ISSN: 2594-0252

AGRO PRODUCTIVIDAD

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Mycelial growth of the edible wild *Floccularia luteovirens* mushrooms in different culture mediums and pH

pág. 33

Año 13 • Volumen 13 • Número 10 • octubre, 2020

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Año 13 • Volumen 13 • Número 10 • octubre, 2020

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

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https://doi.org/10.32854/agrop.v13i10.1749

First report on *Plodia interpunctella* (Lepidoptera: Pyralidae) in stored Amaranth grains (*Amaranthus* spp.)

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ABSTRACT

Objective: To identify and describe the species of lepidopteran that causes damage to amaranth grains.

Design/Methodology/Approach: During six months, amaranth cereal bars were obtained to collect the larvae of the lepidopteran, a pest that consumes the grains. Larvae were kept under laboratory conditions until adults emerged. For the identification of the adults, the genitalia were extracted and photographs were taken by confocal laser scanning microscopy; and those of abdominal termination, egg and larvae of the first stage, by scanning electron microscopy.

Results: The first report on the appearance of the flour moth *Plodia interpunctella* in stored amaranth grains in Mexico is presented. This is the first known record of damage to amaranth grains by this pest in the world.

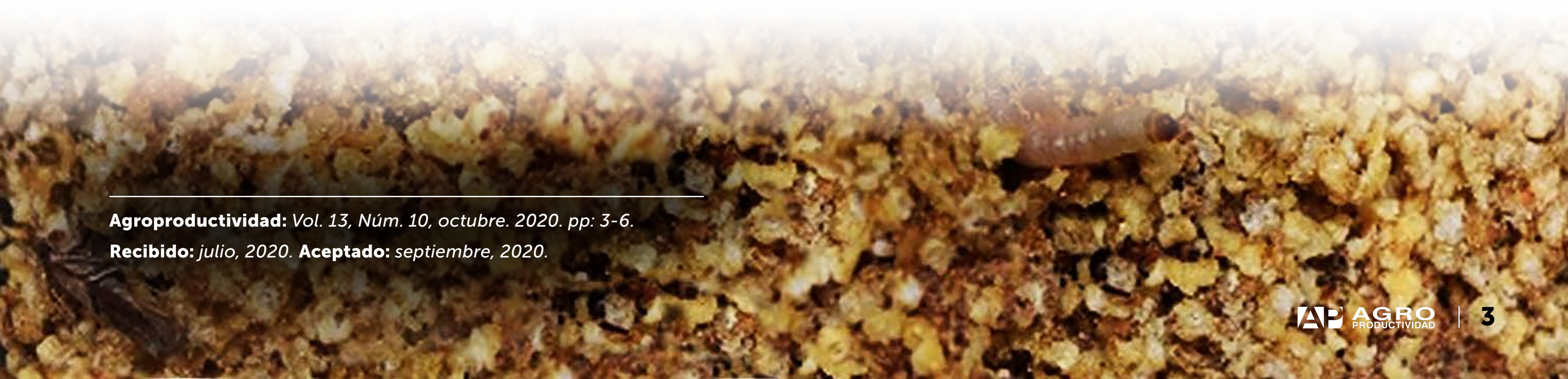
Study limitations/implications: In the Amaranth-producing region of Morelos, Mexico, there is no information on the pests of stored amaranth grains. Therefore, the need arises to deepen the study of the reproductive biology and ecology of *Plodia interpunctella* on this new host.

Findings/Conclusions: The identification of *Plodia interpunctella* in amaranth grains will allow the development of a management strategy to prevent the spread of this new pest in the Amaranth-producing region.

Keywords: flour moth, *Amaranthus*, cereal.

INTRODUCTION

Amaranth is a crop that is booming around the world because of its nutritional properties and contributions to food safety (Nampeera *et al*., 2019). In Mexico, the species *Amaranthus caudatus* and *A. hypocondriacus* are the most cultivated (Gimplinger *et al*., 2008) with a production of more than seven thousand tons (SIAP, 2018). In certain regions, amaranth is consumed as a cereal and it is considered an important income source (Gimplinger *et al*., 2008; Kagali *et al*., 2013). Pest insects are the major factor affecting crop performance and its quality, causing losses up to 100% (Kagali *et al*., 2013). In Mexico, more than ten phytophagous species associated with this crop have been reported (Bautista-Martínez *et al*., 1997; Oliveira *et al*., 2012). However, there is no record of any pest insect reported in amaranth grains. Thus, the objective of this study was to identify and describe the species of Lepidoptera that causes damage to stored amaranth grains in Morelos, Mexico.



Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 3-6.*
Recibido: *julio, 2020.* **Aceptado:** *septiembre, 2020.*

MATERIALS AND METHODS

For six months, recollections of amaranth cereal bars with larvae were made (Figure 1, photographs were taken with a Nikon AF-S DX Nikkor) in the commoners´ locality of Huazulco, Temoac, Morelos (18° 44' 47" N; 98° 46' 58" W at an altitude of 1511 m). Insects were fed with amaranth grains in Laboratorio de Ecología Química of the Escuela de Estudios Superiores Jicarero, under the Universidad Autónoma del Estado de México, at laboratory conditions: 28±2 °C, 50-70% HR and 12:12 h light:darkness.

Obtaining genitalia

To obtain the genitalia, the abdomen was separated from the rest of the body and placed in a beaker with 10 mL of 10% KOH; subsequently, it was heated in a thermal dish for 15 min at 80 °C. The abdomen was placed in a watch glass and distilled water was injected to separate the adipose tissue from the organs, then the genitalia was removed with fine-tipped pliers, under a stereoscopic magnifying glass. Subsequently, the scales and tissue foreign to the genital structure were removed, a drop of clove oil was added to maintain lubrication and stain the tissue (Ramos, 2015). A drop of Hoyer's solution (Anderson, 1954) was placed on a concave slide, and the genitalia was placed, arranged so that the genital structure was facing the front and the valves (in the case of the male) were separated. Once an adequate preparation was made, a coverslip was placed to seal the sample and it was gently pushed to avoid bubbling. In case of the presence of bubbles in the mounting, the slide was heated in a thermal plate for a few seconds until they were eliminated (Ramos, 2015).

Microscopy studies

Photographs of the genitalia were taken under a confocal scanning laser microscope (Carl Zeiss LSM 800 ZEN Software 2.6 Blue Edition, Germany). In addition, other photographs were taken by a scanning electron microscope

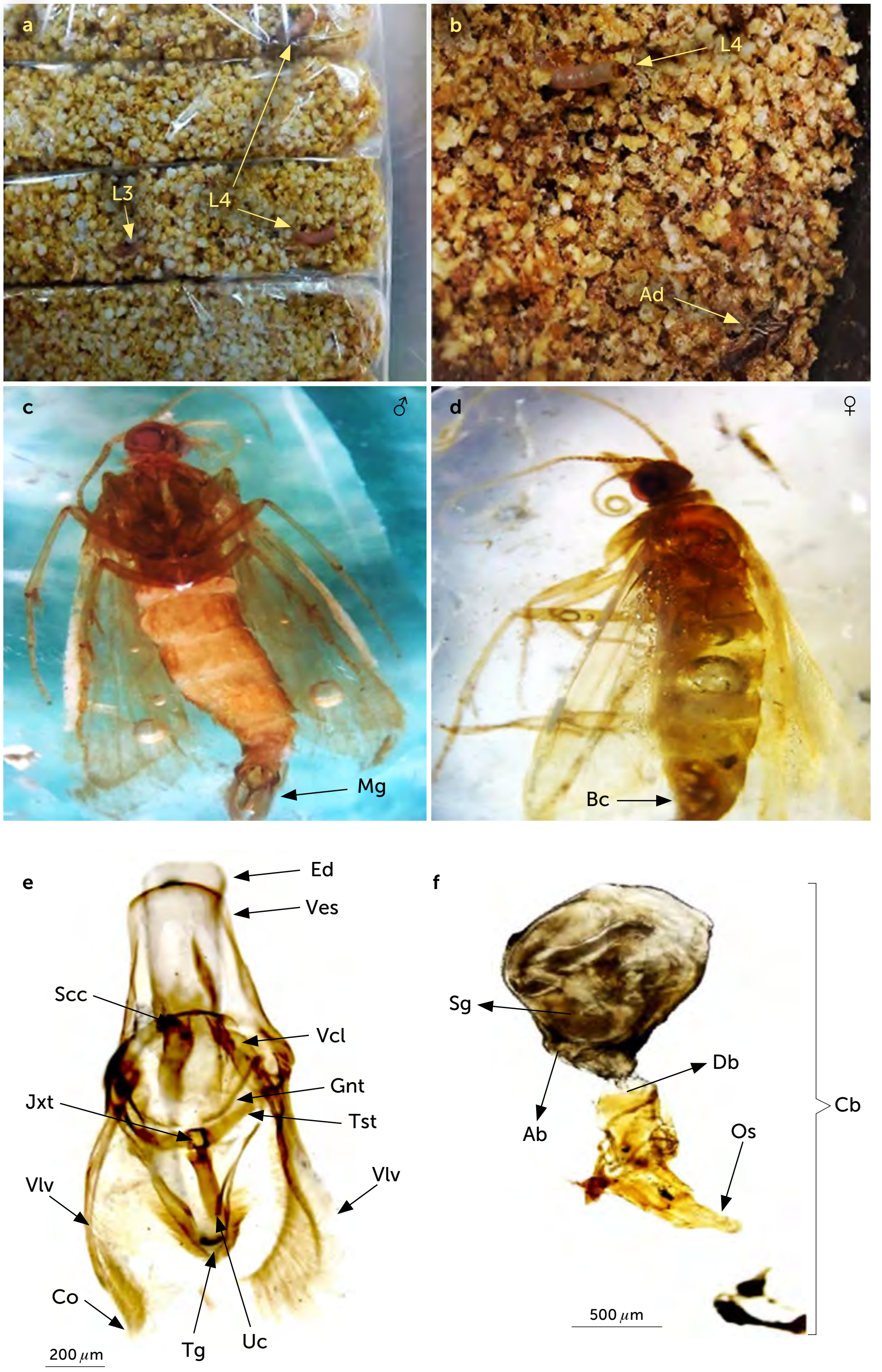


Figure 1. a. Third and fourth instar larvae (L3 and L4) in packages of amaranth cereal bars wrapped in cellophane lining; b. Larva of fourth instar (L4) and adult (Ad) reared on amaranth grains; c. Male Genitalia (Mg); d. Female genitalia, Bc: *Bursa copulatrix* and eggs; e. Internal genitalia, Ed: Edeago, Ves: Vesica, Vcl: Vinculum, Gnt: Ganathos, Tst: Transtilla, Vlv: Valvas, Uc: Uncus, Tg: Tegumen, Co: Cornutos, Jxt: Juxta, Scc: Sacculus; f. Internal genitalia Cb: *Corpus bursae*, Sg: Signum, Ab: *Appendice bursae*, Db: *Ductor bursae*, Os: *Ostium bursae*.

(Zeiss, EVO LS10, Germany) of the abdominal ending of the adults, as well as of the first instar egg and larvae.

RESULTS AND DISCUSSION

In this study, it was determined that the species found inside amaranth cereal bars is *Plodia interpunctella*. When examining the amaranth grain on which the breed was placed, different stages of development of this pest insect were found (Figure 1b). The adults had a white band with dark spots on the forewings. The abdomen showed a brown appearance similar to the wings (Figure 1c and 1d), in the females the presence of eggs was observed (Figure 1d) (García-Barros *et al*., 2015). Also, the internal genitalia of the male (Figure 1e) and the female (Figure 1f) are described; as well as the external genitalia of the male (Figure 2a) and of the female (Figure 2b) (Klots, 1970). The egg had a white oval shape and cell-shaped borders (Figure 2c). The larval development went through five instars, which were differentiated by their color, size and cephalic capsule. The first (Figure 2d) and the second larval stages were characterized by a whitish color, with translucent setae dorsally and laterally. The larvae of the third and fourth instar presented a yellowish body with pink tones and a dark brown cephalic capsule,

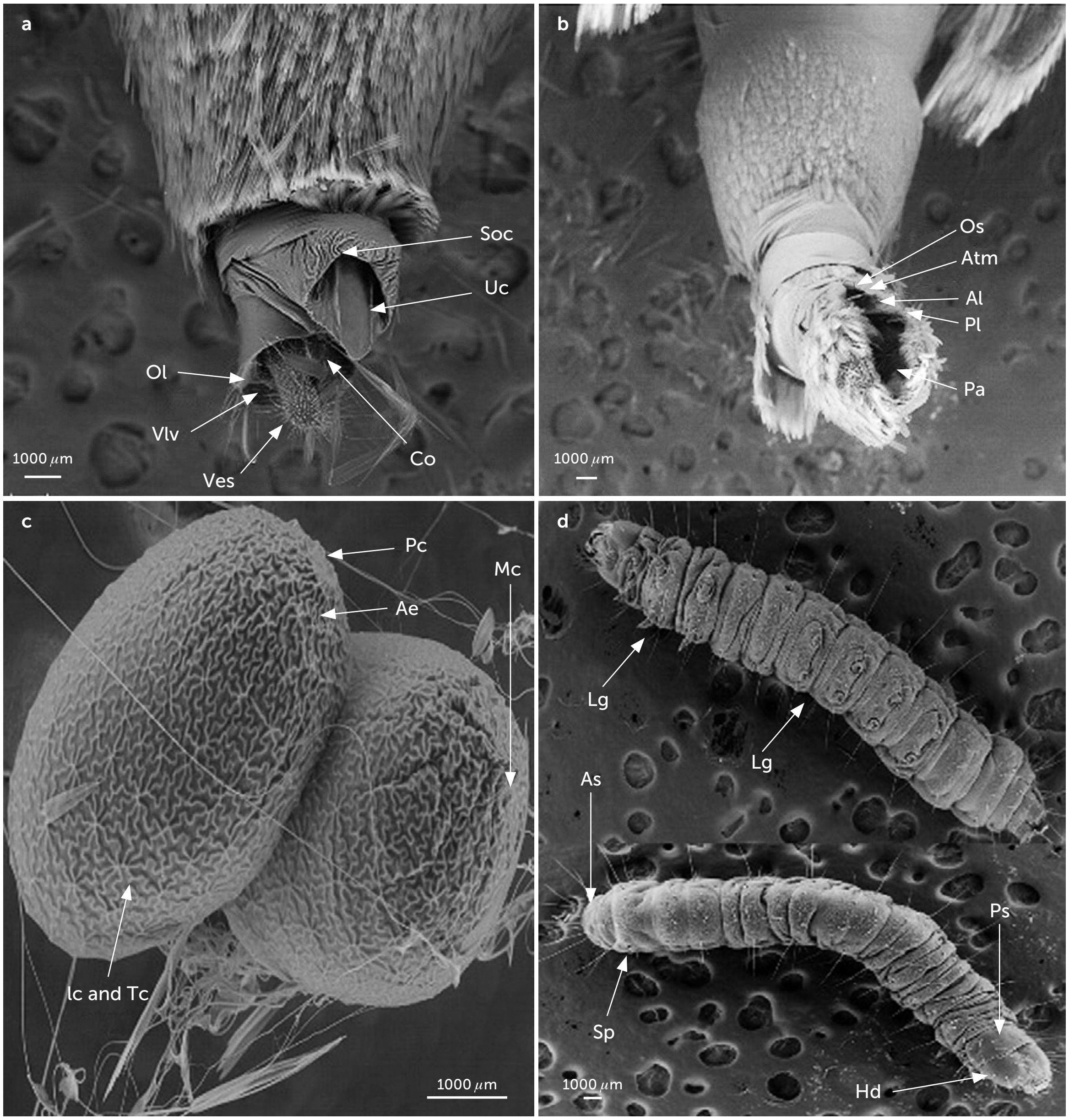


Figure 2. a. Male abdomen, Uc: Uncus, Ves: Vesica, Soc: Socii, Co: Cornutus, Vlv: Valva, Ro Ol: Lining of organs; b. Female abdomen, Os: Ostium bursae, Atm: Antrum, Lav Al: ante vaginal lamellae, Lpv Pl: post vaginal lamellae, Ap Pa: posterior apophysis; c. Egg, Mc: micropyle, Cp Pc: primary cell, Ae: aeropyle, Cl lc and Tc Ct: longitudinal and transverse cells; d. Larva one (L1), Pt Lg: leg, Pf Fl: false leg, Pa As: anal plate, Ep Sp: spiracle, Pp Ps: prothoracic plate, Cb Hd: Head.

while the last instar presented a light brown capsule. At this stage, the insects formed a silk that envelops the pupa; which was characterized by a dark hue.

The specimens described in this study belong to *P. interpunctella*, considered a cosmopolitan pest and of economic importance in stored grains and processed products (Hamlin *et al*., 1931; Heinrich, 1956; Arbogast *et al*., 1980). The survival success of this pest is due to the ability to reproduce on different hosts, where the quality of the food may influence (Marzban *et al*., 2001; Perez-Mendoza and Aguilera-Pena, 2004). It appears than the grain of Amaranth was not the exception, because all the development stages of the moth were observed on this cereal. It was observed that *P. interpunctella* produces a silk on amaranth grains, this factor together with the feces of the larvae causes contamination affecting the quality of the products, causing economic losses (Cox and Bell, 1991). Regarding the larvae found inside the amaranth cereal bars; as one plausible explanation, it may be due to the unnoticed oviposition on the stored grain before making the products. Another explanation would be that, when the female encounters a physical barrier such as plastic linings or paper envelopes, the eggs are deposited on these surfaces close to the food source (Silhacek *et al*., 2003) and, when the larvae emerge, they pierce the packages, or they can also enter through unnoticed existing holes (Mohandass *et al*., 2007); the latter was not observed in this study.

CONCLUSIONS

This is the first study of *P. interpunctella* on amaranth grains in Mexico and it can be considered a potential pest for the cereal; then, there is the need to know more about the species biology and ecology. With this knowledge, a management strategy could be designed against pest insect infestations, not to affect the quality of the stored grain.

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https://doi.org/10.32854/agrop.v13i10.1752

Nematodes in *Sceloporus variabilis* (Squamata: Phrynosomatidae) in localities in the Altas Montañas region of Veracruz, Mexico

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ABSTRACT

Objective: Identify nematodes of the digestive tract of the lizard *Sceloporus variabilis*.

Methodology: In different localities in the Altas Montañas region, 11 individuals of *S. variabilis* were collected and a range of morphological characteristics measured. Their intestines were dissected to identify the nematodes present.

Results: Were identified 17 nematodes belonging to the Oxyuridae family, present in the lizard *S. variabilis*. Not found differences in the frequency of nematodes between sexes and localities.

Implications: Identification of nematodes in *S. variabilis* is basic information for the knowledge of the parasite-host relationship.

Conclusions: The nematodes of the Oxyuridae family may cause mechanical symptoms in *S. variabilis*.

Key words: Reptilia, Squamata, Sauria, Phrynosomatidae, parasitism, host, Oxyuridae.

Diseases and their agents have been little studied in reptiles but some groups, such as the lacertilia, act as reservoirs, mainly of protozoa (Mata, 2018). Studying the influence of parasitic helminths on the life cycle and ecological processes of their hosts, is important in the context of describing parasite-host relationships (Aho, 1990). In addition, identifying endoparasites in reptiles is important to relating aspects of the biology of the species to their environment (Wilson and Carpenter, 1996).

In particular, the helminths in the pink-bellied spiny lizard (*Sceloporus variabilis* Wiegmann, 1834; Sauria: Phrynosomatidae) have not been identified. For this reason, the present study contributes to the knowledge of the diversity of nematodes present in the digestive tract of *Sceloporus variabilis*, a species of wide distribution on the American continent that is tolerant to disturbance of the ecosystems and has adapted to fragmented areas, presenting abundant populations (Chaves *et al*., 2013). The genus *Sceloporus* is considered a biological model with which to study the parasite-host interaction, for which reason it is important to evaluate its role as a transmitter of nematodes towards

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 7-11.*
Recibido: *julio, 2020.* **Aceptado:** *septiembre, 2020.*

other groups of reptiles (Garduño-Montes de Oca *et al.*, 2017).

This study was conducted in three localities in the year 2019 (Figure 1): 1) Peñuela (18° 46' and 18° 58' N; 96° 49' and 96° 58' W; 2) Huiloapan de Cuauhtémoc (18° 48' and 18° 50' N; 97° 06' and 97° 10' W); and Palma Sola (18° 45" N and 96° 47" W). Eleven specimens of *S. variabilis* were collected and transported to the Laboratorio de Microscopia Óptica of the Facultad de Ciencias Biológicas y Agropecuarias, Orizaba-Córdoba region, of the Universidad Veracruzana and sacrificed through hypothermia induced by freezing (García, 2013). The sex of the lizards was determined by sexual dimorphism and the age category estimated from the measurement snout-vent length (SVL) < 42.4 mm for juvenile and > 42.4 mm for adults. A range of morphological characteristics was also recorded in the lizards (Table 1; Serna-Lagunes, 2005). The contents of the digestive tracts of the lizards were prepared in an aqueous solution in Petri dishes and observed under a microscope in order to quantify the frequency of nematodes per age category and sex of the lizard. An ocular micrometer was used to obtain the anatomical characteristics of the nematodes and these were compared to examples in the scientific literature, books, laboratory manuals and specialized keys for the taxonomic identification of the nematodes (Anderson *et al.*, 1989).

The *S. variabilis* lizards evaluated in this study included three females (27% of the total) and eight males (73%). Regarding age category, 10 were adults (90% of the total) and one was juvenile (10%). The lizards presented a higher quantity of nematodes in Palma Sola (n=10) and Peñuela (n=7). In this sense, 17 morphotypes of nematodes were identified as belonging to the family Oxyuridae (Figure 2); four specimens [three males (75% of the total) and a female (25%)] of S. variabilis presented the highest prevalence of helminths (Table 1), while 36% of the lizards presented no nematodes, for which reason it is considered that *S. variabilis* does not act as a reservoir of nematodes. No association was presented between the nematodes and the sex, age category and localities of the lizards (X^2; P>0.05).

On comparison of the results with those of Breves *et al.* (2011), studying Iguana iguana in Brazil, three males and three females presented more than 100 endoparasites belonging to the family Oxyuridae. This family coincides with that reported in this study for *S. variabilis*. Salizar (2008) studied parasitic helminths of the Peru desert tegu (Dicrodon guttulatum Dúmeril and Bibron, 1893) in Peru and, of 38 hosts sampled, 34 (89.47%) were parasitized with 7929 (99.85%) nematodes, with the males presenting greater parasitism (75.76%) than the females (15%). Comparing this with the case of *S. variabilis*, there was no significant frequency between sexes, which could be due to the difference in the number of hosts collected in each study.

On the other hand, Stephen *et al.* (2003) found 404 endoparasites (seven nematodes) in seven lizard species of the genus *Sceloporus* (including *S. variabilis*) of Mexico, a frequency

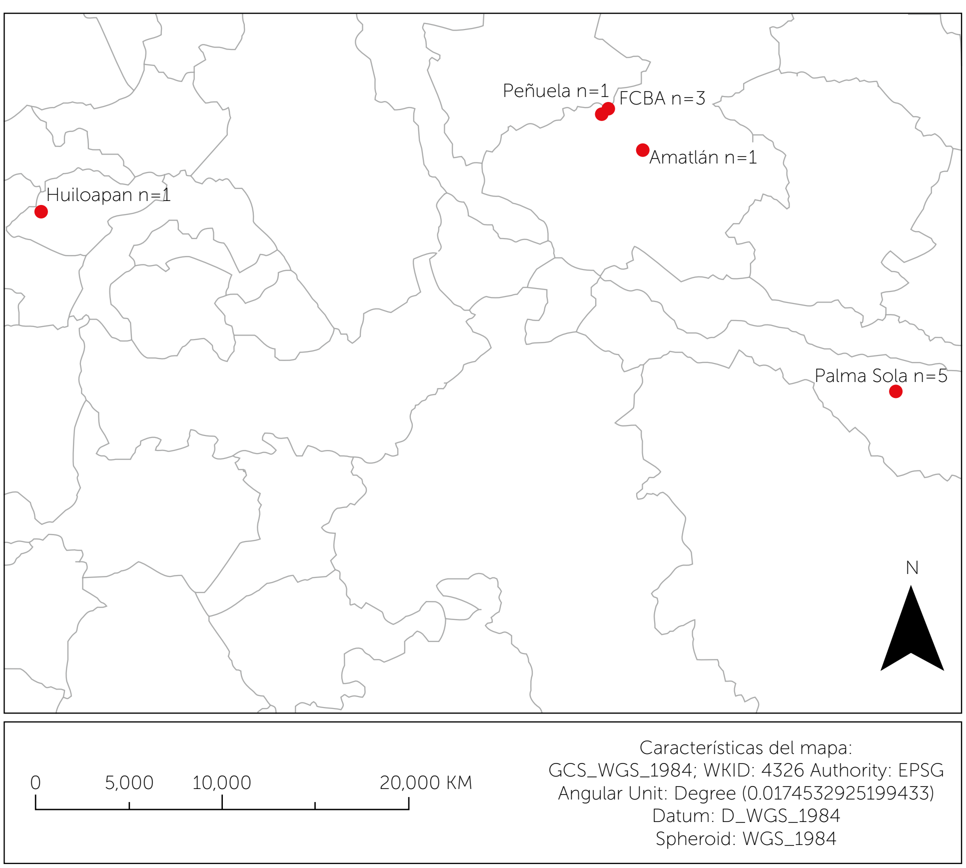

Figure 1. Sites at which the prevalence of nematodes was determined in *S. variabilis*. The map shows the sample size (n) at each sampling location.

Table 1. Morphometric variables of the pink-bellied spiny lizard *S. variabilis* and prevalence of nematodes (counts of nematodes present per individual).

No. Ind.	Male of *S. variabilis*	Total length (TL) cm	Snout-vent length (SVL) cm	Head width (HW) cm	Head length (HL) cm	Left leg length (LL)	Prevalence /absence of nematodes
1	Adult	13.5	6	1	2.1	1.7	0
4	Adult	9	4.5	1.5	2	0.5	5
5	Adult	13.2	5	1.2	2	2.7	0
6	Adult	13	4.5	1.5	3	2	0
7	Adult	9	4.5	0.5	1.5	3	3
8	Adult	15.5	7	1.7	1.5	3.5	0
9	Juvenile	4.5	2.5	0.5	0.8	1	0
10	Adult	12.5	5	1	1.5	1.5	0
	Mean	11.28	4.88	1.11	1.80	1.99	
	Standard deviation	3.30	1.22	0.42	0.60	0.96	
No. Ind.	Female of *S. variabilis*	Total length (TL) in cm	Snout-vent length (SVL) cm	Head width (HW) cm	Head length (HL) cm	Left leg length (LL)	
2	Adult	10	7	1.5	2	1.5	0
3	Adult	11	5.5	1	1.5	2	5
11	Adult	12	5.5	1	1.5	3	0
	Mean	11	6	1.16	1.66	2.16	
	Standard deviation	0.81	0.7	0.23	0.23	0.62	

of nematodes that was similar to that reported in this study. For this reason, the analysis of *S. variabilis* conducted in this study confirms the presence of nematodes of the family Oxyuridae, but this presence is not associated with the sex of the lizards.

ACKNOWLEDGEMENTS

Thanks go to the Laboratorio de Helmintología, Instituto Politécnico Nacional, for their help with helminth identification, and to the project "Caracterización de recursos zoogenéticos de las Altas Montañas, Veracruz: aplicación de la filogeografía y modelación ecológica" (PRODEP: 511-6/18-9245/PTC-896) for funding and technical support of the study. Thanks also go to the Secretaria de Medio Ambiente y Recursos Naturales (SEMARNAT) for granting a scientific collection license for the purposes of teaching: SGPA/DGVS/001894/18 allowing collection of the studied specimens.

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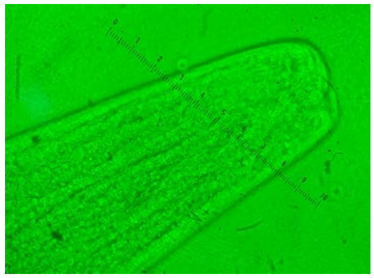
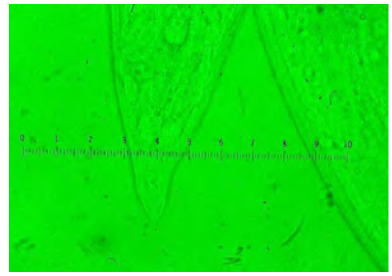
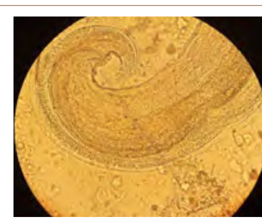
No.	Nematodes observed under a phase contrast microscope at magnification 400 X	
1	 Head of nematode	 Tail of nematode
	Nematodes observed under a compound microscope	
2	 Head of nematode (100X)	 Complete body of nematode (100X)
3	 View of the head (100X)	 View of the tail (100X)
4	 Observation of the head (400X)	 Observation of the tail (400X)
5	 Observation of the head (400X)	 Observation of the tail (400X)
6	 Observation of the head (100X)	 Observation of the tail (100X)

Figure 2. Specimens of nematodes of the family Oxyuridae in the intestine of *S. variabilis*. A): Head. B): Tail. Observation under a microscope at 100X and 400X.

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https://doi.org/10.32854/agrop.v13i10.1742

Reproductive phenology of Uspí tree *Couepia polyandra* (Kunth) Rose (Chrysobalanaceae) in Campeche, Mexico

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ABSTRACT

Objective. To describe reproductive phenological stages, since the formation of flower buds, flowering, fruiting, up to the formation of the ripe fruit and foliation of 21 *Couepia polyandra* trees; and correlation of allometric data of tree height, diameter at breast height (DBH) and crown diameter, as well as the correlation of precipitation with reproductive phenology data.

Methodology. The description of the reproductive phenology (foliage, formation of flower buds, flowers, fruits) was done by direct monthly observation with a digital camera (Canon SX60HS, 65). The correlation among precipitation, allometry, and types of soils where the trees grow was calculated by recording data from each tree by measuring height, diameter at breast height (DBH) and foliage.

Results. The highest tree was 21.0 m; the widest DBH measured 68 cm; and the greater crown diameter was 34.45 m; the overall averages were 10.38 m, 33.17 cm and 9.37 m, respectively. A significant correlation was found between height and DBH ($r=0.91$, $p<0.05$); the correlations for the variables Height-Crown and DBH-Crown were not significant ($p>0.05$).

Conclusions. Water as a factor is responsible for the formation of inflorescence and fruit; determining that these phenological events are dependent variables with precipitation, highlighting a mean positive relation with the growth of flowers.

Keywords: Uspí, southeast, backyard orchards, flowering, fruiting, precipitation.

INTRODUCTION

Despite the importance of phenological knowledge of tree species, to date in Mexico only those species with the highest commercial value have been studied in detail (Salinas-Peba & Parra-Tabla, 2007) and dry forests are not the exception (Porter-Bolland, 2003) from the Yucatán Peninsula in southeastern Mexico. Authors such as Valdez et al. (2010) point out differences in the phenology of tree species between subsequent years, and suggest that rains occurrence, as well as the duration of periods without rain, may play a more important role in phenology than the total annual precipitation. Differences among sites suggest a strong effect on the phenology of trees, and finally Rozendal and Zuidema (2011) confirm that these specific responses of

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 13-18.*
Recibido: *junio, 2020.* **Aceptado:** *septiembre, 2020.*

tree growth to climate variability for each species could be a projection of the differences in growth strategies among species. The species *Couepia polyandra* (Kunth) Rose (Chrysobalanaceae) is a tree known by the name of Uspí (Pennington & Sarukhán, 2005) in the Yucatán Peninsula, Mexico; and it is the only identified species of the genus growing in Mexico (Ojasti, 2001). It is native to the country and has a neo-tropical distribution extending from Mexico to Costa Rica (Durán-Espinosa and Lorea, 2010). *C. polyandra* inhabits jungles, from medium sub-evergreen to low deciduous, preferably along river banks (Pennington & Sarukhán, 2005; Vázquez *et al*., 2010), and at altitudes 0 to 600 m (Durán-Espinosa & Lorea, 2010; Lascurain *et al*., 2010). It is a tree that can reach heights from 6 to 30 m (Figure 1) with a diameter at breast height (DBH) up to 40 cm (Pennington & Sarukhán, 2005; Durán-Espinosa & Lorea-Hernández, 2010). It has axillary, terminal and paniculate inflorescences with white flowers. The fruit is an ellipsoid drupe (up to 45 × 25 mm), green when immature and orange when ripe. It is important to highlight the fresh consumption of ripe fruits in the states of Veracruz, Tabasco and Yucatan (Martinez *et al*., 2007; Lascurain *et al*., 2010; Magaña, 2010; Vázquez *et al*., 2010; Román *et al*., 2016). It is worth mentioning that Ruenes-Morales *et al*., (2016) report Uspí as a floristic element of the Yucatan jungles, cultivated in an isolated or tolerated manner in domestic plots or backyard orchards, in addition to being considered underused because its market is just local. Given this, studies evaluating natural history data of *C. polyandra* are scarce. However, there are researches with other species of the Chrysobalanaceae family, among which there is the one by Knowles and Parrotta (1997) who studied the characteristics of the flowering of 160 species in the heart of the Amazon in Brazil, among them *Couepia longipendula*, *Couepia* sp., *Licania heteromorpha*, and *L. micrantha*, pointing out that tree species show an inverse relationship with rainwater, and flowering peaks occur during the first months of the dry season (July-October). Fruiting trends closely follow annual rainfall patterns, with a peak in the number of fruiting species occurring during the wet season (December-May).

Figure 1. Uspí (*Couepia polyandra*) from solar del Cuyo, Palizada, Campeche, Mexico (Photo: SolMejenes 2017).

Ruíz and Alencar (1999) who documented the foliation, flowering and fruiting of five species (*Couepia longipendula*, *C. robusta*, *Licania heteromorpha*, *L. longistyla* and *L. octandra*) in the middle of the Brazilian Amazon rainforest, thus establishing that some climatic factors are involved with the interaction factors of the biotope environment and physiological factors causing long periods of intervals in flowering and fruiting. Ortiz *et al*. (2016) relate eleven species by their responses to environmental-abiotic variables in two ways: with a stronger correlation to water variables and the second, where thermic variables showed greater influence on growth. *Licania intrapetiolaris* was the species exhibiting the second response. Ríos-García *et al*. (2017) provide data on the durability of flowering and fruiting events, related to temperature and precipitation of *Licania arborea* in Chiapas, Mexico. They stated that flower production in the tree is influenced by a positive mean relationship with temperature, highlighting that both variables are dependent. The present study describes the different reproductive phenological stages since flower buds production, through flowering and fruiting to the formation of mature fruits and foliation of 21 *Couepia polyandra* trees grown in domestic plots and orchards, and associated with mango trees (*Mangifera indica*) in Palizada, Campeche, Mexico, during April 2016 to October 2017, correlating allometric data of tree height diameter at breast height (in Spanish, DAP) crown diameter, and reproductive phenology to precipitation data.

MATERIALS AND METHODS

Description of the study area

Field study was conducted in Palizada municipality, southwest of Campeche state (Figure 2). Elevations range from 0 to 40 m above sea level (Mendoza-Vega & Kú-Quej, 2010). Original vegetation according to Miranda and Hernández X. (1963) is medium sub-evergreen forest. The predominant climate is warm humid with abundant rains in summer A (m), and average annual temperature of 26.7 to 28 °C (INEGI, 2015). Average annual precipitation is 1200-2000 mm with a rainy period from May to October; the highest precipitation occurs in the months of July, August and September (García, 1981; INEGI, 2011). Dry season is relatively more pronounced; it begins in January and ends in April, and the driest month is March (García, 1981).

A mid-summer drought occurs during July to August (Gío-Argáez, 1996; Mendoza-Vega & Kú-Quej, 2010). The Palizada river and its tributaries, the Viejo river and the Limonar river, run across this municipality. The hydrological potential of this municipality is constituted by the Palizada River. According to the FAO / UNESCO classification, the soil types are Vertisols, Gleysol, Luvisols; and Leptosols in a minimal proportion (Bautista-Zuñiga *et al*. 2010).

Tree structure

Records were made monthly and were carried out during April to December, and from January to October, of 21 individuals of *C. polyandra* marked for identification in each of the visits that were made to plots and orchards with associated mango trees (*Mangifera indica*). Each tree had a descriptive card (passport data) which included name of the tree, tree number, location defined by GPS, initial date, diameter at breast height (DBH) measured at 1.30 m height using a caliper, tree height recorded with a SUUNTO clinometer, and crown width of each individual (Table 1); also, the subsequent data collection on reproductive phenology and foliage description. Leaves were cut into branches and flowers collected to be deposited in herbaria (CEDESU-UAC and UADY) and fruits were recollected for further germination study (Mejenes-Lopez *et al*., 2019).

Flower buds, flowering and fruiting

Observations were made directly on a monthly basis, with a photographic camera (Canon SX60HS 65, using Zoom); recording in the individual formats, observation date and changes according to reproductive phenological stage of flower buds setting, flowering and fruiting, recorded in percentage with the method described by Fournier (1974), modified by Newstrom *et al*. (1994) and Pineda-Herrera *et al*. (2012), where a scale is used according to the percentage of presence, in events that range from 0 to 4, as follows: total absence of the event = 0, 1–25% = 1, 26–50% = 2, 51 - 75% = 3 and 76 - 100% = 4.

Figure 2. Location of individuals of *Couepia polyandra* (Kunth) Rose in the municipality of Palizada, Campeche, Mexico.

Foliage

For this variable, presence of leaves was observed in each tree crown and the percentage was evaluated using the method described for reproductive phenology (Op. Cit).

Statistical analysis/Correlation

Data matrices were constructed for the correlation analysis among the variables height, DBH and crown diameter (statistical software STATISTICA V.7.1, Stat Soft, 2005); as well as for the correlation between reproductive phenological stages and monthly total precipitation (mm) (INEGI 2016, 2017). They were evaluated with comparison of Tukey means $p>0.05$ (Spearman) (Gotelli & Ellison, 2004).

RESULTS AND DISCUSSION

Tree structure and correlation of allometric data

The highest tree was 21.0 m and the shortest, 5.75 m with an average height of 10.38 m. The highest recorded DBH was 68 cm while the lowest was 15 cm, with a general average of 33.17 cm. Regarding crown diameter, the smallest was 4.6 m and the largest was 34.45 m, average width was 9.37 m. A significant correlation was found between tree height and DAP ($r=0.91$, $p<0.05$);

Table 1. Locality, UTM coordinates and morphometric data of the Individuals of *C. polyandra*. The records are presented in the order that they appeared during sampling.

Number	Locality	Coordinates (UTM)	Height (m)	Diameter at breast height DBH (cm)	Cup diameter (m^2)
1	San Isidro, puente de palizada	2018752.275-596421.464	5.75	54	10.22
2	Rivera Santa Isabel, Km.13. Highway Palizada Santa Isabel, S/N.	2027940.549-596610.364	10.92	21	5.1
3	Rivera Santa Cruz, Highway Palizada-Santa Isabel	2023030.86-594556.25	10.80	68	10.79
4	Rivera Alamilla, Highway Santa Isabel	2020773.432-596152.973	7.90	60	9.62
5	Rivera Santa Isabel	2027603.843-595660.992	1.05	16	7.6
6	Rivera Santa Isabel	2027607.034-595684.459	11.79	30	7.4
7	Rivera El Cuyo. Domicilio Conocido, Km. 1.3	2019984.784-596412.378	13.45	28	34.45
8	Rivera El Cuyo. Domicilio Conocido	2020021.316-596341.719	9.58	27.5	7.8
9	Rivera El Cuyo, Km. 2	2020251.296-596231.922	11.69	27.8	8.89
10	Rivera El Cuyo, Km. 2.1	2020306.665-596240.455	6.00	16	3.25
11	Rivera Santa Cruz	2021767.603-595187.864	8.00	21.1	6.56
12	Rivera Santa Cruz	2022099.105-595095.206	8.77	22.2	7.5
13	Highway La Viuda	2019355.894-597877.965	2.10	53.5	8.07
14	La Isla, San Isidro	2018572.986-596833.504	9.94	23.1	10.71
15	Rivera San Juan, Highway Palizada-Santa Isabel	2027196.272-598944.853	9.12	15	4.6
16	Rivera Santa Cruz Highway Palizada-Santa Isabel	2022642.129-594825.343	14.13	35	9.58
17	Rivera Santa Cruz, Highway Palizada-Santa Isabel	2022738.875-594747.354	12.53	34	11.25
18	Rancho El Triunfo, Km 9, Highway Palizada-Jonuta	2013650.323-592846.398	10.58	56	7.75
19	Rancho El Triunfo, Km 9, Highway Palizada-Jonuta	2013650.014-592846.106	7.92	23	7.63
20	Rancho El Triunfo, Km 9, Highway Palizada-Jonuta	2013649.705-592845.814	7.45	24	9.95
21	Highway La Viuda a 1.6 km de la Parroquia San Joaquín	2019260.914-597755.987	10.25	41.5	8.12

correlations for the variables Height-Crown and DAP-Crown were not significant ($p>0.05$).

Reproductive Phenology and phenological calendar

The development of phenological events of *C. polyandra* in the study area during 18 months (2016-2017) presented the same pattern (Table 2). Flower buds appear from April to July, while flowering also begins in April, but its maximum representation occurs in May and June, and decreases in July and August. Fruiting develops between May and June, with the highest value in August. The vegetative phenological stage of foliation was the most notable event, since individuals maintain their foliage throughout the year. This fact qualifies this species as an evergreen tree (Pennington & Sarukhán, 2005) despite it is a cultivated species.

Phenology related to precipitation

According to precipitation data during the study period, variations were registered each month of the sampling period, in June 2016 (223 mm) and 2017 (210 mm). As well as in November 2016, the highest (289.5 mm) precipitation occurred; and the lowest (345.4 mm) was in September 2017. This may indicate that phenological stages of the *C. polyandra* species are related to precipitation. Since flower bud production, flowering and fruiting occur on these precipitation events, we suggest that those stages are favored by the season (Figure 3). While with high rainfall no presence of any phenological stage. Precipitation of 34.14 ± 6.78 mm was recorded during the dry period, while during the rainy season, the recorded value was 204.49 ± 23.76 mm.

All individuals were reproductive from the minimum height of 5.75 m. DBH has a correlation with height which define this tree species as sexually mature for producing fruits, *C. polyandra* resulted a species of annual reproduction.

Table 2. Summary of the phenology pattern of *C. polyandra* individuals during the years 2016 and 2017.

	Leaves	Flower bud	Flowering	Fructification
jan^{2017}	4			
feb^{2017}	4			
mar^{2017}	4			
apr$^{2016-2017}$	4	2	2	
may$^{2016-2017}$	4	3	4	1
jun $^{2016-2017}$	4	3	4	2
jul$^{2016-2017}$	4	2	4	3
agu$^{2016-2017}$	4		2	4
sep$^{2016-2017}$	4			1
oct$^{2016-2017}$	4			
nov^{2016}	4			
dec^{2016}	4			

Escale: 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%), 4 (76-100%).

Through phenological analysis, it is recognized that the species has a suitable period for the collection of seasonal fruits in the months of June, July and August; when fruits show maturity and are recollected from the ground for self-consumption, that is the moment of peak maturity (Chi-Saénz, 2016; Ruenes Morales *et al*., 2016; Mejenes-López *et al*., 2019).

This study area has well-defined climatic seasons (dry and rainy); its influence was recording in the expression of flower buds, flowering itself, and fruiting of *C. polyandrous* that occurs in early June. This suggests that the water factor can be a strong trigger to the formation of inflorescence and fruit, as it was indicated by Rios-Garcia *et al*. (2017) for L. arborea, pointing out that flowering and fruiting events, as well as their relationship with temperature and precipitation, are dependent variables; and temperature registered a positive average relationship with the production of flowers in the tree.

CONCLUSIONS

C. polyandra species shows a pattern in reproductive phenological stages, showing a duration of flowering (April to August) and fruiting (May to September), five months for both events. Phenological traits are referred to the period and site of study; therefore, it is recommended to continue studying wild populations of the Pacific slope, in order to build the foundation needed to clarify the complex physiological processes and their relation to phenology.

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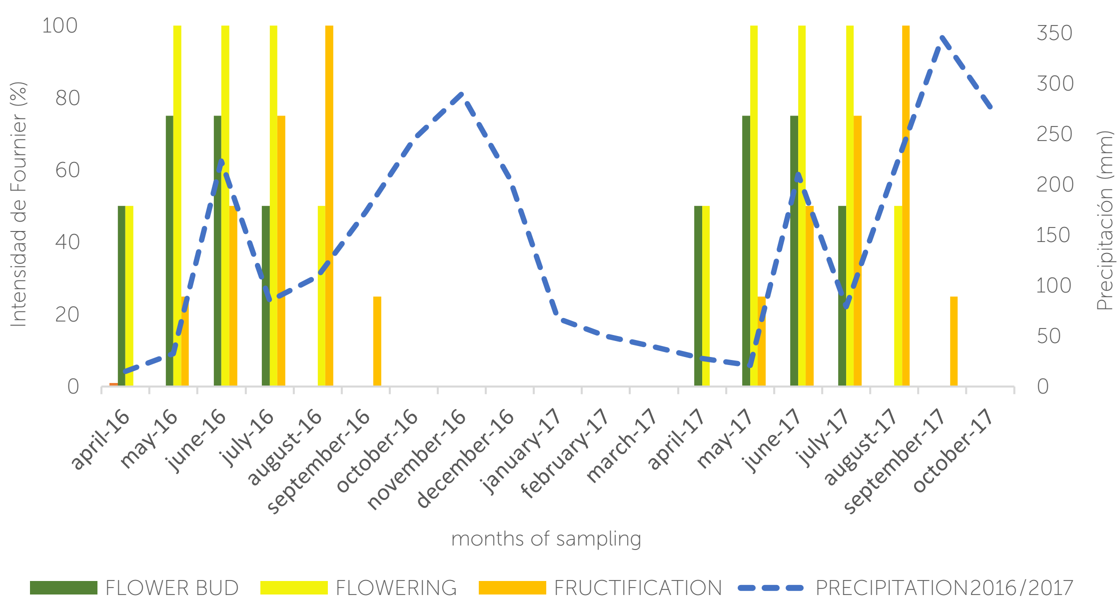

Figure 3. Phenology vs. precipitation of the year 2016 and 2017.

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Agro-Climatic factors required for the correct development of pineapple (*Ananas comosus* (L.) Merr.) cultivation

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ABSTRACT

Objective: To compare the agro-climatic factors: soil, rainfall, sunshine duration (sun-hours) and temperature existing in the municipality of Tecomán, Colima, Mexico, to some optimum values, in order to determine the development of the MD2 pineapple (*Ananas comosus* (L.) Merr.) plantation crop.

Design/Methodology/Approach: For the soil factors, texture and pH, data were obtained in laboratory to determine soil physical and reaction analyses. For temperature and rainfall, data were obtained from the Sistema Meteorológico Nacional (SMN), and to obtain the optimal values, different authors cited in the bibliography were considered.

Results: the soil factor presented a sandy loam texture with a pH of 7.9. From January to June and from October to December, a monthly precipitation deficit (mm) in percentage was evidenced respect that required by pineapple cultivation; and a percentage of excess in monthly precipitation (mm), from July to September. For monthly sunshine duration, an excess between 200 and 300% of sun-hours was evidenced with respect to that required by pineapple. For minimum, maximum and average temperature, monthly variations were evidenced, but within acceptable ranges according to those determined for cultivation development.

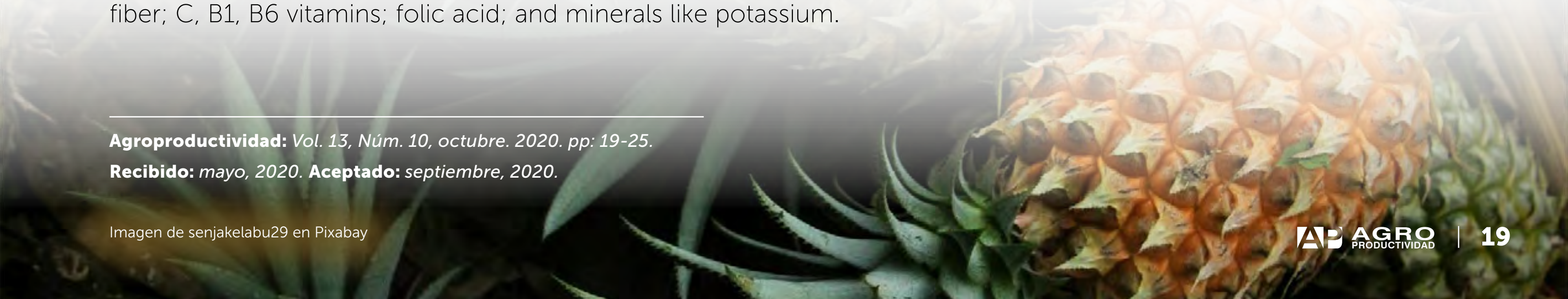
Limitations of the study/Implications: No scientific information was available regarding pineapple cultivation evaluated at the research area.

Findings/Conclusions: It was determined that the evaluated pineapple plantation crop will be able to develop in the production area, by adjusting monthly precipitation deficit through an irrigation system and the excess of sunshine duration, through the use of a shade cloth.

Keywords: Alternatives, tropical crops, agro-climatic factors.

INTRODUCTION

Pineapple (*Ananas comosus* (L.) Merr.) is native to tropical areas of South America, such as Brazil and Paraguay. It is not known in a truly wild state and it does not seem to be derived from the other species of edible fruits of the genus *Ananas* (L.) Merr., (Bromeliaceae), such as *A. bracteatus*, *A. fritzmuelleri*, *Actifolia* and *A. ananasioides*, which produce very small fruits with few seeds (Avelino *et al*., 2009). In addition, according to Cerrato (2013a), the market of this fruit has increased due to consumer demand for healthy foods. As it is the tropical fruit with the highest demand in the world due to its pleasant taste and higher contents of fiber; C, B1, B6 vitamins; folic acid; and minerals like potassium.



Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 19-25.*
Recibido: *mayo, 2020.* **Aceptado:** *septiembre, 2020.*

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In Mexico, pineapple cultivation of the MD2 variety, also known as Super Sweet pineapple, is produced under irrigated or rain-fed (the water required for the crop is provided by rains) conditions, but the rain-fed system is predominant. From 2001 to 2008, more than 98% of the area planted with pineapple was under this condition. According to the Servicio de Información Agroalimentaria y Pesquera (SIAP, 2017), in 2016 those five states with the highest volume of Mexican pineapple production were Veracruz (65%); Oaxaca (13%); Tabasco (6%); Quintana Roo (5%) and Jalisco (4%) that jointly contributed 93% of the total national production. For the proper development of pineapple cultivation, it is necessary to have suitable values of several agro-climatic factors. In this research, emphasis is placed on the type of soil (texture and pH), rainfall, sunshine duration, and temperature. The aim of this study was based on identifying if pineapple cultivation would achieve adapting to those agro-climatic conditions present in the study area. And on implementing those agricultural management or techniques to accomplish proper plantation crop development. Based on the above, agro-climatic factors: soil, rainfall, sunshine duration, and temperature existing in Tecomán, Colima, Mexico, were evaluated to compare them with some cited optimum values of those same factors to determine the adequate development of the MD2 pineapple plantation crop.

MATERIALS AND METHODS

The research was carried out at La Parota site (18° 54.12 N, and 103° 50.21 W) (Google Earth, 2017), during 2017 and 2018. According to the Instituto Nacional para el Federalismo y el Desarrollo Municipal (INAFED, 2017), the specific conditions of the area include an altitude of 33 m above average sea level; an average temperature of 26 °C and annual rainfall of 484.9 mm. An identification of the soil, texture and pH, was determined through physical analyses carried out on the soil samples obtained in the study area and processed in a certified laboratory (Agrolab) in the state of Colima. The analyses consisted of determining the texture and reaction of the soil, and to establish the pH value of the soil (Water 1: 2; $CaCl_2$ 1: 2). Regarding agro-climatic factors, for rainfall and temperature, data were obtained from the Sistema Meteorológico Nacional (SMN) for the period 1963 - 2017. The optimal data of the agro-climatic parameters studied were obtained from different authors and organizations with specific information for pineapple cultivation, such as Doorenbos and Kassam (1979), Bartholomew *et al*. (1985), FAO (1994), Sánchez and Caraveo (1996), Castellanos *et al*., (2000), Sandoval and Torres (2011) and others indicated in the bibliography. The scatter plots of the different factors evaluated were done by using Infostad® and numerical tables were processed on Microsoft Excel™ 2016.

RESULTS AND DISCUSSION

Soil physical analysis determining soil texture, recorded 51% sand, 15% clay and 34% silt, thus classifying the soil as sandy loam (USDA, 1993). With this result, it can be indicated that the area under study presents an adequate soil texture for the development of pineapple plantation crop; as compared with that indicated by Bartholomew *et al*. (1985), who mentioned that the pineapple cultivation requires soils with medium texture, sandy loam, or clay loam. In addition, Dávila (2016), also indicates that pineapple cultivation requires soils with a sandy texture, sandy loam or clay sand. Results of the soil reaction analysis and pH determination of the samples indicated that the soil has a pH (Water 1: 2) value of 7.9 which is considered mildly alkaline, and the result of the reactive pH ($CaCl_2$ 1: 2) showed a value of 7.26 that is cataloged as Neutral. When comparing these results with the optimal ranges established by FAO (1994), it is possible to note that pH can be limiting, because although the plantation crop can develop in soils with pH values 3.5 to 8.0, the optimum value is 5.9. In addition, comparing this result with that indicated by Doorenbos and Kassam (1979) we note that neither is in the range considered as optimum by these authors, which is between 4.5 to 6.5. Whereas Castellanos *et al*. (2000) set it between 5.0 - 6.0, and Dávila (2016) between 4.5 and 5.5. Thus, with a pH from neutral to mildly alkaline, the plantation crop would have to develop exposed to a range outside the optimum mentioned above and, in this particular case, higher than that recommended. As indicated by Acosta (2006), the solubility of nutrients could be decreased, specifically iron, phosphorus, manganese, zinc and copper, which, if not considered during the establishment of the plantation crop, can lead it to nutrition and development problems. Table 1 shows that there is a deficit percentage of monthly precipitation (mm) at Tecomán, regarding the monthly average required for pineapple cultivation in the months of January (−78.44%), February (−94.75%), March (−93.58%), April (−99.55%), May (−84.29%), June (−8.24%), October (−27.07%), November (−83, 71%) and December (−89.19%). In addition, it is possible to determine that there is an excess percentage in monthly precipitation (mm), respect that required, in the months of July (20.05%), August (36.77%) and September (53.94%).

This behavior of monthly rainfall deficit and excess at Tecomán (Figure 1), especially in the months January - June and October-December, need to be supplied / controlled by irrigation, as indicated by Dominguez (1985).

Table 2 and Figure 2 show a percentage of excess in monthly sun-hours at Tecomán respect the monthly sun-hours required for pineapple cultivation in every month. This excess accounts for: January (245.34%), February (217.10%), March (273.09%), April (270.65%), May (297.27%), June (300.08%), July (298.97%), August (285.64%), September (276.35%) October (254.02%), November (241.40%) and December (247.20%). Thus, exceeding the 100 sun-hours indicated by Sandoval and Torres (2011).

By noticing the above and acknowledging that there is a high sunshine duration at Tecoman, it is considered

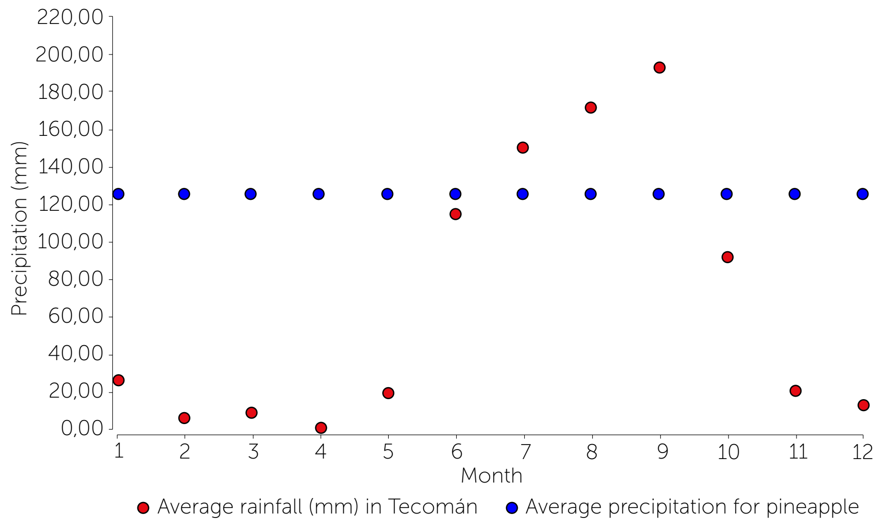

Figure 1. Monthly precipitation (mm) of Tecomán versus the ideal for pineapple cultivation. Source: (Own elaboration, 2018 according to the data of the National Meteorological System SMN).

Table 1. Description of monthly precipitation data (mm) for Tecomán versus the ideal for pineapple cultivation.

Concept	January	February	March	April	May	June	July	August	September	October	November	December
Years with data.	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00
Minimum monthly precipitation (mm) of Tecomán.	0.00	0.00	0.00	0.00	0.00	2.10	21.70	38.00	1.20	0.00	0.00	0.00
Maximum monthly precipitation (mm) of Tecomán.	498.90	115.80	160.50	19.00	212.90	445.30	517.20	405.70	648.50	421.50	316.00	97.20
Average monthly precipitation (mm) of Tecomán.	26.95	6.56	8.03	0.44	19.64	114.70	150.06	170.96	192.42	91.16	20.36	13.51
Standard deviation.	77.68	21.08	30.82	2.62	45.62	85.48	101.01	95.14	136.08	94.87	52.04	25.42
Average monthly precipitation (mm) required by pineapple cultivation.	125.00	125.00	125.00	125.00	125.00	125.00	125.00	125.00	125.00	125.00	125.00	125.00
Difference of mean monthly precipitation (mm) of Tecomán versus required by pineapple cultivation.	-98.05	-118.44	-116.97	-124.56	-105.36	-10.30	25.06	45.96	67.42	-33.84	-104.64	-111.49
% Variation.	-78.44%	-94.75%	-93.58%	-99.65%	-84.29%	-8.24%	20.05%	36.77%	53.94%	-27.07%	-83.71%	-89.19%

Source: Own elaboration, 2018 according to the data of the National Meteorological System SMN.

Table 2. Description of the data of Hours of monthly light of Tecomán versus the ideal one for pineapple cultivation.

Concept	January	February	March	April	May	June	July	August	September	October	November	December
Hours of monthly light in Tecomán.	345.34	317.10	373.09	370.65	397.27	400.80	398.97	385.64	376.35	354.02	341.40	347.20
Hours of monthly light required by growing pineapple.	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Difference of monthly light hours of Tecomán versus monthly light hours required by the pineapple crop.	245.34	217.10	273.09	270.65	297.27	300.80	298.97	285.64	276.35	254.02	241.40	247.20
% Variation.	2.45	2.17	2.73	2.71	2.97	3.01	2.99	2.86	2.76	2.54	2.41	2.47

Source: Own elaboration, 2018 according to data from Weatherspark.

important to protect the fruit, by using shade cloth to avoid affecting the quality of the product, due to sunburn problems to the shell. According to Cerrato (2013b), this shading method has resulted effective for pineapple producers.

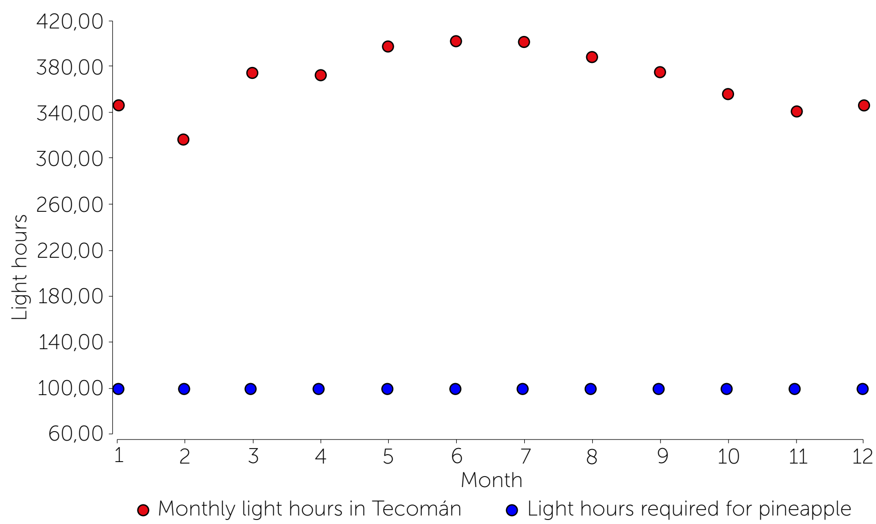

Figure 2. Hours of monthly natural light in Tecomán versus the ideal for pineapple cultivation. (Own elaboration, 2018).

Table 3 and Figure 3 show deficit in monthly maximum temperature (°C) at Tecomán concerning the maximum monthly temperature tolerated by pineapple cultivation in every month (January −6.69%, February −6.91 %, March −6.74%, April −5.97%, May −3.83%, June −2.43%, July −1.86%, August −2.14%, September −3.49%, October −2.03%, November −2.66%, and −5.09% in December).

The behavior of deficit in the monthly maximum temperature (°C) at Tecomán with respect to the maximum temperature (°C) tolerated by pineapple cultivation, can be observed in Figure 4, where the maximum monthly temperatures are in every month below the maximum that the pineapple plantation crop could resist, which is why the crop would adjust well to this parameter.

Minimum temperature

Table 4 and Figure 4 provide evidence of a deficit percentage in minimum temperature (°C) at Tecomán, regarding the minimum temperature suitable for pineapple cultivation in the months of January (−16.85%), February (−18.75%), March (−17.75%), April (−12.05%) and December (−10.20%). Also, it was determined an excess percentage in the monthly temperature at Tecomán, in relation to that required, in the months of May (0.65%), June (15.40%), July (16.40%), August (15.50%), September (14.65%), October (12.15%) and November (0.90%).

According to Pinto (2012) and Sanchez and Caraveo (1996), low temperatures retard growth and development, and can set floral differentiation in advance.

Average temperature

Regarding the adequate average temperature of 25-27 °C for pineapple cultivation, it was observed through

Table 3. Description of monthly maximum temperature (° C) data for Tecomán versus the monthly maximum temperature (°C) supported by pineapple cultivation.

Concept	January	February	March	April	May	June	July	August	September	October	November	December
Years with data.	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00
Minimum monthly maximum temperature (°C) of Tecomán.	29.69	30.24	29.69	28.70	30.37	31.63	31.18	31.95	31.55	32.32	31.90	30.84
Maximum monthly maximum temperature (°C) of Tecomán.	35.73	35.59	35.89	35.62	37.10	36.85	37.06	37.81	36.98	38.65	37.55	36.58
Average monthly maximum temperature (°C) of Tecomán.	32.59	32.59	32.64	32.91	33.66	34.15	34.35	34.25	33.78	34.29	34.07	33.22
Standard deviation.	32.59	32.59	32.64	32.91	33.66	34.15	34.35	34.25	33.78	34.29	34.07	33.22
Average monthly maximum temperature (°C) supported by pineapple cultivation.	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Difference of mean maximum temperature (°C) of Tecomán versus supported by the pineapple crop.	-2.41	-2.42	-2.36	-2.09	-1.34	-0.85	-0.65	-0.75	-1.22	-0.71	-0.93	-1.78
% Variation.	-6.89%	-6.91%	-6.74%	-5.97%	-3.83%	-2.43%	-1.86%	-2.14%	-3.49%	-2.03%	-2.66%	-5.09%

Source: Own elaboration, 2018 according to the data of the National Meteorological System SMN.

analysis, monthly deficit in the months of January (−1.56%), February (−2.32%) and March (−1.72%). Also, it was determined an excess percentage in monthly average temperature (°C) at Tecomán, regarding that required, in the months of April (1.00%) May (7.60%), June (14.44%), July (15.28%), August (14.68%), September (13.40%), October (13.44%), November (8.48%) and December (2.36%).

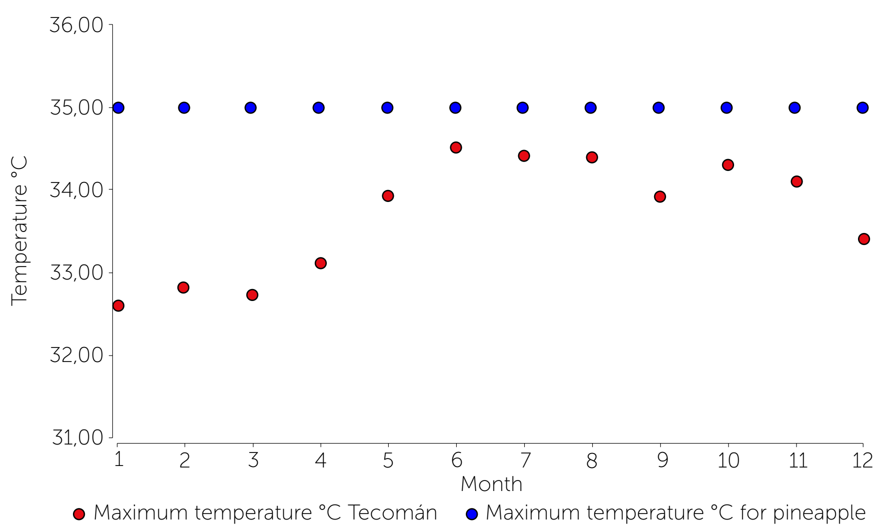

Figure 3. Maximum monthly temperature (°C) of Tecomán versus the ideal monthly maximum temperature (°C) for pineapple cultivation. Source: (Own elaboration, 2018, according to data from the National Meteorological System SMN).

CONCLUSIONS

Regarding the parameters of soil texture and temperatures (minimum, maximum and average), the pineapple plantation crop can adapt. However, soil pH showed neutral to mildly alkaline values, outside the optimal range, which could cause a decrease in the solubility of nutrients, specifically iron, phosphorus, manganese, zinc and copper. On the rainfall factor, it was determined that the amount of annual precipitation does not cover the requirements for pineapple plantation during eight months of the year, thus, recommendation is to implement an irrigation system to supply the water needs in those months with deficit. In addition, on the subject of sun-hours, the site study presented values almost three

Table 4. Description of monthly minimum temperature (°C) data for Tecomán versus the ideal minimum temperature (°C) for pineapple cultivation.

Concept	January	February	March	April	May	June	July	August	September	October	November	December
Years with data.	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00	54.00
Minimum monthly minimum temperature (°C) of Tecomán.	12.13	12.00	12.40	12.07	16.35	16.67	21.35	20.81	20.20	20.16	16.30	14.37
Maximum monthly minimum temperature (°C) of Tecomán.	20.34	19.86	19.77	21.42	24.69	25.47	24.76	24.60	24.32	24.92	22.70	21.13
Monthly minimum temperature (°C) of Tecomán.	16.63	16.25	16.49	17.59	20.13	23.08	23.28	23.10	22.93	22.43	20.18	17.97
Standard deviation.	1.67	1.80	1.53	1.93	1.60	1.27	0.68	0.76	0.71	0.92	1.17	1.47
Minimum monthly temperature (°C) required for pineapple cultivation.	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Minimum temperature difference (°C) of Tecomán versus required by pineapple cultivation.	-3.37	-3.75	-3.51	-2.41	0.13	3.08	3.28	3.10	2.93	2.43	0.18	-2.04
% Variation	-16.85%	-18.75%	-17.55%	-12.05%	0.65%	15.40%	16.40%	15.50%	14.65%	12.15%	0.90%	-10.20%

Source: Own elaboration, 2018 according to the data of the National Meteorological System SMN.

times those required by the pineapple plantation crop, which can affect the quality of the fruit, as it is exposed to damage during flowering and development stage due to sunburn. MD2 pineapple cultivation would reach full development, as long as some adjustments on the limiting factors, rainfall (deficit) and sun-hours (excess) are made in order to achieve good-quality commercial productions.

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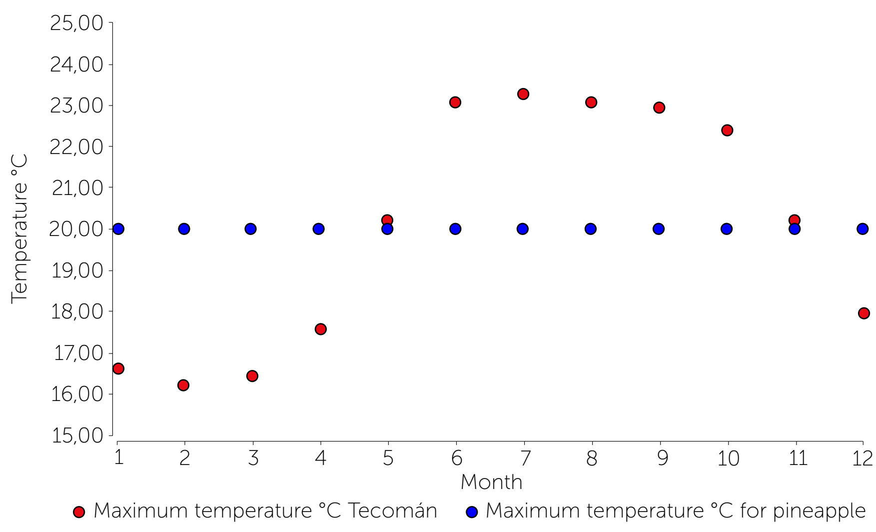

Figure 4. Monthly minimum temperature (°C) of Tecomán versus the ideal monthly minimum temperature (°C) for pineapple cultivation. Source: (Own elaboration, 2018, according to data from the National Meteorological System SMN).

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AGRO PRODUCTIVIDAD

https://doi.org/10.32854/agrop.v13i10.1722

Yield of *Solanum lycopersicum* l. by applying nutrients with or without fertigation interacting with an analogue brassinosteroid

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ABSTRACT

Objective: To evaluate the effect of fertigation and granular fertilizer to the soil interacting with foliar application of analogue brassinosteroids in *Solanum lycopersicum* L.

Design/Methodology/Approach: The treatments were Fertigation (F), Fertigation plus brassinosteroids (F + BR), Granular fertilization (FG) and Granular fertilization + brassinosteroids (FG + BRs). A completely randomized design with four replicates was used, where one plant from each treatment represented the experimental unit. Plants (45 days-old) were sprayed with 2 mg L^{-1} of Brassinosteroid CIDEF-4 and subsequent applications were made every 28 d. Plant height and stem diameter were measured 92 d after transplantation, while the yield was obtained by weighing completely red fruits. An analysis of variance was performed using the GLM procedure and the Tukey means comparison test ($p \leq 0.05$) with the SAS 9.3 program.

Results: Fertigation induced greater plant growth and reproduction of the tomato compared to the granular fertilizer with, or without foliar application of the analogue brassinosteroid. Treatments with granulated fertilizers decreased plant height by 10.36% and 10.45% compared to fertigation. Tomato yield with granulated fertilization, with or without Br, corresponded to 76.6% and 75.80% of what was achieved with Fertigation + Br.

Study limitations/Implications: The absence of a stressful environment did not favor the application of the analogue brassinosteroid.

Findings/conclusions: Fertigation induces greater growth and yield of tomato under field conditions; and environmental temperature homogeneity did not favor morphological nor physiological expression of the analogue brassinosteroid.

Keywords: Fertigation, mineral nutrition, yield.

INTRODUCTION

Water and synthetic chemical fertilizers used in agriculture have become two key factors of study due to their influence on growth and crop productivity (Wang and Xing, 2017). They are essential inputs to face the seasonal distribution of precipitation, which generates drought in certain stages of the cultivation growth and to satisfy the nutritional needs in plants.

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 27-31.*
Recibido: *junio, 2020.* **Aceptado:** *septiembre, 2020.*

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Fertilization constitutes one of the essential management practices for the sustainable management of vegetables (Hernández *et al*., 2009). Drip irrigation has contributed to improve the efficient use of water by supplying small and frequent amounts of the liquid directly to the root zone (Karam *et al*., 2007; Machado *et al*., 2003) and with fertigation, understanding this as the application of chemical fertilizers through irrigation, which supply nutrients in quantities and frequency required by the specific phenological stage of the crop (Guzmán, 2004). The fertigation is a successful technique. However, it is essential to solve some nutritional problems in tropical regions (Hernández *et al*., 2009; Bravo *et al*., 2016). Tomato cultivation requires differentiated fertilization for each region (Silva *et al*., 2011) and it is important to update the levels of mineral fertilization in tomatoes to make their application more efficient, and favor production without deterioration of natural resources, facing the wide availability of cultivars for planting.

With fertigation, the availability of water and nutrients in the root zone is maintained. However, in the aerial part, the plants face contrasting environments of increased temperature and low humidity, which frequently expose the plant to thermal stress (González *et al*., 2006) and in this case, it has been shown that brassinosteroids (Br), plant hormones that favor the polarization of the cell membrane (Singh and Shono, 2005), and induce resistance to biotic and abiotic stresses (Reyes *et al*., 2008; Nie *et al*., 2017), help to improve plant adaptation to local conditions. Also, BRs can influence growth when exogenous applications are made. In addition, they improve crop yield (Nie *et al*., 2017), as in lettuce (*Lactuca sativa*) (Serna *et al*., 2012), in fertile trilinear hybrids of *Zea mays* L. (Torres-Ruíz *et al*., 2007) or increased leaf area, as well as increased content of stevioside, rebaudioside and steviol in *Stevia* (Aguirre-Medina *et al*., 2018), particularly in high temperature conditions, hydric, saline or other abiotic stress (Núñez *et al*., 2001). In tomato (*Solanum lycopersicum* L.), BR was associated with the production of ethylene, with increasing the content of lycopene and carbohydrates (Vardhini and Rao, 2002). In transgenic tomatoes, the number of fruits and yield was increased, but decreased the individual weight of the fruit; likewise, it resulted in early flowering and a significant reduction in maturation time (Nie *et al*., 2017). Thus, fertigation and granular fertilization were evaluated in tomato cultivation, with/without the foliar application of one analogue brassinosteroid in plants of *Solanum lycopersicum* L.

MATERIALS AND METHODS

The experiment was established during the autumn-winter cycle of 2019 in the Experimental Field of the Facultad de Ciencias Agrícolas, Campus IV, located at the junction of the coastal road and Huehuetán Station, municipality of Huehuetán. The type of climate Aw_2 i g, which corresponds to tropical warm-humid with rains in summer. The average annual rainfall of 2,415 mm between the months of May to November. During the research, there was no precipitation, and the averages of maximum, minimum and mean temperatures were 35.0, 20.6 and 27.8 °C from November to March.

The soil is of a sandy-loam texture (66.48% sand, 23.72% silt, 9.80% clay), 1.7% organic matter, electrical conductivity of 0.03 dS m^{-1}, 6.25 meq 100 g^{-1} capacity of cation exchange, pH 5.7, 0.07% of N, P 15.45 mg kg^{-1}, K 238.0 mg kg^{-1}, Ca 704 mg kg^{-1}, Mg 157.5 mg kg^{-1} and Na 168.0 mg kg^{-1}. The cultivar 'Pony Express' was used for *S. lycopersicum* (Harris Moran®), of a determined early Saladette type, with uniform fruits in size, shape and deep red color.

The seeds were germinated in polystyrene trays of 200 cavities and 25 mL of volume in each cavity in Growing Mix® peat-moss as substrate. The transplant was carried out 35 d after sowing in the open field by means of fertigation, which was applied with a strip 20 cm apart between drippers, in furrows 0.8 m wide and 0.50 m apart between plants. The plants were driven to a single stem, removing the lateral shoots. Only three bunches per plant were harvested, when the fruits were ripe.

In fertigation, the Steiner nutrient solution was used (Steiner, 1984) and its base composition was in molc m^{-3}: 12 NO_3^-, 1 $H_2PO_4^-$, 3.5 SO_4^{2-}, 7 K^+, 2 Mg^{2+} y 4.5 Ca^{2+} with 5.8 pH and electrical conductivity of 2 dS m^{-1}. Granulated fertilization was performed by applying deep fertilization with 20 g of diammonium phosphate (DAP) and subsequently, soil applications were made every 8 d with 15 g of urea and 4 g of potassium chloride.

The soluble Brassinosteroid CIDEF-4™ (Natura del Desierto, SA. de CV.) with 80% steroidal content and 10% active ingredient, 2 mg L^{-1} were sprayed every 28 d to the point of dripping per plant.

Four treatments were established: Fertigation (F), Fertigation plus brassinosteroid (F + BR), Granular fertilization (GF), and Granular fertilization + brassinosteroid (GF + BRs). The experiment was conducted as a completely randomized design with four replicates; one plant from each treatment formed the experimental unit.

Variables were, plant height (cm), recorded with a tape measure from the root crown to the apical bud; and stem diameter, measured at a height of 5 cm from the root crown up, with a digital Vernier (Sure Bilt. USA) at 92 d after transplant. Tomato yield was obtained by weighing the fruits per bunch on a digital scale (ADIR, model 1676). An analysis of variance was performed using the GLM procedure and the Tukey mean comparison test ($P \leq 0.05$), using SAS 9.3 program (SAS Institute, 2010).

RESULTS AND DISCUSSION

The height and thickness of the stem increased in the treatments with fertigation and were statistically different from the rest ($p \leq 0.05$) (Table 1). Treatments with granulated fertilizers decreased 10.36% and 10.45% when compared to the best treatment. Analogue brassinosteroid did not show a positive effect in either of the fertilization forms. This is attributed to temperature uniformity during the study period. Maximum temperature values were 34-35 °C and the minimum average temperature ranged from 19.9 to 20.3 °C.

In another similar sowing, with the application of the brassinosteroid BIOBRAS-6, Mazorra and Núñez (2003) did not register differences in the length of the stem when it was applied to tomato seeds var. Amalia, at two concentrations of 0.01 and 0.05 mg L^{-1}. In contrast, at a 10^{-8} M concentration of Brasinosteroid applied to the foliage, Hasan *et al.*, (2014) referred increases of 57.56% in the length of tomato plant. Vardhini and Rao (2001) cited the same result of increasing in plant size (30.57% and 33.95%, respectively) when 28-homobrasinolide (HBL) and 24-epibrasinolide (EPL) were applied to the foliage at 3.0 μM concentration (Vardhini and Rao, 2001).

Fruit yield

Differences in yield were mainly due to the contribution of nutrients and the method of application. In fertigation, more nutrients were provided for the better development of plants compared to granular fertilization. There were highly significant differences ($p \leq 0.05$) in fruit yield attributed to the treatments. The yield with GF + BR and GF correspond to 76.6% and 75.80% of what was achieved with F + BR (Table 1). The difference in yield between the two forms of fertilization is attributed to the ample supply of nutrients that fertigation provides in comparison to granular NPK fertilization; and according to Sainju *et al.* (2003) nutrient concentration in the tomato cultivation influences the yield.

Authors such as Dabire *et al.* (2016) stated, by comparing organic fertilization with mineral fertilization on *S. lycopersicum* var. Mongal F1, that there was no significant effect on the chemical compounds contained in the fruit, with the mineral application. The absence of response in tomato yield to the application of the Brassinosteroid CIDEF-4 is related to the absence of an environmental temperature stress, which was otherwise expected, due to the recurrent increase in temperature that has characterized the region in recent years, which induces flower drop. However, other reported results are 10.32% increment in yield when the signaling gene BRI1 is used (Nie *et al.*, 2017). It was also indicated that the use of HBL (28-homobrassinolide) and EBL (24-epibrassinolide) increased yield in tomato varieties K-25 (49.12%; HBL and 52.63%; EBL) and Sarvodaya (34.38%; HBL and 41.67%; EBL) (Hassan *et al.*, 2014). Similarly, the foliar application of brassinolide, 28-homobrasinolide and 24-epibrasinolide in 3.0 μM concentration increased fruit yield by 53.98%, 70.15% and 67.37%, respectively (Vardhini and Rao, 2001).

In relation to homobrassinolide, and according to Hernández-Silva and García-Martínez (2016), they do not have much effect on plant growth when growing conditions are optimal. Although BRs

Table 1. Yield, plant height and stem thickness (at 92 das*) of Pony Express tomato plants.

Treatment	Yield (kg plant^{-1})	Plant height (cm)	Stem diameter (cm)
F + Br	1.24 a^{Z}	114.35 a	0.90 a
F	1.25 a	115.27 a	0.90 a
GF + Br	0.95 b	102.50 b	0.82 b
GF	0.94 b	102.40 b	0.82 b
^{Y}HMSD	0.4 9	3.14	0.06
^{X}CV (%)	2.1 2	1.38	3.70

^{Z}Different letter values, within columns, are statistically different (Tukey, $p \leq 0.05$); YDMSH = Honest minimum significant difference; XCV = Coefficient of variation; F + Br = fertigation plus brassinosteroid; F = fertigation; GF + Br = Granular fertilization plus brassinosteroid; GF = Granular fertilization. *(das) days after sowing.

are considered a kind of hormone with great potential to increase the yield of crops (Vriet *et al*., 2012), foliar application of BRs aims to improve plant response to stress, since BR promote resistance in plants against low temperature and low light, increasing fresh and dry weight, and maintaining photosynthetic activity (Shu *et al*., 2016); generating basic thermic tolerance in pollen germination (Sing and Shono, 2005) and accumulating ethylene under salt stress (Zhu *et al*., 2016).

Authors such as Corbera and Nunez (2004), by evaluating analogue brassinosteroid Bb-6 on *Glycine max*, inoculated with *Bradyrhizobium japonicum* and arbuscular mycorrhizal fungus, mentioned that the variables, plant and weight of 100 seeds were little influenced by the application of mycorrhiza or the co-inoculation with *Bradyrhizobium* strain; likewise, with the application of the analogue brassinosteroid.

Regarding the fertilization method, fertigation increased tomato yield in 21.7%, 10.5% and 25.3% and it was associated with a greater number and size of fruits per plant, compared to the direct application of solid fertilizers to the soil (Badr *et al*., 2010; Hebbar *et al*., 2004; Shedeed *et al*., 2009). In okra (*Abelmoschus esculentus*), under fertigation, there was an increase of 25.21% and 16.5% in two production cycles, saving 40% of fertilizers compared to the traditional method (Patel and Rajput, 2004).

Fruit weight

The fruit weight showed highly significant differences ($p \leq 0.05$) in the three bunches evaluated. In fertigation treatments, a higher fruit weight was observed, as compared to treatments with granulated fertilizer (Table 2).

It is evident that the form of fertilization induces greater growth of the tomato and affects yield directly. Kumar *et al*. (2013) and Zhang *et al*. (2010) cited differences with the application of different levels of fertilizer in the Azad T-6 tomato variety.

CONCLUSIONS

Fertigation induces greater growth and yield in tomato at field condition. The homogeneity of ambient temperatures did not favor the expression of the analogue brassinosteroid.

Table 2. Fruit weight per cluster of Pony Express tomato plants.

Treatment	Fruit weight (g)		
	Cluster 1	Cluster 2	Cluster 3
F + Br	77.50 a^{Z}	89.65 a	82.55 a
F	82.85 a	83.35 b	84.60 a
GF + Br	64.15 b	63.70 c	62.55 b
GF	64.00 b	62.70 c	61.60 b
^{Y}DMSH	5.35	4.47	5.12
^{X}CV (%)	8.93	7.18	8.48

^{Z}Different letter values, within columns, are statistically different (Tukey, $p \leq 0.05$); YDMSH = Honest minimum significant difference; XCV = Coefficient of variation; F + Br = fertigation plus brassinosteroid; F = fertigation; GF + Br = Granular fertilization plus brassinosteroid; GF = Granular fertilization.

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Mycelial growth of the edible wild mushrooms *Floccularia luteovirens* in different culture mediums and pH

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ABSTRACT

Objectives: To evaluate mycelial growth and biomass production of *F. luteovirens* in different culture mediums and pH values.

Design/methods/approach: The study was carried out in two stages. In the first stage, the amount of biomass produced and growth rate was measured in five generalist culture mediums. During the second stage, the pH was adjusted to 4, 5, and 6 in three of the five mediums, in order to increase biomass and growth speed shown in the first stage.

Results: pH is an influential factor in the availability of nutrients needed by the fungus, which is shown by the increase or inhibition of mycelial growth and biomass production. During the first stage, coconut and malt extract agar were the most favorable for mycelial development, while corn meal agar was the least favorable. When the pH was modified, malt extract agar with a pH of 4 was the most efficient in terms of growth rate, while coconut agar demonstrated the most biomass production regardless of pH.

Study limitations and implications: The growth of cultures in vitro is slow when compared with other mushrooms species.

Findings/Conclusions: *Floccularia luteovirens* cultivation represents an alternative to obtain food with a high nutritional value, safeguard germplasm, and increase and diversify species cultivated; since it is edible, with high nutritional content and has medicinal properties.

Key words: strain, biomass, in vitro culture, golden mushroom, culture mediums.

INTRODUCTION

Worldwide, it is calculated that until 2010, 1.3 % of the global fungal biodiversity was conserved *in vitro*, with a calculated 1.5 million species (Morales *et al*., 2010). The loss of natural habitats has had an unfavorable impact on the population of wild species, and therefore fungal culture collections acquire a more significant relevance for *in situ* conservation (Salmones and Mata, 2012).

In Mexico, different collections safeguard 1639 strains of edible species for their study and cultivation, and include local organisms as well as from other countries (Salmones and Mata, 2012). Despite this great genetic potential, the number of strains safeguarded does not have a positive correlation with the number of species that are currently cultivated on a commercial or

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 33-38.*
Recibido: *junio, 2020.* **Aceptado:** *septiembre, 2020.*

industrial level, and experimentation on new species is scarce. Unlike Asian countries where more than 20 species are cultivated commercially, or the USA and Canada with supermarket sales of numerous cultivated and wild species, the majority sold in Mexico are of Asian origin (Garibay-Orijel *et al*., 2010). Because of this, using wild mushroom strains represents an alternative in order to take advantage of local genetic resources.

Floccularia luteovirens [whose synonyms are *Agaricus luteovirens*, *Armillaria luteovirens*, *Floccularia straminea*, *Tricholoma luteovirens* (Fungorum Index, 2019)], is a reported edible mushroom in Asia and Mexico. In Asia it is commonly known as "Golden mushroom", and is valued as an important ecological and medicinal species (Malaisse and Mathieu, 2008). In Mexico, its consumption has been reported in the state of Tamaulipas (Garcia, 2013), Morelos (Avila *et al*., 1980), Puebla and Estado de México; in the latter, in Amanalco, Zinacantepec, Toluca (Burrola-Aguilar *et al*., 2012; Franco *et al*., 2012; Arana-Gabriel *et al*., 2014), Chalco, Amecameca, and Ozumba (Estrada *et al*., 2009). In Estado de México it is commonly known as "hongo de yema", "canario" and "hamburguesa". Davis *et al*. (2012) report it as a mycorrhizal mushroom, while Evenson (1997) and Garibay Orijel and Ruan Soto (2014) suggest it is saprobic with fructification in coniferous forests, specifically in *Abies religiosa* and *Pinus*-*Quercus* forests (García, 2013; Arana-Gabriel *et al*., 2014). *Floccularia luteovirens* is a good source of protein (41.7 %); it contains minerals (P, K, Ca, Mg, Na, S, Cl, Fe, Mn and Zn), amino acids (tyrosine, valine, isoleucine, leucine, histidine, lysine, and tryptophan) and acids such as palmitic, stearic, oleic, and linoleic (Malaisse and Mathieu, 2008). Among its medicinal properties it is antioxidant, anticancer and antitumor, due to the presence of gastrodin, exopolysaccharides, betulinic acid, and lectins in the mycelium and in the fruiting body (Hai-Feng *et al*., 2008; Liu *et al*., 2011). Cultivation of *F. luteovirens* is an example of strategies which implicate conservation, research, innovation and the development of genetic resources of native fungi (Morales *et al*., 2010). Taking into account biotechnological implications, it is important to determine the conditions in which the mycelium develops under *in vitro* conditions. Therefore, the objective of the present study was to evaluate mycelial growth and biomass production of *F. luteovirens* in different culture mediums and pH values.

MATERIALS AND METHODS

The IE 5034 strain of *F. luteovirens* was used, deposited in the ceparium in INECOL (Institute of Ecology), which was isolated by Arana-Gabriel *et al*. (2014). For the characterization and evaluation of mycelial growth, five culture mediums were used: 1.- Water Agar (WA) (15 g of Agar-BD Bioxon^{MR} per liter of distilled water), 2.- Potato Dextrose Agar (PDA) (39 g of PDA-BD Bioxon^{MR} per liter of distilled water), 3.- Malt Extract Agar (MEA) (33.6 g of MEA-BD Bioxon^{MR} per liter of distilled water), 4.- Corn Meal Agar (CMA) (15 g of MASECA® corn meal, 15 g agar BD Bioxon^{MR}, 2 g BD Bioxon^{MR} yeast, and 1 g of peptone per liter of distilled water), 5.- Coconut Water Agar (CWA). A 0.5 mm diameter piercer was used to place agar with mycelium in the center of each Petri dish, which were incubated in the dark at 18 °C. Each treatment was done with five replicas.

Rate of mycelial growth (Gr) in different culture mediums

Every third day the diameter of mycelium growth was measured with help of a Vernier scale. Once mycelial growth was completed, Gr was calculated (according to Sinclair and Cantero, 1989):

$$Gr = (Df - Di) / (Tf - Ti)$$

Where Gr = growth rate; Df = final growth diameter; Di = initial growth diameter; $Tf-Ti$ = days of mycelial growth.

Biomass (B)

The mycelium grown in Petri dishes was washed three times with hot water; once the agar was completely eliminated, it was placed on aluminum foil in an oven at 60 °C for 24 hours, and the dry B was weighed on an analytical scale (Arana-Gabriel *et al*., 2014).

Morphological and microscopic characterization

During the exponential growth stage, a sample of mycelium was taken. Preparations were made with 10% Red Congo. The preparations were observed at a 100x microscope and photographs were taken of the hyphae with the digital Motic microscope DMB3-223 software. At the end of the mycelial growth, the shape, margin, type of growth, texture, surface and color of the colony were registered.

Statistical analysis

Initially, ANOVA was done (Statgraphics Centurion XVI), taking into consideration as a factor the type of medium, and Gr and B as variables. Afterwards, Tukey's test was applied with a cutoff of $p \leq 0.05$ as significant, in order to establish significant differences between culture mediums. Once those results were obtained, three mediums were selected whose pH was modified in order to increase Gr and B. Subsequently, MANOVA (Statgraphics Centurion XVI) was carried out, with mediums and pH as factors, and Gr and B as variables. Tukey's test was applied with $p \leq 0.05$ as significant in order to establish the difference between culture mediums and pH.

Gr in different culture mediums at three pH levels

The culture mediums CMA, CWA, MEA were adjusted at a pH of 4.0, 5.0 and 6.0, by adding HCl and KOH 1N (Vázquez-García *et al.*, 2002). With a piercer, 5 mm diameter of agar with mycelium was taken and placed in Petri dishes (five of each), which were then incubated at 18 °C. Gr was measured, dry B was quantified, and the mycelium was characterized morphologically and microscopically with the methods previously stated.

RESULTS AND DISCUSSION

Due to the fact that the nutritional requirements of *F. luteovirens* are unknown, PDA and MEA were used as generalized conventional mediums; CMA and CWA as generalized non-conventional mediums, and WA as control. CWA is a culture medium used for *in vitro* cultivation of plants and there are no reports of it being used for cultivating mushrooms; however it was used due to the amount of nutrients reported in the liquid endosperm of the coconut, making it a source of carbon, nitrogen and minerals (Krikorian, 1991), and contributing all the nutritional requirements necessary for good mycelial development. Morphologically, the strain of *F. luteovirens* in the five different medium cultures presented circular growth, fimbriated edges, submerged growth, velvety texture, flat surface, and a cream color. Microscopically, they presented twisted and branching hyphae, round terminal hyphae, frequent and abundant clamp connection. The diameter of the hyphae varied between culture mediums: in WA the average diameter is 1.84 μm, in PDA 2.9 μm, in MEA 3.0 μm, in CMA 2.5 μm, and in CWA 2.6 μm.

Table 1. Growth rate and average biomass of *Floccularia luteovirens* in different culture mediums.

Culture medium	Growth day (mm/day)	Biomass (g) (g/Petri dish)
WA	0.32 c*	0.001 a*
PDA	0.24 b	0.135 b
MEA	0.32 c	0.130 b
CMA	0.16 a	0.162 bc
CWA	0.30 c	0.249 c

*Different letters in the same column for the same variable indicates significant differences ($p<0.05$). WA: Water Agar; PDA: Potato Dextrose Agar; MEA: Malt Extract Agar; CMA: Corn Meal Agar; CWA: Coconut Water Agar.

The ANOVA showed significant differences with respect to Gr in the culture mediums analyzed ($p<0.05$) and in B ($p<0.05$). The highest Gr was seen in the control medium WA with 0.32 mm day^{-1}, invading the complete diameter of the Petri dish in 114 days; then, MEA 0.32 mm day^{-1} in 111 days, and CWA 0.30 mm day^{-1} in 123 d; while the CMA showed the slowest Gr at 0.16 mm day^{-1} (Table 1). Arana-Gabriel *et al*. (2014) isolated *F. luteovirens* in cultures of CMA and MEA, at 18 °C, but they did not evaluate Gr or B due to its slow growth. The Gr of *F. luteovirens* strain is considered slow, when compared to other fungi such as *Pleurotus* sp. which takes approximately 8-9 days (Gaitán-Hernández, 2005), or other strains of wild mushroom species, such as *Flammulina mexicana* which takes 13 d, *Lyophyllum* sp. 21 d (Arana *et al*., 2014), *Suillus luteus* 38 d, or *Scleroderma citrinum* 105 d (González *et al*., 2015).

In terms of B, the least amount produced was in the WA culture (without nutrients), and CWA had the most production with 0.249 g of mycelium. CMA, which had the slowest Gr, produced the most B, only second to CWA (Table 1).

The nutritional contents of each of the cultures analyzed was different, the principal sources of carbon were maltose (MEA), dextrose or glucose (PDA and CWA), sucrose (CWA), fructose (CWA), and starch (CMA). The culture mediums are sources of carbon that the mushroom uses; however, the results in each medium were different, and the medium which showed the best results was CWA, with the fastest Gr and highest B production. This could be because this culture has a mixture of carbon sources and when compared to the other mediums it contains eight of the ten vitamins needed by basidiomycota (Chang and Miles, 2004); among these, thiamine and biotin are the most important. Thiamine acts as carboxylase and biotin as a coenzyme for the carboxylase in the regulation of carbohydrate metabolism, therefore since these vitamins are present, the mushrooms metabolic rate is increased, reflected in B and Gr.

The CMA medium also contains riboflavin, thiamine and nicotinic acid, although the medium's main source of carbon is from starch, which is a large polymer. The mushroom's metabolism takes longer to absorb it when compared to more simple molecules such as sugar, and this explains why it was the second medium with the most B. For the second stage, the MEA, CMA and CWA mediums were chosen and the strain showed the most growth after 30 days of incubation (Figure 1). With the pH value adjusted to 4, 5 and 6, there were no morphological changes to the strain, contrary to what was reported by Vázquez-García *et al*. (2002) where the adjusted pH can modify the hyphae branching pattern for other strains, such as *Laccaria bicolor* where presented scarce and lax mycelium at a pH of 8.0, and at a pH of 6.0 the colony was more compact and had abundant airy mycelium; or *Terfezia olbiensis* which presented a lax and weak colony at a pH of 5.0, but presented a denser colony with abundant airy mycelium at a pH of 8.0.

The multivariate analysis of variance (MANOVA) for growth rate showed significant differences related to culture medium ($p<0.05$) and pH ($p<0.05$), and a significant interaction between the two factors ($p<0.05$). In terms of B there was only a difference with the culture medium ($p<0.05$), while the pH value variable did not show any difference ($p>0.05$) nor did the interaction between the two factors ($p>0.05$). In Table 2 the results of the Tukey test of the different culture mediums and pH are shown with respect to Gr and B.

The MEA medium with a pH of 4 presented the fastest Gr, and invaded the Petri dish completely by 98 days, while the CMA culture with a pH of 6 showed the slowest Gr. None of the CMA cultures invaded the Petri dishes completely. Regarding B, the CWA medium with distinct pH values showed the highest value with an average of 0.23 g, while the CMA medium showed the least B.

In the CWA, the Gr did not change when the pH value was adjusted. With reference to B, this diminished with a pH of 4 (Table 2). In the CMA culture, Gr increased from 0.16 mm day^{-1} to 0.245 mm day^{-1} with a pH of 4, and B decreased in all three pH values from 0.162 g to 0.4 g Petri dish^{-1}. In the MEA culture, the Gr increased from 0.3 mm day^{-1} to 0.4 mm day^{-1} and there were no significant changes in B with a pH of 4; with a pH of 5, there were no significant changes in Gr and B was reduced by half, and with a pH of 6 growth was inhibited (Table 1 and 2).

There are distinct factors which can influence the *in vitro* behavior of mushrooms, such as culture medium, temperature, and pH (Vázquez-García *et al*., 2002). Specifically, this last factor can cause significant changes even in the production of secondary metabolites. Some

Table 2. Growth rate and average biomass of *Floccularia luteovirens* in different medium cultures with different pH levels.

Culture medium	pH	Gd (mm/day)	B (g/Petri dish)
MEA	4	0.406 e*	0.17 bc*
	5	0.28 d	0.07 ab
	6	0 a	0 a
CWA	4	0.286 d	0.21 c
	5	0.281 d	0.24 c
	6	0.2916 d	0.25 c
CMA	4	0.245 c	0.04 a
	5	0.216 b	0.06 ab
	6	0.208 b	0.05 ab

*Different letters in the same column for the same variable indicate significant differences (p<0.05). MEA: Malt Extract Agar; CMA: Corn Meal Agar; CWA: Coconut Water Agar.

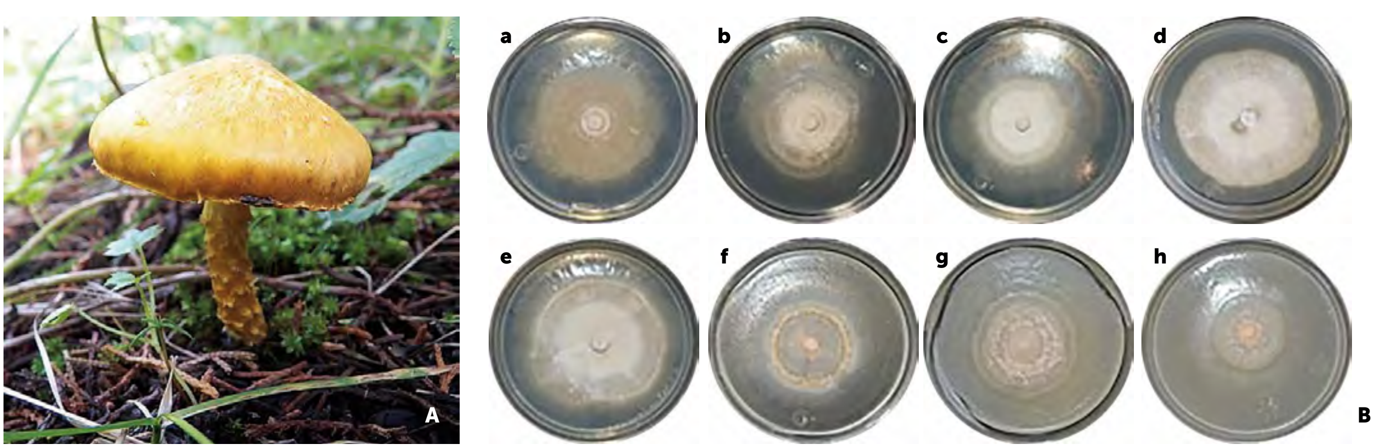

Figure 1. *Floccularia luteovirens*. A. Sporocarp. B. Strain developing in three culture mediums with three pH values at 18 °C. a) MEA, pH 4; b) MEA, pH 5; c) CWA, pH 4; d) CWA, pH 5; e) CWA, pH 6; f) CMA, pH 4.

species which have reported higher radial growth rates when the pH value of the culture was modified are *Laccaria bicolor* (Santiago-Martínez *et al.*, 2003), *Suillus luteus*, and *Scleroderma citrinum* (González *et al.*, 2015). Various studies have shown the potential of using native wild mushroom strains, since they have found the right conditions for mass use of strains or for their experimental cultivation (Carreño-Ruiz *et al.*, 2014; Arana-Gabriel *et al.*, 2014; González *et al.*, 2015; Díaz Talamantes *et al.*, 2017). These were done with the goal of offering an alternative for the diversification of cultivated species, conducive to their development in certain environments, and with the resources available in the region of origin of each strain. This is important since these are species valued by the local population, which permits them to take advantage of and appraise their natural resources.

CONCLUSIONS

The IE 5034 *F. luteovirens* strain showed an increase in Gr and B when pH value of the culture medium was modified; however a medium that was conducive to the right conditions in order to produce more B in a short period of time was not identified. Even though the strain reached a Gr of 0.406 mm per day in the MEA medium with a pH of 4, which is higher than that reported in previous studies, it is slow when compared to the development of other mushroom strains, cultivated or wild. In terms of B, 0.23 g Petri dish^{-1} was produced on average in the CWA medium with a pH of 4, 5 and 6. Describing the behavior of wild strains leads to the identification of the nutritional and environmental requirements for the conservation and characterization of native germplasm; as well as knowing the necessary conditions for the optimization of *in vitro* growth in studies focused on biotechnological aspects, such as the collection of metabolites or experimental cultures where short cycles with high B are required.

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https://doi.org/10.32854/agrop.v13i10.1659

Yield of "Pipiana" squash (*Cucurbita argyrosperma* Huber) in Vertisol soil due to the effect of biostimulants

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ABSTRACT

Objective: To evaluate the effect of liquid (supermagro) and solid (bocashi) biostimulants on the yield of Pipiana squash (*Cucurbita argyrosperma* Huber) in a Vertisol soil.

Design/Methodology/Approach: The research was carried out at "Central Flores" farm, in May-August 2018. Treatments were: T1 = Control, T2 = 400 g of bocashi + supermagro 1:20 v/v, T3 = 400 g of bocashi + supermagro 1:30 v/v, T4 = 500 g of bocashi + supermagro 1:20 v/v, T5 = 500 g of bocashi + supermagro 1:30 v/v, with four replicates (plots) each. For the statistical analysis, 11 variables were recorded in plants, flowers and fruits, and 12 variables in seed, the experimental design used was completely randomized. An ANOVA and a means comparison test (Tukey, $\alpha \leq 0.05$) were performed with the Statistical Analysis System version 9.1 (SAS, 2003).

Results: The plants of treatment 4, showed the highest values in most of the variables evaluated, the yield of fresh fruit and dry seed was 4.48 t ha^{-1} and 1.76 t ha^{-1}, respectively, higher results than those reported in the scientific literature.

Limitations/Implications: No limitations were found in this study.

Findings/Conclusions: The application of foliar and soil bio-stimulant increases quantity and quality of fruits and seed of *Cucurbita argyrosperma* Huber.

Keywords: Supermagro, bocashi, nutrition, organic fertilizer.

INTRODUCTION

The production of (*Cucurbita* sp.) squash seed in Mexico in 2017 accounted a sown area of 68,637.90 ha and a value of $23,915.79 pesos MX per ton (SIAP, 2017). Seed represent the most important food and commercial product obtained from this species (Ireta-Paredes *et al.*, 2018; Kates, 2019); since they are consumed directly, and are the main ingredient for the preparation of meals known in Mexico as "pipianes" (Lira *et al.*, 2016; SR Lira, 1995; Villanueva, 2007). Seed contain the main saturated fatty acids: palmitic (21.5%), stearic (11.6%), oleic (37.0%), linoleic (28.1%) and linolenic (0.3%) (Ribeiro *et al.*, 2018).

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 39-43.*
Recibido: *abril, 2020.* **Aceptado:** *septiembre, 2020.*

Traditionally, squash is grown using chemical fertilizers, which cause increase of nitrate in the soil, and indirectly inhibit decomposition of organic matter (Nicholls, Altieri, & Vázquez, 2017; Zhou *et al*., 2018), since they cause the absence of edaphic microorganisms (bacteria and fungi) and leaf litter degrading enzymes (Zhang *et al*., 2018). As a consequence, the soil aggregates lose their stability (Cardona, Benavides, & Montoya, 2016); therefore, alternatives have been sought, such as sustainable agriculture, which establishes friendly relationships with natural resources (FAO, 2017; SEMARNAT, 2019). This type of agriculture is based on the use of organic fertilizers as a means for crop nutrition, also seeking to increase water retention, contribute to organic matter, and a greater microbial activity (Czekała, Jezowska, & Chełkowski, 2019; Ireta *et al*., 2018; Piya *et al*., 2018). However, the volume required to meet the needs of macroelements in crops is high and consequently expensive. Another solution is the use of organic stimulators, which promote biochemical and physiological mechanisms in plants that culminate in an increased productivity (Ertani *et al*., 2013; Garcia-Martinez *et al*., 2010). This latter can be attributed to peptide, amino acids, polysaccharides, humic acids and phytohormones contents (Parrado *et al*., 2008). Based on the above, the effects caused by the application of a liquid and a solid biostimulant were evaluated on the yield of Pipiana squash (*Cucurbita argyrosperma* Huber), grown in a Vertisol soil in Quintana Roo, Mexico.

MATERIALS AND METHODS

The study was conducted from May to August 2018 at the Central Flores property in Quintana Roo, Mexico (18° 40' 16.3128" N and 88° 44' 44.4456" W). The climate according to the Köppen system and adapted by García (2004) is AW_1 (sub-humid warm, with a cumulative rainfall regime of 1306.1 mm in summer and winter), average annual temperature of 27.5 °C and maximum of up to 32.5 °C (CONAGUA, 2018). As part of the sowing, the land was prepared with a heavy harrow pass, 20 experimental plots (12 × 6 m) were established, with five treatments: T1 = Control, T2 = 400 g of bocashi + supermagro 1:20 v/v, T3 = 400 g of bocashi + supermagro 1:30 v/v, T4 = 500 g of bocashi + supermagro 1:20 v/v, T5 = 500 g of bocashi + supermagro 1:30 v/v, with four replicates (plots) per treatment. Seed were obtained from own farm's harvest in August 2017 and two seeds were deposited per strain no deeper than 5 cm in a plantation squared frame arrangement 2 × 2 m. Weed removal was carried out manually. To control pests and diseases, an insecticide-fungicide based on sulfur and lime (CaS) called calcium sulfide broth, was applied weekly at a dose of 2.5% v/v. Bioindicators were recorded in plants, flowers, fruits and seed, and a completely randomized experimental design was used. For the statistical analysis, 11 variables were registered in plant, flowers and fruits, and 12 in seed, of 10 plants per plot, chosen at random and ANOVA and comparison test of means by Tukey's method ($\alpha \leq 0.05$) were carried out with the Statistical Analysis System program version 9.1 (SAS, 2003).

RESULTS AND DISCUSSION

Among treatments were recorded highly significant differences ($P \leq 0.01$) according to ANOVA results from variables: number of male and female flowers, number of vines, fresh weight and dry fruit, polar and equatorial fruit diameter, pulp thickness, and number of fruits per plant; and treatment four (500 g of bocashi plus 1:20 v/v of supermagro) was the one that registered those highest values for all the aforementioned variables (Table 1). The positive effect to use of supermagro in squash pipiana has been reported by Gonzáles, Mosquera & Trujillo (2015) in watermelon cultivation of and by Favor *et al*. (2019) in corn (*Zea mays* L.); because supermagro can be a foliar biostimulant. In this regard, Yakhin *et al*. (2017) indicate that fermented fertilizers contain a mixture of substances (peptides, amino acids, polysaccharides, humic acids and phytohormones) in addition to microorganisms (bacteria and fungi) that stimulate biochemical and physiological processes in plants; As suggested by Huang *et al*. (2010) these processes unleashed mechanisms that help protect bodies photosynthesis, conferring tolerance to abiotic stress and increment an efficiency of the N.

At the cellular level, it has been shown that fermented fertilizers due to their content of humic substances, inhibit the activity of IAA-oxidase, thereby contributing a higher concentration of IAA in the tissues (Mato, Olmedo & Méndez, 1972), in similar way with the synthesis of auxin transporters in the plant (Du Jardin, 2015), as electron donors-receptors, intervening in the cellular respiration chain and increasing the energy supply to the cells (Csicsor, Gerse & Titkos, 1994; Nadporozhzkaya, 1996) and increasing chlorophyll levels due to the greater availability of (Fe) iron chelates, and the uncoupling of oxidative phosphorylation (Albuzio *et al*., 1994). In this regard, Jindo *et al*. (2012) observed in corn than humic acid applied to root resulted in higher elongation due to activation of ATP-ases mediating proton pumping in the plasma membrane, thus increasing the permeability of the cell wall. Other authors such as Gonzáles *et al*.

(2015) indicated that foliar application of supermagro, stimulates the vigorous growth of the plant due to the presence of micro and macro nutrients; composition described by Peñafiel & Ticona (2015): nitrogen (0.209 kg m^{-3}), soluble phosphorus (0.168 kg m^{-3}), total phosphorus (0.437 kg m^{-3}), soluble potassium (1.807 kg m^{-3}), sodium soluble (1.019 kg m^{-3}), soluble calcium (0.465 kg m^{-3}), soluble magnesium (0.388 kg m^{-3}), sulfides (2.778 kg m^{-3}) and sulfates (1.173 kg m^{-3}), all elements necessary for the physiological activity of the cultures.

Table 1. Differences of mean values for the seed variables in Pipiana squash cultivation.

T	NMF	NFF	NG	LG	FWF	DWF
T1	47.78 b	9.87 b	9.30 c	4.08 cb	1.25 d	0.07 d
T2	52.29 b	11.03 b	12.45 b	4.00 c	1.56 c	0.08 d
T3	51.87 b	10.92 b	12.55 b	4.39 cb	2.06 b	0.12 c
T4	71.09 a	19.01 a	18.78 a	5.37 a	2.70 a	0.20 a
T5	54.08 b	9.35 b	14.73 b	4.56 b	2.22 b	0.16 b
MHSD	10.615	3.839	2.648	0.532	0.253	0.026
T	PDF	EDF	PTF	FPT	NFPP	
T1	14.70 d	15.46 c	2.10 a	15.23 dc	3.33 c	
T2	15.88 c	16.28 c	2.06 a	17.34 d	3.85 cb	
T3	17.26 b	18.07 b	2.17 a	20.72 ba	4.18 cb	
T4	19.33 a	20.35 a	2.30 a	23.48 a	6.63 a	
T5	18.10 b	18.97 b	2.09 a	20.12 bc	5.03 b	
DMHS	0.961	0.921	0.330	3.312	1.201	

T = Treatment; NMF = Number of male flowers; NFF = Number of female flowers; NG = Number of guides; LG = Length of guides, m; FWF = Fresh weight of fruit, kg; DWF = Dry weight of the fruit, kg; PDF = Polar diameter of the fruit, cm; EDF = Equatorial diameter of the fruit, cm; PTF = Peel thickness of the fruit, cm; FPT = Fruit pulp thickness, cm; NFPP = Number of fruits per plant. MHSD = Minimal Honest Significant Difference. Values with equal letters between columns are not statistically different (Tukey, $P \leq 0.05$).

For seed variables, the analysis of variance showed highly significant differences ($P \leq 0.01$) among treatments on total fresh weight, total ambient dry weight, full and empty seed dry weight, number of full and empty seeds, polar and equatorial seed diameter, seed thickness, weight of seed coat, weight of cuticle and weight of cotyledons. Treatment four (500 g of bocashi plus 1:20 v/v of supermagro) was the one that showed the highest values for all the studied variables except empty seed weight and number (Table 2).

Table 2. Differences in average values for the seed variables in Pipiana squash cultivation with organic fertilizers.

T	TFWS	TDWSE	DWFS	DWVS	NFS	NVS
T1	100.70 c	46.54 c	42.12 c	4.42 a	179.25 c	16.63 ba
T2	123.65 c	60.18 c	56.05 c	4.13 a	228.18 bc	22.63 a
T3	158.78 b	76.19 b	71.75 b	4.44 a	274.30 ba	18.95 ba
T4	201.10 a	106.33 a	105.6 a	0.73 b	300.75 a	5.33 c
T5	171.08 b	82.92 b	81.83 b	1.09 b	204.45 c	9.08 bc
MHSD	24.848	14.524	13.946	2.935	51.642	10.208
T	PSD	ESD	ST	WSSC	CUW	COW
T1	25.83 d	12.86 c	2.75 dc	0.04 c	0.04 a	0.17 b
T2	26.33 cd	13.08 c	2.54 d	0.04 c	0.04 a	0.17 b
T3	27.60 cb	14.23 b	2.91 bc	0.05 b	0.06 a	0.19 b
T4	29.01 b	14.94 ba	3.22 a	0.05 b	0.08 a	0.24 a
T5	30.71 a	15.39 a	3.01 ba	0.06 a	0.08 a	0.25 a
MHSD	1.425	0.773	0.236	0.006	0.046	0.024

T = Treatment; TFWS = Total fresh weight of seed, g; TDWSE = Total dry weight of seed to environment, g; DWFS = Dry weight of filled seed, g; DWVS = Dry weight of vain seed, g; NFS = Number of full seeds; NVS = Number of vain seeds; PSD = Polar seed diameter, mm; ESD = Equatorial seed diameter, mm; ST = Seed thickness, mm; WSSC = Weight of the seed seminal cover, g; CUW = Cuticle weight, g; WCO = Cotyledons weight, g; MHSD = Minimal honest significant difference. Values with equal letters between columns are not statistically different (Tukey, $P \leq 0.05$).

The dry squash seed yield in Mexico for the year 2017 was 0.56 t ha^{-1} and 0.58 t ha^{-1} in the state of Quintana Roo (SIAP, 2017), Días-Nájera *et al*. (2019) reported 0.380 t ha^{-1} by applying Bayfolan® as foliar fertilization, lower yields than those obtained in our research, where it was recorded over 1.7 t ha^{-1}. This positive response both of fruit and seed yield, is justified due to the immediate foliar incorporation of some essential nutrients for growth and development, as it was argued by Trejo-Tellez *et al*. (2003), in cultivation of jalapeño pepper (*Capsicum annuum*), cucumber (*Cucumis sativus*) and pepper (*C. annuum*); in addition to the supply of humic substances that stimulate those metabolic functions described paragraphs above.

Other authors such as Devi *et al*. (2018), when studying physical characteristics in Pipiana squash seeds collected in local markets, found the following average values: polar (16.81 mm) and equatorial (8.87 mm) seed diameter, seed thickness (2.75 mm) and dry weight of seed coat (0.054 g), all values lower than those obtained in our study. Differences are attributed to intra population variation and the production mode (without application of soil or foliar fertilizer).

CONCLUSIONS

Best treatment for estimated yield of fresh and dried fruit- and seed of Pipiana squash per hectare was treatment T4: 500 g of bocashi + supermagro 1:20 v/v in three application periods (at planting, growth and flowering start), with weekly application frequency during crop cycle, obtaining an estimated yield of 4.48 t ha^{-1} of fresh fruit, and 1.76 t ha^{-1} of dry seed, exceeding the state and national average by more than 50%.

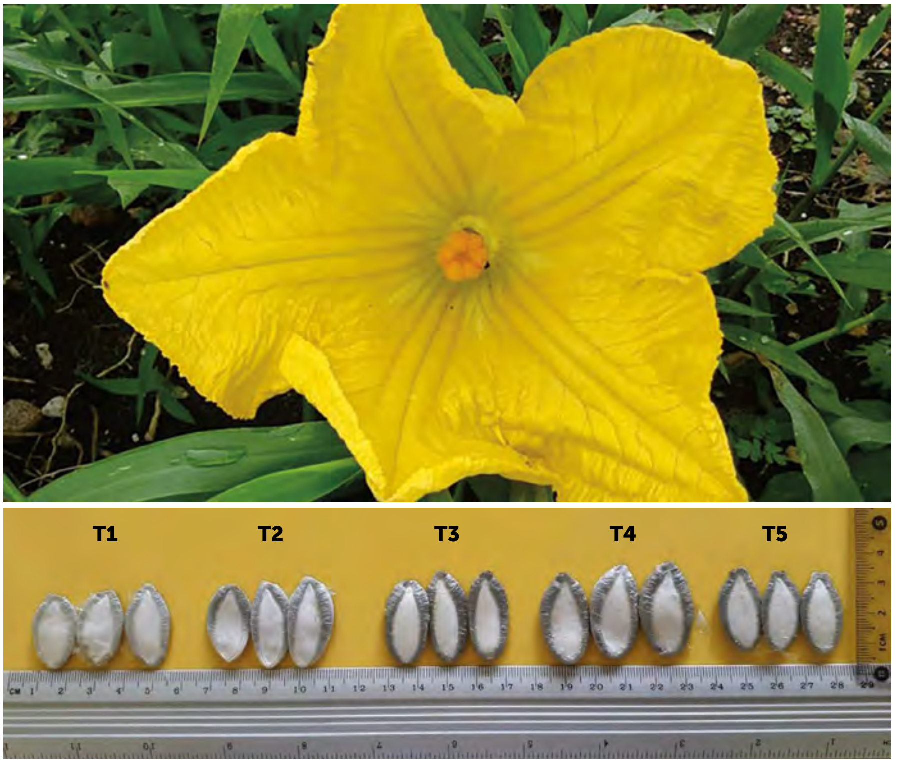

Figure 1. Flower and seed of Pipiana squash (*Cucurbita argyrosperma* Huber).

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https://doi.org/10.32854/agrop.v13i10.1743

Effect of *Bacillus* spp. on the germination and growth of roselle plants (*Hibiscus sabdariffa* L.)

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ABSTRACT

Objective: To analyze the effect of three native strains of *Bacillus* spp. on roselle (*Hibiscus sabdariffa* L.) germination and plant growth of the tecoanapa variety in greenhouse conditions using the Bio-priming method.

Design/methodology/approach: The identity of the Bacillus strains was verified using PCR technique with the universal primers 27F and 1492R for the amplification of the 16S rDNA gene. The roselle seeds were treated with bacterial cells of *Bacillus* spp. with Bio-priming method, evaluating the effect on germination and plant growth. The percentage of germination was evaluated, as well as plant height, root length, and dry matter of plants and roots.

Results: Molecular identification of *B. velezensis* (T1), *B. amyloliquefaciens* (T2), and *B. subtilis* (T3) was carried out. The three treatments caused an increase in germination percentage, root length and plant height, and there was also an increase in dry matter weight of plants and roots, with a significant difference between treatments 1, 2, 3 and the control.

Study limitations/implications: Strains of *Bacillus* spp. must reach commercial production for field applications.

Findings/conclusions: *B. velezensis* is the species that demonstrated the highest percentage of germination and a growth-promoting effect, followed by *B. amyloliquefaciens* and *B. subtilis* respectively.

Key words: Bio-priming, seed, growth

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) (Malvaceae) also known as Jamaica rose, Abyssinian rose or Jamaica flower, originated in tropical Africa, although it is now cultivated in Mexico, Central and South America and Southeast Asia (Morton, 1987; Sayago and Goñi, 2010). Their calyces are the most useful and of socioeconomic importance, since they are used to obtain extracts with applications in the pharmaceutical and food industries (Galicia *et al.*, 2008).

In Mexico presently 18,654 ha of roselle are grown, with the state of Guerrero occupying first place as producer with 76% of that surface. The plant grows better in regions with tropical and subtropical climates in the municipalities of Tecoanapa, Ayutla de Los Libres, Acapulco de Juárez, San Luis Acatlán, Juan R. Escudero, and San Marcos (SIAP, 2019).

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 45-48.*
Recibido: *junio, 2020.* **Aceptado:** *septiembre, 2020.*

There is a recent demand in production for the development of new systems aimed at a lower environmental impact. The genus *Bacillus* is considered a plant growth-promoting bacteria (PGPB), and contributes benefits to plants since it has the capacity to exert a positive effect on plant growth by various mechanisms including the production of phytohormones, solubilization of phosphate, nitrogen fixation and biological control of pathogens (Lugtenberg and Kamilova, 2009). Based on this, this study had the objective of analyzing the effect of three native strains of *Bacillus* spp. on the germination and plant growth of the tecoanapa roselle variety (*Hibiscus sabdariffa* L.) in greenhouse conditions, using the Bio-priming method, which consists of coating the seeds with the bacterial agent in conjunction with a hydration process before sowing.

MATERIALS AND METHODS

Bioassays were carried out with three *Bacillus* isolates obtained from the collection at the Bacteriological Laboratory of Colegio de Postgraduados. The strains are native to roselle cultures, isolated from calyces and previously identified as *B. subtilis*, *B. amyloliquefaciens* and *Bacillus* sp. (Rendón, 2014). For the inoculations the tecoanapa roselle cultivar was used.

Molecular identification of *Bacillus*

The identity of the three strains of *Bacillus* was verified using PCR technique. The DNA extractions were carried out using the commercial kit Wizard Genomic DNA Purification, following the supplier's instructions. The universal primers used for the amplification of the 16S rDNA gene were 27F (5´AGAGTTTGATCATGGCTCAG) / 1492R (5´GGTTACCTTGTTACGACTT) (Lane, 1991; Turner *et al*., 1999). The amplifications were achieved with an Eppendorf Nexus thermocycler with the following program: a denaturalization cycle for 3 min at 94 °C, followed by 25 three-step cycles: denaturalization for 30 s at 94 °C, 30 s annealing at a recognition temperature for each primer pair, and an extension cycle for 1 min at 72 °C. The PCR amplifications were analyzed by electrophoresis in agarose gel. The PCR products were sent in for sequencing to Macrogen Korea (http://www.macrogen.com/eng/). The consensus sequence was submitted for alignment and homology analysis with the bioinformatic tool BLAST (Basic Local Alignment Search Tool) in GenBank from NCBI (https://www.ncbi.nlm.nih.gov/genbank).

Inoculation of *Bacillus* in roselle seeds

The seed treatment was carried out using the Bio-priming method (Rivera *et al*., 2017) with some modifications. A suspension of *Bacillus* cells was used at a concentration of 108 CFU/ml, obtained from nutritional agar plates after 2 d of growth. The effect of the inoculation with bacteria was compared with seeds treated with distilled water. Four treatments were established (T1: *B. subtilis*; T2: *B. amyloliquefaciens*; T3: *Bacillus* sp.; T4: control).

Effect of *Bacillus* on germination and growth of roselle plants

The treated seeds were sown in plastic trays with 200 cavities with peat moss (European Kekkila Peat moss) sterilized at 121 °C for 1.5 h. The trays were stored in greenhouses, where daily germinated seed counts were carried out. Thirty days after sowing, the plants were transplanted to 30 × 30 cm black polyethylene bags with peat moss (European Kekkila peat moss) and organic material (sheep manure) in a 90:10 ratio respectively. The variables evaluated were: germination percentage, plant height, root length and dry matter in plants and roots.

RESULTS AND DISCUSSION

Molecular identification of *Bacillus*

The results of the consensus submitted for homology analysis with BLAST demonstrated identity percentages above 99% for all of the samples when compared with the NCBI database. The preliminary identification of *B. subtilis* was corrected for *Bacillus velezensis* and it was verified that the second strain corresponded to *B. amyloliquefaciens*. The third strain determined for genus showed a 100% match with *B. subtilis* for the consensus sequences of primers 27F and 1492R (Table 1).

Effect on germination

The first seeds to sprout were those treated with *B. velezensis* (T1), *B. amyloliquefaciens* (T2) and *B. subtilis* (T3), and by the fourth day after sowing they reached 80% of total germination. The control (T4) at day six had not initiated germination,

Table 1. BLAST analysis results.

Variable	Primers	Result BLAST	Accession	Percent identify
B. subtilis	27F/1492R	*Bacillus velezensis*	MT538583.1	100.00%
B. amyloliquefaciens		*Bacillus amyloliquefaciens*	JF802170.1	99.05%
Bacillus sp.		*Bacillus subtilis*	MN417010.1	100.00%

suggesting that the bacterial inoculum accelerated the germination process of the seeds. T1 was the best with 95.63% of seeds germinated, followed by T2 with 94.38%, T3 with 93.75%, and T4 with 80%. Final germination was reached 8 d after sowing for T1, T2, and T3, and 15 d later for T4. This coincides with what was reported by Mojica *et al.* (2009), who described an increase in germination percentage with inoculation treatment with *B. thuringiensis* on *R. solani*.

Growth-promoting effect

It was demonstrated that T1, T2 and T3 promoted an increase in root length and plant height, as well as an increase in dry matter weight of plants and roots, with a significant difference between treatments 1, 2, 3, and the control (T4) (Table 2). Previous studies have reported the *Bacillus* genus as bacteria that are beneficial to improve plant growth (Rojas *et al.*, 2016).

The results showed that *B. velezensis* is the species with the most potential as a growth promoter, followed by *B. amyloliquefaciens* and finally *B. subtilis* (Figure 2). Recently there have been reports on many strains of *Bacillus* which promote plant growth and monitor biocontrol, including *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Bacillus velezensis* (Wang *et al.*, 2020).

The *Bacillus* genus is one of the most studied due to its capacity to promote plant growth of important crops (Rojas *et al.*, 2011; Hernández *et al.*, 2014; Cabra *et al.*, 2017).

Recent Bio-priming studies, dealing with mechanisms in the use of plant growth-promoting bacteria, have described *Bacillus* strains with the capacity to produce auxins that stimulate plant growth and phosphate solubilization (Tejera *et al.*, 2012). Together with this study's results, they suggest a plant-bacteria interaction that improves plant development. The results obtained in the greenhouse study reveal that the bacteria evaluated manifested growth promoting activity. The three strains of *Bacillus* belong to the plant growth-promoting bacteria (PGPB) rhizobacteria group (Wang *et al.*, 2020).

CONCLUSIONS

B. velezensis is the species that showed the highest germination percentage and growth-promoting effect, followed by *B. amyloliquefaciens* and then *B. subtilis*.

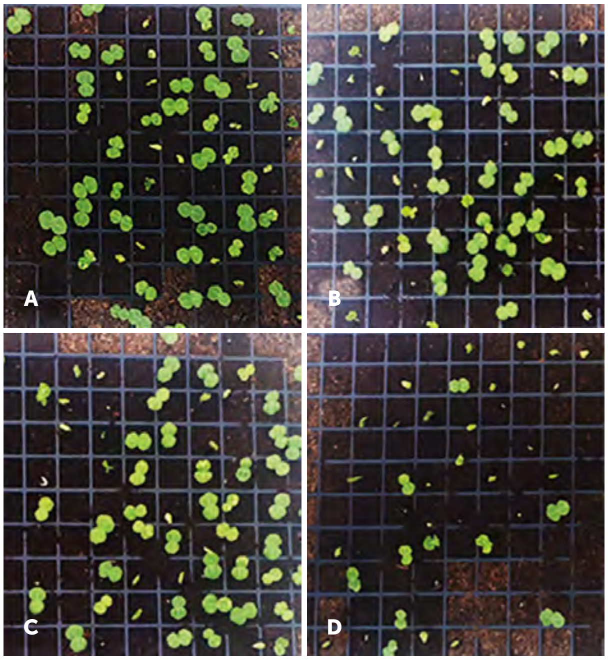

Figure 1. Germination of *Hibiscus sabdariffa* L. seeds eight days after sowing and inoculated with: A) T1: *B. velezensis*. B) T2: *B. amyloliquefaciens*. C) T3: *B. subtilis*. D) T4: control.

Table 2. Statistical values of treatment for growth variables of *Hibiscus sabdariffa* L. plants inoculated with bacteria.

Treatment	AP	PSP	LR	PSR
T1: *Bacillus velezensi*	38.48 a	5.57 a	31.83 a	0.912 a
T2: *Bacillus amyloliquefaciens*	37.07 a	5.21 ab	27.60 b	0.738 b
T3: *Bacillus subtilis*	34.93 b	4.77 b	26.33 b	0.707 b
T4: control	30.67 c	2.71 c	16.20 c	0.264 c
DMSH	1.84	0.66	3.91	0.110

AP Plant height; PSP Plant dry matter; LR Root length; PSR Root dry matter (All initials based on Spanish terms).

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Figure 2. Differences in plant height, root length, and root volume of *Hibiscus sabdariffa* L. inoculated with A) T1: *B. velezensis*. B) T2: *B. amyloliquefaciens*. C) T3: *B. subtilis*. D) T4: control.

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Quality and yield of the *Cucumis sativus* var. Jawell crop under two pest control systems in the Sonoran desert, Mexico

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ABSTRACT

Objective: To compare a commercial pest control program vs a biological pest control program in cucumber (*Cucumis sativus* var. Jawell) cultivation, evaluating quality and production standards.

Design/methodology/approach: The study was carried out in high-tech glass greenhouses, under a cooling system with damp walls and extractors, heating by irradiation, and automated irrigation. Two treatments were evaluated: biological pest control in area A and a commercial control program in area B, both in Persian cucumber (*Cucumis sativus*) of the Jawell variety; each area of 160 m^2, separately, and 41,600 plants for each area. For biological control, the mite (*Amblyseius swirskii*) was released for the control of thrips; the wasp (*Aphidius colemani*) was released for aphid control; the mites (*Phytoseiulus persimilis*) and (*Amblyseius californicus*) for red spider control and application of the entomopathogenic nematode (*Steinernema fetiae*). The chemical control was in accordance with COFEPRIS (2019). The variables were fruit quality total production and incidence of pests in a completely randomized experimental design, and the t-student statistical test and Mann-Whitney test were done for the variables weight quality and loss ($P \geq 0.05$). A correlation was made between the incidence of thrips (*Thrips tabaci*) and the incidence of biological control.

Results: The biological control method was just as efficient as the chemical control method, in variables such as fruit weight, number of boxes obtained of quality cucumber, and incidence of pests and their biological control.

Study limitations/implications: It is important to perform more studies under field conditions where biotic and abiotic factors are different and in other regions, in addition to testing other registered biological products.

Findings/conclusions: Biological and chemical control are complementary, an integrated control would help to slowly adapt a company for a subsequent application of biological control, easing regularization and certification procedures that involve the use of chemicals. A more continuous release of *A. swirskii* is proposed and distributed during the cultivation weeks.

Keywords: management, phytosanitary, pests, system, production, organic.

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 49-55.*
Recibido: *julio, 2020.* **Aceptado:** *septiembre, 2020.*

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INTRODUCTION

Pests such as fungus gnat (*Bradysia* sp), thrips (*Thrips* spp), aphids (*Aphis* sp, *Myzuz persicae*), red spider (*Tetranychus urticae*), among the most common, are present in cucumber cultivated in greenhouses, which if not controlled can cause damage to the crop. Therefore, proper phytosanitary management within the greenhouse is essential. There is commonly the application of commercial pest control programs where only chemical products are used (Rubio and Fereres, 2005; Carrero and Planes, 2008); however, the demand to reduce the use of agrochemicals has prompted the study of other alternatives, such as biological control, in which natural enemies are used. One of the main advantages of greenhouse production is that it is possible to maintain controlled conditions that favor the release of beneficial organisms to achieve a reduction in the use of phytosanitary inputs (Carrero and Planes, 2008; Blom *et al*., 2010; Bale *et al*., 2008; Aguado *et al*., 2009; Pizano de Márquez, 1997; Roberts and Hutson, 1999; Rogg, 2001; Bealmear, 2010).

Some of the beneficial organisms studied for the pests mentioned are the wasp *Aphidius colemani* for the control of aphids (*Aphis gossypii*); the mite *Amblyseius swirskii* for the control of thrips (*Thrips* spp); the mite *Phytoseiulus persimilis* and the thermite *Amblyseius californicus* for the control of red spider mite (*Tetranychus urticae*); and the nematode *Steinernema feltiae* for the control of larvae of the fungus gnat fly (*Bradysia* sp) (Carrillo *et al*., 2003). With the use of these beneficial organisms, the objective of this study was to compare a commercial pest control program and a biological pest control program in *Cucumis sativus* var. Jawell, under the premise that biological control can replace commercial control, obtaining results of equal fruit quality and equal production.

MATERIALS AND METHODS

This study was carried out in high-tech greenhouses (Casasano IMP, 2019), since they have equipment that conditions the optimal development of the crop (cooling system for damp walls and extractors, irradiation heating system, automated irrigation system, and glass infrastructure which allows maximum use of light). The study area is located at 31° 13′ 11.17″ N, 110° 58′ 24.77″ W, and an altitude of 1241 m. The study evaluated the treatments of biological pest control in area A and commercial control in area B, both in Persian cucumber (*Cucumis sativus*) of the Jawell variety.

The cucumber plant was sown in summer (August) in a greenhouse, in rock wool cubes as an inert substrate of porous consistency with high water retention capacity and a dimension of 10 × 10 × 6 cm (Grodan®). Three weeks later, it was transplanted to inert coconut fiber pillows measuring 100 × 20 × 8 cm (Millennium®). Each experimental area was 160 m^2 separately. The sowing density was 3.42 plants m^{-2} modified from Carrillo *et al*. (2003) due to business requirements. Both treatments, area A (biological control) and area B (commercial control), were managed with the same strategy in relation to humidity, temperature, fertilization, irrigation and cultivation tasks.

Biological control program. It consisted in the release of the mite (*Amblyseius swirskii*) for the control of thrips, the release of the wasp (*Aphidius colemani*) for the control of aphids, the mites (*Phytoseiulus persimilis*) and (*Amblyseius californicus*) for the control of red spider mite, and the application of the entomopathogenic nematode (*Steinernema fetiae*). The organisms and the doses used were based on the recommendations made by the Koppert biological systems company and are indicated for each beneficial organism (Malais and Ravensberg, 2006).

Amblyseius swirskii. The mite *Amblyseius swirskii* was applied to control thrips (*Bemisia tabaci*) (Van Emden and Harrington, 2007; Cédola and Polack, 2011). The dose used was 100 mites per m^2. Two mite releases were carried out, during weeks 40 and 41 corresponding to the first two weeks after transplantation of the cucumber crop, this so that the beneficial organism was established in the crop before the appearance of the pest in week 42, period in which this pest has been already detected in the study area. A total of 2,080 sachets were placed for each release (week 40 and 41).

Aphidius colemani. Application of the wasp *A. colemani* was used to control aphids (*Aphis gossypii*). The dose used was 2 wasps per m^2. Two releases were made during weeks 40 and 43, period in which this pest has already been detected in the study area. The first application was made with the objective of establishing the organism and the second was carried out in the first appearances of the pest (week 46).

Phytoseiulus persimilis* and *Amblyseius californicus. Applications of the mites *Phytoseiulus persimilis* and

Amblyseius californicus were made for control of the red spider mite (*Tetranychus urticae*). The commercial product SPIDEX, which contains *P. persimilis* and the SPICAL product, which contains *A. californicus*, were used. The dose of *P. persimilis* adults was 50 mites per m^2 in all releases. Five releases were made at 41 weeks for the organism to establish itself and at 42, 43, 44 and 45 weeks when the first red spider mite colony was found. The dose used for *A. californicus* was 200 mites per m^2. Two releases were made in weeks 40 and 43. The introduction of *P. persimilis* and *A. californicus* in the greenhouse was carried out by placing 50 points evenly distributed in the biological control area.

Steinernema feltiae. Application of the nematode *Steinernema feltiae* was carried out for the control of fungus gnat (García, 2008). The dose used in this experiment was 500,000 EPNs per m^2. Six applications were made from weeks 40 to 45. The first application was to establish the organism and the others were because of the presence of the pest to be controlled. The introduction done manually to all the plants in the biological control area (Area A), since it could not be carried out by the irrigation system because when the organism passes through the sand filter, the population decreases and this affects its efficiency.

Traditional commercial control. It consisted of a conventional pest control management program used in local greenhouses according to COFEPRIS (2019). The thrips control program was the only one where biological release of the mite *Amblyseius swirskii* was carried out in addition to the application of chemicals. This is because the thrips pest is the one that requires more attention, since it causes malformations to the fruit, affecting its quality and causing greater economic loss. The chemicals Actara (Thiametoxam), Beleaf (Flonicamid), Confidor 350 sc (Imidacloprid), Plenum 50 gs (Pymetrozine) and Talstar 100 CE (Bifenthrin) were applied for control of aphids. Tracer (Spinosad), Exalt (Spinetoram) and Beleaf (Flonicamid) were used for control of thrips. Agirmec (Abamectin) and Talstar (Bifenthrin) were applied for control of red spider mites, and finally, Trigard (Cyromazina) for the control of fungus gnat larvae.

Control of aphids. The chemical products used were: Actara (Thiametoxam), Beleaf (Flonicamid), Confidor 350 sc (Imidacloprid), Plenum 50 gs (Pymetrozine) and Talstar 100 CE (Bifenthrin). The doses applied were those as low as recommended in the commercial product; this criterion is the one applied for every product evaluated in this study. The application was made in week 40 and 46, period of aphid presence.

Control of thrips. The release of the organism *Amblyseius swirskii* was carried out in the same way and distribution as explained previously in the biological control. For control of thrips, the commercial chemical products used, normally managed by the company in which the experiment was carried out, were: Tracer (Spinosad), Exalt (Spinetoram) and Beleaf (Flonicamid). The application was carried out on weeks number 2, 45, 46 and 52, when more than 60 thrips were quantified in the total count of the traps in the compartment.

Control of red spider mite. To control the red spider mite pest, commercial chemical products were used normally managed by the company where the experiment was carried out, the chemical products were Agrimec (Abamectin) and Talstar (Bifenthrin), the application of the products was carried out for both based on commercial specifications. The application was made in week 48 and 49, in the presence of the first spider colony.

Control of fungus gnat. To control the fungus gnat fly in the commercial pest control greenhouse area, the commercial chemical product normally managed by the company where the experiment was carried out was used; the product was Trigard (Cyromazina). The application was carried out in weeks 42, 45, 47, 50 and 51, when the presence of the first larva was observed in any of the cubes.

Variables evaluated

The parameters evaluated to determine the effectiveness of cucumber pest control methods under greenhouse conditions were: fruit quality, total production and incidence of thrips.

Fruit sampling. In order to eliminate the variability and keep the criteria in fruit sampling constant, a single person was assigned to carry out this task. 10 boxes were cut daily in the biological control area and 10 boxes daily in the commercial control, during the 77 days that the crop was in production. The size of the cucumber for cutting was measured according to the commercial characteristics; a minimum length of 5'', maximum of 6'', a minimum diameter of 1'' and a maximum of 1¼'', medium green color, and oblong shape, established by the company Mastronardi Produce México S.R. de C.V. The cutting box was considered complete when it was filled with two batches of cucumber, placed across the width on each side, as managed by the company to avoid damaging the fruit.

Fruit quality. As previously described, during the 77 days of crop production, 10 boxes were taken from the biological control area and the commercial control area daily, these boxes were weighed to obtain their net weight, then the fruit considered waste was removed, which did not have the required physical quality, such as deformed fruit, overripe fruit (excess size), mechanical damage (scars, bumps, bruises), damage from thrips (scar). This fruit, removed due to quality deficiency, was weighed to obtain the weight loss. The rest of the fruit that met the quality characteristics was weighed and considered quality weight.

Production. The production yield was calculated by counting the total boxes obtained during the 77 days of production. To determine the total kilograms of quality production and yield per square meter, the average quality weight of the boxes evaluated was calculated and multiplied by the total boxes produced.

Incidence of thrips. The presence of thrips in the cucumber crop is an indicator of production risks, since this pest is the one that causes the greatest loss to the producer, by deforming the cucumber fruits, affecting their commercial quality, considered waste by not complying with the characteristics of commercial quality, so it is important to evaluate the incidence of thrips (*Bemisia tabaci*). In the biological control program and the commercial control program, the incidence was determined by monitoring the thrips population during the 12 weeks of production. In the same weeks of production, the population of *Amblyseius swirskii* was analyzed to relate the behavior of the pest to its predator. Twenty HORIVER® adhesive traps were used for monitoring, which were placed in the treatment area and in the control area. The Koppert Company recommends up to 5 traps per 1,000 m^2. The traps were strategically distributed to be monitored once a week. The traps were placed at the height of the head of the plant. The monitoring consisted of quantifying the thrips adhered to the trap (Figure 1).

In addition to the trap count, three plants were inspected, the one located under the trap and the two continuous. The inspection consisted of checking a flower and the underside of a leaf from each of the three plants with the help of a 10 × 22 mm plastic 5X magnifying glass. The flower was selected by checking the middle part of the plant of each of the three plants and to inspect the leaf, a leaf from the low stratum was selected in the first plant, in the second plant a leaf from the middle stratum, and in the third plant a leaf from the upper stratum (Garza and Molina, 2008). This was done once a week.

In this study, only sporadic focus points of aphids and red spider mite were presented, which did not represent a risk to the crop, since they were controlled without major problems by removing leaves in cultivation practices, which were part of the crop management without being related with pest control, so only the incidence of thrips (*Bemisia tabaci*) was evaluated.

Statistical analysis. A completely randomized experimental design was applied. To analyze the total number of boxes produced, the t-student statistical test was used for normal data between two independent samples and the Mann-Whitney test was used to compare the variables weight quality and weight loss with the data obtained during 77 days, with a level of significance of $P \geq 0.05$ to determine differences. A correlation (r) was made between the incidence of thrips (*Thrips tabaci*) and the incidence of the biological control *Amblyseius swirskii*, to compare the biological control (A) and the commercial control (B) (Cervantes *et al*., 2011).

RESULTS AND DISCUSSION

Based on the method described and under the conditions described, the results obtained in this study are indicated below.

Fruit quality. During the 77 days that the fruit harvest lasted, a record was kept of the quality weight and weight loss of 10 boxes of the commercial control treatment and 10 boxes of the biological control treatment. The average boxed quality weight obtained was 4.95 kg in the boxes from production under biological control and 4.89 kg in the boxes from production under commercial control (Figure 1). There was no statistically significant difference between the treatments ($P \leq 0.05$). The average quality weight did not show differences ($P \leq 0.05$) in both treatments, results compared with those obtained by Cervantes *et al*. (2011) and Paredes *et al*. (2013).

Among the advantages in the use of biological pest control, there is to reduce the problems of regulation and certification that the use of chemical products involves, by reducing the number of applications and avoiding the excessive use of chemical products in biological control as shown in reports by Grijalva *et al*. (2011), where the use of biological control reduces chemical residues in vegetables (Pardo, 2010), favorable cost/benefit ratio and the ease of performing releases (Leigh *et al*., 2010). The average weight loss per box harvested from the biological control treatment was 0.46 kg. In the case of the boxes from production under commercial control, it was 0.47 kg (Figure 1).

There was no statistically significant difference (P≤0.05) between the weight loss of the harvested boxes from the biological control and the weight loss obtained from the boxes from the commercial control. In the results obtained in this study, no significant differences were found in the weight loss (P≤0.05) in both treatments, commercial control versus biological control of pests. Cervantes *et al*. (2011) report a greater amount of weight loss in biological control than chemical control in their study with tomato. On the contrary, in this study, even when there were no statistically significant differences, the value of the weight loss was higher in the chemical control. This is possibly due to the fact that the evaluation in this study is broader in terms of time, during the entire production cycle corresponding to 77 days, while in the study reported by Cervantes *et al*. (2011) it is only for a short period. **Total production**. The evaluation of production during the 77 days after cutting the fruit, the total of boxes obtained during this period, as well as the total weight of the boxes, were measured from the production of the compartments with biological treatment and commercial control. The total of boxes obtained during the period mentioned in production under biological pest control was 27 239 boxes, while the production under commercial control was 28 124 boxes. There was no statistically significant difference between the treatments (P≤0.05) (Table 1). The total weight of the boxes obtained during the entire period of 77 days of cutting for the 27 239 boxes from production under biological control was 134,796.20 kg and the weight of the 28 124 boxes from production under commercial control was 137,416.10 kg, without showing significant statistical difference (Table 1).

The total weight obtained gives an average of 11.13 kg/m^2 in the production under biological control and 11.34 kg/m^2 in the production of commercial control. The production obtained in both controls did not show statistically significant differences (P≤0.05). Studies by González (2009) mention that, despite not presenting differences, production under biological control could be favored in the long term, mainly in

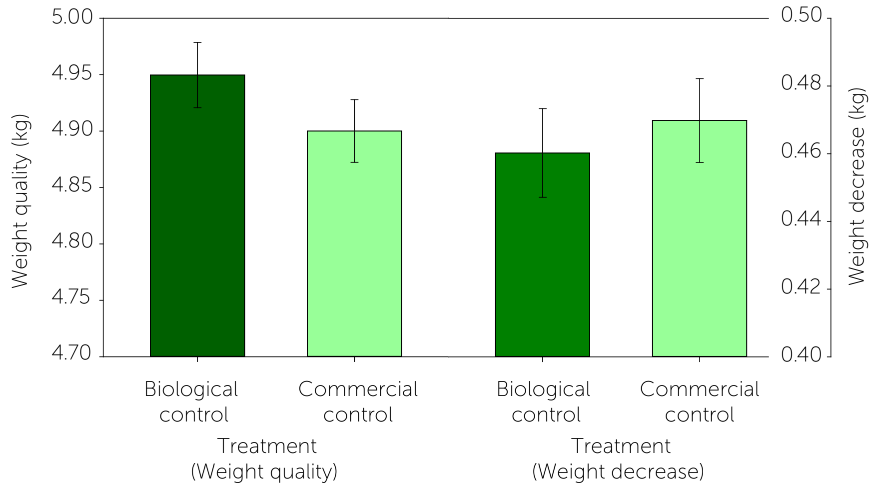

Figure 1. Average weight of the boxes obtained from the fruit quality and average weight of the boxes obtained from the fruit decrease from production under commercial and biological pest control. The bars represent the mean value ± EE of ten repetitions.

Table 1. Statistical values of the data obtained from the total number of production boxes and total quality weight of the boxes obtained during the harvest from the biological control and commercial control treatment for 77 days.

Control	Number of boxes and kg	Mean	Mean deviation	Range	Standard error
Commercial control boxes	28, 124	365.25	58.88	305	6.71
Biological control boxes	27, 239	353.75	51.31	294	5.85
kg commercial control	137, 416.10	1784.62	328.0	1318.54	37.4
kg biological control	134, 796.20	1750.6	470.56	1965.61	53.6

the reduction of the use of chemicals, helping to avoid generating resistance from pests towards chemicals and obtaining a biologically controlled production (Mason and Huber, 2001; Lorca, 2009; Devine *et al*., 2008). The conversion from commercial control to biological control turns out to be somewhat gradual in most cases, where some producers reduce the application of broad spectrum chemicals to use the introduction of beneficial organisms and chemical products that are compatible with the beneficial fauna (Nicholls, 2008ab; Devine *et al*., 2008; Pardo, 2010). In this study, only sporadic outbreaks of aphids and red spider mites took place, which did not represent a risk to the crop as considered by the company where the experiment was developed, since they were controlled without major problems by removing leaves in cultivation practices, which were part of the crop management without being related to pest control, so only the incidence of thrips (*Bemisia tabaci*) was evaluated.

Thrips. The chemical productss Belef (Flonicamid), Tracer (Spinosad) and Exalt (Spinetoram) were applied in weeks 45 Belef (Flonicamid), 46 Tracer (Spinosad), 52 Exalt (Spinetoram), and 2 Tracer (Spinosad). The application of the products was decided based on monitoring thrips in traps. A correlation was made to evaluate the biological control and commercial control treatments, observing the behavior of the thrips pests and its biological control, *Amblyseius swirskii*, where the biological control, when applying a linear regression (Table 2), shows that there is a relationship between the decrease in the *A. swirskii* population and the increase in the thrips population (Figure 2ab). In week 43 there is concurrence with the second release of the beneficial organism where the population of *A. swirskii* increased, which when feeding on thrips larvae caused a decrease in the thrips population in the following weeks. In the commercial control treatment, when applying a linear regression (Figure 2ab), the populations of *A. swirskii* are observed in the graph, where there is the same negative trend in both commercial controls with r^2=0.18272

Table 2. Statistical values of the data obtained from the correlation made in the biological control between the thrips plague and its biological control *A. swirskii* from week 42 to week 2.

Biological control	Media	Mean Deviation	Standard Error
Trips (*Bemisia tabaci*)	4.77	2.17	2.01
Amblyseius swirskii	0.74	1.15	1.17

and in the biological control with r^2=0.2731 (Figure 2ab) from week 48, indicating that with less *A. swirskii* there is larger population of thrips. In week 43, which consisted of the second and last release, there is the presence of *A. swirskii* and decreasing the presence of thrips, but in the course of the following weeks it is observed that as there was no constant release of *A. swirskii*, the population of thrips had an upward trend, showing the highest presence in weeks 48 and 2, reaching up to 8 thrips on average per trap (Figure 2ab).

The thrips pest presented a positive correlation against its natural enemy *A. swirskii* in both biological control and commercial control treatments. Arthurs *et al*. (2009), in a greenhouse pepper crop, show how *A. swirskii* controlled the thrips infestation for a test period of 28

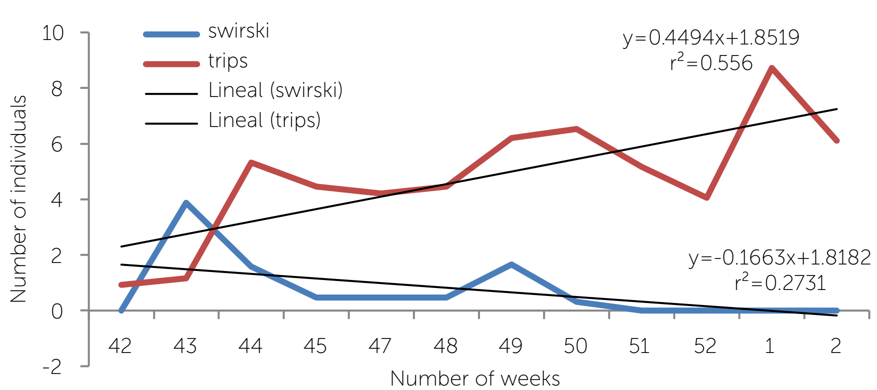

Figure 2a. Incidence of thrips and *A. Swirskii* in treatment under biological control of pests in cucumber cultivation.

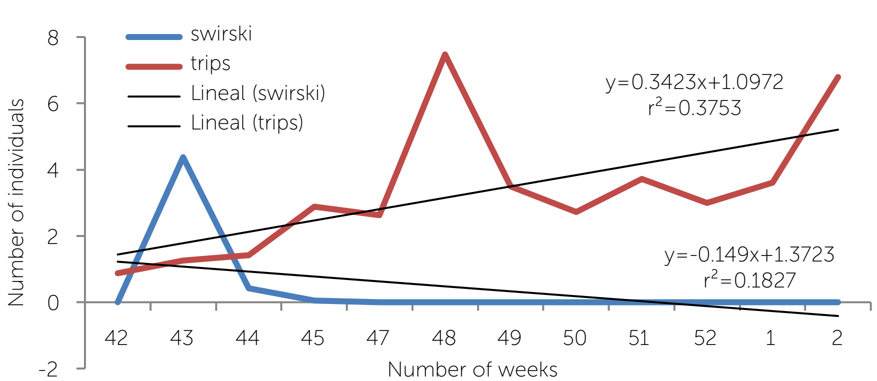

Figure 2b. Incidence of thrips and *A. Swirskii* in treatment under commercial control of pests in cucumber crops.

days. In this study, up to nine on average were found per trap in biological control and eight in commercial control; this increase could be due to the releases of the beneficial organism. A similar behavior was reported by Belda and Calvo (2006), where from week two after the release of *A. swirskii*, the thrips pest was kept under control with no more than an average of 1.8 thrips per leaf evaluated. On the other hand, *A. swirskii* reached 1.96 individuals per leaf on average using the same dose used in this thesis (100 individuals m^2).

CONCLUSIONS

Biological control is a favorable investment in the long term, which requires an initial expense to establish the beneficial insects, whereas chemical control is a constant investment that can generate the appearance of resistance and that requires new doses or products. The biological control program evaluated was just as efficient as the chemical control used in a conventional way. However, in biological control, a release of *A. swirskii* in a more continuous and distributed way is proposed during the weeks of cultivation, which will maintain more uniform and constant populations, to avoid the development of large populations of thrips and allow better control. It is necessary to evaluate both control programs during the spring-summer season to be able to compare their efficiency for each season and annually.

ACKNOWLEDGMENTS

To the National Council of Science and Technology in Mexico, for the scholarship for the Master in Science degree which was essential to carry out this work. To the Ganfer Company for the infrastructure and services provided to carry out the research.

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https://doi.org/10.32854/agrop.v13i10.1746

Edible, medicinal wild mushrooms: A study in Estado de México

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ABSTRACT

Objective: To identify edible mushroom species with medicinal uses in Estado de México.

Design/Methodology/Approach: Based on the bibliographic review of local ethnomycological inventories, weekly market visits, semi-structured interviews with vendors, and the collection and taxonomic identification of mushrooms, information was gained regarding medicinal mushroom nomenclature, local knowledge and cultural importance.

Results: A list of 36 edible mushroom species was compiled, which can be divided into 11 categories of use, mainly in treating digestive and circulatory illnesses.

Study Limitations/Implications: This is a descriptive and exploratory study of edible mushrooms with medicinal uses, and therefore it is necessary to include more empirical and scientific evidence from other similar cases.

Findings/Conclusions: There is limited knowledge on medicinal fungi species, implying that they are underexploited as a resource among local inhabitants. *Lycoperdon perlatum* and *Lactarius indigo* were cited as having the highest quantity of medicinal uses.

Key words: Traditional knowledge, ethnomycology, macrofungi, order of mention.

INTRODUCTION

It is estimated that between 70 % and 80 % of the population in developing countries depends on plants and mushrooms as an alternative for treating various diseases (Luitel *et al*., 2014). Mushrooms have been widely used in traditional oriental medicine, which has had a global reach (Yu-Cheng *et al*., 2009). Medicinal wild mushrooms are part of Mexico's biological and cultural diversity. Their use has been recorded since pre-Colombian times and is manifested in practices still preserved by some groups who consume mushrooms or use them for their stimulating, medicinal, and hallucinogenic properties (Herrera 1992; Ruan-Soto and Ordaz-Velázquez, 2016).

In recent years, more ethnomycological studies with biological, ecological or cultural aspects have been carried out in temperate and tropical regions. However, inventory work is still far from complete. From this stems the importance of recording the diversity of medicinal mushrooms (Aguirre-Acosta *et al*., 2014) and therapeutic treatments based on their use (Barros *et al*., 2008).

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 57-62.*

Recibido: *julio, 2020.* **Aceptado:** *septiembre, 2020.*

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Approximately 371 species of wild mushrooms are consumed in Mexico (Garibay-Orijel *et al.*, 2014) and 252 species in Estado de México (Burrola-Aguilar *et al.*, 2012). Regardless, their medicinal knowledge and use have been scarcely documented. Bautisa-González (2013) reports the use of 121 species in six localities in the country; Guzmán (2008) describes 73 species for treating 46 illnesses. Vázquez (2012) mentions 21 species in the Northern Sierra of Puebla; Jiménez *et al.*, (2013) highlight three species in Oaxaca. With respect to ethnomycological studies in the area, various contributions have been made to medicinal knowledge. In Estado de México, 16 species have been reported (Guzmán *et al.*, 2009), three species in Acambay, (Estrada-Torres and Aroche, 1987), and one species in Ocoyoacac (García, 2009). Facing this lack of information, it is important to document the traditional knowledge associated with local fungi resources, not only because they contribute biodiversity, but also because they are part of the country's culture (Heywood, 2011). Because of this, the objective was to identify species of edible mushrooms with medicinal uses in Estado de México as a contribution to regional inventories about their use, value, and protection, not only of the mushrooms themselves, but also of their habitat and of the culture where they are used.

MATERIALS AND METHODOLOGY

The information on edible mushrooms and their medicinal use was obtained from the following literature: Estrada-Torres and Aroche (1987); Nava and Valenzuela (1997); Juárez (1999); Mariaca *et al.* (2001); Arteaga and Moreno (2006); Pérez-Moreno *et al.* (2008); Estrata *et al.*, (2009); Frutis and Valenzuela (2009); García (2009); Guzmán *et al.* (2009); Franco *et al.* (2012); Bautista-Gonzales (2013); Lara-Vázquez *et al.* (2013); San Román (2014); Domínguez *et al.* (2015); and Jasso-Arriaga *et al.* (2016, 2019). Information regarding treated illnesses was organized according to the Manual of the International Statistical Classification of Disease, Injuries, and Death Causes (WHO, 2016).

To collect additional information on medicinal mushrooms in the central region of Estado de México and areas with high traditional use, visits were carried out to weekly markets in Amanalco, Villa Cuauhtémoc and Santa María del Monte during the months of August to October. During field visits, semi-structured individual interviews were conducted with vendors and collectors to gain information on nomenclature and local knowledge. The reported medicinal mushrooms were collected, dehydrated and characterized macroscopically and microscopically. Keys, specialized literature, and mycological guides were used for taxonomic identification. Order of mention was used to estimate the cultural importance of the mushrooms, obtained according to Moreno-Fuentes (2006).

RESULTS AND DISCUSSION

In all, 36 species of edible and medicinal wild mushrooms were registered, corresponding to 21 families (Table 1). These species represent 15.8% of the total reported for Estado de México (Burrola-Aguilar *et al.*, 2012) and can be considered functional foods (Ruan-Soto and Ordaz-Velázquez, 2016).

Eleven categories of use were identified, the most frequent being those related to diseases of the digestive tract, including signs and symptoms. Metabolic, dermatologic, inflammatory, and nervous system conditions were mentioned to a lesser degree. The mushroom species could have from one to eleven different uses. Eighty-three percent (83%) of species were used in more than one way, and from those, 52% were used for four or more purposes. *Lycoperdon perlatum* took first place in terms of medicinal uses (nine different illnesses), followed by *Lactarius indigo* with eight uses.

The more frequently treated conditions were related to the digestive tract (28 species). Twenty-seven (27) species were useful for treating fever, pain on different parts of the body, nausea, etc., associated with different diseases or ailments (signs-symptoms). Regarding genitourinary problems, 19 species were registered.

Various species described as medicinal in this study have also been reported in other research. Bautista-Gonzales and Herrera (2019) suggest that in Mexico, more than 350 fungi species are used in traditional medicine to treat 150 conditions of different systems of the body and ailments related to maternity, "the evil eye," and fright or shock.

In Santa María del Monte, 11 interviews were carried out, of which just one person used *Cantharellus subalbidius* (white chanterelles known as "calabacitas") to treat digestive problems. For this, the mushroom is grilled and eaten twice a day. In Villa Cuauhtémoc, of the 13 interviews conducted, five people mentioned

Table 1. Edible mushroom species with medicinal uses reported in the literature.

Family	Species	Medicinal use	Family	Species	Medicinal use
PHYLUM ASCOMYCOTA					
Pyronemataceae	*Aleuria aurantiaca*[F]	0	Morchellaceae	*Morchella esculenta*[A]	5
Helvellaceae	*Helvella crispa*[F]	1,3,5,8,9	Pyronemataceae	*Otidea onotica*[A]	0
	Helvella lacunosa[F]	1,3,5,8,9	Pezizaceae	*Peziza vesiculosa*[A]	0
PHYLUM BASIDIOMYCOTA					
Amanitaceae	*Amanita basii*[F]	1,5,9,11.	Hygrophoropsidaceae	*Hygrophoropsis aurantiaca*[F]	3,5,9
	Amanita tecomate[D,E]	11	Russulaceae	*Lactarius deliciosus*[E]	4,5,6,8,9,10
	Amanita vaginata[F]	1,5,9.		*Lactarius indigo*[B,E,F]	1,2,3,5,8,9,10,11
Diplocystaceae	*Astraeus hygrometricus*[E]	2,7		*Lactarius pubescens*[F]	4,5,6,8,9,10,11
Boletaceae	*Boletus aestivalis*[E]	1,4,5,6,8,9,10		*Lactarius salmonicolor*[E]	4,5,6,8,9,10,11
	Boletus edulis[E,F]	1,4,5,6,8,9,10		*Russula* sp.[F]	3,5,9
	Boletus pinophilus[E,F]	1,4,5,6,8,9,10	Lycoperdaceae	*Lycoperdon caudatum*[E,F]	2,3,5,8,9,10
	Leccinum rugosiceps[F]	5,8,9,10		*Lycoperdon perlatum*[B,E,F]	2,3,5,6,7,8,9,10,11
Agaricaceae	*Calvatia cyathiformis*[B,E,F]	2,5,9,11	Lyophyllaceae	*Lyophyllum* secc. *Difformia*[F]	3,5,9
	Macrolepiota procera[F]	3,5,9	Pleurotaceae	*Pleurotus opuntiae*[E]	4,9,10
Cantharellaceae	*Cantharellus cibarius*[F]	3,5,8,9,10		*Pleurotus smithii*[F]	4,9,11
Gomphidiaceae	*Chroogomphus rutilus*[E]	3,5,8,9,10	Gomphaceae	*Ramaria* sp.[E,F]	5,9
Tricholomateceae	*Infundibulicybe gibba*[E,F]	1,3,4,5,8,9,10	Suillaceae	*Suillus granulatus*[F]	5,10
	Gymnopus sp.[C]	0		*Suillus luteus*[F]	5,11
Geastraceae	*Geastrum triplex*[E]	2,3,6,10			
	Geastrum triplex[E,F]	2,3,6,10			

Source: [A]Chio *et al*., 1988; [B]Estrada-Torres and Aroche, 1987; [C]Villarruel-Ordaz *et al*., 1993; [D]García, 2009; [E]Guzmán *et al*., 2009; [F]Bautista-Gonzales, 2013.

Medicinal use: 0. Undefined; 1. Musculoskeletal; 2. Skin; 3. Respiratory; 4. Metabolic; 5. Symptoms; 6. Culture-bound syndrome; 7. Nervous; 8. Urinary; 9. Digestive; 10. Circulatory; and 11. Anti-inflammatory.

using *Amanita* sp. and *A. novinupta* ("tecomates") to treat renal problems: "The mushroom is boiled and the cooking water is drunk at room temperature". *Boletus edulis*, known as "cemita", is used to alleviate digestive problems, chiefly in children with upset stomachs; it is prepared by dehydrating and then grinding it, then "the powder is taken with one teaspoon of olive oil, twice a day." It is also used to reduce cholesterol, whether grilled or sautéed, boiled with epazote, tomato, onion, and chili pepper (green or dried). In Amanalco, 14 interviews were conducted and only two people reported the medicinal use of "tecomate" (*Amanita* sp. and *A. novinupta*), but did not specify preparation. In general, the interview respondents indicated that they have heard of the medicinal uses of mushrooms, but did not know them, as was reported by Lara-Velázquez *et al*. (2013).

The potential of the mushrooms studied can be deduced from research showing their high content in B2, B3, and D vitamins, iron, fiber, iodine, potassium, and water. Their consumption may be beneficial for healthy skin, hair, nails, bones, teeth, eyesight, as well as the nervous and digestive systems (Cano-Estrada and Romero-Bautista, 2016). Their medicinal value includes anticancer, antibiotic, antioxidant, antithrombotic, and antidiabetic properties, as well as cholesterol and hypertension-reducing properties (Chang and Miles, 2004).

In that regard, the use of Gasteromycetes stands out for treating skin conditions (Guzmán, 2008; Bautista-Gonzales, 2013; Bautizta-Gonzalles and Herrera, 2019). Boletes are used for conditions of the skeletomuscular system, such as rheumatic diseases or pain in the tendons of extremities and fingers (Guzmán, 1994; 2008; Bautista-Gonzales, 2013). *Boletus edulis*, *B. aestivalis* and *B. pinophilus* are used for headache, rheumatism, and as purgative (Guzmán, 2008; Bautista-González, 2013). *Lactarius deliciosus* demonstrates antimicrobial activity (Barros *et al*., 2007).

Mushrooms of the genus *Amanita*, mentioned both in interviews and in bibliographic searches and considered favorites of traditional gastronomy (Romero-Bautista, 2016), possess anti-inflammatory properties (Guzmán, 1994; 2008; Bautista-González, 2013). *Cantharellus subalbidius* is used for stomach ailments. Bautista-González (2013) reports that *C. cibarius* is used for treating gastrointestinal disorders, headache, and fever, which agrees with the information obtained, in addition to possessing antitumor, antioxidant, and antibacterial properties (Barros *et al*., 2008; Queiros *et al*., 2009; Yu-Cheng *et al*., 2009).

With regards to traditional mycological knowledge in communities, learning occurs at an early age. Eighty percent of interviewees learn about mushrooms between the ages of 5 and 13, during which time the children acquire identification skills and general knowledge, although adults can still gain knowledge. Collecting season is from May to October. The interview respondents indicated that the mushrooms are harvested directly and are consumed once a week (21%), two to three times per week (58 %), or more than three times per week (21%). The mushrooms are prepared by sautéing (66%), in stews (24%), and eaten raw (10%). For preservation, *Helvella* spp. *Infundibulicybe* spp. and *Morchella* spp. are strung together.

The harvesting process varies according to localities. In Amanalco and Santa María del Monte, women are in charge of this process (65 and 60%, respectively). Meanwhile, in Villa Cuauhtémoc, men make up the majority who carry out this activity (55 %). The collectors directly commercialize the mushrooms, and in general, women are in charge of sales (80%) and are the knowledge-bearers, having learned from their parents (67%), grandparents (31%), and aunts or uncles or other relatives (2%). As Jasso-Arriago *et al*. (2019) mention, culinary knowledge is shared by older women during commercialization. Income is used to cover food costs or to buy objects of personal use (Estrada-Flores *et al*., 2019). Order of mention (OM) was used to estimate cultural importance and to identify the most relevant mushrooms for the population (Table 2). For Villa Cuauhtémoc, "enchilado" mushrooms had the highest value with 40.12%, while in Amanalco, the white "gachupines" (31.47%), and in

Table 2. Traditional names with greatest cultural importance in study sites.

	Villa Cuauhtémoc		Amanalco		Santa María del Monte	
	Common name	Order of mention	Common name	Order of mention	Common name	Order of mention
1	Enchilado (spicy)	40.12	Gachupín blanco (white gachupin)	31.87	Pata de pájaro (bird foot)	20.48
2	Mazorca (corncob)	31.95	Gachupín negro (black gachupin)	30.62	Oreja (ear)	16.74
3	Mantecado café (brown buttery)	30.35	Tecomate amarillo (yellow tecomate)	27.72	Enchilado (spicy)	15.03
4	Oreja (ear)	28.96	Trompetas (cornet)	26.25	Clavo (nail)	14.82
5	Mantecado amarillo (yellow buttery)	28.33	Mantecados (buttery)	23.16	Señorita (miss)	14.44
6	Amargo (bitter)	26.75	Cemas	22.63	Pancitas (belly)	12.96
7	Cemita	22.91	Patitas de pájaro (bird foot)	22.5	Tecomate amarillo (White tecomate)	11.94
8	Tejamanil	18.1	Clavitos (nail)	22.08	Corneta (cornet)	6.6
9	Clavo (nail)	17.46	Orejas ears)	21.25	Bolita (small ball)	6.26
10	Cornetita (cornet)	6.86	Elotitos (corncob)	20.85	Elote (corn cob)	3.67
11	Soldadito (little soldier)	5.62	Ternerita	18.85	Xocoyol	3.52
12	Gachupín	4.7	Bombón (marshmallow)	18.7	Güila	3.02
13	Pata de pájaro (bird foot)	4.37	Xocoyol	18.05	Escobeta (broom)	2.09
14	Escobeta (broom)	3.1	Señorita (miss)	17.55	Trompeta (cornet)	2.06
15	Oyamel (sacred fir)	1.58	Enchilado (spicy)	15	Gachupín güero (blond gachupin)	2.01
16	Queta	1.2	Amarillo (yellow)	14.02	Ternerita	1.34
17	Duraznillo	0.69	Enchilado azul (blue spicy)	7.11	Gachupín negro (black gachupin)	1.18
18	Negrito (black)	0.44	Oreja blanca (white ear)	4.93	Tejamanil	1.1
19	Pajarito (birdie)	0.28			Colita de ratón (mouse tail)	0.69
20	Sopita (soup)	0.26				

Santa María del Monte, the "patas de pájaro" (20.48%). The cultural importance of mushrooms is associated with their nutritional importance, and those who are knowledgeable describe them as "natural meat" with a flavor similar to chicken, fish, or frog, depending on the species (Jasso-Arriaga *et al*., 2019).

Despite the high diversity of edible wild mushrooms in Estado de México, this resource is not used by local residents for medicinal purposes. This is evidenced by the low number of species mentioned in the interviews, which is consistent with other localities. Ruan-Soto *et al*. (2009) found that the Lacandon people in Chiapas only use one medicinal species; or in Oaxaca, Garibay-Orijel *et al*. (2006) mentioned that the Zapotec do not know any medicinal uses for mushrooms.

CONCLUSIONS

There is limited knowledge concerning the number of fungi species with medicinal uses in Estado de México, implying that this resource is under-exploited by its inhabitants. The 36 medicinal species that are used represent an alternative for treating problems related to the digestive tract, the circulatory system, and signs and symptoms; all highly prevalent diseases and some with grave health consequences and high medical treatment costs. Unfortunately, knowledge of medicinal properties in mushrooms is being lost with the passing of generations, therefore additional studies are needed to supply more information on the value of species of medicinal interest, in addition to phytochemical and pharmacological analyses to insure their efficiency and innocuity.

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AGRO PRODUCTIVIDAD

https://doi.org/10.32854/agrop.v13i10.1739

Collective efficiency of the soy cluster (*Glycine max* L.): Tamaulipas Case, Mexico

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ABSTRACT

Objective: To analyze the collective efficiency (CE) of the soy cluster (*Glycine max* L.) in the southern region of Tamaulipas, Mexico.

Design/methodology/approach: The Localization Coefficients and Gini indexes were calculated for the soy sector in the state, based on information obtained from SIAP (2018). The Collective Efficiency Index was obtained from the soy cluster, through the application of a questionnaire to producers in the region for the measurement of external economies and joint actions in the agglomeration.

Results: The indicators allowed identifying a high concentration of the soy sector in the southern region of Tamaulipas, in addition to the participation of a high proportion of the municipalities in production, production value, surface sown, and surface harvested. It was identified that the producers in the soy conglomerate have achieved a middle level of CE, with a higher presence of Joint Actions (JAs) than External Economies (EEs).

Study limitations/Implications: It is a transversal study, because the CE was only studied in the soy cluster during a specific period.

Findings/Conclusions: Although the soy cluster in the region evaluated offers its members various benefits, the maximum development of their CE has still not been attained, which is why there is still a need to continue strengthening the sector by the state government, through programs that promote individual and collective development of soy producers and organizations related with the sector located in the region.

Keywords: agriculture, collaboration, business agglomeration, collective efficiency.

INTRODUCTION

Presently, soy (*Glycine max* L.) is considered as the most important oleaginous plant worldwide, having as main producers the United States, Brazil, Argentina and China (CIMA, 2019). This crop is recognized for its high nutritional value and can be consumed directly or in presentations such as flour or vegetable oil, in addition to being used in the elaboration of cosmetics, soaps and biofuels (WWF, 2014). According to the Ministry of Agriculture and Rural Development (2019), although in Mexico 334,011 tons of this crop were produced in 2018, imports had to be carried out to satisfy the domestic demand of the crop by importing 5,230,000 tons, so it ranked as the third largest importer of soy in the world.

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 63-68.*
Recibido: *junio, 2020.* **Aceptado:** *septiembre, 2020.*

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At the national level, Tamaulipas has historically been leader in soy production, although, according to SIAP (2018), the state produced 78,915.62 tons of this crop and its production value reached $528,680,392.90, exceeded by Campeche which obtained $617,500,433.00 from the sale of 86,934.06 tons. At the state level, the municipalities with higher participation in the soy subsector are González, Altamira, Aldama and El Mante, located in the southern region of the state, which as a whole concentrate 86.96% of the total production of the crop (SIAP, 2018). Although the soy subsector in the state has been a study object in various studies (Maldonado, Ascencio and García, 2017; García *et al*., 2018), little has been analyzed about the collective efficiency with which the oleaginous-production businesses operate in the region and the advantages that have been achieved because of their location. The objective was to analyze the CE of the soy cluster in the southern region of Tamaulipas, given that this concept explains the benefits obtained by the members of a business agglomeration, whether they are sought or not (Di Tommaso, 1999; Giuliani, Pietrobelli, and Rabellotti, 2005). In this regard, according to Schmitz (1997), the CE encompasses two important dimensions, external economies (EEs) and joint actions (JAs). The EEs refer to the unsought advantages by the cluster members which anyhow exist in the region and which the companies can appropriate. In turn, the JAs are related to benefits derived from the interrelation between the different agents of business agglomeration, such as those carried out with other producers, with clients, with suppliers, and with organizations related to the sector (Di Tommaso, 1999).

MATERIALS AND METHODS

To identify the cluster and measure the collective efficiency in the southern region of the state of Tamaulipas, Mexico, three indicators were used: coefficient of localization (CL), Gini index (Ig) and Collective Efficiency Index (CEI).

Coefficient of Localization. This indicator provides information regarding the degree of productive specialization that a territory has in the development of a sector or an industry (Kopczeska, Churski, Ochojski and Polko, 2017), and it is determined in the following way:

$$CL_{ij} = \frac{X_{ij} / X_j}{X_{in} / X_n}$$

Where: CL_{ij} = coefficient of localization of the activity i sector in the region J, X_{ij} = production of the sector of activity i in the region j, X_j = total production of the region j (state, region, municipality), X_{in} = production of the sector of activity i in all of the regions (national), and X_n = total production in all of the regions (national).

It is considered that if $CL_{ij}>1$ it means that there is privileged localization of the sector i in the geographic area j. With higher CL_{ij} there is higher concentration of the sector. If $CL_{ij}<1$ there is a lower localization of the sector i studied in the geographic area j. When CL_{ij} = 1 the regional participation of sector i is the same as the national participation (Kopczeska *et al*., 2017).

Gini index. The *Ig* measures the degree in which a distribution function is distanced from the uniform distribution function of such a variable, and for its determination the difference between two types of distributions *Pi* and *Qi* is divided by the total distributions of *Pi*, with the following formula:

$$Ig = \sum_{i=1}^{k}{}^{1}(Pi - Qi) / \sum_{i=1}^{K}{}^{1}Pi$$

$$Ig = \frac{\sum (Pi - Qi)}{\sum Pi}$$

Where *Ig* denotes the Gini index and *Pi* refers to the frequencies accumulated of the data of one distribution divided by the total observations times 100, which is expressed as:

$$Pi = \frac{Ni}{m} * 100$$

Where *m* corresponds to the total frequencies accumulated. *Qi* is equal to the frequencies accumulated of the distribution data divided by the total observations times 100, represented as:

$$Qi = \frac{Hi}{H} * 100$$

Where *H* is equal to the total frequencies accumulated from the data. Based on Asuad (2001), if *Ig* is close to zero, then the concentration is nearly non-existent and, on the other hand, if *Ig* is close to 1 there is high concentration.

Collective Efficiency Index. A questionnaire was elaborated to calculate the CE index, based on the model proposed by Giuliani *et al*. (2005). The instrument used included 56 items; the first 28 gathered the elements of the EEs, regarding the specialized market of the workforce, specialized market of inputs, access to information and access to markets. The next 28 items referred to the JAs, for example the relationship with suppliers, relationship with clients, relationship with producers and multilateral relationships (Table 3). For the calculation of CEI the sum of 0.5 of the results at the level of EEs plus 0.5 of the degree of JAs is considered. In this sense the CEI formula is represented as

$$CEI = (0.5 * IEE) + (0.5 * IJA)$$

For the interpretation of this indicator, three levels of CE are established based on Giuliani *et al*. (2005): ≥9.5 = High; 5.1>Medium<9.5; and ≤5 Low.

RESULTS AND DISCUSSION

Comparatives between coefficients of localization of the soy cluster

For this study, the CL was calculated by considering the production variables, production value, surface sown, and surface harvested from the soy sector in the southern region of Tamaulipas, made up of the municipalities of Aldama, Altamira, El Mante and González, based on statistical information obtained from SIAP (2018). Table 1 shows the CL for the production variable (tons) which obtained a value of 67.4005, the highest coefficient of the four variables. This result is derived from the fact that the in zone studied the soy production represented 3.73% of the total production, much higher proportion than what this same activity represented at the national level with 0.05%. The production value (thousands of pesos) variable had the second highest CL reaching 40.2489, due primarily to the volume of the crop produced in the region.

Table 1. Coefficients of Localization for the soy cluster in Tamaulipas, Mexico.

Variable	LC
Production	67.4005
Production value	40.2489
Sown area	32.7156
Harvested area	33.3070

Source: own elaboration with data from the Agri-Food and Fisheries Information Service (SIAP, 2018).

Table 2. Gini indexes for the soy cluster in Tamaulipas.

Variable	GI
Production	0.1888632
Production value	0.1888614
Sown area	0.1924682
Harvested area	0.1924695

Source: own elaboration with data from the Agri-Food and Fisheries Information Service (SIAP, 2018).

In the case of the sown and harvested surface, their CLs were lower than the first two variables; this shows that the rest of the states in the country have greater participation in terms of the surface devoted to this crop. However, the southern region of Tamaulipas has achieved higher efficiency in the sector that is reflected in greater production and profits from its sale.

The CLs calculated for the soy sector in the southern region of Tamaulipas showed higher values than those obtained by other studies (Vidal and Pezoa, 2012) for outstanding sectors and industries at the national and international level. According to Kopczeska *et al*. (2017), when CLs higher than 1.25 were found in a region, it can be considered a potential exporter; however, this differs from the soy sector in Tamaulipas because according to the state's Ministry of Rural Development (2017), in recent years imports have been made to supply local consumption, importing up to three million tons of soy from the United States annually.

Comparative between Gini indexes of the soy cluster

The Gini indexes (*Ig*) were determined for the soy cluster in Tamaulipas, taking into consideration the production variables, production value, surface sown and surface harvested, based on data obtained from SIAP (2018). Table 2 shows that the highest *Ig* of the soy sector was the one that corresponded to the surface harvested with 0.1924695. This value was close to zero, which indicates that an important number of municipalities of the state in addition to devoting hectares to growing the crop also harvest it. In this regard, according to figures from SIAP, 19 municipalities presented a harvested soy surface in 2018; however, it stands out that González, Aldama, Altamira and El Mante covered 55,406 ha of the 62,910.32 ha harvested at the state level, which represented 87.56%.

The variable with the lowest *Ig* was the production value with an index of 0.1888614; however, a noticeable difference was not found with the highest *Ig*. In this sense, according to SIAP, in 2018 the value of soy production in Tamaulipas was distributed among 44.18% of the municipalities; however, it is highlighted that the study region concentrated $454,505,465.60 (85.97 %) of the $528,680,392.90 that this activity contributes to the state economy.

The variables of production and surface harvested attained *Ig* of 0.1888632 and 0.1924682, respectively, showing a similar behavior in their distributions. In both variables a high participation of the municipalities was found in the distributions, but this highlighted their concentration in a small group of municipalities, mainly those belonging to the southern region of the state. The *Ig* calculated for the soy sector in Tamaulipas showed weighting below those obtained by Sobrino (2016), for the important sectors in the country such as hide and leather (0.634) and the basic metallurgic (0.408). This is a result from the fact that although a high percentage of municipalities in the state are devoted to this economic activity, they do it at a small scale, and therefore its participation in it is relatively low.

Calculation of the Collective Efficiency Index of the cluster

For the analysis of the CEI of the soy subsector, the model proposed by Giulani *et al*. (2005) was used, and for this purpose a sample of ten companies from the oleaginous-production sector in the region were surveyed. In this regard, the CEI of the soy subsector cluster was 7.35 (Table 3), and a greater presence was identified of the JAs (7.53) than of the EEs (7.17).

In the dimension of the EEs, access to specialized workforce was the component with the highest index (2.05), with it being the one that offers the highest benefit to the cluster producers, such as the identification of qualified staff in the sector (2.22) and the ease to hire human resources in the region (2.16). In contrast, the access to markets was the component that presented the lowest weighting (1.48), because the cost reduction of equipment and raw materials (1.08) and the access to new local, regional and national markets (1.14) were identified as the least exploited elements by soy producers in the region.

The other two components: access to prime materials (1.66) and to technical knowledge (2.16), obtained intermediate scores. The results from the IEE reflect that the producers from the soy subsector have managed to appropriate the benefits that the business agglomeration offers, although they perceive that their localization has still not translated into great benefits referring to access to new markets, as well as acquisition of machinery and prime materials.

In terms of the dimension of JA, the greatest advantage perceived by oleaginous-production businesses in the cluster was associated with the component of relationship with the clients (1.94), highlighting that consumers have a high valuation of the entirety of regional products that the oleaginous subsector offers in the region (2.16). The least weighted segment of the JA was that of relationship with other producers (1.82), which can be derived from the mistrust to collaborate with other companies considered rivals in the market. The components of relationship with suppliers and bilateral and multilateral actions obtained scores of 1.88 and 1.89, respectively. The IJA value (7.53) denotes that the participants of the soy cluster have managed for promotion of work with the rest of the agents of the agglomeration to be articulated as an important source of advantages in the sector.

The mean value of CEI for the cluster of the soy sector in Tamaulipas reflects that the EEs and JAs have not been consolidated; however, the index is above the one obtained for other agglomerations (Pietrobelli and Rabelloti, 2005), which indicates that the oleaginous-production businesses in the region have effectively achieved benefits because of their localization, which they probably would not have reached in an isolated way.

CONCLUSIONS

The existence of the soy cluster was identified in the southern region of Tamaulipas, and its CE level was defined. The existence of a high concentration of the soy sector could be observed in the zone comprised by González, Altamira, Aldama and El Mante, which indicates that this activity has greater participation in the region's economy compared to other states. It was identified that a high percentage of the municipalities have participation in the production, production value, surface sown and surface harvested. However, all the variables are concentrated mainly in the municipalities of the study region. Although it was identified that the soy cluster offers benefits to its participants, highlighting the predominance of the JAs over the EEs, the maximum development of its potential EEs and JAs has still not been achieved.

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Table 3. Collective Efficiency Index of the soy cluster in Tamaulipas.

Component	Concept	Average	Index
External economies			
Access to skilled labor	Skilled labor	2.22	2.05
	High experience	1.86	
	Hiring local staff|	2.16	
	Local education	1.98	
	Attendance at training courses in institutions in the region	2.1	
	Training by regional suppliers	1.68	
	Shared work experiences	1.92	
	Learning through formal and informal networks	2.16	
	Learning collaborations	2.22	
	Learning through coexistence	2.16	
Access to raw materials	Outsourcing of activities	1.56	1.66
	Second hand machinery and equipment	1.92	
	Market of inputs and intermediate inputs	1.92	
	Market of inputs and specialized inputs	1.86	
	Reduction of input costs	1.38	
	Outsourcing of complementary activities	1.32	
Access to technical knowledge	New companies from former workers	1.56	1.99
	Transmission of information and knowledge	2.1	
	Technical information	2.04	
	Generation of innovation for competitiveness	2.16	
	Formal and informal channels	1.92	
	Collaboration between companies	1.98	
	Conversion of ideas	2.16	
Access to new markets	Access to new clients	2.1	1.48
	Ease of access to markets	1.14	
	Low transportation costs	1.08	
	Adequate regional infrastructure	1.38	
	Support and auxiliary companies	1.68	
EXTERNAL ECONOMIES INDEX			7.17
JOINT ACTIONS			
Vertical actions with suppliers	Bargaining power of suppliers	1.32	1.88
	Competition for the acquisition of inputs and resources	1.62	
	Formal relationship with suppliers	2.28	
	Inventory management	2.16	
	Reciprocity with suppliers	2.04	
Vertical actions with clients	Bargaining power of customers	1.86	1.94
	Bargaining power company-client	2.1	
	Contracts with clients	2.04	
	High reputation for regional products	2.22	
	Company reputation	1.5	
Horizontal actions with producers	Collaboration agreements and stable alliances	1.8	1.82
	"Enemy" companies	1.14	
	Marketing and sales relationships	1.98	
	Relationships in high demand markets	2.04	
	Share production costs	1.68	
	Share management and administration costs	1.68	
	Dispute and conflict resolution	2.16	
	Mutual understanding between companies	1.86	
	Joint participation in fairs and exhibitions	2.04	
Bilateral and multilateral actions	Programs to promote and help SMEs	1.2	1.89
	Institutions that provide information on resources	2.1	
	Institutions that provide information on customers and markets	2.04	
	Importance of regional institutions	2.28	
	Research links	2.1	
	Assistance and training by institutions	1.62	
	Promotion of regional institutions	1.38	
	Participation in business and professional associations	2.16	
	Collaboration between institutions	2.1	
Joint action index			7.53
Collective efficiency index			7.35

Source: prepared by authors with field information.

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AP AGRO PRODUCTIVIDAD

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Productive performance and egg physical characteristics of Tufted Creole and Marans hens

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ABSTRACT

Objective: The present study was conducted to evaluate the productive performance and the egg physical characteristics of two backyard-type breeds of birds.

Design/methodology/approach: Tufted Creole (13 hens and a rooster) and Marans (13 hens and a rooster) hens were used in this study. Live body weight, feed intake, egg production, egg weight, egg length and egg width were measured weekly for eight weeks. Collected data were analyzed using a two-way variance analysis; the main factors were breed, week, and their interaction.

Results: Marans hens were heavier and had higher feed intake than Tufted Creole (TCH) ($P<0.05$). The TCH egg was smaller ($P<0.05$) in weight and length with respect to that of Marans hens. TCH produced more eggs than Marans hens ($P<0.05$). The week factor was significant ($P<0.05$) for body weight, feed intake and egg length.

Study limitations/implications: Further studies should be carried out to design a feeding program that would allow both breeds to express their full productive potential and maximize the return on investment in backyard production systems in Mexico.

Findings/conclusions: Marans hens are a heavier breed due to their greater live body weight and feed intake with respect to TCH. TCH are lighter birds but with a higher egg production when compared to Marans.

Keywords: Body weight, egg production, feed intake, poultry, backyard

INTRODUCTION

Backyard poultry farming is an important activity in rural communities of Mexico (Gutiérrez-Triay *et al*., 2017). This activity strengthens the welfare of low-income families, as it provides protein of animal origin and an extra income (Oladunni and Fatuase, 2014). Creole hens (*Gallus gallus domesticus*) are the type of birds that predominate in backyard poultry farming; however, they are being displaced by commercial lines which are genetically selected for high egg production

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 69-73.*
Recibido: *mayo, 2020.* **Aceptado:** *septiembre, 2020.*

Imagen de Varintorn Kantawong en Pixabay but are not well adapted to the outdoor environment and traditional management of typical backyard production systems (Rodríguez- Ortega *et al*., 2019). Some Creole hens have a tuft of feathers on their heads, which is an attractive phenotypic characteristic of these birds for poultry farmers. The shape, color and position of the feathers are important aspects that distinguish different breeds of chickens (Wang *et al*., 2012). Marans hens originated in the city of Marans, France; the most attractive feature of this breed is the production of eggs with dark brown egg shells which appear to be more appealing for consumers. These hens may have black, copper-black, silver, white plumage, and tarsus with or without feathers (Lukanov *et al*., 2015). Despite the desirable rustic characteristics of Tufted Creole and Marans hens, there is very limited information available regarding flock management, performance and egg characteristics. The egg weight, color, length and width are important physical characteristics that describe the breed of hens, age and embryo survival (Narushin and Romanov, 2002; Ikegwu *et al*., 2016). Shell color is an important factor for consumers: a consistent shell color guarantees the purchase of the product. The brown color in the egg is caused by the Protoporphyrin IX pigment (Wilson, 2017), and the deposits of the protoporphyrin-IX are mainly located in the outer epithelial cells within the shell gland (Poole, 1967). The different breeds of hens secrete and deposit pigment at different times giving the egg shells their typical color such as blue, brown or white (Liu *et al*., 2009). Tufted Creole hens as well as Marans are an important genetic resource for the backyard poultry industry in Mexico. Thus, the objective of the this study was to evaluate the productive performance and egg physical characteristics of Tufted Creole and Marans hens in a typical Mexican backyard production system.

MATERIALS AND METHODS

Housing and management. The experiment was carried out at the poultry facilities of the Polytechnic University Francisco I. Madero (UPFIM), located in the state of Hidalgo, Mexico, at approximately 1900 m above sea level. The animals were housed in 6 × 4 m pens, roofed and with dirt/sand floor.

A commercial feed estimated to meet or exceed the nutritional requirements of the birds according to the Poultry NRC (1994) was offered *ad libitum*. Water was offered through plastic bucket-type drinkers. Care to the birds was provided throughout the experiment following the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

Breeds. Two breeds of birds were used in the study: Tufted Creole Hens (TCH), 13 hens and a rooster; and Marans (M), 13 hens and a rooster (Figure 1). These birds were obtained from backyard poultry farming in the Valle del Mezquital, Hidalgo, Mexico. At the time of the experiment, all birds were 60 weeks old.

Figure 1. TCH, Tufted Creole Hens; M, Marans hens.

Data collection. The live body weight (g) of the hens was recorded weekly for all birds. Average feed intake per pen (g), egg production, egg weight (g), egg length (mm) and egg width (mm) were measured on a daily basis for eight weeks.

Statistical analysis. Collected data were subjected to two-way ANOVA using a completely randomized design with the Mixed procedure by SAS v 9.0 (SAS, 2011). The main factors were breed, week and their interaction (breed*week). Significant effects were accepted at $P<0.05$. For live body weight, each individual bird was the experimental unit. While average of sampling per day was the experimental unit for feed intake, egg weight, egg width and egg length. The total number of eggs produced was analyzed using the PROC FREC and PROC GLM procedure of SAS v 9.0 (2011, SAS Institute, Cary, NC).

RESULTS

The eggs of backyard hen breeds evaluated in this study had different shell color (Figure 2); Marans hen

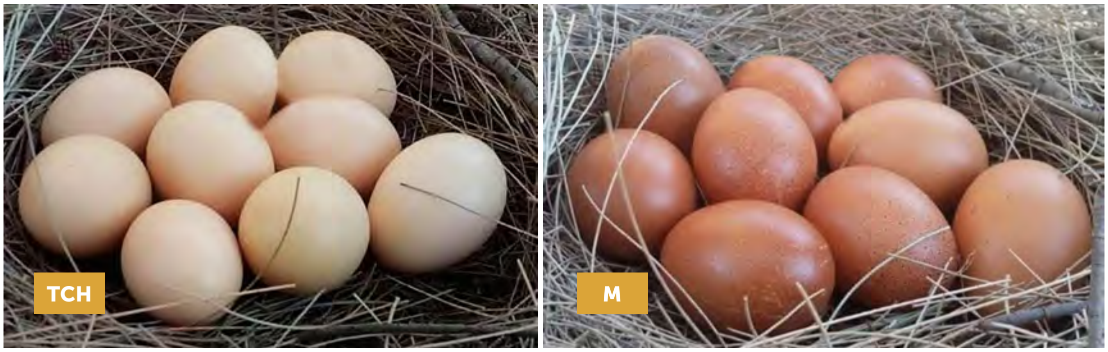

Figure 2. TCH, Tufted Creole Hens; M, Marans hens. Egg color. TCH: beige color egg of Tufted Creole hens, M: egg with shells dark brown color of Marans hens.

eggs were dark brown while Tufted Creole hens laid beige colored eggs.

Overall weekly body weight, feed consumption and egg length of both breeds of hens increased throughout the 8-week trial period. The interaction breed*week for egg length was significant ($P<0.05$) (Table 1). Marans hens were heavier ($P<0.05$) than TCH. Additionally, Marans hens had higher ($P<0.05$) feed intake and egg weight when compared to TCH. For egg production results, TCH laid ($P<0.05$) more eggs than Marans hens. The egg width was not different ($P>0.05$) between breeds and weeks. Egg length was different ($P<0.05$) between breeds; Marans hens had longer eggs compared to TCH (Figure 3).

The color of the shell is highly appreciated by the consumer, which translates into a possible price increase and therefore a higher income for families. Protoporphyrin-IX is the pigment responsible for the brown color of the shell (Samiullah and Roberts, 2013). The intensity of the brown color is determined by the amount of protoporphyrin-IX released in the shell gland (Liu and Cheng, 2010); shell color is a specific characteristic of the genetic variability of laying hens.

The higher body weight of the Marans hens could be due to the fact that these hens were developed to provide meat and eggs; this type of birds is known as dual-purpose. On the other hand, TCH are lighter birds, with slower growth and developed for higher egg production. The Padovanas hens have a feather tuft on the head and a cranial protuberance that increases the size of their tuft (Rizzi, 2018); these birds are light-sized, similar to the TCH hens. De Marchi *et al*. (2005) observed that the Padovana hens had an adult weight of 1328 g, which is similar to the weights registered for the TCH hens in this study. The Marans hens of this study had an average live body weight (2279 g) similar to Rhode Island birds, also considered for dual-purpose. These results are in agreement with Mohammed *et al*. (2013), who reported that Rhode Island Red hens in outdoor production had an average live body weight of 2196 g.

Feed intake results are correlated with body weight. Tufted Creole hens are smaller and had low feed intake. In this study, the TCH had similar feed intake as the Padovana hens used by Tasoniero *et al*. (2018) who observed that Padovana hens had an average feed intake of 72.7 g/d. Feeding management of both breeds of hens was *ad libitum*, which is why both breeds may have increased their live body weight throughout the 8-week trial period. It is important to note that birds were already at their mature live weight (60 weeks old) at the beginning of the experiment.

In the literature, there is very limited information regarding egg production of TCH and Marans hens. The results of this study suggest that the TCH breed is more appropriate for producers whose main focus is total number of eggs produced per hen. The egg weight of Marans hens was similar to the Rhode Island Red and

Table 1. Live weight, feed intake and egg length per week[¥].

Week	Live weight (g)	Feed intake (g)/bird/day	Egg length (mm)
1	2010	126	53
2	2021	135	55
3	2045	137	56
4	2085	138	55
5	2095	137	56
6	2132	141	57
7	2133	141	56
8	2139	146	57
Standard error	56	2	1
Significance	0.041	<0.001	<0.01

[¥]Average body weight, feed intake and egg length of both breeds, Tufted Creole hens and Marans hens.

New Hampshire hens (57.8, 58.3 *vs*. 59 g) as reported by De Witt and Schwalbach (2004). However, the egg weight of 76-week-old ISA Brown hens was higher than Marans and TCH hens (57, 59 *vs*. 62.67 g) as reported by Abou-Elezz *et al*. (2011). Verhoeven *et al*. (2019) reported that egg size is related to body size, and they also observed that the size increases with the age of the birds. In this experiment both breeds were 60 weeks old, however, the TCH were lighter than the Marans, thus, the egg weight was lower. Hanusová *et al*. (2015) reported that the egg width of Rhode Island Red hens was 42 mm, similar to the egg width of Marans and TCH in this study (43, 42 ± 0.13 mm). Ávila (2015) observed that the size of the bird's egg varies fundamentally in relation to the body mass of the adult female and her growth development. The interaction between breed and week may be due to a normal physiological process in birds: as females age, the size and weight of the egg increases (Väisänen *et al*., 1972). The size of the egg in backyard poultry production is a very important characteristic, related to the consumer's preference and the survival of the progeny. Williams (1994) reported that egg size is related to the live weight at birth of the offspring. However, the survival and growth of the chicken is independent of the size of the egg.

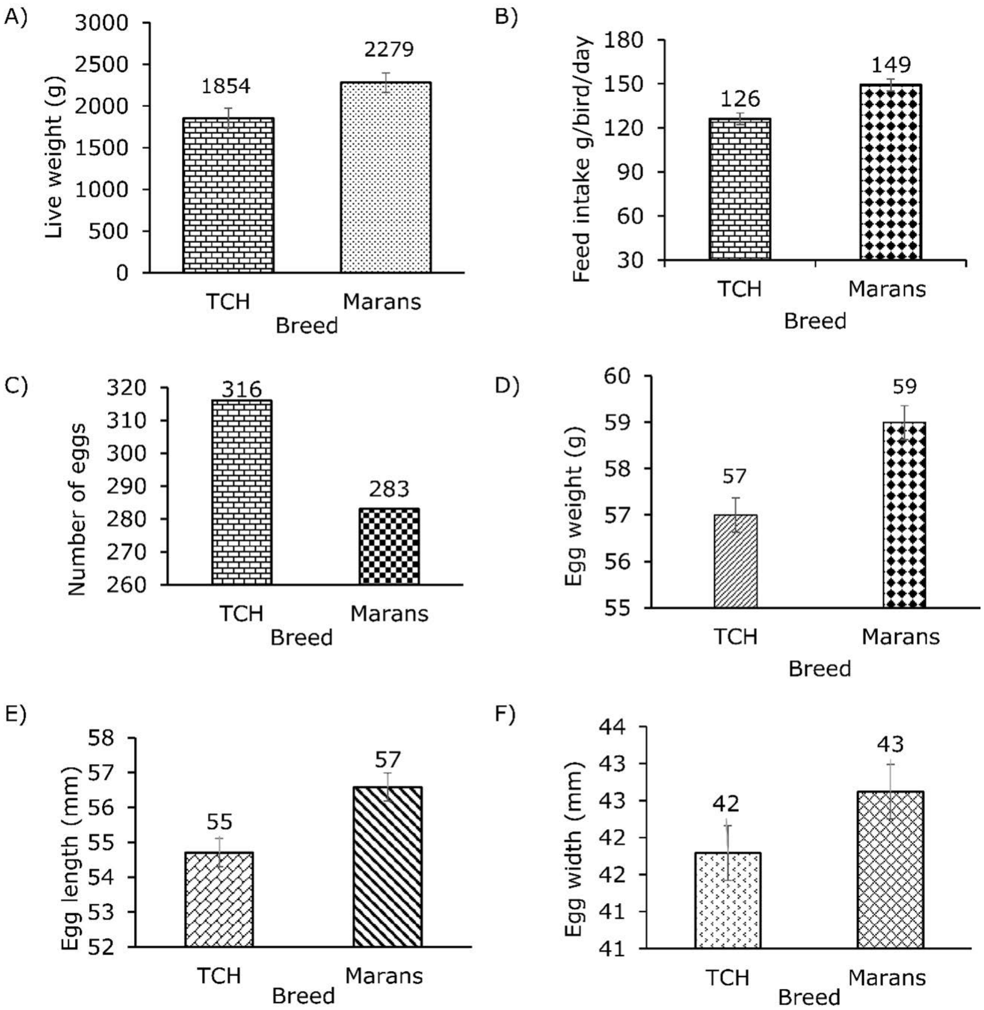

Figure 3. TCH, Tufted Creole Hens; M, Marans hens. A) Live weight of TCH and M hens; B) Feed intake by breed; C) Number of eggs per breed; D) Egg weight of both breeds; E) Egg length; F) Egg length.

CONCLUSIONS

Marans hens are a heavy dual-purpose breed due to their greater live weight and feed intake. Tufted Creole hens are lighter birds with higher egg production. Thus, the latter may be more appropriate for producers that want to focus on number of eggs produced per hen rather than eggs with better external physical characteristics for consumers. Further studies should be carried out to design a feeding program that would allow both breeds to express their full productive potential and maximize the return on investment in backyard production systems in Mexico.

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Dietary alternatives in livestock production for mitigation of greenhouse gas emissions in Mexico

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ABSTRACT

Objective: To analyze the alternative sources of food for livestock production to mitigate greenhouse gas emissions in Mexico.

Design/methodology/approach: A document archive analysis was carried out about the use of certain industrial and agricultural wastes in Mexico in the last decade, and how these can serve as an alternative source of animal feed; these offer certain chemical properties that promote the mitigation of greenhouse gas emissions in livestock systems and also generate an added value in the agricultural and industrial sector.

Results: Agricultural and industrial production in Mexico generates an important amount of wastes that are not exploited; in parallel, livestock production in Mexico is growing exponentially, which demands a surface and consumption of natural resources. Therefore, agricultural and industrial residues in Mexico represent a potential alternative source of animal feed, which offers a reduction in the surface destined to the production of food for animals and gives an added value to the industrial and agricultural residues in the country.

Study limitations/implications: The alternative sources of food adjust to the agricultural and industrial sectors.

Findings/conclusions: The use of agricultural residues, industrial wastes, and other alternate sources of food is suggested as a sustainable alternative in the reduction of GHG in livestock systems and a contribution to the mitigation of climate change in Mexico.

Keywords: ruminants, alternative sources of fodder, methane, ruminal fermentation.

INTRODUCTION

It is estimated that by the year 2050, the Earth's population will be approximately 9 billion (FAO, 2017). This promotes an increase in the livestock sector to supply the dietary needs of the population (Ramírez-Ramírez, 2016). This increase in the production implies an increase in the use of natural resources and spaces, which is why its optimization ought to be contemplated.

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 75-81.*
Recibido: *junio, 2020.* **Aceptado:** *septiembre, 2020.*

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The disposition of natural resources has been affected by climate change, mainly because of the increase in greenhouse gases (GHG) whose origin is anthropogenic. The main gases associated to this effect are carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (NO_2) (Martínez-Prado, 2016).

Ruminants contribute with 53% of the total anthropogenic emissions of CH_4 (Hernández-Medrano, 2018). Methane is synthesized as a product of anaerobic ruminal fermentation, and its synthesis represents a loss of energy for animals, which is quantified in up to 12% (Vélez *et al*., 2014). In addition, the caloric power of CH_4 is 21 times higher than CO_2 (Valencia-Trujillo & Rojas-López, 2019). In Mexico, the agriculture and livestock activity contributes with close to 71% of the total emissions of CH_4 (Saynes-Santillán *et al*., 2016). In this sense, the farming sector covers 73% of the national territory. The economic activity of livestock production has a population of 33.8 million bovines and represents 3.8% of the national GDP (SIAP, 2018). Therefore, sustainable alternatives are sought that can serve as feed for livestock, and which in addition can cover the basic nutritional requirements of the animal, established by the National Research Council (White *et al*., 2017). Additionally, it is hoped that the alternative sources suggested do not represent a direct competition in the use of resources for human consumption and, at the same time, can reduce GHG emissions through modifications in the diet that ensure changes in ruminal anaerobic fermentation (Buitrago-Guillén *et al*., 2018). As a result of this, diverse methods and systems have been proposed, as well as alternative food sources that contribute to the mitigation of GHG in livestock systems, which are already in use or can be applied in Mexico. Based on this, alternative sources of food for livestock production were analyzed with regard to mitigation of greenhouse gas emissions in Mexico.

Silvopasture systems

Livestock production in Mexico is carried out by different systems: intensive, extensive and mixed. In the first, producing animals are stabled in an area that does not constitute the land where their main sources of food are sown, so they depend on being supplied with feed, energy and other necessary inputs for livestock production; in the second, the producing animals are kept in large land extensions and are fed mainly on seasonal grasses and fodders, with which they are in constant contact; meanwhile, in the third type of exploitation, the crops of animal and human food sources and the productive animals, are in constant contact. This last system produces more than 50% of total meat production and more than 90% of the total milk production in the world (FAO, 2014a). In fact, the silvopasture system is included among mixed production systems. The silvopasture system (SPS) is one where forestry, agricultural and livestock production activities are combined, and which also contributes to mitigate the effects of climate change (Arciniega-Torres and Flores-Delgado, 2018).

SPSs can be systematic (as live fences, pastures, passages, etc.) or non-systematic (disperse in pasturelands or grazing) with broad forest density (Buitrago-Guillén *et al*., 2018; Cisneros, 2019). The types of trees that can be included in this system are varied, since they can be native species or else introduced.

In this sense, the species selected for the SPS are distributed in a way that they take advantage of the characteristics of the land, obtaining benefits in the sectors implicated, such as the livestock contention fences and the exploitable forest volume, which improves water retention in the soil and decreases erosion (Canizales-Velázquez *et al*., 2019). This allows for a regeneration of nutrients and microorganisms in the soil, in addition to regulating the temperature in the zone (Villanueva, 2018).

Authors like Cuartas (2014) and Molina *et al*. (2015) mention tree species like *Leucaena leucocephala*, which are used in SPSs due to their protein content (27% raw protein). The same authors mention that animals fed with *L. leucocephala* presented reductions of 21% in methane production when compared with animals that consume the traditional diet.

There are studies that report a decrease of 20% in the production of CH_4 in diets with the inclusion of mulberry (*Morus Alba*) compared to diets based on star grass (*Cynodon plectostachyus*) (Rodríguez-Quiros *et al*., 2013). In regions like Africa, India and South America, the use of mesquite (*Prosopis* spp.) is recurrent in the SPSs. Mesquite has around 50 different species, of which all have a high protein value and are consumed by bovine, ovine and caprine livestock, among other ruminants; in addition, they are species resistant to extreme climate conditions, since they adapt to arid climates and their production is considerably high (Armijo-Najera *et al*., 2019). In addition, mesquite leaves and pods have shown to decrease the production of CH_4 *in vitro* compared to species like African moringa (*Moringa stenopetala*) or French sesban (*Sesbania sesban*) (Melesse *et al*., 2017). This is due to the content of secondary metabolites and phenolic compounds that affect the synthesis of ruminal methane and, therefore, its production (Ramírez-Rojo *et al*., 2019).

Likewise, white oak (*Quercus* sp.) is another species with high possibility of being incorporated to a SPS, since its leaves show high concentrations of secondary metabolites like condensed tannins (CT), which show positive effects on the consumption and ruminal fermentation of a fodder (Tavendale *et al*., 2005). The presence of CT contributes to the decrease in the synthesis of enteric methane in diets where the oak leaf is included (Torres-Fraga *et al*., 2018). In addition, certain studies show a reduction of 21.24% in the production of CH_4 in the enteric fermentation of ruminants *in vitro* when ground leaves of *Quercus* spp. are supplied instead of fodder of *Avena sativa* L. (Rajkumar *et al*., 2015).

Agricultural residues

In recent years, the resulting stubbles or residues from harvests have emerged as an alternative to feed livestock and decrease the production of enteric ruminal CH_4 (Caballero-Salinas *et al*., 2017). In addition, these residues tend to have a high fiber content, which represents an obstacle in the diet of humans, but not in that of ruminants. Supplementation with this type of residues decreases the costs of food without increasing the use of natural resources, thus achieving an integral use of the harvest.

In most Asian countries, production of rice (*Oryza sativa* L.) is high; the total production in Asia in 2014 was estimated at 538.8 million tons of rice straw, which is used to feed mainly the livestock in the region (Devendra and Thomas, 2002; FAO, 2014b). However, this residue presents high fiber content, which is why it is treated previously with additives like commercial urea or fibrinolytic enzymes that increase their degradability, or else, it is supplied as a dietary supplement (Phongphanith & Preston, 2016; Nguyen *et al*., 2020). The objective of pretreatment with urea, in addition to improving the digestibility, is decreasing the generation of ruminal enteric CH_4, since highly fibrous foods tend to increase the production of methanogenic bacteria (Yanti-Yayota, 2017). In this sense, Mexico cultivates close to thirty thousand ha of rice, with a yield of 4.9 t ha^{-1}, and it is expected that this production will continue in this direction until the year 2050, which generates an array of possibilities (Delgadillo-Ruíz *et al*., 2016).

In Mexico, one of the most common residues for bovine livestock feed is maize (*Zea mays* L.) stubble, which has considerable nutritional characteristics for the diet of ruminants (Prieto *et al*., 2016); in addition, maize stubble represents up to 40% of the total residue fodder in the dry season (Caballero-Salinas *et al*., 2017). This residue is offered to the animals as a dry fodder or in ensilage (Gómez-Gurrola *et al*., 2016). Authors like Carro (2018) reported a decrease of CH_4 of 13% per unit of dry matter ingested in animals fed with maize ensilage. Additionally, ensilages have been generated with maize stubble and by adding nopal (*Opuntia ficus indica*) fodder and yeasts, whose mixture causes a reduction in ruminal methanogenesis (González-Arreola *et al*., 2019).

Other elements like cotton seed meal, a yield from residues of the cotton (*Gossypium hirsutum* L.) harvest which is commonly used in ruminant diet, is supplied as paste or protein supplement (Muro-Reyes *et al*., 2017). However, its production is costly and not very accessible for livestock producers of low financial income, which is why substituting them by residues or other more affordable additives and which also help to decrease GHG would be highly desirable. In this regard, there are various studies that have considered the incorporation of other sources of fodder of high protein content that show a decrease in the production of ruminal enteric methane (Pámanes-Carrasco *et al*., 2019).

Weeds and shrubs

Some plants considered as weeds present particular characteristics inherent to the plant, such as production of secondary metabolites, which can influence the fermentation of foods consumed by the ruminant (Lezcano-Más *et al*., 2016).

In Colombia, extensive production presents low production of fodders for bovine livestock, which is why botón de oro (Mexican sunflower, *Tithonia diversifolia*) is studied as an alternative source of fodder. Although this plant is considered a weed in this country, it can present raw protein contents of 17-29%, as well as a digestibility of up to 76%. Its high contents of DM, paired with the values of digestibility, present possibilities in the decrease of production of CH_4 through the reduction of microorganisms in charge of ruminal methanogenesis

(Londoño *et al*., 2019); the inclusion of 20% of this plant as part of the fodder fraction of the total portion has managed to decrease the population of methanogens up to 50% (Mahecha-Ledesma *et al*., 2018). In Mexico, botón de oro is used in the diet of small ruminants such as sheep. There are previous studies that describe the supply of botón de oro as part of the fodder fraction in 10% and managed to manipulate the ruminal biota through the reduction in the population of methanogens and protozoa, and as consequence, the emissions of enteric CH_4 due to the increase in the population of cellulolytic bacteria (Rodríguez-García, 2017).

Forest residues

The forestry sector in Mexico generates approximately 2.8 million m^3 of residues per year, mostly sawdust. The use of sawdust as a food source in livestock production is not prohibited; however, depending on the case and the characteristics of the productive herd, it could result in a very costly practice. There are few studies that endorse the use of wood carbohydrates as a source of energy for ruminants; however, their use in cases of emergency where there is no source of fiber for the animal diet is perfectly justified; in addition, this scenario is highly probable if climate change continues with its current course (Rinne and Kuoppala, 2019). On the other hand, it has been shown that the use of sawdust favors weight gain (Fregoso-Madueño *et al*., 2017). In addition, the smaller the size of the particle is, the lignocellulose will be degraded in less time, favoring the production of volatile fatty acids (VFAs) and promoting the decrease of CH4 (Hamano *et al*., 2017).

Agroindustrial residues

The industry of transformation of agricultural products generates residues as part of its production line. The characteristics of these residues allow them to emerge as potential dietary complements in ruminant production. In this sense, Colombia is an important producer of mango (*Mangifera indica* L.) concentrate and juice; however, its seed, skin and pulp are discarded and can be susceptible of ensilage and increase the nutritional value of the fodder due to its high content of fiber carbohydrates (Borrero-Manrique *et al*., 2017). Despite there being no studies that back the effect of these residues in the production of ruminal enteric methane, it could be inferred that the use of these ensilages can decrease the production of CH_4 due to the high digestibility and high content of soluble carbohydrates that they have (Guzmán *et al*., 2019). Just in Mexico, around 54 million tons of organic residues of fruits and vegetables are generated, among which there are lime (*Citrus* spp.), pear (*Pyrus communis* L.), mango, papaya (*Carica papaya* L.), pineapple (*Annanas comosus*), banana (*Musa paradisiaca*), orange (*Citrus sinensis*), carrot (*Daucus carota*), tomato (*Solanum lycopersicum*), lettuce (*Lactuca sativa*), potato (*Solanum tuberosum* L.) and cabbage (*Brassica napus* L.) (Valdez-Vazquez *et al*., 2010). Thus, any industry that generates organic wastes becomes a potential supplier of alternative sources of feed for ruminants.

In the year 2006, the use of glycerol was legally allowed as a safe animal food according to the United States Food and Drug Administration (FDA, 2006). In addition, its inclusion in the diet of ruminants can be done through pellets, extruded or raw in a mixture with the fodder, where the concentration supplied varies according to the purity of glycerol (Hidalgo-Hernández *et al*., 2017). Likewise, glycerol has been included in the diet of ruminants as substitute of molasses (non-crystallized honeys from the process of sugar production), which generates similar concentrations of volatile fatty acids. The catabolism of glycerol in the rumen increases the formation of propionic acid, which promotes weight gain of the animal. In addition, propionate is an H^+ ion sink, which generates a natural competition with the synthesis of CH_4, favoring propionate synthesis (Martin-Nazly *et al*., 2019).

The coffee industry generates residues that could be used in animal feed. Such is the case of coffee pulp (*Coffea* sp.). Previous studies show that when coffee pulp is ensilaged with sugarcane (*Saccharum* spp.), molasses and urea, the fodder acquires nutritional values of 30 to 33% of raw protein and fiber, respectively (Flórez-Delgado and Rosales-Asensio, 2018). These characteristics allow for it to be incorporated in up to 30% of the total dry base portion without affecting the production and integrity of the livestock. However, there are no studies that encourage the effects of this type of ensilage on methanogenesis or ruminal methanogenics.

Aquatic macrophytes

The term aquatic macrophytes is given to plants that have at least the vegetative part of the plant visible at plain view, on the surface of the aquatic bodies where they grow permanently or periodically (Thomaz *et al*., 2008). In addition, macrophytes do not represent a direct competition to the diet of humans, nor do they hoard the use of natural resources. In this way, plants like aquatic lily (*Eichhornia crassipes*) present comparable nutritional values to those of a fodder of medium quality (Murillo-Ortíz *et al*., 2018); there are even studies that indicate the content of raw protein and fiber is 15.5 and 15%, respectively (Rúales *et al*., 2018). Likewise, other species such as *Oryctolagus cuniculus* have a content of raw protein and neutral detergent fiber (NDF) of 18 and 34%, respectively, in addition to a digestibility of up to 90% (Silva-Borges *et al*., 2019). Presently, the use of macrophytes happens as a source of fodder in the diet of rabbits. In a similar way, *Limnobium laevigatum* has a protein content of 16% and a fibrous part of 7% (Aponte, 2017); both species are highly reproductive in tropical zones. Additionally, Murillo-Ortíz *et al*. (2018) reported a decrease in methane when they included aquatic lily as part of the fodder fraction in *in vitro* diets. Because of this, it is possible that the inclusion of macrophytes represents a real alternative in the mitigation of GHG in livestock systems. However, the cultivation of these plants in natural aquatic bodies represents a latent risk for the existence of flora and fauna because the stagnation of these on the surface of the aquatic body prevents the passage of light and photosynthesis taking place in plants adequately; in addition, their presence consumes much oxygen, which decreases the availability of it for the native flora and fauna (Zingel *et al*., 2006). Although there are no studies that guarantee increases in the production of aquatic lily under hydroponic cultivation, there are studies that suggest its feasibility (Giri and Patel, 2012).

CONCLUSIONS

Industry in Mexico, as well as forestry and agricultural production, generate wastes whose aim is uncertain. However, the use of these can find an integral and sustainable use in livestock production. The exploitation of the diverse sources of feed can represent a sustainable alternative in the mitigation of GHG in Mexico, as well as a decrease in agricultural and industrial wastes. Additionally, the use of these residues allows for surfaces devoted to sowing sources of food for animals to change crops or decrease, as a feasible alternative against the erosion of cultivation lands. In addition, the incorporation of silvopasture systems in livestock production in Mexico could emerge as a viable option in the reduction of lands for livestock exploitation, as well as in their erosion.

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https://doi.org/10.32854/agrop.v13i10.1700

Resilience and Livelihoods; A community development model

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ABSTRACT

Objective: Formulate a community development model to contribute to rural resilience at the states of Campeche, Chiapas, Tabasco, Yucatán and Quintana Roo, Mexico.

Design/methodology/approach: The project execution considered the incursion in high and very high marginalization communities with populations between 300 and 3,800 inhabitants, throughout five states. Sustainable livelihoods and the logical framework made it possible to systematize and analyze the collected data to characterize the potential territorial development, carried out with a secondary sources review and a field phase. A social innovation agenda was formulated with descriptive files of projects and potential financing sources.

Results: 93 localities established in 14 micro-regions in five states were intervened. Ninety extension workers were trained in community development, 216 training actions took place, 90 community databases compiled, 90 community development plans, 90 integration acts of community consultation and planning bodies (CCPB) and 14 acts of integration of extension groups for the microregional development (EGMD).

Limitations of the study/implications: The duration of the project prevented the implementation of community development plans.

Findings/conclusions: The present model consider the individuals participation as the basis for the life quality improvement of the community, based on territorial appreciation and the collective identity framed in participatory processes.

Keywords: Quality of life, sustainable livelihoods, social network analysis.

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 83-89.*
Recibido: *mayo, 2020.* **Aceptado:** *septiembre, 2020.*

INTRODUCTION

In Mexico, 73.03% (3,888,764) of the country's rural economic units relate to family farming schemes (subsistence) with no market linkage (SAGARPA-FAO, 2014); however, the public policy promoted in the sector shows a hegemonic character with the purpose of promoting agricultural exports specialization (Reynolds *et al*., 1993; Appendini, 1995). Unfortunately, the strategy has contributed to the polarization of production systems in the Mexican countryside; including, on the one hand, the agro-export sector, producers in transition, and, on the other, the producers who are the target population of assistance programs to combat poverty. Usually the latter linked to smallholder production schemes (52.81 million Mexicans; approximately 44.60% of the population during the 2008-2018 period) concentrated in the South-Southeast Region, particularly in Veracruz (4.54 million), Chiapas (3.93 million), Oaxaca (2.60 million), Guerrero (2.36 million) among others (CONEVAL, 2019). Paradoxically, the South-Southeast Region has more than 70% of the biodiversity of North America and is part of the Mesoamerican Biological Corridor, so it is convenient to question the logic followed in development policies from above (top down), which propose schemes hegemonic valid for all the territories of Mexico (SEDATU, 2013; Rózga, 2013). This document is framed as a development proposal from below (bottom up), where the micro-social space or community makes it possible to focus attention on the internal interactions between actors and their arrangements as a basis to cement development strategies or sustainable livelihoods (SL) as an alternative to the hegemonic agricultural policy implemented in the sector (Rózga, 2013; Méndez, 2015). The SL are based on the development potential of the territory, and involve natural, productive resources, anthropogenic activity related to the use, conservation and exploitation of resources, therefore, it is the basis for the generation of income and satisfaction of the needs of the rural population (Vázquez-Barquero, 2007).

The capitals that make up the SL are human, which represents all those elements linked to the rural population such as health status, population growth, migration, and social capital. This involves the relationships or links established by the inhabitants, natural capital, which relates the natural resources of the territory (land, flora, fauna, bodies of water, etc.), the physical capital, which considers the basic infrastructure and those production goods used by the populations to satisfy their basic needs and carry out their productive activity, and the financial capital, which considers access to markets, the construction of rural and complementary income, but also the availability of money or equivalent (Alobo, 2015). It is worth mentioning that the conversion of assets to capital through the production of goods and services is of vital importance, with a view to contributing to the improvement of the quality of life of the rural population within the framework of community development. The above as a process of social construction that pursues the development and strengthening of rural resilience based on the development potential of the territory (natural resources, productive resources and anthropic activity) from the perspective of the community (Carlson *et al*., 2017; Pastor, 2015; Zarazúa and Gómez-Carreto, 2014). Rural resilience, therefore, is the ability of a rural territory to positively adapt its economic, social, natural structure, etc., based on the identified livelihoods, and to maintain continued development over time in the face of adverse situations that generate serious impacts (Sánchez-Zamora *et al*., 2016; Méndez, 2016). Therefore, a community development model was formulated to contribute to rural resilience in the states of Campeche, Chiapas, Tabasco, Yucatán and Quintana Roo, Mexico.

MATERIALS AND METHODS

The execution of the project considered the incursion into communities of high and very high marginalization with a population of between 300 and 3,800 inhabitants, established in five states, between July 2017 and March 2018, under the auspices of the Instituto Nacional para el Desarrollo de Capacidades del Sector Rural, A.C. (INCA Rural, A.C.) within the framework of the Extension Center for Community Development (PM171032) project for Campeche, Chiapas, Tabasco, Yucatán and Quintana Roo.

The methodological tools used were sustainable livelihoods and the logical framework, which allowed the systematization and analysis of data collected in the characterization of the development potential of the territory (natural resources, productive resources and anthropogenic activity), carried out with a review of secondary sources and phase field. Subsequently, a community development plan was formulated with descriptive sheets of projects and potential sources of financing.

The methodological proposal proposes the participation of the individual as a basis to contribute to the improvement of the quality of life of the community, for which the integration of the consultation and community planning bodies (CCPB) and of the extension groups for micro-regional development (EGMD) are vital. For the purposes of this model, the CCPB was contextualized as a space for permanent participation of community actors that allows the development and consolidation of the processes of empowerment and construction of Roadmap Agendas of intervention strategy. The integration of the CCPB considered the identification of key actors with the applicability of social network analysis, dissemination of the call for the integration of the CCPB, the planning of the participatory assembly, the signing of the act of installation and compilation of the personal file of the members of the CCPB and finally, the protest of the members of the CCPB. Meanwhile, the EGMD as multi-community bodies will seek to develop and strengthen the capacities of the members of the CCPB, in such a way that these bodies analyze, prioritize, promote and promote community development plans that contain the strategies identified from the means of life and community optics (Alamilla *et al*., 2018).

RESULTS AND DISCUSSION

In this study 93 localities, established in 14 microregions, in five states were intervened. Ninety extension workers were trained in community development, 216 training actions carried out, 90 community databases, 90 community development plans, 90 acts of integration of CCPB, and 14 acts of integration of EGMD (Figure 1 and Table 1).

In total, 26 follow-up or training events were held, among them: workshops to introduce methodologies and tools of the community intervention strategy to state trainers, follow-up meetings and presentation of extension workers, among others (Figure 2).

One of the neuralgic points that the project faced was the identification of the target population, so we proceeded to integrate the unique databases of beneficiaries or BDU (Annex II of the SAGARPA operating rules, 2017), with documentary supports that guarantee the authenticity and veracity of the information. The extension model evaluated in this work showed that the relationship with an educational and research institution provides important contributions to the model since that is where novel information is found. However, not having control over the hiring of extension agents, it hinders the formation of interdisciplinary groups for technology transfer, to form; for example, groups of extension agents with agronomic, livestock, social, administrative and technological profiles, in such a way that the production systems improve, working with the beneficiaries that facilitates the generation of new local entrepreneurs and can migrate to economically higher social strata. In this regard, Cadena-Iñiguez *et al*. (2018) pointed out that the incorporation of ICTs in agricultural production systems is an innovation that needs to be adopted by producers and break the paradigms that technologies are not applicable to the field and are very expensive. Like Landini (2014), the highest percentage of extension workers were men (62%), with university studies (100%) and 5% with postgraduate studies. The average age was 30 years. Although the extension agents were trained in the community development extension model, Landini (2014) recommends training them in service and management of social processes, especially group management, participatory process management, ability to teach and empathize, etc. in order to improve the extension processes. The foregoing agrees with the model presented in this work. Finally, this model has methodological tools that lead communities to value themselves for what they are and have and to identify their productive potential with a focus on equity and gender (Figure 3).

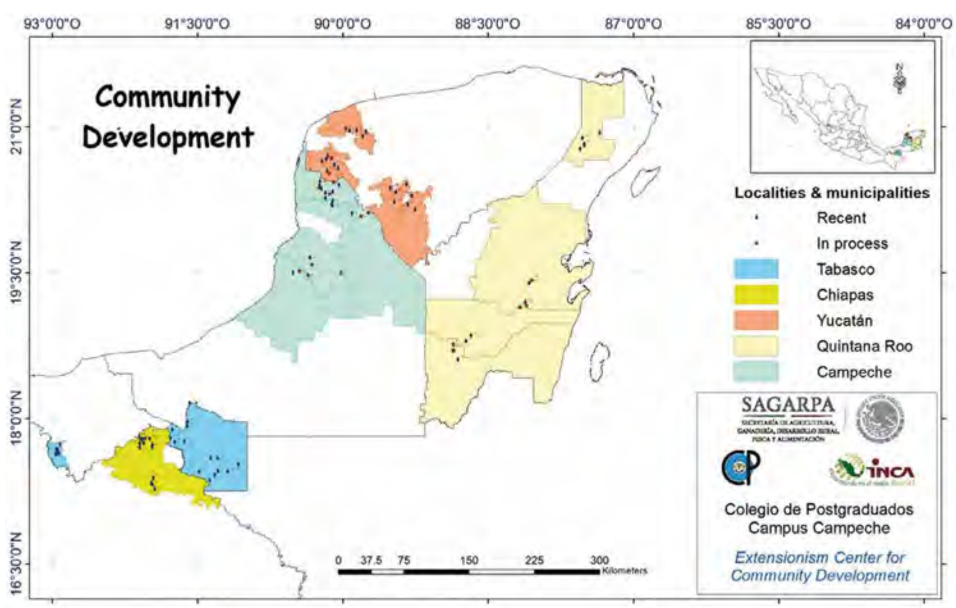

Figure 1. Map of intervened localities. Data from INEGI, CONAPO.

Table 1. List of intervened localities in selected states, ordered based on the microregion to which they belong (Alamilla *et al*., 2018).

State	Municipality	Micro-area	Locality	Habitants
CAMPECHE	CALKINI	Camino Real	Concepción	351
			San Agustín Chunhuás	401
			Pucnachén	865
			San Antonio Sahcabchén	1858
			San Nicolás	369
			Santa Cruz Ex-Hacienda	1255
			Santa Cruz Pueblo	1908
			Tepakán	1895
			Santa María	236
	HECELCHAKÁN		Pocboc	1624
			Santa Cruz	1118
			Dzitnup	891
	CAMPECHE	CAMPECHE CENTER	Nohakal	880
			Pich	1756
			Tixmucuy	497
			San Luciano	319
			Adolfo Ruiz Cortínez	378
	CHAMPOTÓN		Hool	1181
			Santo Domingo Kesté	3763
	HECELCHAKÁN	CHENES	Nohalal	522
	HOPELCHÉN		Chunyaxnic	364
CHIAPAS	CATAZAJÁ	CATAZAJÁ	Agua Fría	571
			Emiliano Zapata (San Joaquín)	377
			Loma Bonita	1071
			La Tuza (Maceo)	437
			Ignacio Zaragoza	963
			Santa Cruz 2da. Sección de Loma Bonita	390
			Cuauhtémoc	728
			Punta Arena	1365
			El Rosario	666
	PALENQUE	PALENQUE ALTOS	Belisario Domínguez Norte	379
			San Antonio	428
	SALTO DE AGUA		América Libre	1263
			Estrella de Belén	396
			Nuevo Mundo	353
QUINTANA ROO	FELIPE CARRILLO PUERTO	CARRILLO PUERTO	Noh-Bec	2045
			Uh May	480
			X-Hazil Sur	1422
			Andrés Quintana Roo	346
			Reforma Agraria	314
	OTHON P. BLANCO	OTHÓN P. BLANCO	Altos de Sevilla	605
			San Pedro Peralta	766
			Lázaro Cárdenas Segundo	699
			San Román	530
			Morocoy	1293
			La Libertad	421
	LÁZARO CÁRDENAS	LÁZARO CÁRDENAS	Cristóbal Colón	341
			San Cosme	361
			San Francisco	767
			San Juan de Dios	360
			Ignacio Zaragoza	2213

Cuadro 1. Continuación.

State	Municipality	Micro-area	Locality	Habitants
YUCATAN	HALACHÓ	HALACHÓ	Cuch Holoch	2017
			Kancabchén	460
			Santa María Acu	1437
	MAXCANU		Granada (Chican Granada)	476
			San Rafael	1252
			Chunchucmil	1091
			Paraíso	656
			Santa Rosa (Santa Rosa de Lima)	913
			Coahuila (Santa Teresa Coahuila)	626
	MANI	PUUC	Tipikal	951
	OXKUTZCAB		Emiliano Zapata	1350
	TEKAX		Canek	308
			Manuel Cepeda Peraza	573
			Pencuyut	1524
	TICUL		Pustunich	2480
			Yotholín	2267
	HUNUCMA	TETÍZ	Hunkanab	466
	TETIZ		Tetiz	3939
			Nohuayún	777
	UMAN		Dzibikak	1388
			Oxcum	1175
TABASCO	TEAPA	TEAPA	Ignacio López Rayón 1Ra. Sección	552
			José María Morelos y Pavón 1ra. Sección	422
			Mariano Pedrero 1Ra. Sección (La Providencia)	381
			José María Morelos y Pavón (Las Delicias)	815
			José María Morelos y Pavón (Santa Rita)	489
			Las Lilias	365
	BALANCÁN	BALANCÁN NORTE	El Pípila	512
			Constitución	523
			Miguel Hidalgo y Costilla	292
			Ingeniero Mario Calcáneo Sánchez	319
	EMILIANO ZAPATA		Emiliano Zapata (Sección Jobal)	344
			Nuevo Chablé	407
	BALANCÁN	TENOSIQUE	Mactún	1055
			Jolochero	737
			Arroyo el Triunfo 2Da. Sección	342
	TENOSIQUE		Canitzán	308
			Emiliano Zapata 2Da. Sección (El Carmen)	443
			Ignacio Zaragoza	357
			San Isidro Guasiván	361
			Los Rieles de San José	336
			Crisóforo Chiñas	353

CONCLUSIONS

The innovation in this model is to use a series of analysis tools to carry out a diagnosis focused on the capital available to producers and to focus their requirements from their perspective and not from a vertical vision of the extensionist or government program. It proposes to generate groups of extension agents from different disciplines that allow meeting the needs of rural actors, leading producers to be agricultural microentrepreneurs, and leading them towards a vision of wealth from different points of view.

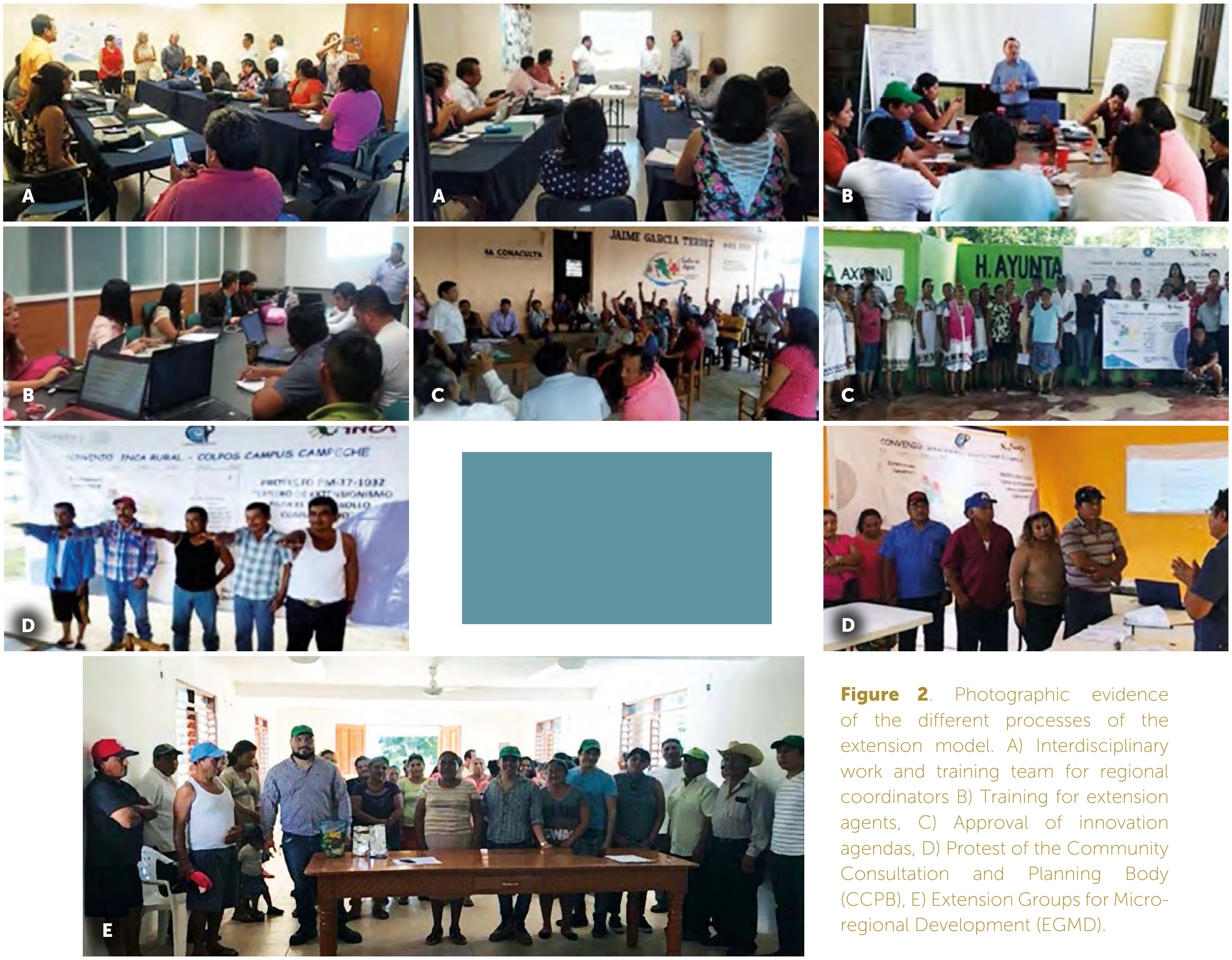

Figure 2. Photographic evidence of the different processes of the extension model. A) Interdisciplinary work and training team for regional coordinators B) Training for extension agents, C) Approval of innovation agendas, D) Protest of the Community Consultation and Planning Body (CCPB), E) Extension Groups for Micro-regional Development (EGMD).

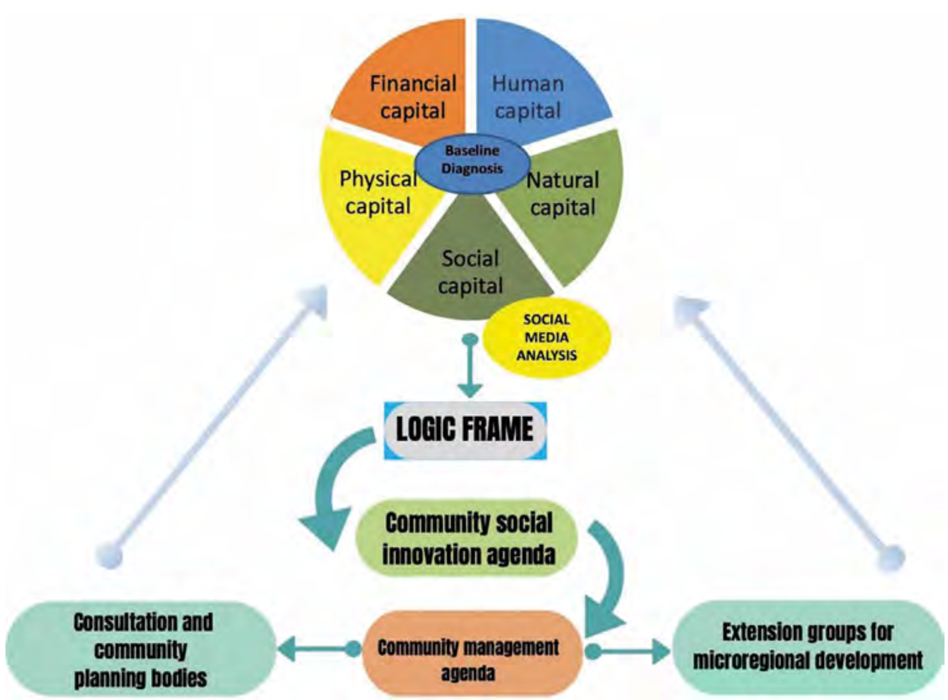

Figure 3. Community Development Model (Alamilla *et al*., 2018).

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https://doi.org/10.32854/agrop.v13i10.1846

Acondicionamiento y evaluación de tratamientos de desinfección para la micropropagación de *Sagittaria macrophylla* zucc. procedente de las Ciénegas del Rio Lerma

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ABSTRACT

Objetivo: Evaluar el método de acondicionamiento y desinfección de *Sagittaria macrophylla* zucc. (Alismataceae) de la Cienega de Lerma, para su propagación y conservación.

Diseño/metodología/ aproximación: Se estableció en invernadero un diseño completamente al azar con tres repeticiones para evaluar dos tipos de sustrato para *S. macrophylla*, se evaluó el número y longitud de brotes sanos. El primer sustrato consistió en una mezcla de suelo y agrolita (2:1) (S1); el segundo sustrato se consideró el control ya que consistió en sedimentos de la Ciénega del Rio Lerma (S2). Se evaluó un método de desinfección donde los tratamientos consistieron en: lavados + etanol al 70% + cloro comercial (T1) y lavados + microdin-jabón líquido + etanol al 70% + cloro comercial (T2). Las condiciones de fotoperiodo (luz y oscuridad) representaron los bloques del diseño experimental.

Resultados: En el experimento de tipo de sustrato se obtuvieron diferencias estadísticas ($P<0.05$) entre tratamientos, donde S1 mostro 2.4±0.24 brotes en promedio con longitud de 2.5 cm en 30 d y con tendencia a incrementar. El experimento de desinfección mostró diferencias estadísticas ($P<0.05$); sin embargo, el fotoperiodo no presento diferencias ($P>0.05$). El T2 presento el menor porcentaje de contaminación 31.25%.

Limitaciones del estudio/implicaciones: los resultados presentados son avances de un experimento a largo plazo.

Hallazgos/conclusiones: El acondicionamiento de plantas madre de *S. macrophylla* es favorable en condiciones de invernadero en un sustrato compuesto por suelo negro y agrolita 2:1 permitiendo brotes sanos. El mejor método de desinfección consistió en un enjuague a chorro de agua con jabón en polvo, 30 min con 100 mL de agua destilada más una gota de microdin comercial y dos de jabón líquido, 2 min en agitación constante en etanol al 70% y 20 min en cloro comercial (6%) al 30% v/v, utilizando como explantes las yemas axilares de los tubérculos sin importar el fotoperiodo en el cuarto de incubación.

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 91-96.*
Recibido: *julio, 2020.* **Aceptado:** *octubre, 2020.*

Palabras clave: papa de agua, peligro de extinción, sustrato, desinfección, cultivo *in vitro*.

INTRODUCCIÓN

Las ciénegas de Lerma en el Estado de México han provisto de diferentes servicios a la población, mismas que por un lapso permitieron el desarrollo de algunas prácticas agropecuarias ribereñas importantes para el sustento de la comunidad aledaña (Albores, 2002). La biodiversidad de las lagunas de la Ciénega de Lerma se caracteriza por su flora y fauna muy peculiar. Gran parte de la variedad de especies que componen la vegetación de las lagunas son plantas acuáticas endémicas de México. Algunas de ellas se encuentran en categoría de riesgo derivado de la pérdida y deterioro del hábitat (Sánchez-Reyes *et al*., 2012; Zepeda-Gómez *et al*., 2012 a y b; Zepeda *et al*., 2014). La especie *Sagittaria macrophylla* zucc. (Acuitlapalli) (Figura 1) en náhuatl significa atl=agua, cuitlac=planta, tierra y palli=barro negro (papa de agua), es una de las 19 especies acuáticas de la familia Alismastaceae que habitan en México, y es una planta emergente endémica que crece en zonas poco profundas como charcas, zanjas y márgenes de cuerpos de agua dulce (Zepeda y Lot, 2005), Esta especie es conocida en México como "cola de pato", "cucharilla", "flecha de agua" y "hoja de flecha". Crece entre los 2100 y los 2700 m de altitud, principalmente en la Ciudad de México y los estados de Hidalgo, Jalisco, México y Michoacán (Miranda, 2019).

Está catalogada por la Norma Oficial Mexicana-059-SEMARNAT-2010 (SEMARNAT, 2010) como especie amenazada (A), debido a la reducción y contaminación de su hábitat. De acuerdo con González *et al*. (2011) algunos pobladores de San Pedro Tultepec de Quiroga, aún se adentran a la laguna de Chimaliapan y la recolectan para autoconsumo o comercialización ya que esta especie forma parte de la gastronomía de las poblaciones ribereñas pese a que la laguna se encuentre contaminada (Viesca *et al*., 2011). Este tubérculo es relevante ya que forma parte de la dieta de algunas especies animales que habitan dentro de las lagunas como es el caso del pato mexicano (Anais dazi) en la laguna de Chimaliapan (Zepeda-Gómez *et al*., 2012 a y b). Una de las formas de conservar especies que se encuentran en alguna categoría de riesgo es mediante la propagación in vitro, de esta manera se puede preservar y multiplicar el número de individuos de una especie en particular en un área reducida para que después se puedan reproducir naturalmente y mitigar la perdida de cierta especie. En este tenor, se han logrado desarrollar protocolos de propagación mediante esta técnica en especies que se encuentran en alguna categoría de riesgo, entre las familias más estudiadas están Orchidaceae (Menchaca *et al*., 2012; Azofeifa *et al*., 2019; Dolce *et al*., 2020), Cactaceae (Téllez-Román *et al*., 2017; Ramírez-Gonzáles *et al*., 2019), Nolinaceae (Flores *et al*., 2019; Reyes-Silva *et al*., 2013; Martínez-Peña *et al*., 2012). Se evaluó un método de acondicionamiento y desinfección de *Sagittaria macrophylla* zucc. (Alismataceae) de la Cienega de Lerma, para su propagación y conservación en condiciones de invernadero.

Figura 1. *Sagittaria macrophylla* zucc. (A) parte aérea de la planta tallo y hoja, (B) parte baja de la planta raíces, tubérculos y estolones.

MATERIALES Y MÉTODOS

Se obtuvieron cuatro plantas completas (planta madre) donadas por los comuneros de la "Laguna Chimaliapan" y sustrato de la laguna. Las plantas se transportaron al invernadero de biotecnología forestal conjunto SEDAGRO Metepec Estado de México en 20 L de agua para su posterior manejo. En un invernadero, las plantas se colocaron en tinas de aproximadamente 1 × 0.5 m.

Acondicionamiento de las plantas

Para evaluar el acondicionamiento de las plantas se evaluaron dos tipos de sustrato, el primero consistió en una mezcla de suelo negro y agrolita en proporción de 2:1, respectivamente (S1); el segundo sustrato se consideró el control ya que consistió en sedimentos de la Ciénega del Rio Lerma (S2), posteriormente se les agrego agua hasta que los tubérculos quedaran inundados, se dejaron en el invernadero durante 30 d con el objetivo de obtener nuevos brotes.

Desinfección del material vegetal

Se evaluó un método de desinfección para obtener material vegetal sano y micropropagarlo, para tal evaluación se estableció un diseño de bloques completos al azar en el laboratorio de biotecnología forestal en donde se evaluaron dos tratamientos. El primero (T1) consistió en lavados + etanol al 70% + cloro comercial, y el segundo (T2) consistió en lavados + microdin (plata coloidal) + jabón líquido + etanol al 70% + cloro comercial (Cuadro 1) con ocho repeticiones cada uno. Las condiciones de luz, oscuridad (fotoperiodo) representaron los bloques del diseño experimental.

Las yemas axilares de los tubérculos (explantes) fueron sembrados en condiciones asépticas en medio de cultivo Murashige y Skoog (1962) MS al 100% de la concentración de sus sales minerales, adicionado con 30 g L^{-1} de sacarosa y 7 g de agar bacteriológico. Se ajustó pH a 5.7±0.1 y se esterilizo durante 15 min a una temperatura de 120 °C, para posteriormente evaluar el porcentaje de contaminación del material vegetal. Las siembras se colocaron en el área de incubación donde se les proporciono un fotoperiodo de 16 h luz y 8 h de obscuridad colocándolos en una caja completamente cerrada en el mismo cuarto de incubación a temperatura de 24-26 °C.

Análisis estadístico

Los variables para evaluar el acondicionamiento de las plantas se estableció un diseño completamente al azar con tres repeticiones. Las variables respuesta fueron el número y longitud de brotes dependiendo del sustrato empleado (S1 o S2). El modelo general lineal fue:

$$Y_i = \mu + T_i + e_j$$

en donde, Y_i fue la variable respuesta del *i*-esimo tratamiento, μ la media, T_i el *i*-esimo tratamiento y e_i el error experimental.

Para evaluar la desinfección de las plantas se estableció un diseño de bloques completos al azar con ocho repeticiones. Las variables que se evaluaron fueron el porcentaje de contaminación, contaminación por hongos (el explante es cubierto por un cuerpo algodonoso), bacterias (aparición de un cuerpo lechoso de olor fétido cubriendo el explante) y el porcentaje de oxidación del material (más del 50% del explante toma un color marrón-café). El modelo general lineal fue:

$$Y_{ij} = \mu + T_i + B_j + e_{ij}$$

en donde, Y_{ij} fue la variable respuesta en el *i*-esimo tratamiento y en el *j*-esimo bloque, μ la media, T_i el *i*-esimo tratamiento, B_j el *j*-esimo bloque y e_{ij} el error experimental. Se consideraron diferencias estadísticas significativas entre tratamientos cuando ($P<0.05$), posteriormente se realizó la prueba de comparación de medias de Tukey (Steel y Torrie, 1980) empleando The SAS System for Windows 9.0.

RESULTADOS Y DISCUSIÓN

Las plantas madre en el invernadero comenzaron a presentar nuevos brotes a los 30 d después de haber sido colocadas en los sustratos evaluados, en ambos sustratos se observaron brotes. Se presentaron diferencias estadisticas ($P<0.05$) entre S1 y S2 (Figura 3). Aunque en S2 se presentaron en promedio 2.58 brotes, los brotes más jovenes se vieron afectados por la necrosis causada por el tipo de sustrato (Figura 2). No se presentaron diferencias estadísticas ($P>0.05$) para la longitud de brotes.

Cuadro 1. Tratamientos de desinfección de material vegetativo de *Sagittaria macrophylla* zucc. para ser evaluadas en cultivo *in vitro*.

Tratamiento 1	Tratamiento 2
• Se enjuagó el tubérculo a chorro de agua con jabón en polvo y cepillo de dientes para eliminar el lodo • Se dejó por 2 min en agitación constante en etanol al 70% y se enjuagó tres veces con agua destilada. • Después se mantuvo en agitación constante por 15 min en cloro comercial (6%) al 30% v/v y enjuagó tres veces con agua destilada. • Para finalizar, en campana de flujo laminar se lavaron cinco veces con agua destilada esterilizada.	• Se enjuagó el tubérculo a chorro de agua con jabón en polvo y cepillo de dientes para eliminar el lodo. • Por 30 min, se mantuvo en agitación constante, con 100 mL de agua destilada más una gota de microdin comercial y dos de jabón líquido, al finalizar se enjuago con agua destilada tres veces. • A continuación, se dejó por 2 min en agitación constante en etanol al 70% y se enjuago tres veces con agua destilada. • Se dejó en agitación constante por 20 min en cloro comercial (6%) al 30% v/v y enjuago tres veces con agua destilada. • En campana de flujo laminar, se hicieron tres lavados con agua destilada esterilizada.

Figura 2. Brotes de *Sagittaria macrophylla* zucc después de 30 días establecidas bajo condiciones de invernadero. (A) Brote originado de un rizoma, (B) Brote en S1 (suelo negro y agrolita 2:1). C. Brote en S2 (sustrato original de Laguna Chimaliapan).

Los brotes en S1 compuesto por suelo negro más agrolita, registró 2.4±0.24 brotes en promedio (Figura 3) y se observó una coloración blanquecina en éstos (Figura 2). La longitud de los brotes en este tratamiento incremento 0.38 cm en 30 d. Los brotes en S2 (compuesto por sedimento del sitio de recolecta de la planta) presentaron reducción en la longitud del brote (0.23 cm) desde su emergencia conforme los días transcurrieron.

Se resalta que para *S. macrophylla* solo se encuentran investigaciones de propagación mediante el uso de sustratos bajo condiciones ambientales de invernadero (asexual), en este sentido, Miranda (2019) reporto que mediante la mezcla de 70% de composta elaborada con *Eichhornia crassipes* y 30% de grava volcánica se logran obtener hasta tres brotes por planta en 45 d, resultados que son similares con los obtenidos en el presente estudio. Sánchez-Reyes *et al*. (2012), reportaron que plantas de *S. macrophylla* producen hojas con láminas de mayor superficie cuando son inundadas 5 cm por encima del sedimento.

Desinfección de material para el cultivo *in vitro*

Respecto al porcentaje de contaminación se presentaron diferencias estadísticas ($P<0.0001$) entre tratamientos de desinfección, presentando mayor contaminación el T1 con 93.75%, y menor en T2 (31.25%). En ambos tratamientos se presentó la contaminación por hongos y bacterias, siendo más notable la contaminación por bacteria (Cuadro 2), los porcentajes de contaminación en T2 fueron de 0% para hongo y 31.25% en bacteria, presentando los menores valores. Respecto al porcentaje de oxidación no se presentaron diferencias estadísticas ($P>0.05$) entre tratamientos de desinfección, el T2 presento menor oxidación 19% que T1 (Cuadro 2).

Respecto al fotoperiodo en el cuarto de incubación, no se presentaron diferencias estadisticas respecto al porcentaje de contaminación y al porcentaje de contaminación por bacteria (Cuadro 2). De acuerdo con la revision de literatura cientifica, no existen investigaciones de cultivo *in vitro* en *Sagittaria macrophylla*, los resultados obtenidos en el presente trabajo son alentadores para investigar y evaluar su multiplicación, enraizamiento y aclimatación, similar al trabajo reportado para *Sagittaria latifoloa* (Hoang, 2016) donde se evaluó el método de propagación *in vitro* mediante la multiplicación de tubérculos obteniendo de 2 a 3 brotes por explante.

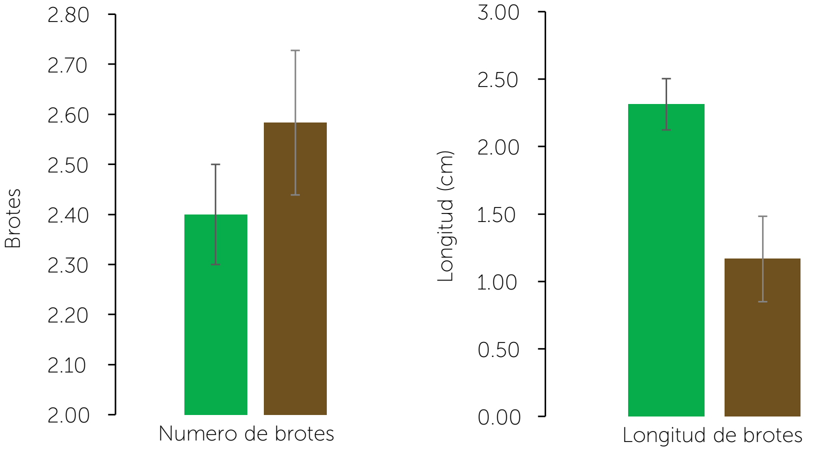

Figura 3. Promedio del número y longitud de los brotes a partir de plantas madre de *Sagittaria macrophylla* zucc. en invernadero durante 30 días. S1, barra verde y S2, barra café.

CONCLUSIONES

El acondicionamiento de plantas madre de *S. macrophylla* es favorable en condiciones de invernadero en un sustrato compuesto por suelo negro y agrolita 2:1 cuando este es inundado con 5 cm de agua. El tratamiento que permite obtener hasta 2.58 brotes y sanos por planta para ser cultivados *in vitro*, fue desinfección enjuague a chorro de agua con jabón en polvo, 30 min con 100 mL de agua destilada más una gota de microdin comercial y dos de jabón líquido, 2 min en agitación constante en etanol al 70% y 20 min en cloro comercial (6%) al 30% v/v, sin tener un efecto del fotoperiodo en el cuarto de incubación.

Cuadro 2. Promedios (n=16) de porcentajes de contaminación general, hongo, bacteria y oxidación de *Sagittaria macrophylla* zucc para el establecimiento *in vitro*.

	Contaminación %	Hongos %	Bacterias %	Oxidación %
Luz				
T1	93 aA	12 a	81 a	25 aA
T2	31 bB	0 b	31 b	19 bA
Oscuridad				
T1	62 aA	12 a	50 a	44 aA
T2	62 aA	0 b	62 a	0 bB
E.E.M.	6	7	9	10
valor–p para:				
tratamientos	***	*	***	*
fotoperiodo	*	NS	NS	***

Letras minúsculas y mayúsculas diferentes entre filas difieren entre tratamientos y bloques, respectivamente, *:P<0.05, ***: P<0.001. NS: no significativo (P>0.05). E.E.M.: Error estándar de la media.

AGRADECIMIENTOS

A los comuneros de la laguna de Chimaliapan por donar las plantas madre de *Sagittaria macrophylla*. A la Protectora de Bosques del Estado de México PROBOSQUE y al Departamento de Ciencias de la Alimentación de la UAM Unidad Lerma por el apoyo brindado para la realización de la investigación para fortalecer los procesos biotecnológicos en cultivos *in vitro* para proteger y propagar especies en peligro de extinción de la región mexiquense.

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AP AGRO PRODUCTIVIDAD

https://doi.org/10.32854/agrop.v13i10.1872

Assessment of land-use change in coastal areas through geographical information systems

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ABSTRACT

Objective: This article describes and analyzes the main concepts of coastal ecosystems, these as a result of research concerning land-use change assessments in coastal areas.

Design/Methodology/Approach: Scientific articles were searched using keywords in English and Spanish. Articles regarding land-use change assessment in coastal areas were selected, discarding those that although being on coastal zones and geographic and soil identification did not use Geographic Information System (GIS).

Results: A GIS is a computer-based tool for evaluating the land-use change in coastal areas by quantifying variations. It is analyzed through GIS and its contributions; highlighting its importance and constant monitoring.

Limitations of the study/Implications: This research analyzes national and international scientific information, published from 2007 to 2019, regarding the land-use change in coastal areas quantified with the digital GIS tool.

Findings/Conclusions: GIS are useful tools in the identification and quantitative evaluation of changes in land-use in coastal ecosystems; which require constant evaluation due to their high dynamism.

Keywords: GIS, vegetation cover, anthropogenic deforestation, urbanization, coastline.

INTRODUCTION

Coastal areas contribute to the socioeconomic evolution of humanity (Gómez *et al.*, 2016) due to their productive potential, where fishing, industry, tourism, and transport are developed as economic activities that address conflicts arising from the use and exploitation of their natural resources, such as soil, water, and landscape (Lara-Lara *et al.*, 2008).

Changes in coastal geomorphology increase when phenomena caused by population growth and socio-economic development coincide with environmental factors of aquatic ecosystems (De la Lanza *et al.*, 2013). In this sense, urban expansion entails an important land-use change in coastal areas (Barragán & de Andrés, 2016) causing effects that deteriorate its ecosystem resources.

Geographic Information Systems (GIS) are technological tools that display spatial information on land-use changes, providing quantitative information related to geographic information on coastal areas.

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 97-111.*
Recibido: *julio, 2020.* **Aceptado:** *octubre, 2020.*

Therefore, this research analyzes national and international scientific information, published from 2007 to 2019, regarding the land-use change in coastal areas quantified with the digital GIS tool, through integration, selection, and comparison of scientific articles available in databases of specialized data; to recognize and define knowledge gaps in the dynamics of these ecosystems.

MATERIALS AND METHODS

Scientific articles were searched using keywords in English and Spanish such as "land-use change in coastal areas", "geographic information systems in coastal areas" and "GIS in coastal areas" in Google Scholar and databases of scientific journals, as a network of Scientific Journals of Latin America and the Caribbean (Redalyc), Elsevier, Scopus, Web of Science, Scientific electronic library online (Scielo).

Articles regarding land-use change assessment in coastal areas were selected, discarding those that although being on coastal zones and geographic and soil identification did not use GIS. Thirty articles were selected, in which the aim of the study, and the main results were identified. Subsequently, a matrix was integrated with the variables: year, author, research institution, DOI/URL, place, title, contribution, line of research, objective, variables, cartographic inputs, type of analysis, GIS program, formulas, and method. Finally, the spatial distribution of the investigations was identified by georeferencing the reported coordinates.

RESULTS AND DISCUSSION

General features: coastal areas

Coastal areas have extensive interaction between the marine and terrestrial environment, where "dry" and "wet" portions of the territory interact through the coastline (Ortiz *et al*., 2010). This, derived from environmental, social, and economic services that this territory provides, having a wide demand for occupation, due to the development of food production, through fishing or aquaculture, transport, urbanization, construction and administration of ports, and industry, among others. Consequently, its growth and expansion directly affect associated ecosystems (Ponce & Botello, 2005).

Coastal lagoons

Diverse coastal ecosystems make up the Mexican coastline, such as lagoons, estuaries, swamps, and bays established in 11,592.77 km Among these, the coastal lagoons cover an area of 15,000 km^2 and, according to their hydric dynamics, there were identified between 125 and 130 bodies of water (Lara-Dominguez *et al*., 2011).

Coastal lagoons are semi-closed bodies of water, parallel to the coastline, with a permanent or seasonal marine communication, protected from the sea through of a bar, while sometimes a river flows into its headwaters (De la Lanza *et al*., 2013). A lagoon can integrate several bodies of water, including the estuary and one or more swamps originated as abandoned beds, which form a chain of bodies of water closed by modification of the channel of rivers or low dynamics lagoons and/or flooded by tides (De la Lanza *et al*., 2013).

The origin of the lagoons is recent and short-lived geological phenomena. The constant interaction with external phenomena, such as erosion, sediment deposition, fluctuations in sea and land levels, as well as river discharges and tidal ranges, prevent these complex, short-lived systems from reaching a final state of equilibrium (Farrera, 2004).

Lagoons are open systems with high primary productivity. Naturally, they are areas of refuge, feeding, and reproduction of around 50% of the species that constitute littoral fisheries (Toledo, 2005). In this sense, they are economically important by providing food resources, in addition to tourism and communication resources (De la Lanza *et al*., 2013; Farrera, 2004).

Vegetation

Mangrove forests or mangroves stand out as abundant vegetation of coastal lagoons, estuaries, and bays (Toledo, 2005). This vegetation includes perennial, bushy, or arborescent plants that reach up to 25 m in height (Ellis & Martínez, 2010). Around 64 mangrove species are known worldwide (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, 2008). Spalding (2010) presented the book "World Atlas of mangroves" where he recognized 73 species including some hybrids. The representative families of species from the Pacific Northwest are *Avicenniaceae*, *Combretaceae*, *Meliaceae*, *Rhizophoraceae*, and *Sonneratiaceae*. The Eastern Pacific species are *Avicenniaceae*, *Combretaceae*, *Pellicieraceae*, and *Rhizophoraceae*.

López *et al*. (2010) mentioned that mangroves develop in flooded soils, periodically or permanently with waters ranging from brackish to saline. Yáñez-Arancibia *et al.* (2014) pointed out that the function and environmental quality of tropical estuarine ecosystems depend on the variety of their biodiversity components.

Seagrasses (*Halodule beaudettei*, *H. wrghtii*, *Syringodium fiiforme*, *Thalassia testudinum*, and *Ruppia maritima*) are other vegetation associated with coastal areas (Toledo, 2005). These grasses are a functional group of approximately 60 species of underwater plants (Green & Short, 2003). Den Hartog (1970) carried out a worldwide geographic distribution and regionalization of seagrasses, identifying seven genera in tropical coasts, mainly *Thalassia*, *Halophila*, *Syringodium*, *Haladla*, *Cymodocea*, *Thalassodendron*, and *Enhalus*. The first four genera have representatives in both the tropical Atlantic and the Indo-Pacific, the remaining genera are restricted to the western Indo-Pacific. The high productivity and ecological value of seagrass meadows classify them as important ecosystems within coastal areas (Millán *et al.*, 2016).

Hydrophilic vegetation, popal, and tular type is another type of abundant vegetation in coastal ecosystems.

The first is a community of herbaceous plants from 1 to 3m in height composed of geniculata and plants of the genera *Calathea* and *Heliconia*, which cover swampy or stagnant freshwater surfaces from 0.5 to 1.5m deep (Ellis & Martínez, 2010; Rzedowski, 2006). The second is made up of herbaceous plants rooted on the shores of lakes, lagoons, or marshy lands, which have long, narrow leaves known as tulles; the genera present are *Typha*, *Scirpus*, and *Cyperus*, as well as the species *Phragmites communis* and *Arundo donax* knew as "reedbeds" (López *et al.*, 2010). Also grouped as hydrophilic vegetation are those plants that float on the surface of fresh and brackish water such as *Pistia stratiotes*, *Salvinia*, *Eichornia crassipes*, and different species of the genus *Nymphaea*, *Brasenia*, and *Nymphoides*, as well as small plants such as *Lemna*, *Spirodela*, and *Wolffia* (Ellis & Martínez, 2010).

Economic activities

The ecosystems of the coastal area play a historical and cultural role in the development and support of world societies. The wealth in natural resources of the coasts develops diverse activities of economic importance (Tovilla *et al.*, 2010). In the municipalities, along the coast, fishing predominates as a productive activity, in addition to being combined with agriculture, livestock, tourism, and industry (Ortiz *et al.*, 2010).

Fishing

Artisanal, riverine, or small-scale fishing is carried out in coastal waters, lagoons, and rivers; using artisanal fishing gear on smaller vessels equipped with outboard motors or artisanal propulsion systems (Marín, 2007). Among the fishing gear are gill nets, hand lines, longlines, and traps; built with a variety of materials, dimensions, and operating systems according to the species being fished (Jiménez, 2006).

Small-scale fishing is characterized by low investment in equipment and low potential for the catch. This activity is often referred to as "artisanal," meaning that the fishermen make their fishing gear. However, "small scale" describes fishing carried out in small boats, with or without a motor, with fishing gear manufactured outside their communities or handcrafted (McGoodwin, 2002).

Small-scale fishing is a productive activity that generates food and employment. In 2016, the Food and Agriculture Organization (FAO, 2016), reported a world fish catch of 90.9 million tons obtained through the work of 40.3 million fishermen, estimating participation of small-scale fishing in 90% of the catches and the jobs created (FAO, 2019). In 2018, 40.3 million people participated (full-time, part-time, or occasionally) in the primary sector of capture fisheries (FAO, 2018); reducing unemployment and alleviating poverty in some developing countries and regions (Soto & Quiñones, 2013).

A long chain of intermediaries responsible for the supply of inputs, transformation, and commercialization of the product, such as boat builders, outboard motors, fishing implements, fishing equipment, and filleting or processing industries (Jiménez, 2006; Ortiz *et al.*, 2010) depend indirectly on fishing.

Land-use change

The accelerated development of economic activities encourages disorderly growth in the coastal area, competition for space and the use of resources creates environmental conflicts that reduce the quality of services (Lara-Lara *et al.*, 2008).

The change in land-use is defined as the territorial use and controlled anthropic exploitation of coastal ecosystems that, due to the productive intensification processes carried out in the territory, are manifested in changes in the modes of productive use, followed by changes in land use (Fernández & Prados, 2010).

The main causes of habitat loss in coastal areas of Mexico are deforestation, land-use change for the urban, port, and tourist developments, as well as extraction of materials used as fill in construction (Lara-Lara *et al.*, 2008). The different environments and their services are maintained on adequate conditions by applying effective and efficient policies, based on evaluation according to the speed of the change that has occurred (López *et al.*, 2015).

Evaluation methods

One way of evaluating land-use change is to measure variations in vegetation and non-vegetation cover. Traditionally, the measurement of changes in vegetation cover and land-use is carried out superimposed on documents generated by remote sensing or thematic coverage mapping (Bocco *et al.*, 2001).

Technological advances in remote sensing equipment determine the precision in the analysis of changes in coverage and land-uses. Aerial photographs gave way to satellite images, which were enhanced using orthophotos and high-resolution satellite images (Fernández & Prados, 2010).

Three methods are available for interpreting remote sensing images: visual interpretation, pixel-based digital image processing, and image segmentation. In visual interpretation, analysts draw polygons around visible differences in satellite images on the computer screen (Puig *et al.*, 2002). Polygons are related to a land cover legend class. An advantage of this method is the possibility of updating recent images, using a base map starting with the initial date, while a disadvantage of this method is its subjectivity because it depends on the criteria of the analyst.

Pixel-based digital image processing performs supervised and unsupervised classifications of soil categories with computer algorithms. Each pixel is considered a unit of soil and is added to groups of similar pixels. When clustering is based on the digital number of the pixel it is called "unsupervised classification", whereas in "supervised classification", an analyst assigns representative pixels of a land cover to a class in the legend, also considering the knowledge that the analyst has of the unit.

In image segmentation, an algorithm assembles groups of pixels based on spectral responses and the set of rules established by the analyst. However, to avoid large-scale errors, careful linking of land cover with land-use field verification information is required (Hyman *et al.*, 2011).

Finally, the evaluation of the dynamics of land-use (time in years) is carried out using cartographic maps modifiable with Geographic Information Systems (GIS) constructed with interpretation of information from images produced by remote sensing (Ramos-Reyes *et al.*, 2004).

Geographic information systems

Geographic Information Systems (GIS) are digital programs that spatially identify and represent areas that are more susceptible to change, and also help to understand processes and dynamics of change that occur in land cover and land-uses of a specific territory (Camacho-Sanabria *et al.*, 2015).

Killeen *et al.* (2005) mention that a GIS integrates geographic spatial referential information, identifying related patterns between different sources of information, applicable to any work with geographic representation. A GIS is a base of information related to the coordinates of the shape X (longitude), Y (latitude), and Z (depth), which improves the user's decision-making capacity on research, planning, and management (Bhardwaj, 2009). Through GIS, changes in land-use are evaluated with the support of cartographic inputs, such as aerial and satellite photographs. The error in maps of changes in the classification of images is minimized by corroborating current land-uses in the field.

Trends in GIS research

The articles included in the database are (43%) in Spanish and (57%) in English; (27%) at the national level and (73%) at the international level. The cartographic inputs used are mainly images from the Landsat satellite (44%), followed by territorial maps (23%), and high-resolution aerial images (14%), the remaining percentage is made up of images from the Spot satellite, various satellite images, orthophotos, printed and digital cartography.

Managed GIS evaluate information through visual interpretation analysis

(57%), pixel-based digital image processing (30%), and image segmentation (13%). The most used programs are ArcGIS (47%), ENVI (12%), ArcView (10%), IDRISI (8%), in addition to QGIS, Quantum GIS, PCI Geomatics, ERDAS, ILWIS. The formulas applied are: FAO deforestation rate (10%), vegetation improvement indices (10%) location stability, and residence stability (7%), among others; the formulas were not specified in 47% of the articles.

The general process identified for quantifying land-use changes using GIS was:

- Data collection: obtaining cartographic inputs to be used in each study and supports for land-use change maps, such as cartographic maps, satellite images, or national maps.
- Image pre-processing: geometric, atmospheric corrections, or georeferencing with GIS.
- Image classification: identification of soil classes, image interpretation, and selection of methods: visual interpretation, pixel-based digital image processing, and image segmentation.
- Maps of change: once the land-uses have been classified in the images, maps of the period to be analyzed are obtained.
- Change map precision evaluation: verification of control points in the field to determine map reliability.
- Changes matrix: list of quantitative changes of soil classes when comparing in the evaluation period.
- Statistical analysis or rates of change: calculation of deforestation rates by type of class or some relation of the degree of fragmentation, or other formulas.
- Changes in land-use and land cover: obtaining results in changes and percentages of losses and gains.

GIS evaluate land-use change in all types of regions, however, this research focused on coastal areas, identifying the lines of research developed (Table 1).

Table 1. Research lines developed on the evaluation of land-use change in coastal areas.

1.	Land-use change and ecosystem services
2.	Land-use change on the shoreline
2.1	Land-use change and coastal vulnerability
2.2	Land-use change in coastal dunes and its relationship with water resources
3.	Land-use change on the mangrove surface
4.	Dynamics of land-use change
5.	Land-use and Land Cover (LULC)
6.	Land-use change and infrastructure

Geographical distribution of land-use change studies in coastal areas, through Geographic Information Systems

The geographical distribution of studies in coastal areas is concentrated in tropical regions with ideal climatic conditions for the establishment and development of mangroves; it is where five studies of land-use change were established. Most of the articles and lines of research are located in the Gulf of Mexico, meanwhile, two other studies are presented in the Pacific Ocean. The dynamics of land-use change are the line of research with the most published articles (11), due to the interest in knowing processes that generate changes in land-use and their feasibility of evaluation in any region.

In Asia, studies of the value of ecosystem services and land-use change are prioritized (3), while in the Gulf of Mexico there is an information gap, as there is only one article in this line. Coastline assessment studies are concentrated in Spain (2) and the Gulf of Mexico (1). In South America, two studies that evaluate the coastline with other aspects, one the coastal vulnerability in Peru and the other, the coastal dunes and their relationship with water resources in Argentina. The coastline is an issue to be evaluated, considering the studies described, the decrease in the coastline and its impact on it, coupled with modifying climatic and geomorphological conditions. Changes in the infrastructure of the Havana Malecón in Cuba, related to climatic, geomorphological, and anthropic conditions were studied. This highlights the usefulness of GIS in the evaluation of coastal areas, considering them a facilitating tool for their spatial analysis.

According to institutions of origin of the corresponding author (Figure 1), Geography is the main science applied to these studies in 40% of the participating institutions, Environmental Sciences have 30% of the studies, followed by Ecology, Geomatics and Informatics with 7% each and to a lesser percentage are Agronomy, Aerospace and Civil Engineering with 3% each.

Research lines developed

Land-use change and ecosystem services

Most articles referring to land-use change in coastal areas compare this with its relationship with ecosystem services and effects derived from anthropic changes in natural covers. The Millennium Ecosystem

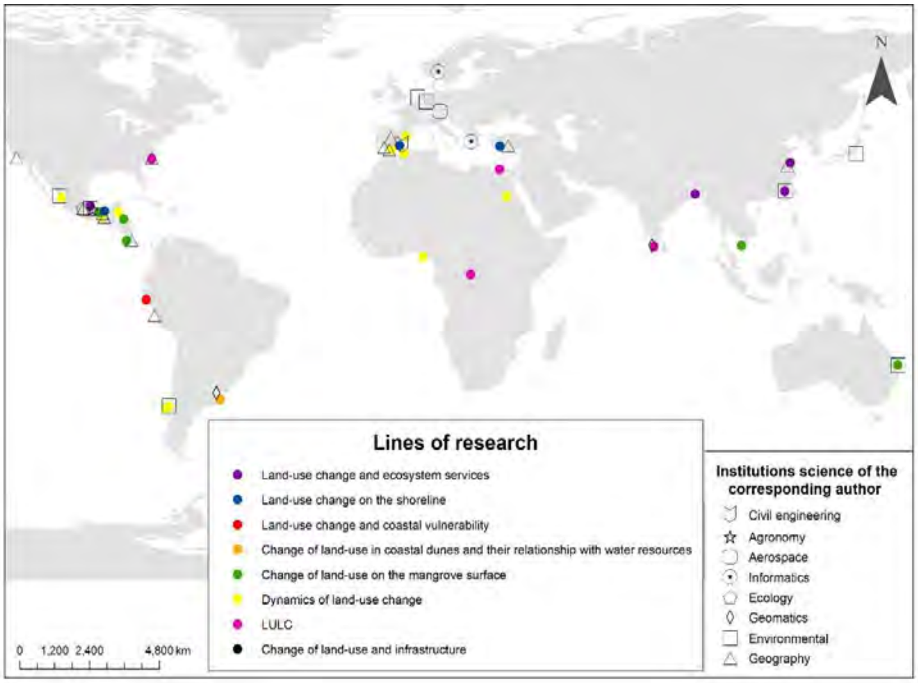

Figure 1. The geographical location of the lines of research and science related to the evaluation of land-use change in coastal areas.

Assessment program (Hassan *et al*., 2005) states that ecosystem services benefit the existence of humanity on the planet, being classified as *Provision*, goods produced by ecosystems; *Regulation*, services obtained from the regulation of ecosystem processes; *Cultural*, non-material benefits enriching quality of life, and *Support*, services necessary to produce all other services.

Lin *et al*. (2013) identified the dynamics of the environment on Xiamen Island, southeast China, by analyzing changes in land-use between 1973 and 2007, impact on ecosystem services and landscape patterns. The urban spatial expansion of the island was maintained using large areas of forests, farmlands, bodies of water, and coastal wetlands; continuously increasing land-use changes, decreasing the value of ecosystem services, and significant fragmentation in the landscape pattern.

Huq *et al*. (2019) conducted an assessment of ecosystem service values in southern Bangladesh from 1973 to 2014, where they analyzed land cover and how its variations affect ecosystem services. They identified 14 ecosystem services (Provision: agricultural inputs, economic benefits, fisheries and aquaculture, food production, medicinal plants, raw materials, water, and biodiversity. Regulation: local climate regulation, soil management, water purification and treatment, regulation of the water flow, extreme event management, Cultural: recreation); in addition to three soil classes (agricultural land, rural vegetation with human settlements and wetlands). The authors concluded that there is an increase in land-use in the first two classes and a decrease in the value of services derived from poor rural agricultural planning.

Xu *et al*. (2016) evaluated, between 1977 and 2014, ecological security, due to changes in land-use in the coastal area of Jiangsu, China; concluding that the land-use types most affected by the increase in the surface of urban areas (area in 2007 from 9.45% to 207.33% in 2014) are the intertidal zone and halophilic vegetation.

Mendoza-González *et al*. (2012) evaluated the effects of land-use change on the Gulf of Mexico coast and its relationship with the decrease in ecosystem service values, between 1995 and 2006. They concluded that the expansion of agriculture, livestock, along with urban expansion directly impacts on ecosystem services and their economic values. The ecosystems with the smallest surface area were beaches, dunes, and wetlands, being, however, the ones with the highest ecosystem value.

Considering the research described, agricultural and urban expansions reduce ecosystem services. Vegetation areas such as forests or wetlands when transformed into land-use, such as urban, agricultural, or livestock areas, do not provide ecosystem services of great value; in this context, the identification of viable maintenance areas in coastal planning will conserve the value of their ecosystem services.

Land-use change on the coastline

Pagán *et al*. (2018) analyzed trends of change in the past, the current state, and the future progression of various sandy beaches of the Levant Mediterranean, between 1956 and 2016. They concluded that historical anthropic pressures reduce the regression of the coastline, affecting its trend. As for artificially regenerated beaches, the original beach width has yet to be reached, showing a lack of adequate beach regeneration techniques.

In México, Hernández *et al*. (2008) determined Spatio-temporal variations of the coastline of the state of Tabasco (1973-2004), caused by variations in the mean sea level and the magnitude of the geographical changes in its territory. They concluded that the morphodynamic processes of the coastal area revealed a predominance of the retreat of the coastline over the effects of accretion processes.

Temiz and Durduran (2016) stated that the changes in the coastline in Lagos can be evaluated, in their study they estimated the changes in Lake Acigöl in Turkey between 1985 and 2015, where they reported a decrease of 129 to 42 km^2 in surface areas of water, losing 67.4% over a period of 30 years. The factors causing the changes were: evaporation of lakes due to heating, reduced rainfall, excessive consumption of water for agricultural use, and formation of other soil covers.

Land-use change and coastal vulnerability

Rondón and Tavares (2018) evaluated erosion vulnerability in the Peruvian coastline, classifying it into three categories, low, medium, and high, under a sea-level rise scenario in a Climate Change context, between 1962 and 2015. They developed four physical variables: *geomorphology*, *beach type*, *beach slope*, and *shoreline* changes and three anthropic variables (*land-use, beach width, and coastal settlement*). The variables with the highest vulnerability were *beach type* (72.2%) and *shoreline changes* (62.6%). Average vulnerability predominated in *beach slope* (50.7%) and *geomorphology* (51.1%). In the *beach width* variable, the situation is similar between medium vulnerability (43.2%) and high vulnerability (44.4%). In *coastal settlement* (81.9%) and coastal land-use (75%) variables, predominated low vulnerability. Using the *beach width* variable, related to the creation of beach infrastructures, a predominantly high or medium vulnerability was identified in all inhabited areas.

Land-use change in coastal dunes and its relationship with water resources

In Argentina, Carretero *et al*. (2014) analyzed the changes in recharge possibilities of the coastal aquifer in the Partido de La Costa, Buenos, Aires, according to the changes in land-use between 1973 and 2010, classified four types of land-use: *mobile dunes*, *semi-fixed dunes*, *fixed dunes*, and *urbanized areas*. The *mobile dune* was reduced, being replaced by settlements of cities located in front of the sea. The *fixed dune* replaces the *semi-fixed dune* in small sectors. A slight advance of a *fixed dune* on *mobile dune* was observed, interfered in certain sectors with *semi-fixed dune*, which is part of the natural process of evolution of the dune barrier. Urbanization and population growth have generated changes in the coastal dune environment. In the *mobile dune*, the infiltration of excess water through the sand is more significant, whereas in the *fixed dune* it is less due to its impermeable, vegetated, or compacted cover.

Considering the above, we point out that coastline vulnerability increases due to urban development expansion. The transformation of coastal dunes to urban areas are geographical modifications reflected in the decrease of the coastline and the reduction of the recharge of aquifers by infiltration.

Change in land-use on the mangrove surface

Sanabria-Coto *et al*. (2018) proposed a comprehensive geographical delimitation of the Mangrove of Nosara, Guanacaste, Costa Rica between 1944 and 2016, through the analysis of physiographic elements typical of the marine/continental context that favor the adaptation and distribution of the mangrove forest, composed of the red mangrove (*Rhizophora mangle*), black mangrove (*Avicennia germinans*) and majagua shrubs (*Hibiscus pernambucensis*). The area was associated with topographic reliefs, hydrographic patterns with obvious lateral changes, and unconsolidated sediments.

Hernández *et al*. (2016) in Tabasco, Mexico, evaluated the change of land-use in the period 1995 to 2008, identifying and quantifying the changes in location and mangrove areas. They identified three types of agroecosystems (AES) surrounding mangroves: coconut, coconut-pasture-livestock, and grass-livestock. In 1995 the mangrove area was 568.49 ha, for 2008 such surface showed an increase of 148.72 ha replacing part of the hydrophytic vegetation that occupied 543.48 ha in 1995 and was reduced to 355.10 ha in 2008. In 1995 the pasture-livestock agro-ecosystem had an area of 487.07 ha, in 2008 it lost 21.19 ha replaced by mangrove. In 1995 the coconut AES had 226 ha, reduced in 2008 to 36.37 ha, of which 189.73 ha were associated with the AES coconut-pasture-livestock and in some cases with mangrove swamps. The increase in the mangrove surface resulted from anthropic disturbances favoring its colonization, negatively impacting the grass-livestock AES.

Tuholske *et al*. (2017) determined the effects of the expansion of urban areas and tourism development in the mangroves of Roatán in Honduras, quantifying the land-use and change of coverage between 1985 and 2015. They concluded that at the end of the period the total coverage of mangroves decreased by 28.21%. The pixel-by-pixel comparison of the classified images indicated that 352.9 ha of *tropical forest*, 486.52 ha of *mixed agriculture*, and *tropical forest* (AMB), and 224.1 ha of *mangroves* were converted into *urban areas*. The conversion of 10% (486.5 ha) of AMB measured in 1985 to an urban area in 2015, suggested that urban development reduced the old agricultural areas. The *tropical forest* 4.3% (271 ha) in 1985 was converted to an *urban* area by 2015. The *urban* class expanded at an annual rate of 22.59 ha for the total of islands between 1985 and 2015. Mangroves throughout the island decreased by 7.50 ha per year. They found a significant relationship between urban growth and loss of mangroves between 1985 and 2015, the model indicated that an increase of 3 ha of an *urban* class is correlated with a loss of 1 ha of mangrove class.

In Australia, Brown *et al*. (2018) described, holistically and in the long term, drivers and processes of change in mangroves of the Maroochy River, Queensland between 1988 and 2016, evaluating the change in mangroves with remote sensing and traditional ecological knowledge. They interviewed seven key participants to describe and identify extensive changes in the distribution and extent of mangroves in the river system. According to participants, mangrove forests extended within the study area before European colonization (1860). Between 1988 and 2016, the total mangrove area decreased by 683 ha in the study area of 5,795 ha, representing a loss of 30%. Drivers of mangrove change in the study area include farming, urbanization, pollution, sewage discharge, and boating.

Hauser *et al*. (2017) mapped the dynamic fragmentation of central mangrove forests in the Ca Mau Peninsula, Vietnam between 2004 and 2013, based on a post-change analysis of land cover. They concluded that mangrove forests decreased from 44% in 2004 to 37% in 2009. The origin of the forest loss was the productive conversion to aquaculture areas from 2004 to 2009. Between 2004 and 2013, around 7,849 ha were reforested and rehabilitated, constituting, in 2013, around 31% of the total mangrove forest cover. For the subsequent years, 2009 and 2013, these "restored" forest patches were established by conversion of aquaculture land cover (6,933 ha).

By the above, irrational forest exploitation, hydrological modification due to deviations of river channels, dredging, or filling of mangrove areas for port construction, aquaculture, and tourism infrastructure, are highlighted among the causes of the physical destruction of the mangrove habitat (Beltrán *et al*., 2005). Since anthropic factors affect the ecosystem services of the environments, their evaluation and monitoring become even more relevant.

Dynamics of land-use change

In Spain, Ramírez-Cuesta *et al*. (2016) explained changes in the mouth of the Ebro River, between 1957 and 2013, establishing evolutionary trends determined by a modification of various geomorphological units. They concluded that the geomorphological units with the greatest increase in area in the period 1957-1984 were those occupied by *anthropized deltaic plain* (ADP) and *sandbar*, *active spit* (S), the diminished class was *fixed dune & vegetated Aeolian mantle* (FD). In the 1984-2013 period, the *littoral ridge* (LR) and FD categories increased, while *interdune depression* (ID), *beach* (B), and S decreased. The most significant processes during the study were marine erosion and agricultural expansion, whose greatest magnitude was during 1957-1984. Concerning to geomorphological changes, the period 1957-1984 was more active than during 1984-2013.

Peña-Cortés *et al*. (2011) analyzed the relationship between geographical distribution, environment, and anthropogenic factors, in the coastal area of the Araucanía Region, in Chile, between 1968 and 2009. They identified 427 fragments of swamp forest with sizes between 0.25 ha and 936 ha, representing a total area of 7,675 ha, equivalent to 4.6% of the evaluated territory. The small size and irregular shape of the vegetation fragments, in addition to the use of surrounding agricultural land, place high pressure on most of the forest distribution.

Berlanga *et al*. (2010) analyzed patterns in vegetation cover and land-use change in the north coast of Nayarit, Mexico, in the period 1973-2000, the percentage of change was 30% at the regional level. The dominant class was the forests, covering in 1973 (48%) and in 1990 (40%) of the total area, this condition changed in 2000, where agriculture dominated by 41%.

Concerning to natural wetlands, these covered 19% of the total area every year, while artificial wetlands, represented by shrimp farming (they were recorded from 1990) covered a small percentage of the region, the same as in 2000. The secondary vegetation class had a lower capacity to stay in the same place during the analyzed periods.

In Kenya, Were *et al*. (2013) characterized land cover dynamics, between 1973 and 2011, in the eastern Mau forest and the Lake Nakuru basin. They concluded that the main land cover types in the lake's drainage basin were forest-shrublands, grassland, croplands, built-up land, bare land, and water bodies. The first period was from 1973 to 1985, the second from 1985 to 2000, and the third from 2000 to 2011. Forest- shrublands, grassland, and cropland had higher magnitudes of change compared to built-up land, bare land, and water bodies during the three periods. Forest- shrublands and grasslands decreased, 428 km^2 and 258 km^2 respectively, at annual average rates of 1% each, while cropland and built-up land expanded by 660 km^2 and 24 km^2 at average annual rates of 6% and 16% respectively. Built-up land was the most dynamic, growing at average annual rates of 2%, 17%, and 5%, in the three periods, respectively.

Fernández and Prados (2010) evaluated land-cover and land-use changes in the Guadalfeo river basin, Spain (1975-1999). The results of which are as follows: in 1975, the dominant categories were shrublands-grassland and conifers with an area of 78,395.81 ha, which represents 60% of the total area. The rainfed and irrigated categories with 43,032.14 ha, followed by rainfed olive groves (2,372.69 ha) and vineyards (770.96 ha) made up the second group. In 1999, the dominant category in coverage and land-use was shrublands-grassland, representing 66% of the surface, with an area of 84,875.14 ha. In 1975 the area of this category doubled to 44,739.18 ha. The next category in order of extension is dry land, with an area of 15,708.69 ha and representativeness of 12%. The rest of the categories, up to eleven different coverage, were dispersed in the basin with less than 5% representation. The southwestern area was more dynamic and prone to the introduction of changes in agricultural land-uses, in turn, the northeast strip tends to the stability of uses in by the conservation objectives. However, progressive shrublands were observed at the expense of the coniferous patches existing in 1975.

In Mexico, Rosete *et al*. (2009) analyzed land-use and vegetation change in the Baja California Peninsula (1978 to 2000), at the level of vegetation types and a 1:250 000 scale. They identified that 92.3% of the territory remained unchanged between 1978-2000, while 7.7% presented some change in vegetation cover. At the regional level, the main land-use and vegetation changes are of anthropic origin caused by urban growth and the expansion of agricultural activities, mainly in the extreme north and south. Types of vegetation and land-use with the greatest decrease were xerophilous scrub, grasslands, rainfed agriculture and irrigation, and humidity agriculture. Most of the changes were in the coastal plain, associated with agricultural areas and human settlements, as well as in adjacent areas with arboreal vegetation (forests and low forests).

Meanwhile, Peña-Cortés *et al*. (2009) geospatially evaluated the change in land-use (1980 and 2004), on the coastal edge of the La Araucanía region in Chile. The most important land-use categories in 1980 were native forest and agricultural matrix (with 21% and 44% respectively). In 2004, forest plantations underwent the most important factor of change, starting at 1% and reaching 9% of the surface with the growth of over a thousand percent. The original land cover of the coastal edge of La Araucanía changed dramatically; initially, as a result of the authorization of land for agricultural-livestock activity and later, due to the impact of forest plantations.

Kuenzer *et al*. (2014), between 1986 and 2013, characterized the geography of the Nigerian Delta using variables such as land surface dynamics, coastline, creation of channels for oil exploitation, and gas flaring. They concluded that urban areas expanded 1,516 km^2 from 1986 to 1,730 km^2 by 2013 and the agricultural area increased 31,700 km^2 to 33,895 km^2 respectively. The area of forests and swamps decreased from 18,325 km^2 to 15,408 km^2, while the mangrove forest remained with 10,311 km^2 in 1986 and 10,072 km^2 in 2013, with a period of decline until 2003 and stabilized afterwards thanks to protection activities and rehabilitation. Analysis of shoreline dynamics revealed that annual accumulation rates were slightly higher than erosion rates, however, starting in 2003, they increased in almost all coastal states. Annual rates of coastal change at discrete locations vary from 64.8 m of coastal erosion to accretion rates of 59.8 m. Highlighting that the impact of the oil industry activities in the Delta is remarkable.

The network of access channels, within the mangrove ecosystems of the three, affected Delta states, expanded from 230.4 km in 1986 to 269.5 km in 2003, and 349.3 km in 2013.

For their part, Díaz-Gallegos and Acosta-Velázquez (2009), in the Chetumal Bay in Mexico, determined the magnitude, dynamics, and distribution of processes of land cover and use changes in the landscape surroundings of the bay from 1990 to 2005. The natural covers dominated the distribution of land-use and vegetation around the bay, with the natural vegetation class being the largest (291,305 ha), followed by wetlands and mangroves. Agricultural and livestock activities were concentrated in the south-western part of the Bay. The medium and lowland semi-deciduous forests decreased about 950 ha each year, with a rate of −0.32% during the analysis period. Mangroves that registered the highest annual change rate (−0.65%), decreased approximately 300 ha each year, being concentrated in the south of the Bay. During the study period, the agricultural / livestock classes, human settlements, and secondary vegetation increased their areas. The secondary vegetation class was less stable in its location (51%), so most of its patches were not spatially maintained in the area; this class displaced natural vegetation (8,225 ha) and agricultural / livestock (1,907 ha) classes. Likewise, the agricultural / livestock class was displaced by the natural vegetation and secondary vegetation classes, showing the temporal dynamics of agricultural activity in the study area.

Gordillo-Ruiz and Castillo-Santiago (2017) recognized factors causing changes in the Sabinal river basin in Chiapas, between 1992 and 2009, based on socioeconomic trends and landscape patterns, through the evaluation of the change in coverage of land-use. Their results showed that 72% of the basin territory has the same type of land cover and 28% had some type of change. The forests showed a decrease of 663 ha, which represents 8.7% of the original area and a deforestation rate of −0.5%. Human settlements expanded by 4,331 ha, equivalent to a growth of 63%. The most important land-use change processes in the basin were the forest for agricultural land and the expansion of the urban area in areas of secondary vegetation and crops.

Ramos-Reyes *et al*. (2016) identified the dynamics of land-use change, between 2000 and 2010, in Comalcalco, Tabasco, Mexico. In 2000, the authors identified 6 land-uses: *grasslands*, *agriculture*, *hydrophilic vegetation*, *mangroves*, *bodies of water*, and *urban areas*. There were specifically located, to the north, mangroves and lagoon bodies; to the northeast, a small portion of *hydrophilic vegetation*, *agricultural use*, and *grasslands*; the central and southern portion was less diverse in terms of land-uses, it is limited to *grasslands*, *agricultural*, and *urban areas*, while the most extensive uses are grassland and agriculture. In 2010, the same six land-uses of 2000 and a greater number of urban centers were identified, highlighting the expansion of the municipal seat in places corresponding to agricultural and livestock use. The main land-uses were conserved both in number and in area, although the change in the use of *grasslands* in *agriculture* and *urban areas* and from *hydrophilic vegetation* to *grasslands* stands out.

We consider that the dynamics of land-use change is the area where the most published articles were found, due to the interest in identifying patterns or transitions of land-use change that generate these transformations. In them, the substantial negative effect exerted by the dynamics of agricultural activity in the study regions is argued, precisely due to the increase in the use of areas of natural vegetation or grasslands as areas of agricultural use; in addition to identifying secondary vegetation as a substitute for other types of vegetation due to its historical displacement capacity.

Land-use and Land Cover (LULC)

In India, Kaliraj *et al*. (2017) evaluated the decadal changes and their transformations of Land-Use and Land Cover (LULC) features, on the South West coast of Kanyakumari, where they identified 12 LULC. The areas of each face land cover, cultivable lands, plantation, fallow land, and barren land are converted into built-ups, the latter with an area of 17.54 km^2 in 2000 and represented 6.01% of the total study area increasing to 39.22 km^2 in 2011 occupying a surface area of 13.43% of the total area. Most of the land cover features were transformed into built-ups and settlements without considering the negative impacts on coastal systems.

In the United States, McCarthy and Halls (2014) evaluated changes on Masanboro Island in North Carolina between 2002 and 2010. Screening results indicated that nearly 20% of the study area experienced change. The soil class intertidal marsh underwent the greatest change among the smallest habitat classes, while the upland scrub underwent 84% changes. The net change (absolute value of the difference in profit and loss) of the Island was 5%, but the swap change (the difference between total change and net change) represented more than 14%.

On the northern Egyptian coast, Shalaby and Tateishi (2007) analyzed changes in land cover and land-use over a 14-year (1987-2001). They concluded that the study area underwent a severe change in land cover as a result of multiple agricultural or tourism development projects. Also, urban settlements and agricultural lands increased in extension considerably, while the area of natural vegetation decreased. The degradation of natural vegetation due to overgrazing, the inter-annual variation in the amount of rainfall, the water record due to poor irrigation management and wind erosion were identified as land degradation processes.

In Greece, Kolios and Stylios (2013) investigated the LULC changes in the southwest part of the Epirus region, called Preveza, between 2000 and 2009. They concluded that the *Barren land and Urban fabric-beaches-roads* classes increased by 4.18% and 1.76% respectively. On the other hand, *Forest-high vegetation* (0.68%) and *Low vegetation* (5.2%) decreased. The *Water land* class remained almost unchanged. The 16.15% and 7.62% classified as *Barren land* in the initial state year (2000), were changed to *Low vegetation* and *Urban fabric-beaches-roads* classes respectively. However, 76.03% of the *Barren* land in the initial state remained in the final state (2009) without changes, determining that the change 16.15% of the spatial change of the *Barren* land class to *Low vegetation* is overestimated due to possible irrigation from agricultural areas, which changed humidity levels on the earth's surface; consequently, their spectral signatures mislead the classifier. There is also a notable change from the *Barren* land class to the *Urban fabric-beaches-roads* class that shows a clear trend towards LULC changes.

By previous works, the LULC verifies the change from natural covers to those transformed to land-uses and the percentage of each one in the study area. Land covers such as vegetation, secondary vegetation, and grasslands are transformed into urban or agricultural land-uses, although the latter use tends to transform from agricultural to urban land.

Change of land-use and infrastructure

In Cuba, Remond *et al*. (2018) mapped the infrastructure of the Havana Malecón and analyzed meteorological events and geographic variables most influential in its deterioration, between 1990 and 2012, using remote sensors, as well as spatial and statistical analysis. The variables analyzed (depth of the sea, the surface of the reef, and distance from the Malecón to the coastline, the height of the Malecón above mean sea level, and orientation of the coast) followed the direction of the prevailing winds on the coast. The Malecón presented some signs of deterioration in 56.9% of its surface, while the most deteriorated areas were located in the northwest of the sector. The orientation of the coast and deteriorated areas showed a high correlation, indicating that the deteriorated areas are perfectly oriented concerning the wave trains caused by the arrival of cold fronts, subjecting the Malecón to an incessant process of wear and tear. The distance from the Malecón to the coastline exerted some influence, since, in sectors of the Malecón separated from the coastline, the energy of the wave trains is dissipated during seasons of cold fronts.

The foregoing shows that the evaluation of coastal areas serves for the preservation or improvement of maritime infrastructure, since environmental factors deteriorate it rapidly, in addition to being applied in determining future impacts with projections of sea-level rise.

CONCLUSIONS

Uncontrolled urban growth and human pressure constantly affect coastal territories, due to the economic, environmental, and social importance of all the ecosystem services provided. Urban settlements and agricultural use are the changes in land-use that cause the greatest impacts on coastlines, whose dynamics begins with the conversion of land from natural vegetation to agricultural use and later to urban or anthropic use; where there is hardly a transition to another use, as in agriculture, which can change to vegetation or mangroves according to the presence of suitable geomorphological conditions.

Environmental impacts in coastal areas are minimized by identifying areas more suitable for different land-uses. These areas often lack a coastal order; if they exist, due to the performance of economic activities, they prioritize the transformation of the territory without considering impacts on the habitat, reducing the quality and value of the ecosystem services provided.

Geographic Information Systems are useful tools in the identification and quantitative evaluation of changes in land-use in coastal ecosystems; which require constant evaluation due to their high dynamism. Updated information is obtained, from baselines or monitoring through change behavior studies, useful in decision-making by those responsible for carrying out adequate mitigation, territorial planning, protection, and preservation actions for ecosystems.

RECOMMENDATIONS

Geographic Information Systems are a tool for evaluating change in study areas of coastal ecosystems. However, to perform more efficient analyzes, technical improvements are required by incorporating statistical analyzes or appropriate extensions to various approaches.

Coastal areas offer diverse ecosystem services that favor urban expansion; however, the anthropic activities carried out affect such services. Various studies evaluate the change in land-use relating to the decrease in the value of these services; China, for example, established the importance of this line of research, unlike Mexico, where studies of this type are needed, as well as the integration and spatial analysis of information through GIS.

After the filling of wetlands or mangroves, the natural habitats of the coastal areas are reduced, changing to urban or agricultural land-uses, causing loss of viable subsistence spaces for native or migratory species. Therefore, it is necessary to identify the relationship between the decline in species of fauna and flora with the change in land-use, since its information is limited.

Climatic and geographical conditions, coupled with the increase in transformations to urban areas, rapidly modify the coastline of coastal areas. According to the studies analyzed, there is a decrease in accretion effects on the coastline; this reduction can affect nearby human settlements. This change is visualized by analyzing the demographic increase and the climatic and geographical conditions that affect the coastline; in addition to serving to carry out a correct territorial organization. Likewise, through GIS, coastal vulnerability indices can be built and areas of a high risk of flooding due to sea-level rise can be detected.

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Seed analysis of *Dichromanthus aurantiacus*, terrestrial orchid from Toluca valley, México

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ABSTRACT

Objective: The objective of this work was to analyze the viability and germination of *Dichromanthus aurantiacus* seeds, a terrestrial orchid from Toluca valley, México.

Design/methodology/approach: The size and color were evaluated. Two methods determined the viability: 1) the tetrazolium test (imbibition for 24 hours in the water, 2 hours in calcium hypochlorite ($CaCOCl_2$), and drops of Tween-80). 2) the asymbiotic seed germination by *in vitro* culture (imbibition for 24 hours in the water, and the concentration of MS medium plus natural extracts).

Results: The seeds of this specie showed approximately 0.2 mm long and 0.05 mm wide; they possess an embryo and a brownish testa. There were significant differences ($P<0.05$) between the treatments finding a positive effect with the tetrazolium test, achieving up to 91.4% viability. In the *in vitro* germination, the imbibition of the seeds favored contamination. The concentration of MS and the addition of natural extract presented significant differences ($P<0.05$), the 50% MS plus 10% of coconut water showed up to 92.8% of germination at 60 days.

Study limitations/implications: The results are preliminary of a long-term experiment.

Findings/Conclusions: The seeds of *Dichromanthus aurantiacus* showed brown testa and an oval embryo with dimensions of 0.2 mm long and 0.05 mm wide. The tetrazolium test's viability showed 91.4% viability when they were soaked in sodium hypochlorite solution ($CaCOCl_2$) for two hours, 24 hours soaking in tetrazolium solution (1%) plus two drops of Tween-80. The asymbiotic *in vitro* culture showed up to 92.8% germination in 60 days using MS medium at 50% enriched with 10% coconut water.

Keywords: *Dichromanthus aurantiacus*, tetrazolium, asymbiotic germination, *in vitro* culture, natural extracts.

Agroproductividad: *Vol. 13, Núm. 10, octubre. 2020. pp: 113-118.*
Recibido: *julio, 2020.* **Aceptado:** *octubre, 2020.*

INTRODUCTION

There are around 35,000 orchids worldwide, of which approximately 60% are epiphytic, and the rest are terrestrial. In Mexico, about 1,400 species are registered, and grouped into 164 genera, around 40% are endemic (Hágsater *et al*., 2015) in the national territory. The states of Chiapas, Oaxaca, Jalisco, Guerrero, Veracruz, Mexico, and Michoacán have the highest number of species recorded (Szeszko, 2011; Castañeda-Zarate *et al*., 2012; CONABIO, 2013; Carvajal, 2014; Hassler and Rheinheimer, 2016). According to (Szeszko, 2011), there are 251 species grouped into 71 genera in the state of Mexico, of which 55% are terrestrial, and the rest epiphytic or lithophytic species. One of these terrestrial orchids is *Dichromanthus aurantiacus*; the botanical characteristics of *Dichromanthus aurantiacus* are: thick and tuberous roots, it has 6 or 7 leaves organized throughout the plant, it has an inflorescence of orange color with 7 to 15 successive flowers up to 2.5 cm long and arranged horizontally; the blooming occurs in from June to August, and it loses the leaves at the beginning of winter. In Mexico, it grows between 1,500 to 3,200 meters, and is located in grasslands, open bushes, and next to the highways (Bertolini *et al*., 2012; García-Martínez and Rodríguez, 2018; Martínez-De la Cruz *et al*., 2018). In the state of Mexico, there are reports of sightings in Zacualpan, San Simón de Guerrero, Zumpahuacán, Tenancingo, Coatepec Harinas, Amatepec, Texcaltitlán, Donato Guerra, Valle de Bravo, Ixtapan del Oro, Almoloya de Alquisiras, Amanalco, Luvianos, Santo Tomás, Villa de Allende, Ocuilan, Temascaltepec, Ixtapan de la Sal, Tejupilco Sultepec, Toluca, Malinalco (Szeszko, 2011; Martínez-De la Cruz *et al*., 2018).

The evolution of this orchid is surprising; its structure has evolved to be pollinated by hummingbirds; however, bumblebees interferes with the pollination process because they are related to the fungus of the genus *Cantharellales*, and fungal genus *Ceratobasidium* and *Thanatephorus* (Rasmussen, *et al*., 2015). In symbiotic germination, the fungi provide the embryo with the necessary nutrients for its development (Rasmussen *et al*., 2015); however, in asymbiotic germination, it is essential to add nutrients to fulfill the same function as the symbiotic fungus germination. These nutrients required for growth are supplied utilizing a culture medium either in solid (agar) or liquid state (De Lucas, 2004; Levitus *et al*., 2010).

In the natural state of orchids, germination occurs in 5 to 10% (Morales, 2018); for this reason, seeds are inoculated with fungi to stimulate plant growth (symbiotic seed germination), or by mechanical scarification, and nutrients added (asymbiotic seed germination). According to CITES (SEMARNAT, 2010), *Dichromanthus aurantiacus* could be threatened because the urban area is expanding. Therefore, this study's objective was to evaluate the viability and germination of *Dichromanthus aurantiacus* seeds for conservation purposes.

MATERIALS AND METHODS

The seeds were donated in December 2019 by the Orchid Seed Germplasm Bank of the State of Mexico (Faculty of Agricultural Sciences of the Autonomous University of the State of Mexico). The seeds were collected in San Cayetano de Morelos Toluca, Mexico, in 2018. They were stored in an amber glass bottle and refrigerated at 10 °C. The shape, size, and weight of the seeds were evaluated (Aguilar-Morales, 2016); for this analysis, 0.2 grams were weighed in triplicate. The length, width, and number of seeds per gram were recorded.

Seed viability

Two tests were carried out; the tetrazolium test (Bohm, 1996; Aguilar-Morales *et al*., 2016) and asymbiotic germination by *in vitro* culture. Briefly, tetrazolium solution 1% (2,3,5-triphenyl-2-H tetrazolium chloride) diluted with distilled water (pH 6±0.1) was stored in an amber bottle and refrigerated until later use. Six treatments were evaluated in triplicate; the seeds were placed in filter paper envelopes by repetition. Treatment 1 (T1): seed soaking for 24 hours in distilled water, then soaking in calcium hypochlorite ($CaCOCl_2$) for two hours to finish with a 24-hour soak in tetrazolium. Treatment 2 (T2): seed soaking for 24 hours in distilled water and subsequently soaking for 24 hours in tetrazolium. Treatment 3 (T3): two hours soaking in $CaCOCl_2$ + 24 hours soaking in tetrazolium + 2 drops of Tween-80. Treatment 4 (T4): 24 hours soaking in tetrazolium + 2 drops of Tween-80. Treatment (T5): 24 hours soaking in tetrazolium. Treatment (T6): 24 hours soaking in distilled water + two hours soaking in $CaCOCl_2$ + 24 hours in tetrazolium + 2 drops of Tween-80. The seeds viability (%) was obtained, dividing the number of embryos stained by the total number of seeds and multiplying by 100. An optical microscope (Leica Microsystems Ltd. Leica Application Suite V4) was used for this purpose, and ten fields were recorded per repetition of each treatment.

Asymbiotic germination

The Murashige and Skoog (1962) culture medium (MS, 100% and 50%) enriched with natural extracts were evaluated: 0% extracts (T), 10% banana, 10% coconut water, and activated carbon 1 g L^{-1} during four germination periods (15, 30, 45, 60 days). All treatments were supplemented with 30 g L^{-1} of sucrose and 7 g L^{-1} of bacteriological agar. The pH was adjusted to 5.7±0.1, and 25 ml of the medium were added to Gerber-type flasks and sterilized for 15 minutes at 120 degrees °C. The seeds were weighed (0.2 g), placed in filter paper envelopes, and disinfected in a 0.5% sodium hypochlorite solution plus a drop of Tween-80 for 10 minutes and rinsed with sterile water until the foam despair. In the laminar flow hood, the seeds were rinsed with sterilized distilled water three times, and the sowing was carried out in the culture media of each treatment. The stage identified as late protocorm was considered as seed germination (Shimura and Koda, 2004).

Statistical analysis

A complete randomized design was used to evaluate the viability of the seeds. The general linear model was:

$$Y_i = \mu + T_i + e_i$$

where μ = is the overall mean, A_i = is the *i*-th treatment effect (*i* = T1, T2, T3, T4, T5, T6), and e_{ijk} = is the *i*-th random error effect. A bifactorial design with completely randomized blocks was used to evaluate the germination percentage in four periods. The general linear model was:

$$Y_{ijk} = \mu + A_i + B_j + C_k + A_i * B_j + e_{ijk}$$

where μ = is the overall mean, A_i = is the *i*-th factor *A* effect (*i* = MS-50%; MS-100%), B_j = is the *j*-th factor effect (*j* = T, P10, C10, CA1), C_k = is the *k*-th random block effect (*k* = 15, 30, 45, 60 days), $A_i * B_j$ = is the i_j-th interaction of the Factor *A* and *B*, and e_{ijk} = is the *ijk*-th random error effect. When the ANOVA identified statistical differences (P<0.05) among the treatments, we applied the Tukey test for multiple means comparisons. The SAS System for Windows 9.0 was used for the statistical analysis.

RESULTS AND DISCUSSION

The seeds of *Dichromanthus aurantiacus* have two basic structures: testa and embryo. This species' seed coat poses brown color that surrounds the embryo; the shape of its cells is hexagonal, forming a network—the analyzed seeds presented 0.2 mm in length and 0.05 mm wide approximately (Figure 1).

These results are in line with a study of four terrestrial species from Argentina (Lallana and Di Persia, 2018); the seeds showed a transparent testa with brownish to dark brown cell walls with dimensions ranging from 0.395 mm to 0.805 mm in length and 0.091 to 0.226 mm wide. The seeds of *Phaius tankervilleae*, a terrestrial orchid (Ranquel *et al*., 2017), presented a transparent testa with cells forming a network and an oval embryo of 0.469 to 0.5597 mm length. For *Prosthechea*, measurements of 0.7 mm in length and 0.03 mm wide (Banda-Sánchez *et al*., 2017). Lallana *et al*. (2016) reported dimensions in ten species of native orchids ranging from 0.202 to 0.805 mm in length by 0.056 to 0.226 in wide.

The tetrazolium test showed no significant differences (P>0.05), suggesting that a similar number of embryonic seeds were under evaluation in the tetrazolium test. There were highly significant differences in the percentage of seeds viability (P<0.0001) among the treatments. The highest percentage of seeds viability was observed in T3 with 91.4%, followed by T4 with 90.5%, and the lowest percentage was observed in T2 with 23.2%.

The embryos' staining presented significant differences (P<0.0001). The imbibition of the seeds 24 hours in distilled water affected the embryos' staining; when they were soaked in water, the viability was 55.5%; on the other hand, if the seeds were not soaked, the viability was 75.6%. It was observed a positive effect of the $CaCOCl_2$ solution, obtaining up to 78% viability compared to not using the solution (52.9%). Similarly, the addition of Tween-80 increased the embryos' staining from 43.6% to 87.5%. For *Prosthechea* sp., an epiphytic orchid (Banda-Sánchez *et al*., 2017), the tetrazolium concentrations and immersion time were evaluated, showing the highest

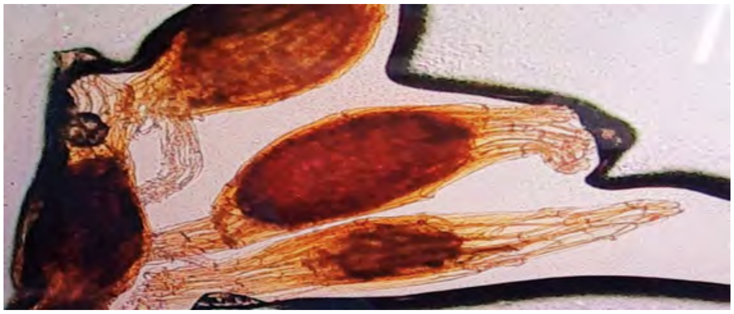

Figure 1. *Dichromanthus aurantiacus* seeds.

percentage of viability (92%) using 1% tetrazolium solution for 120 minutes. In *Encyclia adenocaula*, an epiphyte orchid (Aguilar-Morales *et al*., 2016), the $CaCOCl_2$ solution affected the seeds' viability negatively, decreasing from 50 to 24%, and the Tween-80 did not affect the viability significantly ($P>0.05$). In some species, better precision in the viability has been reported using the tetrazolium test. Mercado *et al*. (2015) performed a study in ten species of orchids (epiphytes and terrestrial) using tetrazolium and indigo carmine tests; his results suggested that tetrazolium test is more efficient in determining seed viability. Karol *et al*. (2015) reported 70% viability in *Comparettia falcata* (epiphyte) employing the 1% tetrazolium. Imbibition for 24 hours in water increased the seeds' viability in *Trichocentrum jonesianum* (Liliana and Garcia, 2013). Ossenhach *et al*. (2007) reported that $CaCOCl_2$ considerably affected the viability of five orchids seeds (all epiphytes); these findings contradict our results in the present study. It cannot be generalized that $CaCOCl_2$ has a positive effect on the viability of terrestrial orchids; however, several studies can be carried out with a considerable diversity of terrestrial orchid seeds due to there are no specific studies regarding this pretreatment for terrestrial orchids.

Undoubtedly, the best orchid seed germination technique is the *in vitro* culture; this could be as asymbiotic or symbiotic approach; the asymbiotic method has shown promising results in several studies (Flores-Escobar *et al*., 2011; Menchaca *et al*., 2012; Salazar and Cancino, 2012; Dalzoth and Lalllana, 2013; Billard *et al*., 2014; Karol *et al*., 2015; Aguilar-Morales *et al*., 2016; Lallana *et al*., 2016; Banda-Sánchez *et al*., 2017). The asymbiotic germination by *in vitro* culture of *D. aurantiacus*, presented significant differences ($P<0.0001$) due to the concentration of the base medium (factor A), MS50% showed up to 44.9% and MS100% up to 22.8% germination at 60 days. Regarding the addition of natural extracts (factor B), there were significant differences ($P<0.0001$) in the four periods; coconut 10% water extract showed up to 78.8% germination at 60 days, and the banana extract considerably affected the asymbiotic germination. There was a highly significant difference among periods (Table 1), MS50-C10 showed similar germination as viability in the tetrazolium tests (90 to 92%); on the other hand, MS100-P10 presented the lowest germination in the four periods (Table 1).

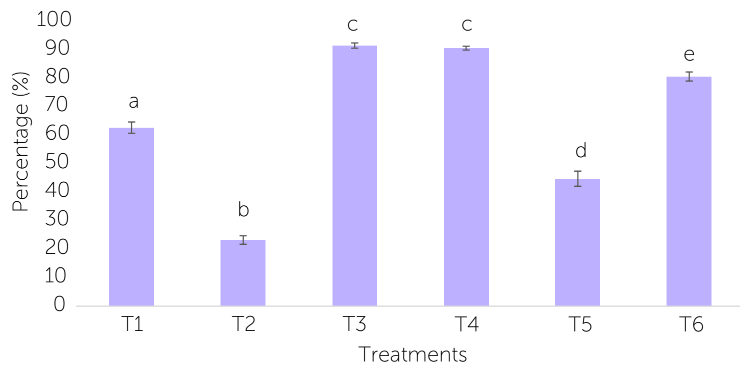

Figure 2. Percentage of seeds viability (*Dichromanthus aurantiacus*) in the tetrazolium test 1% (2,3,5-triphenyl-2-H tetrazolium chloride). T1: 24 hours soaking in distilled water + two hours soaking in calcium hypochlorite ($CaCOCl_2$) + 24 hours soaking in tetrazolium. T2: 24 hours soaking in distilled water + 24 hours soaking in tetrazolium. T3: two hours soaking in $CaCOCl_2$ + 24 hours soaking in tetrazolium + 2 drops of Tween-80. T4: 24 hours soaking in tetrazolium + 2 drops of Tween-80. T5: 24 hours soaking in tetrazolium. T6: 24 hours soaking in distilled water + two hours soaking in $CaCOCl_2$ + 24 hours soaking in tetrazolium + 2 drops of Tween-80.

It is essential to mention that the seeds' germination is asynchronous because it was possible to observe all the stages (Figure 3) for this specie from green seeds to complete seedlings, as mentioned in Aguilar-Morales *et al*. (2016).

The germination was lower in our study than Suárez-Quijada (2010) for the same specie; he observed 74% of germination at 28 days (stage C). Dalzotto and Lallana

Table 1. Asymbiotic germination (%) *in vitro* culture of *Dichromanthus aurantiacus* through four germination periods.

Treatment	Germination periods (days)			
	15	30	45	60
MS50-T	33.80 ^{a}	33.80 ^{a}	40.00 ^{a}	67.60 ^{b}
MS50-P10	2.40 ^{f}	4.80 ^{d}	6.00 ^{d}	9.60 ^{d}
MS50-C10	19.60 ^{b}	27.20 ^{b}	34.80 ^{b}	92.80 ^{a}
MS50-CA1	4.80 ^{d}	4.80 ^{d}	5.20 ^{e}	9.60 ^{e}
MS100-T	0.40 ^{g}	4.80 ^{d}	13.20 ^{c}	18.80 ^{d}
MS100-P10	2.40 ^{f}	2.40 ^{f}	2.40 ^{g}	2.40 ^{g}
MS100-C10	6.40 ^{c}	20.00 ^{c}	40.00 ^{a}	64.80 ^{c}
MS100-CA1	2.60 ^{e}	2.60 ^{e}	2.60 ^{f}	5.20 ^{f}
M.S.E.	4.4	5.3	6.9	11.4
P-value				
Factor A	< 0001	< 0001	< 001	< 0001
Factor B	< 0001	< 0001	< 0001	< 0001
Interaction A*B	< 0001	< 0001	< 0001	< 0001
Blocks	NS	NS	< 001	< 001

Mean values between treatments with different superscript differ significantly ($P<0.05$). M.S.E.: mean squared error.

(2013) achieved 40%, 98%, and 85% germination in 48 days in *Isochilus linnearis*, *Oceoclades maculate*, and *Oncidium viperium*; respectively. Karol *et al*. (2015) established asymbiotic *in vitro* propagation of *Comparettia falcata*, achieving up to 56.6% in MS medium. Lallana *et al*. (2016) recorded asymbiotic germination *in vitro* culture greater than 50% in MS at 50% in ten species of orchids. Banda-Sanchez *et al*. (2017) obtained 29% germination in *Prosthechea* in 90 days of *in vitro* culture using MS medium enriched with 0.003 mg L^{-1} of auxins and gibberellins.

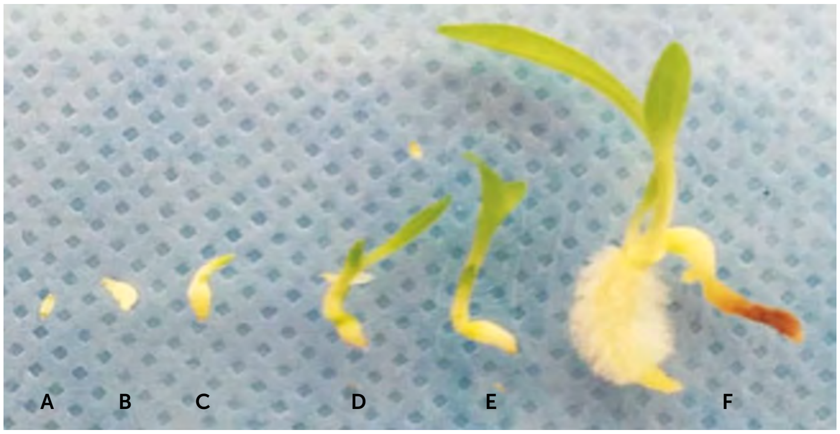

Figure 3. Germination stages of *Dichromanthus aurantiacus in vitro* culture. A: Green seeds; B: Initial protocorm; C: Late Protocorm; D: First leaves; E: Root development; F: Complete plant.

CONCLUSIONS

The seeds of *Dichromanthus aurantiacus* showed brown testa and an oval embryo with dimensions of 0.2 mm long and 0.05 mm wide, approximately. The tetrazolium test's viability showed 91.4% viability when they were soaked in sodium hypochlorite solution ($CaCOCl_2$) for two hours, 24 hours soaking in tetrazolium solution (1%) plus two drops of Tween-80. The asymbiotic *in vitro* culture showed up to 92.8% germination in 60 days using MS medium at 50% enriched with 10% coconut water.

ACKNOWLEDGMENTS

This research was funded by the Departamento de Ciencias de la Alimentacion UAM Lerma and the Protectora de Bosques of the State of Mexico (PROBOSQUE) to protect and propagate endangered species of the State of Mexico.

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