

Population Fluctuation of
Disonycha teapensis
Blake (Coleoptera: Chrysomelidae)
 in *Crotalaria longirostrata* Hook.
 & Arn. (Fabales: Fabaceae) in
 Huimanguillo, Tabasco, Mexico

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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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Photoperiod and its relationship to sheep reproduction

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ABSTRACT

Objective: To describe the seasonal variations throughout the year (day length), as one of the main environmental cues used by ewes to determine the most favorable time for breeding.

Design/methodology/approach: A description of the main factors affecting ewe reproduction (photoperiod, suckling, postpartum period, nutrition, sociosexual effects) was assessed by a review of documentary information.

Results: The inhibition of reproductive activity during one time of the year is a common process in most animal species to prevent births from occurring at an unfavorable time for the survival of the offspring. The seasonality of reproduction (northern latitude) allows births to occur in late winter or early spring when climatic conditions are the most favorable for the offspring development.

Limitations/implications: The duration of the anestrus season (seasonal or postpartum) is influenced by the photoperiod and other factors. In Mexico, a small percentage of Criollo and Pelibuey ewes show a short duration of seasonal anestrus; that is, they show almost continuous annual reproductive activity. Therefore, to improve the reproductive efficiency of ewes, it is important to precisely determine the factors that affect their reproduction to improve management and increase the profits in the production units.

Findings/Conclusions: Photoperiod is the main environmental factor regulating the annual reproductive cycle of the ewes, it occurs through very complex and varied mechanisms that communicate the visual system with the gonads through nervous and endocrine pathways.

Keywords: Photoperiod; seasonality; postpartum; reproduction; ewe; reproduction.

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INTRODUCTION

In sheep, a change from long days (spring) to short days (fall) initiates the reproductive season, the change from short to long days initiates seasonal anestrus (Martin, 2003). Photoperiod is one of the most important environmental factors in a herd's reproductive



management. It controls reproductive physiology through the light captured by the retina and transmitted to the pineal gland (Figure 1), which secretes melatonin in response, at a production rate high at night and low during the day (circadian). The duration of melatonin secretion coincides with the duration of the night and together with estradiol (E) regulates the pulsatile secretion of gonadotropin-releasing hormone (GnRH), which causes changes in the pulsatile secretion of luteinizing hormone (LH) and is responsible for ovulation or non-ovulation (depending on the time of year; Karsch *et al.*, 1984); however, melatonin does not act directly on GnRH-producing neurons, which involves the dopaminergic, serotonergic and excitatory amino acid systems (Malpaux *et al.*, 1999).

In sheep, reproduction is seasonal and is characterized by the alternation of a period of seasonal anestrus, which, at northern latitudes, occurs between spring and summer, and a period of reproductive activity that occurs in autumn and winter. It is important to mention the latitude effect: the higher the latitude, the longer the seasonal anestrus (photoperiod). For example, some European breeds, which develop in latitudes above 60° N, have reproductive activity between November and February, and in latitudes close to 35° N they have anestrus between March and June (Gómez-Brunet and López-Sebastián, 1991) and in tropical regions, where the variation of photoperiod is little (19° N), Creole

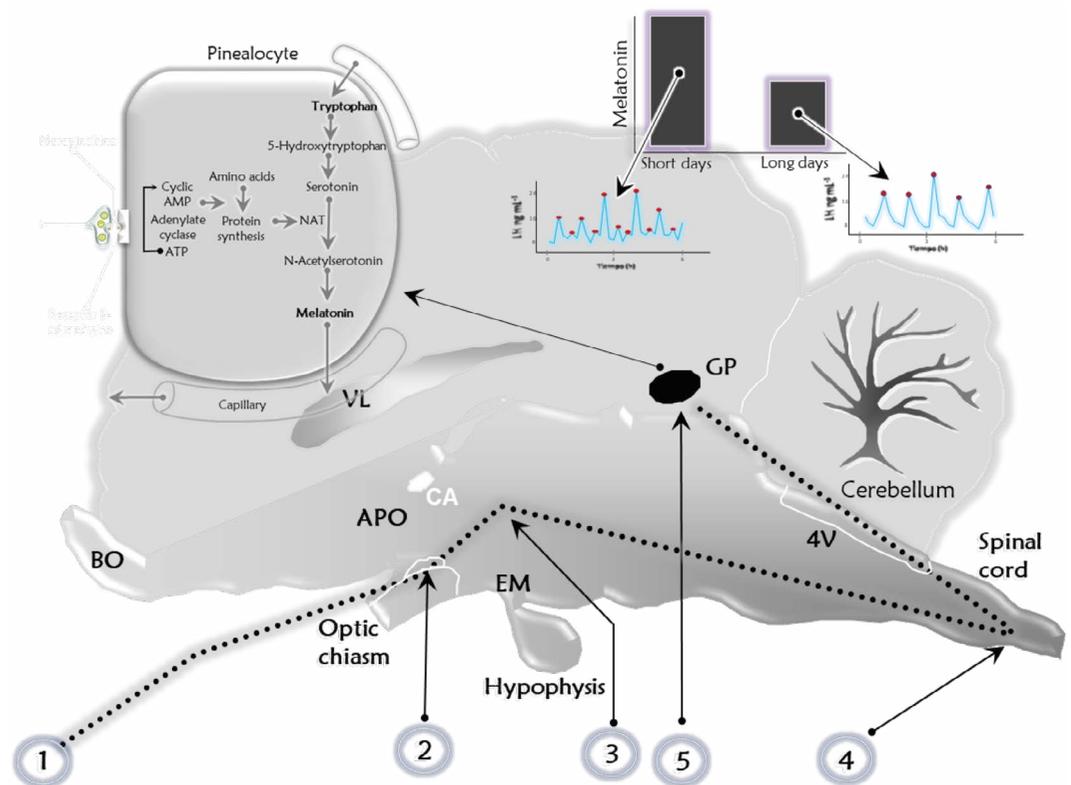


Figure 1. Schematic representation of a sagittal section of the sheep brain. The black dotted line represents the light information path of the eye (1) to the pineal gland (5) through the supraoptic nucleus (2), paraventricular nucleus of the hypothalamu (3) and the superior cervical ganglion (4). Preoptic area (APO), previous commissure (CA), lateral ventricle (VL), median eminence (EM), pineal gland (GP), olfactory bulb (BO), fourth ventricle (4V). Modified from Malpaux *et al.* (1997).

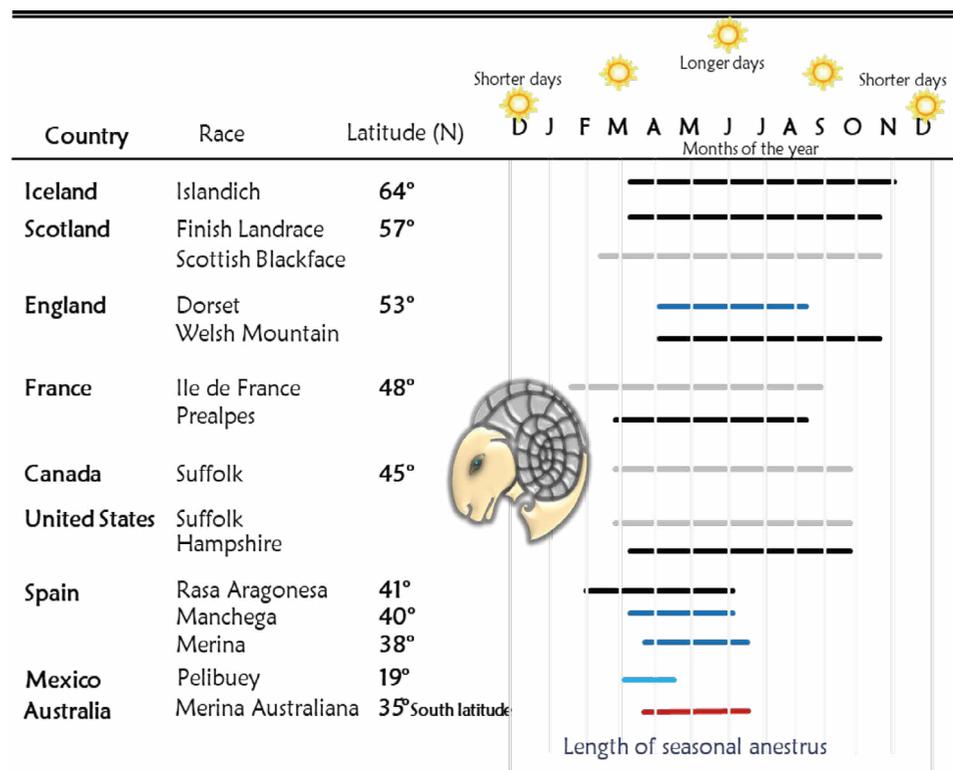
and hair sheep breeds maintain cyclic activity, almost all year round (Arroyo *et al.*, 2007; Arroyo, 2011). In tropical and subtropical regions (latitudes below 30°), there are other environmental factors that determine the reproductive activity of sheep, mainly those related to the rainy season (feeding), “norte” (north) climatic event and temperature.

Reproductive seasonality in sheep

Reproductive seasonality is a strategy to improve reproductive efficiency by matching the time of lambing with the most favorable time of the year, which is spring in northern latitudes, when temperature and food abundance maximize the offspring survival. It is known that photoperiod interacts with breed, postpartum anestrus, nutrition, sociosexual effects and other environmental factors to determine the ideal time to initiate reproduction.

Over time, many breeds have been selected with different reproductive characteristics depending on their place of origin. Many breeds have been and are selected to improve some productive variables such as ovulation rate, prolificacy, milk production, duration of seasonal anestrus, among others. For example, Table 1 shows the duration of the reproductive season in different breeds and different latitudes, it is observed that breeds originating from temperate latitudes (>35°) present a greater reproductive seasonality (López-Sebastián *et al.*, 2005) than breeds of Mediterranean or equatorial origin, which present a reduced seasonal anestrus and sometimes it is nonexistent (See Table 1; Porras, 1999; Valencia *et al.*, 2006; Arroyo *et al.*, 2007; Arroyo, 2011).

Table 1. Duration of the seasonal anestrus in sheep in relation to their breed and latitude (Modified from López-Sebastián *et al.*, 2005).



Modified from López-Sebastián *et al.* (2005)

Postpartum anestrus and its effect on reproduction

It is the period that elapses from the parturition until the female shows her first estrus. The duration of the postpartum period is one of the factors that determines reproductive efficiency and is conditioned by several interactions, among which are suckling, milk production, body condition, time of year at the time of parturition, uterine involution, breed, age and the presence or absence of the male (López-Sebastián *et al.*, 2005). In most sheep breeds located in temperate regions, the timing for lambing is largely determined by the photoperiod (Figure 2); meaning that, when lambing occurs late in the reproductive season, the duration of postpartum anestrus can be confused with seasonal anestrus, whereas when lambing occurs during the reproductive season, postpartum anestrus can last approximately 30 days (Amir and Gacitua, 1987). Also, it is known that if suckling is controlled (decreasing the contact of the calf with the dam), the postpartum anestrus duration in hair ewes decreases (Morales-Terán *et al.*, 2004). In addition, endocrine profiles during postpartum and the response to exogenously applied hormonal treatments in non-lactating ewes have been determined a phase of ovarian inactivity between approximately 21 and 25 days in duration (early postpartum) and a second phase of variable duration that is determined by the effect of continuous nursing have been observed; that is, the presence of the lamb delays the resumption of ovarian activity and its total or partial separation causes the manifestations of estrus and the first postpartum ovulation (Castillo-Maldonado *et al.*, 2013).

Endogenous opioid peptides (EOP's) are known to be released acutely or chronically during alertness periods, stress events and lactation in females; they are implicated in the inhibition of ovulation during the postpartum period, as they act together with E and GnRH-producing neurons, they cause a decrease in the frequency of secretion of GnRH/LH pulses. For example, continuous nursing causes a delay in the reestablishment of postpartum ovarian activity, inhibiting ovulation, compared to ewes lambing at the same time, but weaned (Pérez-Hernández *et al.*, 2009). Also, there is a direct relationship

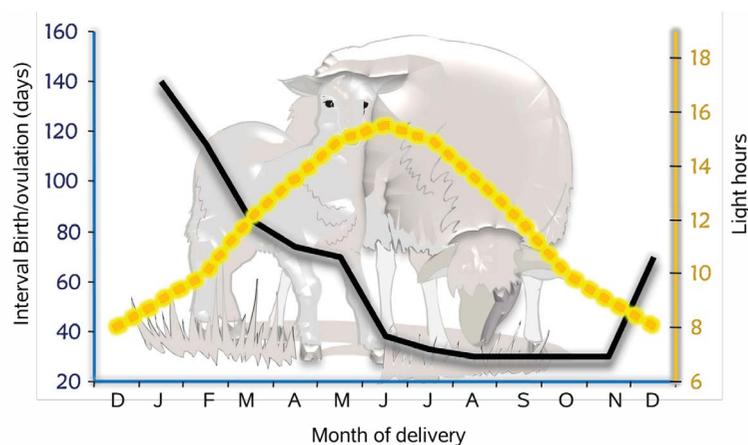


Figure 2. Interval between lambing and first ovulation according to the month in which lambing occurs in Manchego sheep (40° north latitude), it can be observed that ewes lambing in January (E) with approximately 9 hours of daylight per day, the duration of the post-lambing period is approximately 140 days (López-Sebastián *et al.*, 2005).

between the type of weaning, the intensity/frequency of nursing and milk production with the duration of postpartum anestrus (Gómez-Brunet *et al.*, 1992). The reestablishment of cyclic ovarian activity after parturition is frequently preceded by one or more short cycles (short half-life corpora lutea) with low progesterone secretion and ovulations without estrus manifestations (silent estrus). It is now known that normal progesterone concentrations during the luteal phase, are a requirement for the manifestation of estrous signs and occur in weaned females approximately between 20 and 25 days after parturition, in lactating females with controlled lactation (30 min in the morning and 30 min in the afternoon) approximately between 25 and 35 days postpartum and in females with continuous lactation the first ovulation occurs approximately between 45 and 55 days after parturition (Morales-Terán *et al.*, 2004).

In reproductive terms, and taking into account the age of the females at first lambing, the lambing-first estrus interval and lambing-first insemination, are variables that must be taken into account, mainly when analyzing ewe's reproductive efficiency. Some handling strategies can help to reduce the duration of anestrus (seasonal or postpartum), adequate feeding in the last third of gestation or targeted feeding (according to the physiological state of the ewe), early weaning, controlled nursing, avoiding losses of body condition at the end of the reproductive period, stimulating the ewe with the male effect (López-Sebastián *et al.*, 1984; Morales-Terán *et al.*, 2011) are easy to implement practices that reduce the calving interval and do not cause large extra capital outlays.

Nutrition and its effect on reproduction

Sheep feeding, from an economic point of view, is one of the variables that have the greatest impact on production costs, so that overfeeding has an additional cost, so using this strategy should provide maximum benefits to justify its use. Nutritional management (ration balancing based on the physiological state of the females) is a practice that improves the reproductive efficiency of the herd and guarantees efficient ration consumption (Martin *et al.*, 2004). However, it is difficult to dissociate the specific effect of nutrition on reproductive behavior from its effect on reproductive physiology and even more difficult to dissociate the effect of nutrition from the effect of photoperiod on the annual reproductive activity of sheep. For example, the presentation of puberty in ewes depends on their weight and age, in malnourished adult ewes, flushing increases the number of ovulations without the presentation of estrus and reduces the duration of the reproductive season. Yet, its effect is different and largely depends on the breed and photoperiod (long days) inhibits reproductive activity and so far, there is no dietary strategy to reestablish reproductive activity during seasonal anestrus without using exogenously hormones. The strategic use of feed supplements is employed as a tool in sheep production systems, implying that there is a positive correlation between the availability of supplements and the reproductive and/or physiological events that determine the success of a production unit. In such a way, that strategic feeding, refers specifically to feed supplements provided to sheep for a short period of time (3 to 7 days in females and in males from 6 to 8 weeks before mating) and that impact, mainly in a positive way, the reproductive and survival events of the animals and that ensures

a higher economic income for producers (Martin *et al.*, 2004; Martin and Kadokawa, 2006; Martin and Rosales-Nieto, 2014).

Other factors controlling reproductive activity

Humans, through time, have tried to control the environmental factors related to sheep reproduction, since herd's fertility depends on them and to a great extent, on the capability to define and manage those factors.

Temperature and its relationship to reproductive activity

The heat stress effects on animals and production are multifactorial and complex. Variations in temperature are known to have a direct effect on reproduction. For example, prolonged exposure to high temperatures inhibit reproductive activity in rams and ewes, inhibits estrus manifestations or reduces its duration if high temperatures occur at the end of the luteal phase (Maloney, 2003). It is clear that some aspects of heat balance are easier to manipulate than others and that manipulating the animal's response to heat to maintain adequate production can lead to positive feedback cycles (Maloney, 2003). Currently the effect of global warming (direct effects of heat) on animal production implies a negative effect on animal reproduction.

Sociosexual effects on reproduction

Sexual behavior appears during adult life, however, there is evidence that it may begin during the early life and incorporated into, what will be, the final organization (Kendrick *et al.*, 1998). The sudden introduction of rams to the ewe's pens during anestrus (seasonal or postpartum) induces ovulation, a phenomenon known as "male effect". If prior to mating, a group of ewes in estrus are introduced to the rams' pens, the ovulatory response of the ewes' during anestrus is enhanced, suggesting their estrus induction, through the produced pheromones, the reproductive behavior of the rams, which improves their ability to induce ovulation (female effect; Walkden-Brown *et al.*, 1999; Álvarez-Ramírez and Zarco-Quintero, 2001; Martin *et al.*, 2014). However, we must have certain considerations, mainly in natural environments, since reproduction and, in particular that of males, is not equally distributed among individuals in a herd, *i.e.*, dominant males, which are usually the best nourished and the strongest, have priority access to females in estrus (Preston *et al.*, 2003). Sexual behavior expressed by young males or females is less expressive than in adults. In addition, young males are clumsier in mounting females and getting them. Also, pregnant, young females are receptive for less time than adult ewes, however, the receptivity level is similar to that of adult ewes (Fabre-Nys and Venier, 1990). It has also been shown that contact of prepubertal males with females in estrus stimulates their activity (female effect; Price *et al.*, 1996). During sexual behavior displaying, there are other factors that can affect the reproductive efficiency of the production unit, such as the proportion of females (many) per male, avoiding contact between males and females during the development phase, the allocation of fixed spaces during development (unfamiliarity with other pens), the type of grazing area (topography) can have negative effects at the time of displaying all male's sexual behavior (Petherick, 2005).

CONCLUSIONS

The photoperiod is the main environmental factor regulating the annual reproductive cycle of the ewes, occurring through varied and complex mechanisms that communicate the visual system to the gonads through nervous and endocrine pathways. Annual physiological variations in the synthesis and secretion of hormones, neurotransmitters and metabolites influence the onset or termination of the reproductive season. Also, the length of the anestrus season (seasonal or postpartum) is influenced by breed, whether or not the ewe is lactating, the postpartum period, nutrition and sociosexual effects. In Mexico, a small percentage of Creole ewes and Pelibuey ewes have a short seasonal anestrus; that is, they show an almost continuous annual reproductive activity.

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economy, since it is a driving force for economic development; however, to ease this activity, it is necessary to create treaties that allow commercial exchange between Mexico and other countries. Free trade agreements usually incorporate rules on goods trade, services, investment, intellectual property, trade defense mechanisms, and most importantly, dispute settlement. Some recent ones include labor and environmental dispositions (COMEX, 2004). Michael Porter (2007), through his competitiveness diamond model, states that a country's competitive advantage is "...its ability to encourage local and foreign companies to use such a country as a platform for carrying out their activities". Amit and Zott (2001) state that competitiveness is the ability of a company to position and maintain itself in the market in the long term. Therefore, competitive advantages are created by improving efficiency and creating more valuable products. Exports have a certain improvement level, supporting those companies still developing in this area to increase their exporting capacity is important. Park and Urmeneta (2019) point out that a potential cause of export instability is that companies sell only one product to only one destination; both of which are precariousness indicators.

The Social Network Analysis (SNA) attempts to analyze how individuals or companies are connected, to determine the network's general structure, the network's group, and the individuals' position or the company to delve into the social structure that underlies the information flow, interchanges, or power (Menéndez, 2003).

The NEA was created to annually award the effort, tenacity, creativity, and innovation of the companies and educational institutions that participate in exporting. It began with eight categories, but over the years new ones were added and some were eliminated. Currently, 12 categories are available to participate. To give this important recognition to companies, institutions, and leading organizations in foreign trade a new direction and a new impulse, in 2009, Secretaría de Economía (Ministry of Economy), through the Subsecretaría para la Pequeña y Mediana Empresa (Undersecretariat for Small and Medium Enterprises), granted the responsibility for the organization, promotion, and dissemination of the award to the Fundación Premio Nacional de Exportación A.C. (National Export Award Foundation A.C., FPNE, 2009). The FPNE's founding organizations are five of the country's most important and linked to exporting companies: The Consejo Empresarial Mexicano de Comercio Exterior, Inversión y Tecnología (Mexican Business Council for Foreign Trade, Investment and Technology, COMCE), the Confederación de Cámaras Industriales de los Estados Unidos Mexicanos (Confederation of Industrial Chambers of the United States of Mexican, CONCAMIN), the National Agricultural Council (National Agricultural Council, CNA), the Consejo Coordinador Empresarial (Business Coordinating Council, CCE) and the Confederación de Cámaras Nacionales de Comercio y Turismo (Confederation of National Chambers of Commerce and Tourism, CONCANACO SEYTUR) (PNE, 2009). The objective of this research was to analyze the profile of the companies participating in the NEA under category V, through an information collection tool to identify business lines, states of origin, and export destination countries, to develop an improvement strategy that will enable the NEA to achieve better results.

MATERIALS AND METHODS

The data collection was conducted by extracting information from 17 questionnaires applied to the companies participating in category V (Large Agricultural Exporting Companies) from 2010 to 2018 in the NEA. The questionnaire consisted of 10 open-ended questions. A spreadsheet was created to systematize the information about products, amounts, and percentages they represent in the export of the companies, identify the countries where they export to, their logistics, innovations made (on their processes, product or service), among other aspects.

For the data analysis, descriptive statistics were used, and graphics were developed. Using the generated spreadsheet database, the information was also organized to construct an export network through the network generator question: “*principal destination countries for exports*” and through the export network, sub-networks were created to illustrate the export destinations of each line of business. The networks were generated through the NetDraw 2.097 software. The calculation of ARS indicators like network size and the number of links was performed by the UCINET 6 for Windows[®] version 6.288 software.

Likewise, word clouds were created using the data collection tool, to show the most frequently mentioned words by the participating companies under the V category of the NEA. As the questionnaire applied by the award contained open-ended questions, processing them became difficult; therefore, the word clouds were created using the “Wordle” software. The answers given by the participating companies in the category V of the NEA were selected (question by question), then these answers were uploaded into the Wordle software to generate word clouds. A folio was assigned for each company to avoid mentioning names and protect the privacy of the obtained information, as well as the type of exported product (horticultural and animal husbandry).

RESULTS AND DISCUSSION

Attributes of companies participating in the NEA

As for the participation by business line among the companies under the LAEC category, vegetables are in first place with 12 companies, or 70.6% from the total number of participants (17), followed by the swine husbandry sector with three companies, representing 17.6%, and last, the fruits sector with two-company participation, 11.8%. Considering the year of participation within the 2010-2018 interval, the years 2012, 2013 and 2017 stand out with the participation of three agribusinesses for each year, representing 52.9% of the total, followed by 2011 and 2015 with a participation of two agribusinesses each year, *i.e.* 23.5% of the total. Finally, 2010, 2014, 2016, and 2018 with the participation of one agribusiness for each year, representing 23.5% of the total.

As for participation by state, Sinaloa stands out with 35.3%, followed by Mexico City with 17.6% and Baja California with 11.8% (Figure 1).

Activity dynamics

The most exported product by the NEA participating companies in the V category during the 2010-2018 period was tomato, in its different varieties (saladette, ball, cherry, among others), in second place beef, and bell peppers in third place.

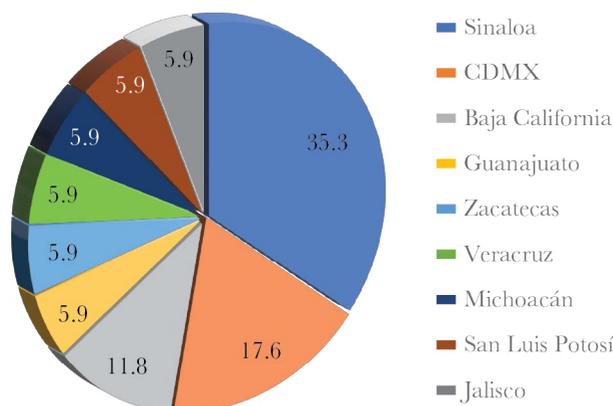


Figure 1. Participation by state in the NEA, category V, 2010-2018.

Regarding the exports destination, during the year each company's participated in the NEA, the results showed that 82.4% of vegetables and fruits were exported to the U.S., pointing out some companies' precariousness. Park and Urmeneta (2019) indicate that a cause of export instability is the fact that companies sell a single product to a single destination, both of which are indicators of precariousness.

Forty-seven percent of the companies participating in the NEA mentioned having certification in Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), and Hazard Analysis and Critical Control Point System (HACCP).

As for the quality control processes of each company, a word cloud was generated, emphasizing answers such as good practices, safety, product, quality.

About the strategy to access new markets, the answers that stood out among the NEA participating companies were: comparison, needs, customers, price, among others.

Innovation dynamics

According to the Oslo manual (2008), a technological innovation comprises new products (goods and services), processes, and significant technological changes to those products and processes. Innovation has been implemented if it has been introduced into the market (product innovation) or used within the production process (process innovation). Therefore, innovations involve a series of scientific, technological, organizational, financial, and commercial activities.

The responses to find out what were the different kinds of innovations implemented by the companies participating in the NEA emphasized the following: production, processes, products, and selection (Figure 2).

The word clouds were helpful because the questionnaires applied by the NEA contain open-ended questions, making it difficult to process them. The word clouds were used to visualize the most frequently mentioned by the companies that participated in the NEA.

Commercial Network Analysis using ARS

A total of 17 horticultural and swine husbandry companies were surveyed. They were asked to enlist the countries to which they export their products, giving a total of 18.

including frozen products, makes it a non-perishable product compared to tomatoes and some fruits or berries, and therefore its network represents a diversity of export destination countries, attenuating the precariousness index that Mexican exports, especially agricultural exports, shown in overall. In the Ahern virtual symposium, Engineer Alfredo Diaz mentioned that “...Mexico needs to be less vulnerable because it depends on a single market, which is the U.S. Of the 80% of agricultural production, 96% is exported to the U.S. and 4% to Canada”. Demonstrating that agricultural exports have only one market, “the American market” (Seeds, 2020).

Figures 4, 5, and 6 show the network divisions that were made. The companies are shown in red and the countries in blue.

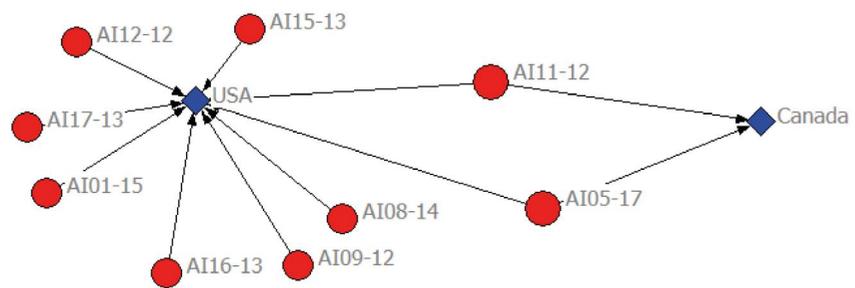


Figure 4. Tomato export network of the NEA participating companies, category V, 2010-2018.

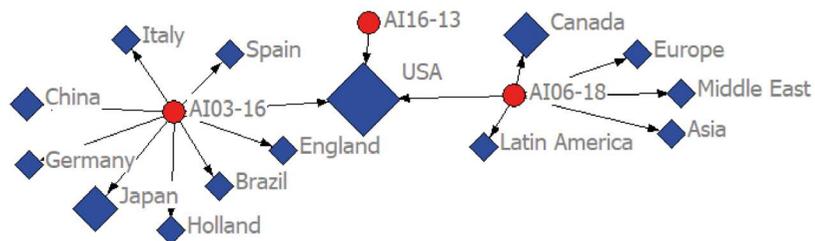


Figure 5. Fruits export network of the NEA participating companies, category V, 2010-2018.

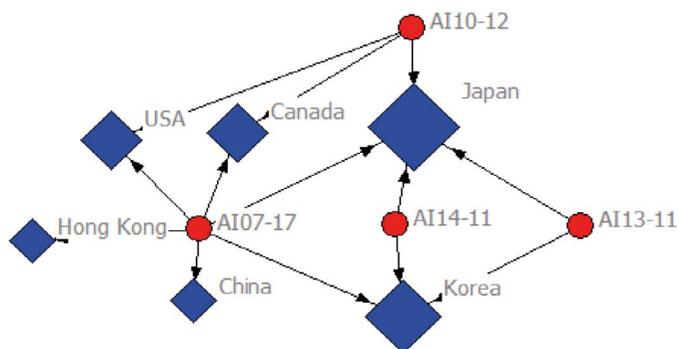


Figure 6. Meat export network of the NEA participating companies, category V, 2010-2018.

CONCLUSIONS

The analysis of the NEA participating companies indicated the precariousness of the agricultural sector as it exports only one product or a reduced number of them to a single country, due to the perishability of most of the products and the geographical proximity to the world's largest market. This one-way flow of Mexican agricultural exports will probably continue or increase due to the pandemic. The same could happen for the rest of them and even for imports. Low participation by States and by business lines was also observed. In the nine assessed years, only nine States and two lines of business participated. The possible reason for this could be the lack of promotion of the NEA.

This study had some limitations, such as the scarce information on the award, which would serve for future research; some companies that won the NEA in the LAEC category did not want to answer the requested survey, this would have been of great help for better understanding the NEA process, and most importantly, the opinion that these companies have about the award. Interviews with other similar awards were also missing to evaluate them and understand what their business model is like and observe opportunity areas for the NEA. These aspects limited the realization and scope of the present research. From the obtained results, it is proposed that the dissemination by social networks be increased, as well as the participation in fairs, forums, expositions, and state awards. This is expected to increase the participation of companies from different sectors and different regions of the country.

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Field production of kale (*Brassica oleracea* var. *Acephala*) with different nutrition sources

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ABSTRACT

Objective: Quantify the development and production of kale (*Brassica oleracea* var. *Acephala* cultivar “dwarf blue curled”) in field conditions with organic and mineral fertilization.

Design/methodology: Kale was sown in a field in a completely rando design with five treatments and five repetitions per treatment (mineral soil fertilization, organic soil fertilization, mineral soil fertilization and foliar fertilization, organic soil fertilization and foliar fertilization and a control). After transplanting (dat) every 15 days *in situ*, the number of leaves was quantified and the phenological stages of the crop were identified. At 116 dat, with destructive sampling, the number length, fresh weight, resistance to penetration and ascorbic acid content in the kale leaves were quantified.

Results: The growth kinetics of the plants in the organic soil and foliar fertilization excelled, compared to the other treatments. In the cuttings, 1.5 to 3 bunches per plant were obtained, the resistance increased in leaves with organic products. Ascorbic acid increased in the leaves with all fertilizations.

Limitations implications: limited knowledge in the crop’s management.

Conclusions: Kale is a crop that responds to different fertilization sources. Organic soil and foliar fertilization are alternatives for kale agroecological production, It’s a vegetable scarcely grown in Mexico. The crop can be produced in the Texcoco area.

Keywords: Kale, organic fertilization, mineral fertilization, ascorbic acid.

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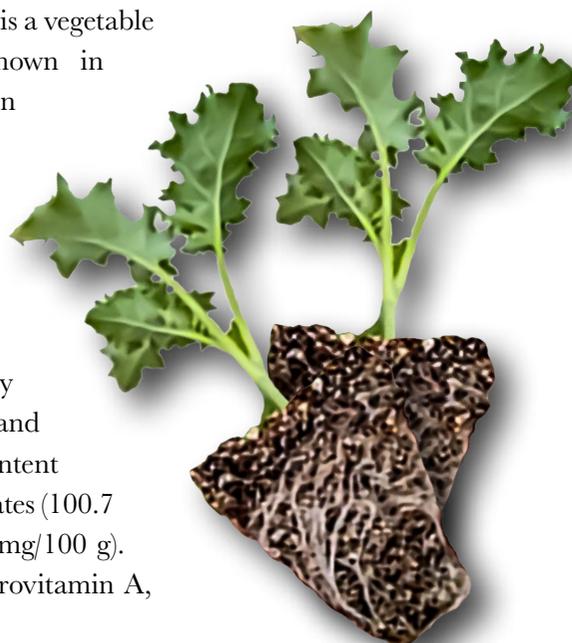
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INTRODUCTION

The kale (*Brasica oleracea* var. *Acephala*) is a vegetable with production potential and little known in Mexico; its consumption has not yet been generalized among the population. Called cabbage or kale, a green leafy vegetable, belongs to the Brassicaceae family such as cabbage, broccoli, cauliflower, and Brussels sprouts (Samec *et al.*, 2019). Due to its high phytochemicals content, it is an important source of secondary metabolites (Reyes-Munguía *et al.*, 2017), and is characterized by a high antioxidants content such as flavonoids (73 mg/100 g), glucosinolates (100.7 mg/100 g), carotenoids, and others (19.61 mg/100 g). It is a vitamin C source (120 mg/100 g), provitamin A,



vitamin K, B6, folic acid, thiamine, riboflavin, pantothenic acid, vitamin E (Samec *et al.*, 2019; USDA, 2016), and minerals such as iron (1.47 mg/100 g), zinc (0.56 mg/100 g), manganese (0.659 mg/100 g), calcium (150 mg/100 g) and magnesium 47 mg/100 g) (USDA, 2016) and It only contributes 49 kcal/100g.

The few kale crops in Mexico, such as those at the “chinampera” area of Xochimilco (Figure 1), are produced with mineral fertilizers using potentially toxic chemical substances, contrary to their nutritional characteristics. Among the main problems for this crop are aphids (*Brevicoryne brassicae*) when crops are developed for leaves cutting.

However, it is possible to produce kale in harmony in a sustainable way using an agroecological system, by changing some of the limitations of conventional ones. This includes a set of practices that promote the proliferation of life throughout the plot, promoting the organic fertility of the soils, plant biodiversity and ecosystemic functions (Restrepo, 2013).

Kale leaves are sold in gourmet and organic markets, in six leaves bunches, their cost between \$ 30 and \$ 45 Mexican pesos, or \$ 150.00 kg. The marketed leaves must be firm and green, without brown edges, an indication of old leaves that should not be consumed, according to the US standard (USDA, 2015). This research aimed to quantify the production and quality of kale produced in the field depending on the sources of fertilization and the application.

MATERIALS AND METHODS

The research was established in the Agroecology and Sustainability module, Colegio de Postgraduados campus Montecillo at the Estado de México (19° 27' 38" N, 98° 54' 11" W). The predominant climate is temperate semi-dry, with an average annual temperature and precipitation of 15.9 °C and 686 mm, each (SMN, 2020). Seeds of kale cv “dwarf blue curled”, Itasco[®] seedbed, were sown in a seedbed in an equal parts mixed substrate of peat moss (Promix[®]), perlite (Agrolita[®]) and vermiculite (Termita[®]). When plants had 10 cm height and four true leaves (Figure 2), they were transplanted to plots in the field.



Figure 1. Kale production in chinampas of Xochimilco, Mexico City.



Figure 2. Kale seedling and seedling production.

At field conditions, the plants were subjected to five treatments (Table 1). Each treatment was replicated five times. The crop was sprinkled watered. The experimental design was of completely randomized plots (DCA). The experimental unit, a plot, consisting of 16 plants at a 0.60 m distance between plants and between rows, in an offset “tresbolillo” arrangement.

Kale is a rarely sown crop in Mexico and therefore has no generalized recommendation for soil mineral fertilization, an equivalent dose to 250-120-100 kg ha of N P K was used for soil mineral fertilization, following recommendations for broccoli (INIFAP, 2008). The sources were urea, DAP (diammonium phosphate) and potassium nitrate. For mineral foliar fertilization, Bayfolan[®] product from Bayer was applied every 15 days after transplant in a 3 L ha dose. The organic fertilization treatment consisted in applying 4 t ha of a sheep vermicompost mixture (C/N ratio 13:1 with 60 days maturity) obtained from the production module of the Agroecology and Sustainability Postgraduate Program. Also, basalt rock flour (BASAGRO) in a 10:1 ratio (vermicompost: rock flour) was included. For foliar organic fertilization, a fermentation of activated mountain microorganisms was used (Castro 2014).

During the kale growth, in the field, different agroecological products were applied, depending on the pests or diseases present. Coconut biozyme was applied as an enzyme activator, calcium sulfide broth to control fungi, and chili, garlic and rue extracts for

Table 1. Established treatments to assess the effect of organic and mineral fertilization on the development and production of kale (*Brassica oleracea* var. *Acephala* auct.) in the field.

Treatment	Description	Abbreviation
1	Control	T
2	Organic fertilization to the soil	FOS
3	Mineral fertilization to the soil	FMS
4	Organic fertilization to the soil + organic foliar fertilization	FOS+FFO
5	Mineral fertilization to the soil + mineral foliar fertilization	FMS+FFM

whiteflies and aphids control. The definition of the phenological stages of kale was based on the plants' physical and physiological changes, from the seed germination until the appearance of the flowers (reproductive stage), registering the dates these were observed.

From transplant to final sampling, the number of leaves per plant was counted (*in situ*) every 15 days to know the kinetics and identify the moment of maximum leaf production per plant. At 116 days after transplant (dat), a destructive sampling was done to assess the leaf production. For this, a scale of sizes (in cm) of the leaves was designed: 4 to 10, 10.1 to 15, 15.1 to 20, 20.1 to 25, 25.1 to 30 cm and 30.1 to 35 cm. Each treatment's commercial yield per cut was obtained, with their leaves of size (20 to 35 cm) and their fresh weight. The firmness or resistance to penetration of the leaves was evaluated with a penetrometer, Grainger 5DPK3 brand, for fruits. The values were reported in kg of force. The ascorbic acid (vitamin C) content was determined with the 2,6-dichloroindophenol method (AOAC, 1998). The data obtained from the variables under study were subjected to a test for normality. The data were subjected to an analysis of variance (ANOVA) with its corresponding homogeneity of variances tests, independence and normality in the residuals, finally, the Tukey's means comparison tests were performed with an $\alpha=0.05$ level significance. The calculations were supported by the statistical package SAS V9.4 for Windows.

RESULTS AND DISCUSSION

The maximum leaf number per plant during the period in which the field experiment was carried out (October 2019-February 2020) was obtained in the penultimate cut at 101 dat (Figure 3). The treatments with the highest number of leaves were organic soil fertilization + organic foliar fertilization (OSF + OFF) with 44.6 leaves, followed by mineral soil fertilization + mineral foliar fertilization (MSF + MFF) treatment, which had an average of 41.2 leaves. In both cases, a higher production compared to the control (T) with 38.5 average leaves.

The crop treated with organic soil fertilization + organic foliar fertilization (OSF + OFF), increased leaf production by 13.7% in the greater plant development stage (penultimate sampling), compared to the mineral soil fertilization treatment + mineral foliar fertilization (MSF + MFF). Applying organic fertilizers to soils has been shown to increase crop productivity and improve soils (Lazcano *et al.*, 2012). In this experiment, organic fertilization applied to the soil as vermicompost and organic foliar increased the number of leaves per plant (Figure 3), compared to mineral fertilization. These results relate to the benefits of vermicompost in the production of vegetables in fields reported by Dinesh *et al.* (2010). These authors indicate that with this strategy the microbial activity of the soil increased by 16%, and with it, the enzymatic activity, and the release of essential macronutrients for plants. Lisiewska *et al.* (2008) reported 25 and 30 leaves per plant production at 70 dat. In our experiment at Montecillo, the average leaves number at 70 dat was 33 to 39, higher than that reported by the above-mentioned authors. This indicates that kale responds to both tested sources of fertilization. The plant development was fast and the crop's evolution was observed in a short time (Figure 4).

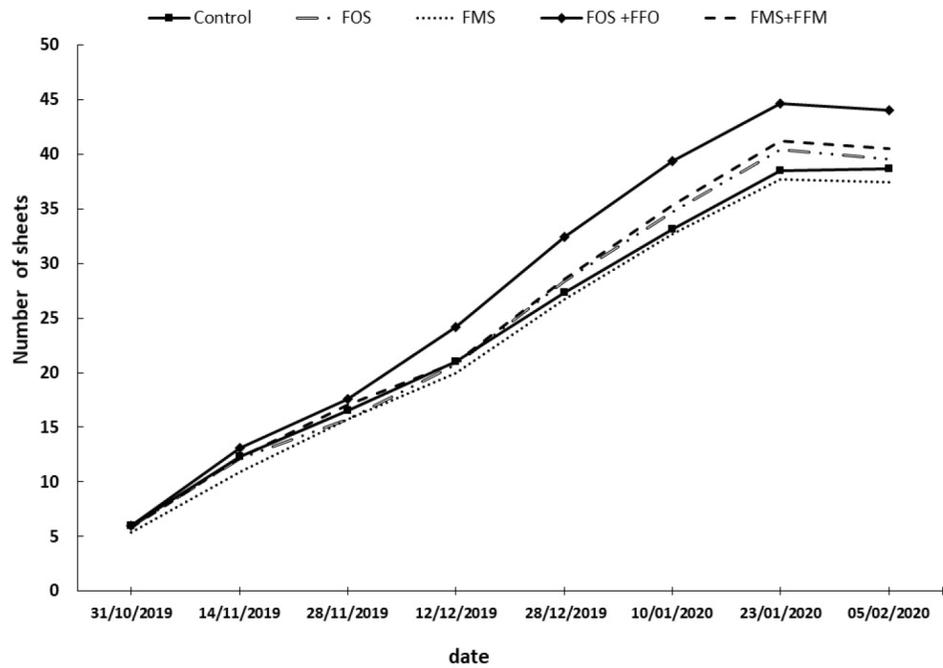


Figure 3. Growth kinetics of kale leaves (*Brassica oleracea* var. *Acephala auct.*) Per plant at 116 dat under five fertilization treatments: TEST=Control; FOS=organic fertilization to the soil; FMS=mineral fertilization to the soil; FOS + FFO=organic fertilization to the soil and foliar; FMS + FFM=mineral fertilization to the soil and foliar.

In Texas, the maximum production of kale leaves occurs at 90 dat, when the harvest begins, with yields of 5 to 6 t ha⁻¹ (Masabni, 2011). In this experiment, the first cut was made at 88 dat and the maximum leaf production was reached at 101 dat, with yields of 5 t ha⁻¹ only considering the leaves of 20-30 cm long intervals that, commercially, are the best.

From the assessed phenological stages, the vegetative stage is the most important for horticultural producers (Fuentes and Pérez, 2003), if the phenology of cabbage (*Brassica oleracea* var. *Capitata*) is taken as a reference since there is no reported phenological scale for kale. We propose six stages, a product of our observations of the species under field conditions (Figure 5).

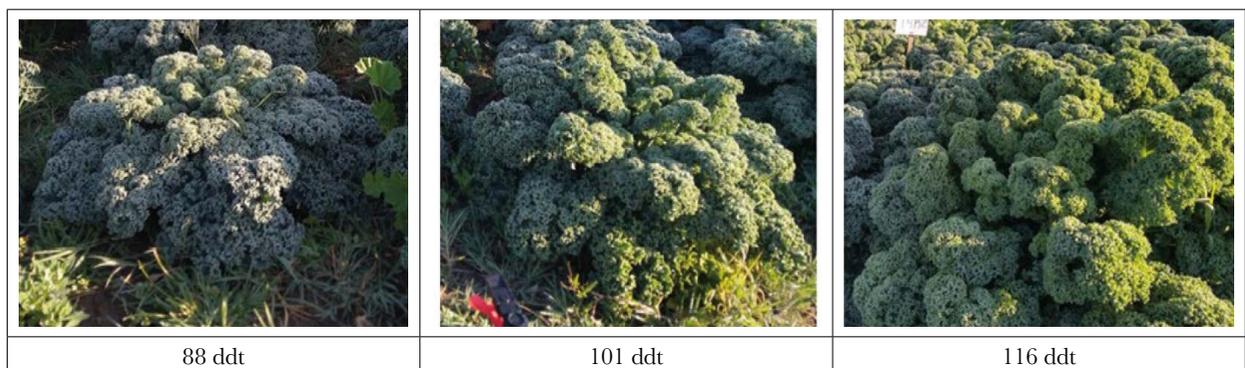


Figure 4. Kale cultivation and development in field conditions on three sampling dates.

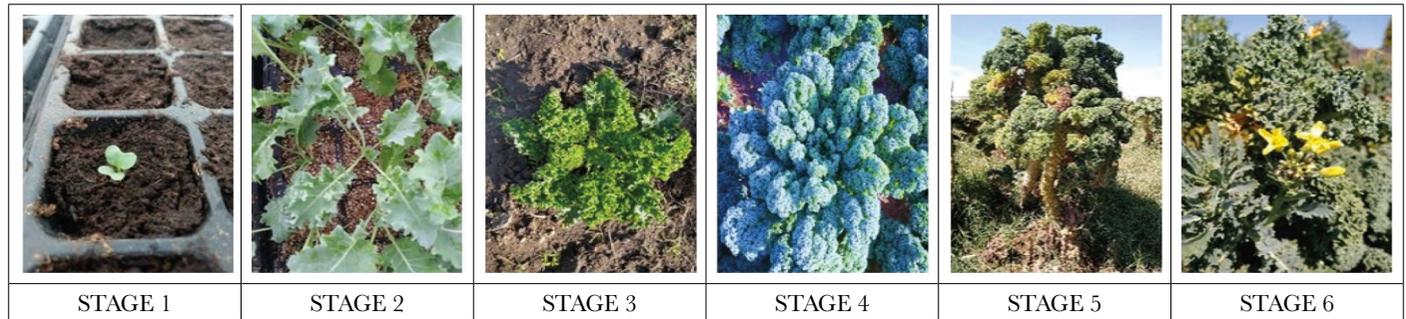


Figure 5. Phenological stages of kale cultivation in the field (*Brassica oleracea* var. *Acephala*).

Stage 1: seed imbibition until cotyledons (seedling) emerge. It occurs from 7 to 10 days. Stage 2 (seedling): begins after the emergence of the cotyledons and ends when the plant has between four to five true leaves (30 days). During this stage, the plants develop their root system and their first true leaves. There is a dull green hue and the leaves begin to present the characteristic kale curl; there is the presence of wax in the cuticle of the leaves, characteristic of brassicas. Stage 3 (vegetative development): starts from transplant to when they have ten to 14 leaves after transplanting, the plants enter a process of rapid biomass increase. The leaf area rapidly increases as well as the root system and the stem. Between 30 and 90 dat, an intense lemon green hue predominates in the leaves and the curling is more consistent. Stage 4 (maximum growth): the plant continues to produce leaves until it reaches its maximum production, that is, 30 to 45 leaves. The appearance rate is six to eight leaves per week. In this period the harvest begins, between 90-120 dat. The tonality of the leaves is an ashy bluish green according to the variety and the curling is intense. Stage 5 (plateau): in this stage, the production of leaves stabilizes, there is no accelerated growth. Harvesting may continue during this stage. From 130-260 dat. The old leaves located at the base of the plant show yellowing. At this point, the plant has the appearance of a palm tree. Stage 6 (reproductive): the plant requires low-temperature stimuli that activate the physiological processes that culminate in the production of inflorescences that are yellow typical of brassicas. From 260-280 dat (Figure 6).

Waterland *et al.* (2019) proposed a phenological classification of kale that consists of only five stages. It begins with an expansion stage of the cotyledon leaves until the presence of eight leaves (approximately 50 days after emergence), from then to an adult plant and flowering is not considered.

The six-length intervals of the leaves proposed in Materials and Methods were recorded. From 4 to 10 cm up to the commercial size of 20 to 35 cm. Fresh commercial-size leaves are heavy, so they are sold in bundles of six leaves or per kilo. This vegetable is usually found in agroecological and organic markets and some supermarkets with gourmet vegetables. For consumers, the cost of a bunch is between \$ 30 and \$ 45 Mexican pesos and the kilo between \$ 120 and \$ 150. However, the intermediaries buy bunches from producers at \$ 5.00 and kilos between \$ 25 to \$ 30. Only when producers directly sell to the consumer can they get a little more for their production. In the present experiment, the five treatments

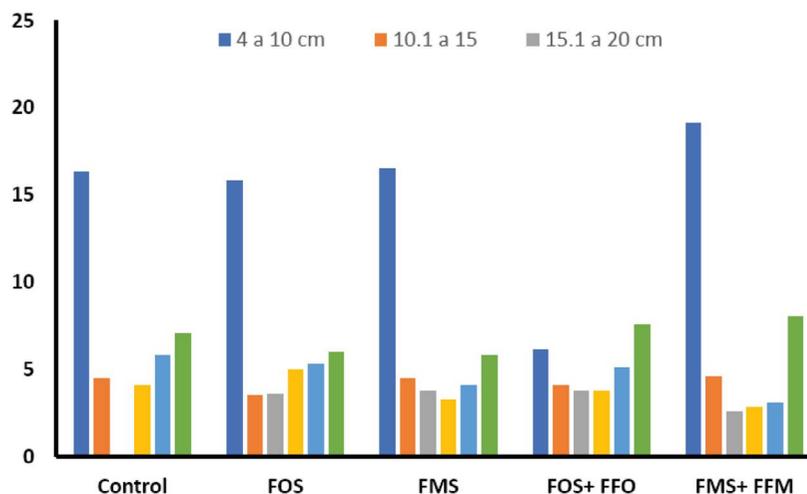


Figure 6. Leaves number per length interval produced per plant with different sources of nutrition at 116 dat.

presented the same trend of the length of the leaves per interval, except for the plants treated with organic fertilizer applied to the soil plus organic foliar fertilizer, the smaller leaves were less (Figure 6).

The leaves of between 20 and 30 cm are the most commercial, found in greater quantity in the control treatment and those where organic fertilization was applied to the soil and the combination to the soil and organic foliar fertilization. In the control and those that were applied to foliage, the number of leaves increased from 30.1 to 35 cm. By analyzing the number per interval, it is possible to divide it into commercial and non-commercial leaves to calculate the income from production, as indicated in Table 2.

Twelve to fifteen percent of the leaves turn out to be non-commercial. With the quantified commercial leaves, the number of bunches per plant was obtained and adjusted to a hectare consisting of 27,500 plants per ha (this value was obtained as a function of the distance sown in the experiment). The number of bunches varied from 1.82 (mineral fertilization to the ground and foliar) to 2.83 (control) and the production per plant from \$ 9.1 to \$ 14.5 from their sale to intermediaries

The income per hectare calculation ranged from \$ 250,250 to \$ 389,125. It seems an acceptable amount for a single cut; however, this crop requires a lot of labor for each cut, which on average it must be done every 7 days from 90 to 100 days, which is the

Table 2. Yield (g) of commercial leaves, number of bunches and income from kale leaves cuttings per plant and hectare developed with different sources of nutrition.

Treat.	Yield (g / plant)			Commercial leaf / plant	Bunch/ plant/ha	Income/ plant/ha \$
	No commercial	Commercial	Total			
Control	35.9	868.5	904.4	17.0	2.83/77,825	14.15/389,125
FOS	124.9	1006.5	1131.4	16.3	2.71/74,525	13.55/372,625
FMS	152.6	746.4	899	13.2	2.2/60,500	11/302,500
FOS+FFO	114.6	962.4	1077	16.5	2.75/75,625	13.75/378,125
FMS+FFM	122.5	1001.5	1124	10.9	1.82/50,050	9.1/250,250

maximum production. The plants in the control treatment were the ones that had more commercial leaves, followed by the plants where organic fertilizer was applied to the soil and foliar, finally the treatments with mineral fertilization to the soil and mineral fertilization to the soil and foliar (Table 2). Our results differ from those reported by Antonious *et al.* (2014), who found a favorable response to any source of fertilization, organic or mineral, which caused an increased yield per plant and per hectare compared to plants without fertilization. Although the control plants are the ones that produce the greatest number of leaves, their resistance to penetration was the lowest (7.51 kg force) compared to the leaves with organic fertilization to the soil and foliar (7.92 kg force). The application of any source of nutrition favored the resistance to the penetration of the leaf, as it increased between 2.6% and 5.4% above the value of the leaves of the control plants without fertilizer. In the experiment, any source of nutrition increased resistance, which favors shelf life (Table 3).

All treatments where fertilizer was applied increased the vitamin C content (Table 3). The plants in the control treatment reported the lowest ascorbic acid values. The mineral fertilization treatment to the soil + mineral foliar fertilization increased ascorbic acid by 61% compared to the control. Aquino *et al.* (2011) indicate that the ascorbic acid content in brassicas is influenced by edaphoclimatic conditions and cultural practices. The ascorbic acid concentration in the two treatments with organic fertilization remained constant and exceeded that of the treatment with mineral fertilization to the soil. When foliar sources are applied, the ascorbic acid value increases, indicating that the plant responded to the application of nutrients. Nepomuseno *et al.* (2020) found 96.7 mg/100g of fresh weight of this compound in 90-day-old kale leaves. In our research at 116 dat, the vitamin C content increased between 60 and 70%.

CONCLUSIONS

Kale (*Brassica oleracea* var. *Accephala*) increases its development with the application of conventional or organic fertilizers. The application of organic fertilizers to the soil and foliar increases the formation of leaves throughout the development of the plant, compared to mineral fertilizers. The amount of ascorbic acid (vitamin C) in kale leaves increases with the incorporation of organic and inorganic fertilizers. On average, a cut of leaves (20 to

Table 3. Resistance to penetration and ascorbic acid content in kale (*Brassica oleracea* var. *Accephala*) leaves of 116 dat developed with organic fertilization to the soil, mineral and foliar fertilization.

Treatment	Penetration resistance (kgf)	Ascorbic acid (mg/100g)
Control	7.51 a	147 c
FOS	7.71 a	189 b
FMS	7.77 a	156 c
FOS+FFO	7.92 a	199 b
FMS+FFM	7.73 a	238 a

Values with different letters in each column represent statistical differences according to Tukey's test ($P < 0.05$).

30 cm) yields 1.5 to 3 bunches of six leaves per kale plant. The quality of the leaf and the nutritional content are a function of the application of fertilizers to the crop.

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Economic profitability analysis of husk tomato (*Physalis ixocarpa* Brot. ex Hornem.) under different silicon dioxide concentrations

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ABSTRACT

Objective: To evaluate the effect of different doses of foliar and soil silicon dioxide fertilization on the economic profitability of husk tomato (*Physalis ixocarpa* Brot. ex Hornem.) cv. ‘Querétaro’ cultivation under plastic paddings and macro-tunnel conditions.

Design/methodology/approach: Ten treatments were evaluated at different concentrations of silicon dioxide fertilization on soil and foliar application: T1: Control treatment (T), T2: Fertilization with silicon dioxide 20, 40, 60 g on soil and 100, 150, and 200 ppm foliar (S20/100F), T3: S20/150F, T4: S20/200F, T5: S40/100F, T6: S40/150F, T7: S40/200F, T8: S60/100F, T9: S60/150F and T10: S60/200, distributed in random blocks. Economic profitability indicators such as Benefit/Cost ratio (B/C), Net Present Value (NPV), and Internal Rate of Return (IRR) were determined.

Results: The research established that the S60/150F treatment was the one with the highest economic profitability because it promoted the highest production per hectare, which was reflected in the NPV (MX\$ 336,932.97 pesos), the IRR (77.3%), and a B/C of MX\$ 1.16 pesos. Also, treatments S40/200F and S60/100F (where SiO₂ was applied) reported positive cash flow, unlike the T.

Limitations of the study/implications: With all research facilities closed due to the COVID-19 pandemic, no evidence related to the contribution of foliar and soil silicon bioassay has been verified.

Findings/conclusions: Using SiO₂ leads to a financial appreciable rebound of vital importance to be included in economic studies to facilitate the efficient management of the available capital to establish a crop whose field productivity is profitable for the producers.

Keywords: Benefit/Cost ratio (B/C), Net Present Value (NPV), Internal Rate of Return (IRR), yields.

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INTRODUCTION

In 2019, husk tomato (*Physalis philadelphica*) production in Mexico was 834,274 t, out of which 160,771 t were produced in Sinaloa, 147,023 t in Zacatecas, and 88,637 t in Jalisco. These three states are where the highest national production was recorded (SIAP, 2020a), while in Veracruz 12,311 t were harvested with a yield of 15.525 t·ha⁻¹ (SIAP, 2020b).

Among the main problems that restrict this crop's field productivity increase is the lack of improved varieties (Peña-Lomelí *et al.*, 2020), phytosanitary problems (Ayala-Armenta *et al.*, 2020), and inadequate agronomic management (López-Ramos *et al.*, 2020). Under protected agriculture conditions, there is greater control of environmental variables compared to open field agriculture conditions (Machorro, 2020). Silicon is a beneficial element for both, open field and greenhouse crops (Tubana *et al.*, 2016; Gómez-Merino *et al.*, 2020) improving growth, development, and production indicators (Asadpour *et al.*, 2020; Bukhari *et al.*, 2020; Shahzad *et al.*, 2021). Silicon is applied to optimize crops profitability beyond the net present value (NPV), payback period (PRI), internal rate of return (IRR), and cost structure, among others. This analysis provides an important point of reference and comparison to assess whether the management implemented for a crop provides adequate returns on the investment made, considering the involved risk (Cano *et al.*, 2013). From such analysis, it is possible to gain certain benefits from the project. The objective of this research was to evaluate the effect different doses of foliar and soil fertilization with silicon dioxide had on the economic profitability of husk tomato (*Physalis ixocarpa* Brot. ex Hornem.) cv. 'Querétaro' cultivation under plastic paddings and macrotunnel conditions.

MATERIALS AND METHODS

Location of the experimental area

The research took place from February to June 2020 in a property near Xalapa, Veracruz state, Mexico, at the geographical coordinates 19° 33' 05.37" N, 96° 56' 40.64" W, and 1,428 m a.s.l. elevation.

The husk tomato crop was established with a drip irrigation system with plastic padding in a macro-tunnel. The production costs and yields of 10 treatments were evaluated and extrapolated to 1 ha, taking into account two production cycles per year. The seeds used were of the cv 'Querétaro' (Optimus Seeds), sown in 200-cavity uncel trays containing peat as substrate (COSMOPEAT[®]). Thirty days after sowing (DDS) the seedlings were transplanted to the field, placing them at a 0.4 m distance between plants and 1.2 m between rows, with a planting density of 20,833 plants. ha⁻¹. The technological package applied in its cultivation was the one recommended by AgroScience (AgroScience[®], 2019).

Treatments and experimental design

Different doses of silicon dioxide (SiO₂) applied to the soil (g·plant⁻¹) and foliage (ppm) were evaluated. The doses applied in the soil were 0, 20, 40 and 60 g SiO₂ per plant, while the doses applied to the foliage were 100, 150, and 200 ppm SiO₂ per plant. There were 10 treatments in total, as described in Table 1.

The experimental design was a completely randomized block. The treatments were distributed in three blocks with 12 plants spaced every 40 cm, in a linear topological arrangement, with a total of 360 experimental units.

Economic profitability analysis

Production costs were classified into fixed and variable costs, and at the same time, fixed assets, deferred assets, and working capital were considered. Depreciation was calculated

Table 1. Silicon dioxide (SiO₂) treatments applied on soil (S) and foliage (F) in husk tomato (*Physalis ixocarpa* Brot. ex Hornem.) in a microtunnel production system at Xalapa, Veracruz, Mexico.

Treatment	Application of SiO ₂ to the soil (g plant ⁻¹)	Application of SiO ₂ to the foliage (ppm)	Key to treatments
T1	0	0	T1 (S00/F00)
T2	20	100	T2 (S20/F100)
T3	20	150	T3 (S20/F150)
T4	20	200	T4 (S20/F200)
T5	40	100	T5 (S40/F100)
T6	40	150	T6 (S40/F150)
T7	40	200	T7 (S40/F200)
T8	60	100	T8 (S60/F100)
T9	60	150	T9 (S60/F150)
T10	60	200	T10 (S60/F200)

T1: Control (without silicon dioxide), T2: Application of 20 g of SiO₂ on soil and 100 ppm of SiO₂ sprayed on foliage, T3: Application of 20 g of SiO₂ on soil and 150 ppm of SiO₂ sprayed on foliage, T4: Application of 20 g of SiO₂ on soil and 200 ppm of SiO₂ sprayed on foliage, T5: Application of 40 g of SiO₂ on soil and 100 ppm of SiO₂ sprayed on foliage, T6: Application of 40 g of SiO₂ on soil and 150 ppm of SiO₂ sprayed on foliage, T7: Application of 40 g of SiO₂ on soil and 200 ppm of SiO₂ sprayed on foliage, T8: Application of 60 g of SiO₂ on soil and 100 ppm of SiO₂ sprayed on foliage, T9: Application of 60 g of SiO₂ on soil and 150 ppm of SiO₂ sprayed on foliage, T10: Application of 60 g of SiO₂ on soil and 200 ppm of SiO₂ sprayed on foliage.

using a linear method, yield and costs were projected to 1 ha area, calculating a five-year production period in the same area unit, based on this, the following indicators were estimated:

Cost/benefit ratio (C/B): this indicator allows valuing investments, considering economic, social and environmental aspects that are considered in the purely financial evaluation (Arévalo *et al.*, 2016). Net present value (NPV): This refers to the monetary value resulting from subtracting the sum of the discounted cash flows from the initial investment (Fajardo *et al.*, 2019). Internal rate of return or internal return rate (IRR): This is the rate that makes the NPV equal to zero (Fajardo *et al.*, 2019), knowing that this does not mean the non-existence of benefits, but that these are barely enough to cover the project's expectations (Mete, 2014). The above-mentioned indicators were determined with the Office software using the Excel program, based on the analysis of the costs of inputs that were used in the production, the generated income based on the yields per treatment, the rest of the production costs, and the depreciation of the assets.

RESULTS AND DISCUSSION

The highest yield (43.8 t·ha⁻¹) was achieved in the S60/150F treatment (T9), which is above the previously reported in 2019 for husk tomatoes (22.75 t·ha⁻¹) grown in irrigated systems (FIRA, 2020). Similarly, the remaining treatments exceeded both, the tonnage referred by the FIRA and that of plants without silicon dioxide (SiO₂) (T1, S00/F00) (Table 2).

Table 2. Yield per plant and unit area of husk tomato fruits (*Physalis ixocarpa* Brot. ex Hornem.) as a response to silicon dioxide (SiO₂) applications on soil (S) and the foliage (F) in a microtunnel production system at Xalapa, Veracruz, Mexico.

Treatment	Yield (kg plant ⁻¹)	Yield (t plant ⁻¹)
T1 (S00/F00)	0.558	15.5
T2 (S20/F100)	1.007	27.7
T3 (S20/F150)	1.474	40.9
T4 (S20/F200)	1.138	31.6
T5 (S40/F100)	0.971	26.9
T6 (S40/F150)	1.052	29.2
T7 (S40/F200)	1.365	37.9
T8 (S60/100)	1.522	42.2
T9 (S60/F150)	1.580	43.8
T10 (S60/F200)	1.184	32.8

T1: Control (without silicon dioxide), T2: Application of 20 g of SiO₂ on soil and 100 ppm of SiO₂ sprayed on foliage, T3: Application of 20 g of SiO₂ on soil and 150 ppm of SiO₂ sprayed on foliage, T4: Application of 20 g of SiO₂ on soil and 200 ppm of SiO₂ sprayed on foliage, T5: Application of 40 g of SiO₂ on soil and 100 ppm of SiO₂ sprayed on foliage, T6: Application of 40 g of SiO₂ on soil and 150 ppm of SiO₂ sprayed on foliage; T7: Application of 40 g of SiO₂ on soil and 200 ppm of SiO₂ sprayed on foliage, T8: Application of 60 g of SiO₂ on soil and 100 ppm of SiO₂ sprayed on foliage, T9: Application of 60 g of SiO₂ on soil and 150 ppm of SiO₂ sprayed on foliage, T10. Application of 60 g of SiO₂ on soil and 200 ppm of SiO₂ sprayed on foliage.

The exogenous application of silicon improves plant performance due to its participation in regulating proteins related to photosynthesis, photosynthetic pigments, increasing nutrient uptake, and activating antioxidant defense systems in plant cells (Liu *et al.*, 2019; Abdelaal *et al.*, 2020). The basic total cost of production represents the total amount of fixed and variable costs that were required in each treatment and is not affected by costs derived from procurement, silicon application, or direct harvesting and packing costs. This variable includes costs that were the same for all treatments: MX \$269,172.00 (Table 3).

Table 3. Variable and fixed costs of husk tomato (*Physalis ixocarpa* Brot. ex Hornem.) cultivation in the control (T1) in a microtunnel production system at Xalapa, Veracruz, Mexico.

Variable Costs	Amount (MX\$)	Fixed Costs	Amount (MX\$)
Insecticides and fungicides	\$28,188	Renta de terreno	\$15,000
Fertilizers*	\$49,684	Renta de tractor	\$2,000
Herbicides	\$1,400	Servicios	\$26,700
Seedlings	\$122,500		
Wooden box and plastic padding	\$8,700		
Land workers	\$15,000		
Temporary landworkers	\$19,800		
Total costs	\$245,272	Total costs	\$43,700.00

* The cost changes depending on each treatment. Costs in Mexican pesos as of June 2020.

Table 4 shows the variation in costs depending on the silicon concentration of each treatment and the yield obtained in each one.

Labor represents the highest expenditure percentage in the costs of production, which, according to the SiO₂ applied dose, was around 33.07%, depending on each treatment's yield. This coincides with the results obtained in other assessments of the economic profitability, such as the one by Ayala *et al.* (2014) in amaranth (*Amaranthus* spp.) cultivation at the central region of Mexico, which influenced jobs generation and prevented migration.

Villa and Barrientos (2012) reported a costs production increase in creole potato (*Solanum tuberosum* Andigena group) crops per unit area due to manganese application, which increased yields and demanded more labor for harvesting and packaging, as well as the direct and indirect costs of using this micronutrient.

It is important to note that in this research, the use of silicon increased yields above the average reported for Veracruz by both SIAP (15.525 t·ha⁻¹) (SIAP, 2020b) and FIRA (22.75 t·ha⁻¹) (FIRA, 2020) (Table 2).

Assets costs were classified as fixed, variable, and working capital (Table 3). Regarding the concept of fixed cost, Baca (2010) states that they are those done by the company and that in short term, or for certain production levels, do not depend on the production volume. The total cost of the assets was MX \$57,042.00, with the irrigation system accounting for 70% of the total. All fixed assets were depreciated using the straight-line method with a useful life of five years, which is the estimated time of the project.

For the scale, a salvage value of MX\$ 1,197.50 pesos was achieved, since its useful life is of 10 years, unlike the rest of the fixed assets whose useful life is equal to or less than five

Table 4. Costs increase by silicon dioxide (SiO₂) application per year of production in a hectare of husk tomato (*Physalis ixocarpa* Brot. ex Hornem.) in the Control (T1) in a microtunnel production system at Xalapa, Veracruz, Mexico.

Treatments	SiO ₂ (Cost)	Land workers	Wooden box
T1 (S00/F00)	\$0.00	\$19,800	\$62,080
T2 (S20/F100)	\$600	\$35,400	\$111,600
T3 (S20/F150)	\$600	\$40,500	\$126,120
T4 (S20/F200)	\$600	\$40,500	\$126,120
T5 (S40/F100)	\$600	\$33,900	\$107,600
T6 (S40/F150)	\$600	\$33,200	\$116,560
T7 (S40/F200)	\$1,200	\$48,000	\$151,240
T8 (S60/100)	\$1,200	\$54,600	\$171,960
T9 (S60/F150)	\$1,200	\$55,500	\$175,040
T10 (S60/F200)	\$1,200	\$41,700	\$131,000

T1: Control (without silicon dioxide), T2: Application of 20 g of SiO₂ on soil and 100 ppm of SiO₂ sprayed on foliage, T3: Application of 20 g of SiO₂ on soil and 150 ppm of SiO₂ sprayed on foliage, T4: Application of 20 g of SiO₂ on soil and 200 ppm of SiO₂ sprayed on foliage, T5: Application of 40 g of SiO₂ on soil and 100 ppm of SiO₂ sprayed on foliage, T6: Application of 40 g of SiO₂ on soil and 150 ppm of SiO₂ sprayed on foliage, T7: Application of 40 g of SiO₂ on soil and 200 ppm of SiO₂ sprayed on foliage, T8: Application of 60 g of SiO₂ on soil and 100 ppm of SiO₂ sprayed on foliage, T9: Application of 60 g of SiO₂ on soil and 150 ppm of SiO₂ sprayed on foliage, T10: Application of 60 g of SiO₂ on soil and 200 ppm of SiO₂ sprayed on foliage. Costs in Mexican pesos (MX\$) as of June 2020.

years. Working capital was estimated at MX\$ 173,294.67 pesos. The total income for the five years of the project was restated at a 20% rate, as shown in Table 5.

Also, for the project purposes, the selling price recorded by the producer was MX\$ 9.00 pesos according to the fruit and vegetable marketing margins reported by the FIRA (2020) for peel tomato for October 2020, giving a total of MX\$ 9,000.00 pesos per ton of fruit. Although the highest yield treatment, and therefore the one with the highest income was T9 (S60/150F), all treatments reported higher cash flows than that of the control.

The NPV of the treatment with the highest income (T9) was MX\$ 336,932.97 pesos, which indicates the gains with regard to the initial investment amount (Table 5) at a discount rate of 20%. Thus, and according to this indicator criteria, the project is profitable, considering that an NPV of less than zero indicates that the project is not.

The economic IRR of a project is the discount rate that makes the present value of the stream of benefits equal to the present value of the stream of costs (Baca, 2010). Then, the IRR for this treatment was 77.3% and is acceptable since it is higher than the expected return rate (20%), so that during the useful life of the project, for each invested peso, a profit of 0.16 Mexican pesos will be obtained (B/C=MX\$ 1.16), so that this treatment does not support a price lower than MX\$ 8,000.00 pesos per ton of fruit.

Table 5 shows that treatments T9 and T8, where high doses of silicon (S60/150F and S60/100F) were used, positive cash flows are obtained. These results concur with those by Mejía and Lopez (2019), who indicate that by applying Si in high doses on King Grass texas-25 (*Pennisetum purpureum*), the gain is higher compared to the absolute control.

Table 5. Total updated income and expenses for each treatment projected to five years, and calculation of NPV, IRR and B/C in different treatments with silicon dioxide (SiO₂) in husk tomato (*Physalis ixocarpa* Brot. ex Hornem.) cultivation in a microtunnel production system at Xalapa, Veracruz, Mexico.

Treatments	Income (\$)	Expenses (\$)	Cash Flow (\$)	Updated income (\$)	Updated expenses (\$)	NPV (\$)	IRR (%)	B/C (\$)
T1	1,436,646	2,493,476	2,493,476	856,670	1,554,573	-697,903.71	% [‡]	0.55
T2	2,582,443	2,867,811	285,368	1,540,092	1,784,655	-244,563.00	% [‡]	0.86
T3	2,918,235	2,979,565	-61,329	1,740,379	1,853,343	-112,964.32	-13.3	0.94
T4	2,918,235	2,979,565	61,329	1,740,379	1,853,343	-112,964.32	-13.3	0.94
T5	2,490,164	2,836,484	346,319	1,485,051	1,765,400	-280,348.57	% [‡]	0.84
T6	2,697,791	2,906,315	208,524	1,608,893	1,808,321	-199,428.44	% [‡]	0.89
T7	3,500,105	3,168,783	331,322	2,087,441	1,969,644	117,796.88	43.1	1.06
T8	3,979,443	3,324,395	655,047	2,373,347	2,065,290	308,057.25	73.2	1.15
T9	4,051,215	3,347,064	704,150	2,416,156	2,079,223	336,932.97	77.3	1.16
T10	3,036,147	3,018,753	17,394	1,810,708	1,877,429	-66,720.95	3.1	0.96

T1: Control (without silicon dioxide); T2: Application of 20 g of SiO₂ on soil and 100 ppm of SiO₂ sprayed on foliage, T3: Application of 20 g of SiO₂ on soil and 150 ppm of SiO₂ sprayed on foliage, T4: Application of 20 g of SiO₂ on soil and 200 ppm of SiO₂ sprayed on foliage, T5: Application of 40 g of SiO₂ on soil and 100 ppm of SiO₂ sprayed on foliage, T6: Application of 40 g of SiO₂ on soil and 150 ppm of SiO₂ sprayed on foliage, T7: Application of 40 g of SiO₂ on soil and 200 ppm of SiO₂ sprayed on foliage, T8: Application of 60 g of SiO₂ on soil and 100 ppm of SiO₂ sprayed on foliage, T9: Application of 60 g of SiO₂ on soil and 150 ppm of SiO₂ sprayed on foliage, T10: Application of 60 g of SiO₂ on soil and 200 ppm of SiO₂ sprayed on foliage.

Benefit / cost ratio (B/C); Net present value (NPV); Internal rate of return (IRR).

[‡]Project data without a return value. Costs in Mexican pesos (MX\$) as of June 2020.

CONCLUSIONS

Product price and yield per hectare represent the main factors affecting the economic profitability of treatments applied to crops. In our research, the best treatment was T9 (S60/150F), reporting a higher cash flow than that of the control treatment. Also, because the present research only considered one price per kilogram of husk tomato, without considering quality variables that could contribute to the commercialization of the fruit of this Solanaceae in specific markets at higher prices, it is recommended to continue with the economic evaluation for several cycles, to corroborate the yield data per treatment and therefore compare the results obtained in the present results.

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First attempt to fill gaps in the feeding of the axolotl

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ABSTRACT

Objective: To determinate the attractability of four oils, fish, chicken, krill, and red crab (*Pleuroncodes planipes*) for *Ambystoma mexicanum* juveniles, evaluating their feeding behavior using a Y aquarium.

Design/Methodology/Approach: Ten axolotls were used per test, fasted for 48 h. Gels with oils were prepared using gelatin and poured into petri dishes and refrigerated until gelation. A recording of the test was made using two video cameras. In the feed chamber the gelled oil was placed and allowed to stand for 15 min. On the other chamber a gelled disk with no other ingredient than gelatin and water was placed. The video recording began once the 15 min of gel permanence had finished, removing the barrier so that the axolotls could move through the rest of the aquarium. All tests were carried out with a recording time of 30 min.

Results: Fish oil demonstrated a lower attraction effect compared to krill, red crab and chicken oils ($P < 0.05$), while chicken oil (30.00 ± 1.73) doubled the attractive effect of krill oil (16.00 ± 1.00).

Limitations of the study/implications: It was necessary to condition a room with controlled environmental temperature for *A. mexicanum* (18 ± 1 °C).

Findings/Conclusions: Krill and chicken oil are good feeding effectors for *A. mexicanum* causing positive feeding behavior. The use of chicken oil is desirable because of its low cost compared to krill.

Key words: axolotl; chicken; krill; chemostimulants.

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INTRODUCTION

Ambystoma mexicanum (Shaw & Nodder, 1798) is an endemic species from Lake Xochimilco, Mexico, that is endangered in its natural habitat. Its population has decreased considerably, it is estimated that today there are less than 35 animals per square kilometer



(Vance, 2017). Due to its biological characteristics in which it stands out the ability to regenerate almost any part of its body, it has been used as a biological model for years on labs, and it is well appreciated as an exotic pet being distributed in great part of the world. Because of that, *A. mexicanum* has been reproduced largely in captivity which has helped to conserve the species (Gresens, 2004; Vance, 2017).

Despite the high degree of scientific and ornamental interest of the Mexican axolotl (*Ambystoma mexicanum*), studies on its feeding and nutrition in captivity, are practically nil. Keeping them in captivity can be expensive because their diet is mainly made up of live food. In the early larval stages, because their gape size is really small, the live feed has an important role in the development of *A. mexicanum* (Chaparro-Herrera *et al.*, 2011) and other species of the same genre like *A. maculatum* (Freda, 1983), *A. altamiranoi* (Lemos-Espinal *et al.*, 2015), *A. granulatum* (Sarma *et al.*, 2017) being zooplankton their main source of nutrients. Gresens (2004) mentions that once axolotls reach 5 cm, they can begin to receive food in pellets in their diet. However, there is the possibility of them not being accepted because they seem unattractive.

Aquatic organisms can detect compounds (amino acids, sugars, fatty acids) dissolved in the environment and identifying them as possible food using different detection methods (Nolasco-Soria, 2014; Villarreal-Cavazos *et al.*, 2017). Axolotls have a vomeronasal system and an olfactory system. It is believed that the former is used to detect large molecules, especially pheromones for reproduction compared to the olfactory system, which detects smaller molecules (Eisthen & Park, 2005).

It is easier to achieve the change in diet from live feeding to pellets using soft pellets, which sink to the bottom and have a strong smell, as this would help the attractability of the feeding increasing the chances of success (Gresens, 2004).

The production of balanced feed aimed at aquaculture species must consider the attractability. Adding attractant compounds is supposed to increase food detectability and intake, optimize feed conversion rates, reduce waste, and help the transition from live food to pellets (Dempsey, 1978; Mendoza, 1999; Gresens, 2004; Montemayor-Leal *et al.*, 2005). Among aquafeed producers, the ingredients most used as attractants are fish meals, soluble extracts from marine fish, shrimp head and squid meals, or squid liver oil (Mendoza *et al.*, 1999). In order to measure the attractant power of a molecule, ingredient or food for a determined species, attractability tests are carried out *in vivo* at the laboratory level or in experimental or commercial feeding bioassays.

To the best of our knowledge, the attractability of feeds or their ingredients for *A. mexicanum* has not been formally subjected to behavioral bioassays, nor has feeding behavior been described. Contrarily, some extensive studies have been carried out on the subject for fish (Oikawa & March, 1997; Kolkovski *et al.*, 2000; Barroso *et al.*, 2013; Sanches-Alves *et al.*, 2019) and crustaceans (Mendoza *et al.*, 1997; Cruz-Suárez *et al.*, 2000; Montemayor-Leal *et al.*, 2005; Montoya-Martínez *et al.*, 2018).

Therefore, the objective of this study was to determine the feeding behavior of these animals by including some oils of animal origin as additives to increase the attractability of feeds, helping to contribute on the design of specific diets for *A. mexicanum*.

MATERIALS AND METHODS

Attractability bioassays were carried out using oils from alternative sources: krill oil (SimiKrill[®]), red crab (*Pleuroncodes planipes*) (obtained from the Centro de Investigaciones Biológicas del Noroeste, SC), chicken oil (Proteínas Marinas y Agropecuarias, S.A. de C.V.[®]) and fish oil (Proteínas Marinas y Agropecuarias, S.A. de C.V.[®]) of conventional use in the formulation of commercial feed for aquatic organisms. The juveniles of *A. mexicanum* were donated by the AXOS-PIMVS production center located in Tepic, Mexico (Bahía de Banderas 62, Lomas de la Cruz, 63037). The smallest axolotls (12.2 ± 1.0 cm) that the producer had, accustomed to feeding with pellets, were selected. The animals were transported in individual bags to the Laboratory for Water Quality and Experimental Aquaculture (LACUIC), belonging to the University of Guadalajara, located in Puerto Vallarta, Jalisco. After an acclimatization period of 30 minutes, they were placed in the laboratory with a controlled environment to guarantee a stable temperature of 18 ± 1 °C. They were placed randomly in two 300-liter ponds and fed high-protein pellets for cold water fish (Silvercup[®], 42% protein, 15% fat) ad libitum during the maintenance period prior to the bioassays. For this study, gels with oils were prepared using the method proposed by Nolasco-Soria (2014). Gelatin was weighed and pre-hydrated with cold water. Afterwards, 5 g of the oil to be evaluated was weighed and 20 mL of water was added and stirred using a heating plate. The gelatin (pre-hydrated and heated in the microwave for 8 seconds) was then added. The homogenized mixture (15 mL) was poured into petri dishes and refrigerated until gelation. For the evaluation, a Y-shaped aquarium (Figure 1. Nolasco-Soria, 2014; Montoya-Martínez *et al.*, 2018) made with acrylic material was used. For the tests, the aquarium was filled with water (6.5 cm deep) at the same temperature as the maintenance tanks. After each test the aquarium was washed with detergent and neatly rinsed with tap water to discard remains of the used oil. Ten axolotls were used per test, fasted for 48 hours, placed in the R region

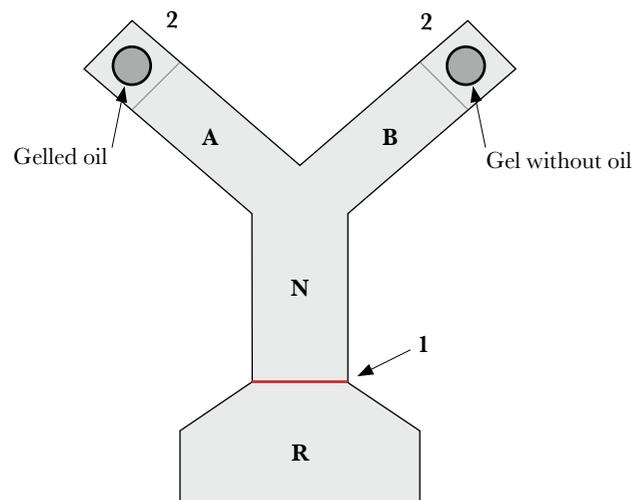


Figure 1. Y system for attractability tests (Nolasco-Soria, 2014; Montoya-Martínez *et al.*, 2018). R: Acclimation chamber, N: Transit zone, A and B: Arms, 1: Barrier, 2: Feed chambers.

of the aquarium, and allowed to acclimate for one hour. Between the R and N regions, a barrier was placed to prevent the axolotls from entering the rest of the system. This barrier is a transparent acrylic square, which snaps into the body of the aquarium so that it does not allow the axolotls to perceive the odor during acclimatization. In the feed chamber of regions A or B (arms), alternating between the tests, the gelled oil was placed and allowed to stand for 15 minutes. On the other chamber a gelled disk with no other ingredient than gelatin and water was placed.

A recording of the test was made using two video cameras (GoPro®). One of these was placed framing both arms of the aquarium and a fraction of the “N” zone, and the second one was installed framing the side of the feeding chamber with the gelled oil. The video recording began once the 15 minutes of gel permanence had finished, removing the barrier so that the axolotls could move through the rest of the aquarium (the room was abandoned to avoid human interference). All tests were carried out with a recording time of 30 min, within the same closed room with controlled temperature. The axolotls used in the bioassays were left in isolation for at least 48 hours before being used again for another test. They were fed *ad libitum* during isolation time.

After having performed all the tests in triplicate, the videos were reviewed and the following indices were analyzed: i) time (s) of the first admission (it is the time required for the first axolotl to enter the feeding chamber of the gel arm once the gate was removed); ii) number of axolotls that entered the gel arm; iii) time of permanence of the axolotl in the feeding chamber (s); iv) number of axolotls that entered the feeding chamber remaining close to the gel; v) number of axolotls that fed on the gel.

All bioassays were carried out in compliance with animal welfare protocols. The animals were not subjected to any kind of suffering in a programmed or innocent way. Once the study was completed, the axolotls were relocated to their original reservoirs under optimal maintenance conditions.

A Kolmogorov-Smirnov test was performed to assure the normality and homoscedasticity of the obtained data. Subsequently an ANOVA analysis was performed. In cases where significant differences were found between treatments ($P < 0.05$), the Tukey test was performed for comparison. These tests were conducted using SigmaPlot software.

RESULTS AND DISCUSSION

The statistical tests indicate that there were significant differences ($P < 0.05$) in the number of organisms that entered the arm containing the gel (far orientation) and number of organisms that entered the feeding chamber remaining close to the gel (close orientation). The response to the fish oil stimulus was lower compared to that of krill, red crab and chicken oils, despite fish oil being the most commonly used for aquatic animal feed. Between chicken oil and krill oil, there were no differences in the number of individuals that accessed the arm with the gel, although the latter attracted twice as many individuals that entered the feeding chamber while remaining close to the gel (Table 1).

Table 1. Average and standard deviation of the effect on feeding behavior of *A. mexicanum* induced by red crab, krill and chicken oils, in 30 min tests.

	Red crab oil	Krill oil	Chicken oil	Fish oil
Time to first admission (sec)	196±129 ^a	284±23 ^a	241±72 ^a	269±62 ^a
Number of organisms that entered the arm with gel	16.67±3.79 ^a	15.00±2.00 ^a	17.67±1.53 ^a	7.67±0.58 ^b
Time of the stay in the feeding chamber (sec)	1719±274 ^a	2942±316 ^a	1994±176 ^a	1941±847 ^a
Number of organisms that entered the feeding chamber	18.33±2.08 ^a	16.00±1.00 ^a	30.00±1.73 ^b	13.67±4.16 ^a
Number of organisms that fed of the gel	4.67±4.619 ^a	6.33±0.58 ^a	6.67±0.57 ^a	3.33±4.16 ^a

* Means with different letters in the same line are significantly different according to Tukey's test ($P < 0.05$).

This may mean that chicken oil and krill oil are good attractants, but when axolotls are near the stimulus, krill oil is the most striking. In addition, despite the fact that no statistical differences were found between treatments in the number of axolotls that fed on the gels, it was observed that chicken and krill oils are good stimulants for the ingestion of the gel and encourage the axolotls to keep feeding. This was evidenced by the higher consumption rate of these gels observed at the end of each test. It was observed that the axolotls explored the arm with the gelled disc without additives, but did not remain in it, so data recording was not considered. This exploration was rapid, which suggested the search for the origin of the attractant molecules, which in fact was in the other arm and in which they entered and remained at the different times that were recorded.

Since no study was found on the attractability of compounds, ingredients, or foods in the family Ambystomatidae (and probably not in the entire order Caudata), the interpretation of the eating behavior responses was based on the generalized behavior pattern for all type of species described by Lindstedt (1971). This author indicates that there are different chemical effectors that can positively or negatively affect animals and are classified according to their effect on their behavior patterns (Table 2). By observing the videos, it is possible to identify the four behavioral responses, starting with the orientation (far away) towards the food, movement for orientation close to the food, start of feeding and continued intake until its completion. For this reason, the "Y" -shaped aquarium can be considered suitable for obtaining favorable results in the attractability tests with *A. mexicanum*. Unfortunately, studies on nutrition in amphibians are scarce as for many species there are no specific commercial feeds, so pelleted feeds designed for other species need be used (Domínguez, 2005). Most of the research carried out with

Table 2. Types of chemical stimuli that affect the response pattern of feeding behavior on all type of species. Beck (1965) modified by Lindstedt (1971).

Response	Evoking stimulus	
	Positive	Negative
Orientation (distant)	Attractant	Repellent
Orientation (close)	Arrestant	Repellent
Initiation of feeding	Incitant	Suppressant
Continuation of feeding	Stimulant	Deterrent

balanced feeds for tadpoles is focused on finding the optimal protein requirements and their effects on development (Carmona-Osalde *et al.*, 1996; Martins *et al.*, 2013; Pinto *et al.*, 2015; Godome *et al.*, 2019), without considering the attractiveness and palatability of the ingredients used.

However, there are several studies evaluating the attractability or responses to food effectors (term called for attractants, initiators, and stimulants), ingredients, foods, commercial attractants, animal and plant extracts, pheromones, etc. in other aquatic animals. In studies carried out with fish (Oikawa & March, 1997; Kolkovski *et al.*, 2000; Barroso *et al.*, 2013; Sanches-Alves *et al.*, 2019) and crustaceans (Costero and Meyers, 1993; Jaime-Ceballos, 2007; Suresh *et al.*, 2011; Sacristán *et al.*, 2014; Montoya-Martínez *et al.*, 2018) the authors seek to increase the attractability of the food supplied to improve intake and therefore obtain better results in food consumption, growth, development and survival of organisms.

In general, the chemoreceptors of aquatic organisms are sensitive to low molecular weight water soluble chemicals (Lee & Meyers, 1996; Nunes *et al.*, 2006). Ingredients originating from aquatic animals (such as flour, soluble fish, mollusks, or crustaceans) have a high content of these compounds, which is why they are considered as excellent attractants (Smith *et al.*, 2005; Ali *et al.*, 2007), while by-products of terrestrial animal origin (such as oils and flours from poultry by-products) contain lower levels of them. Therefore, they are considered to have lower attractability and palatability, but few studies have been carried out to confirm this fact (Suresh *et al.*, 2011). In this context some studies have shown in fish that hydrolyzed krill is a good attractant, since when used as a food additive it increases intake and growth in *Oncorhynchus mykiss* (Oikawa & March, 1997) and in *Perca flavescens*, *Vitreous Stizostedion* and *Coregonus clupeaformis* (Kolkovski *et al.*, 2000). However, similar results have been found with the use of hydrolyzed poultry protein (Sanches-Alves *et al.*, 2019) when including it as a food additive for *Oreochromis niloticus*. In crustaceans (*Litopenaeus stylirostris*), Suresh *et al.* (2011) found that poultry by-product meals showed the highest attractability, but the highest palatability was obtained by krill meal. Smith *et al.* (2005) reported that the inclusion of krill increased feed consumption in *Panaeus monodon* and improved growth (Williams *et al.*, 2005), with similar results obtained in *Panaeus vannamei* (Córdova-Murueta & García-Carreño, 2002).

CONCLUSIONS

In conclusion, krill and chicken oil are good feeding effectors for *A. mexicanum*, causing positive feeding behavior. Based on these results, it is suggested to carry out nutritional bioassays using oils as additives in experimental diets to evaluate the increase in the attractability of foods directed to the species, and to contribute to the design of specific diets that allow better development and health status of axolotls. These results provide essential knowledge to improve the quality of nutrition of this endangered endemic species from Mexico, in captivity conditions, which would allow the design of more effective reproduction and assisted repopulation plans.

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Effect of NPK fertilization on the chemical properties and bioactive compounds of the *cabezona* pineapple fruit

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ABSTRACT

Objective: To evaluate the effect of NPK fertilization on the chemical properties and bioactive compounds of the *cabezona* pineapple fruit.

Design/methodology/approach: 15 NPK fertilization treatments were evaluated and carried out in the field in a random complete block design with four repetitions per treatment. At the end of the crop cycle, we determined the pH, °Brix and citric acid % of the fruits that were harvested, following the methodology established in NMX-FF-028 and 011. The pH of the juice was measured directly using a potentiometer. Ascorbic acid was measured with a SIGMA-ALDRICH® kit, the total polyphenol contents were determined following the methodology of De la Cruz-Ricardez *et al.* (2020), while total flavonoid contents were measured following a modified version of the methodology proposed by Hossain *et al.* (2011).

Results: The mean values for °Brix, citric acid %, and pH were 7.3, 7.1 and 3.4 respectively, without significant differences between treatments. A highly significant difference in the concentration of ascorbic acid was observed between treatments; treatment three had the highest concentration (19.4 mg 100 g⁻¹ FF). There were no significant differences between the fertilization treatments and the control, regarding total polyphenol content (41.34 mg 100 g⁻¹ FF) and flavonoids (1.6 mg 100 g⁻¹ FF) concentrations.

Study limitations/implications: The ripening degree of the pineapple fruit directly influences the chemical properties and the concentration of bioactive compounds.

Findings/conclusions: NPK fertilization had no effect on °Brix and pH. The citric acid percentage and the total polyphenol content increased as the doses of P₂O₅ increased. The ascorbic acid concentration and total flavonoid content are not affected by the NPK fertilization dose.

Keywords: Cabezona pineapple, NPK fertilization, chemical properties, bioactive compounds.

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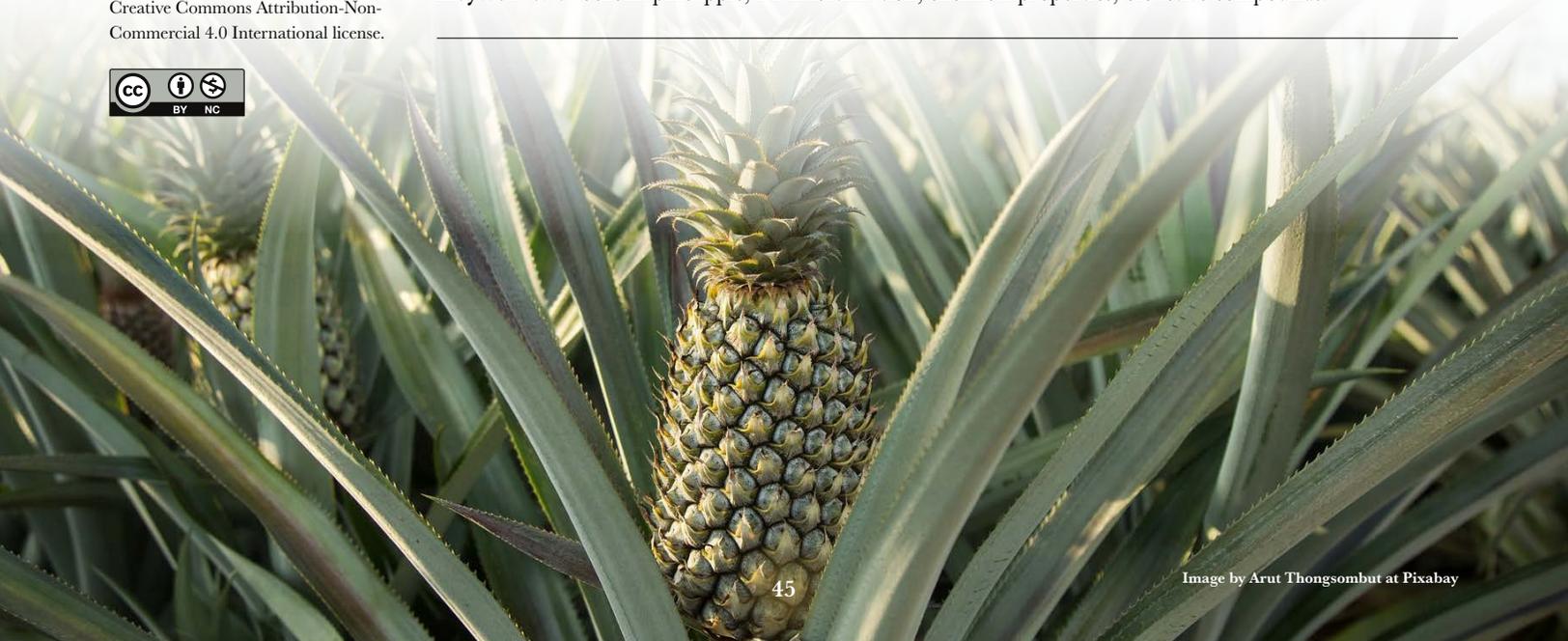
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INTRODUCTION

Pineapple is a source of sugars, organic acids, and some essential minerals for human nutrition (Paull and Chen, 2003); it is also an antioxidant-rich fruit that promotes health; these natural antioxidants include ascorbic acid and phenolic compounds (Hossain *et al.*, 2011; Lu *et al.*, 2014). Ascorbic acid has a high bioavailability and can inhibit the development of cardiovascular diseases and certain types of cancer, protecting the membranes and the lipoproteins from oxidative damage (Gardner *et al.*, 2000; Ferreira *et al.*, 2016). The phenolic compounds are responsible for the sourness, astringency, taste, color, and oxidative stability of fruits and vegetables; these compounds protect health, removing free radicals and inhibiting hydrolytic and oxidating enzymes, and they also have anti-inflammatory functions (Naczka and Shahidi, 2004). The *Cabezona* pineapple is produced and commercialized in La Chontalpa, Tabasco. However, there is no information about its chemical properties and the bioactive compounds content of its fruit.

MATERIALS AND METHODS

Location

The experiment was carried out in a field where Cayenne, MD2, and *Cabezona* pineapples have been grown for many years. This field is located in the Huimanguillo municipality, Tabasco, at 17° 40' N and 93° 38' W, at 17 masl.

Vegetable Material

Basal shoots (also known as spikes or thorns) from pineapple vegetable material were used for this experiment; these basal shoots came from a *Cabezona* pineapple cultivar and weighted 370 g.

Treatments and Experimental Design

The following NPK levels were evaluated: N (urea) (120, 160, 200, and 240 kg ha⁻¹); P (DAP) (70, 110, 150, and 190 kg ha⁻¹ of P₂O₅); and K (KCl) (150, 200, 250, and 300 kg ha⁻¹ of K₂O). Treatments were generated through the Plan Puebla 1 matrix (Turrent, 1985) and were evaluated in a random complete block design with four repetitions.

Study Variables

Fruits with optimal physiological ripeness that had undergone a fertilization treatment were used to determine the following chemical properties: pH, °Brix, and titratable acidity. The following bioactive compounds were determined: ascorbic acid and total polyphenol and flavonoid contents. The pH was directly measured from the juice, using a PC18 Conductronic potentiometer. °Brix were measured directly from the juice, as established in NMX-FF-015-1982, using an Atago[®] handheld refractometer, with a 0-33 °Brix measurement range. Titratable acidity was determined following the methodology established in NMX-FF-011-1982; the results were expressed as citric acid percentage (%). The ascorbic acid concentration was determined using a SIGMA-ALDRICH[®] ascorbic acid detection kit; the results were expressed as mg of ascorbic acid 100 g⁻¹ of fresh fruit (FF). The total polyphenol content concentration was determined following the method

described by De la Cruz-Ricardez *et al.* (2020) with some modifications. The galic acid was used as standard; the results were expressed as $\text{mg } 100 \text{ g}^{-1}$ of fresh fruit. The total flavonoid content concentration was determined following a modified version of the methodology described by Hossain *et al.* (2011), using quercetin as standard; the results were expressed as $\text{mg } 100 \text{ g}^{-1}$ of fresh fruit.

Statistical Analysis

In order to verify the assumption of normality and homogeneity of the variances, a RDBA ANOVA and a 0.05 Tukey test were carried out to analyze all the variables, using the Rstudio statistical kit, version 3.5.1 (Gentleman and Ihaka, 1993).

RESULTS AND DISCUSSION

Chemical Properties

pH

There was no statistical difference between treatments; in a $\frac{1}{4}$ color (NMX-FF-028-SCFI-2008), the *Cabezona* pineapple had a mean pH of 3.4 (Table 1). This pH value is similar to the results reported by Lu *et al.* (2014) for the Smooth Cayenne #1 (3.58), Smooth Cayenne #2 (3.86), Comte de Paris (3.93), and Ripley (3.91) cultivars. This pH value is also similar to the findings of Da Silva *et al.* (2014): Victoria (3.6), Perola (3.8), Gold (3.8), and EC-93 (3.8) pineapples. In their study, Morales *et al.* (2001) reported 3.8-4.10 pH values for the Indian pineapple.

Figure 1 shows the graphic response to NPK fertilization; in the N-110-200 curve the highest pH value was obtained with 200 kg ha^{-1} of N, while in the N-150-250 curve the highest pH values were obtained with 160 and 240 kg ha^{-1} . Meanwhile, the highest pH values were obtained with 70, 110, and 190 kg ha^{-1} of P. Finally, the highest values for the K doses were obtained with 200 kg ha^{-1} . The T6(200-110-250) has an acceptable pH and it was also associated with a higher fresh fruit yield (Pérez-Romero *et al.*, 2020).

°Brix

There were no statistical differences on the NPK fertilization regarding °Brix; the mean value was 7.3 °Brix (Table 1). According to the NMX-FF-028-SCFI-2008, in order to be commercialized, MD2, Cayenne Lisa, and Champala pineapple cultivars must have at

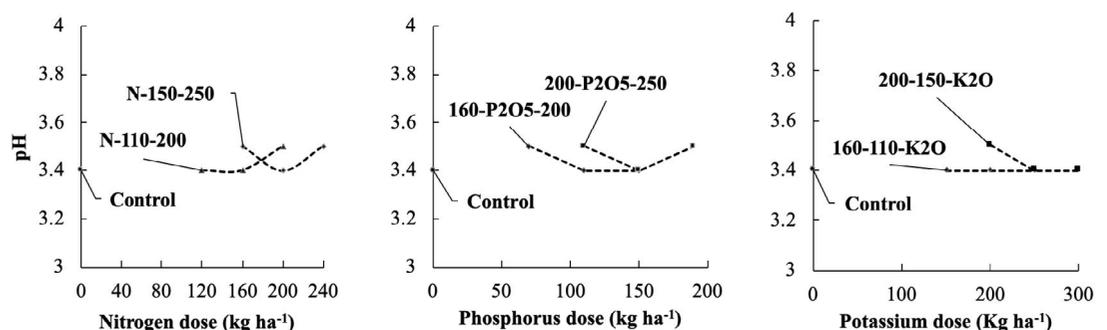


Figure 1. Response of the *Cabezona* pineapple juice's pH to NPK fertilization.

Table 1. Chemical properties (pH, °Brix, and citric acid %) resulting from the different fertilization treatments carried out in *Cabazona* pineapples.

Fertilization treatments (kg ha ⁻¹ de N, P ₂ O ₅ , K ₂ O)	pH	°Brix	Citric acid, %
T1 ₍₁₆₀₋₁₁₀₋₂₀₀₎	3.4a	7.1a	0.77a
T2 ₍₁₆₀₋₁₁₀₋₂₅₀₎	3.4a	7.4a	0.76a
T3 ₍₁₆₀₋₁₅₀₋₂₀₀₎	3.4a	7.2a	0.78a
T4 ₍₁₆₀₋₁₅₀₋₂₅₀₎	3.5a	7.0a	0.71a
T5 ₍₂₀₀₋₁₁₀₋₂₀₀₎	3.5a	6.9a	0.69a
T6 ₍₂₀₀₋₁₁₀₋₂₅₀₎	3.5a	7.7a	0.71a
T7 ₍₂₀₀₋₁₅₀₋₂₀₀₎	3.5a	7.1a	0.67a
T8 ₍₂₀₀₋₁₅₀₋₂₅₀₎	3.4a	7.9a	0.76a
T9 ₍₁₂₀₋₁₁₀₋₂₀₀₎	3.4a	7.4a	0.74a
T10 ₍₂₄₀₋₁₅₀₋₂₅₀₎	3.5a	7.3a	0.75a
T11 ₍₁₆₀₋₇₀₋₂₀₀₎	3.5a	7.7a	0.60a
T12 ₍₂₀₀₋₁₉₀₋₂₅₀₎	3.5a	5.8a	0.68a
T13 ₍₁₆₀₋₁₁₀₋₁₅₀₎	3.4a	7.4a	0.76a
T14 ₍₂₀₀₋₁₅₀₋₃₀₀₎	3.4a	7.8a	0.79a
T15 ₍₀₀₋₀₀₋₀₀₎	3.4a	6.0a	0.61a
Media	3.4	7.3	0.71
C.V. (%)	0.02	0.08	0.08
Prob. De F.	0.28 ^{ns}	0.18 ^{ns}	0.20 ^{ns}
DMS	0.24	2.54	0.27

*Means with the same letters are not statistically different (Tukey, 0.05). C.V.=variance coefficient; DMS=significant minimum difference; Prob. De F.=Fisher probability.

least 12, 11, and 11°Brix respectively, regardless of their ripening degree. This norm does not take into account the *Cabazona* pineapple, probably because it is a cultivar of regional importance. Additionally, the mean value (°Brix) of *Cabazona* pineapples is far below the results reported by other authors, such as Ulloa *et al.* (2015) for Comte de Paris (16.94), Smooth Cayenne #2 (14.95), and Queensland (17.80) cultivars or Da Silva *et al.* (2014) for Victoria (16) and Perola (13.10) cultivars. A possible cause of these low °Brix values in the *Cabazona* pineapple could be the ripening degree and the harvest season, because those values increase as the fruit ripens (Rosas *et al.*, 2011) and they decrease during the season of North's winds (Uriza *et al.*, 2018).

Figure 2 shows, on the one hand, the response of °Brix to the dose of N: when low amounts of fertilization (N-110-200 curve) are used, the °Brix decrease as the doses of N increase. On the other hand, when high fertilization doses (N-150-250 curve) are used, °Brix increase, reaching their maximum level at 200 kg ha⁻¹ of N and then they tend to decrease. Figure 2 also shows the °Brix response to the application of P, where both curves (200-P₂O₅-250 and 160-P₂O₅-200) indicate that °Brix decrease as the doses of P increase. Regarding K, there was little difference in °Brix in the 160-110-K₂O curve when its dose increased. However, when high doses of fertilization (200-150-K₂O) were applied, °Brix increased as the doses of K increased. This behavior has already been reported by Py *et*

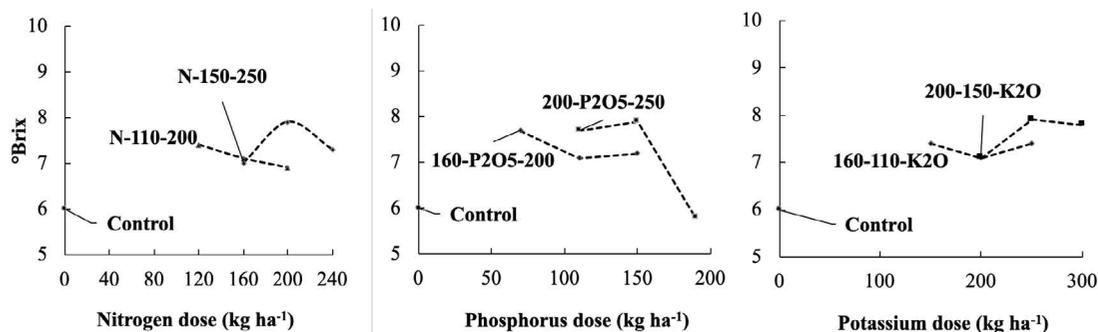


Figure 2. Response of the pineapple cultivar's °Brix to NPK fertilization.

al. (1987), Rebolledo *et al.* (2011), and Uriza *et al.* (2018), who point out that K is directly responsible for the quality of the pineapple fruit.

Citric Acid Percentage

There was no statistical difference between NPK fertilization treatments; the citric acid mean value was 0.73% (Table 1). This value was similar to the results reported by Lu *et al.* (2014) for the Comte de Paris pineapple (0.73%) and the Giant Kew pineapple (0.69%). Additionally, this value falls within the range reported by Rosas *et al.* (2011) for the Esmeralda pineapple (0.4-1.2%). However, this value could increase just like in the case of the Esmeralda pineapple, where the citric acid concentration increases as it ripens (Rosas *et al.*, 2011), contrary to what happens with other tropical fruits such as mango, passion fruit, and papaya (Torres *et al.*, 2013). This study corroborated that citric acid concentration increases during the rainy season and with the use of KCl as source of the K_2O ; fertilization treatments exceed the unfertilized control in one unit (Rebolledo *et al.*, 2011; Uriza *et al.*, 2018).

Citric acid concentration did not show a clear response to the application of high or low doses of N (Figure 3); the highest concentrations in the N-110-200 and N-150-250 curves were obtained with 160 kg ha^{-1} of N and 200 and 250 kg ha^{-1} of N, respectively. Figure 3 shows that citric acid concentration has a positive response to the increase of P_2O_5 doses; in both curves ($160\text{-}P_2O_5\text{-}200$ and $200\text{-}P_2O_5\text{-}250$), the highest concentration was obtained with 150 kg ha^{-1} of P_2O_5 ; concentrations tend to decrease after this dose. Additionally, high doses of K ($200\text{-}150\text{-}K_2O$ curve) can affect the citric acid concentration; concentration increased as the doses changed from 200 to 300 kg ha^{-1} (Figure 3).

Bioactive Compounds

Ascorbic Acid Concentration

Highly significant differences were observed in the concentration of ascorbic acid in the NPK fertilization treatments (Table 2). The Tukey Test establishes five groups: the T13 and T15 treatments obtained the lowest concentration, while the T3, T6, T8, and T10 treatments obtained the highest concentrations. These results match those reported by Samson (1991), who found out that the ascorbic acid of pineapples varies from 8 to $30 \text{ mg } 100 \text{ g}^{-1}$ FF. Several authors have observed wider intervals ($5.08\text{-}62.11 \text{ mg } 100 \text{ g}^{-1}$ FF)

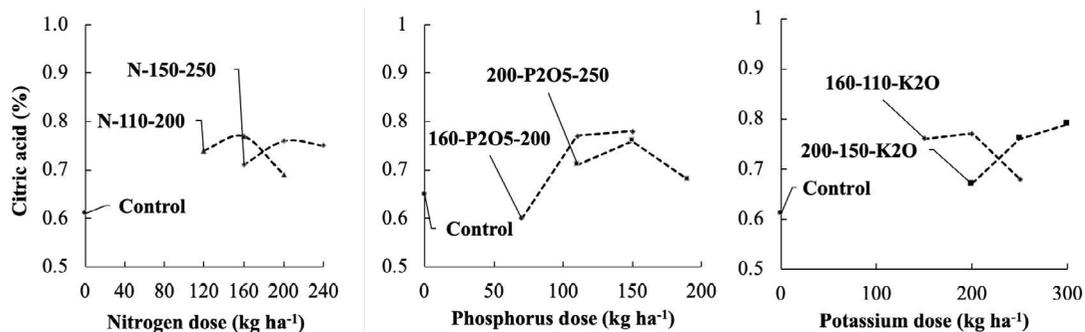


Figure 3. Response of the *Cabezona* pineapple juice's citric acid percentage to NPK fertilization.

Table 2. Bioactive compounds - ascorbic acid concentration in the different fertilization treatments of *Cabezona* pineapple.

Fertilization treatments (kg ha ⁻¹ de N, P ₂ O ₅ , K ₂ O)	Ascorbic acid	Total polyphenols	Total flavonoids
	(mg 100 g ⁻¹ of fresh fruit)		
T1 ₍₁₆₀₋₁₁₀₋₂₀₀₎	14.6abc	41.4a	1.8a
T2 ₍₁₆₀₋₁₁₀₋₂₅₀₎	14.9abc	46.3a	1.5a
T3 ₍₁₆₀₋₁₅₀₋₂₀₀₎	19.4a	43.9a	1.5a
T4 ₍₁₆₀₋₁₅₀₋₂₅₀₎	14.6abc	35.5a	1.4a
T5 ₍₂₀₀₋₁₁₀₋₂₀₀₎	11.8abc	49.3a	1.6a
T6 ₍₂₀₀₋₁₁₀₋₂₅₀₎	19.4ab	40.5a	1.4a
T7 ₍₂₀₀₋₁₅₀₋₂₀₀₎	11.3abc	35.7a	1.2a
T8 ₍₂₀₀₋₁₅₀₋₂₅₀₎	16.0ab	43.8a	1.6a
T9 ₍₁₂₀₋₁₁₀₋₂₀₀₎	14.7abc	41.1a	1.7a
T10 ₍₂₄₀₋₁₅₀₋₂₅₀₎	15.1ab	36.2a	1.6a
T11 ₍₁₆₀₋₇₀₋₂₀₀₎	13.4abc	35.7a	1.9a
T12 ₍₂₀₀₋₁₉₀₋₂₅₀₎	11.3abc	42.2a	1.6a
T13 ₍₁₆₀₋₁₁₀₋₁₅₀₎	6.2c	43.2a	1.9a
T14 ₍₂₀₀₋₁₅₀₋₃₀₀₎	11.8abc	42.7a	1.7a
T15 ₍₀₋₀₋₀₎	10.5bc	42.3a	1.5
Media	13.67	41.34	1.6
C.V.	0.25	0.10	0.11
Prob. De F.	0.001**	0.30 ^{ns}	0.30 ^{ns}
DMS	8.83	14.28	0.82

*Means with the same letters are not statistically different (Tukey, 0.05). C.V.=variance coefficient; DMS=significative mean difference; Prob. De F.=Fisher probability.

for various pineapple cultivars, including: Ferreira *et al.* (2016), who reported 35.88-62.11 mg 100 g⁻¹ FF concentrations; Lu *et al.* (2014) who reported 5.08-33.57 mg 100 g⁻¹ FF concentrations; and Da Silva *et al.* (2014), who reported 7.03-25.48 mg 100 g⁻¹ FF concentrations. We must mention that Morales *et al.* (2001) and Rosas *et al.* (2011) reported that ascorbic acid concentration increases in the Esmeralda and Indian pineapples as their fruit ripens. This effect could provide a possible explanation for the lack of much similarity in the concentrations reported by the authors.

Ascorbic acid concentration does not show a clear response when the N doses increases (N-150-250 curve), although the highest concentration was obtained with 250 kg ha⁻¹ of N per hectare (Figure 4); however, as the doses of N increase, the ascorbic acid concentration shows a clear decrease in the N-100-200 curve. With low fertilization doses (160-P₂O₅-200 curve), the ascorbic acid concentration increases as the dose of P increases, reaching a maximum concentration of P at 150 kg ha⁻¹. The opposite result is obtained with high fertilization doses (200-P₂O₅-250): the ascorbic acid concentration decreases as the doses of P increase, reaching a minimum concentration of 190 kg ha⁻¹. As a result of the positive interaction, the 200-150-K₂O curve, with 250 kg ha⁻¹ of K₂O, has the highest ascorbic acid concentration (Figure 4); meanwhile, the ascorbic acid concentration increases in the 160-110-K₂O curve, with 200 and 250 kg ha⁻¹ doses of K₂O, although this increase is lower than the one reported for the 200-150-K₂O curve.

Total polyphenols content

There were no significative differences between fertilization treatments (Table 2). Total polyphenols content had a mean value of 41.34 mg 100 g⁻¹ FF. This value is closer to the results reported by Lu *et al.* (2014) for various cultivars (37.48-77.55 mg 100 g⁻¹ FF), by Rosas *et al.* (2011) for Esmeralda pineapples (44.78 mg 100 g⁻¹ FF), and by Ferreira *et al.* (2016), likewise for several cultivars (71.08-126.95 mg 100 g⁻¹ FF). These differences can be the result of the type of cultivar and the ripening degree of the pineapple fruits. Polyphenols content—as well as ascorbic acid content—increases in Esmeralda pineapples as the fruit ripens.

The maximum polyphenols content was found at 200 kg ha⁻¹ of N for high and low fertilization doses (N-110-200 and N-150-250 curve); however, polyphenols content shows a drastic decrease with a 200 to 240 kg ha⁻¹ of N increase and high fertilization doses (Figure 5). Increasing doses of P provide a better response: polyphenols content increases as the doses increase, reaching a maximum content with 150 ha⁻¹ of P; however, content tends to decrease with higher doses (Figure 5). Meanwhile, potassium had a poor response: the highest polyphenols content was obtained with 250 kg ha⁻¹ of K₂O for both curves (160-110-K₂O and 200-150-K₂O).

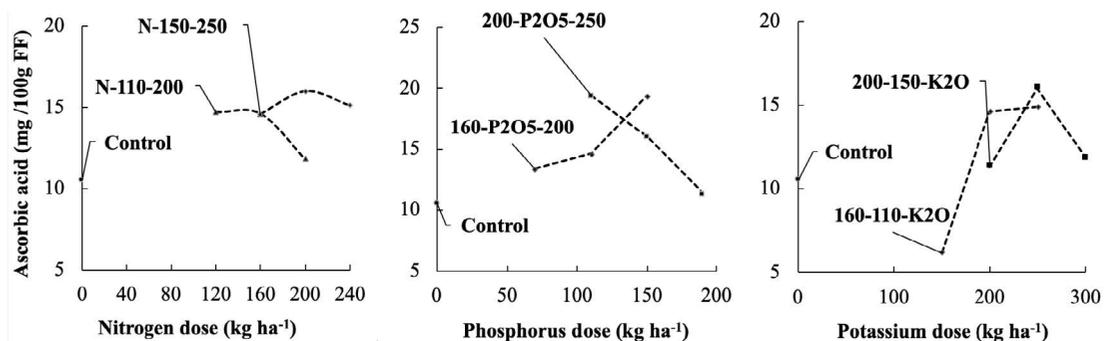


Figure 4. Response of the Cabezona pineapple juice's ascorbic acid percentage to NPK fertilization.

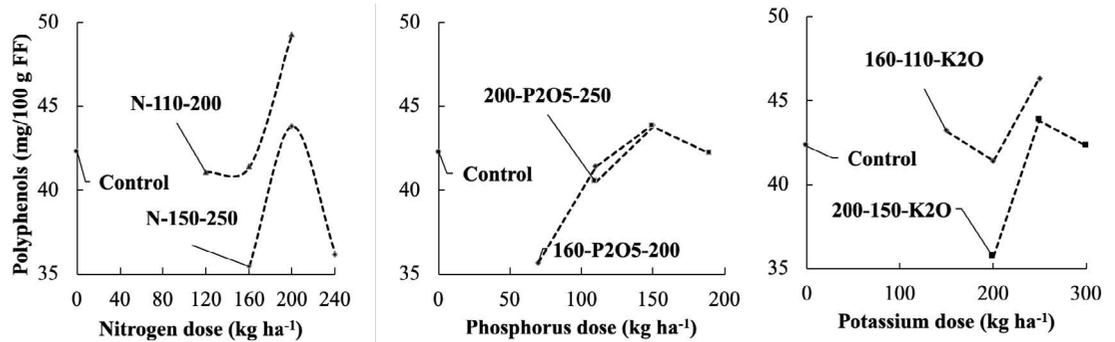


Figure 5. Response of the total polyphenols content in *Cabezona* pineapples to NPK fertilization.

Total flavonoids content

There were no statistical differences between fertilization treatments regarding the content of total flavonoids content (Table 2). The mean concentration was $1.6 \text{ mg } 100 \text{ g}^{-1} \text{ FF}$.

The flavonoids content in *Cabezona* pineapples were different from the results obtained by Lu *et al.* (2014) —who reported 27.3, 34.5, 17.24, and 19.64 $\text{mg } 100 \text{ g}^{-1} \text{ FF}$ for MD2, Comte de Paris, Fresh Premium, and Pearl, respectively— and by Hossain *et al.* (2011) —who reported 51.1 $\text{mg } 100 \text{ g}^{-1} \text{ FF}$ for tropical pineapples. These differences are likely the result of the color of the flesh of the *Cabezona* pineapple, which is paler than the flesh coloration percentage of the MD2 pineapple (63-87%); flavonoids are responsible for the color and taste of fruits and vegetables.

The flesh of the *Cabezona* pineapple could also have a lower browning tendency than other cultivars; therefore, its capacity to synthesize the flavonoids that are involved in the prevention of oxidation would be low and this process allows the protection of existing vitamins and enzymes. Another potential cause could be the low levels of glucose —the precursor molecule of the synthesis of flavonoids— in the flesh (Martínez *et al.*, 2002; Mandalari *et al.*, 2006).

The effect of N doses on flavonoids content is not clear: the contents obtained with low fertilization doses (N-110-200 curve) are higher than the contents obtained with high fertilization doses (N-150-250 curve) (Figure 6). The flavonoids content response to

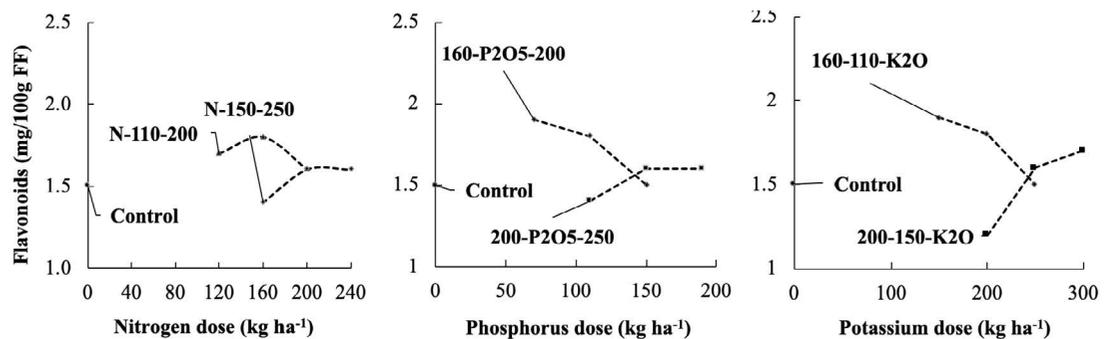


Figure 6. Response of the flavonoids content in *Cabezona* pineapples to NPK fertilization.

increasing P and K doses are similar. Content tends to decrease with low fertilization doses (160-P₂O₅-200 and 160-110-K₂O curve), while it increases with high doses of the same nutrients (200-P₂O₅-250 and 200-150-K₂O curve), reaching maximum P and K levels of 150 and 200 kg ha⁻¹ and 300 kg ha⁻¹, respectively.

CONCLUSIONS

NPK fertilization did not have a significant effect on pH and °Brix. The application of N and K does not affect the citric acid percentage and the total polyphenols content; however, they increase along with the P₂O₅ doses and they reach a maximum concentration at 150 kg ha⁻¹. NPK fertilization did not affect the ascorbic acid and flavonoids concentration.

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Potential of the physical and chemical characteristics of prickly pear (*Opuntia albicarpa* Seheinvar var. Villanueva) seeds in agroindustrial processes

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ABSTRACT

Objective: To verify the physical attributes and assess the chemical quality from prickly pear's seeds (*Opuntia albicarpa* Seheinvar cv. Villanueva), including seeds' size, internal friction, external friction, performance for size reduction, sieving and electric conductivity, soluble protein, total protein, phosphorous, carbohydrates, amount of oil and minerals.

Design/methodology/approach: To verify and compare the physical attributes and chemical parameters using proved and reliable techniques, to explore their impacts on its future potential, when seeds are intended to agro-industrial processes, considering; its size and quality of space storage and their effect when interacting with handling apparatus and the material they are manufactured with.

Results: Selected groups of 100 seeds from four consecutive prickly pears harvests had no differences. While for their chemical analysis, seeds were milled and their revised chemical compounds showed differences between harvest, therefore, drought, management and crop protection significantly affect those attributes. The factors were not included in this study.

Limitations on study/implications: Prickly pears are harvested for 4 months per year. This reduces the availability of raw material from other varieties and from other regions, to expand this study and to compare between diverse parameters.

Findings/conclusions: There were not significant differences in seed size (wide, large, thickness), external friction and electric conductivity; therefore, handling equipment does not need specifications. While, for the internal friction and all chemical compounds analyzed from seeds' flour, significant differences were quantified. The latter means that, it is adequate to homogenize these parameters by using other compounds in the agrifood processes.

Keywords: agroindustry, technological processes, chemical compounds, physical attributes, seeds from cactus prickly pear.

INTRODUCTION

Arid and semi-arid zones comprise about half the Mexican surface. Within it there are grasslands and isolated tree vegetation (Rzedowski, 1988). The main present plant resources include mesquite, candelilla, maguey, jojoba, “vegetable” nopal, “forage” nopal and “tunero” nopal. From them, various products such as fibers, syrups, fermented beverages, and soaps are obtained and prepared (Cervantes-Ramírez, 2005). In the case of the nopal (*Opuntia* spp.), by processing its fruit (prickly pear), honey, cheeses, jellies, jams, wines, shampoos, soaps, creams, colorants, organic acids, construction materials and filters, among others are produced. There is, therefore, high industrial demand for this raw material availability (Mohsenin Nuri, 1986). Therefore, it is a priority that the agro-industrial transformation processes (processing technologies) become increasingly efficient. Due to the above, it is convenient to introduce the concept of Industry 4.0 (Pat Mooney, Grupo ETC 2018), which consists of the digitization of production processes to obtain indicators, with the help of sensors and information systems. Although, it is important to note that the interaction of technical tools with biological material is still incipient, so its digitization represents great challenges. The advancement of new technologies in the industry, in which the digital part is merged with industrial processes (Industry 4.0), today is a necessity to develop technically produced products. Associated with the fact that agribusiness evolves according to the food needs, energy, water, and biomaterials, without neglecting to minimize the environmental impact. A significant advance comes from using raw material by-products, for example, for prickly pear case, using its seeds in industrial processes. Prickly pears contain numerous seeds in their pulp, which are not used and discarded by producers, even though extracting oil from them is feasible. For their industrialization, it's convenient to characterize these inputs potential, from both a physical and chemical perspective, to guarantee adequate and technologically correct processes. For prickly pear seeds, there are no records that involve mechanized industrial uses, for example, that involve the development of machines for harvesting and storing them, especially for the transformation and elaboration of agro-industrial products. The SIAP (2004) indicates that in México, the prickly pear nopal occupies sixth place in fruit production, behind oranges, avocado, bananas, mangos, and apples.

In Mexico, there are about 53,000 hectares of nopal orchards that annually produce 344,000 tons of prickly pear. According to Martínez *et al.* (2010), one of the main problems for nopal producers is the harvest seasonality. The prickly pear is harvested for 90 days, therefore, when the production is high; 60% of the harvest is lost. Sumaya-Martínez *et al.* (2010) detail that it is mainly due to deficiencies in the commercialization, such as: a) lack of an assured market, b) lack of adequate storage and storage spaces for the conservation of the prickly pears, c) lack of transportation infrastructure to mobilize the product and d) disarticulation of the production chain where the production and marketing processes are taken separately. Using the seeds from the losses contributes to reduce and solves some problems, that is why the seeds are a current research object.

Inside the peel and pulp, there is an average of 91 to 388 seeds, with a size between 2.16 mm to 4.34 mm. The prickly pears have a high nutritional value, it is an accessible

product to industrialize; Its characteristics allow them to be used as biomaterials (colorants), although to increase the processes effectiveness, it is necessary to apply better technologies and offer an adequate post-harvest treatment, as well as seek storage options, such as productive processing of both prickly pears and its seeds.

Agribusiness and the food processing industry intend to ensure that products have high nutritional value and pleasant sensory characteristics for the consumers. Therefore, technologies must be used, with which to participate in the fourth industrial revolution or intelligent industry, which promotes the total integration of information and knowledge in all stages of a product life cycle in an automated way, in this case, achieving the interaction of the technical tools with the organic materials. To industrialize the processes, knowledge of the materials is necessary, hence the importance of characterizing them both physically and chemically.

The physical and chemical characteristics of prickly pear seeds represent a guide for its potential to get sustainable processing, in which, these physical parameters do not destroy any chemical or biological characteristics of the product and thus guarantee high-quality products and many of them during their processing.

MATERIALS AND METHODS

In this research, prickly pear seeds from the *Opuntia albicarpa* Scheinvar cv. Villanueva were used, corresponding to different harvests from the municipality of Villanueva, Zacatecas (22° 21' 13" N, 102° 52' 59" W). The research was carried out at Salinas de Hidalgo, San Luis Potosí at the San Luis Potosí Campus of the Colegio de Postgraduados and the Western Altiplano Region Academic Coordination of the San Luis Potosí Autonomous University.

Physical and chemical parameters were analyzed due to the importance of the active contact of the organic material with the engineering tools in agro-industrial processes: a) physical characteristics including 1) size (length, width and thickness), 2) internal and external friction, 3) grinding (particle size), 4) electrical conductivity, 5) screening and b) chemical characteristics including 1) humidity 2) carbohydrates, 3) total proteins, 4) lipids, 5) soluble proteins and 6) phosphorus.

RESULTS AND DISCUSSION

At random, 100 seeds of the *O. albicarpa* Scheinvar cv. Villanueva were collected from four crops. The measurement of each seed's width, length and thickness were made with a digital vernier showing an LCD display and digital calibration (0.001 mm precision). The statistical analysis indicates that none of the fruit harvestings had a significant effect on the cv. Villanueva seeds dimensions; therefore, the width, length and thickness were not statistically different among the four harvests ($p > 0.05$). Table 1 shows the indicators of the values of the three measurements, which allow the selection of technical parameters; for example, the sizes of the holes in the screens or the shape of the slits that the presses used for obtaining oil must have, to be sure of achieving the highest efficiency in agro-industrial processes.

Table 1. Average data of the sizes of prickly pear seeds.

Parameter	Wide	Length	Thickness
	(mm)		
Average	3.58	4.23	2.00
Variance	0.17	0.21	0.11
Standard deviation	0.41	0.46	0.33

In the case of the measurement of the internal friction of the μ_i seeds, the method described in Figure 1 was used. The statistically processed data of the internal friction (μ_i) of the different crops with a $p < 0.0001$, were determined *via* the Tukey’s test (Table 2).

The determination of the angle results from the value of the height (h) divided by $\frac{1}{2}$ of the diameter (d) of the pile of organic material, it results that $\tan \beta = h/r$ and $\tan \beta = \mu_i$.

The values of the internal friction allow selecting, with sufficient technical security, the angle of the cone for the exit of material from a vertical silo (Figure 2). When physical data is applied, it guarantees a continuous flow of the grain material inside the storage container. This means that the material entering the container comes out in the same order it came into the silo, there is no mixing, there are no different storage times and there are no chemical changes in the contents. Hence, the material as a component of a diet is clearly defined and can be planned according to the content of its nutritional values.

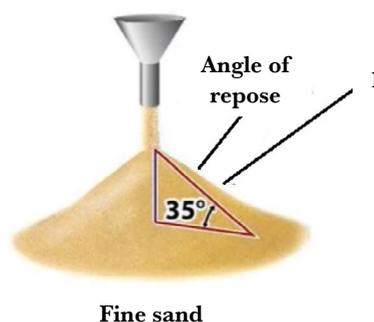


Figure 1. Measurement of internal friction (viscosity of the granular material). Where: 1 Cone of the granular material; β =angle of repose.

Table 2. Internal friction μ_i from Villanueva’s seeds for four different fruit harvests.

$ \mu_i$	$\pm \sigma \mu_i$	Villanueva variety Fruit (harvest)
0.194	0.017	1(2013) a
0.216	0.025	2(2014) a
0.141	0.033	3(2015) b
0.215	0.042	4(2016) a

Letters a,b, indicate that there is statistical difference between fruit harvest.



Figure 2. Array of large vertical steel silos for grain storage.

Regarding the measurement of external friction (μ_e), it presented significant differences during movement due to the gravity of the seeds, which is a function of the different materials on which it made contact, with a $p < 0.05$ determined with the Tukey's test. The external friction measurement values (μ_e) are shown in Table 3. These values are the result of the measurement process with the physical phenomenon of the following equation:

$$F_r = \mu_e * F_n$$

Where: F_r - friction force; F_n - normal force; μ_e coefficient of friction between the organic material (not ideal elastic) and the material of a conveyor belt or plate made of a solid material and with an angle of inclination in relation to the horizontal plane (Figure 3).

For the case of requiring its transportation in the horizontal direction of grains, for example, from a warehouse to a dispenser or mixer due to gravity force, the passive conveyor needs at least one inclination angle, which must result in the type of organic material and the material of the technical conveyor on which said material is placed. This form of

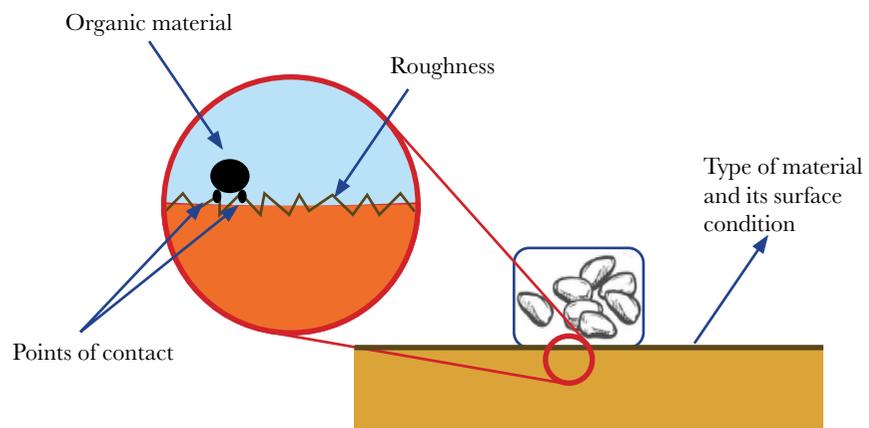


Figure 3. Contact of organic material with a solid surface

transport is a possibility to save technical energy for the process, which can guarantee a continuous technological flow.

The variation of the average values and their respective standard deviation has a very narrow range, which allows the manufacture of technical installations with a high degree of safety without difficulties. Table 3 shows the average friction values of seed flour of the different cv. Villanueva prickly pears harvests obtained during their movement on different materials. If a selection of material is required to manufacture a gravity conveyor, the most convenient angle of inclination is the one with the lowest value, in this case, the glass would be ideal, also the height of the space for a conveyor is smaller compared to that of other materials. The standard deviation values allow confidence in selecting the correct contact material for a conveyor. The friction forces against the movement of flour on a conveyor with a surface made of glass would have a minimum value. Although sometimes, it is convenient to consider other factors such as its price, *e. g.* wood is cheaper and with proper maintenance, its useful life may be lengthened; in addition to being a degradable good, it contributes to environmental preservation. The parameters for flour (Table 3) are high, which is due to its technical processing, while the values of the seeds in Table 3 are lower, largely due to its more natural character.

Figure 4 shows the variation of the external friction values of the seeds and the flour obtained from grinding seeds when both products are being handled through different materials, in which it is observed that they both lack the representativeness of the normal distribution. This situation is characteristic of physical values, which result from a certain process, based on an unnatural parameter, but achieved in a technical or artificial installation. Regarding the seed's size reduction, the process was complicated, due to the thick pericarp that surrounds the embryo, for which two processes were done; first, the pericarp of the seeds was broken in a mortar and subsequently cut into small particles in a coffee bean mill (using the free cutting principle), turning it into a coarse flour.

Table 4 shows that the sieve in which the largest amount of flour accumulates was 419 μm . This result is important since, even when it is complicated to reduce the size of the prickly pear seed particles, giving it the appropriate processing, it is possible to manipulate them in other processes to generate industrial products. Some authors have classified the flours of other products (*e.g.* coffee) according to their average diameter, and mention that the size of the particles has a marked influence on the final product's characteristics

Table 3. External friction (μ_e) of the seed and flour of cv. Villanueva prickly pear seeds for the 3rd (n=300) and 4th (n=400) harvests.

Sample size (n)	Material	Average (μ_e) /-/ flour	Standard deviation (μ_e) /-/ flour	Average (μ_e) /-/ grain	Standard deviation (μ_e) /-/ grain
300	Plastic	0.93	0.02	0.662	0.035
400	Ceramic	0.84	0.03	0.425	0.012
400	Rough wood	0.93	0.05	0.861	0.063
400	Wood	0.98	0.04	0.613	0.061
400	Steel	0.76	0.04	0.433	0.019
300	Glass	0.64	0.02	0.384	0.015

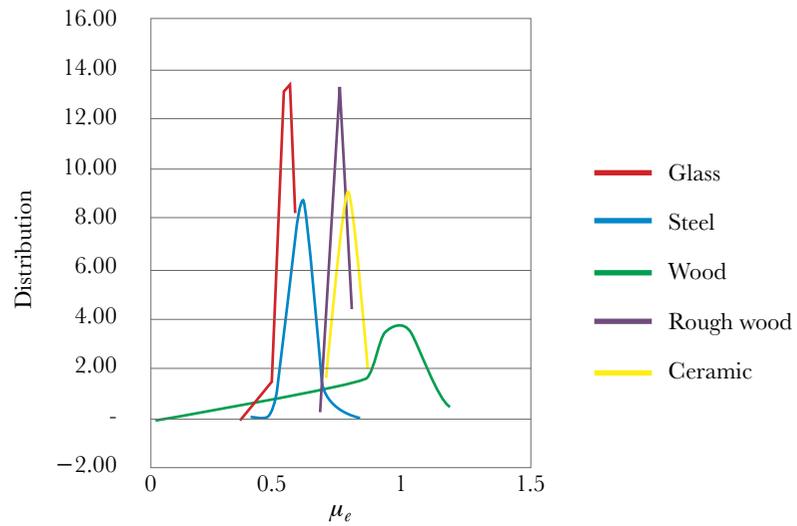


Figure 4. External friction of the flour of the prickly pear seeds.

Table 4. Prickly pear seeds flour particle sizes.

Sieve (m)	Flour of seeds from fruit harvest 1 (g)	Flour of seeds from fruit harvest 2 (g)	Flour of seeds from fruit harvest 3 (g)	Flour of seeds from fruit harvest 4 (g)
1999	10.52	11.561	16.68	14.94
1409	37.74	42.35	51.99	44.72
419	136.27	158.41	154.66	149.71
131	44.57	61.79	54.62	75.36
73	7.58	13.43	7.12	2.08

(Guevara and Cataño, 2005). As previously reviewed, the highest percentage of flour (50-60%) was retained on the 419 μm sieve, as it was ground in a coffee mill without any previous heat treatment (Table 4), and is considered as that generated in a fine grind. The second sieve in which flour was retained was the 131 μm sieve. The 1986 National Technical Standard (ITINTEC .205-027) establishes that fortified wheat flour must pass at least 98% through a 212-micron sieve.

To achieve a suitable mixture with different flours it is important to consider their physical characteristics, such as the size and shape of the particles (Table 4 and Figure 5), substance density, as well as its chemical characteristics, such as humidity, content, and quality of oils, among others. Likewise, it is necessary to assess the affinity with other components of the mixture. For example, once cornflour has been selected, to determine it choosing by affinity degree, the two parts of the mixture are mixed and thus determine its affinity by its grinding degree.

Regarding the electrical conductivity of the seeds, the agro-food industry uses different processes to generate inputs to produce market products such as pastries, “tortillas” or balanced forage for animals. The processing of these products is, in most cases, linked to thermal, hydraulic (solubility), mechanical, optical and chemical phenomena. Electrical conductivity is a general evaluation that involves these parameters since there



Figure 5. Image of prickly pear seeds (left) and their flour (right).

is a determined physically relationship between electrical conductivity values and the mentioned parameters (Table 5).

The results are useful in the biomaterials industry, where the seed can be used as a thermal insulator, in controlling the temperatures of the processes, such as in the piglet beds or the artificial nests of the “escamoles” (ant egg).

To determine the moisture content of the prickly pear seed samples, the AOAC Method was used. 925.10. The protein determination of the flours was carried out based on the Standard (NMX-F-068-S, 1980) following the Kjeldahl method and the soluble protein quantification analyzes were carried out by the Lowry method. The analysis for the quantification of oil was carried out using the Soxhlet technique following the NMX-F-089-1978 Standard. The determination of the carbohydrate content of the prickly pear seeds was carried out based on the NMX-F-312, 1978 Standard. Finally, for the phosphorus analysis, the samples were digested in microwave equipment and their analysis was performed via UV spectrophotometry, based on the NOM-AA-29-1981 Standard.

The use of flours from previously unexplored food sources, or in combination with other commercial flours for the generation of new products can contribute to increase the nutritional value of the products or improve its technological characteristics. However, for flours elaboration, as well as to elaborate the products derived from them, it is important to consider the equipment characteristics and the processes that will be used on them.

Hence the importance of applying technology to preserve the quality of the final product for its nutritional content, in addition to the fact that it must be attractive to the consumers, promoting its sale. This entails the design of machinery and the application of the appropriate processes, both in the production of flour and in that of food products,

Table 5. Values of electrical conductivity from prickly pear seeds flour.

Flour from prickly pear var. Villanueva (year)	Electric conductivity (ms/m)
Fruit harvest 1 a	0.240±0.050
Fruit harvest 2 a	0.293±0.088
Fruit harvest 3 a	0.270±0.050
Fruit harvest 4 a	0.285±0.002

Letter a indicates that there is not statistical difference between crop harvests.

so that, in addition to knowing their physical qualities, the behavior of the chemical characteristics must be understood (Table 6).

There is no doubt that it is feasible to carry out adequate technological processes to avoid nutrients loss from the organic material during their process when there is an understanding of the meaning of the parameters measured in this investigation. Thus, the moisture content of the seeds was between 4% and 6.5%, like that reported by other authors in other fruit seeds (Monroyetal, 2017). The main advantages of a reduced moisture content food are that it tends to have less water activity and these influences reduce physical, chemical, enzymatic and microbiological reactions, related to food spoilage, as well as those that may arise from the interaction with the materials, they are in contact with from which the equipment is made. Regarding the carbohydrate content in prickly pear seeds, values between 7.54% and 19.72% were found, which differed significantly depending on the year of cultivation ($p=0.0001$). In the year 2013, the carbohydrate content was higher, while the 2014 and 2015 crop years had the lowest percentages with 7.54% and 8.07% respectively (Table 6).

Currently, many diets have a high carbohydrate content, so that the energy provided by them is consumed in greater proportion than required by the same diets causing health problems, such as obesity, which can lead to diabetes or other diseases (Piernas and Popkin, 2010). This particular flour has low carbohydrate content and acceptable fiber content, compared with other flours in the market therefore its consumption can be associated with health benefits. As for the total protein, lipid and phosphorus content in the samples analyzed, the values obtained revealed that these nutrients showed significant differences between these years ($p=0.0001$). All the differences between nutrients in the different crop years may be due to the prevailing climatic conditions, the presence of pests and diseases, the period of fruit development and the time the seeds were stored.

This should be verified to ensure that the product market has the same qualities and the same nutritional value. Likewise, it was found that the protein, lipid and phosphorus values of these flours are similar to those of commercial wheat flours. Therefore, when developing equipment for processing prickly pear seed flour and/or mixtures with other flours, as well as for product development, the process temperatures should be considered to avoid color changes in the products because of the darkening reactions due to carbohydrate and protein content, aggregation or denaturation of proteins and nutritional loss (Carbonaro *et al.*, 1997).

Table 6. Selected chemical characteristics for agro-industrial processes.

Fruit harvest (year)	Carbohydrates (%)	Total protein (%)	Lipids (%)	Moisture content (%)	Soluble protein (%)	Phosphorous (%)
1	19.72±0 a	7.20±0.14 b	11.0±4.2a	4.94±0.14 b	0.38±0 a	0.93±0 a
2	7.54±0.09 d	4.99±0.13 d	5.5±0.7 b	4.9±0.05 b	0.29±0 c	0.82±0 e
3	8.07±0 d	7.64±0.04 a	5.0±0 b	5.09±0.03 a	0.34±0 b	0.87±0 b
4	11.42±0.11 c	7.10±0.03 b	3.0±0 b	4.81±0.21 b	0.25±0.01 c	0.84±0 c

The letters a, b, c, d, indicate there is a statistical difference between the materials.

The physical properties are largely influenced by the chemical values, most probably the internal friction values show different behavior in the different harvest years due to the difference in humidity and fat that they present in those years. The internal friction of the material is also influenced by the biomolecules that constitute it. While regarding particle size reduction for flour production, it is also necessary to take into account both moisture and fat content, as these figures should be much less as those for the optimum particle size that was used in this research, as their actual data cause oil syneresis, resulting in caking of the flour.

CONCLUSIONS

The data registered on the physical characteristics of prickly pear seeds allow guaranteeing an integral interaction between the biological material with the equipment materials that will be used to process them agroindustrially, whose purpose is to obtain maximum efficiency of the process with a minimum of losses. Also, the content of chemical components in prickly pear seed flours contributes to increasing the nutritional and/or technological value when mixed in the appropriate proportions with other grain and cereal flours. Regarding the qualities of prickly pear seed flour, since it lacks odor and has high fiber content, it can be used as a thickening agent and cause a pasty texture in consumer products. In addition, due to the content of biomolecules contained in this product, the possibilities of complications during the interaction with the equipment material used in the agroindustrial treatment, as well as in the process during the elaboration of the final products, are reduced.

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Productive behavior of *Dactylopius coccus* in two confinement cultivars of *Opuntia ficus-indica* (L.) Mill

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ABSTRACT

Recently, the commercial breeding of *Dactylopius coccus* in confined environments has been developed not to depend on seasonal production and ensure the supply chain. Previous studies have shown the technical viability of their breeding of *Opuntia* in a repository also called “Nopaloteca”. However, considering the genetic diversity of *Opuntia* in each region, it is necessary to evaluate alternative cultivars to maximize the efficiency of that production system. In this research, the evaluated cultivars were Esmeralda and Villanueva (*Opuntia ficus-indica* (L.) Mill.). Four height levels (m) within the “Nopaloteca” (N1: 0.5; N2: 1.0; N3: 1.5 and N4: 2.0) and two harvest indices (pre and post oviposition) were evaluated regarding their effects on the carminic acid concentration (CAC, %) and fresh weight (FW, g) of *D. coccus*. The results showed no significant difference in the CAC of the *D. coccus* colonies in both cultivars (18%); however, the highest FW was reached in the cv. Esmeralda with 6.3 g per cladode ($p \leq 0.05$). The highest CAC was found in the N4 treatment (2.0 m) with 18.6% ($p \leq 0.05$); while the highest average FW values ($p \leq 0.05$) were in the intermediate levels of the N2 (1.0 m) and N3 (1.5 m) treatments, with 6.4 and 6.1 g per cladode, each. The post oviposition phase harvest of *D. coccus* resulted in a higher CAC concentration (20.4%); meanwhile, in the pre-oviposition phase, 15.6% was harvested ($p \leq 0.05$). Due to the productivity and quality obtained, the Esmeralda cultivar could be used as an alternative host for the intensive breeding of *D. coccus* in confinement, in north-central Mexico.

Keywords: Reared, cochineal insects, quality, hosts, cactus pear.

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INTRODUCTION

One of the trends of consumers worldwide is the demand for natural, safe, and sustainable products, which has shown a greater preference towards natural colorants (Müller-Maatsch *et al.*, 2018), among which those obtained from the carmine cochineal *Dactylopius coccus* stand out. The females of the 11 species of *Dactylopius*, so far recognized,

are characterized by containing a hydroxyanthraquinone linked to a glucose unit, called carminic acid (CA), mainly used in food, cosmetics, pharmaceuticals, and textiles (Cooksey, 2018), as an alternative to some synthetic dyes (González *et al.*, 2010). Both, the European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) have allowed using cochineal extracts and carmines in food products (Rather *et al.*, 2020).

Derived from the above, *D. coccus* is one of the few insects domesticated since pre-Hispanic times by American cultures (Costa-Neto, 2015). It is currently bred in several countries for commercial purposes. At the international level, its intensive breeding has been characterized by unarmed variants, locally or regionally available; although derived from this plant-insect interaction, differences in their quality and yield are generated. Campana *et al.* (2015) pointed out that *D. coccus* can survive in a wide range of host species, to our knowledge, there is little research where its productive behavior is assessed in different cultivars. For example, in southern Peru, the Amarilla, Blanca, and Morada cultivars are used (Anculle-Arenas *et al.*, 2017), although no evidence of their yield and quality is reported. In Mexico, alternative cultivars of fruit, forage, or horticultural importance have been evaluated to generate greater added value to be integrated into the value chain. Previous research indicates that the host used can influence both the development (Tovar *et al.*, 2005; Méndez-Gallegos *et al.*, 2010; Tovar and Moreno, 2010) and the quality of *D. coccus* (Coronado-Flores *et al.*, 2013). In this regard, Chávez-Moreno *et al.* (2011) recorded that in Mexico the predominant host is *Opuntia ficus-indica* (L.) Mill.; the cv Atlixco among those that stand out (Flores-Alatorre *et al.*, 2014) in the center and south of the country, and the cv Villanueva (Coronado-Flores *et al.*, 2013), mainly used in the north-central of Mexico.

Given the currently implemented measures to ensure the safety of the insects and their derivatives for human consumption, mass-rearing systems under adequate sanitary conditions are required (Costa-Neto, 2015), to reduce the dependence on their recollection, or its production seasonality, as is the case of Peru, the world's leading producer of *D. coccus*. There, the agroclimatic conditions allow its cultivation in open-sky throughout the year (Anculle-Arenas *et al.*, 2017). However, in other countries, where they are not available, such as Mexico, intensive breeding has been used in confined systems such as the "Nopalotecas" (Campos-Figueroa and Llanderal-Cázares, 2003; Alonso *et al.*, 2013; Hernández *et al.*, 2017; Zacarías-Alvarado *et al.*, 2020), which ensures its quality, safety, and supply chain. Except for Alonso *et al.* (2013), in all these studies, only performance has been assessed based on the number of females, fresh weight, or dry weight per cladode. However, the final quality of the insect has not been evaluated, regard the carminic acid concentration (CAC) at the different levels of the "Nopaloteca".

Considering that the final price of this product is determined by the CAC, the methods and techniques that favor a higher CAC are required; therefore, the objective of this research was to determine the concentration of carminic acid (CAC, %) and the fresh weight (FW, g) of *D. coccus* in two *Opuntia ficus-indica* (L.) Mill., cultivars, collected in the pre and post oviposition phases in a confined "Nopaloteca".

MATERIALS AND METHODS

Study site

The experimental work was carried-out under semi-controlled conditions in a glass greenhouse, north-south oriented, from November 28, 2018, to May 8, 2019, at the facilities of the Colegio de Postgraduados, Campus San Luis Potosí, located at 22° 37' 55.66" N, 101° 42' 42.96" W and 2078 m altitude.

Characteristics of the breeding system used

The structure of the "Nopaloteca" was built with wood with a of 3×3 m (9 m²) dimension; this was divided into four levels with a 0.50 m separation (Level 1=0.5 m, Level 2=1.0 m, Level 3=1.5 m and Level 4=2.0 m). This setting corresponds to the commonly used levels in the "Nopaloteca" production system in Mexico. Five horizontal lines of 12-gauge galvanized wire were included at each level, at a 0.20 m distance. The cladodes were suspended from the base of the cladode using a wire hook of the same gauge.

Infestation process

The used original seed of *D. coccus* was acquired from the GranaZac S.P.R.R.L. company, located at Villanueva, state of Zacatecas, Mexico. To carry out the artificial propagation of *D. coccus*, initially, the cladodes were carefully cleaned with a brush, to reduce predators and competitor's incidence. Subsequently, these were horizontally placed on the floor and two sieves (40×40 cm) were placed on them, each containing 200 g of gravid females. After 48 h, the infested cladodes were removed and installed in the corresponding levels and rows in the "Nopaloteca".

Experimental procedure

In order to determine if the cultivar used as a food substrate and the height levels within the "Nopaloteca" influence the carminic acid concentration (CAC, %) and the fresh weight (FW, g) of *D. coccus*, 120 whole cladodes were used, between 8 and 12 months old, from the Villanueva (60 cladodes) and Esmeralda (60 cladodes) cultivars, both *Opuntia ficus-indica* (L.) Mill.

In order to determine the biological stage for collecting that allows to obtain a higher CAC (%) and FW (g) of *D. coccus* two harvest indices were evaluated: pre and post oviposition. For the first phase, 60 cladodes from both cultivars were harvested from each row and level at 112 d after the infestation (prior to the egg deposition). In the second, the females of the remaining cladodes were collected at each level and line (60 cladodes in total). In this case, in particular, as soon as more than 50% of the females finished their reproduction was considered as a time index (169 d).

During the development cycle, the maximum, average and minimum temperature (°C) (TMAX, TPROM, TMIN) and the maximum, average and minimum relative humidity (%) (HRMAX, HRPROM, HRMIN) were recorded hourly using a HOBO Pro v2 temp / RH onset sensor, USA[®], located at the center of each level. Also, illumination (lux) was quantified, once a week (8:00 a.m., 2:00 p.m. and 5:00 p.m.), at each level, as well as outside the greenhouse, with an EXTECH Instruments photometer (Nashua[®], USA). The

obtained values throughout the insect development cycle were added to obtain the final values. The exterior light was considered as the total illumination received (% IL), from which the proportion (%) of total illumination that affected each level (ILTOTAL) was acquired.

Carminic acid concentration and fresh weight of *D. coccus* assessment

The dry bodies of the females from the cladodes of both cultivars located in each level and developmental phase under study were pooled in a composite sample. The CAC (%) was determined following the methods by Briseño-Garzón and Llanderal (2008) using a GENESYS 10S VIS Thermo Scientific® spectrophotometer at a 494 nm absorbance. The boiling time established in the mentioned above methodology was increased from 10 to 15 min because it was previously verified that the CAC is maximized in laboratory conditions in which the procedure was carried out. The evaluations were made in triplicate and 10 readings were considered for each sample. The CAC was acquired by substituting the absorbance values in the equation generated from a calibration curve with 90% carminic acid (SIGMA®).

To determine the FW (g) of both evaluated phases, pre and post oviposition, after harvesting the females of the cladodes of both cultivars and each level, the weight of each sample was recorded in an analytical balance Explorer Pro (NJ, USA®) d=1/0.1 mg. Subsequently, the product was placed in a Petri dish and placed in a Maytag® freezer at -10 °C, for 3 days, for its sacrifice. Afterward, the samples were subjected to environmental drying, inside the greenhouse, for 10 d. Finally, each sample was pulverized in a Wiley Mini Mill® (3383-L10 115 V, 60 Hz, Thomas Scientific Swedesboro, NJ 08085-0099-U.S.A.) Reducing it to particles of 40 mesh, for the CAC assessing.

Statistical analysis

A completely randomized design was used, with 15 replicates consisting of a cladode as an experimental unit. To determine the statistical difference among means, the Tukey test was used ($p \leq 0.05$). Also, a simple correlation analysis (Pearson's r correlation coefficient) was carried out between the climatic variables and the evaluated response parameters. For the statistical processing the R Version 3.5.1 software (R Core Team, 2018) was used.

RESULTS AND DISCUSSION

Carminic acid concentration in *D. coccus*

The carminic acid concentration (CAC, %) was statistically influenced by the height at which the level was located within the "Nopaloteca" (gl=3; $Pr > F = 0.001$) and by the developmental phase in which the insects were (gl=1, $Pr > F = 0.001$). The magnitude of the response due to the cultivar effect was reduced since they generated similar values of 18.0% (gl=1, $Pr > F = 0.9464$).

In this research, the CAC in *D. coccus* consistently increased, as the height of the level at which the cladodes were located raised with respect to the ground level. For example, 1% increase in the CAC was registered, when the level height increased from 0.5 m (17.6%) to

2 m (18.6%), these values were statistically different, as well as the obtained values in the intermediate levels 2 and 3, located at 1.0 and 1.5 m (Figure 1).

During the crop cycle in which the research was carried out (autumn-winter) the average values of CAC increased, as the height rises from the floor where the level was located. Similar behavior was recorded by Alonso *et al.* (2013) when evaluating three height levels under the same system, since the CAC, also increased from 16.6% at 0.50 m to 17.5% at 1.5 m. Likewise, the average values of CAC obtained in this research were higher than those reported in previous studies such as those reported by Méndez-Gallegos *et al.* (2014), who obtained average values of 11.5%, but in brood chambers and under different photoperiods. On the contrary, Méndez-Gallegos *et al.* (2010) and Coronado-Flores *et al.* (2015) determined average values in the CAC higher than those registered in this work, with 19.1% and 21.5%, respectively. Although in this last study, even though the breeding was developed under the same system, the CAC was not differentiated between levels.

Regarding the biological phase in which *D. coccus* females were collected, there was a statistical difference ($p \leq 0.05$) between both evaluated indices. When these were harvested in the post-oviposition phase, the highest CAC was acquired, with average values of 20.5%, while the CAC in the pre-oviposition phase was only 15.7%.

Consistently, in both cultivars and in the four height levels of the evaluated “Nopaloteca”, when the females were collected in post-oviposition, it was possible to achieve a 5% CAC. This trend is comparable with that reported by Flores-Alatorre *et al.* (2014) who determined a higher CAC (23.8%) in the post-reproductive stage. Also, Aldama-Aguilera *et al.* (2005) acquired between 19.4 and 22.9%, when females were collected 15 d after the reproduction onset. Similarly, Tello and Vargas (2015) observed that, in collected females, when 50% of them began oviposition a CAC of 23.6% was achieved.

In this research, *D. coccus* collected in the post-reproductive stage could be classified as of first quality, according to the provisions of ITINTEC (1986); but according to Molero

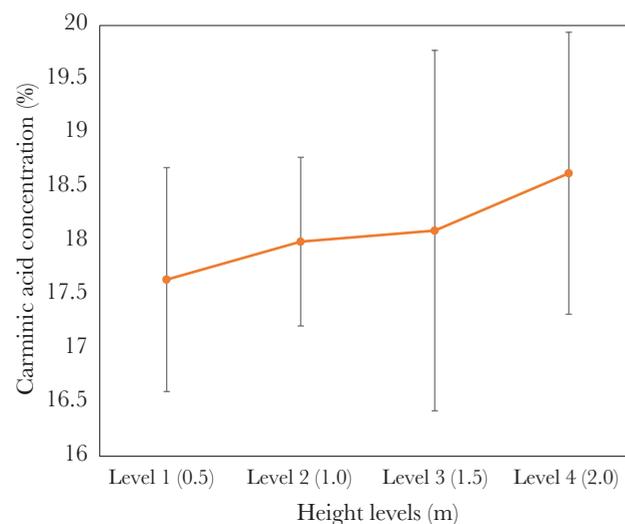


Figure 1. Carminic acid concentration ($\% \pm$ standard error) in *D. coccus*, reared in a “Nopaloteca” with four height levels.

and Herrera (2013), they would be considered of medium quality (20 to 20.75 %). However, it is necessary to consider that, for those collected females in post oviposition, the biological cycle lasted 169 d after the infestation, 70 to 80% longer than a normal cycle. This implies the convenience of increasing the quality or sacrificing another crop cycle. Nevertheless, the reproduction process can be used to obtain new progeny, carry out another infestation for a successive generation, and generate a larger CAC value.

***Dactylopius coccus* fresh weight**

The fresh weight (FW, g) of *D. coccus* was statistically influenced by the cultivars used as hosts for feeding ($gl=1$; $Pr>F=0.001$), by the height of the level within the structure ($gl=3$; $Pr>F=0.001$) and by the development phase in which they are collected ($gl=1$, $Pr>F=0.001$).

Concerning the cultivars used as food substrates, these showed a different behavior on the FW (g) of *D. coccus*. The statistically higher average values ($p\leq 0.05$) of FW per cladode (6.3 g) were attained obtained in the cv. Esmeralda, while the cladodes on cv. Villanueva produced 0.5 g less (5.8 g) (Figure 2).

The average yield of *D. coccus*, based on the FW (6.07 g) reached in both cultivars in this assessment could be considered high, according to the available literature. For example, Tovar *et al.* (2003) when evaluating three cultivars as a food substrate, recorded only 3.3 g per cladode in the cv. Villanueva. Also, these were superior to those registered by Coronado-Flores *et al.* (2015) using the same breeding system. In the case of the cv. Esmeralda, although no evidence was found to allow its comparison, it is possible to recommend it given its ease of handling, high productivity, and its performance in the FW, which is comparable to that reported in the literature.

The FW (g) of *D. coccus* per cladode depends on the height at which each level was located within the “Nopaloteca” ($p\leq 0.05$). The highest average FW value per cladode was reached at Level 2 (1.0 m), with 6.4 g while the lowest value was achieved in Level 1 (0.5 m)

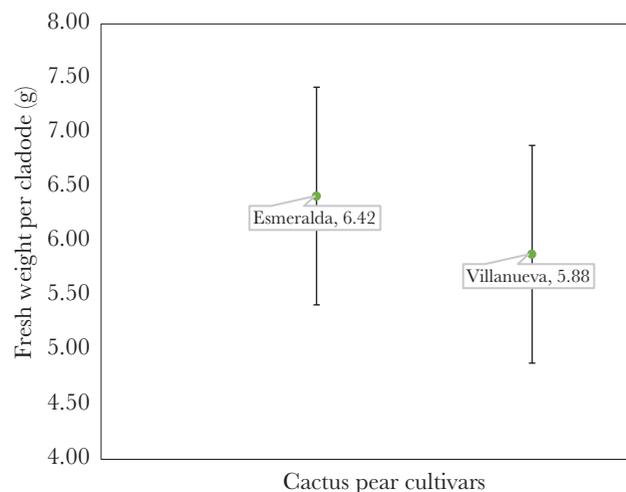


Figure 2. Fresh weight (g \pm standard error) of *D. coccus* obtained in two *O. ficus-indica* (L.) Mill. cultivars used as food substrate in a “Nopaloteca”.

with only 5.7 g on average per cladode. Likewise, the levels located at the extremes at 0.5 and 2 m in height obtained the lowest average FW values with 5.7 and 5.9 g per cladode, indicating that the intermediate levels provided better characteristics for the development of *D. coccus* (Figure 3).

Contrary to the behavior shown in the CAC, the highest average FW values were reached in the middle part of the “Nopaloteca” with values higher than 6 g per cladode, but the CAC in the females from the first three levels were lower at 18%, which shows a divergence between performance and CAC. The observed trend indicates that there are probable complex interactions between TPROM, HRPROM and ILTOTAL that affect each level, with the growth and development of *D. coccus*, suggesting that it has differential requirements for its optimal development, survival, reproduction, and CAC, such and as stated (Méndez-Gallegos *et al.*, 1993; Méndez-Gallegos *et al.*, 2014; Tello and Vargas, 2015; Zhang, 2017).

In this regard, Campos-Figueroa and Llanderal-Cázares (2003) found no difference in FW between the three levels evaluated, although they attained higher mean FW values than in this research (7.8 g per cladode). Similarly, Aldama-Aguilera and Llanderal-Cázares (2003) attained 8.6 g per cladode; in both studies the cv. Atlixco was used. They were also lower than those reported by Hernández *et al.* (2013) who obtained 9.3 g per cladode, under the same system in Peru, using another cultivar and a different protection structure; however, the values reached in this investigation were higher than those registered by Tovar *et al.* (2005) but using a rearing system in microtunnels.

In relation to the development phase in which the adult females of *D. coccus* are collected, it was observed that the detached females after concluding the reproductive process registered the highest average FW values with 6.3 g, statistically higher ($p \leq 0.05$) than the FW of those females collected before the beginning of reproduction (5.8 g), although the difference was only 0.5 g. Contrary to expected values, the average FW values of *D.*

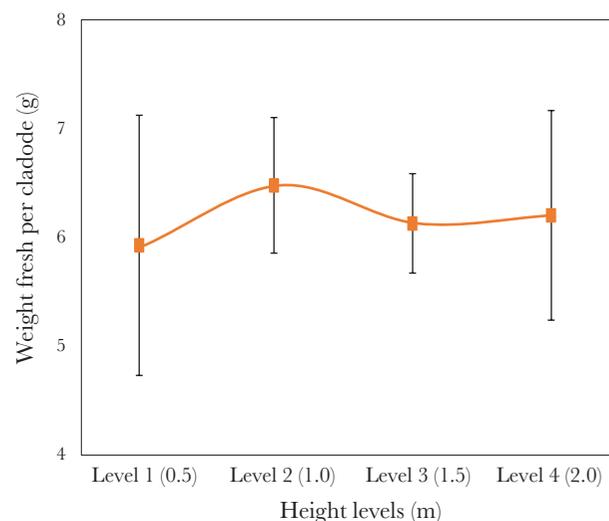


Figure 3. Average fresh weight values ($g \pm$ standard error) of *D. coccus*, reared at four levels of height (m) in a “Nopaloteca”, under confinement conditions.

coccus in the post-oviposition phase were higher, despite having undergone a reproduction process. This could be associated with the fact that the females in this phase did not die, as there was no lethal factor, but rather continued to increase their volume and reproduce for a longer period of time.

Correlation analysis

When studying the association between the evaluated parameters and the climatic variables studied, a positive correlation was observed between total illumination (ILTOTAL), maximum temperature (TMAX), and average temperature (TPROM) with CAC (AC), suggesting that there is a direct relationship of these variables with the CAC. Likewise, the minimum temperature (TMIN) has a positive effect on the CAC up to a certain limit and then decreases. The FW of the adult females of *D. coccus* was positively correlated with the average temperature (TPROM), minimum temperature (TMIN), and with the total illumination (ILTOTAL) also within certain limits (Figure 4).

CONCLUSIONS

The cultivars used as food substrate, the height of the level in the “Nopaloteca” and the collection phase influenced the yield and the CAC of *D. coccus*. From a productive point of

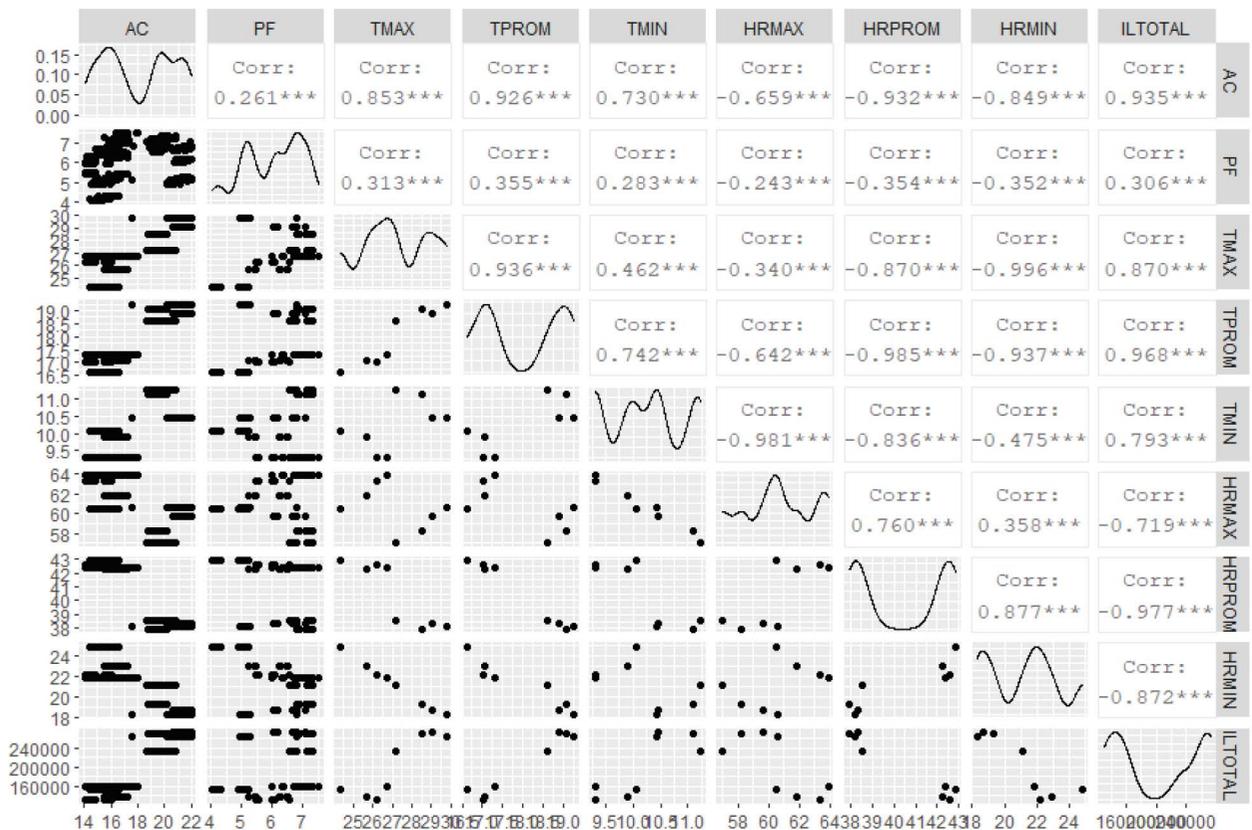


Figure 4. Correlation between fresh weight (FW) and carminic acid (CA) concentration of *D. coccus* and minimum relative humidity (HRM), average relative humidity (HRPROM), maximum relative humidity (HRMAX), minimum temperature (TMIN), average temperature (TPROM), maximum temperature (TMAX) and total illumination (ILTOTAL).

view, the cultivars used could be recommended in the commercial breeding of *D. coccus*, in the absence of other more suitable cultivars. The height of each level tested influences quality and performance, emphasizing that the higher the CAC, the lower the FW of the insect. The harvest, at the end of the reproductive phase, allowed to achieve a higher CAC value. The correlation analysis showed that there are specific limits between the physical factors evaluated and the growth and development of the insect. More detailed studies are needed to define these relationships and thus optimize performance and CAC under “Nopaloteca” conditions.

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Cuajilote (*Parmentiera aculeata* (Kunth) Seem.): A Potential Fruit for Ruminant Feed

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ABSTRACT

Objective: to review previous researches about the use of the fruits of the cuajilote tree (*Parmentiera aculeata*) as ovine feed.

Design/Methodology/Approach: this research was conducted, using all the available sources: science articles, conference reports, and books.

Results: *P. aculeata* (cuajilote) is generally used to provide shade, and as live fence, wood or firewood. However, it can also be used as forage. Both the foliage and the fruit have excellent nutritional qualities; additionally, the fruits are available during the dry season and free grazing cattle avidly eat them. Nevertheless, the nutritional value and the ruminal availability of this fruit's dry matter —during various ripeness stages— are unknown; it is also said to have medicinal properties.

Study Limitations and Implications: the cuajilote fruit was used in in vitro tests to remove protozoa from the ruminal fluid of bovines (defaunation); therefore, there are few in vitro researches about the use of cuajilote in a wholemeal diet as animal feed and about its maximum inclusion tolerance in ruminants.

Findings/Conclusions: as a result of its nutritional and medicinal benefits, the cuajilote fruit shows great potential as livestock feed. Additionally, it is available all year round, particularly during the dry season, when production expenses increase as a result of the lack of other food sources.

Keywords: Cuajilote, fruit, feeding, sheep, *in vitro*.

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INTRODUCTION

Given its natural and cultural diversity, Mexico is one of the world's most fertile fields for ethnobotany researches. There are about 30,000 species of vascular plants, which make this country one of the richest floristic regions of the planet (Rzedowski, 1978). Though the greatest potential is found in species of the Fabaceae family, other species can also be used, particularly if they are native species (López and Del Ángel, 2010). Many trees have leaves that are used as animal feed. Some of them also have fruits, such as the pods that can be used as animal feed. These trees are usually used to provide shade and as live fences, wood, or firewood. However, they can also be used as forage, because both their foliage and their fruits have desirable nutritional qualities (Patiño, 2014). Trees such as cuajilote (*Parmentiera aculeata*) (Bignoniaceae) (CONABIO, 2009) produce fruits that contain water, energy, and minerals. These fruits fall during the dry



season, during which food sources are required in the humid tropics. Usually, they are consumed by free grazing livestock (Jiménez, 1989). Therefore, the characterization and the use of the fruit of the cuajilote tree in the development of ruminant feed could help to decrease the competition for some food sources between domestic animals and people.

Distribution

Cuajilote is native to the tropical deciduous forest that covers wide expanses of land in southern and central Mexico. This tree can grow up to be 12 m tall, it has a wide trunk and furrowed bark. Most of its leaves are trifoliated; a few of them are simple, elliptical, glossy, and have an intense green color. The lumps of its branches have thick, small, curved, and sharp thorns, which can be found underlying each leaf. The flowers of this species grow on the stem (cauliflory) and from the twigs. Its fruit is shaped like a cylinder, it is usually curved and has thick, fleshy, and fibrous ridges. It grows up to 17 cm long and over 3 cm wide (Patiño, 2014).

Habitat and Location

The cuajilote tree is a very abundant species (Figure 1). It provides shade in open grasslands and adapts to several types of soil; however, it thrives on shaded places, in the vicinity of streams, and in warm climates (semi-warm and temperate) (Villar, 2011).

This tree is native to Mexico and northern Central America. It can be found from southern Tamaulipas and San Luis Potosí to the Yucatan Peninsula (in the coast of the Gulf Mexico) and from the center of Sinaloa to Chiapas (in the coast of the Pacific Ocean). It is a part of the deciduous tropical forest and it can adapt to the warm and humid regions of Central America. It is grown at an altitude of 2000-2240 msal and in places where the annual rain fall is higher than 1500 mm. It has a potential yield of 1000-2000 fruits per year (Villar, 2011).

Morphological Traits

The tree can grow from 3 to 15 m high and up to 30 cm wide. Its base has a channel-like structure and an abundance of short thorns, its shapeless crown branches out from the

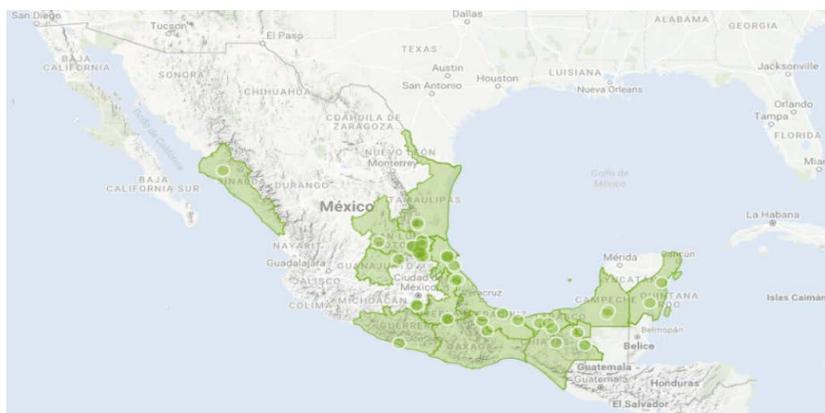


Figure 1. Main states in which the cuajilote tree can be found (*Parmentiera aculeata*) (INEGI 2018).

base, its branches grow upwards, the main trunk is 30 cm wide, it has a yellowish grey-brown furrowed bark with slight cracks, the bark is 6-15 mm thick (Sorela, 2006).

This species blooms all year long in Mexico, Puerto Rico, and Honduras. In that Central American country, the plants begin production 2-3 years after they are transplanted to their definitive location. Cuajilote develops from a flower to a ripe fruit in approximately 40 days (Villar, 2011). It blooms and bears fruit from March to December (Escamilla and Moreno, 2015).

It has several common names, including: *chote*, *cuachilote*, *guajilote*, *cuajilote*, *guetoxiga*, *pepino de árbol* (tree cucumber), *guachilote*, and *chayote*. It can be eaten raw or in various dishes; it is said to cure illnesses and diseases. Unripe berries are green yellow; ripe berries are reddish brown. It has a whitish flesh (endocarp) and numerous black seeds. It tastes like sugar cane (Villar, 2011). The inhabitants of Mexico's Mixteca region eat the fruit raw or boiled, some eat the whole fruit, while others throw out the fibrous interior (Pérez-Gutiérrez *et al.*, 1998; Andrade *et al.*, 2006).

It has hermaphrodite flowers which appear alone or in groups, they grow directly from the trunk or from the twigs, they are 5-8 cm long and have a greenish yellow color with purple striations, they are unevenly divided to one side. The cuajilote flower has a 2-3 cm long green chalice. It has a 4.5-6.5 cm long greenish cream-colored corolla with a wide tubular shape with five giant lobes; four stamens grow from the corolla's base. It has greenish yellow filaments, grey-brown anthers, and a superior bilocular ovary (Sorela, 2006).

The cuajilote trees have 6-15 cm long compound leaves that are made of 2-3 secondary leaves or leaflets. They vary from 3.5×1.5 cm to 8×3 cm. Their terminal or "point" leaflet is usually the biggest leaflet; the whole margin of the leaflets has a green upper side and a pale green underside. These trees lose their leaves once per year (Villar, 2011).

The rope-shaped, pointy, and compressed seeds are dispersed by animals (zoochory). They are 3-3.3 mm long and 2.5-2.8 mm wide. They have a dark, slightly rough, opaque, coriaceous, 0.1-0.3 mm thick seed coat. They have a straight, cordiform, compressed, slightly yellowish cream-colored embryo which takes up the whole cavity of the seeds. They have two flat, fleshy, cordiform cotyledons with two thin, literally round lobes on their upper section. They have a short, erect, lower radicle which faces the filament. They lack an endosperm (Gutiérrez *et al.*, 2003).

Uses in Ruminant Feeding

In vitro Experiments

Various authors, including Ley de Coss *et al.* (2013), have evaluated *P. edulis* (a synonym of *P. aculeata*) in an *in vitro* cultivation medium in order to keep the rumen's ciliate protozoa alive. They have reported that the fruit retains a similar protozoan concentration to the one found in the rumen; this characteristic enables the evaluation of defaunation works. Likewise, they evaluated an *in vitro* culture medium for the rumen's ciliate protozoa in 2015 and reported that it kept a 10⁴ population for three days without altering the fermentation pattern.

In situ Experiments

García-Castillo *et al.* (2008) carried out a research to determine the *in situ* degradation of the dry matter of the *P. edulis* fruit at various ripeness states and determined that the ripe fruit's dry matter (MS) showed greater ruminal degradation than the chilillo or unripe state (unripe fruit); however, the fruit's dry matter shows low ruminal availability and therefore they concluded that it is not a forage material that can substitute tropical forages. Based on their evaluation of the chemical composition and *in situ* ruminal degradation of the sprouts, leaves, and green stalks of the cuajilote tree in Chiapas, Mexico, Pinto-Ruiz *et al.* (2010) point out that it has approximately 15% crude protein, moderate fiber fractions levels, low content of total condensed tannins, and acceptable degradation values (76%).

In vivo Experiments

Moctezuma *et al.* (1993) carried out an *in vivo* digestibility test with wholemeal diets for sheep that included various levels of the cuajilote (*P. edulis*) fruit; they found that it improves digestibility up to a maximum level of 30% and concluded that it is a feed alternative. Additionally, they recommend including it in sheep portions, subject to availability and cost.

CONCLUSIONS

P. aculeata is an introduced species whose fruit has great potential as forage. Further animal nutrition research must be carried out to put to good use the full potential of its alleged nutritional characteristics. It is an alternative livestock feed in places with a harsher dry season.

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Climate and soil effect on oil palm (*Elaeis guineensis* Jacq.) yield

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ABSTRACT

Objective: To determine potential and water-limited yields in oil palm producing areas in the State of Tabasco, México.

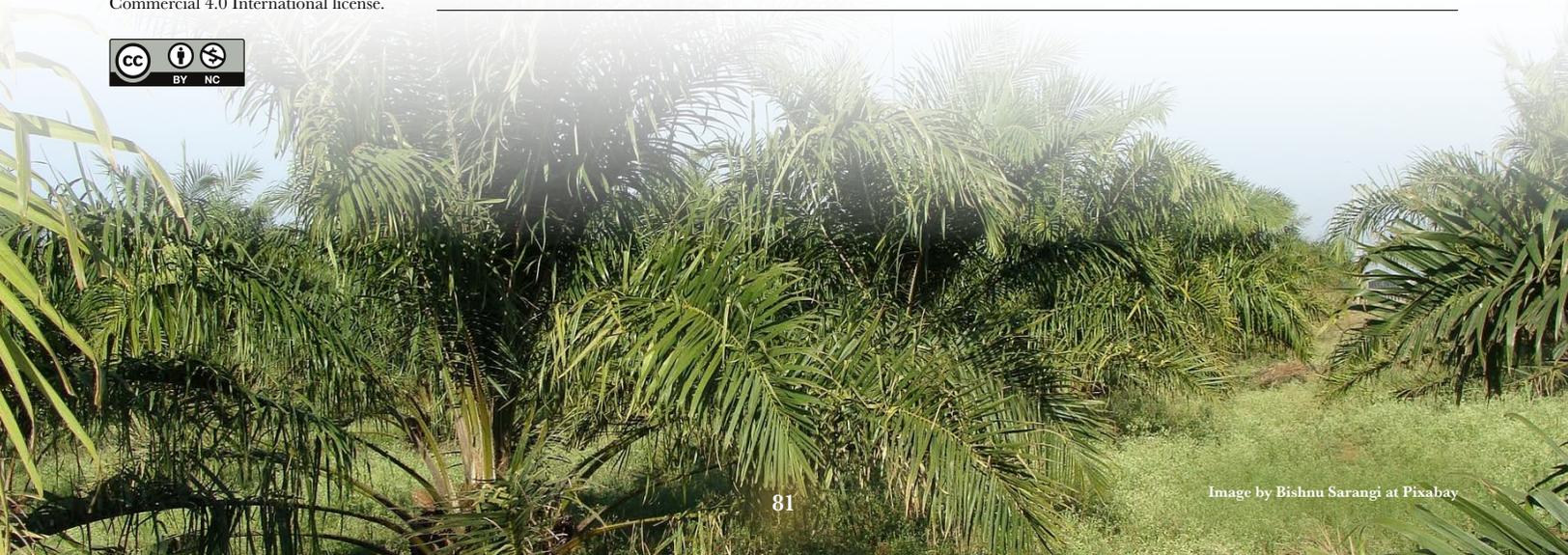
Design/Methodology/Approach: The ERIC III v. 3.2 database (IMTA, 2009) was used to select climatological stations with daily precipitation and maximum and minimum temperature records, going back to more than 20 years. The methodology proposed by the FAO and improved by Fischer *et al.* (2012) was used to estimate the potential yield. The equation reported by Ruiz-Álvarez *et al.* (2012) was used to estimate the annual water deficit from the climatic water balance.

Results: The average potential yield of oil palm with a high level of inputs varies from 35.8 to 40.6 t ha⁻¹ of fresh fruit bunches. The average water-limited yield can vary from 15.6 to 23.5 t ha⁻¹ in plantations of at least 8 years of age, under rainfed conditions. The reduction in the maximum average attainable yield was the result of 19.2-49.5% soil moisture deficits.

Study limitations/Implications: In order to determine their impact on potential and water-limited yields, climate change horizons must be included in future studies; this would enable researchers to establish the future theoretical economic profitability of the crop.

Findings/Conclusions: The analysis between the yields indicates that —if the gap between the current yields and water-limited yields is closed— output and percentage would be 6.5-14.4 t ha⁻¹ and 72.8-129% higher than the potential yield.

Key words: yield gap, annual water deficit, water balance, potential yield.



INTRODUCTION

In the State of Tabasco, 26,718 ha of oil palm are grown in the Sierra and Ríos subregions. The average yield is 15.4 t ha⁻¹ (SIAP-SADER, 2021), almost 42% less than Guatemala's average yield. In tropical regions, such as Tabasco, precipitation is usually the main cause that limits the growth and yield of fresh fruit bunches (Corley and Tinker, 2008).

Oil palm yields are exponentially reduced as the annual water deficit increases. This has allowed other researchers to evaluate and forecast the annual production (Corley and Tinker 2008; Sparnaaij, 1960; Spanaaij *et al.*, 1963; Ochs and Daniel, 1976). The French *Institut de Recherche pour les Huiles et Oleagineus* (IRHO) establishes a maximum attainable yield of 30 t ha⁻¹ for fresh fruit bunches. The maximum attainable yield can face an approximately 10% reduction per 100 mm of annual water deficit (Corley and Tinker, 2008).

Water requirement becomes equal to the reference evapotranspiration value (ET₀); when plants consume water quantities equal to ET₀, a maximum production of dry matter is expected to take place (Dufrene *et al.*, 1992).

Annual water deficit takes place up to two years after the oil palm culture has been subject to water stress (Corley and Hong, 1981; Lubis *et al.*, 1993). The main objective of this study was to determine the potential and the water-limited yields in the oil palm producing areas of the State of Tabasco. Additionally, we analyzed and compared the existing gaps between yields.

MATERIALS AND METHODS

Study Area. The study was carried out in the two oil palm producing subregions of the State of Tabasco. Seven climatological stations were selected: Teapa, Jalapa, Tacotalpa, and Macuspana (Sierra) and Balancán, Tenosique, and Emiliano Zapata (Ríos) (INEGI, 2015).

Climatological Data. Once the seven climatological stations were selected, the ERIC III v. 3.2 (IMTA, 2009) data base was used to obtain their daily data, monthly precipitation, and monthly maximum and minimum temperatures. The daily data records ranged from 20 to 50 years, depending on the selected locations. Only full years were selected for each series. Most of the stations do not record the climate variables, which are necessary to estimate potential and water-limited oil palm yields; instead, temperatures were used to carry out this task.

The monthly average global solar radiation (R_g) data were estimated using the Hargreaves-Samani equation proposed by the FAO when there is no data for this variable (Allen *et al.*, 2006). Equation (1):

$$R_g = 0.16 * \sqrt{T_{\max} - T_{\min}} * R_a \quad (1)$$

Where R_g =global solar radiation (MJ m⁻² d⁻¹) and R_a =extraterrestrial radiation (MJ m⁻² d⁻¹). R_a was estimated using the latitude data of the climatological station and the date (Allen *et al.*, 2006).

The reference evapotranspiration (ET_0) values are essential to carry out a water balance and to determine the annual water deficit. The Hargreaves-Samani (1985) equation was used to carry out the estimate. Equation (2):

$$ET_0 = 0.0023 * \left[(T_{max} - T_{min})^{0.50} \right] * (17.8 - T_{med}) * \left(\frac{R_a}{2.45} \right) \quad (2)$$

Where ET_0 =reference evapotranspiration ($mm\ d^{-1}$), T_{med} =average daily temperature ($^{\circ}C$), T_{max} =daily maximum temperature ($^{\circ}C$), T_{min} =daily minimum temperature ($^{\circ}C$) and R_a =extraterrestrial radiation ($MJ\ m^{-2}\ d^{-1}$).

Yield Type. Scientific literature mentions at least three types of yield: actual yield, water-limited attainable yield, and potential yield. The average or actual yield is the output that producers obtain, and it varies in time. It is limited by nutrients and water. Weeds, pests, blights, and plantation management contribute to its reduction. The attainable yield is only limited by water deficit in the soil and is equivalent to a water-limited potential yield, in rainfed condition areas. Potential yield has no limitations, except those imposed by the CO_2 concentration in the air, global solar radiation, temperature, and crop characteristics (physiology, phenology, canopy structure, etc.). Van Ittersum and Rabbinge (1997), Fischer *et al.* (2012), Fischer (2015), and Woittiez *et al.* (2017) discussed these types of yields in detail.

Actual Yield (2019). The data provided by SIAP-SADER (2021) for the 2010-2019 oil palm yields in the oil palm producing municipalities of the State of Tabasco are shown in Table 1.

Calculation of the reduction of water-limited attainable yield. The reduction of the water-limited attainable yield of oil palm is calculated on the basis of the relation between the annual water deficit (DHA) and the oil palm yield. Several authors have reported this relation: Spanaaij (1960), Sparnaaij *et al.* (1963), Ochs and Daniel (1976),

Table 1. 10-year Evolution of Yields in the Oil Palm Producing Municipalities of Tabasco (2010-2019).

Municipality	Year										Average
	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	
Balancán	4.0	7.0	15.0	17.3	22.8	12.8	12.6	13.0	14.2	14.1	13.3
Centro	20.7	26.0	25.4	21.5	18.0	15.8	17.6	16.9	17.8	17.7	19.7
Emiliano Zapata	4.0	8.0	8.9	16.0	8.0	12.4	14.7	14.0	14.6	16.6	11.7
Jalapa	26.0	28.7	29.3	27.8	23.1	18.4	23.7	20.0	20.5	20.7	23.8
Macuspana	22.0	24.0	25.2	23.2	22.1	13.0	21.0	14.0	16.3	16.4	19.7
Tacotalpa	19.0	20.9	24.4	17.8	15.6	15.4	17.2	18.8	19.3	19.4	18.8
Teapa	25.5	34.0	24.5	31.7	12.3	16.2	18.0	18.5	18.5	18.8	21.8
Tenosique	5.5	7.0	8.9	6.5	9.6	10.5	10.0	9.4	10.1	10.4	8.8
State average	9.8	12.0	16.0	16.4	17.2	13.5	14.8	14.0	14.8	15.5	14.4

Corley and Hong (1981), and Corley and Tinker (2008). This study takes into account the relation reported by Corley and Tinker (2008), which relates DHA with relative yield. The relation has an exponential behavior. Equation (3):

$$Y = 100 * e^{(-0.00169 * X)} \quad (3)$$

Where X =DHA value and Y =percentage decline regarding a 100% maximum potential attainable yield. The relative yield values reported by Corley and Tinker (2008) were obtained from the yield data of the world's main oil palm producing countries. Subsequently, that data —resulting from applying equation (3)— was expressed as a percentage reduction of the maximum yield (100%). The aim was to visualize the percentage reduction of the attainable yield with regard to a maximum yield established according to various DHA values. The resulting equation was the following:

$$Y = 0.1602 * X - 0.00009 * X^2 \quad (R^2=0.999) \quad (4)$$

Where Y =relative reduction of attainable yield (%) and X =annual water deficit (mm).

The IRHO reports that, for every 100 millimeters of DHA, an average 2.88 t ha^{-1} yield reduction takes place in palms that were at least 8 years old and under rainfed conditions.

Potential Yield Calculation. To calculate potential oil palm yield we need to find out the daily and monthly average values of global solar radiation, average temperature, and diurnal temperature, as well as leaf area and crop harvest indexes. The average and diurnal temperatures are obtained based on maximum and minimum temperatures. Diurnal temperature is calculated using the equation (5) proposed by Aceves Navarro *et al.* (2017). Oil palm leaf area and crop harvest indexes for a high supply level is 6.0 and 0.60, respectively (Fischer *et al.*, 2012). Equation (5):

$$T_{diu} = T_{max} + 0.25 * (T_{max} - T_{min}) \quad (5)$$

Where T_{diu} =diurnal temperature; T_{max} =maximum temperature; and T_{min} =minimum temperature.

The potential yield calculation procedure is based on the methodology proposed by the FAO and improved by Fischer *et al.* (2012). Rivera-Hernández *et al.* (2012) described the said calculation in detail.

Annual Water Deficit (DHA) Estimate. Water deficit can be estimated for any period based on a climatic water balance (Ruiz-Álvarez *et al.*, 2012). A one-month period was selected for this study. Equation (6):

$$BH_i = SW_i + PPt_i - ET_{0_i} \quad (6)$$

Where BH_i =climatic water balance of the i (current month) (mm); SW_i =theoretically useful soil moisture reserve, at the beginning of the i period, at a one-meter depth (mm); PPt_i =total precipitation of i (current month) (mm); ET_{0_i} =reference evapotranspiration of i (current month) (mm); and i =selected period (month).

Negative values for the monthly water balance (BH) result in a deficit. An annual water deficit (DHA) consists of the yearly sum of the monthly BH negative values. We calculated the water-limited yield and its relative reduction regarding the maximum attainable yield, taking into account the annual water deficit of every recorded year and using equations (3) and (4).

In the oil palm producing regions of the world, the useful moisture reserve at a one-meter depth in the soil, varies from 83 mm to 167 mm (Corley and Tinker, 2008). To guarantee a 100-mm minimum supply of useful moisture, this study was carried out at a depth of one meter, following the findings of López *et al.* (2007) for the soil textures predominant in the Ríos and Sierra subregions. The BH was carried out during November in order to guarantee that the soil had reached its maximum water retention capacity, as a result of the abundant precipitations of October.

Expected Return Periods. A probability distribution function was fitted to the data resulting from the annual water deficit and the relative annual yield reduction at each selected climatological station, and the exceedance probability and its matching return periods were calculated.

RESULTS AND DISCUSSION

Return Periods

The results show that the annual water deficit and the relative yield reduction at the Jalapa, Balancán, Emiliano Zapata, and Tenosique climatological stations fitted a regular probability distribution function. On the contrary, the Transformation of the Normal (Fourth Root) was a better fit for the Macuspana, Tacotalpa, and Teapa climatological stations. The exceedance probability and return period for the annual water deficit and the relative reduction of the relative yield were calculated based on the probability distribution functions, enabling the definition of annual water deficit values and reduction with a one-year return period (*i.e.*, values that will be recorded every year while a moisture deficit remains). Table 2 shows these values for the chosen stations.

Table 2 shows that there will be yearly reductions in the average attainable yields, as a result of the deficient rainwater supply during the yearly dry periods. This work proves that every year there is a reduction of the water-limited yields with regard to the maximum attainable yields (30 t ha^{-1}) reported by the IRHO.

Actual Yield

From 2010 to 2019, the average yield in the State of Tabasco was 14.4 t ha^{-1} (Table 1). The average yields in the Sierra subregion were higher than the yields reported for the Ríos subregion. There were time-dependent variable yields in both subregions. On the

Table 2. Annual Water Deficit (AWD) and Reduction of the Attainable Yield (RAY) Values with a One-Year Return Period.

Municipality	Securities with a return period of one year	
	AWD (mm)	RAY (%)
Jalapa	364.6	46.0
Macuspana	221.5	31.2
Tacotalpa	172.4	25.3
Teapa	87.5	13.7
Balancán	273.5	37.0
Emiliano Zapata	324.5	42.2
Tenosique	229.4	32.1

one hand, this phenomenon was partly the result of the different plantation ages —since not all of them have reached their maximum production yet and therefore they follow the behavior described by Woittiez *et al.* (2017). On the other hand, this phenomenon is partly the result of the annual rainfall volume variations —which define the annual water deficit differences that have a differentiating impact on yields.

Reduction of the Maximum Attainable Yield

The climatic water balance showed that the average annual water deficit fluctuated between 152.3 and 410.6 mm, in the oil palm producing municipalities of the State of Tabasco (Table 3). Table 3 also shows the average actual attainable yield in tons and its reduction percentage regarding their maximum attainable potential. The average water-limited yield ranges from 15.2 to 23.5 t ha⁻¹. It should be pointed out that average values (50% probability) actually mean the recurrence of the said values with a 2-year return period.

Based on the results shown in Table 3, if auxiliary irrigation was used to close the gap between the maximum attainable yield and the actual water-limited yields, it could be possible to achieve a 5.8-14.8 t ha⁻¹ increase of oil per year —or a 1.5-3.7 ha⁻¹ increase for

Table 3. Annual Water Deficit (AWD) and Actual Average Water-Limited Yield per Ton and Maximum Attainable Reduction Percentage.

Municipality	AWD (mm)	Actual Average Water-Limited Yield	
		(t ha ⁻¹)	Decrease (%)
Jalapa	410.6	15.2	49.5
Macuspana	300.4	18.4	36.9
Tacotalpa	167.1	22.6	23.8
Teapa	152.3	23.5	19.2
Balancán	323.2	17.6	41.3
Emiliano Zapata	330.2	17.5	41.8
Tenosique	294.9	18.6	37.9

(*) AWD: average annual water deficit. (**) Reduction with regard to a 30 t ha⁻¹ yield according to IRHO (Corley and Tinker, 2008).

its equivalent. Table 3 also shows that there will always be an average 150-410 mm annual water deficit, equivalent to 4.3-11.8 ha^{-1} yield reductions.

The analysis of historical weather data from the two oil palm producing subregions of the State of Tabasco proves that there is a moisture deficit every year; therefore, there will always be reductions to the maximum attainable yield, proportional to the annual water deficit value. According to the results of the area covered by the Teapa climatological station, the actual water-limited oil palm yields under rainfed conditions fluctuated between 14.0 and 30 t ha^{-1} (average value: 23.5 t ha^{-1}).

Meanwhile, in the area covered by the Balancán municipality—which includes most of the oil palm producing areas of the State of Tabasco—, an average yield of 13.1 t ha^{-1} has been reported (similar to the national average) (SIAP-SADER, 2021). Clearly, plantation fertilization and weed, pest, and blight control must be improved in order to increase yields. The maximum attainable yield could be achieved through the use of auxiliary irrigation; this would reduce or eliminate water deficit in the oil palm producing areas; the yield could even exceed 40 t ha^{-1} , in those areas which have been established as highly suitable as a result of their soil and climate conditions (Aceves *et al.*, 2008). The municipality of Tacotalpa has the best weather conditions for the rainfed production of oil palms: 2 out of 10 years have no annual water deficit.

Potential Yields

The average potential yields for oil palms are very similar from one subregion to another (Table 4): they have a non-significant 300 kg ha^{-1} difference. The low variation is the result of the high similarity of the space-time distribution of global radiation and diurnal temperature. These average yields must be set as a goal for high-tech oil palm plantations that have appropriate water supply.

Yield Gap Analysis

Table 5 shows the average yield for the various types of yield. The gap that exists between the average actual and water-limited yields can be observed when they are compared with the maximum attainable and potential yields (Table 5). Analyzing these gaps can help to explore ways to improve plantation productivity (van Ittersum and Rabinge, 1997).

Table 4. Average Potential Yields in the Oil Palm Producing Municipalities of the State of Tabasco.

Municipality	Potential yield (t ha^{-1})
Jalapa	40.6
Macuspana	37.3
Tacotalpa	36.4
Teapa	35.8
Balancán	38.2
Emiliano Zapata	36.6
Tenosique	36.9

Table 5. Yield Types and their Average Variation per Tons of Fresh Fruit Bunches(*).

Yield type	Average yield (t ha ⁻¹)
Current average	9.8 a 17.2
Achievable maximum	30.0
Limited by current water	15.6 a 23.5
Potential	35.8 a 40.6

(*) Resulting values under historic environmental conditions.

Additionally, Table 5 shows that —given the prevailing weather, soil, and handling conditions in the State of Tabasco— actual yields only represent 32.7-57.3% of the maximum attainable yield. Consequently, if the gap is closed through auxiliary irrigation and improved handling practices, a 12.8-20.2 t ha⁻¹ increase of actual yields can be expected, just as Donough *et al.* (2009); Cavalche (2013), and van Ittersum and Rabinge (1997) reported. For example, according to De Taffin and Daniel (1976), auxiliary irrigation increased yields from 12 to 31 t ha⁻¹ over a 3-year period. Meanwhile, Cavalche (2013) reports that auxiliary irrigation increased oil palm yield in Ecuador by 21.3% during the first year and that investing in irrigation had an over 2.7 cost-benefit ratio. For their part, Van Kraalingen *et al.* (1989) reported that 6-12-year-old plantations can produce up to 34.1 t ha⁻¹ of fresh fruit bunches; meanwhile, Corley and Tinker (2008) report that the maximum growth rates for mature palm oil plantations with 6-7 leaf area indexes in theory can produce up to 50 t ha⁻¹.

Nevertheless, measures taken to close the oil palm yield gaps require a long time, as a consequence of the time gap between the moment in which the stressor takes place and the moment when it impacts the yields. This time gap can last up to 40 months: the gap between the flower's initiation and the fruit's ripening (Donough *et al.*, 2009).

CONCLUSIONS

The maximum attainable yields will always be reduced —in proportion to the value of the annual water deficit— in the two-oil palm producing subregions. The actual average water-limited yield ranges from 15.2 to 23.5 t ha⁻¹. The average potential yields for oil palms per subregion are very similar (300 kg ha⁻¹ difference). Actual yields represent only 32.7-57.3% of the maximum attainable yield.

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Hedonic perception and preference analysis of double cream cheeses formulated with raw and pasteurized milk

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ABSTRACT

Objective: To compare the characteristics of double cream cheeses made with raw and pasteurized milk per liking and preference level.

Design/Methodology/Approach: Cheeses from three brands were evaluated: Santa Teresa, Montero, and Colegio de Postgraduados (CP). The first two cheeses were formulated with raw milk, while the CP cheese was formulated with pasteurized milk inoculated with lactic cultures. The hedonic perception study was carried out in monadic series with 19 volunteers who blind tasted the three types of cheese. The evaluation attributes were: appearance, taste, aroma, and general acceptability on a 9-point scale; and saltiness, acidity, and creaminess on a 3-point scale (JAR). Preferences were evaluated by rank and multiple comparison tests.

Results: No differences were found in the aroma, taste, and texture liking level of the cheeses ($p > 0.05$); nevertheless, there were differences in the appearance and general acceptance ($p < 0.05$). The general acceptance of the CP cheese was significantly lower than that of the two raw milk cheeses ($p < 0.05$). The penalty analysis showed that low acidity and low creaminess attributes are related to a low general acceptance ($p < 0.05$).

Limitations/Implications: The study has enough evaluations for statistical tests.

Findings/Conclusions: Raw milk cheeses obtained the highest marks in all attributes. Determining if there are other sensory attributes—in addition to those that were the subject of this study—will help to explain the greater preference and global acceptance of raw milk cheeses.

Keywords: pasteurized milk cheese, lactic cultures, raw milk cheese, liking level.

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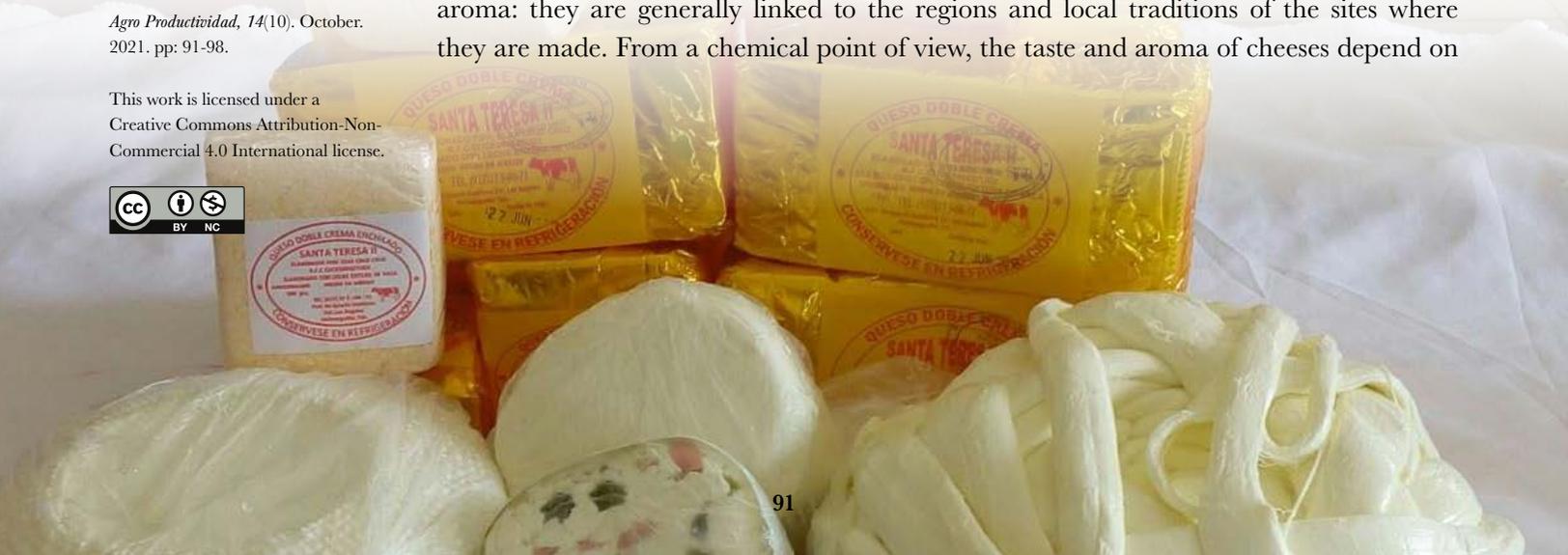
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INTRODUCTION

Raw milk artisan cheeses are appreciated by consumers due to their unique taste and aroma: they are generally linked to the regions and local traditions of the sites where they are made. From a chemical point of view, the taste and aroma of cheeses depend on



the physicochemical and microbiological composition of raw milk and the technological manufacturing characteristics (for example: heat treatment or pressing) of each type of cheese. Beuvier *et al.* (1997) showed that the raw milk of Swiss cheeses contains the microorganisms that generate their typical taste and aroma.

Cheeses made with raw milk generally have greater microbial and sensory diversity than cheeses made with pasteurized milk, which in many cases gives them a sensory advantage (Tadjine *et al.*, 2020) (Table 1).

Pasteurization is a process in which raw milk is heated to 63 °C/30 min or 72 °C/15 min to eliminate pathogenic microorganisms and undesirable bacteria (Yoon *et al.*, 2016). Milk composition, nutritional profile, and sensory properties may be slightly affected (Alegbeleye *et al.*, 2018). Grappin and Beuvier (2007) also mention: elimination of milk microorganisms, inactivation of milk autochthonous enzymes, denaturation of whey proteins, and modification of the curdling properties of milk.

Using a heat treatment to pasteurize the milk that will be used to make cheeses requires harmless acidifying cultures (lactic cultures) that restore some of the microorganisms lost in pasteurization; this guarantees the acidification of the milk (Ramos *et al.*, 2009; Saidi *et al.*, 2020). Lactic acid bacteria are one of the main bacteria that can be safely (GRAS) inoculated into milk to acidify it with lactic acid from lactose. Lactic acid not only give the pleasant taste typical of many cheeses, it also has antimicrobial properties (Pacheco and

Table 1. Pros and cons of artisanal cheeses made with either raw milk or pasteurized milk.

	Cheeses made with raw milk	Cheeses made with pasteurized milk
Pros	<ul style="list-style-type: none"> • Many cheese varieties made with raw milk have better taste, aroma, and organoleptic characteristics than the same cheeses styles made with pasteurized milk. • These cheeses contain many microorganisms that produce flavor and aromas, which are associated with greater consumer preference (Montel <i>et al.</i>, 2014). • Many traditional cheeses are gastronomic elements of great value because their distinctive flavors and textures. • Its production is linked to value chains that generate local employment. • Products for the nostalgic market segment (Beuvier <i>et al.</i>, 1997). • Immigrants away from their home country, do everything possible to consume products that make them to remind their childhood. • Some raw milk cheeses can be legally accepted, under the specifications described in NMX-F-735 (2011). • Some cheeses can be formulated with raw milk with low level of microbial safety concern due to technological particularities such as high acidity and high salty level (Pacheco and Bucio 2010; Bucio <i>et al.</i>, 2021). 	<ul style="list-style-type: none"> • During milk pasteurization, microorganisms of all kinds are eliminated. • The national and international standards of heat treatment are accomplished (Yoon <i>et al.</i>, 2016). • After pasteurization, milk is ready to be inoculated with lactic cultures to make artisan style cheeses or any other type of cheeses, including European style cheeses. • The flavors of cheeses made with pasteurized milk depend mainly on the microorganisms used in the inoculation. • Practically all cheeses sold in self-service stores are made using pasteurized milk. • Cheeses made using pasteurized milk, especially European cheeses have very attractive sensory characteristics for all types of consumers. • Many European artisan cheeses, even with Protected Designation of Origin, are also made with pasteurized milk; or have some heat treatment on the curd, p. Eg in scalding that eliminates pathogenic microorganisms, such as pasta filata cheese.
Cons	<ul style="list-style-type: none"> • In many cheeses, sanitary quality might be variable (Pomeón <i>et al.</i>, 2011). • Some cheese varieties are prompt to contain certain pathogenic microorganisms. • Sales are carried out only in the informal markets. • Many cheeses do not meet national or international standards for sale in formal markets, including self-service chain stores and export markets (Tadjine <i>et al.</i>, 2020). 	<ul style="list-style-type: none"> • If pasteurized milk is not inoculated with the specific flavor-imparting microorganisms in raw milk cheeses, the flavors will be very different. • Some consumers are not used to enjoy the taste, aroma, and texture of many of the cheeses made with pasteurized milk (Alegbeleye <i>et al.</i>, 2018), but they are used to cheeses with raw milk (Villanueva, 2010).

Bucio, 2010). Many raw milk cheeses also contain other microorganisms that produce other types of acids or that have functions other than acidifying, but they are not usually added to the milk that is used to make pasteurized milk cheeses; this could have an impact on taste. For example, double cream cheeses made with raw milk have yeasts in addition to lactic bacteria (Ramos *et al.*, 2009). No studies have been carried out to evaluate the taste of double cream cheeses formulated with raw milk and pasteurized milk to which lactic cultures that include yeasts have been added. Therefore, the hedonic perception and preference analysis of double cream cheeses made with raw and pasteurized milk were compared.

MATERIALS AND METHODS

This research was carried out at the Colegio de Postgraduados, Campus Tabasco. Cheeses from three brands were randomly collected. The cheeses formulated with raw milk were Sta. Teresa and Montero, both regionally prestigious brands. The CP cheese was made by Caobanal, a younger company, which has equipment to pasteurize milk. This company used the following mixed lactic culture, prepared by the Colegio de Postgraduados, Campus Tabasco: $>1 \times 10^8$ /g of *Lactobacillus fermentum*, and $>1 \times 10^8$ /g of *Lactobacillus pentosus/plantarum* (Ramos *et al.*, 2009), as well as a proteolytic yeast strain (Duran, in preparation). The first two strains acidify milk; the third strain hydrolyzes caseins to peptones. Therefore, all of them increase the amino acid availability for milk acidification. The presence of yeast in double cream cheese manufactured with raw milk inspired us to suggest its use (Ramos *et al.*, 2009). This culture was multiplied by the Caobanal company about 6 times; they used it to process 6 sets of 100 kg of milk and they obtained about 600 kg in total. The company was invited to exhibit its cheeses at a CANACINTRA fair.

The consumer perception study was carried out with 19 randomized volunteers who evaluated the three types of cheese in monadic series. The cheeses' identity was unknown to the panelists. The following acceptability characteristics were evaluated: general appearance, aroma, taste, texture, and general acceptance. A nine-point hedonic scale was used (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely) (Frizzi-Amayo *et al.*, 2019). Saltiness, acidity, and creaminess were evaluated on the ideal point scale (Just-About-Right, JAR) (Prinyawiwatkul, 2011; Fernandez-Segobia *et al.*, 2018), with three levels of taste perception for each of these attributes: 1, little; 2, adequate; and 3, exceeded. Histograms were developed, drawing a graph of the response percentage of the scale in the three mentioned levels for each cheese. Student t tests with known mean were carried out in order to evaluate if the responses that did not fall into the "adequate" category were significantly different (Lawless and Heymann, 2010). A penalties analysis was also made, based on the proposals of Lawless and Heymann (2010) and Narayanan *et al.* (2014): the JAR scale is combined with the general acceptance scale to obtain a graph of penalties for the evaluation of the potential impact of being outside the "adequate" level on the product general acceptability. The procedure was as follows (Lawless and Heymann (2010)): (1) the general acceptability \bar{x} of the categories (1,2,3) was obtained; (2) the \bar{x} category 2- \bar{x} category 3 and \bar{x} category 2- \bar{x} category 1 subtractions were made; (3) A table and scatterplot were

developed from the scores of differences resulting from the penalty related to the percentage of the total panel of consumers in each category.

For the rank tests, each panelist evaluated 3 cheese samples to qualify their preference: from 1 (most preferred) to 3 (least preferred). The scores were processed to make multiple comparisons between the data of the three types of cheeses, according to the procedures of Christensen *et al.* (2006).

Statistical analysis

An ANOVA and Tukey mean difference tests were carried out in the SPSS software ver. 15, to determine the significant differences between the sensory attributes of the cheeses: appearance, taste, aroma, and general acceptability. Excel was used to analyze the JAR data. SPSS ver. 15 was used for the one-way *vs.* known mean t-test. Multiple comparisons were made according to the procedures proposed by Christensen *et al.* (2006) for the analysis of the range tests.

RESULTS AND DISCUSSIONS

No differences were found in the aroma, taste, and texture of the cheeses ($p > 0.05$). Montero cheese obtained the highest qualifications for all attributes (Table 2).

The cheeses' characteristics that presented significant differences were appearance and acceptance ($p > 0.05$) (Table 2). The appearance of CP had a lower rating than Montero ($p < 0.05$), but did not have differences with respect to Sta. Teresa ($p > 0.05$). The appearance of CP and Sta. Teresa fell into the category of "I like it moderately", while Montero fell into the category of "I like it a lot". Regarding general acceptance, the CP cheese had a significantly lower acceptance than the Sta. Teresa and Montero cheeses ($p < 0.05$).

Villegas de Gante *et al.* (2011) conducted a global consumer acceptability study on 12 samples of double cream cheeses. The acceptability averages ranged from 5.18 to 6.78. In our study the values are higher, possibly because we restricted the study to the 3 most prestigious companies in the region: Sta. Teresa from Huimanguillo, Montero from Malpaso, and Caobanal. The Sta. Teresa brand is so recognized and appreciated that there are several imitations in the market. Although they did not have such a high acceptability, the CP cheeses obtained a 7.0+ score; therefore, they represent an opportunity to offer this product to the market. This value is higher than the average value of Villegas de Gante's study.

Table 2. Degree of liking of Appearance, flavor, taste texture and overall liking of cheeses made either with raw or pasteurized milk.

Degree of liking	Homogeneity of variances	Significance	CP	STA. TERESA	MONTERO
Appearance	0.20	0.03	7.1 ^a	7.2 ^{ab}	8.0 ^b
Flavour	0.05	0.12	7.1	7.3	7.9
Taste	0.82	0.09	7.1	7.7	7.9
Texture	0.23	0.11	6.5	7.3	7.5
Overall liking	0.61	0.001	6.8 ^a	7.6 ^b	8.1 ^{bc}

*Results with different letters are significantly different ($P < 0.05$). The values represent the mean of the evaluation of 19 consumers using the 9-point hedonic scale.

An analysis of the saltiness, acidity, and creaminess attributes —measured with the JAR scale— is presented in Figures 1, 2, and 3, respectively. The histograms show that the CP and Sta. Teresa cheeses had certain biases in relation to the “adequate” category,

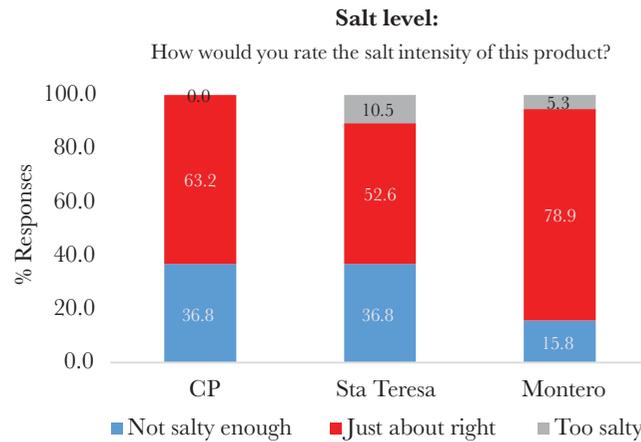


Figure 1. Just-about-right (JAR) scale response percentages for the level of salt in cheese grouped into three levels: not enough, just about right, too much.

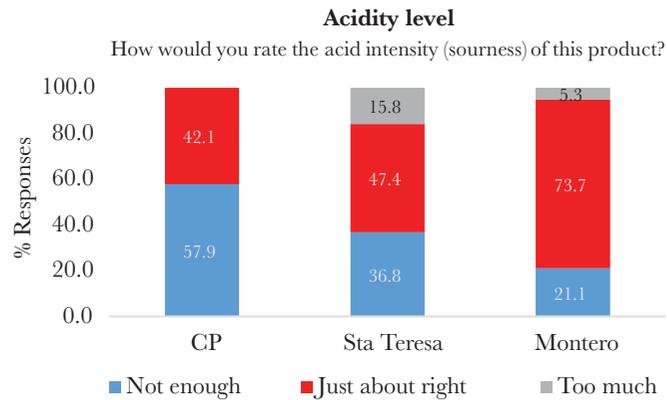


Figure 2. Just-about-right (JAR) scale response percentages for the level of acidity in cheese grouped into three levels: not enough, just about right, too much.

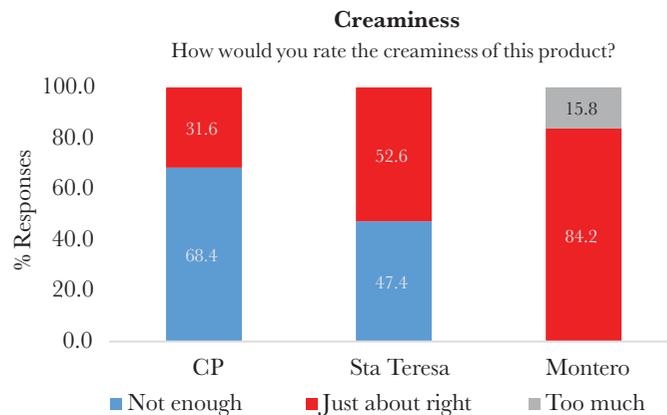


Figure 3. Just-about-right (JAR) scale response percentages for creaminess in cheese grouped into three levels: not enough, just about right, too much.

oriented towards the “low level of salt”, “low level of acidity”, and “low level of creaminess” category. The Student *t* test confirms that the CP cheese was significantly biased from the “adequate” category, as a result of its low salt content ($\bar{x}=1.63$; $p=0.005$), acidity ($\bar{x}=1.42$; $p=0.00$), and creaminess ($\bar{x}=1.31$; $p=0.00$), suggesting that an increase in the saltiness, acidity, and creaminess levels could improve its general acceptability. Sta. Teresa cheese was slightly biased by its low saltiness ($\bar{x}=1.74$; $p=0.1$) and creaminess ($\bar{x}=1.37$; $p=0.0001$) levels, but not by its acidity level ($\bar{x}=1.79$; $p=0.21$). The Montero cheese histograms focused on the “adequate” category and were unbiased with respect to the “adequate” category for any of the three attributes ($p>0.05$): saltiness ($\bar{x}=1.89$; $p=0.33$), acidity ($\bar{x}=1.84$; $p=0.19$), and creaminess ($\bar{x}=2.1$; $p=0.16$). Consequently, we inferred that this cheese does not need changes (Lawless and Heymann, 2010).

A penalty analysis is shown in Figure 4. This is an analysis of the attributes that generated low acceptance ratings (low acidity and creaminess), noted in the upper right-hand quadrant.

Therefore, according to these results, in order to increase the acceptance of the CP cheese, we should focus on increasing its acidity and creaminess.

Most people preferred Montero, followed by Sta. Teresa; CP cheese ranked 3rd (Table 3). This result was expected, since both Montero and Sta. Teresa had higher scores in all the attributes of the hedonic scale and were more focused on the “adequate” level in the JAR scale (especially Montero). These results are likely a consequence of the many years that both products have been available on the market. Like many other cheeses, they are the result of a traditional know-how, which is a local heritage, with a reputation linked to the region (Villegas de Gante and Escoto, 2011). This gives them a competitive advantage with a tendency towards acceptance and consumer preference. It is necessary to provide more than just a sentimental and culinary value to the different varieties of artisan cheeses made throughout Mexico. Although consumers appreciate artisan cheeses, they run the risk of disappearing if the intergenerational knowledge transfer is lost.

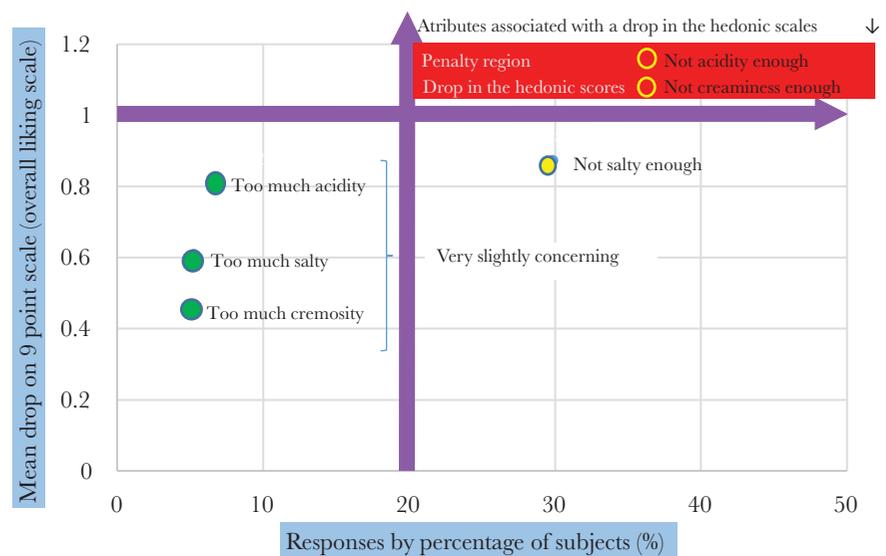


Figure 4. Mean drop analysis as a function of the percentage of subjects

Table 3. Preference test in the consumption of cheeses.

Cheese treatments	Rank sum	Level of preference
CP	47a*	3rd
STA. TERESA	38ab	2nd
MONTERO	29b	1st=most preferred

*Results with different letters are significantly different ($p < 0.05$).

Pomeón *et al.* (2011) carried out a strategic analysis about the production and consumption circuit of artisan cream cheese from Chiapas. These are two of the main threats they detected: conflict of interest between cheese makers and the Secretaría de Salud; and the statement that the introduction of pasteurization and other similar processes would cause these cheeses to lose their identity (Pomeón *et al.*, 2011). This study did not intend to compete with raw milk cheeses, but to develop technology to supply markets that are currently not being served; these cheeses can be included within the local identity.

A good product requires to build an identity that combines the know-how of micro-enterprises (better sensory characteristics) with the knowledge of the institutions (better safety). Buchin *et al.* (1998) compared raw milk and pasteurized milk cheeses; both of them were inoculated with the same amount of starter culture—which is constituted by lactic acid bacteria. Morbier raw milk cheeses from France had a more intense flavor than pasteurized milk cheeses. This difference was the result of the volatile compound profiles. Raw milk cheeses had higher amounts of alcohols, fatty acids, and sulfur compounds than pasteurized milk cheeses—which had higher amounts of ketones.

CONCLUSIONS

Consumers prefer raw milk cheese over pasteurized milk cheese. Less acidity and less creaminess were associated with a low acceptance rating. Further studies are required to establish if other sensory attributes—in addition to those mentioned in this study— could explain the greater preference and global acceptance of raw milk cheeses.

The results of this work show that using pasteurized milk to prepare double cream cheese is feasible. There were no significant sensory differences in taste, aroma, and texture between raw milk and pasteurized milk cheeses, although the consumers preferred artisan cheeses. Determining if there are other sensory attributes—in addition to those that were the subject of this study— will help to explain the greater preference and global acceptance of raw milk cheeses.

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Comparative study of different drying methods regard to the phenols and flavonoids content of dried *Citrus aurantium* L. leaves

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ABSTRACT

Objective: To determine the effects of different thermal drying technologies on the total phenol and flavonoid contents (TPC) and total flavonoids (TFC) in sour orange (*Citrus aurantium* L.) leaves.

Design/methodology/approach: Solar drying was carried out in outdoor sunny conditions using two direct solar dryers; one with natural convection, the other with forced convection. The total phenol and flavonoid contents in gallic acid equivalents (GAE) and quercetin (Q), respectively, of ethanolic extracts of *C. aurantium* were assessed with spectrophotometric techniques.

Results: The results demonstrated maximum phenol values for the direct natural convection solar dryer (161.4 mg EAG/g MS) and minimum values for shade drying (61.43 mg EAG/g MS). As for flavonoids, the highest values were obtained in the direct forced convection solar dryer (32.22 ± 1.6 mg EQ/g MS), while the lowest was registered in the open air sun (11.72 mg EQ/g MS).

Conclusions: Direct solar dryers are technologies effective for maintaining the phenols and total flavonoids content in dried leaves of *C. aurantium*.

Keywords: *Citrus aurantium*, sour orange, flavonoids, phenols, solar drying.

INTRODUCTION

Plants are both agrochemical and food additive sources, as well as pharmaceuticals, yet, their adequate use constitutes a challenge for current pharmacology. Worldwide, there is a wide variety of plants with no researched pharmacological effects which could be crucial in diseases treatment (Ghasemzadeh *et al.*, 2016).



The properties in the leaves of the sour orange tree (*Citrus aurantium* L.), for example, have been scarcely studied; however, their availability year-round is greater than that of the fruits and can even be waste materials from pruning processes, which facilitates using them in obtaining extracts with pharmacological purposes.

Citrus aurantium L. belongs to the *Citrus* genus, which inhabits tropical and subtropical areas (Arendse *et al.*, 2014). It is a thorny small tree of green color; its very fragrant green leaves have an oval limb of complete edges; of white and highly fragrant flowers; spherical fruit with a thick-skinned peel, that protects the edible and juicy parts (Capecka *et al.*, 2005). It is widely used in southeastern Mexico because of its anticarcinogenic, anxiolytic, antiobesity, antibacterial, antioxidant, pesticidal, and antidiabetic properties (Aiello *et al.*, 2020).

Among the bioactive compounds in *Citrus* are the flavonoids, present in thousands of different structural forms in a variety of plants, which provide citrus with antioxidant activity (Pellati *et al.*, 2002, Suryawanshi, 2011). The major flavonoids found in citrus include flavanones (hesperetin and naringenin), polymethoxylated flavones (PMF) (nobiletin and tangeretin) (Horowitz and Gentili, 1977), and glycosylated flavanones such as naringin. At the same time, Naringin is widely used in traditional medicine to have anti-apoptotic, anti-osteoporosis, anti-ulcer, antioxidant, anti-inflammatory, and anticancer properties (Cui *et al.*, 2012).

Remedies made from bitter orange leaves are often consumed in infusions, either from the dried or fresh leaves; yet, drying, extraction, storage, and processing have a significant impact on the quality of the plants (Kunle *et al.*, 2012). That is why they should be dried under specific conditions, to preserve their nutritional quality and avoid contamination, deterioration and protect their phytochemical efficiency (Ghasemzadeh *et al.*, 2016).

Worldwide, several methods are used for plant drying; from those using conventional fuels, such as gas or electricity, to open-air solar drying or via solar thermal technologies. The latter has great advantages over the others, both from an environmental perspective and on the final quality of the dried products (Fonseca *et al.*, 2002).

Given the potential therapeutic value of *Citrus aurantium* and the scarce knowledge about the impact of different drying methods, including solar drying, on the phenolic compounds, the objective of this research was to determine the effect of different thermal drying technologies on the total phenols and flavonoids content of sour orange leaves; to understand the changes during drying for obtaining the required quality characteristics for consumption.

MATERIALS AND METHODS

Samples

The studied vegetal material was collected in a rural plantation located in the municipality of Huimanguillo, Tabasco, Mexico, in November 2016. The sampling site locating at 18° 01' 32.0" N and 93° 39' 05.7" W, 10 m a.s.l. All the material was washed by jet water to remove external impurities, and the leaves were grouped following the following characteristics: maturity, color, and freshness (Castillo *et al.*, 2017). Plant identification was performed by Dr. Eustolia García López of the Colegio de Postgraduados, H. Cárdenas, Tabasco, and a sample was deposited in the departmental herbarium.

Drying

The fresh leaves of *C. aurantium* (20 g) were dried in the facilities of the Solar Drying Laboratory at the Facultad de Ingeniería, Universidad Autónoma de Campeche. The following methods were used: drying in shade, drying in direct solar dryers, open-air drying, and drying in an electric oven with controlled temperature.

- a) Shade drying: It took place during 21 d at environmental temperature, in a dark and well-ventilated room, at an average temperature of 30 °C and 60% relative humidity.
- b) Direct solar drying: Solar drying was performed in two prototype non-commercial direct solar cabinet dryers (Cuernavaca, Morelos, Mexico); one operating with forced convection and the other with natural convection. Both dryers, which have holes to extract humid air, were designed at the Instituto de Energías Renovables (IER) at the Universidad Nacional Autónoma de México (UNAM) and manufactured by Manufacturas Plásticas de Cuernavaca S.A de C.V. in Cuernavaca, Morelos.
- c) Open-air drying. In this process, *C. aurantium* leaves were placed on a suitable piece of plastic. The leaves were placed without overlapping and left until sunset. If by then these were not completely dry, they were collected and stored indoors. This method was daily repeated until the drying process was complete. The total duration was 31.3 h (hours of sun exposure).
- d) Oven drying at controlled temperature: done in a conventional dryer consisting of a non-convective electric oven, Riossa brand (Morelos, Mexico), capable of a 50 °C to 220 °C temperature range, with a ± 2 °C sensitivity. Drying was carried out at 55 °C.

During the drying process, the following variables were assessed: internal temperature, weight, and size of the samples, solar irradiance, relative humidity, air temperature (Castillo *et al.*, 2017). The dried samples were then stored at -20 °C.

Moisture

The moisture was determined before and after each drying method, using two thermobalances with a moisture analyzer, brand Ohaus MB45 (New Jersey, USA), with $\pm 0.01\%/0.001$ g accuracy. The evaluations were performed on 1 g of sample, following the procedure by Castillo *et al.* (2017).

Once the desired moisture values were obtained, extracts were prepared in two stages. A grinding stage of the materials in an industrial mill, Thomas-Wiley Laboratory Mill, Thomas Scientific model (New Jersey, USA), and another of extraction by maceration. A successive cold maceration with 85% ethanol was carried out at a rate of 1 g of plant material for every 10 mL of solvent. They were previously covered with aluminum foil and placed in an IKA shaking equipment, model KS 260 Basic (Wilmington, USA), for 7 d at environmental temperature for subsequent filtration in a Büchner funnel with filter paper attached to its bottom. Then the Büchner funnel was placed on a Kitasato flask connected to a vacuum pump (Stage Vacuum Pump Model RS-4, Zhejiang, China), for solvent removal. Subsequently, the solvent was removed by concentrating with a Rotavapor, Model RE52, Lanphan RE-52 (Zhengzhou, Henan, China) at 40 °C.

Total phenols quantification

The total phenolic content in the ethanolic extracts of *C. aurantium* was assessed following the Folin-Ciocalteu assay (Fonseca *et al.*, 2002). Four hundred μL of the extract (1 mg/mL) were introduced into test tubes. Then, 1 mL of Folin-Ciocalteu reagent (1:10) was added, followed by 1 mL of sodium carbonate (20%). Subsequently, the test tubes were allowed to stand in the dark for 90 min at environmental temperature, before measuring their absorbance using a UV-Vis spectrophotometer (Velab brand, Model VE-5600UV, EU) at 760 nm.

The calibration curve was prepared using aqueous gallic acid with 0.02 mg/mL to 0.1 mg/mL, with an r^2 of 0.999. The results were expressed in milligram gallic acid equivalents per gram of dry material (mgEAG/gMS). Standard gallic acid monohydrate (>99%; CAS registry number 5995-86-8) was used; the coefficient of variation was between 2% to 5% in all cases. Each sample was run in triplicate.

Quantification of total flavonoids

Total flavonoid content was measured using the aluminum trichloride colorimetric method described by Liu and Zhu (2007) with some modifications. One mL of solution containing flavonoids was mixed with a NaNO_2 solution (0.3 mL, 5%); after 6 min of incubation at 25 °C, 0.3 mL of AlCl_3 solution (2%) was added. The mixture was stirred and allowed to stand for 6 min. Subsequently, 1 M NaOH (0.2 mL) solution was added to each extract and incubated for 10 min at environmental temperature. The absorbance was determined at 510 nm against a blank. Quercetin dihydrate (>98%, CAS registry number 6151-25-3) was used as a standard to prepare the calibration curve ($r^2=0.9924$). The results were expressed as milligram quercetin equivalents per gram of dry mass (mgEQ/gDM).

The hydroalcoholic extract sample was prepared at 0.2 mg/mL from each sample dissolved in 70% ethanol. and One mL of reactive grade methanol was added to 1 mL of the sample. All samples were done in triplicate.

Statistical analysis

The Statistical analysis was performed using the OriginPro 2018 software. Data are presented as mean \pm standard deviation (SD) and a $P < 0.05$ was considered as statistically significant. An analysis of variance (one-way ANOVA) was used to compare the means of the phenol and flavonoid content between the different drying methods for multiple comparisons. Holm Sidak's=0.05 test was used to determine significant differences between two groups.

RESULTS AND DISCUSSION

Drying time and moisture content

Table 1 shows that the drying time varied from one method to another, solar drying with natural convection being the shortest (5 h), followed by solar drying with forced convection (6.7 h), and drying in an electric oven at 55 °C (7.7 h). In contrast, the traditional methods of open-air and shade drying showed longer drying durations (31.3 h and 504 h, respectively).

Table 1. Drying time and moisture percentage content of different drying methods in *C. aurantium* leaves.

Drying methods	Drying time (h)	Moisture (X±SD) (%)	
		Initial	Final
Hot air oven drying (55 °C)	7.7	80.68±1.10	8.57±0.30
Direct natural convection solar dryers	5.0	83.67±1.20	8.85±0.50
Direct forced convection solar dryers	6.7	80.73±1.10	8.68±0.16
Open sun drying	31.3	81.20±0.35	9.11±0.18
Shade drying	504 (21 days)	80.50±1.15	12.00±0.20

X: average; SD: standard deviation

No significant differences were observed between drying methods for the moisture content ($P < 0.001$). The highest moisture loss was obtained in the forced convection and natural convection methods (89% each) and the lowest in shade drying (85%). The moisture values achieved in this study were between 8% and 12%, which corresponds to previously established values (9% to 10%) for the commercialization of dried plants such as wormwood, marigold, thyme, sage, oregano, lavender, mint, chili, among others (Ceballos and Jimenez, 2012).

Phenols and flavonoids quantification

Figure 1 shows the concentration in mgEAG/gDM for each of the drying methods. It is observed that phenolic compounds are present in each of them, but with significant differences according to the analysis of variance ($P < 0.001$).

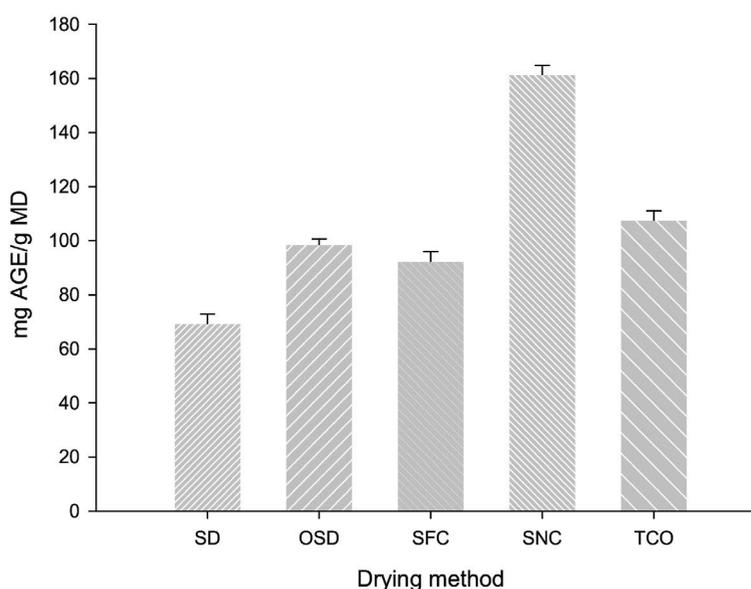


Figure 1. Effect of different drying methods on total phenolic content. Data represent means ± standard deviation of triplicate measurements. $P < 0.001$. S- Shade drying; CA- Open-air drying; SCF- Solar drying with forced convection; SCN- Solar drying with natural convection; HTC- Oven drying with controlled temperature.

The highest phenol content was recorded in SCN (161.40 ± 3.38 mgEAG/gMS) and the lowest content in shade drying, with 69.43 ± 3.48 mgEGA/gMS. It is important to note that shade drying was not only the method that yielded the lowest phenol content, but also the one that showed the longest drying time. Regarding the flavonoid content, the obtained results show differences ($P < 0.05$) between the different drying methods, with a minimum value of 11.72 mgEQ/gMS in open-air drying and a maximum of 32.23 mgEQ/gMS in SCF (Figure 2).

From the obtained results and those published in previous studies, it is to be noted that the observed decrease in the total phenol content in shade drying concurs with Ghasemzadeh *et al.* (2016). This could be attributed to the degradation caused by the Polyphenol Oxidase (PPO) enzyme because the sample's drying process was completed at environmental temperature for 21 d. However, in previous studies, drying lemon, oregano, and mint at temperatures between 25 °C and 32 °C for 10 days, both significant increases and decreases in antioxidant activity were observed, which was related to the phenol content (Capecka *et al.*, 2005).

Likewise, Norhidayah *et al.* (2013) reported that freeze-drying of ginger (*Zingiber officinale* Rosc.) at -40 °C, showed lower phenol content ($P < 0.05$) compared to cabinet drying at a 60 °C temperature, but not in the flavonoids, which, although higher in cabinet drying, were not significant. However, for blackberry (*Morus alba* L.) leaves drying, no differences were found in different drying methods (Katsube *et al.*, 2009). Whereas, for Chinese chasteberry (*Vitex Negundo* Linn), hot air oven drying was not the best method to preserve antioxidant compounds (Rabeta and Vithyia, 2013).

Enzyme degradation and loss of antioxidant activity, related to the decrease of total phenols, have also been reported due to sun drying (Chan *et al.*, 2009). Therefore, it is

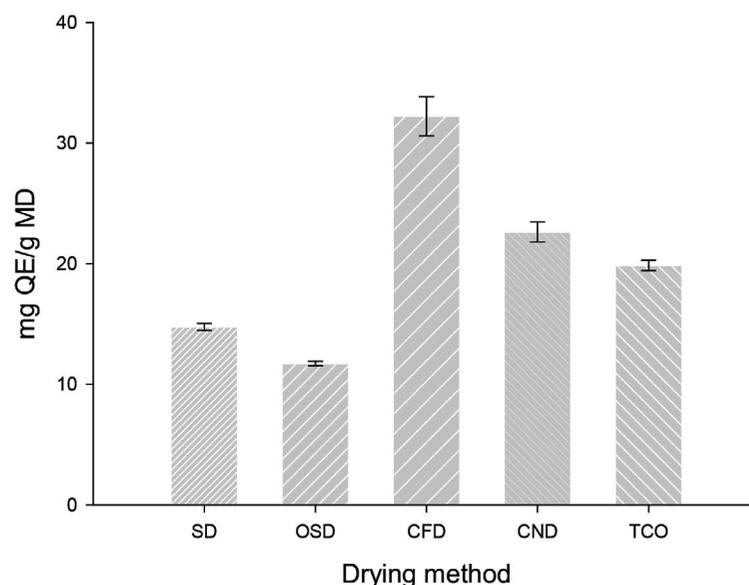


Figure 2. Effect of different drying methods on the total flavonoids content. Data represent means \pm standard deviation from triplicate measurements. $P < 0.001$. S- Shade drying; CA- Open-air drying, SCF-Solar dryer with forced convection, SCN- Solar dryer with natural convection; HTC- Oven drying with controlled temperature.

important to examine the correlation between the content of certain constituents and their biological activity (Ghasemzadeh *et al.*, 2016). Such information could help researchers to establish suitable conditions and techniques to improve these featured compounds. Therefore, it is important to understand how they vary in the *C. aurantium* case with each drying method to predict which would be better.

This is the first study that evaluates the variation of the total phenols and flavonoids content of *C. aurantium* leaves by different drying methods. The results indicate that the solar convection drying method, either natural or forced, preserves the highest content of total phenols and flavonoids, being a suitable method for drying and preserving bioactive compounds of this plant. Also, it is an environmentally friendly method, which contributes to maintaining the pharmacological properties by preserving their secondary metabolites.

The results here are promising and will contribute to broadening the scientific knowledge on the extracts of sour orange leaves, due to their content of phenolic compounds. However, further studies on the chemical composition of these extracts and their relationship with antioxidant activity should follow.

The temperature-controlled electric oven drying method showed values of the pharmacological properties of *C. aurantium*, which are intermediate between those of direct solar drying and the shade and open-air methods. Taking into account this result and the requirement of electrical energy for its operation, which also comes mostly from fossil resources, the viability of using the solar resource for drying medicinal plants is reinforced.

Further analysis of this plant and its effects on humans, for the treatment of cardiac and nervous system disorders, which could be due to the high content of phenolic compounds, would be suitable. Finally, it is desired that the present study serves as a basis for further research aimed at the complete characterization of sour orange leaves.

CONCLUSIONS

These results demonstrated that solar drying by direct dryers is superior compared to other drying methods for preserving the total phenols and flavonoids content in dried *C. aurantium* leaves. The maximum values for phenols were obtained with the direct solar dryer with the natural convection method, while shade drying was the least favorable method. As for flavonoids, the highest values were obtained in the direct solar dryer with forced convection, followed by the solar dryer with natural convection; the lowest values were observed in the open-air drying method.

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Evaluation of Suitable Lands for *Elaeis guineensis* Jacq. Using Geographic Information Systems in the Sierra Region, Tabasco, Mexico

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ABSTRACT

Objective: The objective of this study was to evaluate the edaphic suitability of *Elaeis guineensis* at a semi-detailed scale (1:50000), in the Plains and Terraces of Tabasco, Mexico (PTT).

Design/Methodology/Approach: The edaphic requirements consisted of a semi-detailed soil map. The evaluation of the edaphic suitability was based on the agroecological zoning methodology proposed by FAO and the final 1:50000 scale map was developed using the algebra mapping tool with the ArcGis[®] Geographic Information System.

Results: The Gleysols, Fluvisols, Luvisols, and Lixisols groups dominate the PTT, with 65.1, 16.2, 6.3, and 4%, respectively. Most of the soils (79.95%) have zero suitability for oil palm.

Study Limitations/Implications: Oil palm is the eighth most important perennial crop (over cocoa) for the economy of southeastern Mexico. In this scenario, the expansion of plantations in the country is a possibility; therefore, complying with internal regulations and carry is important.

Findings/Conclusions: The internal drainage of Gleysols soils limits the establishment of *E. guineensis* plantations in the plains and terraces of Tabasco, Mexico.

Keywords: Oil palm, Precision Agriculture, Gleysols, Lixisols, Luvisols.

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INTRODUCTION

Palm oil is being increasingly used on a large scale, driven by the economic development of countries such as India and China (Murphy, 2014). Palm oil is an enormous world industry with an annual value that exceeds US\$50 billion (Paterson and Lima, 2017). Most of the crude palm oil is used to produce food and biodiesel, while palm kernel oil is used



to manufacture detergents, make-up, plastics, and chemical products (Rival, 2017). The large-scale oil palm (*E. guineensis* Jacq.) cultivation has transformed the tropical regions, the lives of people, and the profits of the palm industry. Its high yield, easy establishment, and low costs make this a very profitable crop (Dislich *et al.*, 2017). For example, oil palm is the eight most important perennial crop in southeastern Mexico, surpassing cacao (SIAP, 2021). However, during the last ten years, the fresh fruit bunches (RFF) yield has not increased in Mexico: it remained at 13 t ha⁻¹ from 2009 to 2019 (SIAP, 2021). On the contrary, the main producing countries in southeastern Asia report 17 t ha⁻¹ RFF yields (FAO, 2021). In response to the growing domestic demands and to the high cost of palm oil imports—for example, in 2019, Mexico imported 478,900 t of palm oil at a cost of US \$ 265 million (SIAVI, 2021)—, some South American governments are encouraging national and foreign investors to plant more oil palms (FEMEXPALMA, 2020). Therefore, in view of the potential expansion of these plantations in the country, complying with the domestic regulations and carrying out a sustainable crop management is important to increase local production and to reduce oil imports (Brindis-Santos *et al.*, 2020b).

Evaluating suitable lands availability is necessary to establish crops and to obtain an optimal yield; this information is essential for the development of government policies and for investors (Rhebergen *et al.*, 2016). In this regard, Mexico has made efforts to evaluate land suitability for several crops that have an economic importance for the agricultural sector (Aguilar *et al.*, 2013; González and Hernández, 2016; Ramírez-Sánchez *et al.*, 2019). Specific studies about the establishment of forest species (Aguilar-Rodríguez *et al.*, 2017) and perennial crops (Rivera-Hernández *et al.*, 2012), as well as about alternatives for the production of biofuels (González *et al.*, 2015), have been carried out in Tabasco. Aceves-Navarro *et al.* (2008) zoned areas with high production potential for the cultivation of *E. guineensis*; however, their analysis was carried out more than ten years ago and the evaluation scale (1:250000) was made at reconnaissance level. Consequently, the objective of this research was to carry out a semi-detailed scale (1:50000) evaluation of the edaphic suitability of *Elaeis guineensis* Jacq., in the plains and terraces of Tabasco (PTT), in order to determine potential areas for this crop.

MATERIAL AND METHODS

Study Area. The PTT are located at 17° 31' 57" and 17° 47' 44" N and 92° 42' 55" and 92° 54' 22" W, in a geographical area of 138,143 ha, located in the Llanura Costera del Golfo Sur geomorphological province, in the municipalities of Jalapa and Tacotalpa, in southeastern Tabasco, Mexico (INEGI, 2016). The study zone has three geomorphological landscapes: active alluvial plains (PFA), detrital coastal terraces (TCD), and soft sloping volcanic hills (LSIV). The prevailing landscape is PFA; it covers 82% of the total area; it is a flat area located in the lowest topographic position, over fluvial sediments and marshes from the Quaternary period (Holocene). The remaining 18% of the region consists of terraces and low hills landscapes, over detrital sedimentary rocks and andesite rifts from the Tertiary (Paleocene) and Quaternary (Pleistocene) periods, at an altitude of 7-70 m (Zavala-Cruz *et al.*, 2016). The climates are humid-warm—with abundant rainfall in summer (Am (f))— and humid-warm—with rainfall all year round

(Af(m)). The average temperature is 25 °C and the annual average precipitation is 2,500-4,000 mm (García, 2004).

***Elaeis guineensis* Suitability Requirements (Climate, Topography, and Soil).** Seven edaphic properties of soil were analyzed to determine land suitability: pH, depth, texture, cation exchange capacity (CIC), electric conductivity (CE), base saturation percentage (PSB), and drainage. There are four significant climate and relief variables: temperature, precipitation, height above sea level, and slope. The requirements of the values used to define suitability ranges were based on Ecocrop (2014) and Corley and Tinker (2016); the types of drainage were based on Cuanalo (1990) and Parmananthan (2003). The pH, CIC, CE, and PSB ranges were based on NOM-021-RECNAT (2000) and IUSS Working Group WRB (2015). The lower limits were defined taking into account the information provided by Corley and Tinker (2016), who pointed out that 80% of the *E. guineensis* roots can be found at a depth of 0-50 cm.

Edaphic, Climatic, and Relief Data. The soil units' map (1:50000 scale) and the physical and chemical data were obtained from Brindis-Santos *et al.* (2020a). The precipitation and temperature data were obtained from the cartography of INEGI (2021) (1:250,000 scale); the format was changed from vectors to raster. The relief and slope map was generated based on a LIDAR model, using the ArcGis[®] 9.2 software (ESRI, 2004).

Zonification of the *Elaeis guineensis* Edaphic Suitability

The *E. guineensis* edaphic suitability was evaluated based on FAO's Agro-Ecological Zoning guidelines (1997). The suitability types were grouped in the following categories: 1) high suitability (*i.e.*, the values of the variables found in the optimal range); 2) medium suitability (which gathers the values of the variables found in the absolute suitability range); and 3) zero suitability (which is defined by the variables whose values are lower than those reported as absolute minimum by Aguilar-Rodríguez *et al.* (2017)). Using the map algebra tool—which executes all the spatial analysis operators and functions, adding two or more maps to obtain a polygon mix of the original maps' variables (Rivera-Hernández *et al.*, 2012)—, an edaphic suitability map of *E. guineensis* was generated (1:50000 scale).

RESULTS AND DISCUSSION

Table 1 shows the variables required to determine the suitability of *E. guineensis*: soil, climate, and relief.

Physical and Chemical Characteristic of the Soils in the Sierra Region of Tabasco

Soils from the Gleysols (GL), Fluvisols (FL), Luvisols (LV), and Lixisols (LX) groups prevail in 65.1, 16.2, 6.3, and 4%, respectively, of the Plains and Terraces of Tabasco (PTT)—which were the subject of this study. GL were formed in zones with reducing conditions; FL, in natural levees and meander banks; and LV and LX, in low hills and terraces. The following representative soil units were found: GL- st.eu- lo.oh (50%), GL-hi.oy.eu- cen. lop.hu (12.7%), FL- fve.gl.eu- hu.lo (12.5%), LV-gl- ct.hu-je.lo.qv (3.6%), and LX-ab.cr-ct. df.cen.lop.je.pn (3.8%) (Figure 1).

Table 1. *Elaeis guineensis* Jacq. edaphic requirements in Tabasco, Mexico (Ac=clay; An=sand; F=loam; L=silt).

Factor	Variable	Aptitude		
		High	Average	None
Weather	Type: Tropical	X	X	
	Mean Temperature (°C)	20-35 ¹	12-20 y 35-38 1	<12 y >38 ¹
	Precipitation (mm)	1500-3000 ¹	1000-1500 y 3000-8000 1,2	<1000 y >8000 1
Soil characteristics	pH	4.5-7.5 1,3	3.2-4.5 y 7.5-8 1,3,4	<3.2 y >8 1,3,4
	Depth (m)	>1.5 ¹	0.5-1.5 ¹	<0.5 ¹
	Texture *	L, LC, LSt, LCSt 1,2,3	LCSd, SdL, SdL, CSd ²	Sd, C, St, gravel 1,2,3
	Cation-exchange capacity (Cmol (+) (kg ⁻¹))	>24	14-24 ⁷	<14
	Salinity by electric conductivity (dS/m ⁻¹)	<4 ¹	<4 ¹	>4 ¹²
	Exchangeable bases, %	>50	20-50	<20
	Drainage	Good to average 1,2	Good to excessive, poor, slow, easy to drain 1,2,4	Poor, hard to drain ⁴
Topographical relief	Altitude (meters above sea level)	0-500 ⁵	500-1300 ¹	>1300
	Slope	<12 2,4	12-23 2,4	>23 ⁴

GL and FL have slow permeability (in horizon A) and very slow permeability (in underlying horizons C); they have loamy and loamy clayey textures (FL) and clayey texture (GL). Their pH ranges from moderately acid to neutral. A and C horizons have contrasting organic matter (OM) contents (rich to very poor); nutrients range from high to low (both with assimilated P and CIC), with over 50% with PSB. These highly-fertile soils are suitable for intensive agriculture; however, water excess causes anaerobiosis and oxide-reduction processes in these soils (Palma-López *et al.*, 2017; Brindis-Santos *et al.*, 2020a) (Figure 1). LV and LX share the following characteristics: loamy clay, silty clay, and clayey texture; Bt horizon formed through the accumulation of clay; good to imperfect drainage; rich to poor organic matter content; high to low nutrients with P; cation exchange capacity (CIC); base saturation percentage (PSB); and moderately to strongly acid pH. These conditions limit agricultural capacity (Zavala-Cruz *et al.*, 2016).

Climatic and Relief Characteristics of Tabasco's Sierra Region. Am(f) climate prevails in the center, north, and northeast; while Af(m) prevails in the South and Southeast of the PTT; the average temperature is 25 °C. Fifty-seven percent of the area is suitable for *E. guineensis*, as a result of the average (2000-3000 mm) and moderate (43%; 3000-4000 mm) annual precipitation in southern Tacotalpa. These findings match those reported by Paramanathan (2003), who argues that *E. guineensis* grows better with a 2000-2500 mm year⁻¹ precipitation, evenly distributed throughout the year (no month with <100 mm of rain). Relief and slope intervals have the following characteristics: a) flat to gentle, 0-12% (97% of the PTT); b) moderate to steep, 12-24% (2.5% of the PTT), to the South and Northeast; and c) very steep, >25% (0.07% of the PTT), to the Southwest. Altitude ranges from 7 to 70 masl (Figure 2).

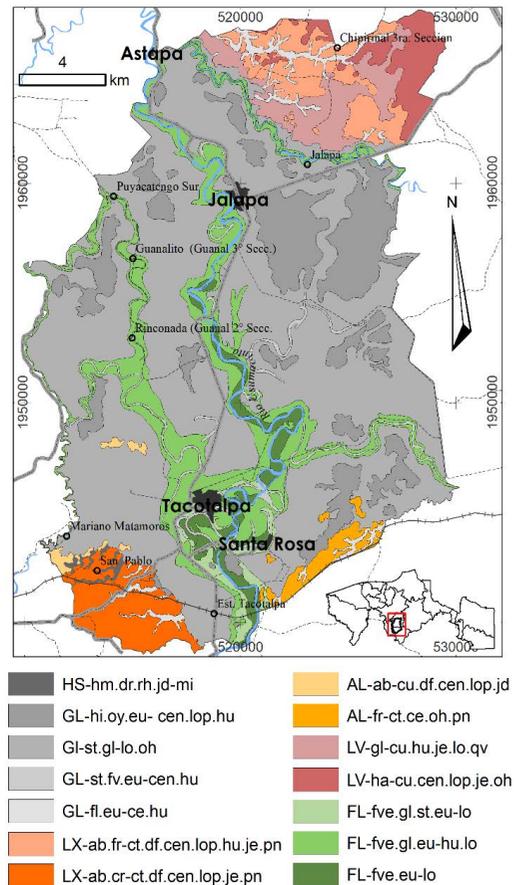


Figure 1. Soil survey in La Sierra, Tabasco, Mexico (Brindis-Santos *et al.*, 2020a). meaning of abbreviations: GL-st.fl.eu.-ce.hu/ Eutric Fluvic Stagnic Gleysol (Clayic, Humic), FL-fve.gl.st.eu-lo/ Eutric Stagnic Gleyic Pantofluvic Fluvisol (Loamic), FL-fve.gl.eu.-hu.lo/ Eutric Gleyic Pantofluvic Fluvisol (Humic, Loamic), FL-fve.eu-lo/ Eutric Pantofluvic Fluvisol (Loamic), GL-st.eu-lo.oh/Eutric Stagnic Gleysol (Loamic, Ochric), GL-hi.oy.eu.-cen.lop.hu/ Eutric Oxigleyic Histic Gleysol (Endoclayic, Epiloamic, Humic), GL-fv.eu.-ce.hu/Eutric Fluvic Gleysol (Clayic, Humic) LV-gl-ct.hu.je.lo.qv/ Gleyic Luvisol (Cutanic, Humic, Hypereutric, Loamic, Protovertic), LX-ab.fr-ct.df.cen.lop.hu.je.pn/ Ferric Abruptic Lixisol (Cutanic, Differentic, Endoclayic, Epiloamic, Humic, Hypereutric, Profondic) AL-fe-ct.ce.oh.pn/ Ferric Alisol (Cutanic, Clayic, Ochric, Profondic), LV-ha-cu.cen.lop.je.oh/ Haplic Luvisol (Cutanic, Endoclayic, Epiloamic, Hipereutric, Ochric), HS-hm.dr.rh.jd-mi/Hyperdystric Rheic Drainic Hemic Histosol (Mineralic), LX-ap.cr-ct.df.cen.lop.je.pn/ Chromic Abruptic Lixisol (Cutanic, Differentic, Endoclayic, Epiloamic, Hipereutric, Profondic), AL-ab-cu.df.cen.lop.jd/ Abruptic Alisol (Cutanic, Differentic, Endoclayic, Epiloamic, Hyperdistric).

Edaphic suitability for *Elaeis guineensis* in the Sierra Region of Tabasco. The PTT has 1532.26 ha that are ideal for oil palms (3.75% of the total surface area). Lands with at least one limiting condition comprised 7875.09 ha (19.29%). It has been determined that this area is non-suitable for the production of *E. guineensis*, as a result of its CIC, interchangeable bases, drainage, and topography. This area comprises 31 414.50 ha. The FL-fve.eu.lo unit has high suitability for *E. guineensis* (Figure 3): it has a deep soil (>100 cm); its surface layer (0-50 cm) has loamy, silty loam, and clayey loamy silt textures; it has a good internal drainage at a depth of 100 cm; it does not have salinity problems; and it has over 50% PSB.

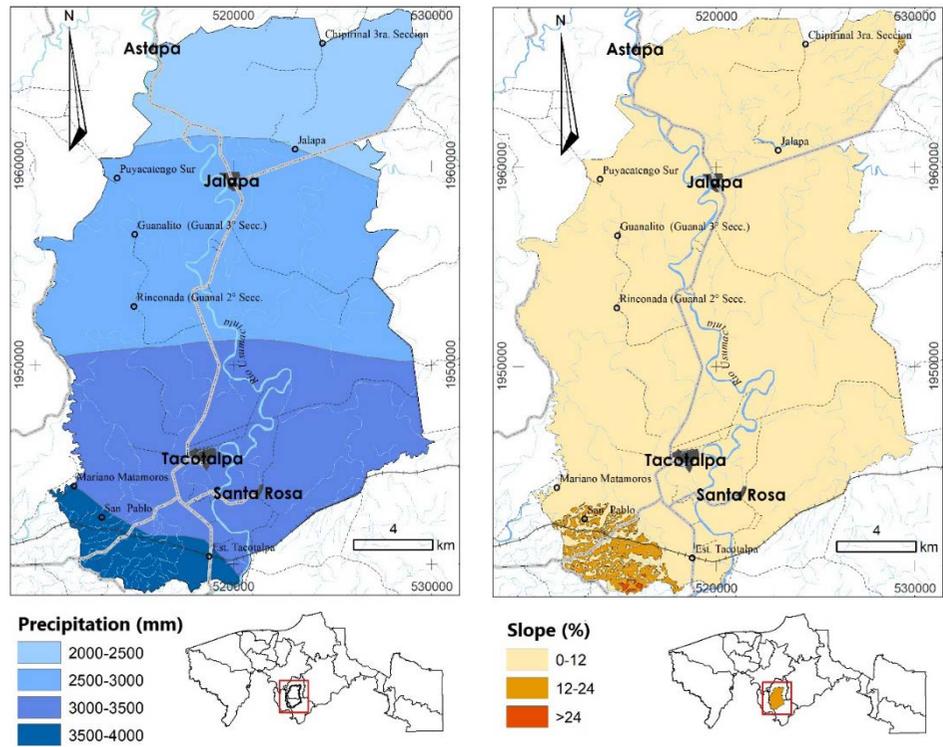


Figure 2. Precipitation and topography in La Sierra, Tabasco, Mexico.

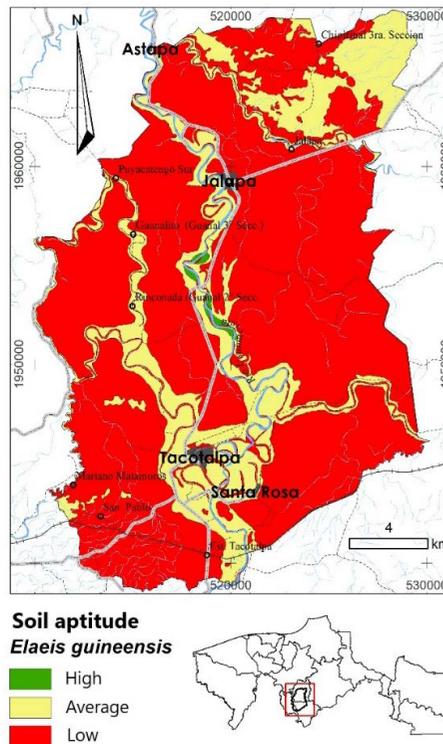


Figure 3. Soil aptitude for *Elaeis guineensis* in La Sierra región, Tabasco, Mexico.

These properties match those of other soils suitable for *E. guineensis* in other tropical regions (Paramanathan, 2003; Corley and Tinker, 2016). Meanwhile, Rhebergen *et al.* (2016) maintain that the soil's physical properties—which control the soil's capacity to provide water for the crops—are more important than its chemical properties—which cannot be easily changed through handling. The soils in the study area have variables with optimum values to expand the surface in which the crop is grown. Low fertility is the edaphic variable that causes an average suitability for *E. guineensis* in the PTT's Lixisols (except in Luvisols with poor drainage). Nutrient availability is not a major limiting factor for the evaluation of land suitability, since handling can be carried out adding nutrients and returning residues to the soil. However, correcting the poor to slow drainage that limits this perennial crop is a difficult task (Rhebergen *et al.*, 2016; Harahap *et al.*, 2018).

Gleysols (GL) with various qualifiers dominate relieves with low topographical position. This zone shows reducing conditions, a gley color pattern, and a 7-150 cm depth, as a result of the high-water table and the water stagnation associated with medium and fine textures (Brindis-Santos *et al.*, 2020a).

GLs with zero suitability for *E. guineensis* cover most of the study area surface (76.95% of the PTT); these soils show slow permeability in horizon A (which is associated with a clayey texture) and moderate permeability in underlying Cg horizons (as a result of the decrease in the clay content). The area has stagnic properties, as a consequence of its high-water table. These soil color reducing conditions can be found on the first 50 cm of depth—as seen in the dominance of grey and deep grey colors. According to their geographical position, these characteristics are divided into decantation basins, floodplains, and cumulative valleys (Zavala-Cruz *et al.*, 2016). However, this unfavorable physical soil conditions limit root growth and function—leading to plants with inadequate growth (Ogunkunle, 1993)—and, even when fertilizers are applied, they reduce nutrient and water absorption—inhibiting microbial growth (Goh *et al.*, 2016).

CONCLUSION

The poor internal drainage of Gleysols in most of the plains and terraces of Tabasco, Mexico, limits the establishment of *E. guineensis* in those areas. Land suitability in the study area is ideal (3.75%), medium (19.29%), and zero (76.95%). Climatic suitability ranges from medium-high to medium as a result of the relief (<70-masl heights and 30% slopes).

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Evaluation of chemical resistance inducers in maradol papaya against *Phytophthora nicotianae* var. *parasitica*

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ABSTRACT

Objective: To test the efficiency of four chemical resistance inducers on Maradol papaya to reduce *Phytophthora nicotianae* var. *parasitica* infections in rainfed crops at Chontalpa, Tabasco, Mexico.

Design/methodology/approach: Three doses of four resistance inducers were tested on 60-day-old papaya plants in a greenhouse with a randomized design, with four replications and 10 plants as experimental plots. Three days after the inducers' application inoculations with mycelium discs were made, there were negative and positive control treatments to evaluating their efficiency by applying Abbott's formula.

Results: The four chemical inducers for resistance (sodium silicate (SS), potassium silicate (PS), potassium phosphite (PF) and acibenzolar-s-methyl (ASM)) were statistically different from the control ($P < 0.0001^{**}$). The inducers SS 1%, PS 1%, FP 0.35% and ASM 0.1 mM showed higher effectiveness (81.2, 75.9, 74.7 and 74.0 %).

Study limitations/implications: The retained effective concentrations were tested in a single application, and their durability is unknown, so this point should be broadened. however, it may be an alternative for repeated use after transplanting.

Findings/conclusions: Optimal concentrations of SS, PS, FP, and AMS, that respond against *P. nicotianae* var. *parasitica* infections can reduce damages in rainfed crops.

Keywords: *Carica papaya*, root rot, *Phytophthora* n. var. *parasitica*.

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INTRODUCTION

Papaya root rot (*Carica papaya* L.) is caused by different *Phytophthora* species and is worldwide recognized as one of the most important diseases of this crop, as it can occur at any stage of development, from seedling to harvest (Vázquez *et al.*, 2010). This disease was first reported in 1916 in the Philippines, later in 1924 in Ceylon, and then after in other countries such as Malaysia, Hawaii, Australia, Brazil, Spain and Taiwan caused by *P. palmivora* (Ho, 1990). Nevertheless, in India, *P. nicotianae* var. *parasitic* was reported as the main causal agent of papaya fruit and root rot (Sukhada *et al.*, 2011).

In Mexico, in 1976, Garcia-Alvarez reported the presence of root and stem base rot caused by *P. parasitica* in Colima, Guerrero, and San Luis Potosi. Likewise, in Tabasco, Saldaña *et al.* (1986), informed the presence of root rot in papaya caused by *Phytophthora* sp. It was not until 2002 that *P. nicotianae* var. *parasitica* was confirmed as the causal agent of the papaya foot rot. Also, Fernández-Pavía *et al.* (2015) included reports of *Phytophthora parasitica* as the causal agent of root and stem base rot on a report of plant species diseases in Mexico (Fernández-Pavía *et al.*, 2015). Vázquez *et al.* (2010) reported the presence of *P. palmivora* at the Huasteca region as the responsible for stem and root rot caused in papaya; although this species is not found in Mexico and is included in the list of pathogens under phytosanitary surveillance (SENASICA, 2016).

In Tabasco, papaya is grown in rainfed conditions, on flatlands with no drainage which is considered as of low technology; using direct sowing or seedlings transplanting, manual weeding control, and a limited application of pest management and disease control (Guzmán *et al.*, 2009). Therefore, during rains, soils are easily saturated and waterlogged, which favors the root rot incidence by up to 11% (Saldaña, 2002).

Currently, there are control alternatives, such as the induction of systemic resistance, which involves activating plants' natural defense mechanisms through chemical inducers of resistance (Ozeretskoykaya and Vasyukova, 2002), by the pathogens themselves, other organisms, or environmental factors (Park and Paek, 2007). The papaya crop grown in rainfed conditions does not have sustainable management strategies to reduce root damage caused by *Phytophthora*, and its cultivation process demands using chemical products with low toxicity for mammals and is environmentally friendly. Under this premise, the present research was conducted to assess the effectiveness of chemical inducers for resistance to *Phytophthora nicotianae* var. *parasitica*, in transplanted (60 days) Maradol papaya.

MATERIALS AND METHODS

Isolation. From November to January, four papaya plantations were located in the municipalities of Cunduacán and Huimanguillo. From these municipalities comes the CPA1504 strain of *P. nicotianae* var. *parasitic*, isolated from Maradol papaya plants with root rot (Figure 1), proven as its causal agent at the Chontalpa subregion, Tabasco (Rodríguez, 2017).

Production of papaya seedlings in greenhouses. The production of papaya seedlings followed the techniques by Rodríguez and Cruz (2003). Plant emergence was carried out in germination trays with 72 wells, filled with a substrate (COSMOPEAT[®] COSMOCEL) sterilized (autoclave at 15 lb per half hour) two days before used and kept cold; one seed was sown in each well at a one-centimeter depth. The germination trays were protected from the sun, under partial shade, keeping a constant substrate humidity; seedling emergence began after three days.

In vivo test effectiveness of the resistance inducers. The experiment was conducted in a greenhouse, on Maradol papaya seedlings, 60 d of growth. Four chemical resistance inducers (RI) and three doses of each, were tested: sodium silicate ([Silicatos y Derivados S.A de C.V. A subsidiary of PQ Corporation, density of 1.5 kg/L] were 0.1, 0.75 and 0.5 % [SS]), potassium silicate ([Silicatos y Derivados S. A de C.V. A subsidiary

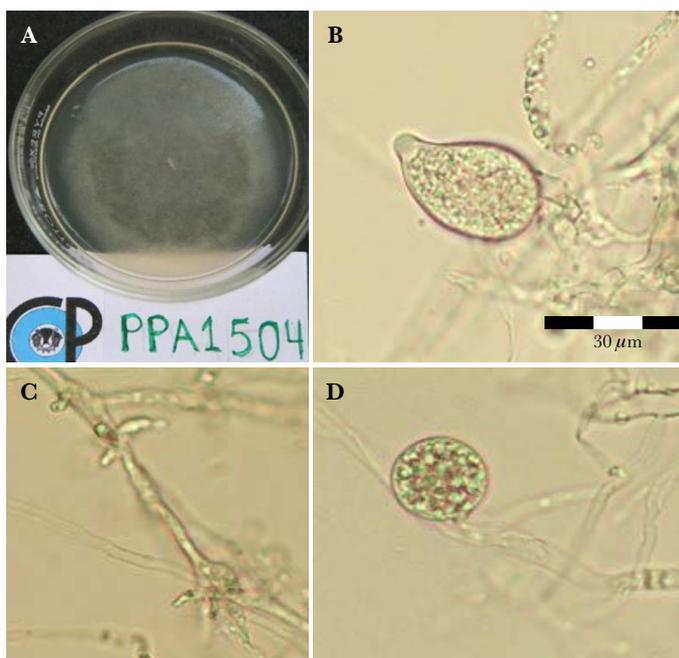


Figure 1. A) General aspect of the CPA1504 colony (*P. nicotianae* var. *parasitica*) 4 days after seeding. B) globose sporangium, limoniform with large papilla C) branched mycelium, turulose and swollen hyphae. D) terminal chlamydospore.

of PQ Corporation, Sil-MATRIX[®], 29.1 % a.i.] were 1.25, 1 and 0.75 % [SP]), potassium phosphite ([ALLETTE[®] WDG, Bayer: FOSETIL-Al 80%. GD] were 0.35, 0.30 and 0.25 % [FP]) and acibenzolar-s-methyl ([Syngenta, Actigard[®] 50 GS] were 1, 0.1 and 0.01 mM [AM]). In addition, a negative control treatment (papaya seedlings sprayed with sterile distilled water, without inoculation [Te]) and a positive control treatment (papaya seedlings artificially inoculated with *Phytophthora* sp. [Te-]) were included.

The experiment was established with 14 treatments, and four replications, in a randomized complete experimental block design, using sets of 15 papaya plants as the experimental units. The treatments were applied by spraying the plants with a manual atomizer, until they reached dew point, three days before the artificial inoculation. The treated plants, before inoculation, were placed in a humid chamber equipped with a Vitalys^{plus} ultrasonic humidifier. Mycelial discs from the CPA1504 pathogen strain were used for artificial inoculation (2.5 mm in diameter) at the base of the papaya seedling stems, placed in a humid chamber, maintaining the humidity for four consecutive days.

Study variables. Daily, for a 10 d period, the number of dead plants was counted and their survivorship and dead percentages, average days to death per treatment, and the efficacy of the inductor were calculated, using the Abbott formulas (1925) corrected by Rosenhein and Hoy (1987).

$$P_{corr} = \left(\frac{P_{sti} - P_{ste}}{1 - P_{ste}} \right)$$

Where: P_{corr} (corrected survival percentage), P_{sti} (survival percentage of treatment with inducer), P_{ste} (survival percentage of the positive control).

Statistical analysis. Survival percentage data were transformed to Arcosine \sqrt{Y} , prior to performing an ANOVA with the SAS V9 statistical software. Statistical significance was considered at a $p=0.05$ level. Tukey's test was used to separate means. The values shown are untransformed means. Covariance tests between survival and height, survival, and leaf area were also performed in the same statistical software.

RESULTS AND DISCUSSION

Table 1 shows that the four evaluated resistance inducers were effective and responded, as indicated by Walter *et al.* (2005), who states that, induced resistance being of broad-spectrum, should not always be expected to completely control the infection. Pointing out that the efficacy of chemical inducers on the causal agent of wheat blight disease varied from 20 to 85%. They emphasize that, when the pathogen is highly aggressive, as is the wheat blight case, resistance inducers such as SS and FP did not reduce the disease severity, as they had done in a previous cycle with a less aggressive version of the disease (Pagani *et al.*, 2014).

Overall, in this research, all treatments with chemical inducers for resistance were significantly effective (37-81% effectiveness) compared to the negative control, where

Table 1. Chemical resistance inducers effect on root rot in 'Maradol' papaya plants.

Treatment*		Survival %	Efficacy %	Days of dead plants
Inducers	Doses			
SS ₁	1.00% ^x	82.17 ab	81.26	8
SS ₂	0.75%	45.72 b	42.95	5
SS ₃	0.50%	71.79 ab	70.35	7
SP ₁	1.25% ^x	76.22 ab	75.01	8
SPi ₂	1%	77.12 ab	75.96	7
SPi ₃	0.75%	71.90 ab	70.47	6
FP ₁	0.35% ^x	75.92 ab	74.70	6
FP ₂	0.30%	53.38 b	51.00	7
FP ₃	0.25%	64.68 ab	62.88	7
AM ₁	1mM ^x	60.65 ab	58.64	7
AM ₂	0.1mM	75.28 ab	74.02	7
AM ₃	0.01mM	40.82 b	37.81	8
Te	0	95.15 a	94.90	0
Te-	0	0.00 c	0.00	5

*Inducers: SS (sodium silicate), PS (potassium silicate), PF (potassium phosphite), AM (acibenzolar-S-methyl), Te (uninoculated control and Te- (negative control). X percentage weight/volume ratio. Groups (a, b, ab and c), equal letters are not significantly different between treatments (Tukey $\alpha=0.05$). The arcsine transformation was applied to the survival percentage values. Untransformed means are shown.

100% of dead plants were quantified in a five-day period. The 1% sodium silicate treatment was the most effective (81%) and delayed the first dead plants eight days (Table 1). Likewise, the 1% dose of the three tested SS treatments was also the most effective against the root rot, suggesting that SS probably acts as a plant growth regulator which is effective at optimal doses, contrary to conventional fungicides, which increase their effectiveness as the dose increase. This result concurs with those by Li *et al.* (2012), who used 100 mM SS, on the postharvest to induce resistance in melon (*Cucumis melon*) to *Trichothecium roseum*, which reduced the diameter of lesions during storage. Additionally, Moscoso-Ramírez and Palou (2013) reported a SS 90% efficacy when used at 1000 mM to green and blue rots in ‘Valencia’ oranges; however, they did not recommend using it, due to the presence of phytotoxicity in the fruit rind during the postharvest at that dose. It is worth pointing out that, in the above studies, higher SS concentrations than those used in this study were used (1%).

Potassium silicate at a 1% concentration was the second-best chemical inducer of resistance in this research, with a 76% effectiveness. In the three tested concentrations, it had a stable effect on the plants, with a survival range of 71 to 77% (Figure 2) and an efficiency of 70 to 76%; although there were no significant differences among them. Likewise, this chemical inducer showed a mean number of dead plants up until the 7th day. Reports mention that PS affects *Sphaerotheca fuliginea* applied on cucumber plants at Culiacán, Sinaloa, with 9.45 g L⁻¹ and 18.90 g L⁻¹ doses, the latter with a 96.4% efficacy, although this dose showed phytotoxicity in the crop (Pérez-Angel *et al.*, 2010). Nevertheless, Ramírez *et al.* (2013) reported that neither SP nor FP had a detrimental effect on *P. cinamomi* applied on avocado, regarding other organic practices.

Papaya plants treated with FP had a 53 to 76% survival rate (Figure 2), the 0.35% dose being the best, with a 75% effectiveness. The 0.30% dose showed lower effectiveness, 51%. Whereas the minimum dose treatment showed 0.25% and 64.6% effectiveness, with an average death of the first plants within seven days.

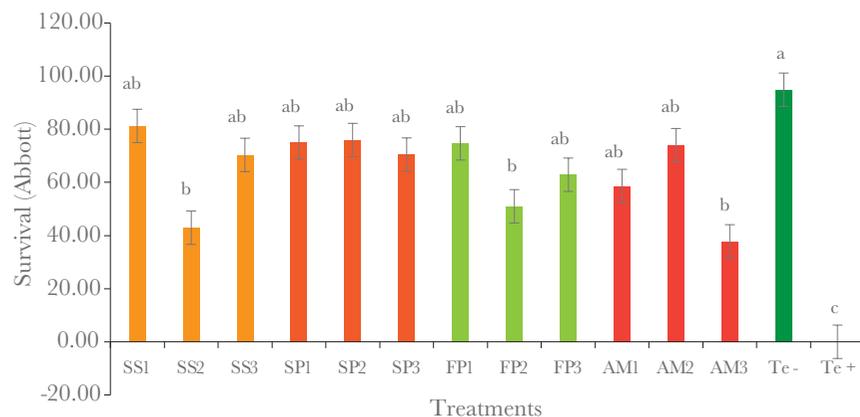


Figure 2. Average survival percentages of 60-day-old Maradol papaya plants, by IR resistance, inoculated with *P. nicotianae* var. *parasitica* Groups (a, b, ab and c), equal letters are not significantly different between treatments (Tukey $\alpha=0.5$).

These results suggest that PF probably worked as a growth regulator, effective at optimum doses (in this case, PF at 0.35%). Multiple researchers show the FP efficacy as a control product for important diseases caused by oomycetes, not only on the *Phytophthora* genus, but also on *Peronospora*, *Plasmopara*, and *Pythium* genera (Groves *et al.*, 2015). Similar results were obtained for wheat blight with PF when the disease is mild; however, its effect is constrained when the pathogen shows greater aggressiveness (Pagani *et al.*, 2014). In plants of *Pinus radiata* other wild species, inoculated with *P. cinnamomi* (soil), at high PF concentrations, showed no significant effect on the pathogen but did cause phytotoxicity on some plants. However, stem injections and PF spraying inhibited *P. cinnamomi* (Shearer and Crane, 2014).

The third most effective treatment (74%) was acibenzolar-s-methyl at 0.1 mM. The three applied concentrations had effectiveness from 37 to 74%, with the first plants dying within seven days, being ASM at 0.1 mM the effective dose in the control of papaya root rot. These results also suggest that this resistance inducer acted as a growth regulator. Our results are similar to those reported by Vawdrey and Westerhuis (2007), who showed that ASM (0.025 g L^{-1}) significantly reduced root rot incidence caused by *P. palmivora* on papaya in Australia. Gilardi *et al.* (2014), on other hand, found that AM at 0.025 and $0.0125\text{ g i.a. L}^{-1}$ applied three times before inoculation, produced 100% efficacy in disease mitigation in the first test. The difference with this research was that it was done with only one application. In the present investigation, the effectiveness (75%) with ASM (0.1 mM) against the infection of *P. nicotianae* var. *parasitica* was higher than that reported by Macedo *et al.* (2009), who observed a survival of 68.3 to 65.3% in the plants, after three days of inoculation, with a previous application of 0.30 g L^{-1} of ASM in the Golden papaya variety against the pseudofungus *P. palmivora*. Concerning the present research, the highest concentration obtained 16% more survival, however, in both cases, the survival was higher than 40% for this resistance inducer. Moscoso-Ramírez and Palou (2013) conducted preventive studies through primary *in vivo* experiments with resistance inducers to *Penicillium* spp. in ‘Valencia’ oranges. They found that ASM at 0.9 mM (0.2 g L^{-1}) reduced the green rot incidence by 15%. Furthermore, this resistance inducer also reduced the severity of green rot, but not of the blue rot causal agent. Despite the above, ASM does not always act as an inhibitor of all pathogens. For example, Méndez *et al.* (2010) evaluated conventional fungicides and resistance inducers among them, where ASM at a concentration of $0.02\text{ kg a.i. ha}^{-1}$, against *Pseudoperonospora cubensis* infection responsible for downy mildew in melon, and the results showed inefficiency and behavior as the positive control.

CONCLUSIONS

Mortality of 60 d of age Maradol papaya plants inoculated with *P. nicotianae* var. *parasitica* occurs on average five days post-infection, demonstrating the high susceptibility of the papaya to this pathogen. The four tested chemical resistance inducers: sodium silicate, potassium silicate, potassium phosphite, and acibenzolar-s-methyl, showed significant efficacy in inducing resistance on 60 d old papaya seedlings inoculated with *P. nicotianae* var. *parasitica*.

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Population Fluctuation of *Disonycha teapensis* Blake (Coleoptera: Chrysomelidae) in *Crotalaria longirostrata* Hook. & Arn. (Fabales: Fabaceae) in Huimanguillo, Tabasco, Mexico

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ABSTRACT

Objective: To establish the population fluctuation of *Disonycha teapensis* adults and larvae and to determine its relationship with the amount of damaged foliage, temperature, and precipitation.

Methodology: The study was carried out in a n=48 *Crotalaria longirostrata* plant crop. The following data was recorded every week: number of adults and larvae, damaged leaflets percentage, plant phenology, temperature, and precipitation. Based on these data, monthly averages were calculated, and correlation analysis were performed between the population fluctuation of *D. teapensis* and the fluctuation of the damaged foliage percentage, temperature, and precipitation.

Results: All the phenological stages of *C. longirostrata* were affected by the pest, which showed a constant population growth. The population fluctuation resembled the fluctuation of the damaged foliage percentage, with a significant positive correlation between both variables. Regarding temperature and precipitation, the correlation was negative but not significant; therefore, the relationship between population fluctuation and these climatic factors is not clear.

Study Limitations: It was not possible to conduct the study for a full year.

Conclusions: The population development of *D. teapensis* depends on the increase in the amount of foliage of its host plant. This insect attacks plants in all their phenological stages and the amount of damaged foliage is considerable.

Keywords: Chrysomeloidea, population, chipilin.

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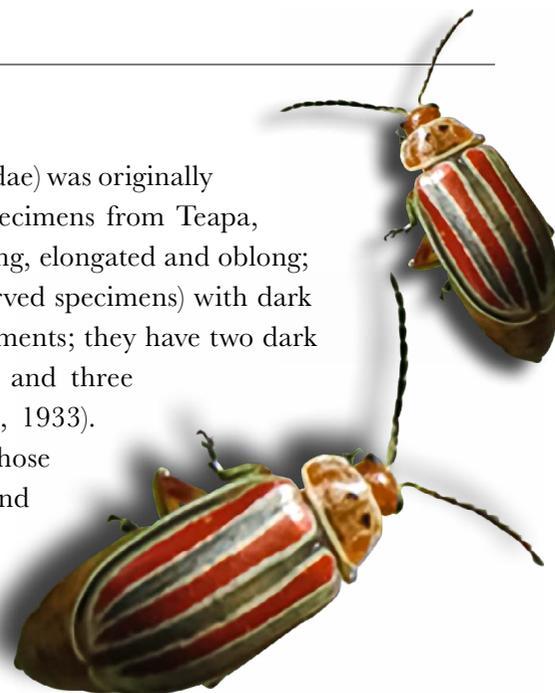
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INTRODUCTION

Disonycha teapensis (Coleoptera: Chrysomelidae) was originally described by Blake (1933), based on adult specimens from Teapa, Tabasco, Mexico. Adults are about 7.5 mm long, elongated and oblong; other aspects include: a yellow body (in preserved specimens) with dark antennae, except for the mostly pale basal segments; they have two dark spots on the anterior part of the pronotum and three dark longitudinal bands on the elytra (Blake, 1933).

D. teapensis belongs to the alternata group, whose females have a similar irregular pronotum and traces of elytral costae; *D. teapensis* is more



similar to *D. pluriligata*, from which it differs by the shape of the aedeagus (Blake, 1955). *D. teapensis* is reported only in Mexico (Blake, 1955), with records in Tabasco (Blake, 1933, 1955), San Luis Potosí (Blake, 1955), Oaxaca (Furth, 2013), and Tamaulipas (Sánchez-Reyes *et al.*, 2015).

In La Chontalpa, Tabasco, adults and larvae of *D. teapensis* damage the leaves of *Crotalaria longirostrata* (Fabaceae), a native species commonly found in home gardens. Its foliage is used in regional gastronomy. Although its cultivation has not yet been developed, it is considered a valuable phylogenetic resource that should be preserved and studied to determine its use and production potential (Lagunes-Espinoza, 2019). Therefore, the objective of this study was to find out the population fluctuation of *D. teapensis*, taking into consideration the phenological stages of *C. longirostrata*, as well as to determine its relationship with the fluctuation of the amount of damaged foliage and the climatic conditions—such as temperature and precipitation—in this area of western Tabasco, Mexico.

MATERIALS AND METHODS

Study Site

The study was carried out on a *C. longirostrata* crop established at El Recinto, Tabasco Campus, Colegio de Postgraduados, Huimanguillo, Tabasco (17° 58' 37.10" N, 93° 23' 10.57" W). The local climate is hot-humid with copious rainfall from June to October. The average temperature is 26 °C and the average annual rainfall is 2000 mm (INEGI, 2001). The crop consisted of n=48 plants arranged in four rows, with 12 plants per row. Each row consisted of a 20-cm high, 20-cm wide, and 10-m long furrow. The distance between rows was 1.5 m and the distance between plants was 0.9 m. Hand weeding was the only method used to maintain the crop; no chemical products were applied.

Insect Count and Damaged Foliage

In order to determine the population fluctuation of *D. teapensis*, weekly counts of the adults and larvae present in each plant were carried out, from the seedling stage to the fruiting stage, from September 24, 2018 to February 27, 2019. At the same time, the weekly damage to the foliage was quantified, counting the total number of leaflets and the number of damaged leaflets in each plant. The rule of three was used to calculate the damaged foliage percentage. *D. teapensis* adults bite the leaflets destroying the leaf blade; they usually start at the edge of the leaflets and then move on to the midrib, on one or both sides of the leaflet (Figure 1A). They also pierce the leaves, but this is less frequent. Small larvae scrape the leaf tissue, while the damage caused by the biggest larvae is similar to the damage caused by the adults (Figure 1B, C).

Phenological Data

The number of plants in the different development stages (seedling, vegetative development, flowering or fruiting stages) was recorded weekly, in order to compare the population fluctuation with the plant phenology. This data was used to determine the percentage of plants in each stage per month. In this study, we considered that the seedling



Figure 1. *Crotalaria longirostrata* leaf damaged by the adult (A) and larvae (B-C) and (D) *Disonychia teapensis* adult.

stage starts at the germination and ends when the first true leaves are formed and that the vegetative development starts when the first leaves are fully developed and ends when the flowering begins.

Temperature and Precipitation Data

The temperature and precipitation data (September 2018-February 2019) were obtained from the meteorological station of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), located approximately 14 km away from the study site.

Statistical Analysis

Monthly averages were calculated based on the weekly data about the number of individuals of *D. teapensis* and percentage of damaged foliage; those averages were used to draw graphs of the said insect's population fluctuation and the damage to the foliage. The daily temperature (°C) and precipitation (mm) data were used to calculate monthly averages, from which fluctuation graphs of these climatic factors were drawn. The Statgraphics Plus Software version 5.1 was used to carry out a Pearson correlation analysis between the population fluctuation values of *D. teapensis* (adults plus larvae) and the fluctuation values of the damaged foliage, temperature, and precipitation percentages.

RESULTS AND DISCUSSION

Presence of *D. teapensis*

During the study period, 1,048 adults and 100 larvae of *D. teapensis* were counted. We were unable to determine the instar or stage of the registered larvae; however, based on their size, most of the individuals were in an advanced stage of development. Smaller (especially recently-hatched) larvae may have gone unnoticed during plant review; therefore, they were not accounted for in the weekly counts. Consequently, the number of larvae registered could have been lower than the number of adults. In addition, the adults are more visible as a result of their coloration, size, and greater mobility. However, it is likely that more

adults were registered than larvae mainly as a result of the possible increase in the adult population. Considering that this species is capable of flight, this increase could have been the result of the arrival of individuals from other sites. Additionally, in a study carried out in Florida, United States, *D. leptolineata* Blatchley larvae were observed only once on *Itea virginica*, their host plant (Watts, 1990).

Population Fluctuation

Although less larvae than adults were registered, the population behavior of both development stages was similar (Figure 2A). When comparing the population fluctuation (Figure 2A) with the development phases of *C. longirostrata* plants (Figure 2B), we observed that *D. teapensis* was present in all phenological stages, although only some adults were observed in the seedling stage. Adults began the colonization of the plants in the month of October, when 50.36% of the plants already had fully developed leaves. This behavior is similar to that of *D. leptolineata* in Florida, whose adults begin to feed on the leaves of *I. virginica* when the plants are in their initial phase of development (Watts, 1990). The *D. teapensis* larvae were first observed in November, when the plants were just over 30 days old and 89.82% were in the vegetative development phase (Figure 2A, B). The initial larvae population—in the first stages of development stage— might have been present since mid-October, since the larvae registered in November were in an advanced development stage and the larval stage of other *Disonycha* species lasts 13 to 36 days (Cordo *et al.*, 1984; Watts, 1990).

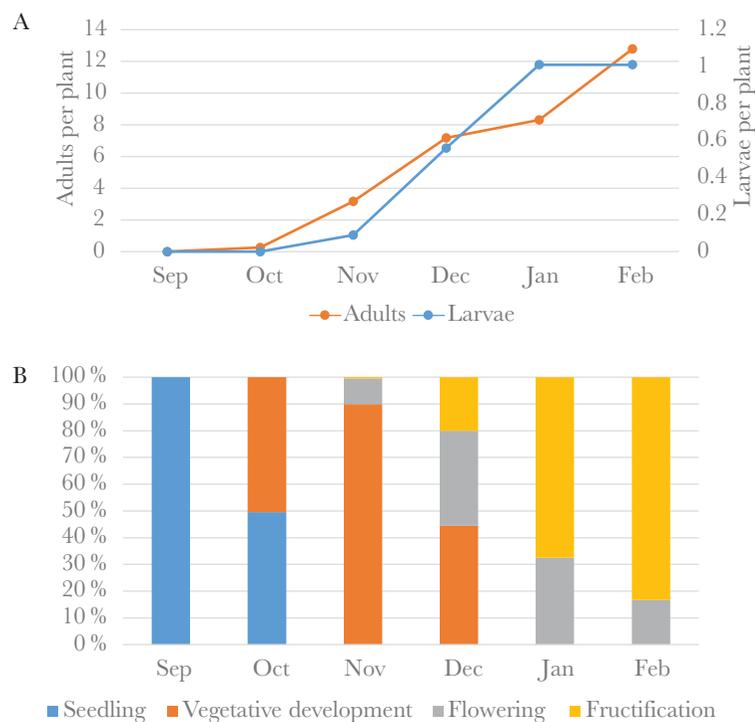


Figure 2. Population fluctuation of *D. teapensis* adults and larvae (A). Percentage of *C. longirostrata* plants per phenological stages during the study period (B). Huimanguillo, Tabasco, September 2018 to February 2019.

From November, the population of adults and larvae began to increase steadily (Figure 2A), as a result of the increase in the amount of foliage, a product of vegetative development (Figure 2B). From December —when 55.56% of the plants were already in the flowering and fruiting stages—, the amount of foliage available allowed the constant growth of the *D. teapensis* population; it kept growing, until it reached its highest density at the end of February —when 83.33% of the plants were in the fruiting stage and the experiment ended (Figure 2A, B).

The Pearson correlation analysis showed a highly significant positive correlation between the insect population fluctuation and the fluctuation of the damaged foliage percentage (Table 1, Figure 3A). This means that there exists a direct relationship between the two variables; therefore, the amount of damaged foliage increased as the quantity of insects increased.

Temperature and precipitation had a negative correlation without statistical significance (Table 1); therefore, there is no clear relationship between the population fluctuation of *D. teapensis* and these climatic factors. Long-term studies —or at least a full year study— are necessary to determine if there is a relationship between climatic factors and the impact of this insect. Therefore, the temperatures (23-29 °C) and the rainfall (7-30 mm) that were recorded (Figure 3B) did not limit the life cycle and population development of *D. teapensis*. During the study, a maximum of up to 30% of damaged leaflets was recorded; however, the percentage may be higher, if we take into account that the pest population continued to grow during the last month of the study (Figure 3A).

Table 1. Pearson’s correlation analysis between the population fluctuation of *Disonycha teapensis* and the fluctuation of the percentage of damaged foliage, temperature, and precipitation.

Variable	Correlation coefficient	Probability
Damaged foliage (%)	0.946	0.004
Temperature (°C)	-0.646	0.165
Precipitation (mm)	-0.202	0.701

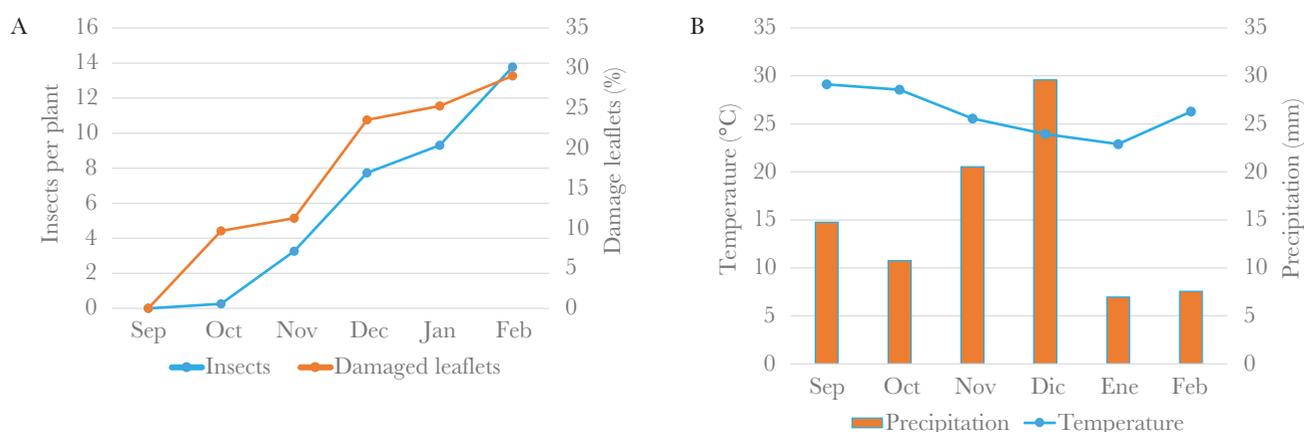


Figure 3. Population fluctuation of *D. teapensis* (adults plus larvae) and the damaged leaflet percentage (A). Temperature fluctuation and precipitation (B). Huimanguillo, Tabasco, September 2018 to February 2019.

CONCLUSIONS

Since *D. teapensis* feeds on the leaves of *C. longirostrata*, the development of its populations depends on the increase in the amount of the foliage of its host plant. *D. teapensis* attacks plants in all their phenological stages and it causes a considerable amount of damage to their foliage. We suggest continuing with this type of studies, throughout all the seasons of the year, in order to clarify the relationship between this insect's population fluctuation and the temperature and precipitation fluctuations.

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Animal welfare during transport and slaughter of beef cattle

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ABSTRACT

Objective: To review how transport and stunning of cattle affect animal welfare.

Approach: During the transport of beef cattle to slaughter plants, several factors affect animal welfare, such as travel time, stress, and load density. Additionally, the correct stunning of cattle helps comply with the animal welfare guidelines established by different protocols such as Welfare Quality[®].

Study limitations/Implications: Meat quality is affected by several factors, being of utmost importance the way animals are transported to the slaughterhouse, and they are stunned. Therefore, it is critical to perform these stages properly to obtain good quality meat; besides, it is a welfare issue.

Conclusions: It is critical to comply with transport and slaughter procedures that guarantee good beef meat quality and ensure animal welfare to avoid stress in cattle as possible.

Keywords: Beef cattle, loading density, stress, animal welfare, stunning.

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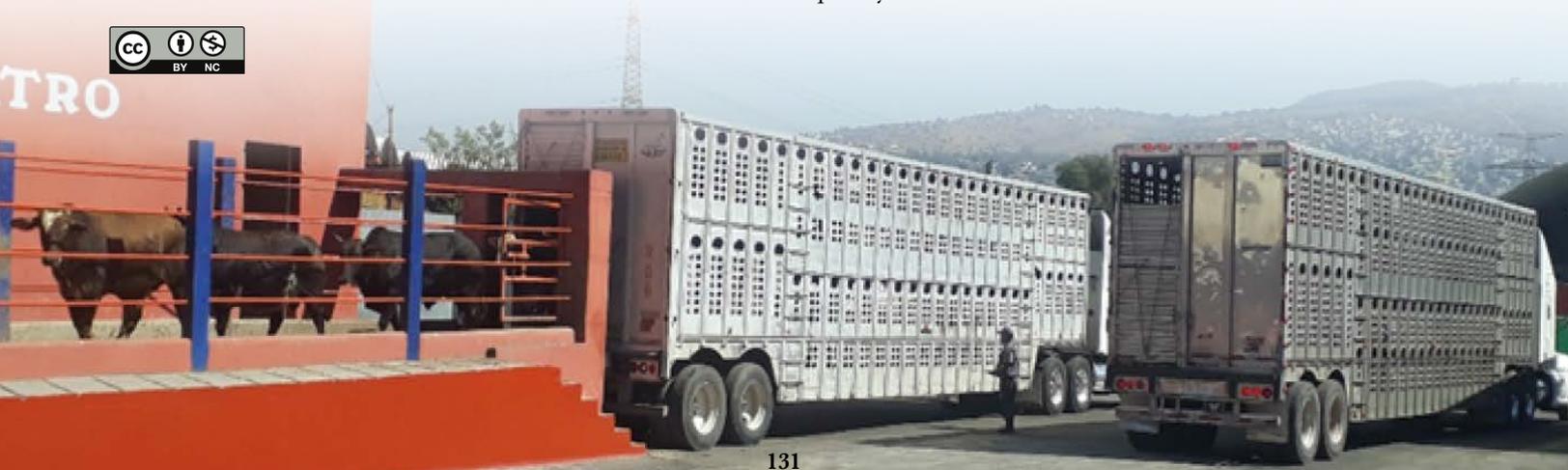
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INTRODUCTION

Red meat is part of the human diet worldwide since it provides good quality proteins (McNeill and Van Elswyk, 2012) and minerals such as iron (Kongkachuichai *et al.*, 2002).

After fattening, bovines are transported for slaughter. Transport can be stressful for the animals, affecting their well-being (Romero *et al.*, 2010; Van De Water *et al.*, 2003), which manifests in the meat, like an increase in pH (Gallo *et al.*, 1998), decrease in muscle luminosity (Gallo *et al.*, 2003), and presence of lesions when using inappropriate vehicles (Huertas *et al.*, 2010). All these represent economic losses for the meat industry (Mach *et al.*, 2008). Therefore, it must be ensured that the animals are transported under animal welfare conditions to obtain better meat quality.



Animal Welfare

Animal welfare or well-being is defined in different ways. Broom (1986) defines “the welfare of an animal and its state as regards its attempts to cope with its environment.” The World Organization for Animal Health (OIE) states that animal welfare “is an animal’s physical and mental state with the conditions in which it lives and dies”. It also mentions that an animal must be healthy, well-fed, safe, protected, in addition to having proper and humane handling at the time of slaughter (OIE, 2011). Different protocols have been developed as the one established by Welfare Quality[®], designed to integrate animal welfare into the food chain (Welfare Quality Network, 2009).

Transport and animal welfare

During the transport of beef cattle, factors such as load density (Gallo *et al.*, 2005), travel time (Gallo *et al.*, 1998), experience and attitudes of the driver (Valadez *et al.*, 2018), vibrations, and road conditions (Gebresenbet *et al.*, 2011), type of vehicle used, and environmental factors, can affect animal welfare (Schwartzkopf-Genswein *et al.*, 2016).

Animal transport requires trained personnel. Animals are to be protected from the weather. Upon arrival at the slaughterhouse, they must be provided with good quality water and forage and comfortable and clean pens with an area of 3 m² per animal for resting (OIE, 2011; FAO, 2004). Electric prods should not be used for herding, the vehicle must have good ventilation, and overcrowding should be avoided.

Load density and travel time

The allocation of sufficient space for animals during transport should be considered a code of practice and regulation to guarantee the humane treatment of animals (Whiting, 2000). It is necessary to use vehicles designed to transport them properly to avoid injuries and the stress caused by the movement of the vehicle (Santurtun and Phillips, 2015), which can increase blood cortisol levels (Leme *et al.*, 2012).

Load density is the available space that the animals have when transported and is expressed in kg of the animal per m² or m² per animal. The FAO establishes average load rates for the transport of adult beef cattle by land of 1.0-1.4 m² per animal of floor area for beef cattle (FAO, 2001a). The European Union establishes in its regulations for the protection of animals during transport load densities for the transport of bovines according to their weight (Table 1). The same is true in countries such as Australia and New Zealand, which have regulations to determine load density.

Petherick and Phillips (2009), by means of an allometric equation, calculated the space necessary for the transport of livestock. For animals that remain standing during the journey, they determined an area of (m²)=0.020 W^{0.66} of the animal, and for animals that are allowed to lay down during the journey, it is given by the equation:

$$area (m^2) = 0.027 W^{0.66}$$

where: *W* is the LW of the animal.

Table 1. Load density established by different countries.

Department of the Local Government and Regional Development of Western Australia (2003)		European Union (2005)	
Cattle body weight (kg)	Floor area (m ² / animal)	Cattle body weight (kg)	Floor area (m ² / animal)
250	0.77	50	0.30-0.40
300	0.86	110	0.40-0.70
350	0.98	200	0.70-0.95
400	1.05	325	0.95-1.30
450	1.13	550	1.30-1.60
500	1.23	>700	>1.60
550	1.34		
600	1.47		
650	1.63		

Broom (2008) recommends that for a 500 kg LW bovine, with a route of less than 12 h, a floor area of 1.35 m² should be assigned, and if the route is longer than 12 h, the floor area should be 2.03 m².

In Mexico, beef cattle are generally transported from feedlots to slaughter plants in potbelly cages (Figure 1), loading 45 to 50 bovines per cage, weighing 550-700 kg LW, and a travel time that ranges from 1-15 h. Moreover, livestock traders have chosen to have personnel dedicated exclusively to the loading and unloading of livestock as a strategy to reduce the stress caused by this activity.

Teke *et al.* (2014) carried out a study in Turkey, transporting Simmental beef cattle over a distance of 1800 km, with a load density of 2.8 m² per animal, with a resting time before slaughter of 24, 48, and 72 h. The authors concluded that after a prolonged transport, it is necessary to rest the animals for 72 h since this shows better pH 24 values in the meat (5.48). In a study conducted by Gallo *et al.* (2005), they surveyed two regions of Chile, where load densities fluctuated between 457 ± 6.6 kg/m² and 453 ± 9.7 kg/m². The authors

**Figure 1.** Transport and unloading of cattle at the slaughterhouse (Image of the authors).

observed that if the travel distance was long, the load density increased, exceeding that allowed by the country's legislation, which is 500 kg/m^2 , resulting in more significant stress in the animals and a high incidence of muscle contusions.

Factors such as the absence of loading and unloading facilities, transporting animals on hot days, and the stress caused during cattle slaughter can cause depletion of muscle glycogen having low postmortem lactic acid production, which results in a high pH of the meat (Mounier *et al.*, 2006). Therefore, it is necessary to transport and unload the cattle in the mornings without extending the rest period for more than 4 h before slaughter in hot seasons (Pérez-Linares *et al.*, 2015).

Stunning and slaughter

Effective stunning can be defined as rendering the animal unconscious or insensitive to pain immediately. The physical signs are that the animal collapses, does not breathe rhythmically, and has no corneal reflex, a relaxed jaw, and a hanging tongue (HSA, 2016). Likewise, some authors have used other signs to assess animals' stunning, such as floppy heads and blank stares. There is often limb movement after stunning, but it is not considered a sign of a return to sensation (Grandin, 1998). There are different stunning methods, mechanical such as a captive bolt, electrical, gas, and others such as lethal injection and puntilla. The captive bolt and puntilla are the most used to stun beef cattle in Mexico.

Captive bolt

The primary function of stunning cattle with a penetrating captive bolt, or stun gun, is to cause a forceful and irreversible concussion (Gregory *et al.*, 2007).

The device (Figure 2) consists of a trigger or contact-operated pistol containing a bolt or projectile, which is propelled by the detonation of a cartridge or compressed air (FAO, 2001b).

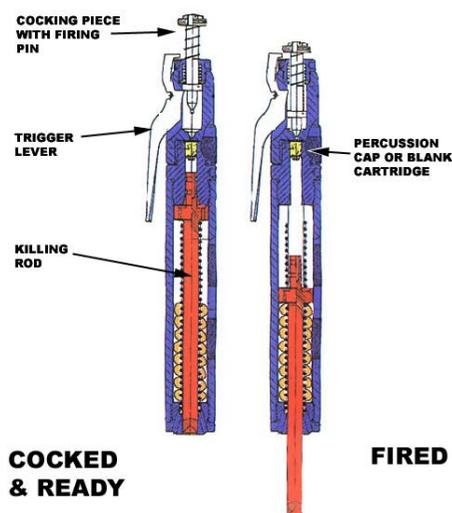


Figure 2. Penetrating captive bolt (Firearms History, Technology & Development, 2011). Firearms History, Technology & Development. (2011). Utility Firearms: Meat Processing Industry, March 11, 2011. <http://www.firearmshistory.blogspot.com/2011/03/utility-firearms-meat-processing.html>

The device penetrates the skull, producing a concussion by injuring the brain or increasing intracranial pressure (Gregory *et al.*, 2007), causing internal cerebral hemorrhage (Atkinson *et al.*, 2013), followed by a tonic phase or contraction of the extremities lasting from 10 to 20 seconds. Subsequently, there is the clonic phase or involuntary movement of the extremities, which gradually decreases. Otherwise, if an animal sign kicking or row when collapsing, it indicates that it did not receive a correct stun (HSA, 2016).

The functionality and effectiveness of the captive bolt depend on several factors, like the animal's breed and the length of the captive bolt. Martin *et al.* (2018) indicate that Holstein breed animals present more significant limb movement activity than zebu breed animals. Furthermore, animals stunned with a 15.24 cm long bolt tend to present more movements in the slaughter track.

The accuracy and direction of the shot are critical in determining the efficiency of the stun. The Scientific Panel on Animal Health and Welfare (AHAW) recommends that for a good stunning, the shot (Figure 3) should be placed at the crossing point of imaginary lines drawn between the base of the horns and eyes, not exceeding a radius greater than 2 cm from this point (AHAW, 2004). Thus, the imprecise use of the stun gun could affect the stunning efficiency. Therefore, evaluating the impact points and the firing direction can be taken as standards for stun control (Fries *et al.*, 2012).

Some authors attribute the failure to stun animals with captive bolts to the operator's lack of experience, gun maintenance, and cartridges storing in humid places. Animal's size is also crucial because they have thicker skulls that are more difficult to penetrate, the caliber of the cartridge used, and the frequency of use of the gun (Gibson *et al.*, 2015; Grandin 2002).

Puntilla

Published information about stunning with a puntilla is limited. The OIE does not have the puntilla method approved because it is considered an ineffective and inhumane method of animals' slaughter (OIE, 2011).

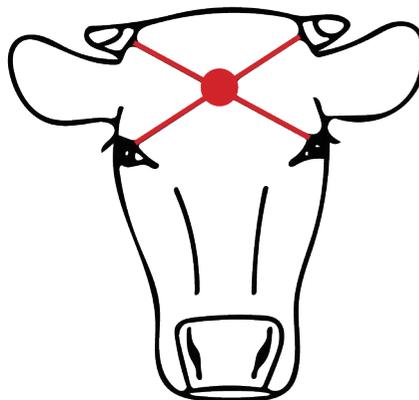


Figure 3. Shot position with a penetrating captive bolt (HAS, 2016).

Limon *et al.* (2009) examined the puntilla method applied in llamas ($n=20$), where they reported that in 45% of the cases, it was necessary to repeat the stunning and 95% of the animals presented palpebral reflex, concluding that it is difficult to achieve a good stunning with a single puntilla stab (Limón *et al.*, 2009).

In another study, the application of puntilla was evaluated in beef cattle ($N=309$), reporting that it was necessary to repeat the stunning in 24% of the animals, occurring more frequently in heavier animals (>380 kg), concluding that the nerves often remain functional after stabbing 8. (Limón *et al.*, 2010). Therefore, it is very likely that the animals are still conscious during slaughter.

There are no evaluations about the brain and spinal cord activity after using the puntilla. Therefore, it is necessary to determine a strategy to use an effective method for stunning humane and accessible to use daily in slaughterhouses (Limon *et al.*, 2012). Other factors must be considered, such as sex, breed, animal live weight, body condition, and the operator's experience.

CONCLUSIONS

The beef production system must consider procedures that guarantee the welfare of the animals during the transfer from the feedlot to the slaughterhouse since it can be very stressful for the animals. The load density, travel time, and how animals are slaughtered play an essential role in the meat production chain. Currently, in Mexico, studies related to animal welfare are beginning to be carried out during the transport and slaughter of beef cattle, trying to determine the critical points that generate more stress in the animals.

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Soil fertility classification for sugarcane in supply areas of a sugar mill

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ABSTRACT

Objective. To prepare the fertility classification for the sugarcane-cultivated soils in the Pujiltic Sugarcane Mill (PSM) supply area in order to improve decision-making.

Design/methodology/approach. The soils were classified according to their fertility (FCC), using a system based on the quantifiable parameters of the upper soil layer and some characteristics of the subsoil directly linked to the growth of sugarcane.

Results. Six factors limited the agricultural potential of the PSM soils: alkalinity, water excess or deficit, clay content, erosion, nutritional deficiencies, and low CEC, which alone or in groups act in detriment of soil fertility.

Limitations/implications. Solving these problems requires a comprehensive analysis that considers crop type, planting season, and technology availability.

Findings/conclusions. The soil fertility classification system enabled the classification of 11 soil subunits of the PSM area.

Key words: Fertility, System, Classification, Sugarcane.

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INTRODUCTION

The Pujiltic Sugarcane Mill is the most important of its kind in the State of Chiapas. It has a cultivated surface of 17,100 ha and uses auxiliary irrigation, with a yield of 90.60 t ha⁻¹ of sugarcane and a factory yield of 12.01%, according to the Comité Nacional para el Desarrollo Sustentable de la Caña de Azúcar (CONADESUCA, 2019).

The agricultural production of sugarcane is affected by droughts and a diminished soil fertility. As an alternative, production can be boosted through the increase of the cultivable surface and —according to studies about the yields of sugarcane—, these can be increased through adequate soil fertility management practices and the use of improved

varieties (Pérez *et al.*, 2019). Local experiments have shown that the integration of soil fertility and nutrient management is an advanced approach that serves as a resource to increase or maintain soil fertility throughout time (Salgado-García *et al.*, 2008; Salgado *et al.*, 2013; Salgado-Velázquez *et al.*, 2020). However, a diagnosis is needed in order to identify limitations. Buol *et al.* (1975) developed a system to classify soils according to their fertility (Fertility Capability Classification or FCC), with the aim of closing the gap between the classification and soil fertility subdisciplines. As a technical soil classification system, the FCC has a specific use, derived from natural classification systems such as soil taxonomy (Soil Survey Staff, 2014) or the World Reference Base for Soil Resources (IUSS Working Group WRB, 2014). The FCC's categories indicate the main limitations of the soils according to their fertility, which can be interpreted in relation to the crops of interest. Since its publication in 1975, the FCC has been assessed and applied in several countries. As a result, the definitions of several modifiers have changed and new ones have been therefore included to improve the system (Sánchez *et al.*, 1982). The system is a good starting point to study the suitability of tropical soils. In the Mexican tropic, particularly in the State of Tabasco, this system has only been applied in three regions, resulting in good agronomic management recommendations (Salgado and Palma, 2002; Salgado and Obrador, 2012; Salgado-Velázquez *et al.*, 2017). Knowing the FCC's classes allows us to identify the fertility limitations and—given the importance of sugarcane in the State of Chiapas—generating the said information is necessary. Moreover, a soil study of 33,974.7 ha is available for the Pujiltic Sugar Mill (Salgado-García *et al.*, 2006). Therefore, the objective of this article was to develop the fertility classification of the sugarcane-cultivated soils in the PSM supply area.

MATERIALS AND METHODS

The study area covered a surface of 33,974.7 ha, divided in 11 soil subunits (Figure 1), all of which are cultivated with sugarcane (Figure 2). The physical and chemical properties data of the first two horizons of each soil subunit in the PSM supply area was taken from the soil study conducted by Salgado-García *et al.* (2006). The system to classify soils according to their fertility (FCC) was used. This system comprises three categories which, in turn, comprise different classes. The combination of these classes makes up the FCC units:

Type. The texture of the plow layer or the top 20 cm, whichever is shallower.

S: Sandy topsoil: loamy sands and sands (Soil Survey Staff, 2014).

L: Loamy topsoil: <35% clay, but without loamy sand or sand.

C: Clayey topsoil: >35% clay.

O: Organic soils: >30% organic matter (OM) up to a depth of 50 cm or more.

Substrata type (subsoil texture). This is only used when there is a marked textural change relative to the surface or if a hard layer hinders root growth up to the first 50 cm of the soil.

S: Sandy subsoil: same as in type; **L:** Loamy subsoil: texture similar to type;

C: Clayey subsoil: texture similar to type; **R:** Rock or another hard layer hindering root development.

Modifiers. When more than one criterion is indicated for each modifier, only one needs to be known. Ideally, the first criterion mentioned should be used if data is available. The following criteria are presented for those cases in which identifying the first one is impossible (Sánchez *et al.*, 1982): **g** (gley), **d** (dry), **e** (low cation exchange capacity), **a** (aluminum toxicity), **h** (acid), **i** (high P-fixation by iron), **x** (short-range-order minerals), **v** (vertisol), **k** (low K reserves), **b** (basic reaction), **s** (salinity), **n** (natric), **c** (cat clay), ‘ and ‘ (gravel), % (slope).

Procedure. Soils are classified according to these parameters by determining if the characteristic is present or not. Most quantitative limits are criteria found in the Soil Taxonomy and the *World Reference Base for Soil Resources* (Soil Survey Staff, 2014; IUSS *et al.*, 2014). FCC units list the type of texture and substrata (if they differ) in capital letters, the modifiers in lowercase, the gravel modifier with a prime symbol (‘), and the slope in parentheses, if so wished.

RESULTS AND DISCUSSION

Soil Study

Table 1 presents the chemical and physical properties of the first two horizons of the soil subunits and Figure 1 shows the representative profiles. The FCC classification for each soil subunit is presented below.

Cb. Chernic Chernozem (CHch). This soil subunit has a mollic horizon, it is deep and well structured, has a high base saturation (80% or more), high content of organic matter (2.5% or more), and a high biological activity. These soils have clayey textures in most horizons, with a moderate permeability. The irrigation availability allows yields of up to 116 t ha⁻¹ (Salgado *et al.*, 2014).

Lb (12%). Hypocalcic Calcisol (CLccw). This subunit presents a medium infiltration index and a medium water retention capacity; it has secondary carbonate concentrations up to a depth of 100 cm from the soil surface. Its rapid permeability allows a good drainage. The pH is generally considered as moderately alkaline; the electric conductivity (EC) is 0.20 dS m⁻¹, which indicates that no salinity problems are present; the OM is very rich in the plow layer and poor in the last horizons (5.33-1.24%). Given the calcareous nature of these soils, the use of fertilizers derived from phosphate rock or other non-water-soluble phosphates must be avoided. Its iron, boron, and zinc deficiency must be supplemented through chemical or organic fertilization (Salgado *et al.*, 2010).

Cbv (<5%). Vertic Calcisol (CLvr). This subunit has a vertic horizon up to a depth of 100 cm from the soil surface. These soils present a subsurface clayey horizon, as a result of expansion and contraction. Its slickensides or structural aggregates have 30% more clay throughout its thickness. Most of this soil profile shows >60% clay contents; however, these clay contents and the bulk density (BD) (1 g cm⁻³) do not cause compaction problems, likely as a result of its high organic matter and calcium contents. Based on field observations, these soils have cutans as a result of the accumulation of carbonates, which react strongly to HCl. Moreover, they have soft, small, cream-colored CaCO₃ nodules; the profile presents a good drainage, although permeability goes from moderate to slow, which is attributed to a high clay content (Salgado *et al.*, 2006).

Cb (<1%). Mollic Cambisol (CMmo). This subunit has a low infiltration index and good water retention capacity. Its soils have a high base saturation (>50%) and a high OM content. They have a moderately alkaline pH and no salinity problems ($EC < 1 \text{ dS m}^{-1}$).

Cbeg (5%). Calcaric Fluvisol (FLca). This subunit has a low infiltration index and good water retention capacity. It is a calcareous soil at least up to a 20-50 cm depth from the soil surface. Based on field observations, the water table was found at a depth of 150 cm; the gleyic processes at this depth gives the soil a grey color. These soils have an average BD of 1 g cm^{-3} and have no compaction problems. They have a moderately alkaline pH and no salinity problems, because their EC ranges from 0.13 to 0.47 dS m^{-1} . The OM has an irregularly arranged profile: it is rich in the first horizon, decreases as the depth increases, and increases again at a still lower depth (3.2-0.4-3.1%), as a result of the continuous alluviation processes. The subunit has a low cation-exchange capacity (CEC), which favors lixiviation, especially in the cases of K, Ca, and Mg. Therefore, we recommend using 10 t ha^{-1} of compost in order not to limit crop development (Salgado *et al.*, 2014). This soil presented a lower K content than Vertisols, contrary to the results reported by Bolio *et al.* (2008) for the sugarcane soils in Chontalpa, Tabasco.

Cbg (<1%). Mollic Gleysol (GLmo). This subunit's soils have high nutrient and organic matter contents; they present loamy textures in the surface that overlie silty clay textures (C horizon). Occasionally, the bottom of the profile may present sandy textures. These soils are deep, although most of the year the water table is found near the surface, causing the sugarcane rooting depth to be less than 60 cm. These soils are predominantly characterized by a clayey texture, grey colors due to gleyization processes, and poor drainage. Therefore, drainage is necessary to avoid altering the crop's physiology and to allow it to ripen in optimal conditions (Méndez-Adorno *et al.*, 2016). The 1 g cm^{-3} BD does not indicate compaction problems and most horizons maintain a strong reaction to HCl. These soils have a moderately alkaline pH and the EC does not indicate salinity problems ($< 3 \text{ dS m}^{-1}$). Most of the profile has a very high CEC and, in order to improve the OM content, vinasse and compost must be applied in 150 m^3 and 10 t ha^{-1} doses, respectively (Hernández *et al.*, 2008).

Cb (<.1%). Rendzic Leptosol (LPrz). The main characteristics of these soils are related to their low depth and calcareous rock origins, which provides them with very significant properties such as good OM contents, good nutrient contents, a good infiltration or permeability, a good soil structural development and soil structure stability. However, they have significative problems related to the slope, including: a low radicular volume, a tendency to erosion, a difficult accessibility, and the Ca and Mg saturation. Phosphorus fixation phenomena and iron deficiencies take place when the said saturation reacts to calcium, during the early development stages of the sugarcane crop. The pH is alkaline and the EC does not indicate salinity problems (0.12 dS m^{-1}). The CEC is very high ($52.2 \text{ cmol}(+) \text{ kg}^{-1}$) and the water availability allows the sugarcane crop to achieve yields in excess of 90 t ha^{-1} .

Cb. Pachich Leptic Phaeozem (PHphle). Similar to the Rendzic Leptosol (LPrz), this soil achieves its best yield in the Mex 79-431 cultivar, using the fertilizer dose recommended

by the SIRDF (*Integrated System for Recommending Fertilization Rates in Sugar Cane*): 120-60-60 kg ha⁻¹ of N, P₂O₅, and K₂O, 92.01 t ha⁻¹ (Pérez *et al.*, 2019).

Lbe. Calcaric Regosol (RGca). These Regosols have calcareous properties at least between 20 and 50 cm from the soil surface. They have good permeability and drainage. They show a strong reaction to HCl in all their horizons and are also very rocky soils, with pebbles and gravel throughout the profile. The BD does not reflect compaction problems (1 g cm⁻³); these soils have a moderately alkaline pH and there are no salinity-related effects (average EC of 0.2 dS m⁻¹). The superficial horizon of these soils is rich in OM, which diminishes along with the depth of the profile (6.6-1.1%). These Regosols present a low CEC, which favors lixiviation, particularly in the cases of K, Ca, and Mg.

Cbev. Eutric Vertisol (VReu). This subunit has a >35% clay layer which covers the whole profile. It has a high-water table that remains flooded during rainy season, causing the stems to die, as a consequence of the saturation of the pores of the soil. Since these soils present denitrification problems due to an anaerobic subsoil, a superficial drainage is recommended. It has a moderately alkaline pH and no salinity problems, as a result of its ECof <2 dS m⁻¹. These soils have a low CEC that favors lixiviation, particularly in the cases of K, Ca, and Mg. During the sugarcane cultivation, the soil did not accumulate potassium, as indicated by Bolio *et al.* (2008) for Vertisols in Chontalpa, Tabasco. Low K contents—compared with the high Ca and Mg contents (Table 1)—account for the foliar deficiency of K (<1.0% in leaf 4) in the sugarcane crops of the Pujiltic region. This phenomenon took place in spite of the application of the fertilizer dose recommended by SIRDF and/or 10 t ha⁻¹ of compost, which allows a yield of 60-101.8 t ha⁻¹ (Salgado *et al.*, 2014).

Cbve (3%). Pellic Calcic Vertisol (VRpecc). Similar to Eutric Vertisol (VReu).

CONCLUSIONS AND RECOMMENDATIONS

Six factors limit the potential production rate of soils in the area where sugarcane used in the PSM is cultivated: soil alkalinity, water excess or deficit, clay content, erosion, nutrient deficiencies, and a low cation exchange capacity. These factors, alone or grouped together, act in detriment of soil fertility.

Knowledge about the relation between soils, plants, and atmosphere allows us to consider agricultural drainage, irrigation, and fertilization with macro- and micronutrients as agronomic practices that would improve the conditions of Gleysol, Vertisol, Fluvisol, and Cambisol units.

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Table 1. Physical and chemical properties of the superficial horizons of the soil subunits cultivated with sugarcane in the Pujilic Sugarcane Mill, Chiapas.

Subunit	Horizons (cm)	pH (H ₂ O) rel. 1:2	EC (µS m ⁻¹)	OM %	P (mg kg ⁻¹)	K	Ca	Mg	Na	CEC	D _a (g cm ⁻³)	Clay				Texture
												Clay	Silt	sand	(%)	
CLccw		7.70	0.59	9.55	37.18	0.76	64.2	10.86	0.97	55.3	1.03	63	13	24	Clayey	
	A2 (25-62)	7.51	0.43	4.25	2.54	0.44	36.7	6.91	0.77	55.3	1.02	83	5	13	Clayey	
	Ap (0-26)	7.96	0.23	5.32	7.04	0.29	22.3	7.88	0.11	17.2	1.02	27	40	32	Loamy	
	A2 (26-43)	8.02	0.22	4.23	2.89	0.22	20.5	7.88	0.04	14.7	1.2	31	41	28	Clayey Loam	
CLvt	AP (0-20)	7.59	0.51	4.11	5.35	0.55	53.6	11.35	0.74	45.1	1.01	57	17	27	Clayey	
	A2 (20-56)	7.52	1.88	2.12	3.66	0.41	85.7	8.39	1.31	40.6	1.15	79	3	18	Clayey	
CMlmo	Ap (0-13)	7.22	0.75	3.28	18.87	0.67	29.0	6.41	0.53	29.9	1.04	35	23	43	Clayey Loam	
	A2 (13-55)	7.24	0.32	3.85	2.54	0.23	38.2	4.11	0.30	26.9	1.01	40	17	43	Clayey	
FLca	Ap (0-20)	8.06	0.47	3.2	5.44	0.19	20.1	10.67	0.33	13.2	1.02	29	55	16	Silty Clay Loam	
	A2 (20-40)	8.11	0.39	2.33	2.49	0.10	18.6	7.88	0.39	11.2	1.01	29	54	17	Silty Clay Loam	
GLmo	Ap (0-22)	7.77	0.57	6.50	7.75	0.48	69.1	14.31	0.37	52.7	1.03	67	19	14	Clayey	
	A2 (22-42)	7.64	2.55	2.92	1.83	0.32	82.3	18.91	1.41	45.1	1.02	67	19	14	Clayey	
LPz	Ap (0-37)	6.75	0.12	8.7	1.97	0.47	49.7	3.52	0.06	52.2	1.02	69	15	16	Clayey	
	Ap (0-22)	7.42	0.45	6.90	2.39	0.43	51.6	9.70	0.76	54.3	1.24	81	9	10	Clayey	
PHphle	A2 (22-62)	7.49	0.56	8.89	16.06	0.83	54.9	10.69	0.90	59.3	1.02	63	17	21	Clayey	
	Ap (0-31)	7.94	0.26	6.63	34.65	0.15	38.9	3.22	0.13	12.2	1.01	30.7	31.4	37.9	Clayey Loam	
RGca	C (31-65)	8.02	0.24	1.39	4.37	0.05	31.2	1.02	0.05	2.0	1.01	24.7	24.7	50.6	Sandy Clay Loam	
	Ap (0-21)	8.10	0.21	4.73	8.71	0.27	60.5	13.47	0.26	8.6	1.02	55	36	9	Clayey	
VRcu	A2 (21-44)	8.10	0.29	2.53	0.28	0.09	71.2	13.63	0.41	16.2	1.01	55	36	9	Clayey	
	Ap (0-20)	8.07	0.19	3.60	3.80	0.17	31.9	13.4	0.14	24.3	1.02	41	27	40	Clayey	
VRpcc	A2 (20-49)	8.02	0.33	1.33	2.68	0.18	61.8	16.92	0.26	23.8	1.01	43	28	29	Clayey	

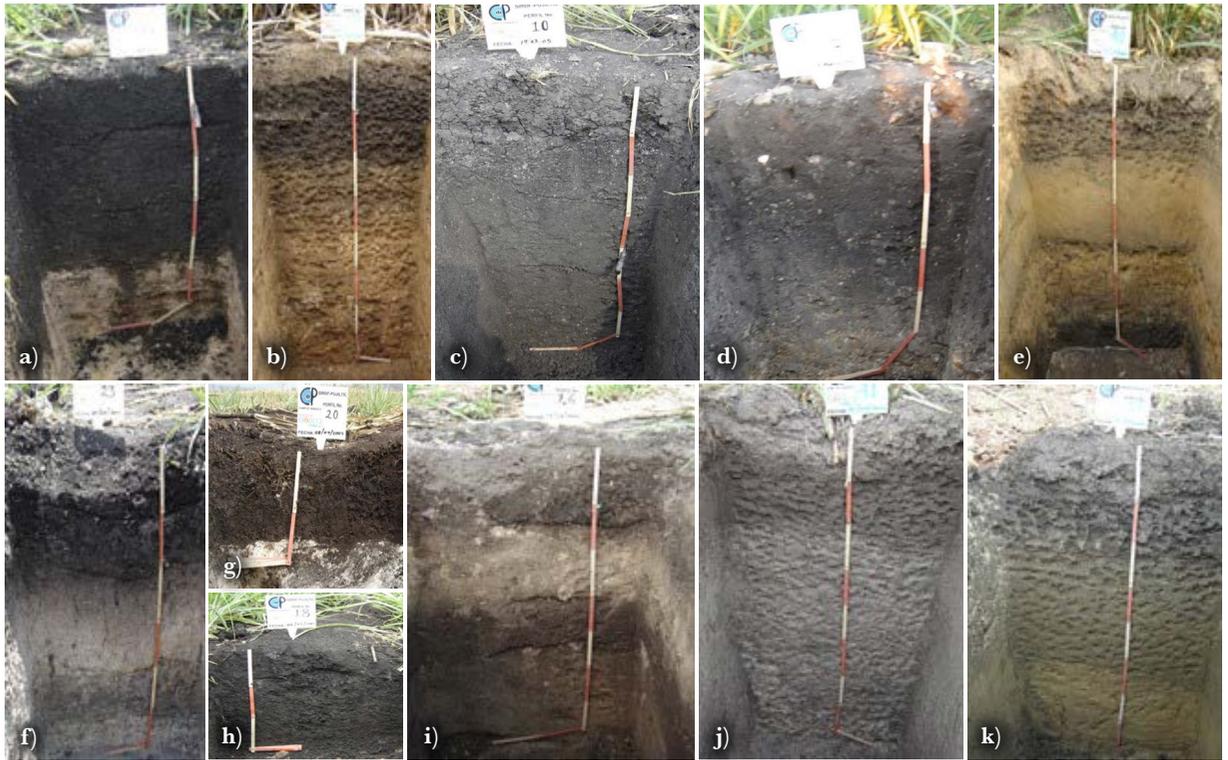


Figure 1. Details of the profiles of sugarcane-cultivated soils at the Pujlitic Sugar Mill: a) Chch, b) CLccw, c) CLvr, d) CMmo, e) FLca, f) GLmo, g) LPrz, h) PHphle, i) RGca, j) VReu, and k) Vrpecc.

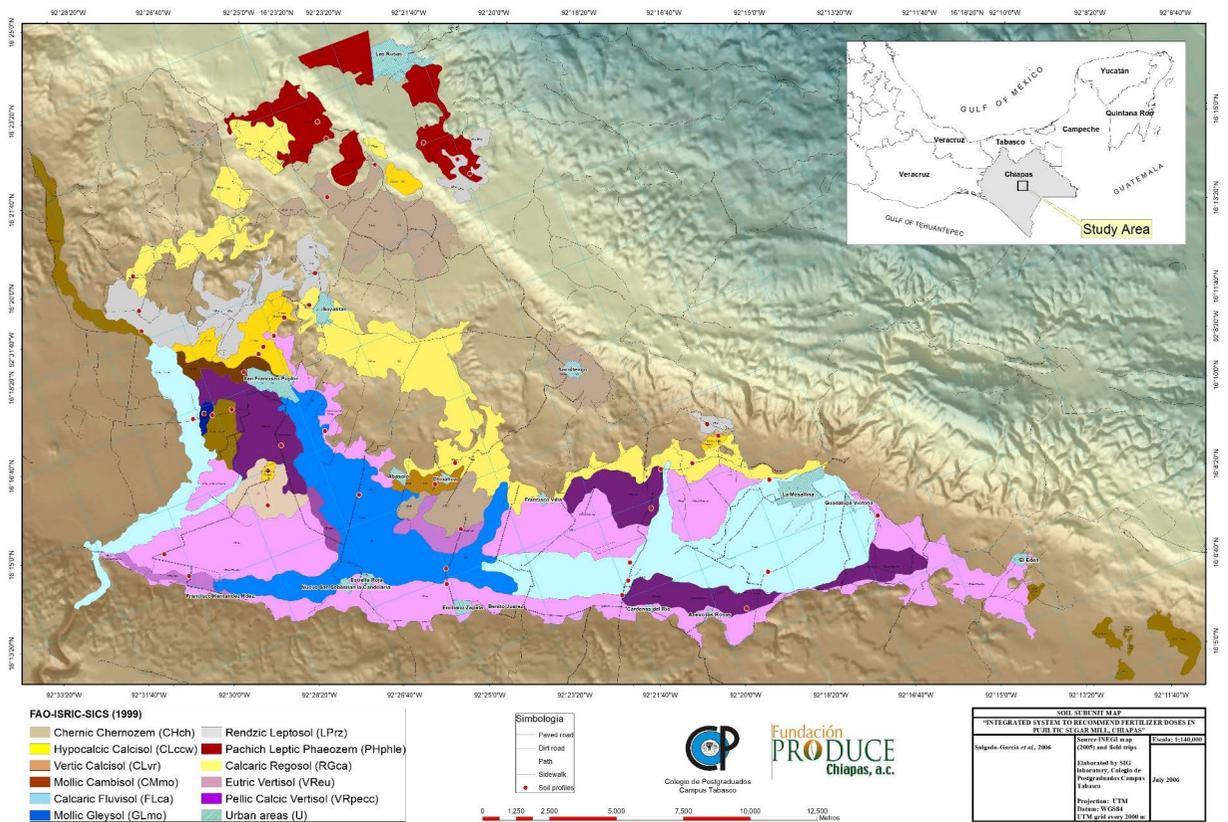


Figure 2. Map of soil subunits at the Pujlitic Sugar Mill, Chiapas.

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Plant quality index of caracolillo (*Ormosia macrocalyx* Ducke), in nursery stage with controlled release fertilizers

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ABSTRACT

Objective: To evaluate, in a nursery, the effect of two controlled release fertilizers in three concentrations on the growth components and quality index in caracolillo seedlings.

Design/Methodology/Approach: A factorial arrangement (2×3) housed in a completely randomized design was used, with seven treatments and twelve repetitions. For three months, the growth of caracolillo plants maintained in 310 cm³ polyethylene tubes, with a substrate of peat moss, vermiculite, agrolite and soil (2:1:1:1), plus controlled release fertilizer (CRF) Basacote[®] 9M (16-8-12) and Multicote[®] 12M (18-6-12), in three doses each: 10 (low), 20 (medium) and 30 kg m⁻³ (high), in addition to a control without conventional fertilization (CF) 17N-17P-17K in doses of 10 kg m⁻³. The variables height, diameter, leaf number, robustness index (RI), aerial/root dry biomass ratio (ADB/RDB R) and Dickson's quality index (DQI) were measured. The means were compared by Tukey's test at a 5% confidence level.

Results: Basacote[®] and Multicote[®] fertilizers in low doses allowed an adequate growth and development in caracolillo plants, which was reflected in the variables height, diameter and number of leaves. The RI and DQI indices presented values within the normal ranges, while those of ADB/RDB R were rather low, but in the three cases there were no statistical differences.

Study Limitations/Implications: Based on the results obtained, it is recommended to continue monitoring the studied plants in the field, which would allow adjustments in the initial fertilization doses.

Findings/Conclusions: The low doses (10 kg m⁻³) of the controlled release fertilizers Basacote[®] and Multicote[®] showed the best results in the growth and development of caracolillo in the nursery stage, but the RI, ADB/RDB R and DQI indices did not present statistical differences.

Keywords: Robustness, Basacote, Multicote, doses.

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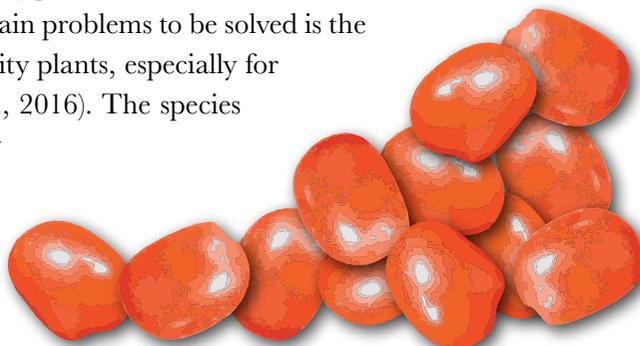
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INTRODUCTION

Southeastern Mexico is one of the regions with the greatest forestry vocation, reason why there is a significant increase in areas with forest plantations, which implies an increase in the production of nursery plants (Escamilla-Hernández *et al.*, 2015). In this sphere, one of the main problems to be solved is the production of vigorous and high quality plants, especially for native forest species (Carpenedo *et al.*, 2016). The species *Ormosia macrocalyx* Ducke, commonly known as caracolillo, is listed in NOM-059 (SEMARNAT, 2010) as an endangered species



(P). From an ecological perspective, it is important because it reaches nodulation rates by nitrifying bacteria of 48.9 mg g^{-1} , and as a seedling it responds favorably to high concentrations of CO_2 (700 ppm approximately) (Vargas-Simón *et al.*, 2018). It produces bright red seeds that are used to make necklaces, bracelets, earrings, curtains and ornaments, and its wood is considered valuable (SERFOR, 2016), important in cabinetmaking and carpentry, in the construction of bridges, canoes, railroad sleepers and house constructions (Vargas-Simón *et al.*, 2018). In livestock farming, it is used as shade for the livestock and, to a lesser extent, for round wood posts (Pérez-Hernández *et al.*, 2011).

The success of forest plantations depends on the quality of the plants produced in the nursery (Landis *et al.*, 2004), which Prieto and Sáenz (2011) define as the capacity of the plants to adapt and develop to the climate and soil conditions of the plantation site, and depends largely on their morphological and physiological genetic characteristics. Morphological attributes such as height, root collar diameter, root and stem dry weight, together with the relationships that can be established with these attributes, called morphological indices, among which robustness, proportion of aerial and root parts, and Dickson's index stand out, allow characterizing the quality of the plant produced in nursery in a quantitative way; this, in turn, allows making better decisions on the desired characteristics for the production of quality plants (Prieto *et al.*, 2009; Sáenz *et al.*, 2010).

At the national scale the survival rate of plantations is 50% one year after their establishment in the field, and the main causes of death are associated with the low quality of plants produced in nursery (Orozco-Gutiérrez *et al.*, 2010). After irrigation, fertilization is the cultural practice that most directly influences this key attribute of container-grown plants (Oliet *et al.*, 1999). According to Castro-Garibay *et al.* (2018), in most nurseries plant nutrition is generally done with water soluble fertilizers (WSF) applied with irrigation. This form of fertilization has disadvantages, such as nutrient loss by leaching, which can sometimes cause over-fertilization and promote an imbalance between the roots and the rest of the plant. Therefore, to solve this problem, controlled release fertilizers (CRF) are applied, which gradually transfer nutrients to the substrate, minimizing the risk of toxicity and reducing losses from leaching. In Mexico, the use of CRF in the production of forest plants is still incipient, although they are gradually entering the market. It must be considered that not all plants have the same nutritional needs, which is why applying and checking doses of these fertilizers through trials allows establishing their best use and at the same time determining nutritional requirements for each species (Terán-Soto, 2018). Based on the above, the effect of two controlled release fertilizers on *Ormosia macrocalyx* Ducke plants during their growth in the nursery stage was determined. Specifically, the effect of three concentration levels of two CRFs on morphological attributes and plant quality indices was evaluated.

MATERIALS AND METHODS

The study was carried out in the area of the facilities of Colegio de Postgraduados Campus Tabasco (Figure 3), located at Periférico Carlos A. Molina s/n, km. 3.5 Huimanguillo, Tabasco, Mexico. According to the Köppen classification system, the climate is classified as tropical Am (g) "w" (warm-humid) with abundant summer rains, with average annual

temperatures of 26.7 °C, and precipitation of 2,240 mm with a dry season in the months of March and April where less than 50 mm falls per month, and other rainy months such as September and October where precipitation is close to 400 mm per month (Palma-López *et al.*, 2007).

The seed came from a collection made in the area of the Experimental Field of the Tabasco-CP Campus, located at km 21 of the Cárdenas-Coatzacoalcos Federal Highway. Seed germination was conducted in greenhouse in cotyledon stage (seedlings), where it was scarified to accelerate its germination, and 15 days after emerged the seedling was transplanted to 310 cm³ polyethylene tubes, placed in polypropylene trays for 54 tubes, using 12 of them to place the experimental units corresponding to each treatment, leaving a central row of six cells for the separation between plants (Carpenedo *et al.*, 2016), placing them on a wooden structure inside the greenhouse (Castro-Garibay *et al.*, 2018).

A factorial arrangement (2×3) was used in a completely randomized design, with seven treatments, of which six resulted from the factorial combination of two controlled release fertilizers: Basacote[®] 9M (16-8-12) and Multicote[®] 12M (18-6-12), considering three doses: low, medium and high (10, 20 and 30 kg m⁻³ respectively) for each one, plus a control without fertilization. There were 12 replicates per treatment, each plant being an experimental unit, for a total of 84 experimental units.

Seedlings were evaluated for three months. Height was measured in centimeters (ruler ±0.01 cm), from the base of the stem to the growing apex. For diameter, a QL-V series digital Vernier caliper of 0-150 mm/0-6, resolution 0.01 mm, accuracy ± 0.02 mm, was used; the root collar was measured in millimeters (Villalón-Mendoza *et al.*, 2016). The number of leaves was obtained by counting each of the leaves of the plants in the treatments. The aerial and root biomass was quantified at the end of the experiment (Maldonado-Benítez *et al.*, 2011).

The aerial part (leaves and stems) was separated from the radical part (roots) in paper bags that were placed in a SHEL model CE5F oven with forced circulation for 72 hours at a constant temperature of 60 °C. At the end, each part was weighed on a VELAB model VE-5000 analytical balance with accuracy of 0.0001g (González-Orozco *et al.*, 2018). With the data of these variables, the calculation of plant quality indexes was performed: robustness index (RI), aerial and root dry biomass ratio (ADB/RDB R) and Dickson's quality index (DQI) (Reyes-Castro *et al.*, 2020), whose values were classified as: high, medium and low (Table 1).

The calculation of the indices and their classification was based on Escamilla-Hernández *et al.* (2015).

$$ADB / RDBR = \frac{\text{Aerial Dry Biomass (g)}}{\text{Root Dry Biomass (g)}}$$

$$RI = \frac{\text{Height (cm)}}{\text{Root Collar Diameter (mm)}}$$

$$DQI = \frac{\text{Total dry weight of the plant}}{\frac{H (cm)}{RCD (mm)} + \frac{ADB (g)}{RDB (g)}}$$

Where: *H* is height, in cm; *ADB* is aerial dry biomass; *RDB* is root dry biomass; *RCD* is root collar diameter.

The data were analyzed with variance (ANOVA) with the factorial design. Those variables that showed significant differences were subjected to Tukey's multiple means comparison test, with a significance level of $P \leq 0.05$, using Statistica, 2003.

RESULTS AND DISCUSSION

The results showed that the fertilizers evaluated in their different doses had a significant influence ($P \leq 0.05$) on the variables related to the growth and development of caracolillo plants (height, diameter and number of leaves). The best results were obtained with the application of Basacote[®]. Although Multicote[®] in its low dose showed statistically equal results to the first one. Regarding application rates, the three variables evaluated were significantly higher ($P \leq 0.05$) when applying the lowest (10 kg m^{-3}), and decreased as the dose increased. Fertilization in its different doses had a statistically significant effect on the growth in height of the caracolillo plants. Figure 1 shows the behavior of this variable in the last measurement (week 15), where it can be clearly observed that the highest growth in height was obtained with the low doses (10 kg m^{-3}) of both fertilizers (Basacote[®] and Multicote[®]), and in the case of Basacote[®] this same result can be observed in its medium dose (20 kg m^{-3}), showing significant statistical differences in relation to the control treatment, the remaining treatments and the control were statistically equal. According to Morales-Pérez (2018), the height of tropical forest plants should range between 15 and 30 cm so that they can be catalogued with good development in the nursery stage, which ensures their survival in the field; in this sense, the evaluated plants presented mean values within the suggested range (17 to 20 cm) for their release to the field.

Regarding diameter, in this last measurement, the low doses (10 kg m^{-3}) of both fertilizers were the ones with the greatest increase (Figure 2), showing significant statistical differences in relation to the other doses (except Basacote in the medium dose) and the control, which, according to the results, did not show significant statistical differences between them.

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

Quality index	High	Medium	Low
RI	<6.0	6.0 a 8.0	>8.0
BSA/BSR	1.5 a 2.0	2.0 a 2.5	>2.5
DCI	>0.5	0.2 a 0.5	<0.2

RI=robustness index; ADB/RDB=aerial and root dry biomass; DCI=Dickson's quality index.

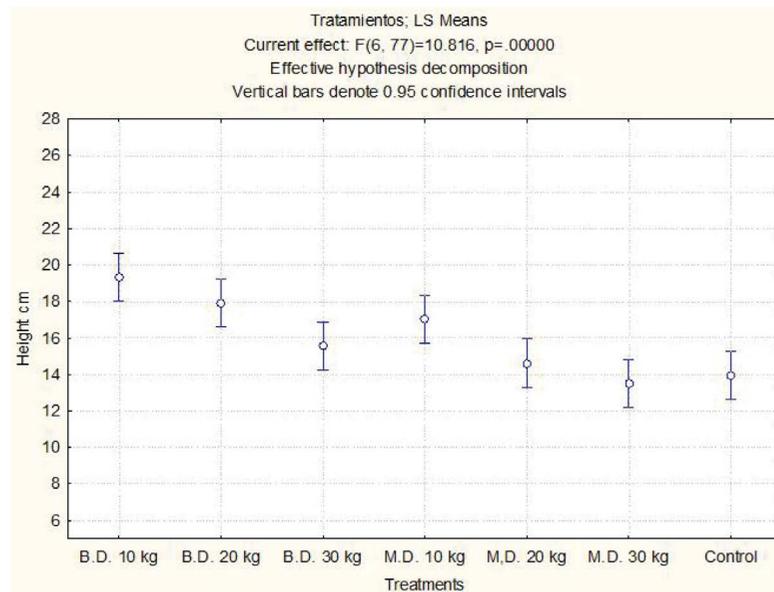


Figure 1. Height (cm) of caracolillo plants (nursery stage) of 15 weeks, with three doses of the controlled release fertilizers Basacote® and Multicote®. Vertical bars indicate the confidence interval (0.95).

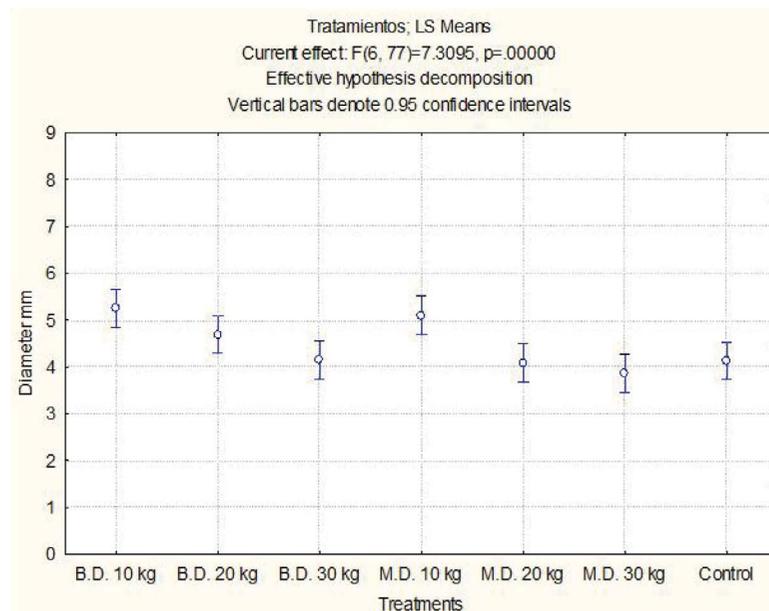


Figure 2. Diameter of caracolillo plants (nursery stage) of 15 weeks, with three doses of controlled release fertilizers, Basacote® and Multicote®. Vertical bars indicate the confidence interval (0.95).

Plants with diameters of 5 to 6 mm are more resistant to bending and tolerate better damage from pests and harmful fauna (CONAFOR, 2009). For the case of caracolillo, the average diameter of the low doses fluctuated between 5 and 6 mm, which can be considered as good, since the average value was above the suggested value (5 mm) (Sáenz *et al.*, 2010). On the contrary, the control treatment and the high doses presented values below this range. The CRFs have the advantage of releasing nutrients at a rate that

coincides with the plant's demand, thus avoiding losses from decreasing the frequency of fertilization, or from leaching, volatilization, or degradation, favoring plant quality (Reyes-Castro *et al.*, 2020).

Regarding the number of leaves, the low doses of both fertilizers presented the highest number, in relation to the control treatment (Figure 3); the latter was statistically equal to the medium and high doses of Multicote[®] fertilizer, but different from the low doses of both fertilizers, as well as the medium and high doses of Basacote[®] fertilizer. The plants had an average of seven and ten leaves in each treatment. The number of leaves is related to various physiological processes such as photosynthetic efficiency and transpiration area, in addition to representing the plant's capacity to store carbohydrates (Prieto *et al.*, 2009).

As for the quality indices, the RI varied between 3.4 and 4.0, an interval that corresponds to high quality plants in all treatments, although there were no statistical differences between them (Figure 4). Basacote[®] in its low and medium doses showed the highest average values. Prieto *et al.* (2009) indicate that plants with values lower than six are considered to be of high quality, robust, with vigorous stems, and apt to develop in sites with moisture limitations, in addition to being an indicator of the plant's resistance to desiccation by the wind.

The values of ADB/RDB R denote low quality plants (>2.5) in all cases (Figure 5), with the high dose of Basacote[®] fertilizer being the treatment that presents the lowest quality interval (highest value of ADB/RDB R); the value obtained in the control treatment presents the best quality characteristic (lowest value of ADB/RDB R), although statistically equal to the other treatments, except for Basacote[®] in its high dose (Reyes Castro *et al.*, 2020).

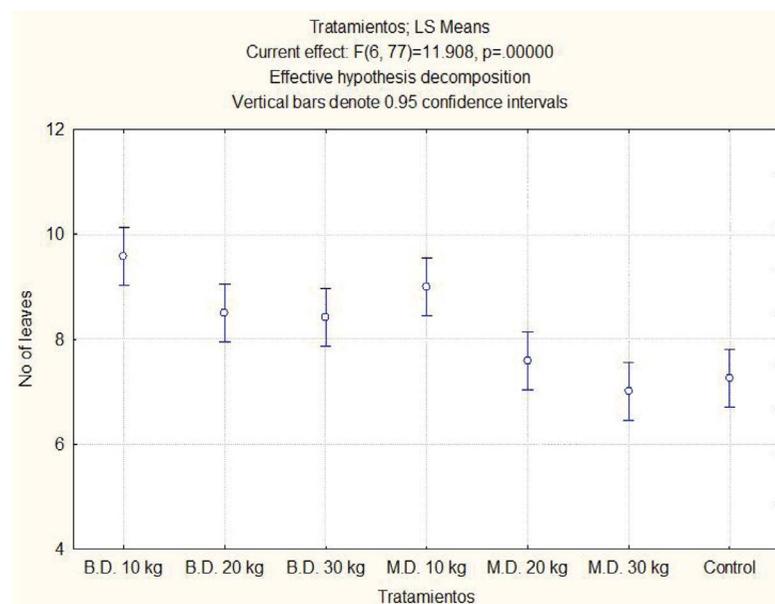


Figure 3. Statistical analysis of number of leaves in caracolillo plants (nursery stage) of 15 weeks, with three doses of controlled release fertilizers, Basacote[®] and Multicote[®]. The vertical bars indicate the confidence interval (0.95).

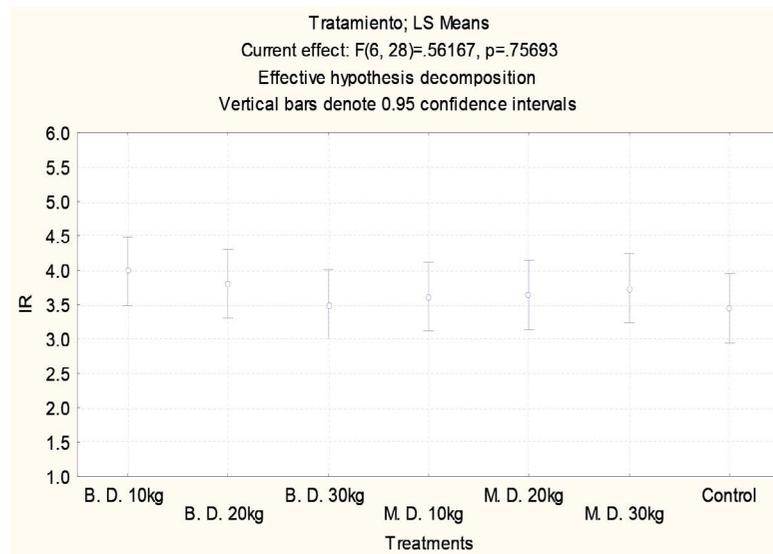


Figure 4. Statistical analysis of the robustness index in the nursery stage caracolillo plants, at week 15. Vertical bars indicate the confidence interval (0.95).

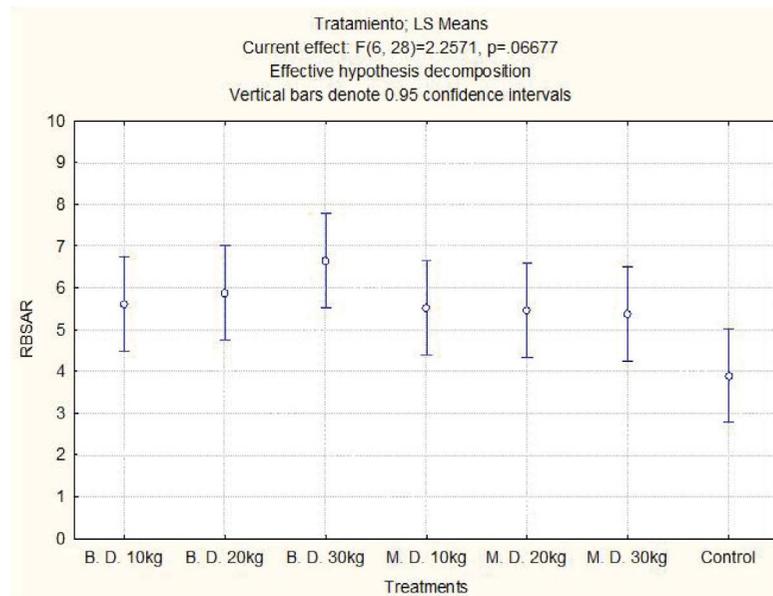


Figure 5. Effect of fertilizer doses on the aerial dry biomass/root dry biomass relationship of caracolillo plants nursery stage, in week 15. Vertical bars indicate the confidence interval (0.95).

In Dickson’s quality index (DQI), the results did not show significant statistical differences between treatments (Figure 6); they all corresponded to high quality plants. However, the best results were obtained with the low and medium doses of Basacote[®] fertilizer, with the low dose having the best average value, followed by the low dose of Multicote[®] and the control treatment, which presented values within the intervals suggested by Sáenz *et al.* (2010), who indicate that high quality plants are those with indexes >0.5.

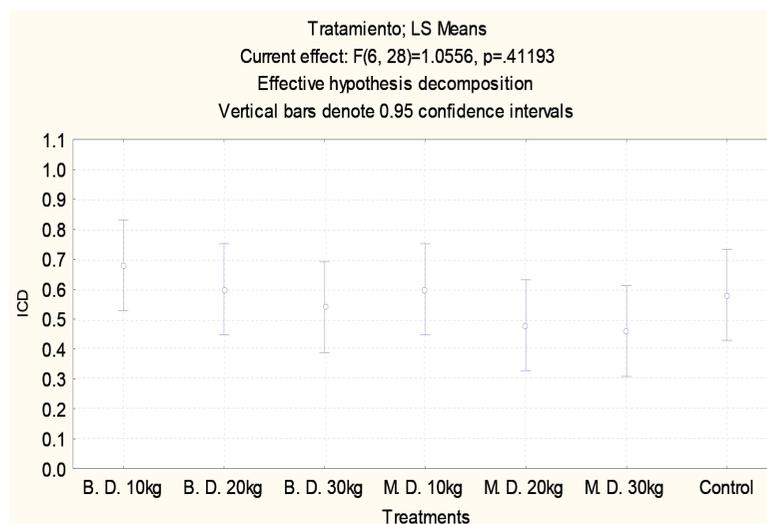


Figure 6. Effect of the fertilizer dose on the ratio of DQI in caracolillo plants (nursery stage) in week 15. The vertical bars indicate the confidence interval (0.95%).

CONCLUSIONS

The controlled release fertilizers Basacote[®] and Multicote[®] in their low doses (10 kg m⁻³) were, in general, the treatments that showed the best results in growth and development of caracolillo in the nursery stage. However, with respect to the robustness index (RI), aerial/radical dry biomass ratio (ADB/RDB R) and Dickson quality index (DQI), the treatments did not show significant statistical differences.

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Bovine milk production in Campeche: prospects and challenges for rural producers' development

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ABSTRACT

Objective: To characterize at the municipal level the productivity of the bovine-milk sector in the state of Campeche, Mexico; to determine the profitability of the sector and to propose improvement schemes in order to add value to the commercialization of the product.

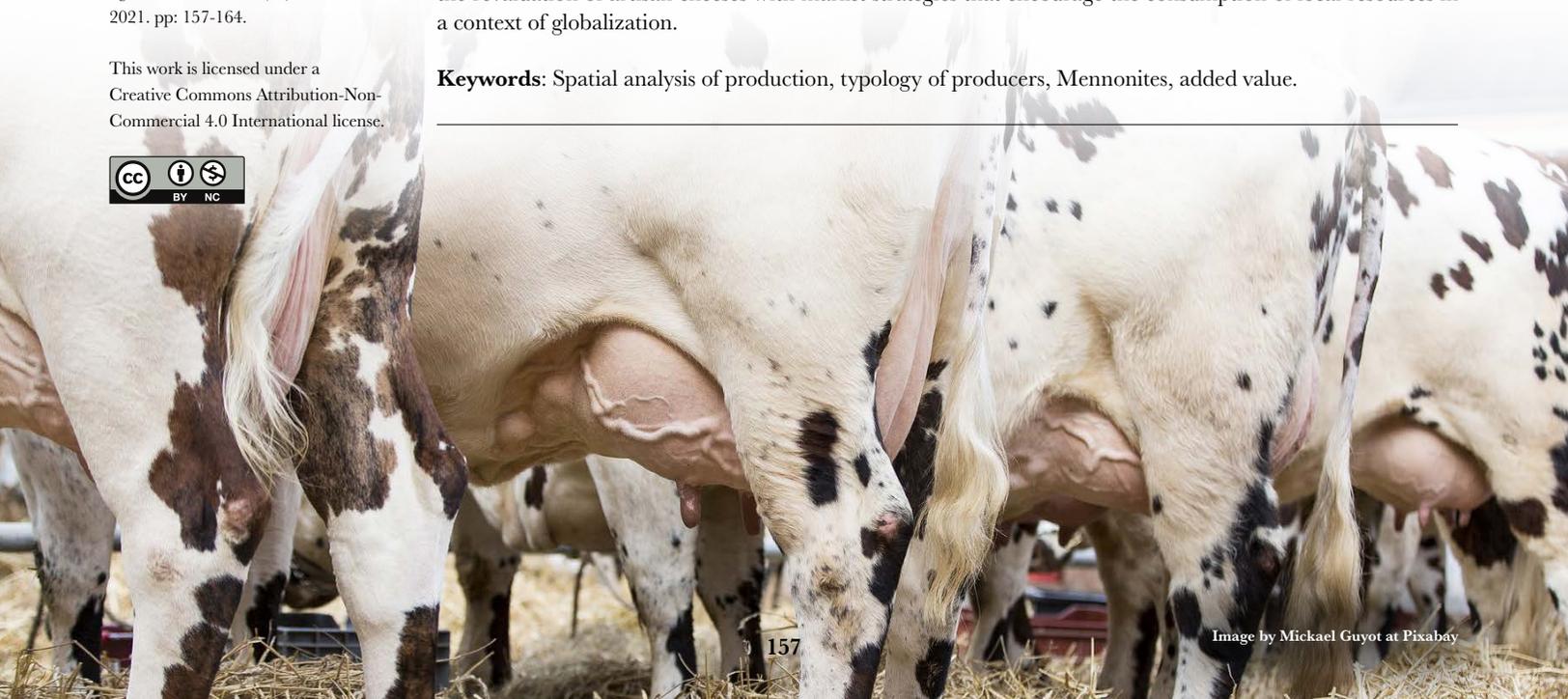
Design/Methodology/Approach: From the Agricultural and Fisheries Information System, the following variables were obtained: Production (thousands of L), Price (\$ L⁻¹) and Value of Production (thousands of \$) from 2006 to 2018. Through multivariate statistics and analyses, the profitability of the bovine-milk sector from 2013-2018 compared to 2007-2012 was determined. The analyses were completed by an interview with 12 milk producers who gave added value through the manufacture of cheeses.

Results: A drop in profitability (-8.8%) was found in the sector from 2013-2018 compared to 2007-2012, despite the increase in production of 6.2% that did not compensate for the fall in prices (-14.2%). The regions with the highest productivity were located from the center to the south of the state: Champotón (24.62%), Escárcega (17.36%), and Campeche (16.63%).

Study Limitations/Implications: Adding value to the milk commodity is a priority need in the short term, to avoid abandoning the activity, which could compromise the economy of rural producers.

Findings/Conclusions: The Mennonite settlements in Campeche have managed to add value to the bovine-milk sector through the manufacture and sale of artisan cheeses. However, the great challenge of the sector is the revaluation of artisan cheeses with market strategies that encourage the consumption of local resources in a context of globalization.

Keywords: Spatial analysis of production, typology of producers, Mennonites, added value.



INTRODUCTION

Mexico produces annually around 12 billion liters of milk of bovine origin, and the main producing states are Jalisco (20.27%), Coahuila (11.27%) and Durango (10.19%); Campeche contributes 0.37% which places it in 23rd place nationally (SIAP, 2018). The production of bovine milk in the state of Campeche is a secondary activity, which complements the dual-purpose livestock farming that predominates in the center-south of the state (Loera and Banda, 2017). Uzcanga *et al.* (2015) attributes the low productivity of bovine-milk in the state of Campeche to the deficiencies and in some cases lack of infrastructure, equipment for animal management, and fodder; in addition to insufficient technical assistance and training. Adding to this scenario is the fact that milk is marketed as a commodity product in which quality is not differentiated nor added value provided (Loera and Banda, 2017).

Milk constitutes a basic food basket product in the diet of the Mexican population and is considered key in nutrition for food security (Aguilar, 2015). According to Rodríguez-González *et al.* (2015), backyard livestock production in rural communities contributes to food security in developing countries. For their part, Loera and Banda (2017) consider that backyard livestock production should be complemented with marketing schemes that add value to products and improve producers' profitability. In this context, the objective of this study was to characterize the productivity of the bovine-milk sector in the state of Campeche at the municipal level, using multivariate statistics and spatial analysis of production, to determine the profitability of the sector and eventually to propose schemes to improve the commercialization and marketing of the product.

MATERIALS AND METHODS

Study Area

This study considered bovine-milk productivity from 2006 to 2018, from the state of Campeche in southeastern Mexico, according to statistics from the Agrifood and Fisheries Information Service (Servicio de Información Agroalimentaria y Pesquera, SIAP, 2018).

Bovine-milk productivity in the state of Campeche

From SIAP (2018), the following variables were obtained: Production (thousands L), Price (\$ L⁻¹) and Production Value (thousands \$) at the state level and by municipality, from 2006 to 2018. Temporal regression models were adjusted to determine the evolution in the production and profitability of bovine-milk at the state level (Gujarati, 2007).

Municipal characterization of bovine-milk productivity

The differences in productivity by municipality from 2006 to 2018 were performed with a Multiple Variance Analysis (MANOVA) and Tukey's means tests with a reliability level of 95%. To determine the regions with the highest bovine-milk productivity in the state of Campeche, annual production by municipality was summed for the period 2006-2018. The results were grouped into five class intervals: very low, low, medium, high and very high production, and spatially represented with the help of ArcMap 10.3 software (ESRI, 2015).

In addition, a cluster analysis was used to establish a typology of municipalities; for this purpose, the averages of the variables Production, Price and Production Value were standardized, and with the help of the statistical software R (R-Studio, 2020), the differences between the elements were calculated using the Euclidean distance method and the grouping by Ward's method.

Profitability of bovine-milk productivity at the municipal level

The profitability of bovine-milk productivity from 2007 to 2018 by municipality was analyzed with the decomposition model of production growth factors proposed by Gómez (1994). The model determines how profitable bovine-milk production is from a base period (2007-2012) with respect to a final period (2013-2018), according to the percentage variations of Production (L) and Price (\$).

Value added to the milk commodity through cheese production

Through targeted sampling, between January and March 2021, 12 bovine-milk producers were interviewed who have managed to add value to the commodity system by making cheese. The producers were selected based on their availability, leadership and positive references as cheese producers in the state. The 12 producers interviewed were located in the Mennonite farm El Temporal, in the municipality of Hopelchén. The interview was directed at defining the cost-benefit of cheese production and the main points of commercialization.

RESULTS AND DISCUSSION

Bovine-milk productivity in the state of Campeche

Bovine milk production in the state of Campeche presented a growing linear trend from 2006 to 2018 (P-Value <0.0001; $R^2=0.9096$). According to SIAP (2018), this increase coincides with an increase in the number of animals of 3.5% per year sustained by Campeche since 1980. However, as shown in Figure 1, the increase in production was accompanied by a fall in prices that impacted the production value. Loera and Banda (2017) found that bovine milk production in Campeche occurs as a secondary activity where meat production is prioritized in the framework of dual-purpose livestock farming; this explains why the activity is maintained even when it seems unprofitable (García *et al.*, 2018).

Municipal characterization of bovine-milk productivity

The highest indicators of bovine-milk productivity and profitability were obtained in three municipalities, which accounted for 58.61% of productivity: Champotón (24.62%), Escárcega (17.36%), and Campeche (16.63%) (Table 1). By associating productivity spatially with the hierarchical cluster analysis, two groups were differentiated: the municipalities in one group are similar regarding their productivity, but different from the municipalities in the other group (Pardo and Del Campo, 2007) (Figure 2).

Figure 2 shows that the highest bovine-milk productivity in Campeche was located in the center-south of the state; this is explained by the fact that, historically, livestock

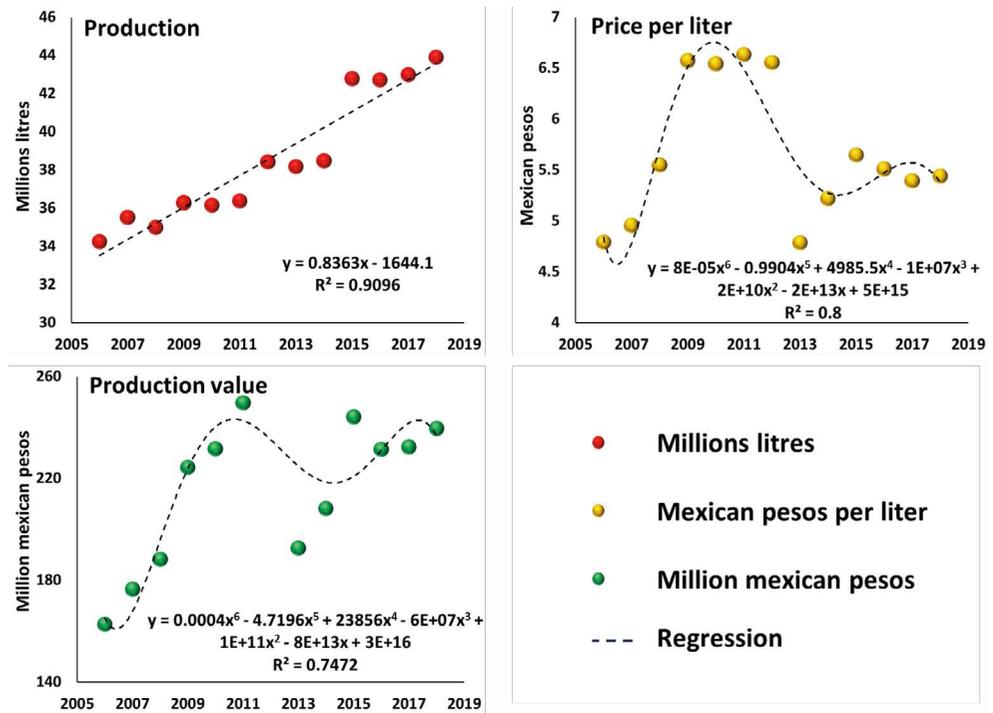


Figure 1. Evolution of the production, price, and value of the production of the bovine-milk system in the State of Campeche from 2006 to 2018.

Table 1. Indicators of bovine-milk productivity and profitability from the municipalities in the state of Campeche for the period 2007-2018.

Municipalities	Production (Thousands litres)	Price (Mexican pesos per liter)	Production value (Thousands Mexican pesos)
Calakmul	1399.31 E	5.22 A	7311.74 E
Calkiní	88.45 F	5.32 A	485.13 E
Campeche	6413.13 B	6.13 A	38790.79 B
Candelaria	4950.90 C	5.87 A	29081.74 C
Carmen	4703.61 C	6.02 A	28253.96 CD
Champotón	9492.40 A	5.16 A	49061.18 A
Escárcega	6692.69 B	5.66 A	37899.63 B
Hecelchakán	686.62 EF	5.56 A	3760.49 E
Hopelchén	446.11 F	6.05 A	2674.07 E
Palizada	3633.79 D	5.82 A	21306.15 D
Tenabo	53.53 F	5.10 A	272.85 E

Means followed by the same letter in the column do not differ statistically (Tukey, $\alpha=0.05$).

activities have been developed in the south and agricultural activities in the north, with the center being a transition region (Uzcanga *et al.*, 2015). However, according to Loera and Banda (2017) in the south of the state cattle ranching for meat production predominates and in the center-north, dual-purpose cattle production.

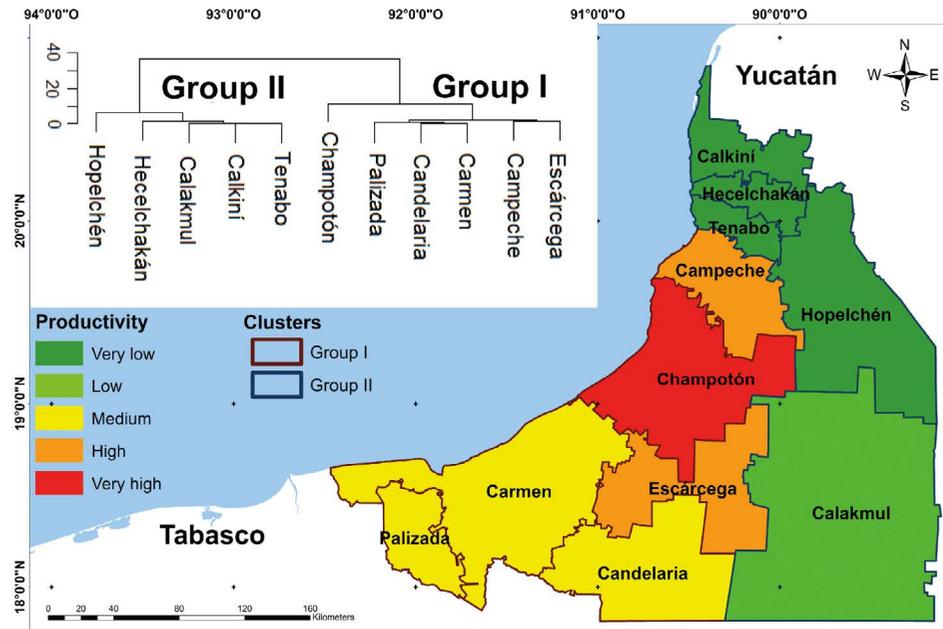


Figure 2. Spatial representation of the typology in bovine-milk productivity at the municipal level in the state of Campeche from 2006 to 2018.

The hierarchical analysis differentiated Champotón as the municipality with the highest productivity in Group I and Hopelchén in Group II. The high productivity of Champotón is explained by the reconversion of agricultural areas to livestock production (Santillán-Fernández *et al.*, 2020). In the case of Hopelchén, despite not having the same productivity as the central-southern municipalities, it stands out among the municipalities in the north, and this coincides with the settlement of Mennonite groups (Porter-Bolland *et al.*, 2008).

Profitability of bovine-milk productivity at the municipal level

By the factor decomposition model of production growth (Gómez, 1994), it was found that in 2013-2018 the profitability of producing bovine-milk in the state of Campeche was lower by -8.8% compared to the period 2007-2012, despite the fact that there was an increase in production of 6.2% that failed to cushion the fall in prices (-14.2%). According to Loera and Banda (2017) the loss in profitability of the dairy sector can be improved by adding value to the product, and gradually abandoning markets that privilege the purchase and sale of milk as a commodity.

Figure 3 shows that only the municipality of Palizada reduced its production, while the remaining municipalities increased it. However, the fall in prices that the sector has maintained since 2013 (SIAP, 2018) caused only Champotón, Calakmul and Tenabo to have an increase in the profitability of producing bovine-milk; Hopelchén turned out to be the municipality with the lowest profitability. According to Vargas-Godínez and García-Ortega (2018) the low profitability of milk production is a factor that explains cheese production in Mennonite communities of Hopelchén which seek to add value to the bovine-milk product system.

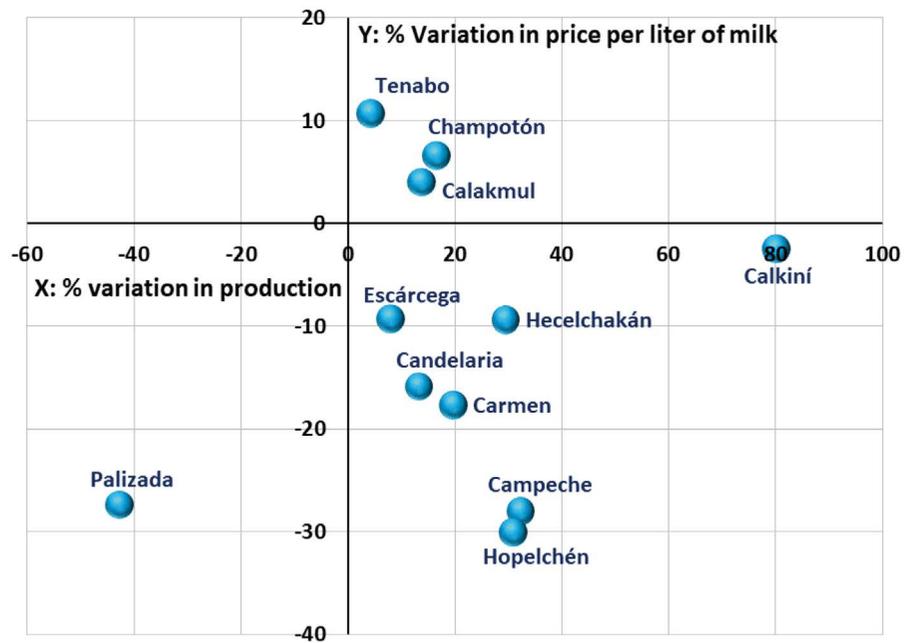


Figure 3. Profitability in bovine-milk production by municipalities in Campeche in 2013-2018 compared to 2007-2012. Municipalities above the x-axis presented an increase in their profitability.

Value added to the milk commodity through cheese production

The interview with 12 bovine-milk producers in the Mennonite farm El Temporal, in the municipality of Hopelchén, allowed learning that the strategy followed by this community in the face of falling milk prices has been the manufacture of Chihuahua-type cheese or also known as Mennonite cheese. López-Díaz and Martínez-Ruiz (2018) consider that the tradition that Mennonite groups have in the manufacture and marketing of cheese has allowed them to open market niches in southeastern Mexico.

According to the results of the interview, 6 to 8 L of milk are invested for each kg of cheese, and a profit/cost of 1.78 is achieved, as a result of a production cost of \$42.00 and a sales price of \$75.00. The cheeses are marketed mainly in Mérida, Yucatán (80%) and the remaining 20% is distributed in the state of Campeche, with the main destinations being the cities of Campeche, Champotón and Hopelchén. The producers agreed that without cheese elaboration, milk production would be an unsustainable activity for the Mennonite community. Villegas-de Gante and Cervantes-Escoto (2011) consider that southeastern Mexico has great potential for the production of genuine cheeses from cow's milk. Although Campeche has little tradition in cheese making (Agudelo-López, 2015), Grass-Ramírez *et al.* (2015) found that the transformation of milk into cheese is the easiest way to add value and improve the profitability of rural dairy farmers.

CONCLUSIONS

The bovine-milk sector in the state of Campeche shows a loss in profitability from 2012-2018 compared to 2007-2012, as a consequence of a reduction in prices per liter of milk. Given this scenario, adding value to the product seems to be a priority in the short term,

to avoid the abandonment or reconversion of the activity to other production systems, which could compromise the economy of rural producers in a context of food security. The cheese production and marketing model implemented by the Mennonite community in the north of the state of Campeche can help to improve the profitability of the bovine-milk product system. However, it should be considered that the great challenge for the sector is the revaluation of artisanal cheeses with market strategies that encourage the consumption of local resources in face of a context of globalization.

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Environmental services in home gardens of la Chontalpa, Tabasco, Mexico

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ABSTRACT

Objective: To identify the environmental services provided by home gardens in nine communities of La Chontalpa region in Tabasco, Mexico.

Design/methodology/approach: Having previously identified home gardens, a visit route to nine communities was drawn using cartographic and demographic information. Three home gardens per community were randomly selected. Interviews about the use of home gardens were conducted. Physical environmental factors were measured inside and outside the gardens and soil samples were taken to determine organic matter and carbon contents in the soil. According to their category, the environmental services provided by the home gardens in each community were identified and classified into four types: provisioning, supporting, cultural, and regulating services.

Results: Thirteen environmental services were identified as being provided by home gardens. Provisioning and cultural services are present in all localities. The most frequent category is the provision of food, followed by the provision of raw materials and education. Supporting and regulating services were observed in most communities —except for climate regulation, since the environmental conditions only allow gardens to provide the said service in four of the evaluated communities.

Limitations on study/implications: Knowledge about the benefits of building home gardens suggests that these agrosystems provide environmental services. Categorizing the environmental services provided by home gardens is therefore important.

Findings/conclusions: Home gardens in La Chontalpa, Tabasco, do not only provide provisioning services, but also cultural, supporting, and —to a lesser extent— regulating services.

Keywords: Provisioning Services, Supporting Services, Agrosystems, Rural Communities, Welfare.

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INTRODUCTION

The environmental services topic is particularly related to large surface ecosystems and agroecosystems (Ruiz *et al.*, 2007; Palma-López *et al.*, 2011). Paying for these services has rarely been considered relevant in the case of home gardens (HG). Home gardens are



considered by definition to be sustainable agroecosystems (Colin *et al.*, 2012; Montañez *et al.*, 2012), since they provide different environmental services, such as: food, raw materials, preservation of local and regional biodiversity, reservoirs of germplasm of both flora and fauna, and soil fertility preservation, among others (Alayón and Gurri-García, 2009; Sol, 2012).

Home gardens in Tabasco, Mexico, have long achieved transcendence, as a consequence of their worldview (Van der Wal *et al.*, 2011; Mariaca, 2012), and they maintain their own characteristics. In order to adopt a more global approach for the implementation of home gardens in the tropic, identifying the environmental services that they provide must be the first step. The objective of our research was to highlight the importance of home gardens by identifying and characterizing the different environmental services that these systems provide.

MATERIALS AND METHODS

The study area was located in the region of La Chontalpa, in the State of Tabasco, Mexico, and included the municipalities of Huimanguillo, Cárdenas, and Comalcalco (Figure 1). The communities were selected based on maps: a transect line was drawn in three representative physiographic zones of the region, focusing on rural communities (INEGI, 2010). Other factors considered were: distance (km), accessibility, and —first and foremost— the localities' availability and willingness to cooperate. Three communities per physiographic zone were chosen and, ultimately, nine localities were studied: Villa Guadalupe, La Soledad, Gustavo Díaz Ordaz, Pedregalito, Tierra Nueva 3ra sección, Miguel Alemán Valdez, El Suspiro, Ignacio Zaragoza, and Ejido C-22 (Lic. José María Pino Suárez). Subsequently, three home gardens per community were chosen and georeferenced (Figure 1).

Soil samples were taken from each intervened home garden at a depth of 0-20 cm, from five randomly selected points. Approximately one kilogram of soil sample was extracted from each sampling point and homogenously mixed in plastic bags to be transferred to the laboratory. Additionally, sunlight incident, environmental temperature, and humidity percentage values were registered within and outside every sampled home garden, in order to obtain an environment control, with the help of a Kestrel 4000 portable weather station.

The dry soil samples were grinded and put through a 2-mm mesh sieve. Afterwards, they were sent to the Laboratorio de Análisis de Suelos, Plantas y Agua (LASPA), where their organic matter, pH, texture, carbon, and bulk density values were determined, based on the methods established by the NOM-021-RECNAT-2000 (DOF, 2002); and those values were later used to estimate the organic carbon sequestration.

Subsequently, with the help of georeferencing, soil analysis and environmental parameters, the environmental services provided by the home gardens of each visited community were identified and —based on the classification proposed by the Millennium Ecosystem Assessment (MEA, 2005)— they were classified according to their function as follows: Provisioning Services, Supporting Services, Cultural Services, and Regulation Services.

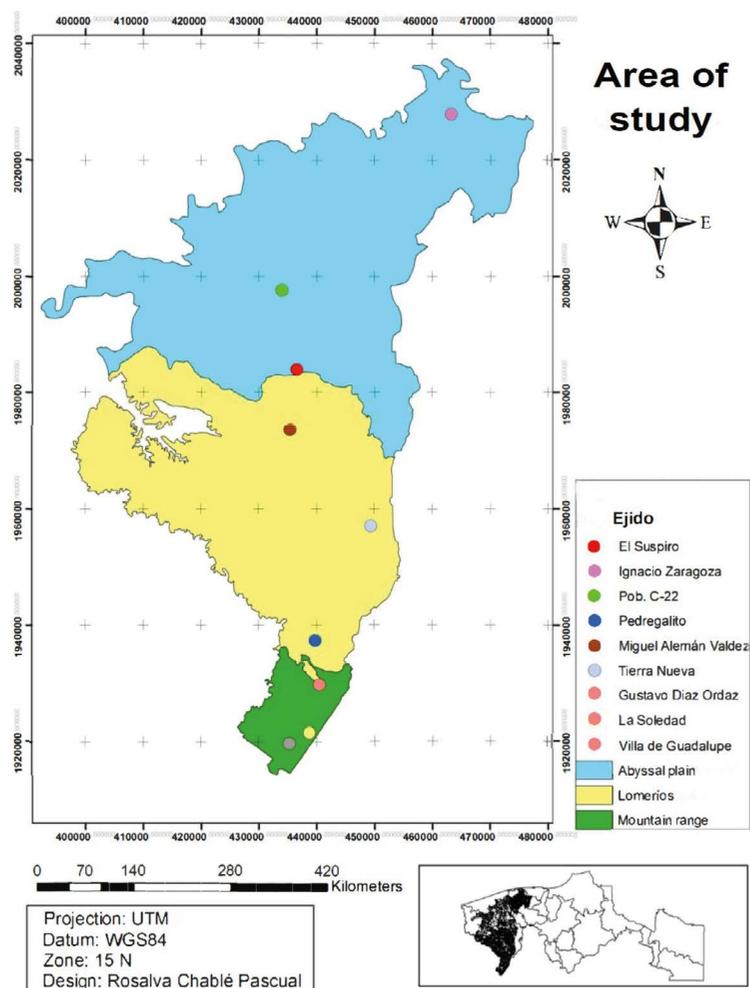


Figure 1. Cartography of the study area and geolocation of intervened communities.

RESULTS AND DISCUSSION

Physical and demographic factors. The physical environmental factors (*i.e.*, more vegetation) make home gardens ideal places for the comfort of their inhabitants (Mariaca, 2012). The sunlight incident, temperature, and humidity results provide a general picture of how these factors change throughout the day.

Regarding the light, an important decrease of the incident was observed throughout the day within the home gardens, when compared to the surrounding exterior areas. In Villa Guadalupe, for instance, an incident of $1,882 \mu\text{E m}^{-2} \text{s}^{-1}$ was recorded outside the gardens (the values increased from 12:00 to 15:00 h); during the same period, values of $14\text{-}99 \mu\text{E m}^{-2} \text{s}^{-1}$ were recorded inside the gardens (a significantly lower figure). In La Soledad, the light incident outside homes garden reached $2000 \mu\text{E m}^{-2} \text{s}^{-1}$ (these values increased after 12:00), while the variation inside the home gardens was lower, reaching a maximum of $260 \mu\text{E m}^{-2} \text{s}^{-1}$ between 12:00 and 13:00 h. The lowest sunlight incident values for home gardens were recorded in Gustavo Díaz Ordaz, where the highest value was $89 \mu\text{E m}^{-2} \text{s}^{-1}$ (HG1) at noon and the lowest, $14 \mu\text{E m}^{-2} \text{s}^{-1}$ (HG3).

With regard to the environmental temperature within the home gardens, similar values were registered among the assessed communities, where 27.9-29.9 °C average values were recorded throughout the day —except in Pedregalito, where the recorded average was 34.4 °C at HG2. The average temperature recorded in all communities were the same inside and around the visited home gardens. The highest temperature points were observed between 13:00 and 16:00 h, with a temperature drop starting from 18:00 h onwards.

The average relative humidity values exceeded 60.8% and were similar inside and outside the HG. At the Ejido C-22 and Ignacio Zaragoza, the average relative humidity exceeded 85% in the four sampled zones. The highest values were registered between 02:00 and 06:00 h and started to drop from 14:00 h onwards in all sampled sites.

Based on the analysis of the environmental data for all home gardens, the light reached its maximum level between 12:00 and 13:00 h, with an average value of $195 \mu\text{E m}^{-2} \text{s}^{-1}$. This confirms that the light incident is mitigated in areas with more trees. Temperature and relative humidity remain similar inside and outside the HG, although humidity varies according to the light incident: the higher the light incident, the lower the humidity. Mariaca (2012) states that home gardens protect houses against direct sunshine, cooling the space down and preventing abrupt and excessive temperature and environmental humidity changes. Likewise, Poot-Pool *et al.* (2012) and Sol (2012) mention that backyard crops bring comfort to the household, since their configuration and structures help regulate the house's temperature, an assumption that could allegedly be extrapolated to the microregion where the crops are located.

In average, home gardens represent 23% of the village surface. The ratio of home gardens to other ecosystems is highest in the community of Ignacio Zaragoza (29%) and lowest in La Soledad. The average ejido surface is 1038 ha: the smallest is found in Ignacio Zaragoza (80 ha) and the largest in C-22 (4171 ha). The average village surface is 83 ha, the smallest being El Suspiro and La Soledad with 42 ha. The most populated village is C-22 (INEGI, 2010) (Figure 2a).

Soil analysis. The analysis of carbon (C) and organic matter (MO) in the soil of the sampling areas presented mixed results (Table 1). The highest values were found at the



Figure 2. Home gardens in the alluvial plain physiogeographic zone: a. Ejido C-22 (Lic. José María Pino Suárez); b. El Suspiro.

Table 1. Soil analysis results and sequestered carbon calculation in the visited home gardens.

Ejido	HG	OM (%)	OC (%)	BD	Depth (m)	Kg C m ²	t ha ⁻¹	CO ₂ SEQ
Villa Guadalupe	1	5.9	3.0	1.2	0.2	0.7	7.1	26.0
	2	4.8	2.4	1.2	0.2	0.6	5.8	21.1
	3	4.1	2.1	1.1	0.2	0.5	4.5	16.6
Soledad	1	6.4	3.2	1.25	0.2	0.8	8.0	29.4
	2	5.1	2.6	1.25	0.2	0.6	6.4	23.4
	3	4.1	2.1	1.25	0.2	0.5	5.1	18.8
Gustavo Díaz Ordaz	1	2.8	1.4	1.15	0.2	0.3	3.2	11.8
	2	1.5	0.8	1.25	0.2	0.2	1.9	6.9
	3	1.1	0.6	1.25	0.2	0.1	1.4	5.0
Pedregalito	1	3.8	1.9	1.2	0.2	0.5	4.6	16.7
	2	2.6	1.3	1.15	0.2	0.3	3.0	11.0
	3	1	0.5	1.15	0.2	0.1	1.2	4.2
Tierra Nueva 3 rd section	1	5.6	2.8	1.25	0.2	0.7	7.0	25.7
	2	3	1.5	1.25	0.2	0.4	3.8	13.8
	3	2	1.0	1.25	0.2	0.3	2.5	9.2
Miguel Alemán Valdez	1	2.8	1.4	1.25	0.2	0.4	3.5	12.8
	2	2	1.0	1.25	0.2	0.3	2.5	9.2
	3	1	0.5	1.25	0.2	0.1	1.3	4.6
El Suspiro	1	4	2.0	1.1	0.2	0.4	4.4	16.1
	2	2	1.0	1.1	0.2	0.2	2.2	8.1
	3	1.1	0.6	1.15	0.2	0.1	1.3	4.6
C – 22	1	3	1.5	1.1	0.2	0.3	3.3	12.1
	2	2.6	1.3	1.25	0.2	0.3	3.3	11.9
	3	1.8	0.9	1.15	0.2	0.2	2.1	7.6
Ignacio Zaragoza	1	2.3	1.2	1.25	0.2	0.3	2.9	10.6
	2	1.5	0.8	1.15	0.2	0.2	1.7	6.3
	3	1.8	0.9	1.2	0.2	0.2	2.2	7.9

HG: Homegarden; OM: organic matter; OC: organic carbon; BD: bulk density; SEQ: sequestration

Villa Guadalupe and La Soledad communities (MO= 4.1–6.4%; C= 2.1–3.2%), while the Miguel Alemán Valdez and Ignacio Zaragoza villages presented the lowest figures (MO= 1-2.8%; C=0.5-1.2%).

Potential carbon sequestration values are not high in home gardens (<10 t ha⁻¹); however, the said values are considerable for environmental services payment (ESP) benefits (Hernández *et al.*, 2016). The low C content in the soil might be the result of constant cleaning at home gardens (Cotler *et al.*, 2016). The low MO contents in the soil might be the result of the high use of nutrients made by cultivated plants and of the tendency of MO to oxidize —which releases nutrients (Rimski-Korsakov and Álvarez, 2016).

Identification and characterization of environmental services. Cultural services (CS) were identified in all gardens of the visited communities (Table 2). SC quantification is

Table 2. Identification of environmental services per type in every assessed community according to MEA (2005).

Community	PS			SS				CS		RS			
	1	2	3	4	5	6	7	8	9	10	11	12	13
Villa Guadalupe	X	X	X	X	X	X		X	X	X		X	X
La Soledad	X	X	X	X	X	X		X	X	X		X	X
Gustavo Díaz Ordaz	X	X		X	X	X		X	X	X		X	X
Pedregalito	X	X		X	X	X	X	X	X	X	X	X	X
Tierra Nueva 3 rd section	X	X		X	X	X	X	X	X	X	X	X	X
Miguel Alemán Valdez	X	X	X	X	X	X	X	X	X	X	X	X	X
El suspiro	X	X		X	X	X	X	X	X	X	X	X	X
C-22	X	X		X	X	X		X	X	X		X	X
Ignacio Zaragoza	X	X	X	X	X	X	X	X	X	X		X	X

PS: Provisioning services, SS: Supporting services, CS: Cultural services, RS: Regulating services. 1: Food, 2: Raw materials, 3: Genetic material, 4: Soil formation, 5: Pollination, 6: Biological control, 7: Home, 8: Education, 9: Culture, 10: Gas regulation, 11: Weather regulation, 12: Erosion mitigation, 13: Nutrient cycle regulation.

more subjective (De Groot *et al.*, 2010), but those services are easier to identify (Rodríguez *et al.*, 2016; Merlotto and Verón, 2019). In the case of home gardens, educational CS are very important, because knowledge about crop development can be taught to family members from an early age, thus generating cultural growth among households (Palma-López *et al.*, 2011; Mariaca, 2012).

Food and raw materials were identified among the provisioning services (PS) in all communities. This matches the findings of Palma-López *et al.* (2011), who emphasize that, with regard to their own concept, home gardens offer these kinds of ecosystemic services to each household where they are implemented. Genetic material PS were only found in Villa Guadalupe, La Soledad, Miguel Alemán Valdez, and Ignacio Zaragoza, probably as a result of the greater plant diversity of these communities' gardens.

Supporting (SS) and regulating services (RS) were identified in almost every HG of the communities. However, the capacity to provide habitat SS and climate RS was only observed at the communities of Pedregalito, Tierra Nueva 3ra Sección, Miguel Alemán Valdez, and El Suspiro (Figure 2b). Environmental conditions for these services within HG were only found in the aforementioned communities, primarily as a result of the stability of the said conditions and a greater difference compared to the conditions outside the HG. Miguel Alemán Valdez and El Suspiro are very likely to benefit from the environmental services payment (ESP) program, just as was pointed out by Palma-López *et al.* (2011) and Chablé-Pascual *et al.* (2015).

The environmental conditions of home gardens generate many benefits for households (Mariaca, 2012; Poot-Pool *et al.*, 2012). In this respect, environmental services are an important alternative for the inhabitants of the visited communities (Colin *et al.*, 2012). The economy of most of the households in the sampling communities that own HG depends on primary production activities (Chablé-Pascual *et al.*, 2015). These gardens increase the

households' comfort, not only with the on-farm consumption of its produce, but also with socioeconomically and environmentally healthier surroundings.

The most important environmental service present in the sampled HG was food provisioning. Alayón and Gurri-García (2009), Sol (2012), and Vázquez *et al.* (2012) state that species used as food are key resources in the HG, since they have a positive impact on the household diet and nutrition. Juan (2013) reports that the use of produce obtained from HG is very important in the household diet and that 63% of the said produce is cooked by the owners of the HG.

The raw materials provisioning service is essential in the sampled HG, whose owners mentioned the use of resources such as composts for other crops, natural or herbal medicine, fodder, and handmade products such as jams and spicy sauces. Pérez *et al.* (2012) and Sol (2012) mention that one social function of the HG is satisfying the inhabitants' needs, not only with food, but also with raw materials for fuel and construction, as well as fodder for the farm animals that live within the garden. Guzmán *et al.* (2012) report that, in the HG of Olcuatitán, Nacajuca, Tabasco, only 35% of garden products are used as food; the use of raw materials for other purposes is more important. Those purposes include: non-timber products (13%), construction (7%), medicine (29%), fuel (85%) and others (16%).

CONCLUSIONS

Promoting the implementation of home gardens and extending the value chains derived from these sustainable agrosystems is beneficial for the life quality of the inhabitants of the rural communities of La Chontalpa, Tabasco, Mexico. Home gardens provide several environmental services in the visited communities —although provisioning and cultural services stand out. We recommend transferring the knowledge about environmental services in home gardens, in order to increase the commitment of the owners of these systems to maintain and diversify the products they offer.

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Nutritional Chemical Analysis of Taro (*Colocasia esculenta* Schott) Accessions from the State of Tabasco, Mexico

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ABSTRACT

Objective: To determine the main nutritional properties and content of bioactive compounds of five varieties of taro, grown in the municipalities of Huimanguillo and Cunduacán, Tabasco.

Methodology: The Campechana, Chiapaneca, Tabasqueña, Dulce (P1 and P2) varieties were evaluated to determine their proximal chemical composition, non-nutritional compounds, and antioxidant activity. An analysis of variance and the Tukey test were applied to the data obtained.

Results: These varieties had a protein, lipid, ash, and moisture content of 1.13-3.95, 0.64-2.94, 1.58-7.32, and 2.28-5.34%, respectively. In all cases, the starch content stood out with 62.7-74.7% values. This compound is important in the food industry because its functional properties enable its use in various food systems or other applications. Among the non-nutritional compounds, the following concentrations were identified: phytates (0.235-0.40 Eq/g sodium phytate/100 g sample); total phenols (0.54-5.76 Eq/g gallic acid/100 g sample); condensed phenols (1.20-1.51 Eq/mg catechin/100 g sample); saponins (0.37-1.02 Eq/mg Diosgenin/100 g sample); and trypsin inhibitors (0.00-0.70 UIT/100 g sample). Finally, the antioxidant activity had 0.29-0.57 Eq/mg of trolox/g of flour values.

Limitations: This work had no limitations.

Conclusions: The varieties evaluated presented low concentration of non-nutritional compounds and adequate antioxidant activity; therefore, the taro varieties from the State of Tabasco can be considered sources of starch and compounds with antioxidant activity.

Keywords: *Colocasia esculenta*, antioxidants, taro, nutritional, starch.



INTRODUCTION

Taro (*Colocasia esculenta* Schott) is an annual herbaceous with a perennial life cycle (Araceae); it is native to Asia, Africa, and Oceania. It has round ovoid tubers, starchy flesh, and dark brown skin (Viloria and Córdova, 2008), it is both used as food for humans and animals, although it also has industrial uses (Madrigal *et al.*, 2018; Temesgen, 2015). The value of taro as a food is attributed to the easy digestibility of its starch granules, its high energy value, and its content of vitamins A and C, as well as of some elements of the B complex and minerals, such as calcium, phosphorus, and iron (Onwueme, 1999; Temesgen, 2015). Before they are consumed, they must be baked, roasted or fried; its new leaves and petioles can be consumed as vegetables in traditional meat and fish dishes (Lamrot, 2018; Púa *et al.*, 2019).

In Mexico taro is grown in Puebla, Sinaloa, and Oaxaca; however, it has only prospered as a commercial product in Veracruz. The cultivation of taro has increased in the State of Tabasco. Some producers in the municipalities of Cunduacán and Huimanguillo, Tabasco, have used some hectares to grow taro, but they are still not enough. Considering the agroclimatic conditions, the agronomic management is inadequate. Therefore, producers should become acquainted with the nutritional potential of this species, especially its bromatological characteristics and its functionality, in order to increase the value chain that will support greater commercialization and consumption in Tabasco.

Some producers have begun to use taro to make breadmaking flour mixed with wheat flour or to prepare a traditional drink known as horchata; as a result of this situation, the objective of this study was to determine the proximal chemical composition of non-nutritional compounds and the antioxidant activity of the following taro varieties: Campechana (white), Chiapaneca (purple), Tabasqueña (Coco) and Dulce (P1 and P2). The cultivation took place in in the Cumuapa 2nd settlement of the municipality of Cunduacán, and the Ejido José Santamaría and Tierra Nueva 3rd Section settlements of the municipality of Huimanguillo in the State of Tabasco.

MATERIALS AND METHODS

Sampling Sites

The sampling sites were: R/A Cumuapa 2nd section, Cunduacán, Tabasco, México (18° 01' 1.23" N, 93° 10' 11.2" W); R/A Ejido Francisco Santamaría Chontalpa Huimanguillo (17° 63' 43" N, 93° 49' 60" W); R/A Tierra Nueva 3rd section, Huimanguillo, Tabasco (17° 73' 025" N, 93° 46' 23" W). The samples were analyzed in the Laboratorio de Suelos, Plantas y Agua del INIFAP-Campo Experimental Huimanguillo (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias).

Sample Preparation

Two corms from each of the five accessions of taro —Campechana, Chiapaneca (purple), Tabasqueña, and Dulce (P1 and P2)— were washed with distilled water; the corms were peeled, and they were cut into 0.5 cm thick slices; subsequently the slices were dried at 75 °C for 24 h; finally, they were ground using a commercial blender to

obtain flour (60 mesh) which was stored in hermetic bags. The percentage of dry matter and its moisture content was calculated using the following equation:

$$\% \text{ materia seca} = \frac{Pi \text{ (peso después de la desecación)}}{p \text{ (peso antes de la desecación)}} \times 100$$

Proximal Chemical Analysis

Its bromatological composition was determined according to the following AOAC methods (2005): humidity (934.01), ash (942.05), proteins [976.05, using as conversion factor ($N \times 6.25$)], and lipids (2003.06). The Fehling method—established in the NMX-F-277-1991 standard—was used to quantify the total starch.

Non-Nutritional Compounds

The trypsin inhibitors activity was determined according to Liu and Markakis (1989) and it was reported as UIT/100 g of sample. Phytic acid content was determined using the colorimetric procedure described by Vaintraub and Lapteva (1988). The results were expressed as Eq/g sodium phytate/100 g sample, using phytic acid as standard (phytic acid sodium salt hydrate, P8810; Sigma, St. Louis, MO, USA).

Saponins were quantified using the spectrophotometric method described by Hiai *et al.* (1976). The concentration of saponins was read from a standard curve of different concentrations of diosgenin (D1634, Sigma, St. Louis, MO, USA) in 80% aqueous methanol and expressed as the equivalent of diosgenin. Finally, the content of total phenolic compounds was determined using the Folin-Ciocalteu method (Singleton *et al.*, 1999), with gallic acid as standard for the calibration curve. The results were reported as Eq/mg of gallic acid/100 g of sample.

Antioxidant Activity

The antioxidant activity was determined based on the ABTS⁺ radical according to the findings of Kuskoski *et al.* (2004) and it was carried out using the ethanolic extracts of taro flour (1:10 flour: ethanol, ambient T, 16 h). The absorbance values were obtained from a trolox curve (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the results were reported as Eq/mg of trolox/g of flour.

Statistical Analysis

The Statistical Analysis System (SAS) software V.6.12, USA, was used to carry out an analysis of variance (ANOVA) and the Tukey test in order to analyze the data obtained. All parameters were tested for significance ($P \leq 0.05$).

RESULTS AND DISCUSSION

Proximal Chemical Analysis

According to Table 1, the Chiapaneca accession presented the highest moisture content (84.7%), while the Dulce P1 and P2 samples showed the lowest moisture values (62.7 and

Table 1. Dry Matter and Moisture in Five Taro Accessions.

Accessions	Dry matter (%)	Moisture (%)
Campechana	25.06 ^b	74.9 ^b
Chiapaneca	15.3 ^c	84.7 ^c
Tabasqueña	28.62 ^b	71.3 ^b
Dulce P1	37.3 ^a	62.7 ^a
Dulce P2	37.0 ^a	63.0 ^a

* Means with the same letters in each column are statistically equal (Tukey, $P \leq 0.05$).

63.0%, respectively). In order to obtain a higher taro flour yield, taro must have a higher dry matter content: the Dulce P1 and P2 samples, followed by the Tabasqueña sample showed the highest dry matter content. Ferreira *et al.* (1990) reported similar values to those found in this work: 80% humidity. This is a typical characteristic of tubers.

On the one hand, starch is the main component of taro (Table 2) and it represents 62-74% of its dry weight; these values are close to the 65-80% starch content reported by Madrigal *et al.* (2018). On the other hand, Rodríguez-Miranda *et al.* (2011) reported approximately 88% starch content, highlighting that taro starch has good water solubility capacity and good water and oil absorption, and that it also functions as an emulsifier; therefore, it could be used as raw material in the food industry.

The protein content of the taro samples ranged from 1.13 to 3.95 g/100 g; these results match those reported by Torres *et al.* (2013) and Antonio *et al.* (2009) who found a protein content of 2.08 and 1.35 g/100 g. These values are relatively low; therefore, using taro as a source of starch would provide a purer carbohydrate.

The lipid concentration ranged from 0.64 to 2.94 g/100 g. These values were similar to the findings of Madrigal *et al.* (2018) (1 and 1.26 g/100 g) and Rodríguez-Miranda *et al.* (2011) (0.79 g/100 g). The lipid concentration was higher than 2 g/100g in the Dulce (P1 and P2) and the Campechana varieties; therefore, studying the shelf life of these flours would be an important topic for future research.

Regarding the ash content, the results ranged from 1.91 to 7.32 g/100 g. Púa *et al.* (2019) and Madrigal *et al.* (2018) reported similar results: 7.65 and 3.23 g/100 g, respectively. Potassium has been reported as one of the minerals with the highest concentration; it is an important element in plant metabolism, since it is essential for the biosynthesis of

Table 2. Chemical Composition of the Different Taro Accessions (g/100 of sample B. S).

Accession	Protein	Lipids	Ash	Moisture	Starch
Campechana	1.13±0.02 ^c	2.11± 0.02 ^c	2.47±0.11 ^c	5.11±0.05 ^b	74.7±0.01 ^a
Chiapaneca	2.36± 0.01 ^b	0.64±0.01 ^c	7.32± 0.06 ^a	2.28±0.02 ^c	62.7±0.02 ^c
Tabasqueña	3.95±0.18 ^a	1.42±0.03 ^d	4.13±0.13 ^b	5.34±0.00 ^a	72.0±0.01 ^b
Dulce P1	1.42± 0.02 ^d	2.94± 0.03 ^a	1.58±0.05 ^c	3.35± 0.01 ^c	70.1± 0.22 ^c
Dulce P2	1.71±0.01 ^c	2.61±0.12 ^b	1.91±0.01 ^d	3.35±0.01 ^d	68.4±0.01 ^d

* Means with the same letters in each column are statistically equal (Tukey, $P \leq 0.05$).

carbohydrates (Madrigal *et al.*, 2018). Minerals are important for human nutrition, as well as for the vegetable itself, and their concentration is usually higher in the peel than in the flesh (Subramanian *et al.*, 2011).

Non-Nutritional Compounds

The presence of non-nutritional compounds can interfere with the assimilation or digestibility of proteins or carbohydrates. In this regard, the non-nutritional compounds of taro accessions were analyzed. Table 3 shows a significant variation in the activity of trypsin inhibitors ($p \leq 0.05$) in taro accessions: for example, Chiapaneca had a 0.70 UIT/100 g value, while no activity was observed in Campechana. Madrigal *et al.* (2018) found a higher activity of trypsin inhibitors (1424.58-2262.98 UIT/g).

The total phenol content varied significantly ($P \leq 0.05$) among the taro samples (0.54-5.76 g gallic acid/100g). These values are higher than 0.19 Eq/g gallic acid/100 g (Madrigal *et al.*, 2018) and similar to 5.15 Eq/g of gallic acid/100 g (Eleazu *et al.*, 2016). Eleazu *et al.* (2016) have reported that cooked potato has a higher concentration of total phenolic compounds than raw potato, as well as a higher concentration of phenolic compounds in the tuber peel. Genotypic variation is the predominant factor that affects the tuber's phenolic contents compared to other parameters such as location and time of year (Ji *et al.*, 2012). Keutgen *et al.* (2019) reported that the composition of phenolic compounds of potatoes depends on the growing site and the variety. Meanwhile, the concentration of condensed phenolics was 1.20-4.65 g Eq/g (+) catechin/100g.

The phytic acid value of the analyzed samples ranged from 0.22 to 0.40 g Eq/g sodium phytate/100 g; these values are higher than those reported by Rodríguez *et al.* (2011) for taro flour (0.6 g/100 g). Phytic acid is a known chelator, forming protein and phytic acid mineral complexes, resulting in a decrease in the bioavailability of both proteins and minerals (Feizollahi *et al.*, 2021). Low phytate concentration has been proven to have a beneficial effect on their activity as antioxidants and anticarcinogens and they can potentially play an important role in controlling chronic non-communicable diseases such as hypercholesterolemia and atherosclerosis (Gemedede *et al.*, 2014; Thakur *et al.*, 2019).

The content of saponins ranged from 0.37 to 1.02 Eq/g diosgenin/100 g sample; Campechana had the highest value. These values are higher than the 0.02 g/100 g values reported by Caicedo (2013). Saponins are heat-labile and water-soluble compounds, therefore it is possible that they were lost as a result of these treatments (Güçlü-Üstündağ and Mazza, 2007). As we have already mentioned, in small amounts, the so-called non-nutritional factors are compounds that can help to prevent diseases; therefore, they have been called bioactive compounds. One of its main biological activities is its antioxidant function (Vargas *et al.*, 2019).

Antioxidant Activity

Table 4 shows that the antioxidant capacity of the ethanolic extracts of taro flour varied from 348.63 to 573.13 Eq/mg of trolox/g of flour: the Chiapaneca accession registered the lowest value (matching the phenolic compound content). The antioxidant activity is mainly the responsibility of the phenols—particularly the flavonoids—, since they confer a strong

Table 4. Antioxidant Activity of Taro Flour from Four Accessions.

Accession	Antioxidant activity (mg eq of trolox / g of flour)
Campechana	348.63±0.39 ^b
Chiapaneca	294.12±8.237 ^c
Tabasqueña	517.09±1.79 ^a
Dulce P1	519.20±1.04 ^a
Dulce P2	573.13±2.58 ^a

*Means with the same letters in each column are statistically equal (Tukey, $P \leq 0.05$).

antioxidant activity. In this work they are mainly attributable to this type of compounds (Baião *et al.*, 2017).

Another important function of antioxidant molecules is their impact on health. Although humans have endogenous antioxidant systems, having more oxidant molecules than antioxidant molecules cause oxidative stress (OS). An exogenous antioxidant-rich diet can help to prevent OS damage (Pisoschi and Pop, 2015, He *et al.*, 2017). Including taro in the diet —combined with other foods such as fruits, cereals, and fabaceae— could contribute both to a varied and balanced diet and to a better health, as they reduce the risk of such situations as: premature aging, cancer, diabetes, inflammation, liver disease, cardiovascular disease, cataracts, nephrotoxicity, and neurodegenerative disorders (Neha *et al.*, 2019).

CONCLUSIONS

In addition to moisture, starch is the main component of taro; therefore, it represents an economical and sustainable alternative to obtain raw materials of interest to the food industry. The accessions evaluated had low concentrations of non-nutritional compounds, however, they had phenolic compounds that provide them with antioxidant activity. The characterization of these accessions opens a potential alternative use that provides an added value, which is beneficial for the producer.

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