is the amount of workdays linked to this agricultural activity and the value of this production. In addition, tobacco production has developed under a contract agriculture scheme, which is not only still maintained but rather becomes a key piece both for the organization of production in peasant families devoted to this crop and for its living and working conditions (Pacheco, 2003).

According to the study by Madera and Hernandez (2016), the level of personal occupation in the industry continues to be very low compared to the last century, and actions have been implemented to improve this situation; results are slightly noticed, although they are still not as substantial so as to take them into account.

The tobacco industry, due to the adverse consequences generated by its consumption on health, has been levied with taxes that discourage its production and consumption in Mexico. It is taxed with a Special Tax on Production and Services (*IEPS*) and VAT, the first has an ad-valorem component of 160% and another specific one of $0.35 MX per cigarette. One of the objectives of the taxes is to reduce the consumption of tobacco in order to lower the incidence of diseases associated to smoking (Ibarra, Fuente and Miravete, 2021). Estimations were made which showed that an increase in the price of cigarettes of 10% could reduce the consumption in 2.5% ($p<0.05$) and increase tax collection in 16.11%; the result confirms the effectiveness of taxes as a tool for tobacco control in Mexico (Olivera *et al.*, 2010).

The tobacco industry is present in other states of the country, although the number of jobs linked to it is not significant in relation to the total; however, something that cannot be sidestepped is that employment in the rural sector linked to the agricultural activity of tobacco farming is important in terms of training, technical assistance, technological development, and the social benefits received by day laborers and their families (Waters *et al.*, 2010). The objective of this study is to analyze productivity of tobacco in Mexico during the 1980-2020 period, through the KLEMS methodology.

MATERIALS AND METHODS

KLEMS Model

Measuring productivity of the different factors that intervene in the production process has been included in the agenda of economic themes since Robert Solow (1957) and his "Technical change and the function of added production"; a natural and intuitive evolution of the function has been the KLEMS methodology which allows understanding the productive factors that contribute to the economic growth and production, as well as their performance in the productive process, which for decision makers is very important for the design of public policies in the country (INEGI, 2021).

Various authors have applied the method, for example Timmer, Mahony and Van Ark (2007). The accounts of growth and productivity of the European Union KLEMS include measurements of production growth, creation of employment and abilities, capital formation, and multifactorial productivity (MFP) in the industrial level for the member states of the European Union since 1970. The entry measures include several categories of capital inputs (K), labor (L), energy (E), material (M) and services (S). O'Mahony and Timmer (2009) describe the contents and the construction of the KLEMS growth and productivity accounts; the database contains measurements of production, inputs and productivity at the industrial level for 25 European countries, Japan and USA, for the period of 1970 onward; the methodology used in the construction of the database and shows how it can be useful to compare productivity trends.

In Poland, the hypothesis was suggested that through the use of some innovative but acceptable evaluation techniques of missing data, it is possible to provide enough data for Poland for the accounts mentioned, after a general description of the KLEMS economic productivity accounts and the relevant fundamental methodology; the article presents more about how problems that have emerged of specific data have been solved (Kotlewski and Błażej, 2018). In addition, Goldar, Krishna and Aggarwal (2017) analyze the growth, structural change and advance in productivity of the Indian economy in the 1980-2014 period; the KLEMS approach takes into account the role that the inputs of capital, workforce, energy, materials and services have on the growth of production in the industries.

In Latin America, it was applied to analyze the economic growth, the productivity and its determinants in five countries, Argentina, Brazil, Chile, Colombia and Mexico, during the 1990-2010 period. The results showed that the slow economic growth is basically driven by the negative contribution of the total productivity of the factors (TPF) in all the countries and nearly all the sectors, despite the investment effort carried out in the last 20 years (Hofman, Mas, Aravena and Guevara, 2017). In Mexico, it was used according to Ibarra and Ros (2019) to estimate equations of the accumulation rate of the non-residential private capital in manufacture, tradable and non-tradable, in Mexico during the 1992-1994 period.

Banco de México (2015) found a positive correlation between growth of the TPF and that of production, as a result of this pattern, and causality tests were conducted for panel data in order to determine a possible causality between these two variables; it was found that the hypothesis of causality in both directions cannot be rejected at conventional significance levels. Another pattern resulting from INEGI data also show is that growth of the TPF, in addition to irregular, tends to concentrate in a few sectors. Since the structural changes in the Mexican economy in the decades of the 1980s and 1990s, a successful insertion into the global markets has been experienced, although the growth in productivity has been modest, which has led to low and volatile economic growth; despite a significant reassignment of hours worked between industries, their added impact has been obstructed by the prevalence of flows in the sectors with a high increase in labor productivity towards those with a lower or decreasing productivity growth, and the production factors are highly qualified both in workforce and capital have not shown a significant contribution to the increase in added value (Padilla and Villarreal, 2017).

Linear correlation coefficient

A positive Pearson's (1895) correlation between economic growth and flow of remittances, the correlation coefficient can be calculated through the following equation:

$$r = \frac{\sum xy}{\sqrt{\left(\sum x^2\right)\left(\sum y^2\right)}}$$

Where: $\sum xy$: is the covariance between *X* and *Y*; $\sum x$: is the typical deviation of *X*, and $\sum y$: of *Y*.

Pearson's linear correlation coefficient in the population will always be unknown and it will be estimated through the one obtained in the sample. An equivalent expression (Martínez *et al.*, 2014) is:

$$r = \sum\left(xi - \bar{x}\right)\cdot\left(yi \ n \ i = 1 - \bar{y}\right)\sum\left(xi - \bar{x}\right)2\cdot\sum\left(yi - \bar{y}\right) n2 \ i = 1 \ n \ i = 1$$

The other method is by Luquez, Gómez and Hernández (2021), with the aim of characterizing the production and generating a general outlook of the situation of different competitors. The growth rates of the indicators chosen were calculated for the analysis of exports with the following formula:

$$Tc = \left(Vf - Vi / Vi\right) * 100$$

Where: *Tc*: is the growth rate; *Vf*: at final value; *Vi*: refers to the initial value.

The research process was affected importantly by the global COVID-19 pandemic, which limited the physical consultation of documents, economic atlas, industrial census, economic census in libraries, and other institutions that protect these materials. All of the data were obtained virtually (INEGI, 2022), which impacted the selection of the time period to be studied, that is, from 1980 to 2020, and the databases were elaborated to then load and process them in the STATA12.0 software.

RESULTS

Data analysis was carried out, finding the correlations represented in Table 1. The gross production is positively related with the number of businesses; they have a correlation of 40.82%, which means that as the number of businesses devoted to the harvest, production and commercialization of tobacco increases or grows the gross production available to be offered also increases. At the same time that the relationship between the number of businesses and the gross production is positive, the latter maintains a stronger although positive relationship with the number of jobs used in all the levels necessary to make tobacco reach households or businesses of consumers; the correlation found in the period analyzed between these two variables is 71.55%, and in

Table 1. Correlations by tobacco variables.

	Gross production	Number of companies	Number of employees	Gross value added	Total energy costs	Ttotal expenditure on raw materials	Total fixed assets	Total expenses in services	Total advertising expenses
Gross production	1								
Number of companies	0.7155	1							
Number of employees	0.4082	−0.0526	1						
Gross value added	0.4919	0.6924	−0.459	1					
Total energy costs	−0.6128	−0.9388	−0.1188	−0.5203	1				
Total expenditure on raw materials	0.5093	0.5208	−0.2125	0.921	−0.3987	1			
Total fixed assets	0.9468	0.6707	0.4835	0.2557	−0.594	0.2156	1		
Total expenses in services	−0.4714	−0.8617	0.0211	−0.4935	0.849	−0.3121	−0.4634	1	
Total advertising expenses	−0.4352	−0.7826	−0.1297	0.3283	0.7826	−0.2054	−0.4469	0.8864	1

Source: Prepared by the authors.

terms of the gross production and the added value, they have a correlation of 49.19% between both variables.

Gross production has a solid correlation that amounts to 94.68% with the total fixed assets, and 50.93% of positive correlation with the variable total expenditure in raw materials, showing that when there is a higher amount of fixed assets, it is the originator to produce a higher amount of product without leaving aside all the expenses in necessary inputs to comply with these high production quotas. The existing relationship between gross production and the expenditures in energy is negative. The correlation between these two variables is 61.28%, which implies that when a greater amount of energy can be consumed to produce, it is used with negative productivity, attaining a growth rate under zero.

The relationship there is between the variable gross production and the variables expenses in services and expenses in advertising is negative in the gross production with both variables, although it was certainly not as strong as the case of other variables; regarding the expenses destined to services, there is a correlation of 74.14%, while for the expenses focused on promotion, advertising and sponsorships, the correlation reaches 43.52%, showing that as the production grows, it is necessary to invest increasingly less in advertising to reach markets, even those unknown, than what is required as the part referring to services within the intermediate consumption.

The number of employees variable maintains a negative relationship with four variables among the nine studied; the variables number of businesses, total expenditure on energy, total expenses in services, and total expenses in advertising, has a correlation of 93.88% with the total expenditure in energy while the correlation is 86.17% with total expenses in services, and in addition to these ratios, the number of employees in the industry has a correlation of 78.26% with total expenses in advertising and 5.26% with the number of businesses in the sector. This entire situation shows a lack of social policies in order to promote jobs, particularly for rural communities, although at the same time, the presence of new machinery that consume a higher amount of energy and a lower number of workers is undeniable.

Positive ratios were obtained of the employees with the rest of the variables studied: gross added value, total expenses in raw materials, and total fixed assets; the correlations with these variables mentioned are 69.24%, 52.08% and 67.07%, respectively, manifesting with the figures that as the number of employees increase, the total expenses in raw materials also increase. This takes into account that not all of it us used in the productive process as such, but that the consumption of materials also increased because since there are more workers in the industry there must be more mistakes at the time of consumption, among other elements that somehow spend inputs only for the employees; the total fixed assets also increase, since the higher number of workers bring with them the need to obtain a greater amount of productive tools and elements. Table 1 shows the correlation matrix.

Some other variables that were analyzed were the number of businesses, fixed assets and total expenses in services; the correlation is positive with each of them, of 48.35% and 2.11%, respectively. This denotes that as the number of businesses increase in the Mexican tobacco industry, an increase is also manifested in the amount of assets available for production. The relationship between businesses and employees behaves negatively and is almost inversely proportional; something similar happens between the number of businesses and the gross added value, and the total expenditure in energy, the variables total expenses in raw materials, and total expenses in advertising, promotion and sponsorship. The relationship with these four variables is negative, although it is certainly not as strong as the existing correlation between the total expenses in raw materials and total expenditure in energy, showing a correlation of 21.25% and 11.88%; the correlation between number of businesses and gross added value reaches 45.95%, demonstrating that as the number of businesses in the sector increases, an increase in the value added to the production is not seen, as it was expected to happen.

The gross added value has a negative relationship with most of the variables related; regarding the total expenditure in energy, a negative correlation of 52.03% was obtained, which is explained by the fact that as the value added to the final product increases, a lower amount of energy destined to achieving this productive and commercial purpose is necessary in most cases. With the total expenses in services variable, it maintains a correlation of 49.35%, it is directly related to the expense in intermediate consumption necessary to increase the value added to the production, when investing less time and intermediate goods of service in attaining an increase in the added value to the product, the investment in advertising, marketing and promotion destined to this aim would also have a negative relationship.

The gross added value is positively related with the amount of total fixed assets, the correlation is 25.57% which evidences the need to acquire more fixed assets to the extent that the purpose is to increase the value that should be added to the production; the ratio with the total expenses is higher, with 92.10%, and as the gross added value to the product increases, the total expenses in raw materials increase almost to the same measure. The total expenditure in energy manifests negative relationships with the consumption of energy, and in total expenses in raw materials and total fixed assets, the correlation is 39.87% and 59.40%, respectively, expressing negative parameters since there is no need to have a higher amount of machinery and equipment because there is less machinery but which consume less amount of inputs even when they consume more energy of every type.

The existing correlation between the consumption of materials and inputs with total fixed assets is 21.56%; this ratio is positive and reflects the need for consumption in resources to produce increasingly more as the amount of machinery destined to production increases; the correlations with total expenses in services and total expenses in advertising are negative, of 31.21% and 20.54%, respectively, implying that as the total expenses in raw materials increase, the expenses in services and advertising also increase, although in an inversely proportional manner. The total fixed assets have a negative ratio with the variables expenses in services and total expenses in advertising, where the correlation is 46.34% and 44.69%, respectively, implying less expenses in both variables while the amount of fixed assets increases, and this is reflected in lower levels of production and therefore lower expenses in intermediate consumption destined to services to produce what is required by the demand. Figure 1 illustrates that throughout the last 30 years large amounts have been sustained destined to advertising, promotion and sponsorship, variation rates are present mostly increasing, ranging from 2% until reaching a level of 45%, going through intermediate levels but at the same time showing some value that denotes a decreasing variation that ended in the first decade of 2000; a growing trend is seen in the progress which refers to the amounts spent in fulfilling the sales goals both at the national and the international level.

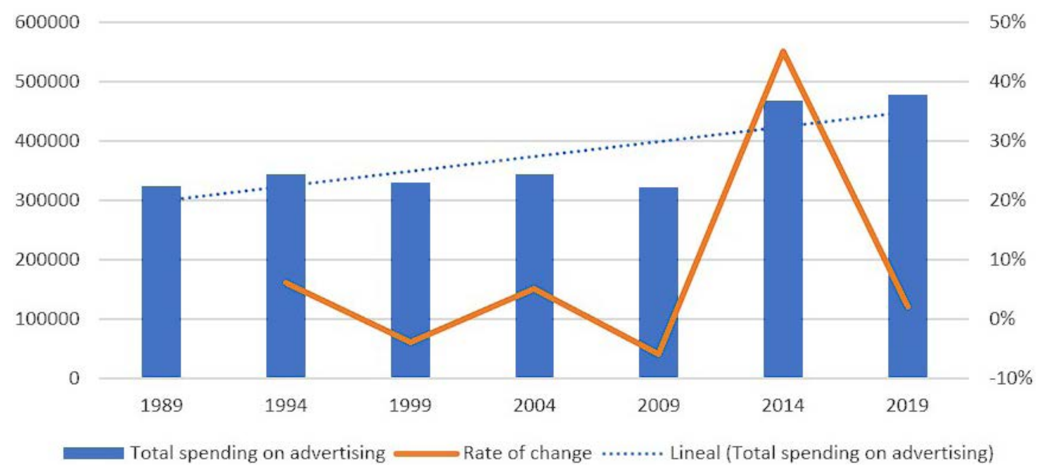

Figure 1. Total expenses in advertising, their variation rates and tobacco trends. Source: Prepared by the authors with data from INEGI.

Regarding the intermediate consumption, mostly increasing variation rates are seen which range from 1% to a level of 15% going through intermediate levels, but at the same time a value is seen that denotes a marginally decreasing variation that ends in the first decade of 2000. Figure 2 shows the peaks, both increasing and decreasing, in the percentage evolution of total expenses destined in services to produce.

Equipment and machinery are acquired with the aim of increasing production and utilities. Next, the variation rates are presented graphically, which were mostly increasing and range from 1% to a level of 206% passing through intermediate levels, and there were also negative variations. Figure 3 shows how the total expenses destined to having a higher amount of fixed industrial assets have increased, with the aim of reaching the productive goals planned in sectorial strategies.

Figure 4 shows the behavior of the acquisition of raw materials and inputs, which show mostly constant variation rates that range from 0% to a level of 3%, passing through

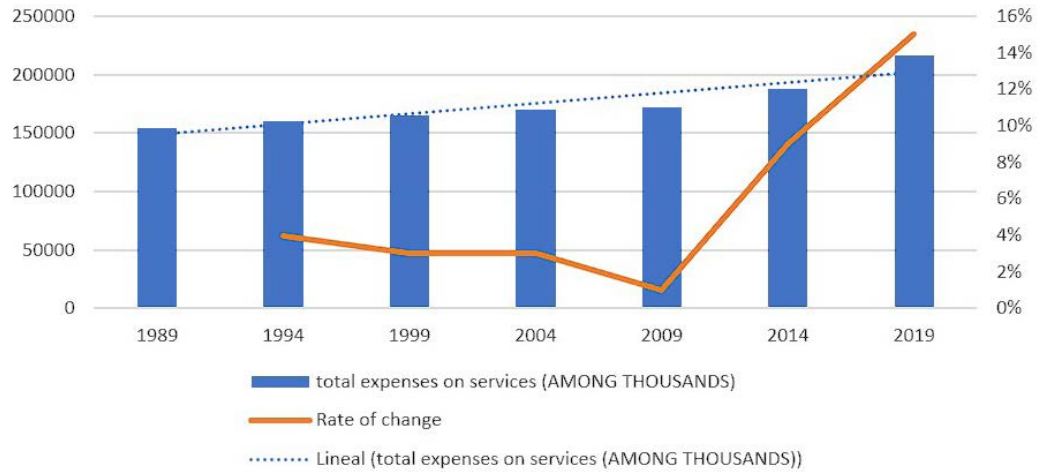

Figure 2. Total expenses in services, their variation rates and tobacco trends. Source: Prepared by the authors with data from INEGI.

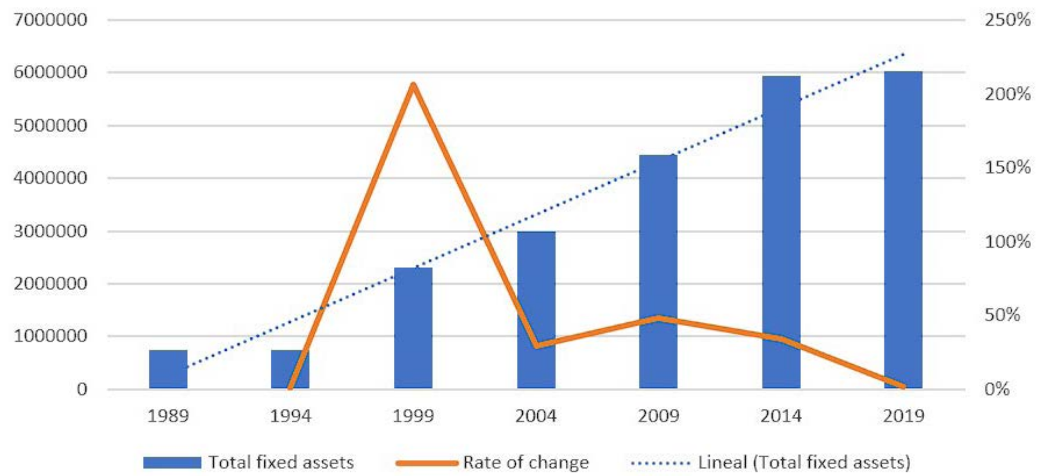

Figure 3. Total fixed assets, their variation rates and tobacco trends. Source: Prepared by the authors with data from INEGI.

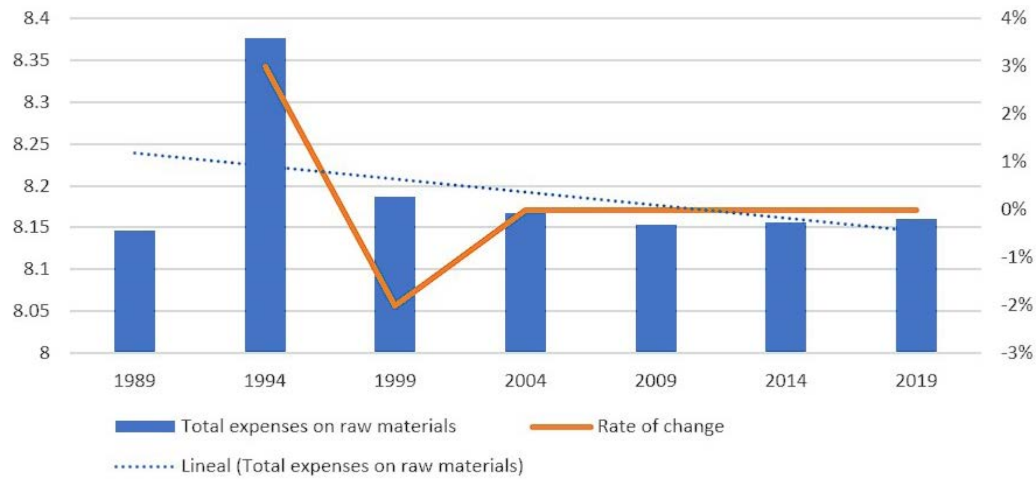

Figure 4. Total expenses in raw materials, their variation rates and tobacco trends. Source: Prepared by the authors with data from INEGI.

intermediate levels that are rather discouraging; the continuous line shows both increasing and decreasing peaks in the percentage evolution of expenses in raw materials, and a decreasing trend is seen which has implied a decrease in general of production in the sector; the general trend is decreasing.

The energy consumption has increased with the aim of increasing production and utilities. Figure 5 presents the variation rates that are mostly increasing, which range from 2% to a level of 68%; at the end of the second decade of 2000, some negative values are seen, and this is shown by a discontinuous line in general with an increasing trend with a very accentuated slope; both the increasing and decreasing peaks are shown.

It was identified that there are increasingly less investments to add the best level possible to the final product; mostly decreasing variation rates are seen, which range from 140% to a level of 199% passing through intermediate levels, although at the same time, there are some values that denote a decreasing variation from the beginning of the

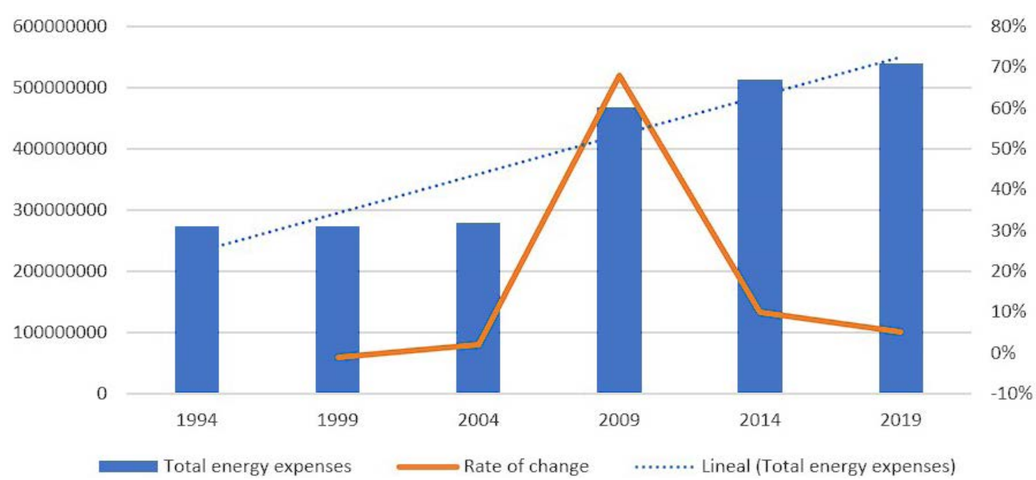

Figure 5. Total expenditure in energy, their variation rates and tobacco trends. Source: Prepared by the authors with data from INEGI.

second decade of 2000. In the four decades analyzed, a very strong decreasing trend is seen in the evolution referring to the amounts spent in adding value to the production, as can be seen in Figure 6.

Throughout the four decades analyzed, a very strong descending trend is seen in the evolution referring to the amounts generated by the total gross production, as can be seen in Figure 7. In these years lower and lower investments are seen, destined to producing a higher amount of the final good, to increase the utilities in the Mexican tobacco sphere. In this sector, mostly decreasing variation rates are seen which range from 109% to a level of 199% passing through intermediate levels, although at the same time, there are some values that denote a decreasing variation from the beginning of the second decade of 2000.

In this sector, mostly decreasing variation rates are seen, which range from 3% until reaching totally negative levels; they reach 23% and pass through intermediate levels,

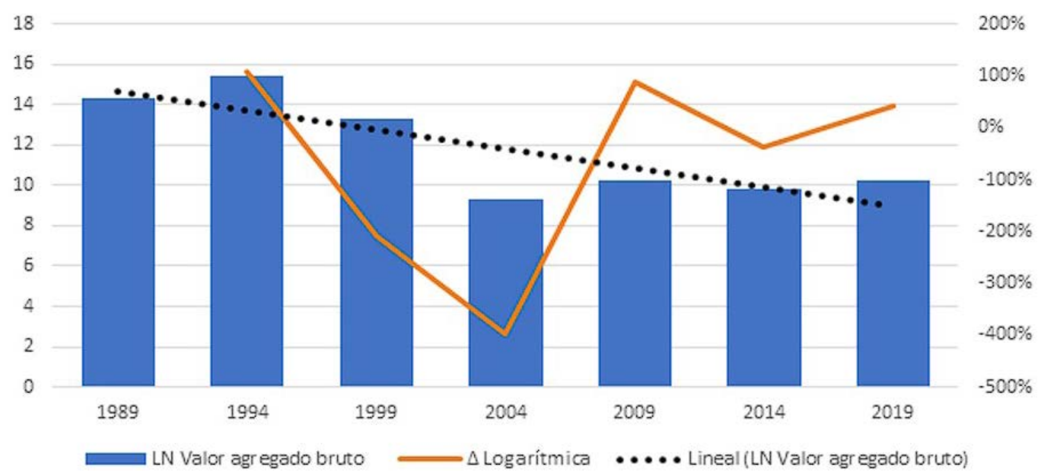

Figure 6. Gross added value LN, variation rate and trend line of the gross added value. Source: Prepared by the authors with data from INEGI.

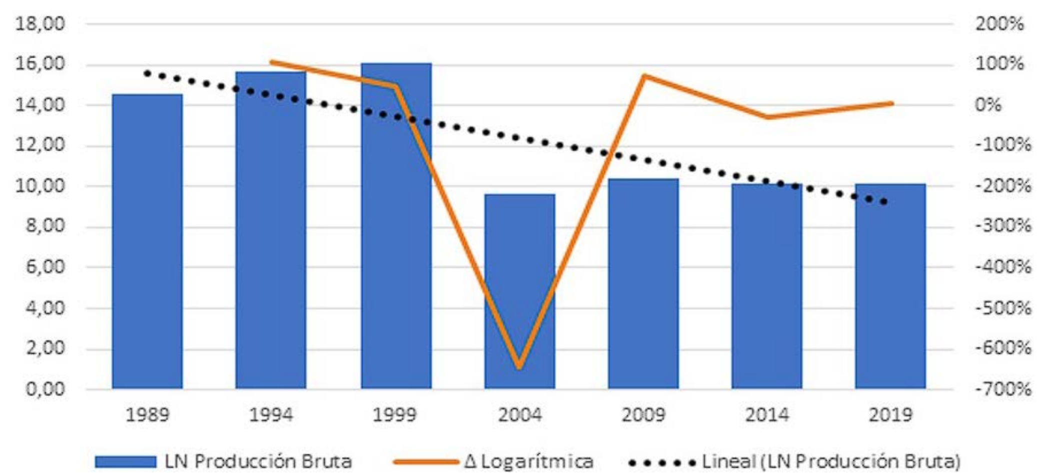

Figure 7. Gross production LN, variation rate and trend line. Source: Prepared by the authors.

rather discouraging, values that denote decreasing variations throughout the entire period studied. Throughout the four decades analyzed, a rather decreasing trend is observed in the evolution referring to the numbers of employees used for production, as seen in Figure 8.

As Figure 9 shows, quite decreasing levels have been maintained in the number of businesses. In this sector there are variation rates that are mostly decreasing, which range from 23% to a level of 95% passing through intermediate levels, although at the same time, there are some values that denote really worrying decreasing variations since the year 2000 began. Throughout the four decades analyzed, a very stable trend is observed, although increasing in the evolution referring to the number of businesses operating in this sector, as can be seen in Figure 9.

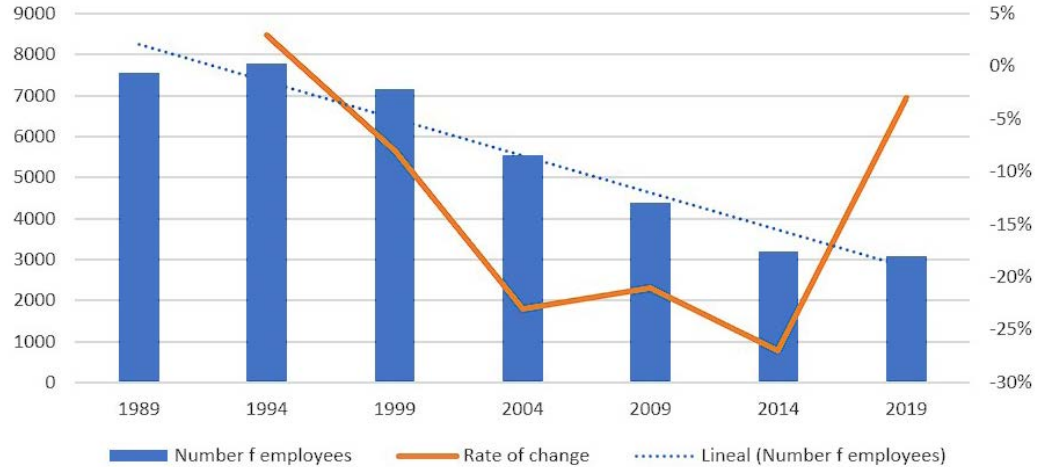

Figure 8. Number of employees, their variation rates and tobacco trends. Source: Prepared by the authors.

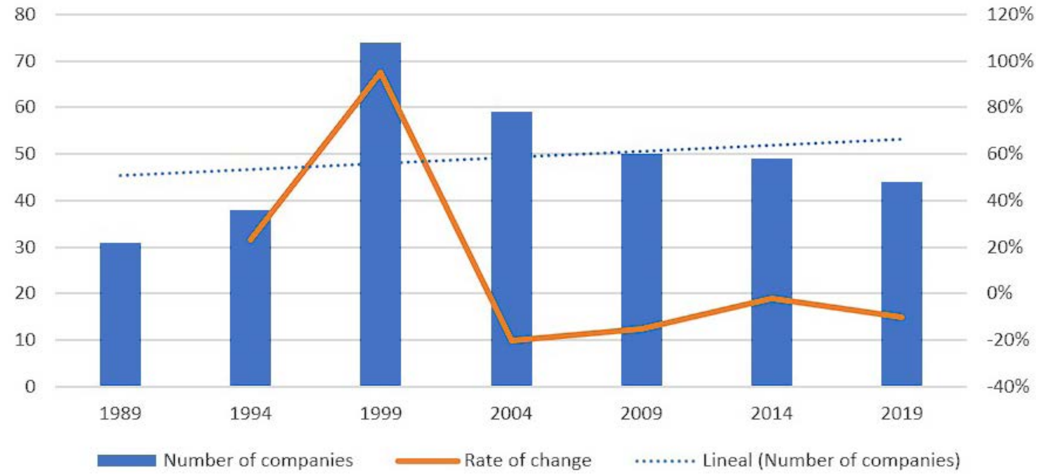

Figure 9. Number of businesses, their variation rates and tobacco trends. Source: Prepared by the authors.

CONCLUSIONS

Throughout the last four decades, the Mexican tobacco industry has contracted, especially the number of employees, and in this sector negative variations have been seen since the middle of the 1990s and until today. The conclusion is that the level of productivity in the tobacco sector has been kept in constants, with both positive and negative peaks with a strong attraction to decrease, particularly in the last 25 years, regardless of the amount of investment applied in the industry. Practically half of the variables and indicators have an inversely proportional relation to the capital destined to this national product.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Vitrification of White-tailed Deer (*Odocoileus virginianus*) oocytes with sucrose or trehalose for *in vitro* maturation and fertilization

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ABSTRACT

Objective: Evaluate the White-tailed Deer (WTD) *in vitro* embryo production (IVP) and oocytes vitrified with Trehalose (TH) or Sucrose (SC).

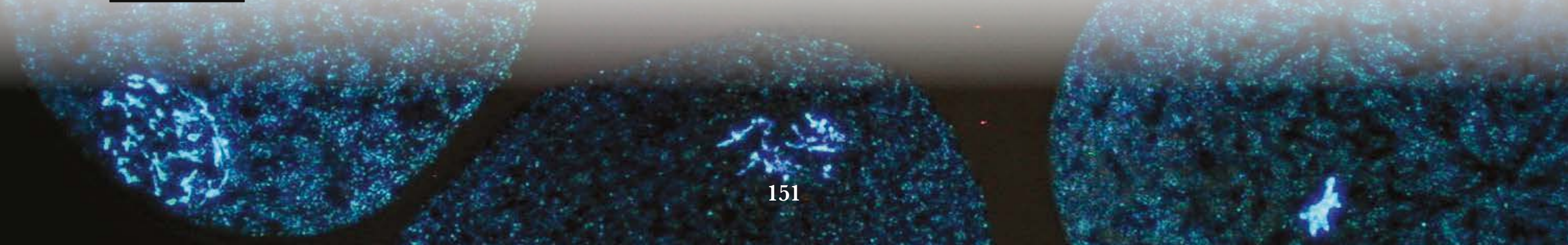
Design/methodology/approach: Total vitrified oocytes were placed into two different groups: TH (n=60) and SC (n=61). Samples were selected and analyzed for viability evaluation TH (n=5) and SC (n=5), nuclear status (NS) TH (n=4) and SC (n=5), Germinal Vesicle (GV), Metaphase I, or not evaluable (NE) after warming. *In vitro* maturation (IVM) was conducted for 36 h in supplemented TCM-199 medium. Immediately afterwards, oocyte NS was evaluated (n=88) [(GV, MI=immature), (MII=mature)]. *In vitro* fertilization (IVF) was performed in supplemented TALP medium for 24 h using frozen WTD semen (3×10^6 sperm/mL), NS was classified [Fertilized (F), Not fertilized (NF), or NE].

Results: After warming, viability for the TH group (n=5) was 60% versus 40% for SC group (n=5), however, oocytes in both groups were immature (GV and MI stage). For IVM, NS evaluations of the TH group (n=38) revealed no maturation versus 2% in the SC group (n=50) (MII stage=matured). IVF evaluations for the TH group (n=10) revealed no fertilization compared to 20% in the SC group (n=5). A statistical difference ($p>0.05$) was not found between the TH and SC groups.

Limitations on study/implications: White-tailed Deer *in vitro* embryo production is not well documented.

Findings/conclusions: Future research with a larger number of WTD oocytes is needed for further evaluation of oocyte vitrification IVP techniques as a model for endangered cervids.

Keywords: White-tailed Deer, *in vitro* embryo production, solid surface vitrification.



INTRODUCTION

The WTD is a wild ruminant that with its physical characteristics, and availability, can be considered as a model animal for those deer species in danger of extinction, *e.g.*, Florida Key Deer, regarding reproductive studies associated with conservation (Somfai *et al.*, 2014; Gastal *et al.*, 2018). Further, semen processing, oocyte cryopreservation/vitrification, IVF, embryo cryopreservation, and embryo transfer allow the selection and use of animals that are genetically superior for increasing the productive parameters that are of economic importance, thus also decreasing the risk of spreading diseases (Clemente-Sánchez *et al.*, 2017).

Given the high demand for breeding deer of superior genetics and trophy bucks, *e.g.*, hunting ranch breeding programs and the availability of a hunting season, ART can be an effective method for deer conservation and deer production programs (Maraboto *et al.*, 2022).

Vitrification is defined as a solidification similar to the vitreous state. Through high concentrations of cryoprotectants and an ultra-rapid cooling rate (Begin *et al.*, 2003) reaching high cooling rates of 16,700 °C/min (Criado-Scholz, 2012). Vitrification is a proven technique capable of preserving the integrity of cell structures (Gastal *et al.*, 2018). Solutions that contain SC or TH are widely used for cryopreservation of oocytes and embryos of mice, cattle, horses, pigs, and sheep (Tian *et al.*, 2015). SC is a disaccharide formed by glucose and fructose, and TH is a disaccharide of glucose, both are non-permeable cryoprotectants that maintain the extracellular osmotic gradient to prevent crystallization for oocytes and embryos of various species such as humans, bovine and murine (Chen *et al.*, 2001; Tian *et al.*, 2015).

Solid surface vitrification (SSV), one type of cryopreservation technique, is performed by ultra-rapid temperature reduction on a sterile, cold metal surface (10,000 °C/min) (Somfai *et al.*, 2010). Importantly, it is necessary to carry out a process of elimination regarding the vitrifying solution and at the same time of cellular rehydration. The warming procedure is carried out in several stages, subjecting the vitrified cell to solutions with decreasing concentrations of non-permeable cryoprotectants, so that through the osmotic effect they leave the cell, to prevent sudden water pressure changes (Izaguirre and Díez, 2012).

At present, no scientific literature is available pertaining to WTD vitrified immature oocytes for IVP, and only fresh oocyte IVM has been done for this cervid species (Maraboto *et al.*, 2022). In order to enhance cervid endangered species conservation and embryo production, the aim of the present study was to use vitrify WTD oocytes with two non-penetrating additives TH or SC and evaluate their survival after IVM and IVF.

MATERIALS AND METHODS

The present study was carried out in the Reproduction Management Laboratory of the Autonomous Metropolitan University-Xochimilco Unit in Mexico City.

Ovaries and oocytes collection. The collection of WTD immature oocytes (n=121) was performed according to methods of Siriaroonrat *et al.* (2010). Oocytes were collected from hunter harvested deer (n=18) using the slicing technique with Syngro holding medium (Vetoquinol, France) for oocyte collection, and not more than 2 h postmortem.

Vitrification. The WTD oocytes were then cryopreserved using a SSV technique (Somfai *et al.*, 2014). The equilibrium medium consisted of base medium (PBS) supplemented with 4 mg/mL bovine serum albumin (BSA), 2% propylene glycol (PG) and 2% ethylene glycol (EG; Sigma-Aldrich, Mexico). The vitrification solution was composed of base medium supplemented with 4 mg/mL BSA and separated into two different groups: TH (n=60) (0.3 M) (TH, 17.5% EG, 17.5% PG) and SC (n=61) (0.3 M SC, 17.5% EG, 17.5% PG). For the SSV method, COCs were placed at the equilibration medium for 15 min, washed three times in 20 μL drops of vitrification solution, then pipetted into a glass capillary tube with respect to each of the groups, and finally, placed into about 2-3 μL of vitrification solution and then dropped onto a piece of aluminum foil (*i.e.*, raft) floating on the surface of liquid nitrogen (Somfai *et al.*, 2010).

Warming. To process the vitrified WTD oocytes that were stored in cryotubes, the microdrops were placed onto an aluminum raft with a nylon net placed on the liquid nitrogen and used to recover oocytes. Thereafter, the nylon net was rinsed with liquid nitrogen to ensure a full recovery of the vitrified oocytes. The oocyte warming (OW) media content was base medium supplemented with 4 mg/mL BSA and TH, which was conducted using 4 different concentrations of the warming medium (OW1 1 M, OW2 0.5 M, OW3 0.25 M and OW4 0 M) (Sigma-Aldrich, Mexico).

In a 4-well dish, the first warming medium (OW1 medium (500 μL) was pre-heated in a water bath at 39 °C, then oocytes were placed in this warming solution for 2 min. In the 4 well dish, the other OW medium 2, 3 and 4 were placed (500 μL) at 38 °C and the oocytes from OW1 were placed in each medium for 1 min. A 35 mm Petri dish was prepared separately for the washing medium prior to IVM (Somfai *et al.*, 2010; Zhang *et al.*, 2017).

Warming Viability and Nuclear Evaluation. A sample from the warmed oocytes was used to evaluate viability from group TH (n=5) and SC (n=5). The cumulus cells were removed by flushing the oocyte in PBS. A drop of MTT stain [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], 0.5 mg/mL) (Sigma-Aldrich, Mexico) was placed into a four well dish with the oocytes for evaluation, and after 2 h viability results were obtained. Oocytes with purple coloration were considered viable and the non-colored ones were non-viable (Lestari *et al.*, 2018; Figure 1a). For NS evaluation oocytes from TH (n=4) and SC (n=5) groups were stained with DAPI stain, and oocytes were classified into germinal vesicle stage GV, MI, and NE. For DAPI staining (4α,6-diamidino-2-phenylindole, 1 μg/mL) (Sigma-Aldrich, Mexico), oocytes were transferred to 2% paraformaldehyde (HYCEL, Mexico) for 15 min, washed 3 times at IMACELL (IN VITRO, Mexico), followed by DAPI staining with 1 μg/mL, and mounted with minimal medium onto a clean glass slide, for evaluation using epifluorescence microscopy (Nikon eclipse E600 20x) to determine oocyte nuclear maturation (Siriaroonrat *et al.*, 2010).

***In vitro* maturation and evaluation**. The remaining oocytes were used for IVM, and incubation of oocytes took place at 38.5 °C with 5% CO_2 for 36 h in maturation media TCM-199 (IN VITRO, Mexico) supplemented with human menopausal gonadotropin (75 IU/mL) (IBSA Institut Biochimique SA. Switzerland) and epidermal growth factor (10 ng/mL) (Sigma-Aldrich, Mexico). From those oocytes used for IVM (n=88), a sample

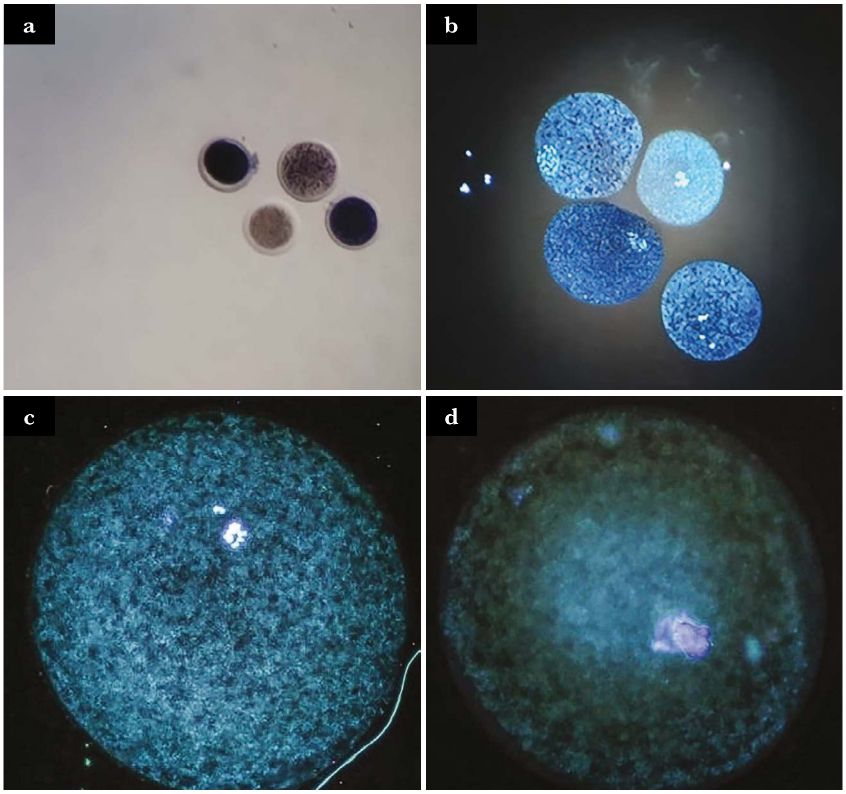


Figure 1. a) White-tailed Deer (WTD) (*Odocoileus virginianus*) oocyte viability post-warming evaluation with MTT stain; purple stained oocytes indicate active mitochondria (*i.e.*, considered alive); unstained oocytes indicate no mitochondrial activity (*i.e.*, considered dead oocytes), b) WTD oocyte nuclear status after warming, c) WTD oocyte after *in vitro* maturation Metaphase II showing the nuclear status with DAPI stain, and d) WTD oocyte after *in vitro* fertilization showing the pronuclear status.

was used to evaluate NS using DAPI stain, defining the oocyte stage as GV, MI and NE [considering these three stages as immature, and MII as mature] (Izaguirre and Díez, 2012; Siriaroonrat *et al.*, 2010; Locatelli *et al.*, 2005).

***In vitro* fertilization and evaluation**. For IVF, frozen conventional WTD straws of semen were used and capacitation was induced with "swim-up" technique using modified TRIS media (Locatelli *et al.*, 2005). IVF took place in TALP media supplemented with heparin (10 μg/mL) (PISA, Mexico), penicillamine (0.075 mg/mL), hypotaurine (10 M), epinephrine (1 μM) and BSA fraction V (0.4%) (Sigma-Aldrich, Mexico), with a final semen concentration of 3×10^6 sperm/mL (Locatelli *et al.*, 2006). After 24 h of incubation at 38.5 °C with 5% CO_2, and humidified air, nuclear status evaluation was performed using DAPI stain with 1 μg/mL to evaluate WTD oocyte fertilization. The aforementioned resulted in oocytes being classified as F, NF, or NE (Berg *et al.*, 2002).

Theory/Calculation. The WTD oocyte vitrification can serve as a model to assist with genetic improvement and conservation of the other cervid species, a promising tool to gather genetics, *i.e.*, cryobank oocytes, for future IVF and cloning projects. In order to apply IVP techniques as a model for other endangered cervid species, we used the technique developed for bovine oocyte vitrification and IVF and applied it to WTD.

Statistical analysis. A Fisher's exact test (SAS, version 9.0 for Windows) was used to determine statistical differences $p<0.05$ in the different stages of the study, concerning vitrified oocyte warming, IVM and IVF.

RESULTS

Warming. Viability evaluation for the TH group (n=5) after warming was 60%, and 40% for the SC group (n=5) (Figure 1a). In addition, for nuclear status evaluation (Figure 1b) TH (n=4) was 50% GV, 50% MI, which were considered immature oocytes, and for the SC group (n=5) results were 60% GV, 20% MI, immature oocytes and 20% NE (Table 1).

After 36 h IVM (Figure 1c) in the TH group (n=38) the nuclear status evaluation was conducted (66% GV, 24% MI, 0% MII and 10% NE), which demonstrated that no maturation took place in the sample used and for the SC group (n=50) (84% GV, 10% MI, 2% MII and 4% NE), even though the sample was small, matured oocytes were obtained. No statistical difference ($p>0.05$) was found between the TH and SC groups in viability, warming, IVM and IVF evaluations (Table 2).

For IVF, after 24 h (Figure 1d), in the TH treatment (n=10) nuclear status was 0% F, 60% NF, and 40% NE and no positive fertilization rate was obtained versus the SC treatment (n=5) with (20% F, 40% NF, and 40% NE), representing a small percentage, but opening the possibility to further studies for replicating the procedure with a 20% fertilization rate, although no statistical difference ($p>0.05$) was found (Table 3). Notably, a statistical difference ($p>0.05$) was not found between the TH and SC groups with regard to post-warming IVM, and IVF viability.

Table 1. Effect of two different sugars on White-tailed Deer (*Odocoileus virginianus*) vitrified oocyte nuclear status after warming.

	Nuclear Status					
	GV	**(%)**	**MI**	**(%)**	**NE**	**(%)**
TH	2	50	2	50	0	0
SC	3	60	1	20	1	20

No difference ($p<0.05$) between treatments Trehalose (TH) or Sucrose (SC) using a Fisher's exact test. Germinal Vesicle (GV), Metaphase I (MI) and Not Evaluable (NE).

Table 2. Effect of two different sugars on White-tailed Deer (*Odocoileus virginianus*) vitrified oocyte nuclear status after *in vitro* maturation.

	Nuclear Status							
	GV	**(%)**	**MI**	**(%)**	**MII**	**(%)**	**NE**	**(%)**
TH	25	66	9	24	0	0	4	10
SC	42	84	5	10	1	2	2	4

No difference ($p<0.05$) between treatments Trehalose (TH) or Sucrose (SC) using a Fisher's exact test. Germinal Vesicle (GV), Metaphase I (MI), Metaphase II (MII), and Not Evaluable (NE).

Table 3. Effect of two different sugars on White-tailed Deer (*Odocoileus virginianus*) vitrified oocyte nuclear status after *in vitro* fertilization.

	Nuclear Status					
	F	**(%)**	**NF**	**(%)**	**NE**	**(%)**
TH	0	0	6	60	4	40
SC	1	20	2	40	2	40

No difference ($p<0.05$) between treatments Trehalose (TH) or Sucrose (SC) using a Fisher's exact test. Fertilized (F), Not Fertilized (NF) and Not Evaluable (NE).

DISCUSSION

Warming viability and nuclear evaluation. In the cryopreservation of mature oocytes at MII, irregularities have been found in the meiotic spindle, resulting in a disruption in the alignment of the chromosomes and disorganization in the microfilament network. Cryopreservation of oocytes may affect de Ca^{++} signaling, mitochondrial membrane potential and membrane proteins. On the other hand, in immature oocytes, such as in the GV stage, they do not have an organized meiotic spindle, so oocyte cryopreservation at this stage could be an alternative to avoid damaging the meiotic spindle (Zhang *et al.*, 2017). In bovine oocytes, vitrification and the warming process did not affect the morphology nor viability (Serra *et al.*, 2020). In goat, Gonzales-Silvestry *et al.* (2022) observed that vitrified cryoprotected exhibited a higher degree of alterations in terms of oocyte cleavage, +6 cells stage at 72 h post-fertilization.

***In vitro* maturation nuclear status**. Siriaroonrat *et al.* (2010), used fresh WTD oocytes for IVM, where nuclear maturation was observed in 24 h. Vitrified oocytes at MII stage might end up with a depolymerized spindle, but it can be restored after cryopreservation; however, this process may take a longer period of time. Therefore, extending incubation time for IVM for vitrified oocytes might improve maturation results, as the regeneration of organelles after vitrification may be an important role in the developmental ability (Somfai *et al.*, 2010). In the present study, vitrified WTD oocytes were used for IVM, with vitrified oocytes of WTD at GV stage 36 h for incubation was used. Oocytes vitrified by the SSV technique in the present study had low developmental competence, and this can be attributed to damage to the meiotic spindle and to the chromosome configuration generated by the vitrification, based on previous research from other studies. For example, in one study using pre pubertal goats (Menéndez-Blanco *et al.*, 2020), fresh oocytes were compared to vitrified oocytes after IVM, results stated that vitrified and warmed oocytes showed higher reactive oxygen species levels compared to that of the fresh oocytes. According to the results of the present study, the oocyte competence may be affected by the vitrification process and that increases the number of dead oocytes and more reactive oxygen species.

Berg *et al.* (2002), analyzed the time taken for *in vitro* nuclear maturation in Red Deer. The aforementioned study revealed that oocytes freshly subjected to IVM remained in the GV stage for the first 6 h of incubation. The nuclear membrane began to fade after 6 h and at 10.6 ± 0.6 h, 75% of the oocytes showed breakdown of the GV. The average time in which 50% of the oocytes reached MI was 11.7±0.4 h and MII was 24.8±0.9 h. The full expansion of the cumulus cells was at 18 h. In nuclear maturation, a similar period is indicated for ovine and bovine fresh oocytes. Based on this, the incubation time for IVM of vitrified oocytes in WTD was adjusted to 24 h; however, when evaluated, there was no activity in the nuclear maturation in WTD oocytes. Therefore, in the present study, after 36 h of incubation in WTD vitrified oocytes a possible injury to the meiotic spindle might cause a delay in the incubation time needed for maturation.

***In vitro* fertilization nuclear status**. In the ZP there can be structural changes, such as hardening or membrane characteristics, promoting an early exocytosis of cortical granules caused by cryopreservation. Moawad *et al.* (2012), carried out IVF of vitrified ovine oocytes with TH and obtained a significantly low fertilization rate with 39.3% *vs.* the control group with 64.7%. With unfertilized oocytes, the VSS group was higher at 55.3% compared to 20% in the control group. Therefore, in vitrification a depolymerization of the meiotic spindle can occur, orienting it towards aneuploidy, an early release of cortical granules, causing hardening of the ZP, as well as alterations in glycoproteins, especially in ZP2, which is responsible for this hardening and does not allow fertilization.

Cryopreservation can compromise oocyte quality and future embryo development. TH and SC are two non-penetrating cryoprotectant additives that are suggested for the vitrification of immature oocytes. Zhang *et al.* (2017) found that the maturation rate, fertilization rate and embryo development in humans were comparable for SC and TH, *i.e.*, no significant difference.

CONCLUSIONS

Additional research with larger samples of immature or mature oocytes is warranted for further evaluation of both TH and SC in WTD oocyte survival after vitrification. Further investigation is needed for WTD oocyte vitrification using cytoskeleton stabilizers to improve maturation rates and apply IVP techniques as a model for other endangered cervid species. The imminent danger that cervids are exposed to the spread of diseases, such as Chronic Wasting Disease, can be prevented and provide the ability to cryopreserve and cryobank oocytes and embryos of cervids. Such aforementioned gamete and embryo cryopreservation could be very advantageous for certain cervid species and threatened populations.

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