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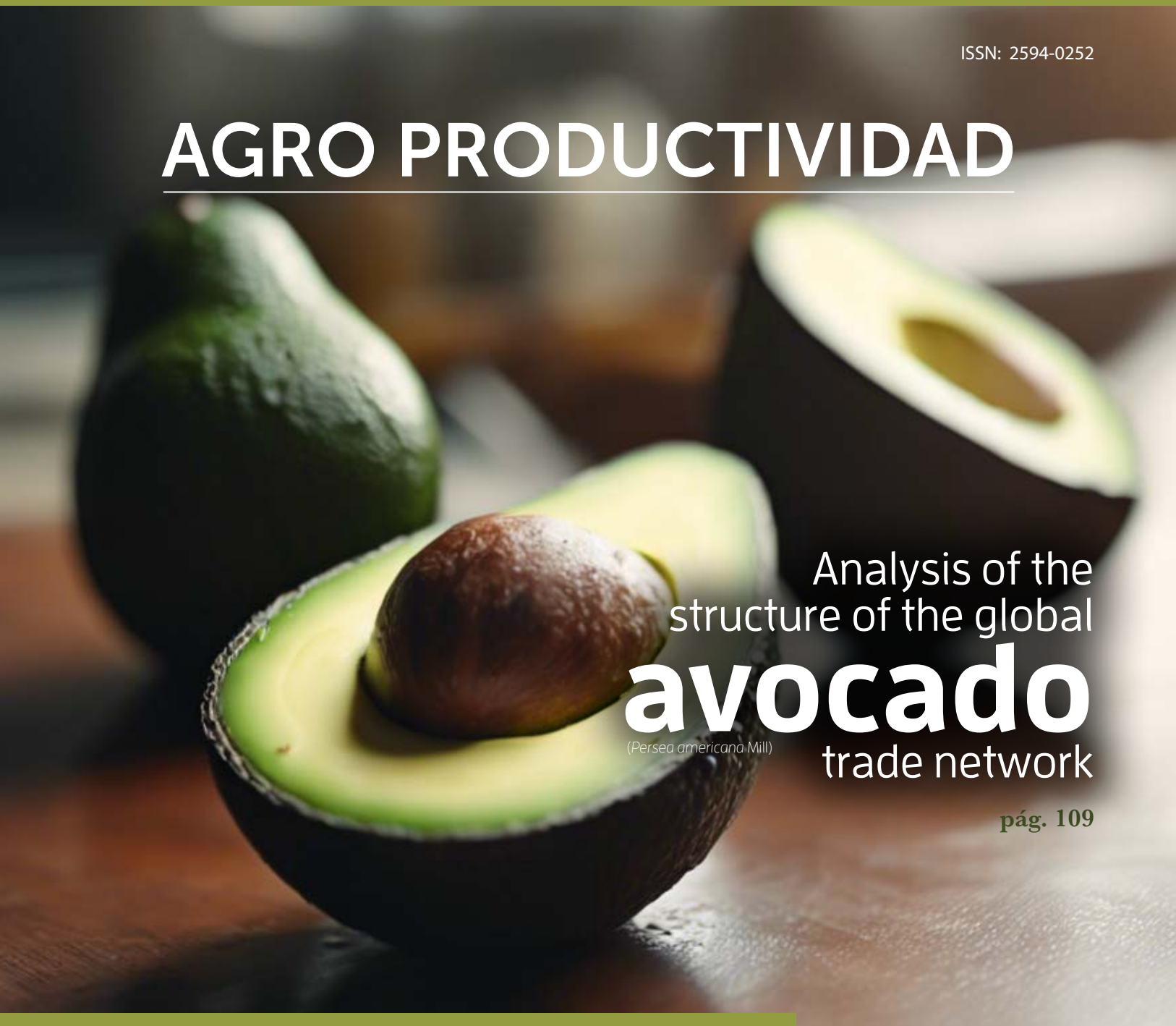

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Palabras clave y Keywords: Se deberá incluir una lista de 3 a 5 palabras clave en español e inglés que permitan identificar el ámbito temático que aborda el artículo.
Introducción: Se asentará con claridad el estado actual del conocimiento sobre el tema investigado, su justificación e importancia, así como los objetivos del trabajo. No deberá ser mayor a dos cuartillas.
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> Ogata N. (2003a).
> Ogata N. (2003b).

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Effectiveness of feed restriction to improve feed efficiency in finishing pigs

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ABSTRACT
Objective: To review the effects of feed restriction in finishing pigs on production efficiency and meat quality.
Design/methodology/approach: A bibliographic review of feeding management in pigs fed *ad libitum*, restricted, and their combination was carried out.
Results: Feed restriction decreases the growth rate, but if the restriction is moderate, better feed efficiency can be obtained. Feed restriction followed by feed *ad libitum* results in compensatory growth, which equals or improves continuous free access feeding.
Study limitations/implications: Implementing feed restriction can be useful to improve feed efficiency and in periods that it is necessary to slow down growth.
Findings/conclusions: Moderate feed restriction (−10%) of finishing pigs improves feed efficiency. Feed restriction of less than 20% for 30 days, followed by ad libitum feed, promotes compensatory growth and improves feed efficiency.

Keywords: feeding strategy, feed efficiency, compensatory growth, finishing pigs.

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INTRODUCTION

There are different feeding plans (*ad libitum*, restricted or a combination of both) that are implemented with the aim of increasing or decreasing the growth rate, age and live weight (LW) at slaughter, or manipulating meat quality (Carco *et al.*, 2018 a,b; Schiavon *et al.*, 2018; Poullet *et al.*, 2019).

Under free access feeding conditions, pigs are encouraged to maximize feed consumption, which can decrease the digestibility of nutrients (Njoku *et al.*, 2015a) and the feed efficiency (Dalla Bona *et al.*, 2016; Njoku *et al.*, 2018; Schiavon *et al.*, 2018).

Feed restriction (FR) consists in offering the animals a regulated amount of feed, normally lower than what the pigs are capable of ingesting voluntarily (Brustolini *et al.*, 2019). Depending on the percentage of FR, it is possible to improve the feed efficiency and the meat quality, since severe FR can drastically affect the growth rate and the fat content in the meat (Njoku *et al.*, 2013; Kim *et al.*, 2014; Dalla Bona *et al.*, 2016; Carco *et al.*, 2018ab).

FR followed by a period of free access feeding leads to the compensatory growth mechanism, with which greater weight gain and better feed efficiency are observed during this refeeding period, making it possible to attain optimal weights at the time of slaughter, with high feed efficiency (Lovatto *et al.*, 2006; McEwen *et al.*, 2009; Poullet *et al.*, 2019), without a negative effect on meat quality (Wiecek *et al.*, 2011; Pugliese *et al.*, 2013). The purpose of this review is to summarize studies that show the effectiveness in the implementation of FR in finishing pigs on the productive efficiency and meat quality.

Feed restriction (FR) in fattening pigs

One of the main challenges to make pig production systems more efficient is to define the strategic feeding plan according to the objective of production (Njoku *et al.*, 2015a). FR can be a useful strategy to improve meat quality and, at the same time, to improve feed efficiency by reducing the waste of feed and improving the digestibility of the nutrients consumed, accompanied by a reduction in the needs for maintenance and higher energetic efficiency for the synthesis of protein and fat (Lebret *et al.*, 2001; Njoku *et al.*, 2015a; Poullet *et al.*, 2019).

Feed restriction and productive behavior

Pigs with FR present better weight gain than pigs fed with free access; however, the feed efficiency (FE) in pigs subjected to FR is not affected or improved (Kim *et al.*, 2014; Dalla Bona *et al.*, 2016; Carco *et al.*, 2018a; Brustolini *et al.*, 2019). The implementation of a FR regime of 7% in finishing Talent line Topigs pigs reduced weight gain (3.2-3.5%) and feed consumption (7.4%), although the FE increased (2-4%) (Dalla Bona *et al.*, 2016; Schiavon *et al.*, 2018). Njoku *et al.* (2018) observed that 10% FR did not affect the LW at slaughter. More encouraging data show that a FR of 15% increased the FE in 8%, although it reduced the weight gain (Kim *et al.*, 2014).

The amount of feed offered, the frequency and the feeding rate (consumption of feed per visit/duration of the visit) in pigs can restrict or decrease the feed consumed, using these as variants of the FR regime, modifying the consumption behavior to compensate the restriction and increasing the feeding speed or the time devoted to feeding (Carco *et al.*, 2018a). Feed restriction (*ad libitum vs.* 2.5 and 2.75 kg) in pigs (22-114 kg of LW) reduced the feed consumption between 15 and 22%, which resulted in lower weight gain, without a negative effect in feed conversion (Quiniou *et al.*, 2012).

On the other hand, the pigs responded to FR by increasing their feeding rate (Carco *et al.*, 2018a; Schiavon *et al.*, 2018). Manipulating the feeding rate through FR affects feed consumption, and consequently the growth, although it has low influence in feed conversion (Njoku *et al.*, 2013; Colpoys *et al.*, 2016; Carco *et al.*, 2018b). The pigs with a higher feeding rate (7% FR) had better LW (16%) and weight gain (27%) than the pigs without restriction (feeding *ad libitum*) (Carco *et al.*, 2018b). In a similar study, Njoku *et al.* (2013) found that the final LW, weight gain and FE improved when feed was offered more times (1-3 times) with FR (1.5, 2.0 and 2.5 kg), observing higher weight gain and FE with 2 or 2.5 kg of feed divided into three servings.

As is suggested in the aforementioned, FR can be an effective feeding strategy; however, there must be care not to make it too severe, since there could be some irreversible negative effects in the productive behavior. Njoku *et al.* (2013; 2015b) observed that pigs fed with 2.0 and 2.5 kg/d obtained similar weight gains and feed conversion, although with negative effects for animals fed with 1.5 kg. Lebret *et al.* (2001) observed that the reduction of 25% in the feed offered to pigs decreases weight gain in 25%, which represented that the LW for market would be reached 30 days later than the animals fed at free access; no effect was found in the FE. This information agrees with what was found by Boddicker *et al.* (2011) who indicate differences in the FE of growing pigs between the *ad libitum* feeding regime and FR of 25%, although FR of 45% decreased the feed efficiency.

Feed restriction, characteristics of the carcass and meat quality

FR in pigs increases feed efficiency, although the negative response that there may be in weight gain must be considered, as well as the characteristics of the carcass, and the quality and chemical composition of the pig meat (Njoku *et al.*, 2015 a,b; Dalla Bona *et al.*, 2016; Njoku *et al.*, 2018).

Nevertheless, some studies report that FR does not affect meat quality (Dalla Bona *et al.*, 2016; Njoku *et al.*, 2015b; 2018), although it can lead to changes in the growth of internal organs (Njoku *et al.*, 2015b; 2018). In addition, FR (7%) can negatively influence the percentage of fat, lean meat, and protein synthesis (Colpoys *et al.*, 2016), and the characteristics of the carcass (Njoku *et al.*, 2015b; 2018; Dalla Bona *et al.*, 2016; Schiavon *et al.*, 2018; Brustolini *et al.*, 2019). However, Carco *et al.* (2018b) observed that FR of 7% in pigs causes greater accumulation of protein (22%) and lipids, higher carcass weight (16%), weight of lean cuts (14%), and weight of fatty cuts (21%). Another aspect of FR in pigs is that the carcass weight has a low coefficient of variation, important in production chains given the economic value of this aspect (Gallo *et al.*, 2015; Schiavon *et al.*, 2018).

The feeding program in pigs affects the lipid body composition. FR reduced the dorsal fat content (Quiniou *et al.*, 2012) and intramuscular lipids (Lebret *et al.*, 2001; Minelli *et al.*, 2019), while it increased the concentration of polyunsaturated fatty acids (Wiecek *et al.*, 2011; Dalla Bona *et al.* 2016; Minelli *et al.*, 2019) and omega-3 (Wiecek *et al.*, 2011; Minelli *et al.*, 2019) in the meat fat.

Feed restriction and refeeding period

The pigs show an accelerated growth rate or compensatory growth when they are provided feed at free access after a period of FR; in addition, the weight gain accumulated in both feeding regimes can be higher or the same as with continuous *ad libitum* feeding, reaching optimal weights at sacrifice and a good FE (Kristensen *et al.*, 2004; McEwen *et al.*, 2009). It has been found that FR followed by compensatory growth does not affect the meat quality (Pugliese *et al.*, 2013). Compensatory growth could be a feasible strategy when the cost of raw materials and the price for market of the pigs are disadvantageous for the producer.

The compensatory growth in pigs depend on the age, the level of restriction, and the duration of the FR period, from the moment when FR begins, and the duration of the refeeding period (Therkildsen *et al.*, 2002; Lovatto *et al.*, 2006; Poullet *et al.*, 2019). Therkildsen *et al.* (2002) studied the effects of the duration of the refeeding period after FR in pigs, which were 70 days away from slaughter. The pigs received feeding *ad libitum* or 60% of the *ad libitum* level for 28, 43, 52 and 60 days, followed by feeding *ad libitum* (42, 27, 18 and 10 days, respectively) during the rest of the fattening period and until sacrifice. FR resulted in a lower weight in comparison to the pigs fed *ad libitum*; however, the pigs showed compensatory growth in the subsequent period of feeding *ad libitum*. The pigs refed *ad libitum* for a minimum of 27 days before slaughter had similar carcass weight and muscular mass than the pigs of the control treatment, and the meat quality was not affected. McEwen *et al.* (2009) indicated that limited feeding (70-85%) in pigs followed by feeding *ad libitum* improved feed conversion, reduced feed consumption (7.5%), and increased weight gain, presenting similar days to sale compared to pigs fed *ad libitum*. A better FE was also confirmed by the studies carried out by Daza *et al.* (2007). These results show an increase in feed efficiency and suggest an economic benefit by limiting feeding and applying the effect of compensatory growth.

For their part, Poullet *et al.* (2019) studied the effect of FR of short duration and severe (restriction of 50% during 6 days) and refeeding (14 d) on the production and metabolism of pigs. FR reduced the growth rate and the FE which was quickly compensated with refeeding. In a similar design but with shorter refeeding (7 days), Lovatto *et al.* (2006) did not observe compensatory growth. In the case of severe FR in pigs, the reduction in weight gain is expected to be proportionally higher than the reduction in feed consumption, due to greater relative importance of the necessary nutrients for maintenance (Lovatto *et al.*, 2006; Poullet *et al.*, 2019).

A period of FR that is too long must the managed carefully. A study by Serrano *et al.* (2009) assessed pigs in the finishing stage that had previously been feed restricted (72-82%) for 100 days and refed for 54 days before sacrifice; they found increased feed consumption (9%) and weight gain (20%), and improved feed conversion (13%) in comparison to pigs fed *ad libitum* during the stage of refeeding. Although at the end of the trial the pigs fed *ad libitum* weighed 9.7 kg more than the restricted pigs, the feed efficiency and the meat quality were not affected with any feeding regime.

FR followed by a period of feeding at free access allows directing the high supply of energy when the pig is adult, due to the limited growth and biological rhythm in the previous phase, which could alter the fat deposition and the meat characteristics. McEwen *et al.* (2009) indicated that FR (70-85%) of pigs during the growth phase followed by feeding *ad libitum* reduced intramuscular fat, compared to pigs fed *ad libitum*. Kristensen *et al.* (2004) observed that the compensatory growth improved meat tenderness when pigs are subjected to a restriction regime of 31% during 52 or 62 days and a refeeding period of 60 to 70 days. However, other studies (Daza *et al.*, 2007; Lebret *et al.*, 2008; Serrano *et al.*, 2009; Wiecek *et al.*, 2011) in different refeeding periods (21-63 days) did not find a positive effect in the meat quality or the fat content.

Effect of feed restriction on metabolism

The compensatory growth due to FR implies a greater replacement of muscular proteins, increasing the activity of proteases (calpains) after the pigs are returned to feeding *ad libitum*; this activity can continue until the post mortem period, which presents the potential of increasing meat tenderness (Kristensen *et al.*, 2004; Therkildsen *et al.*, 2002); however, Therkildsen *et al.* (2002) observed that a greater activity of calpains and collagen (rechange and resynthesis) did not improve meat tenderness.

Feeding *ad libitum* tends to promote the synthesis of body fat that is inefficient in terms of feed conversion (Njoku *et al.*, 2013). Kim *et al.* (2014) inform that FR of 15% in the diet of growing pigs altered the expression of the fat tissue of key enzymes that regulate acetyl-CoA carboxylase, the fatty acid synthase, hormone-sensitive lipase, and the lipoprotein lipase during the finishing period; this suggests a decrease in the capacity of de novo synthesis of fatty acids and an increase in lipolytic activity. The effect of FR decreases the availability of energy for de novo synthesis, primarily of saturated fatty acids, which leads to greater unsaturation of the lipids, in addition to an increase in polyunsaturated fatty acids (Daza *et al.*, 2007).

The change in growth rate and FE during FR of pigs suggests changes in the metabolism and use of nutrients, which shows modifications in the levels of metabolites and hormones. FR reduced blood urea (Lovatto *et al.*, 2006; Schiavon *et al.*, 2018; Poullet *et al.*, 2019), which is associated to lower feed consumption and better balance of the N ingested; a reduction in the cholesterol level is also reported (Poullet *et al.*, 2019).

In terms of the effect of FR on the hormonal level, leptin was reduced and ghrelin increased (Barretero *et al.*, 2010; Poullet *et al.*, 2019); it increased the growth hormone and decreased insulin and glucose (Barretero *et al.*, 2010). The concentration of IGF-1 was reduced in pigs with FR, although they returned to normal levels during the period of feeding *ad libitum* (Therkildsen *et al.*, 2004; Chaosap *et al.*, 2011). The liberation of growth hormone by the pituitary gland stimulates the production of IGF-1, stimulating bone and muscular growth (Schiaffino *et al.*, 2013).

Lower weight gain during FR can be due to a lower availability of nutrients for growth and to a higher proportion of the energy being used for maintenance (Lovatto *et al.*, 2006). However, a higher FE during FR could reduce the metabolic rate related with a lower weight of the entrails during compensatory growth (Koong *et al.*, 1983; Hornick *et al.*, 2000), and a more efficient use of nutrients (Lovatto *et al.*, 2006; Poullet *et al.*, 2019).

Factors that affect the response to feed restriction

Feed consumption in pigs is influenced by the genotype (including the sex, the breed), the health status, the diet's characteristics, environmental factors and possible interactions (Schiavon *et al.*, 2018). The degree of response during compensatory growth after FR could be due to differences in the genotype of pigs, since feed restriction is especially appropriate for pigs of unimproved genotypes with low FE (Kristensen *et al.*, 2004), since the level of FE between genotypes is associated to the difference in fat and protein deposition (Poullet *et al.*, 2019). Also, the percentages of FR should be applied in function of the sex, so that

they are higher as the growth rhythm is higher, since there are marked differences between males and females (McEwen *et al.*, 2009; Serrano *et al.*, 2009).

The thermal environment has an important effect on feed consumption, growth rate and FE (Cervantes *et al.*, 2016). Feeding *ad libitum* can predispose the animals to obesity, thermal stress, high incidence of limping, high morbidity and mortality due to skeletal disorders, and heart failure (Njoku *et al.*, 2018). The FR protocol in pigs reduces the body temperature (around −0.4 °C) and respiratory frequency; this taking into account that when there is an overconsumption of energy, it is channeled to the increase in body temperature (Njoku *et al.*, 2013; Cervantes *et al.*, 2016; Poullet *et al.*, 2019). Therefore, the reduction in metabolic heat production leads to a better FE and reduction of caloric stress (Lovatto *et al.*, 2006).

CONCLUSIONS

Applying feed restriction of less than 10% can be viable in finishing pigs since it improves feed efficiency without affecting the characteristics of the carcass and the meat. The implementation for a period of 30 days with a restriction lower than 20%, followed by feeding *ad libitum*, is a useful strategy to promote compensatory growth and to improve feed efficiency.

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Colegio de Postgraduados

The contribution of herbal medicine in health problems of the indigenous community of Oaxaca, Mexico

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ABSTRACT

Objective: To characterize the ways and magnitude in which herbal medicine mitigates health problems and represents a source of savings; as well as to identify the most common ailments treated with herbal medicine and the most used medicinal plants in Santiago Ixtaltepec, Nochixtlán, Oaxaca.

Design/methodology/approach: The study was conducted in 2022, with a quantitative approach and a descriptive-correlational scope. Data were obtained through a survey, applied through direct interviews. Once the data were obtained in the field, they were systematized and processed in Excel databases and descriptive statistical analysis was performed to determine frequencies, percentages, means and other measures of dispersion.

Results: The use of herbal medicine is relevant as an alternative in health care for the community's population. The most common ailments treated with herbal medicine are stomach aches, postpartum treatment, cough, fever, and bad breath, and the most commonly used medicinal plants are coyote grass, horehound (*Marrubium vulgare*), cancer grass, puli (*Pinaropappus roseus*), and juniper (*Juniperus deepeana*).

Limitations on study/implications: As this is a case study, the findings are not generalizable but specific to the community of study.

Findings/conclusions: Herbal medicine contributes to the mitigation of health problems and its use has an impact on the economy of families. There is a direct relationship between the most common symptoms and the most used plants in the community.

Keywords: Herbal medicine, health, Mixtec community, common ailments.

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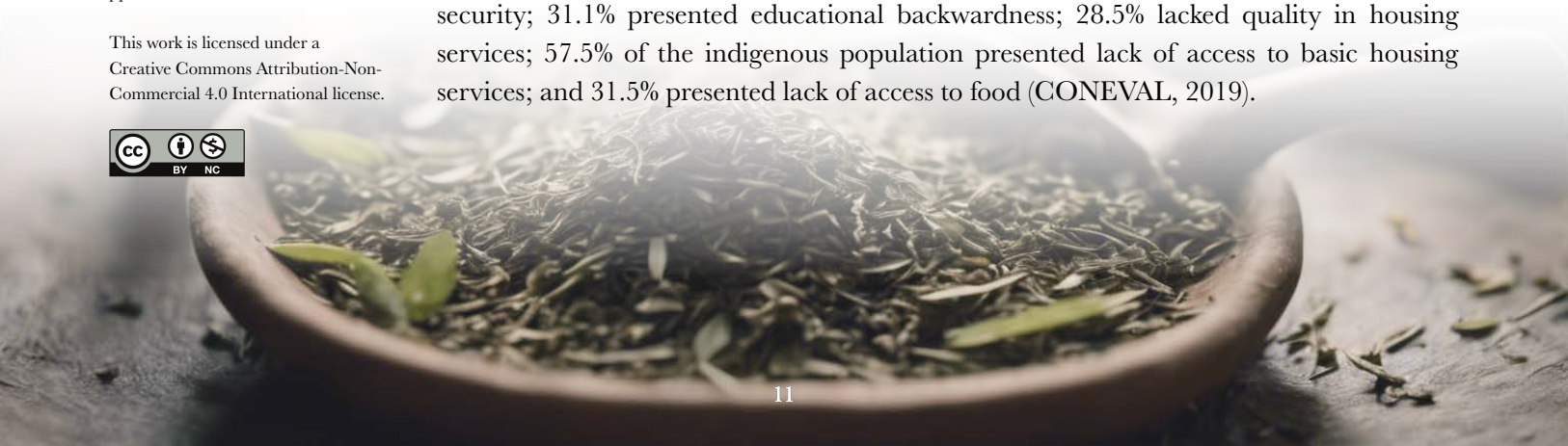
INTRODUCTION

Indigenous peoples are subjected to important economic, social, cultural and political inequalities that keep them in conditions of poverty and exclusion. In 2018, 15.4% of the indigenous population lacked access to health services and 78.2% did not have social security; 31.1% presented educational backwardness; 28.5% lacked quality in housing services; 57.5% of the indigenous population presented lack of access to basic housing services; and 31.5% presented lack of access to food (CONEVAL, 2019).



In Mexico, indigenous communities live under a system historically dominated by racism, which has been defining in the living conditions and access to services of their population, constantly victims of structural violence, understood as differentiation made from social stratification and because of which the most vulnerable sectors are violated in their access to basic rights, such as health care. Institutional racism has ideologically justified the inequitable access of indigenous peoples to the satisfaction of their rights, which is visible because: a) there is no program or specific resources for the attention to cultural differences of indigenous communities, such as the existence of translators, linguistic or cultural interpreters; b) there is difficulty in access to the health care system, hospitals are concentrated in cities, leaving aside rural and indigenous populations, and c) there is discrimination from health staff and users toward the ethnic condition based on stereotypes (Cortez *et al.*, 2020).

The social determinants of these inequalities are linked at three levels: structural, institutional, and of livelihoods; that is, the limited access to basic satisfiers such as food, work and housing (drinking water reduces gastrointestinal diseases and the construction materials are determinant for the prevention of respiratory illnesses); the limited access to health care benefits due to geographic conditions, and the limited access to education. Knowledge about health allows better decision making, in addition to speaking Spanish, which is fundamental for the access to services (Freyermuth, 2017).

Presently, herbal medicine is a cultural element to which indigenous peoples have access; it represents an alternative to face health problems and the current context of institutions (Cortez *et al.*, 2020). It is a component of traditional medicine and can be defined as the practice and knowledge of the use of herbal medicines, which due to their value are used by the community for the attention, prevention, promotion, and/or treatment of health problems (Lima *et al.*, 2019).

It is calculated that about 10 thousand species in the world are used in a medicinal or therapeutic way, and around 80% of the population in developing countries depends nearly completely on the use of plants as home therapy to cover basic health needs (Prieto *et al.*, 2004). Their broad use is attributable to their easy access, affordability and efficiency to treat chronic ailments, diseases that are not serious, and minor or moderate symptoms.

Mexico stands out for its great floristic diversity, its contribution to herbal medicine is calculated conservatively in more than 5,000 species, which corresponds to 15% of the total vegetation in the country (Bellucci, 2001) and Oaxaca is one of the states with greatest biodiversity and pluriculturalism of the country; the Sierra Mixteca and its vegetation are among the most studied, yet there is scarce information regarding its ethnobotany and the medicinal use of its herbs, although they represent an important component for health care and the treatment of ailments among its inhabitants (Bellucci, 2001).

In terms of health, the Mixtec peoples have a strong connection to their traditions and culture, so the causes of their physical or emotional illnesses are related to the fluctuation of the cold-heat relation of the body, from dietary disorders, sudden movements, alterations in the vital force, and in general from the imbalance suffered in the body because of

natural phenomena (empacho) or socially caused (fright, evil eye). These communities "have a tradition of health attention through medicinal plants, cures, massages, cleanses and *temazcal* baths" (París-Pombo, 2008, p. 65).

According to the study by Valdés (2013) carried out in several communities of the zone, among them Santiago Ixtaltepec, it was identified that among the diseases of greatest importance in the region there are the respiratory, caused by changes in temperature; the gastrointestinal, derived from insalubrity; malnutrition in children; and those caused by excess work. The use of medicinal plants is common to "cure discomfort and ailments such as colds, coughs, diarrhea, stomach pain, fever and body ache", and most of the plants that are conserved and used are native (Valdés, 2013, p. 94).

Thus, this study has the hypothesis that traditional herbal medicine represents an important component in the health care of the Mixtec community of Santiago Ixtaltepec; it is a therapeutic option and an alternative for disease prevention, which complements the lack of access and deficiencies in conventional health care systems. Therefore, its objective was to characterize the ways and magnitude in which herbal medicine mitigates health problems and represents a source of savings; to identify the most common ailments treated with herbal medicine and the most frequently used medicinal plants in Santiago Ixtaltepec, Nochixtlán, Oaxaca.

MATERIALS AND METHODS

Located in the Mixtec region, the municipality of Asunción Nochixtlán is located in northeastern Oaxaca; the climate is mainly semi-dry temperate (52.74%) and temperate sub-humid with summer rains (21.37%), with a temperature that ranges between 16 and 22 °C, and a rainfall range of 400 to 1,000 mm (INEGI, 2006). The predominant vegetation is oak (*Quercus* spp.), ocote pine (*Pinus* spp.) and juniper (*Juniperus* spp.) forest (Alvarado and Martínez, 2017).

The locality of Santiago Ixtaltepec is 15.1 Km southeast from the municipal township (Figure 1); its geographic location is at longitude 97° 06' 51.0", latitude 17° 32' 36.0" and altitude of 2,264 meters above sea level. It has a population of 116 inhabitants (31 of age zero to 14 years; 63 from 15 to 64 years; 22 older than 64 years), according to the INEGI 2020 census.

The methodological approach of the study was quantitative with a descriptive-correlational reach (Hernández-Sampieri *et al.*, 2014), and the contribution of herbal medicine to the wellbeing of inhabitants of the community of Santiago Ixtaltepec was characterized in terms of health and the savings that it can represent for the economy of families; the most common diseases and the most frequently used medicinal plants in the community were identified through the application of a survey, with a questionnaire made up of 21 items, divided into three sections: 1) the socioeconomic aspects of the participant and his/her family; 2) the impact of herbal medicine on the health and economy of families; and 3) the herbal medicine practice.

For the application of the questionnaires in the field, a probabilistic sample was obtained previously through the formula:

$$n = \frac{\frac{z^2 xp(1-p)}{e^2}}{1+\left(\frac{z^2 xp(1-p)}{e^2 N}\right)}$$

where N=Total population, z=Level of confidence (1.96), p=Proportion expected (0.95), and e=Margin of error (0.05) considering that in Santiago Ixtaltepec there is a universe of 58 families, and according to the resulting sample 32 questionnaires were applied to men and women heads of household, distributed in the different places of the whole community. Its application was through direct interviews in the month of September, 2022.

Once the data were obtained in the field, they were systematized and processed in Excel databases with which the basic statistical analysis for the determination of frequencies, percentages, means and other dispersion measurements was conducted.

RESULTS AND DISCUSSION

Sociodemographic profile of the population of study

In the survey applied to 32 heads of family, 75% were women and 25% men; the age range in the case of men ranges between 48 and 81 years with an average of 64 years, while for women the average age was 63 years, the minimum 38 and the maximum 91.

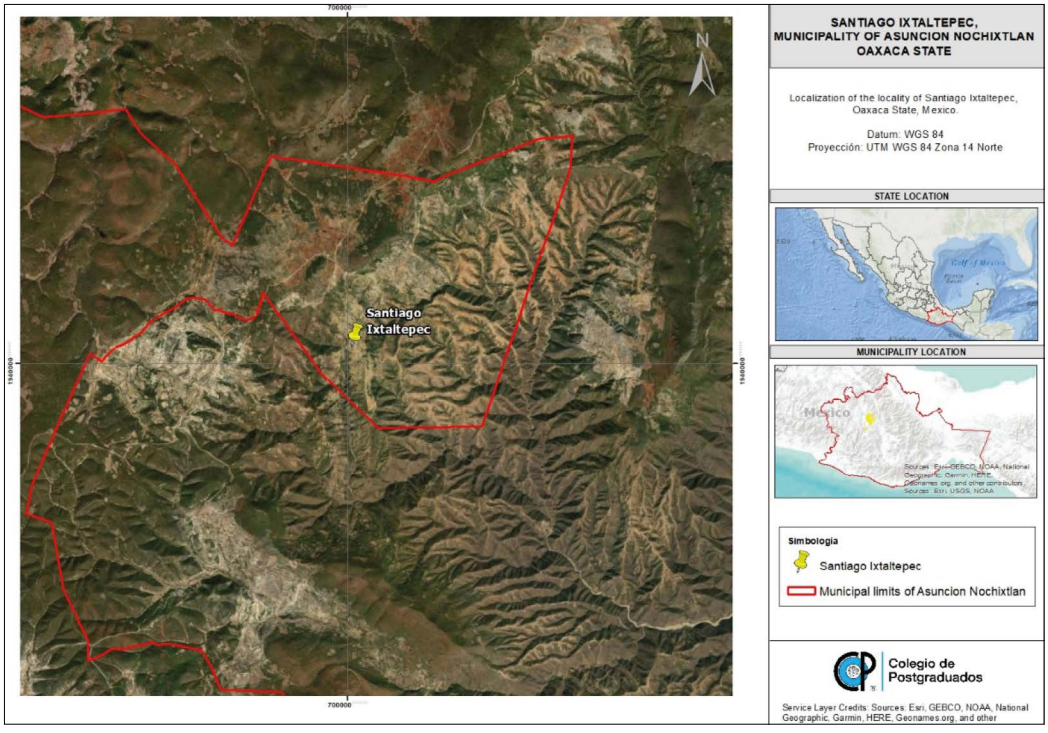


Figure 1. Location of Santiago Ixtaltepec, Nochixtlán, Oaxaca, Mexico.
Source: Prepared by the authors.

Regarding the level of literacy, 100% of the men interviewed know how to read and write, they have an average schooling of 5.5 years, while in the case of women, 21% do not know how to read or write, and the 79% that do have average schooling of 6.4 years. All (100%) of the men surveyed speak Mixtec, while among women it is 92%; one survey respondent (4%) understands it but does not speak it and only one (4%) does not understand or speak it.

Regarding the occupation of participants, among men, 100% are devoted to farming activities, production of corn, bean, vegetables, sheep breeding and other livestock, basis of the diet. Of all the women, 71% are devoted to the home, 21% to farming, 4% are paid workers, and the remaining 4% traders. Of the women, 50% mention having a secondary activity related to the farmland.

The incomes in households are limited, the main activity carried out is farming, and this is basically to satisfy the need for sustenance of the family, occasionally complemented with activities that some members of the family perform outside the community whether temporally or permanently, and with government supports; therefore, as Banerjee and Duflo (2012) mention, among families that live under conditions of poverty, the presence of a serious disease can become the event that ruins them or leads to the loss of the scarce stability they have, since the diseases that require specialized medical attention or displacement of the ill person imply an important expenditure that could gravely impact the welfare of the family, even more if the ill person is the main provider for the household.

The impact of herbal medicine on health

The most common ailments in Santiago Ixtaltepec, according to 72% of the participants, are colds caused by changes in temperature; cough and body aches occupy 9% and 6%, respectively, which agrees with what was exposed by Valdés (2013). Of the participants, 75% reported that they prefer to use herbal medicine first to treat their ailments, and resort to the doctor only if it does not work, or if the disease is more serious, of greater urgency or requires specialized attention. The remaining 25% of the participants resort immediately to medical attention and the use of allopathic medications.

In the last year (August 2021 to July 2022), 91% of the interview respondents or a member of their family suffered an ailment; 45% of these correspond to non-serious diseases such as colds (42%) and tonsillitis (3%), 31% to chronic illnesses such as constant pains in a body zone (22%), diabetes (3%), anemia (3%), and liquid retention (3%), and 24% to serious diseases such as fractures (7%), one eye surgery (7%), one vesicle surgery (7%), COVID (3%) and pneumonia (3%).

Of all the interview respondents with an ailment in the last year, 28% were treated only with herbal medicine (21% for non-serious diseases and 7% for chronic illnesses), 14% complemented medicinal plants with medications (all with chronic illnesses). Of the respondents, 58% had access to allopathic medicine immediately after presenting symptoms (24% linked with serious illnesses, 10% with chronic, 24% for non-serious diseases).

Of the total families that had some ailments during the last year, 72% resorted to the use of medications whether immediately or in combination with the medicinal plants, and this could be because of its unavoidable need to use them in the case of serious illnesses, because the medications are easy to use and they tend to combat the symptoms in shorter time, and due to their easy access since they are supplied in the clinic and often they have them at home leftover from other times.

Herbal medicine is a valuable resource that is part of the attention and care that is provided within the household when an illness presents, it represents an alternative to combat common symptoms, of immediate acquisition and without cost (many plants are found in the backyards, others have to be collected again, and some species will be available after being collected during a specific season of the year and conserved for their later use), which is why the use of medicinal plants can avoid the difficulty involved when the person who presents the common symptoms has to travel from their household to the clinic in the center of the community, to Nochixtlán or Oaxaca.

Site and cost of medical attention

From the medical attention received during the period of August 2021 to July 2022, 47% was in the clinic of the community itself to treat chronic diseases (14%) and non-serious illnesses (29%), 24% in Nochixtlán to treat chronic (14%), serious (5%) and non-serious (5%) diseases, and 29% in Oaxaca where they go to mainly for serious illnesses (28%).

As was established by Cortez *et al.* (2020) and Freyermuth (2017), the accessibility of inhabitants from the community is a determinant factor in health care in the indigenous communities and although Santiago Ixtaltepec has its own Rural Medical Unit from where they obtain medical assistance and free medications. This clinic does not have the necessary staff; for example, since the month of February 2022 until the date of the study (September, 2022), when the resident doctor concluded his service, only a nurse has been present, who is available only from Monday to Friday, which is why to treat serious illnesses or during the weekend, it is necessary to go to Nochixtlán, where they can gain access to medical attention but the community is located more than 15 km away from the municipal township and there is no public transport that connects them. This is an important factor that affects the access and the economy of families when dealing with health issues, since in addition to the doctor's fees and the cost of medications, the inhabitants of Santiago Ixtaltepec can pay between $150 and $300 for transport only to leave the community in a situation of emergency, and given the economic limitations of these families this turns out to be a factor of great relevance.

Regarding the last year, the families that presented some serious illness had an average expenditure of $17,685.00, a minimum of $2,800.00 and a maximum of $40,000.00; in chronic diseases, the average investment was $1,333.00, with minimum of $0.00 and maximum of $6,000.00; meanwhile, for non-serious illnesses, most of the families didn't have to pay, so the average expense for their attention is $161.00, the minimum is $0.00 and the maximum $800.00.

In the case of the families that required being treated in Nochixtlán, the average cost was $2,520.00, with minimum $800.00 and maximum $6,000.00, while those who went to Oaxaca paid a price that ranges from $3,000, from a knee condition, to $40,000 in the case of a vesicle surgery, with an average of $17,714.00.

According to the result from the interviews, 100% of the participants considered that medicinal plants are a good alternative for health care and that their use allows savings in the care of non-serious illnesses, on average $1,212.00 for transport, fees and medications.

Herbal medicine practice

The members of the community have broad knowledge about the plant resources available to them for health care, and the interview respondents mentioned 19 plants on average; it should be mentioned that the plants come to mind according to the need for their use, when a member of the family presents a symptom and the woman or person in charge of caring for the ill does not know what plant to use; they resort to a family member or neighbor to ask which is the adequate plant to treat the illness and the way to use it, so it is possible to state that the knowledge remains latent among members of the community. On the other hand, after specific plants are used to treat specific illnesses, whether of their own or of a member of the family, the user acquires knowledge around this remedy, becoming a specialist in the use of useful plants for that illness.

The 32 people surveyed mentioned 103 different species with medicinal use in total, with the following being the most frequent: *hierba de coyote* (*Calea ternifolia*), horehound (*Marrubium vulgare*), *hierba de cáncer* (*Tournefortia mutabilis*), *puli* or *espule* (*Pinaropappus roseus*), juniper (*Juniperus deepeana*), *chamizo* (*Barkleyanthus salicifolius*), arnica (*Heterotheca inuloides* Cass.), wormwood (*Artemisia absinthium* L.), rue (*Ruta graveolens* L.) and aloe (*Aloe vera* L.) (Table 1).

Based on the survey conducted in the community of Santiago Ixtaltepec, it was found that the most common ailments treated with various medicinal plants are: stomach pain, post-partum treatment, cough, fever, mal de aire (bad breath), cold, diarrhea, bile, wounds, kidney discomfort, and evil eye (Table 2).

For stomach pain, 24 different plants were mentioned, with the most popular being hierba de coyote and wormwood or hierba maestra (*Artemisia absinthium* L.).

In the community, applying herbal baths is still very common after the woman has given birth, the most common plants in this treatment are: reed (*Phragmites australis*), *cachovenado* (*Dodonadea viscosa*), juniper (*Juniperus deepeana*), *vergonzosa* (*Mimosa albida*), *chamizo* (*Barkleyanthus salicifolius*), *hierba de cáncer* (*Tournefortia mutabilis*) and arnica (*Heterotheca inuloides* Cass.). The medicinal plants used for this treatment help in closing wounds, for tissues to contract, to expel the cold from inside the body, and to prevent infections.

For the treatment of cough, 18 plants were mentioned, the most relevant due to the frequency of mentions are: eucalyptus (*Eucalyptus globulus* Labill), bouganvillea (*Bougainvillea glabra*), lime (*Citrus aurantifolia*), mullein (*Verbascum thapsus*), and tandede (*Gymnosperma glutinosum*), and it is common for these plants to be consumed in a mixture of two or more, a compound tea.

Table 1. Most mentioned plants.

Common Name	Scientific Name	Frequency	%
Coyote grass	*Calea ternifolia* Kunth	39	6.44%
Horehound	*Marrubium vulgare* L.	29	4.79%
Cancer herb	*Tournefortia mutabilis* Vent.	29	4.79%
Puli	*Pinaropappus roseus* (Less.) Less.	28	4.62%
Juniper	*Juniperus deepeana* Steud.	27	4.46%
Chamizo	*Barkleyanthus salicifolius* (Kunth) H. Rob. & Brettell	26	4.29%
Arnica	*Heterotheca inuloides* Cass.	23	3.80%
Wormwood	*Artemisia absinthium* L.	20	3.30%
Rue	*Ruta graveolens* L.	19	3.14%
Aloe	*Aloe vera* L.	18	2.97%
Cachovenado	*Dodonadea viscosa* (L.) Jacq.	17	2.81%
Tandede	*Gymnosperma glutinosum* (Spreng.) Less.	15	2.48%
White sapote	*Casimiroa edulis* La Llave	15	2.48%
Smells at night	*Cestrum nocturnum* L.	12	1.98%
Ink	*Justicia spicigera* Schltdl.	12	1.98%
Rosemary	*Rosmarinus officinalis* L.	11	1.82%
Maguey papalome	*Agave nuusaviorum* García-Mend.	10	1.65%
Angel grass	*Ageratina tomentella* (Schard.) R. M. King & H. Rob.	9	1.49%
Eucalyptus	*Eucalyptus globulus* Labill.	9	1.49%
Garlic	*Allium sativum* L.	8	1.32%

Source: Self elaborated with field data.

Table 2. Most common diseases.

Disease	Frecuency	%
Stomachache	73	11.99%
Postpartum Treatment	45	7.39%
Cough	45	7.39%
Fever	40	6.57%
Bad breath	39	6.40%
Flu	31	5.09%
Diarrhea	29	4.76%
Bile / Cholera	28	4.60%
Wounds	27	4.43%
Kidney discomfort	22	3.61%
Evil eye	20	3.28%
Infections	18	2.96%
Stroke / Hematoma	17	2.79%
Diabetes	15	2.46%
Scare	11	1.81%
Gastritis	8	1.31%
Inflammation	7	1.15%
Hypertension	7	1.15%
Muscle Pain	6	0.99%

Source: Self elaborated with field data.

CONCLUSIONS

The conclusion is that herbal medicine contributes to the mitigation of health problems and is a source of savings, since it represents an important alternative for health care, for the prevention and treatment of diseases. The most common ailments identified treated with herbal medicine are stomach pain, post-partum treatment, cough, fever, bad breath, cold, diarrhea, bile, wounds, kidney discomfort, and evil eye; the most used medicinal plants in the community Santiago Ixtaltepec are *hierba de coyote* (*Calea ternifolia*), horehound (*Marrubium vulgare*), *Hierba de cáncer* (*Tournefortia mutabilis*), *puli* or *espule* (*Pinaropappus roseus*), juniper (*Juniperus deepeana*), *chamizo* (*Barkleyanthus salicifolius*), arnica (*Heterotheca inuloides* Cass.), wormwood (*Artemisia absinthium* L.), rue (*Ruta graveolens* L), and aloe (*Aloe vera* L.).

A growing use of allopathic medications is seen among inhabitants of the community of Santiago Ixtaltepec, access to them without cost, the need, the practicality, and the ease in use are some of the factors that influence the preference of inhabitants for their use; this situation reflects the advantage of the presence of the health system within the community, and although there are deficiencies, such as there not being a permanent doctor or women having to be transported to distant shelters to give birth, the attention that the clinic offers is valuable and necessary, and a right of this and any other community in the country.

The family medical unit, the presence of doctors and nurses, and the use of allopathic medications do not reduce the importance of herbal medicine for the attention of various symptoms and ailments so the members of the community are commonly affected. Their use and that of traditional medicine has an impact on the economy of the families in Santiago Nochixtlán, and this is not necessarily linked with preventing the use of allopathic medications, since these, are regularly received free of charge, as has been mentioned. The attention to symptoms and common illnesses with medicinal plants implies significant savings when avoiding the need to exit the community to receive medical attention, since the costs related to this need are mainly connected with mobility, situation given directly by the geographic location of the community and the lack of public transport, so health recovery through the use of herbal medicine implies considerable savings in this sector for the families.

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Sensitivity to fungicides of *Botrytis cinérea* (Pers.) isolated from raspberry (*Rubus idaeus* L.)

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ABSTRACT

Objective: To evaluate the sensitivity of *B. cinerea* isolated from raspberry to nine fungicides alone and in combination with potassium silicate.

Design/methodology/approach: The study evaluates the sensitivity of four isolates obtained from four raspberry plantations in October 2022, which were identified in a previous study as *B.cinerea* based on morphological, morphometric and molecular characteristics, to nine fungicides alone and combined with potassium silicate. *B. cinerea* was planted in PDA culture medium modified with fungicides plus potassium silicate, and mycelial growth and mycelial growth inhibition percentage (PICM) were evaluated. A completely randomized design with six repetitions and two controls was used, an ANOVA and Tukey's mean comparison test were performed.

Results: *B. cinerea* isolated from CITAP showed lower growth with fluazinam (PICM=100), while with azoxystrobin it presented a PICM equal to 0. *B. cinerea* isolated from Paso del Cristo and Sierra Negra 2 had lower growth with fluazinam (PICM=100), while with boscalid and azoxystrobin it showed a PICM equal to 0. In Sierra Negra 1, iprodione controlled *B. cinerea* better with a PICM equal to 100, while azoxystrobin showed a PICM equal to 0. All the isolates were sensitive when fungicides were combined with potassium silicate.

Findings/conclusions: All the isolates were sensitive to the fungicides fluazinam, fenhexamid, thiophanate methyl, captan, pyrimethanil, fludioxonil and iprodione. The isolates from Sierra Negra 1 and CITAP were sensitive to boscalid, while those from Sierra Negra 2 and Paso del Cristo were insensitive; 100% of the isolates were insensitive to azoxystrobin, which suggests that they could be resistant; finally, potassium silicate potentiates the effect of fungicides.

Keywords: potassium silicate, isolates, mycelial growth, PICM.

INTRODUCTION

Raspberry (*Rubus idaeus*) cultivation is produced in different parts of the world and approximately 886 538.5 t are harvested, where the main producer is the Russian Federation, followed by Mexico with a production of 165 677 t (FAOSTAT, 2023); the main producing states are Jalisco, Michoacán, Baja California, Guanajuato and Puebla (SIAP, 2023); the most important disease in pre- and post-harvest of this crop is gray mold induced by *Botrytis cinerea*, where the most severe damage is in the fruit (Fillinger and Elad, 2016), the symptoms are brown soft rotting, and conidiophores and conidia are formed which together form the gray mold and then the infructescence dries up and mummifies (Carisse *et al.*, 2015). If the crop is not protected with fungicides of different modes of action, the fungus could generate resistance in addition to destroying the crop in a matter of weeks (Fillinger and Elad, 2016; Nieto *et al.*, 2022). Chemical control is the main efficient means against gray mold, and currently there are seven modes of action of single-site fungicides in addition to the multi-site fungicides (Fillinger and Walker, 2016); the application of these can increase the production costs and generate selection of resistant populations (Fernández-Ortuño, 2014). One alternative to counteract this is the use of alternative products which, when combining with different fungicides, are efficient in potentiating their effect; this is the use of Si (Nieto *et al.*, 2022), which is the natural form in which plants accumulate it in intercellular spaces, the cell wall, the cell lumen, epidermis and cuticle, and which possibly acts as an adverse factor in the adequate nutrition of fungi (Datnoff *et al.*, 2011). Jennings (2007) mentions that when the Si adheres to the cell wall, the enzymes from the hyphae do not act efficiently in unfolding the cellulose to glucose, and the fungi nutrition is affected negatively so the disease decreases; therefore, Si reduces the impact and severity of diseases of the plants caused by biotrophic, hemibiotrophic and necrotrophic pathogens (Lopes *et al.*, 2014; Rodrigues *et al.*, 2015, Nieto *et al.*, 2022), in addition to activating the natural defenses of plants, primarily the expression of resistance genes (Fauteux *et al.*, 2005). Different *in vitro* studies of chemical control of *B. cinerea* have been conducted where the action mechanism of fungicides are known exactly, but information of *in vitro* control with potassium silicate is limited; it is believed that Si is toxic to it, and in its presence the fungus cannot absorb the sources of carbon from the culture medium reducing its hyphae growth rate (Velazquez *et al.*, 2016). Because of the aforementioned, the objective of this study was to evaluate the sensitivity of *B. cinerea* isolated from four commercial crops of raspberry in the state of Puebla, to nine fungicides of different chemical families, alone and in combination with potassium silicate.

MATERIALS AND METHODS

Origin of the isolates

Four isolates were used selected from 40 obtained from four raspberry plantations were used: Centro de Innovación Tecnológica en Agricultura Protegida (CITAP) in the Universidad Popular Autónoma del Estado de Puebla (UPAEP) (18.930912, −98.398468), Paso del Cristo (18.885798, −98.443146), Sierra Negra 1 (18.503310, −97.464932), and Sierra Negra 2 (18.8754977, −98.4087937). They were characterized by a previous

study and correspond to *Botrytis cinerea* GenBank (ITS: OQ618427; G3PDH: OQ630985; HSP60: OQ630986; RPB2: OQ630987).

Sensitivity to fungicides and potentiation with potassium silicate

The sensitivity of four isolates to nine fungicides of different chemical groups and modes of action, alone and combined with potassium silicate to potentiate their effect with the mycelial growth inhibition method (Table 1), was evaluated in Petri dishes (Fernández-Ortuño, 2014; Fillinger and Walker, 2016).

Preparation of modified culture media and *B. cinerea* sowing

A modified PDA culture medium was used with fungicides alone and combined with potassium silicate; they were emptied into Petri dishes of 100×15 mm. For azoxystrobin, hydroxamic salicylic acid was added at a concentration of 100 ppm to inhibit the alternate oxidative respiration; a disc of 5 mm of PDA was sown with seven-day-old *B. cinerea* growth and incubated in the darkness at 20±1 °C.

Determination of percentage of inhibition

The diameter of the colony was measured every 24 h during seven days in two perpendicular directions using a digital Vernier (Truper®, Mexico) and it was used to calculate the growth inhibition percentage (GIP) with the Abbot (1925) formula:

$$GIP = \left(\frac{Dc - Dt}{Dc} \right) \times 100$$

where: *GIP*: radial growth inhibition percentage; *Dc*: diameter of the control; *Dt*: diameter of the treatment.

Experimental design

A completely randomized design with six repetitions and two controls was used, where each Petri dish was a repetition. The experiment was repeated twice.

Data analysis

An ANOVA was carried out and a multiple means comparison test with the data obtained, using Tukey's honest significance difference method with a level of significance of 5% in the SAS V.9.1 software for Windows.

RESULTS AND DISCUSSION

Sensitivity of *B. cinerea* to fungicides alone and combined with potassium silicate

According to the statistical analysis, differences were found (α=0.05) (Pr≤F=0.0001) between treatments in the mycelial growth diameter (MGD) and the GIP of *B. cinerea* isolated from the four sampling sites, when the fungicides were evaluated alone and when they were combined with potassium silicate.

For the CITAP site, based on the means comparison analysis, it was found that fluazinam was statistically lower in MGD (0 mm) and statistically higher in GIP (100%) than in the rest of the treatments, and azoxystrobin had the highest MGD (100 mm) and showed the lowest GIP (0%) compared to the rest of the treatments (Table 1). When the fungicides were combined with potassium silicate, based on the means comparison analysis, it was identified that fluazinam plus potassium silicate and azoxystrobin plus potassium silicate were statistically lower in MGD (0 mm) and higher in GIP (100%) than the rest of the treatments, while potassium silicate presented the highest MGD (50.16 mm) and the lowest GIP (49.84%) compared to the rest of the treatments (Table 1).

For the Paso del Cristo site, when the fungicides were applied alone, with the means comparison, it was found that fluazinam was statistically lower in MGD (0 mm) and statistically higher in GIP (100%) than the rest of the treatments, and boscalid and azoxystrobin presented the highest MGD (100 mm) and the lowest GIP (0%) compared to the rest of the treatments (Table 1). In the combination of fungicides plus potassium silicate, based on the means comparison analysis, it was seen that the fungicides fluazinam, azoxystrobin and iprodione, all in combination with potassium silicate, were statistically lower than the rest of the treatments in MGD with a value of 0 mm and higher in GIP (100%), while potassium silicate presented the highest MGD (52 mm) and lowest GIP (48%) compared to the rest of the treatments (Table 1).

In the Sierra Negra 1 site, in the treatments with fungicides alone, based on the means comparison analysis, it was found that iprodione was statistically lower in MGD (0 mm) and higher in GIP (100%) than the rest of the treatments and azoxystrobin presented the highest MGD (100 mm) and the lowest GIP (0%) compared to the rest of the treatments (Table 1). In the treatments where the fungicides were combined with potassium silicate, the means comparison analysis found that fluazinam, azoxystrobin and iprodione in combination with potassium silicate were statistically lower in MGD (0 mm) than the rest of the treatments, and higher in GIP (100%) than the rest of the treatments, while fludioxonil showed the highest MGD with a value of 51.5mm and lowest GIP (48.5%) compared to the rest of the treatments (Table 1).

In the Sierra Negra 2 site, based on the means comparison analysis, it was identified that fluazinam was statistically lower in MGD (0 mm) and higher in GIP (100%) than the rest of the treatments, while boscalid and azoxystrobin presented the highest MGD (100 mm) and lowest GIP (0%) compared to the rest of the treatments (Table 1). When combining with potassium silicate, the means comparison analysis showed that fluazinam and azoxystrobin plus potassium silicate were statistically lower in MGD (0 mm) and higher in GIP (100%) than the rest of the treatments and potassium silicate alone, and presented the highest MGD (55.5 mm) and lowest GIP (44.5%) compared to the rest of the treatments (Table 1).

The sensitivity of nine fungicides of different chemical group and mode of action (Fillinger and Walker, 2016) alone and in combination with potassium silicate was evaluated, with the principle of mycelium inhibition (Fernández-Ortuño, 2014); in this regard, Shao *et al.* (2015) found five isolates of *B. cinerea* resistant to fluazinam associated to mutations, the values of CE_{50} varied from 0.23 to 0.44 μg mL^{-1}. Based on this study, the four isolates used

Table 1. Mycelial growth diameter (MGD) and growth inhibition percentage (GIP) of *B. cinerea* isolated from four sampling sites controlled with nine fungicides alone and in combination with potassium silicate.

Fungicides alone									
Fungicide	**Concentration μg mL^{-1}**	**CITAP**		**Paso del Cristo**		**Sierra Negra 1**		**Sierra Negra 2**	
		MGD (mm)	**GII (%)**	**MGD (mm)**	**GII (%)**	**MGD (mm)**	**GII (%)**	**MGD (mm)**	**GII (%)**
Fluazinam	0.08	0i*	100a	0h	100a	15.33g	84.67b	0h	100a
Fenhexamid	0.01	71.17c	28.83g	69.83b	30.17g	65c	35f	74.5b	25.5g
Tiofanato metil	0.15	38.18f	61.83d	36.33e	63.67d	42.17e	57.83d	37.67e	62.33d
Captan	3.5	47.83e	52.16e	43.5d	56.5e	51.67d	48.33e	44.83d	55.17e
Pirimethanil	0.08	29.33g	70.67c	25.33f	74.67c	31.5f	68.5c	26.5f	73.5c
fludioxonil	0.004	60d	40f	56.33c	43.67f	64c	36f	59.83c	40.17f
Boscalid	0.4	80.17b	19.83h	100a	0h	91.33b	8.67g	100a	0h
Azoxystrobin	0.2	100a	0i	100a	0h	100a	0h	100a	0h
Iprodiona	0.15	15.5h	84.5b	14g	86b	0h	100a	15.67g	84.33b
LSD		2.78	2.78	3.25	3.25	1.99	1.99	1.8	1.8
Fungicides plus potassium silicate (500 μg mL^{-1})									
Fluazinam	0.08	0g	100a	0f	100a	0g	100a	0h	100a
Fenhexamid	0.01	28.67d	71.33d	51.83a	48.17f	50.33a	49.67g	32d	68e
Tiofanato metil	0.15	27.5d	72.5d	24.67d	75.33c	33.67d	66.33d	25.67e	74.33d
Captan	3.5	35.17c	64.83e	35.17c	64.83d	40.83c	59.17e	34.67c	65.33f
Pirimethanil	0.08	22.83e	77.17c	17.33e	82.66b	21.5f	78.5b	17.67f	82.33c
fludioxonil	0.004	44.17b	55.83f	43.67b	56.33e	51.5a	48.5g	49.33b	50.67g
Boscalid	0.4	20.67e	79.33c	36.67c	63.33d	27.17e	72.83c	35.17c	64.83f
Azoxystrobin	0.2	0g	100a	0f	100a	0g	100a	0h	100a
Iprodiona	0.15	10.5f	89.5b	0f	100a	0g	100a	8.5g	91.5b
Silicato de Potasio	500	50.17a	49.83g	52a	48f	47.5b	52.5f	55.5a	44.5h
LSD		3.28	3.28	2.43	2.43	2.49	2.49	1.36	1.36

*The mean values followed by the same letters in the same column are statistically equal (Tukey α=0.05) according to Tukey's honest significant difference.

in this study could be considered sensitive to fluazinam; the isolates from the CITAP, Paso del Cristo and Sierra Negra 2 sites presented a GIP of 100%, being the best treatments; the isolate from Sierra Negra 1 presented a GIP of 84.66% at a concentration of 0.08 μg mL^{-1}. Few cases of resistance to fluzinam have been reported for *B. cinerea* (Fillinger and Walker, 206). Regarding the fungicide Fenhexamid, Fernandez-Ortuño *et al.* (2014), in strains of *B. cinerea* isolated from strawberries, obtained a CE_{50} between the discriminatory doses of 25 and 100 μg mL^{-1} and the isolates with a $CE_{50} \geq 50$ μg mL^{-1} were considered resistant. On the other hand, Esteriol *et al.* (2017) reported different degrees of resistance associated to mutations in the isolates of *B. cinerea* from grapevine, with a $CE_{50} \geq 1.25$ to 299 μg mL^{-1}. Based on these studies, the isolates used in this study could be considered sensitive to fenhexamid since they presented GIP of 25.5% (Sierra Negra 2) to 35% (Sierra Negra 1) at a concentration of 0.01 μg mL^{-1}. Studies that define the base line of sensitivity

of *B. cinerea* to methyl thiophanate showed that isolates with values of CE_{50} higher than 50 μg mL^{-1} are resistant. Based on a discriminatory dose of 50 μg mL^{-1}, Mercier *et al.* (2010) found that 92% of the isolates were resistant to methyl thiophanate in strawberry fields of California, USA, and based on these criterion the isolates of this study could be considered sensitive, because their growth was inhibited with 0.15 μg mL^{-1} presenting a GIP of 57.83% (Sierra Negra 1) to 63.66% (Paso del Cristo). Resistance to multi-site fungicides has been observed only in some cases in *Botrytis* spp., and it seems to imply the detoxification (Fillinger and Walker, 2016). In this study it was found that the four isolates evaluated can be considered sensitive to captan since they presented GIP that varied from 48.33 % (Sierra Negra 1) to 56.5 % (Paso del Cristo), with a concentration of 3.5 μg mL^{-1}. Esteriol *et al.* (2017) detected that five out of 10 isolates of *B. cinerea* obtained from grapevine showed resistance to pyrimethanil with values of CE_{50} from 10.2 to 62.1 μg mL^{-1}, associated with mutations, and based on these studies 100% of the isolates from this study could be considered sensitive because they presented a GIP that varied from 68.5% for the Sierra Negra 1 site to 74.66% for Paso de Cristo with a concentration of 0.08 μg mL^{-1}. Fernandez-Ortuño *et al.* (2014) obtained a CE_{50} in isolates of *B. cinerea* obtained from strawberry between the discriminatory doses of 0.5, 1 and 2 μg mL^{-1} and isolates with $CE_{50} \geq 0.5$ μg mL^{-1} were considered resistant to fludioxonil; based on this criterion, all the isolates of this study could be considered sensitive since they obtained GIP that ranged from 36% (Sierra Negra 1) to 43.66% (Paso del Cristo) using a concentration of 0.004 μg mL^{-1}. Esteriol *et al.* (2017) detected that the strains of *B. cinerea* isolated from grapevine that grew between CE_{50} 15.04 to >1000 μg mL^{-1} were resistant to boscalid in different degrees of resistance associated to mutations. Fernandez-Ortuño *et al.* (2014) identified isolates of *B. cinerea* resistant to boscalid at $CE_{50} \geq 75$ μg mL^{-1}, and based on this the isolates from the CITAP and Sierra Negra 1 sites could be considered sensitive since they presented GIP of 19.83% and 8.66%, respectively; however, the isolates of the Paso del Cristo and Sierra Negra 2 sites could be considered resistant because they presented GIP equal to zero, that is, there was no *in vitro* control using a concentration of 0.4 μg mL^{-1}. Regarding the fungicide iprodione, Myresiotis (2007) in strains of *B. cinerea* isolated from different vegetables, obtained a CE_{50} in the range of 0.1 to 1.42 μg mL^{-1} and the isolates with a $CE_{50} \geq 1$ μg mL^{-1} were considered resistant. Grabke *et al.* (2014) consider that the isolates of *B. cinerea* obtained from strawberry and blackberry were moderately resistant to resistant if they grew at concentrations higher than 5 μg mL^{-1} of iprodione; based on the studies mentioned, all the isolates evaluated in this study could be considered sensitive since they presented GIP that fluctuated from 84.33% (Sierra Negra 2) to 100% (Sierra Negra 1) at a concentration of 0.15 μg mL^{-1}. Esteriol *et al.* (2017) and Yin *et al.* (2012) detected that the isolates of *B. cinerea* from grapevine and apple, respectively, which grew in CE_{50} between 102 to >1000 μg mL^{-1} were highly resistant to azoxystrobin associated to mutations, and based on these data, 100% of the isolates from this study could be considered resistant to azoxystrobin since they presented a GIP of 0% using a concentration of 0.2 μg mL^{-1}; this strobilurina acts in the complex III of respiration (Qols), and this category of fungicides presents a high risk of resistance development, since the target which is the cytochrome b is codified by a mitochondrial gene (Villani and Cox, 2014); these results can

be partially explained by the management and the applications that producers make in the areas of origin of the isolates, which can be related not only to the number of applications of fungicides by cycle (15 to 20) but also to the intrinsic biological aspects of the populations of pathogens in this area.

The combination of the nine fungicides with potassium silicate decreased the *in vitro* growth in the four isolates since they presented lower MGD and therefore higher GIP than when the fungicide was used alone, and in fact, possibly resistant isolates were not identified, so it can be said that potassium silicate potentiated the effect of the fungicides; it is thought that Si is toxic and that in presence of Si the fungus cannot adequately absorb the sources of carbon from the culture medium, reducing its mycelial growth rate (Velázquez *et al.*, 2017). This could be the explanation for the low growth values of isolates in presence of potassium silicate and it is possible to hypothesize that it is an effect from toxicity, which increases the effect of fungicides.

CONCLUSIONS

All of the isolates (100%) were sensitive to the fungicides fluazinam, fenhexamid, methyl thiophanato, captan, pyrimethanil, fludioxonil and iprodione, showing different GIP. The isolates obtained from Sierra Negra 1 and CITAP were sensitive to the fungicide boscalid, while Sierra Negra 2 and Paso del Cristo were insensitive with GIP values equal to 0; 100% of the isolates were insensitive to the fungicide azoxystrobin with 0% of GIP; the isolates that were insensitive to boscalid and azoxystrobin could be considered resistant because they grew in the culture medium modified with these fungicides and presented 0% of mycelial growth inhibition; however, when these fungicides were combined with potassium silicate, all the isolates were sensitive, and this suggests that the potassium silicate potentiates the effect of fungicides.

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Colegio de Postgraduados

Evaluation of the influence of ph modification on food proteins structure by FT-IR AND AFM

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ABSTRACT

Objective: The related research of proteins is important due to their wide applications in food science. The aim of this study was to evaluate the influence of pH variation (3.6, 4.6 and 5.6) on morphometric parameters and the secondary structure of proteins (ovalbumin and gliadin). The arithmetic mean roughness (Ra) and agglomerate size (AS) of the proteins were analyzed by atomic force microscopy (AFM), while their secondary structure was analyzed.

Design/methodology/approach: by Fourier transform infrared spectroscopy (FT-IR), both at different pH. Subsequently, a correlation analysis of the morphometric changes of the proteins with their secondary structure was performed.

Results: Highlighting that it was found that, protein agglomerate size is influenced by changes in β-sheets and turn conformations.

Findings/conclusions: The novelty of this contribution consists in demonstrating that there is a close structure-functionality relationship between the morphometric parameters of proteins and their secondary structure, combining microscopy and spectroscopy techniques. This allows a clear and deep understanding of protein behavior to select the appropriate pH conditions to improve the properties of many foods.

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INTRODUCTION

The development of research related to proteins in general plays a fundamental role not only because they are essential for human growth, but also because proteins provide the structural basis for several functional properties of foods that have a profound impact on food quality. They commonly serve as gelling, binding, emulsifying and foaming agents, which are functions that modify processing and behavior in food systems (Yada, 2007).

Although it is important to consider that some protein properties are affected by various factors, such as pH, protein concentration and ionic strength (Chang *et al.*, 2016; Mine *et al.*, 1991). An important protein to study is ovalbumin, the most abundant protein in egg white is useful for its good functionalities, including gelling properties and foaming activities. It is a glycoprotein consisting of 385 amino acids (mostly of which are hydrophobic). Due to its surface hydrophilic nature, it is not a good emulsifier in its raw state (Mine, 1995; Mleko *et al.*, 2007). But the emulsion stability and emulsifying activity of this protein are considerably lower than those of β-lactoglobulin, bovine serum albumin, κ-casein, soybean β-conglycinin and blood globin (Kato *et al.*, 1983; Nakamura *et al.*, 1984). However, the pH-dependent structural changes of ovalbumin lack characterization. Although, it is known that the structural state of ovalbumin is highly sensitive to pH variation because of surface charge alteration (Chen *et al.*, 2018).

Likewise, another protein of importance for its study is gliadin, which is the major storage protein in the byproducts of wheat starch. Wheat gliadins are monomeric, disulfide-bonded proteins, which are abundant in α-helices and β-turns (Tatham & Shewry, 2012). Besides, it should be noted that the peculiar sequence (abundant in prolines and glutamines) and structure of gliadin has already been proven to be responsible for severe anaphylaxis such as celiac disease and food allergy (Kim *et al.*, 2004). Moreover, the gliadin protein has the property of generating foam such as ovalbumin but also has viscoelastic and mechanical properties that are implemented in bread that provide the extensibility to stretch the bread dough without tearing (Quester *et al.*, 2014; Thewissen *et al.*, 2011). But, compared to other proteins, some properties of gliadin are still poorly explored (Wan *et al.*, 2015).

In addition, non-destructive techniques, such as molecular spectroscopy, are available to determine the behavior of proteins at the molecular scale. Different spectroscopy techniques (X-ray diffraction, nuclear magnetic resonance, Fourier transform infrared (FT-IR) and Raman spectroscopy) have been widely used for decades to elucidate the molecular structure of different types of proteins (Horne, 2002; Wang *et al.*, 2017). As a result of the development of high analytical capacity and the ease of acquisition of spectroscopy equipment, its applications have increased dramatically within the food industry. To evaluate the conformational changes of proteins produced by their processing and pH variations, which has been useful to understand their structure-functionality relationship. It should also be noted that FT-IR spectroscopy has been suitable for assessing conformational modifications of secondary and tertiary structures of food-derived proteins (Carbonaro & Nucara, 2010). On the other hand, atomic force microscopy (AFM) has been used to determine protein topography, as reported by McMaster *et al.* (1999), who have shown that the dimensions of A-gliadin fibers can change at different pH values and concluded that fiber shape and size depend on pH. Furthermore, Vié *et al.* (2002) demonstrated by AFM that the diameter of casein micelles is reduced due to their hydrophilic and lipophilic properties. Likewise, Grácia-Juliá *et al.* (2008) studied the dispersion at high pressures of whey protein and reported that the size distributions at 200 MPa ranged between 5 and 8 nm, while at >250 MPa between 60-170 nm and concluded that at pressures >250 MPa the protein agglomerates. Therefore, to have a better understanding of the influence of pH on protein structure and the development of proteins as multifunctional components

for the food industry, the conformational and morphometric changes produced in ovalbumin and gliadin were evaluated in the present study. With the main objective of unveiling the importance of pH-dependent structural features for their properties using AFM microscopy and FT-IR spectroscopy as tools to deepen the analysis of this type of biomolecules, because there is a certain complexity of such systems in terms of composition and spatial organization.

MATERIALS AND METHODS

Samples

Ovalbumin was acquired by the supplier Hycel (Cat. 568, México) and Gliadin was acquired by Sigma-Aldrich as wheat gliadin (G3375-25G, USA). Gliadin and ovalbumin were used 1% w/v dissolved in MiliQ water; pH adjusted to corresponding using 0.1 M HCl at room temperature (25 °C). The pH values for proteins studied were 3.6, 4.6 and 5.6. The solutions were mixed by a sonicator (VCX130, SONICS Vibra cell™, 90 Newton CT, USA) with 50% of amplitude at 20 kHz and 130 W with a 120 V generator (CV13) and was used a standard probe of the sonicator (length of 113 mm and diameter of 6 mm).

Atomic force microscopy

Proteins were observed by AFM (Bruker, Bioscope Catalyst ScanAsyst, USA). AFM images of gliadin and ovalbumin were acquired at different pH values (3.6, 4.6 and 5.6 pH). Samples were placed on a glass slide, dried at room temperature for 10 min and mounted on to equipment. ScanAsyst mode was used, $5 \times 5\ \mu m^2$ scans were performed and 3 images of each sample were selected from the different pH values. The study was carried out at ambient conditions and the cantilevers used in this study were silicon cantilevers (DNP-10A) with a spring constant of 0.540 Nm^{-1} and a resonance frequency of 1 kHz. For image processing the software NanoScope Analysis v2.0 (Bruker Nano, Santa Barbara, CA, USA) was used.

Fourier transformed infrared spectroscopy

The analysis of the secondary structure of the proteins was carried out by FT-IR spectroscopy (Agilent Cary model 630, USA). A small amount of each sample was placed on the attenuated total reflectance (ATR) diamond crystal of the analyzer. The samples were pressed against the diamond crystal using the attached pressure clamp with a slip clutch press on the clamp that prevents over-tightening. It was operated in the 1000 to 1800 cm^{-1} wavenumber ranges and 64 scans at 4 cm^{-1} of resolution at room temperature (25 °C). For evaluation, a necessary baseline and smoothing correction was performed on the spectrum using OriginPro 8 software (v8.0724, USA). Finally, the deconvolution method was used to evaluate the areas of the regions of interest.

Statistical analysis

Measurements were expressed as mean values with a precision of standard deviation. The data obtained by the previous analyses were compared using the ANOVA-Tukey test, and significant differences were considered significant when $p<0.05$. Both statistical

analyses were performed using SigmaPlot v.12 software (Systat Software Inc. USA). In addition, using XLSTAT software (2020.1.3, Addinsoft, USA) a Pearson analysis was performed on all acquired variables and a visualized correlation matrix was produced.

RESULTS AND DISCUSSION

Analysis of morphometric parameters by AFM

Figure 1 shows a selection of AFM height images for protein solution at different pH values: 3.6, 4.6 (close to its isolelectric point) and 5.6. These pH values considered are within the range used in food systems (Mleko *et al.*, 2007; Thewissen *et al.*, 2011). Figures 1(a-c) correspond to AFM height images of ovalbumin, where it is noted that larger agglomerates are formed at pH 4.6 compared to pH 3.6 and 5.6. This is considered to be because the protein is quite close to its isoelectric point and therefore most likely has minimal solubility and possesses a neutral charge. While at pH 3.6 and 5.6, although they also present less agglomeration, at these pH values they are mostly soluble, and their surface is more uniform. The agglomeration size (AS) in a certain way presents relationship, as shown also in Figures 1(a-c), with the average roughness values, where the Ra values are higher at the same pH of 4.6 (4.05±2.60 nm), due to the increase of agglomeration. Since, as observed the Ra at pH 3.6 was 3.40±0.54 nm and that at pH 5.6 was 2.59±0.84 nm. Which suggests that the protein is immobilized with a slight net positive charge (Lahiri *et al.*, 1999).

Mine, (1996) reported the particle size of ovalbumin with a mean of 317.3 nm to 752.6 nm due to using pH 7.5. And in a different study of the particle size of the mixture of proteins including ovalbumin, finding Ra values of 70-295 nm because the treatment was at pH 3 and with thermosonication. Previous research reported the globular structure of ovalbumin and the size of about 24-25 nm because the protein was subjected to heat

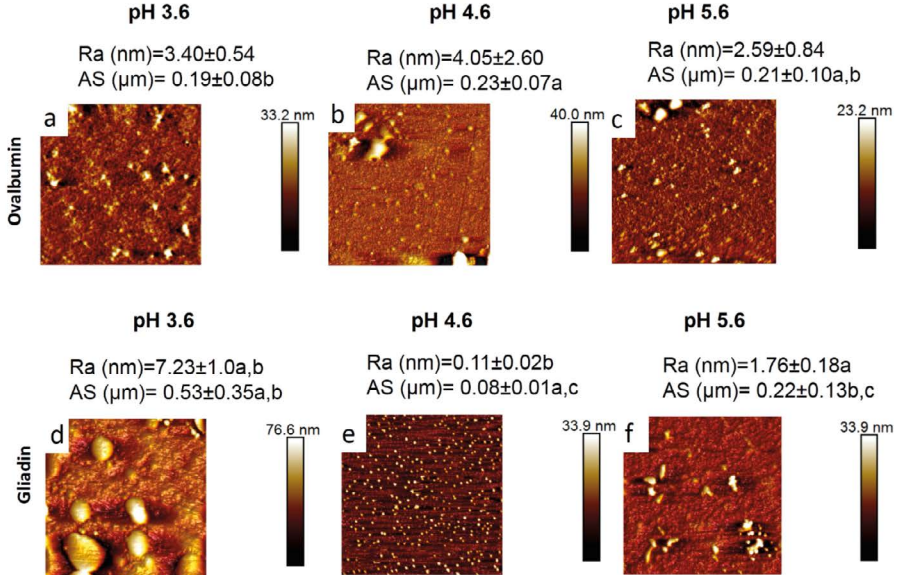

Figure 1.

treatment (Najbar *et al.*, 2003). Another study likewise confirmed the shape of the globular structure of ovalbumin and the size of 78-143 nm (Taheri *et al.*, 2012).

For gliadin, the same pH values were used and in the AFM height images shown in Figures 1(d-f) it is observed that the largest Ra values were at pH 3.6, since this is where larger and heterogeneous agglomerates are present compared to pH 4.6 and 5.6. When the protein is close to the isoelectric point (pH 4.6) it has homogeneous agglomerates of 0.08 ± 0.01 μm and larger agglomerates than at pH 3.6 and 5.6 due to protein precipitation. The Ra values vary as a function of the number of gliadin agglomerates that are not dissolved in solution. This is consistent with the characteristic size found for each pH condition. Therefore, these results show, that the size of the protein as well as the topographical characteristics depend on the specific pH value used for its precipitation and affect the different properties for food use (Moitzi *et al.*, 2011).

Research has reported average 100 nm sphenoidal structures of gliadin mixed at 70% aqueous ethanol by AFM analysis (Terence J. McMaster *et al.*, 1999). In addition, work found gliadin aggregates in egg cake substitution around 100-200 nm (Lin *et al.*, 2017). While McMaster *et al.* (2000) found α-gliadin fibrils at pH 3 with a width of 15 to 80 mm and a length of 100 nm to 2 μm. Since the agglomerate size observed in the present work is similar to that reported by previous literature, it is likely that the change in morphology of ovalbumin and gliadin as size and shape at different pH values is associated with reassembly at the molecular level.

Analysis of secondary structure by FT-IR

Proteins dissolved at different pH values exhibit modifications in morphology and solubility properties through protein rearrangement at the molecular level, but their correlation remains unknown. Consequently, the secondary structure of proteins was studied to associate these behaviors with their morphometry of the protein evaluated at different pH values. Figure 2 shows the FT-IR spectra with the characteristic bands of the protein. There is information concerning amide vibrational bands and stretching, bending and other values. The first band observed in the spectrum of ovalbumin is due to stretching of the C-C and C-O bonds (1100 cm^{-1}).

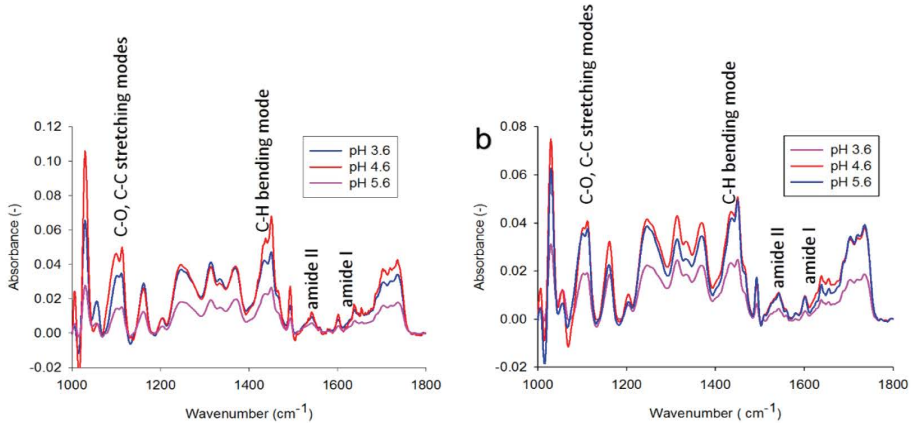

Figure 2.

The corresponding intensity is higher at pH 4.6 compared to those at pH 3.6 and 5.6. (Carbonaro & Nucara, 2010). A similar behavior had to C-H bending (1420 cm^{-1}) the lowest intensity found at pH 5.6 following that at pH 3.6. The band of amide I of ovalbumin shows a reduction of the area under the curve when pH decreases (Figure 3).

Amide II has observed a high valley on the far right generated by all peaks. Table 1 shows that at pH 4.6, ovalbumin decreases the percentage of α-helix conformation and increases the β-antiparallel conformation compared to pH 3.6 and 5.6. It is concluded that at pH 4.6 ovalbumin unfolds due to decreased solubility and hydrophobic, covalently bound interactions (Wang *et al.*, 2017).

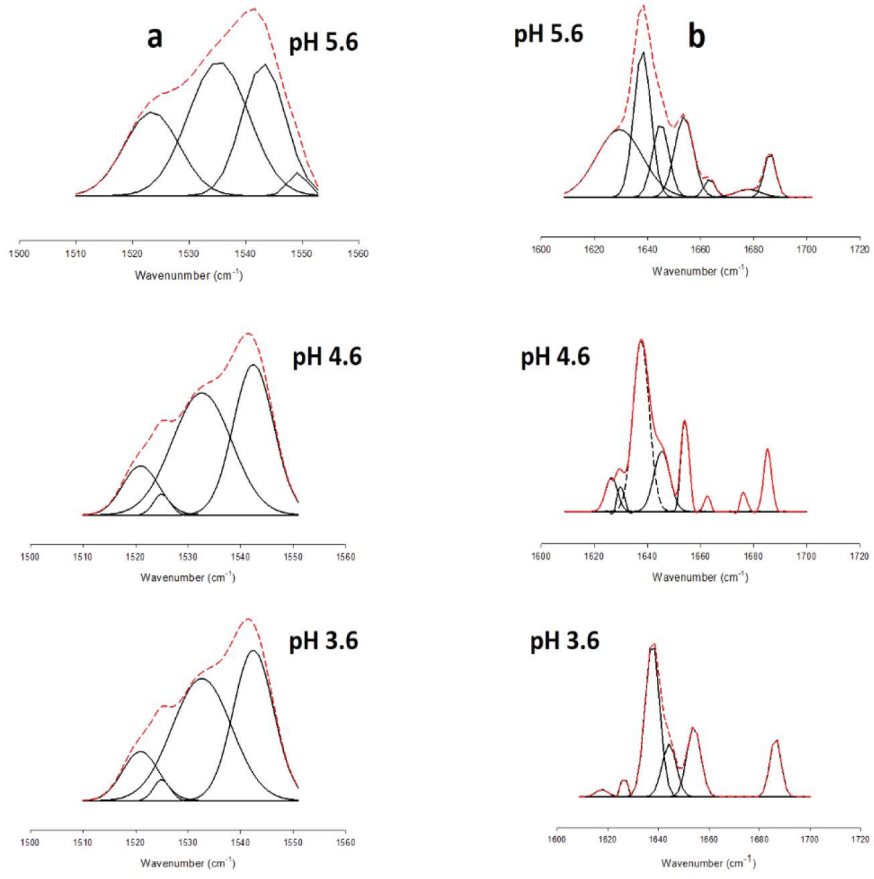

Figure 3.

Table 1. Determination of the secondary structure percentages by FT-IR of the ovalbumin solution (0.01%) at different pH values.

Protein structures	pH 3.6 (%)	pH 4.6 (%)	pH 5.6 (%)
β-Antiparallel	2.75	21.53	16.09
β-Parallel	17.26	45.46	50.14
Turn	0	3.65	4.30
Random coil	15.47	16.76	12.97
α-helix	20.21	12.57	16.48

The previously mentioned characteristics allow water retention and increase gelling properties (Li *et al.*, 2014). Due to the exposure hydrophobic groups and sulfhydryl groups (Campbell *et al.*, 2003). On the other hand, at pH 3.6, the results are different, they do not have a turning percentage but develop a higher content of the α-helix structure. This most probably means that the protein is folded. This most likely means that the protein is folded and disordered. At pH 5.6, the results are different; it develops a higher content of the β-parallel and twist structures. There is also a reduction of the α-helix conformational structure, so the protein was found to be unfolded. While in gliadin the bands show the lowest intensity for 3.6 pH compared to 4.6, and 5.6 pH these behaviors affect the bands C-H bending mode, C-O and C-C stretching mode. Gliadin had similar behavior in the amide I and amide II regions. Figure 4 shows that the amide I region at 4.6 pH and 3.6 pH, have similar behavior compared to pH 5.6 which has a heterogeneous distribution. In amide II a high valley was observed at the right end generated by all the peaks. At pH 4.6, ovalbumin increases the α-helix but decreases the β-parallel, since the protein was partially disordered and folded and when the protein is folded, it acquires energy transfer properties due to the closeness of protein-protein interaction (Chakraborty & Basak, 2007).

While the pH of 5.6 and 3.6 of gliadin (Table 2) decreases the α-helix and decreases the β-parallel conformations. The protein was partially disordered and unfolded.

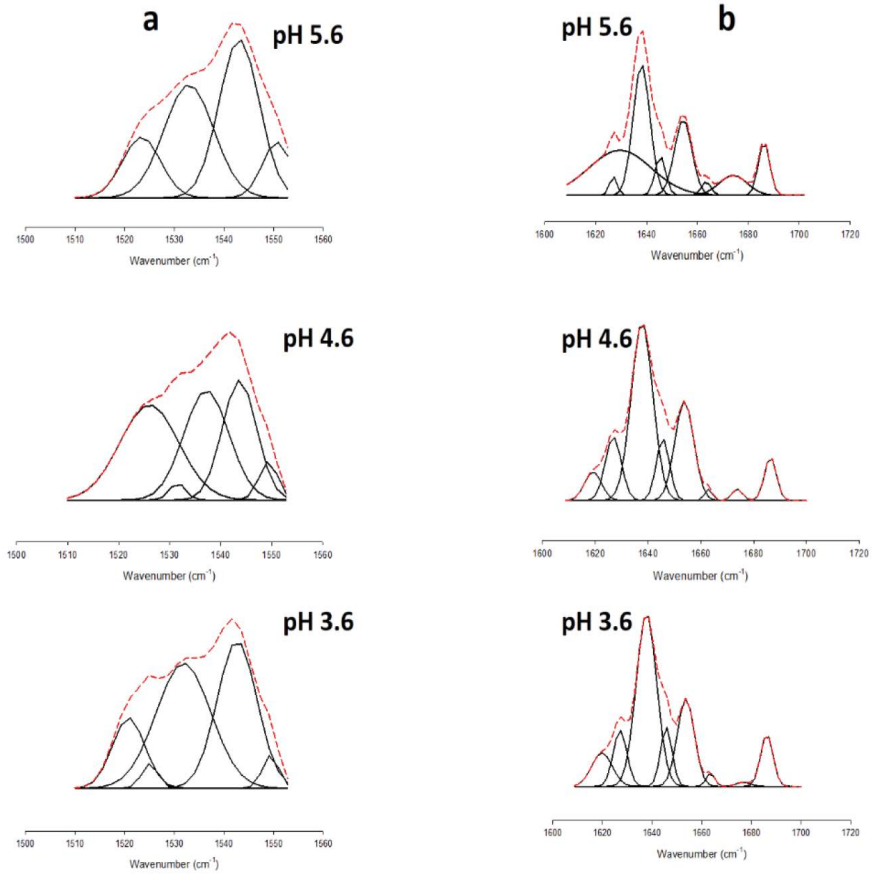

Figure 4.

Table 2. Determination of the secondary structure percentages by FT-IR of the gliadin solution (0.01%) at different pH values.

Protein structures	pH 3.6 (%)	pH 4.6 (%)	pH 5.6 (%)
β-Antiparallel	11.88	23.90	10.20
β-Parallel	58.65	42.87	60.31
Turn	2.20	2.43	8.34
Random coil	8.47	9.34	5.07
α-helix	18.78	21.43	16.05

Correlation analysis

Pearson's analysis provided a representation of the correlation of morphometric parameters with protein secondary structure. Figure 5 shows an image of a Pearson correlation matrix, where the range of its correlation coefficients is indicated. The intensity of the green and blue colorings shows which variables have the highest positive or negative correlations (green(α)=1 to 0.81, red (α)=−1 to −0.81) with F (dotted red line). Pearson matrix of ovalbumin observed positive correlation random coil conformation with roughness protein and agglomerate size with β-Antiparallel, β-Parallel and turn conformations. The negative correlation agglomerated protein with random coil and α-helix conformations. Roughness and agglomerate size also have negative correlation with α-helix.

The secondary structures of ovalbumin are correlated with each other, the positive correlation was β-Parallel with β-Antiparallel and twist conformations. In addition, the negative correlation was α-helix with β-Antiparallel. In contrast, the Pearson matrix of gliadin has positive correlation agglomerate size with β-Parallel conformation, but agglomerate size has negative correlation with β-Antiparallel. Secondary structures there are correlations with each other, negative correlation was α-helix conformation with β-Parallel and twist conformations, another negative correlation was β-Parallel with β-Antiparallel. Positive correlations were α-helix with β-Antiparallel, random coil and α-helix, another positive correlation was random coil with β-Antiparallel.

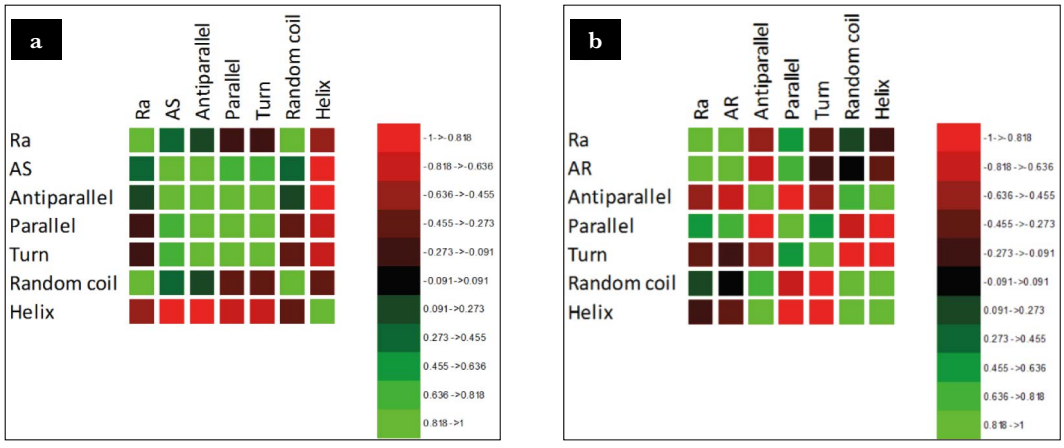

Figure 5.

CONCLUSION

Changes in pH provided ovalbumin and gliadin with useful information to correlate molecular parameters and topographical parameters. The information collected indicates that the regions of secondary structure with the greatest influence on agglomerate size are the β-Parallel conformation for proteins. Additionally, for ovalbumin the agglomerate size was β-Antiparallel and twist conformations. Overall, Ra is shown to have a higher correlation with random coil conformation for ovalbumin. Structural changes at the microstructural and molecular level in proteins and secondary structure at different pH values. This information can be valuable for understanding protein behavior and selecting appropriate pH conditions to improve some food properties.

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Colegio de Postgraduados

Hydroponic corn (*Zea mays* L.) fodder production through the implementation of mineral fertilization: a comparative study

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ABSTRACT

Objective: To produce hydroponic corn forage using a mineral fertilization planning with three different seed types and to determine the optimal concentration for production and livestock feeding.

Design/Methodology/Approach: A completely randomized experimental design was used. The following study variables were included in the design: plant height, root length, stalk thickness, and fresh biomass. Five hundred grams of three different variety seeds were selected, washed, disinfected, soaked, covered, aerated, and placed in plastic trays. Starting from the fifth day, the seeds were watered with formulated solutions until the end of production.

Results: Plant height reached 17.25 cm, at a 50% concentration; root were 12.55 cm long, at a 25% concentration with Sinaloa seeds; stalks were 2.40 mm thick, at a 25% concentration with Bajío seeds; and biomass recorded 1.184 kg at a 50% concentration with Sinaloa seeds.

Study Limitations/Implications: A greenhouse is not essential for fodder production, as long as the minimum necessary conditions (light, humidity, and temperature) are met.

Findings/Conclusions: The 50% nutrient solution had a positive influence on the study variables that used Sinaloa variety seeds. Therefore, hydroponics would be a reliable and practical technique for producers, useful during periods of prolonged drought; it would significantly counteract agroclimatological setbacks in the agricultural, livestock, and environmental sectors.

Key words: nutrient solution, hydroponics, agronomic variables, mineral treatments.

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INTRODUCTION

Hydroponic green fodder (HGF) is obtained from the germination of seeds or grains and can be used as a nutritional supplement for several animal species. It has an excellent protein percentage (Contreras *et al.*, 2015), an adequate balance between soluble and insoluble fiber, high dry matter digestibility, and a good energy contribution (Bedolla-Torres *et al.*, 2015). The intensive production of HGF in a protected environment is less prone to climate change, enables a scheduled and periodical production all year round, making an efficient use of water and reducing the use of agrochemicals and labor (Candia, 2015).

This technique does not require soil, its fodder mass can be fully (100%) consumed, it provides an ongoing supply of goods every day of the year, it prevents digestive alterations, and has lower disease incidents (Suarez, 2015). Several species, including pulses and leguminous plants, have been used for HGF production (Naik *et al.*, 2017).

Hydroponics is an alternative to agriculture: this technique provides the ideal conditions for the development and growth of plants, using only water and a full nutrient solution (Gilsanz, 2007). According to Núñez-Torres and Guerrero-López (2021), watering with a nutrient solution helps to increase biomass production, improves nutritional quality, and optimizes cutting time.

Consequently, the purpose of this project was to compare the effects of the application of mineral nutrient solutions on various morphological variables in three corn varieties.

MATERIALS AND METHODS

The study was carried out in Xalapa, a city located in northern Veracruz, at 1,460 m.a.s.l. In average, the climate is humid-temperate, with a mean temperature of 18 °C. A 4×4 m^2 space was used, with concrete walls, cement tiles, and a 2-m long × 2.19-m tall window. Mean temperatures ranged from 10 to 15 °C and a 20-75% relative humidity were recorded. Seeds from three different varieties of commercial hybrid corn were used (Sinaloa, Bajío, and Exportación), to obtain a total of 24 kg of seed.

Experiment design

HGF was produced according to the method proposed by Reyes *et al.* (2012), with some modifications: seed selection (whole, dry seeds, and 85% germination capacity); prewashing of 500 g of seeds per variety (Mejia-Castillo and Orellano-Núñez, 2019); disinfection with 2% sodium hypochlorite; washing and submersion in water for a full day; then allow aeration and rest for another 24 hours, ensuring the samples are enshrouded in black bags. The seeds were sown in 36 plastic trays watered with 250-ml sprinklers. Only water was used during the first four days, followed by a nutrient solution (NS) from days 5 to 14; treatments with a 0, 25, 50, 75% dilution of NS with minerals (hydroponic nutriments) (Linch) were applied in demijohns labelled A and B (FAO, 2001). A Steren® thermo-hygrometer was used to record the mean temperature (11 °C) and humidity (75%).

Statistical Design

A completely randomized design was used, consisting of four NS treatments (0, 25, 50, and 75%), three repetitions per treatment, and a total of 36 trays. Each treatment was evaluated in two periods (6 and 14 days). Ten seedlings were randomly selected per each NS treatment, corn variety, and repetition. HGF production was determined at 6 and 14 days, considering plant height (cm), root length (cm), and stalk thickness (mm). Fresh weight (g) was determined weighing all the plants per tray per NS treatment and corn variety (N=36). The statistical analyses were carried out using the MiniTab statistical software ver. 21.3 (Minitab, 2023). A three-way ANOVA was used to consider the 0, 25, 50, and 75% NS treatments in the corn varieties as fixed factors, at 6 and 14 days of germination. The interactions between the factors were calculated with post hoc Tukey's HSD tests.

RESULTS AND DISCUSSION

Evaluation of seedling height

Table 1 shows the results of the height evaluation of corn HGF. The Bajío and Sinaloa varieties recorded the greatest increase from day 6 to day 14 of the experiment. The Bajío variety increased by 10.73 cm, while the Sinaloa variety plants grew 10.81 cm. The Exportación variety increased by 8.33 cm. At 14 days, the Sinaloa variety plants were in average 16.05 cm tall, followed by Bajío (15.01 cm) and Exportación (13.26 cm). The tallest plants had been supplemented with 50% NS, followed by plants that received 25% NS (15.07 cm). Plants supplemented with 75% NS recorded the lowest average height (14.36 cm). Statistical comparison suggests that the Sinaloa variety has the best development at 14 days of watering with 25 and 50% NS concentrations. On the contrary, corn plants from the Exportación variety were the smallest at 14 days, with 0 and 75% NS.

Table 1. Plant height in several periods, varieties, and NS concentrations.

Period *Variety (%)*NS	N	Average	Group										
14 Sinaloa 50	10	17.25	A										
14 Sinaloa 25	10	15.97	A	B									
14 Sinaloa 75	10	15.5		B	C								
14 Sinaloa 0	10	15.38		B	C								
14 Bajío 50	10	15.3		B	C								
14 Bajío 75	10	15.1		B	C								
14 Bajío 25	10	15		B	C	D							
14 Bajío 0	10	14.6			C	D							
14 Exportación 25	10	14.25			C	D							
14 Exportación 50	10	13.7				D	E						
14 Exportación 0	10	12.6					E						
14 Exportación 75	10	12.5					E						
6 Sinaloa 25	10	6.87						F					
6 Sinaloa 50	10	6.75						F					
6 Exportación 25	10	6.7						F					
6 Exportación 50	10	5.7						F	G				
6 Bajío 50	10	5.1							G	H			
6 Bajío 75	10	4.5							G	H	I		
6 Sinaloa 75	10	4.42							G	H	I	J	
6 Bajío 25	10	4.2								H	I	J	
6 Exportación 75	10	3.95								H	I	J	K
6 Exportación 0	10	3.35									I	J	K
6 Bajío 0	10	3.15										J	K
6 Sinaloa 0	10	2.82											K

Means without a common letter significantly differ.

Evaluation of root length

The Sinaloa variety recorded the greatest increase in root length: 6.61 cm from day 6 to day 14. During the same period, the Bajío and Exportación varieties increased by 2.23 and 2.36 cm, respectively. The greatest increase regarding root length, from day 6 to day 14, was recorded with 25% (3.38 cm) and 75% NS (3.37 cm). A significative increase was likewise recorded with 25% NS (8.63 cm), followed by 50% (8.35 cm). A significative increased was equally recorded with 25% NS (8.63 cm), followed by 50% (8.35 cm). The tallest plants at 14 days belonged to the Sinaloa variety supplemented with 25 and 50% NS. Finally, the lowest growth was recorded at 14 days by the Bajío variety, with 25 and 75% NS (Table 2).

Evaluation of stalk diameter

The Bajío variety recorded the highest increase in stalk diameter (0.1 cm) from day 6 to day 14. The Exportación and Sinaloa varieties increased by 0.08 and 0.09, respectively. Diameter increased by 0.1 cm with 25% and 75% NS concentrations. For their part, the Bajío and Sinaloa varieties recorded a greater diameter at 14 days with 25% NS. Finally, the Exportación variety recorded the lowest values with 75 and 0% NS (Table 3).

Table 2. Tukey's multiple comparison of root length.

Period *Variety (%) *NS	N	Average	Group									
14 Sinaloa 25	10	12.55	A									
14 Sinaloa 50	10	12.4	A									
14 Sinaloa 0	10	11.55	A	B								
14 Sinaloa 75	10	10.5		B	C							
14 Exportación 25	10	9.5			C							
14 Bajío 0	10	7.3				D						
6 Sinaloa 50	10	7.1				D						
6 Sinaloa 25	10	7				D						
14 Exportación 50	10	6.75				D	E					
6 Exportación 25	10	6.5				D	E	F				
14 Bajío 50	10	5.9				D	E	F				
14 Exportación 75	10	5.8				D	E	F				
6 Exportación 50	10	5.75				D	E	F				
14 Exportación 0	10	5.7				D	E	F	G			
6 Bajío 50	10	4.9					E	F	G	H		
14 Bajío 75	10	4.75						F	G	H	I	
14 Bajío 25	10	3.85							G	H	I	J
6 Sinaloa 75	10	3.46								H	I	J
6 Exportación 75	10	3.35								H	I	J
6 Bajío 75	10	3.05								H	I	J
6 Sinaloa 0	10	2.97									I	J
6 Exportación 0	10	2.7										J

Means without a common letter significantly differ.

Table 3. Tukey's multiple comparison test of stalk diameter.

Period *Variety * (%) NS	N	Average	Group							
14 Bajío 25	10	2.4	A							
14 Sinaloa 25	10	2.39	A							
14 Exportación 50	10	2.37	A	B						
14 Sinaloa 50	10	2.37	A	B						
14 Exportación 25	10	2.33	A	B	C					
6 Sinaloa 50	10	2.31	A	B	C	D				
6 Sinaloa 25	10	2.29	A	B	C	D	E			
14 Bajío 50	10	2.27	A	B	C	D	E			
6 Bajío 50	10	2.27	A	B	C	D	E			
6 Exportación 50	10	2.25	A	B	C	D	E			
6 Exportación 25	10	2.24	A	B	C	D	E	F		
14 Sinaloa 75	10	2.19	A	B	C	D	E	F		
14 Sinaloa 0	10	2.17	A	B	C	D	E	F	G	
6 Bajío 25	10	2.16	A	B	C	D	E	F	G	H
14 Bajío 75	10	2.15	A	B	C	D	E	F	G	H
14 Bajío 0	10	2.13		B	C	D	E	F	G	H
14 Exportación 75	10	2.09			C	D	E	F	G	H
6 Sinaloa 0	10	2.08			C	D	E	F	G	H
6 Bajío 0	10	2.07				D	E	F	G	H
6 Sinaloa 75	10	2.06				D	E	F	G	H
6 Bajío 75	10	2.05					E	F	G	H
6 Exportación 75	10	1.99						F	G	H
14 Exportación 0	10	1.92							G	H
6 Exportación 0	10	1.91								H

Means without a common letter significantly differ.

Evaluation of total biomass

The Sinaloa variety recorded the largest biomass production values at 14 days (787.83 g), followed by Bajío (712.08 g) and Exportación (671.66 g). The greatest biomass was reported with 50% NS (920 g), followed by 25% NS (702.77 g) and 75% NS (630.66 g). The Sinaloa variety, fertilized with 50% NS, showed the highest biomass amount.

HGF production involves a methodology for the development of plant biomass from the early seedling stages (embryogenesis and early progress phases), using seeds with a high germination index to generate a nutritious live fodder with outstanding digestibility, which is adequate for animal consumption (Bedolla-Torres *et al.*, 2015; Contreras *et al.*, 2015).

Consequently, the results of this research suggest that the Sinaloa variety recorded the greatest height (17.25 cm), with only 50% of fertilizer concentration. Tomalá (2021) reported an average height of 25 cm in corn germinules treated with bokashi fertilizers.

For his part, Suarez (2015) reports 28.79 cm at 12 days, using the FAO's nutrient solution. Meanwhile, Víquez and Soto (2017) recorded between 26.38 and 28.13 cm at 14 days with mineral nutrition. In turn, Machaca (2018) recorded 26.9 cm with liquid cattle manure. Likewise, Silva (2017) reported a 5.67-12.80 cm range with 6-12-day fertilizations, using various solutions supplemented with Raizal®, Cytokin®, and Newgibb® phytohormones. Finally, Valverde *et al.* (2017) reported an average of 14 cm at 12 days after germination, watering corn with conventional chemical and organic fertilizers.

Vélez (2020) reported a similar root length (10.99 cm) using a mineral fertilization process. Morales *et al.* (2012) observed 12.3-23.03 cm root growth in 10-14-day cultivars. Likewise, Valverde *et al.* (2017) recorded 12.49 cm in white corn at 12 days after fertilization. The stalk thickness results match the findings of Duran (2007), who reported 2.27 and 2.40 mm at 6 and 9 days after the fertilized germination, respectively. Espinosa (2019) suggests that supplying a nutrient diluted solution through a drip irrigation system would result in an up to 2.66 mm diameter at 12 days. In that sense, Cuadra-Quispe (2019) observed a 2.96 mm growth when certified corn seeds (type: V-53) were irrigated with 3.5% NS minerals. Finally, Varela Rojas (2016) proved that the diameter of HGF produced from certified corn seeds (NB-6, NB-S), using a chemical fertilizer, would reach approximately 3.3 mm at 15 days.

HGF has been established as an innovative technique for the generation of livestock sustenance that remediates inherent setbacks of traditional production, including deprived plots, scarce rainfall, high evaporation, and very low-quality water and soil resources. Consequently, it is taking shape as an alternative to conventional production procedures, contributing to the sustainability of farming activities in arid and semi-arid areas (Varela Rojas, 2016).

The biomass production results of this research were compared with those of previous studies. In the first place, Vélez (2020) recorded 2.11 kg/m^2 of sprouts using a 2 g/L dose of zeatin. Muñoz *et al.* (2008) reported 1.8-2.3 kg/m^2 corn production with several NS types and doses. Likewise, Preciado *et al.* (2014) applied vermicompost to corn, recording 1:7 germination ratios at 14 days; they recorded 1:5-1:6 ratios between native corn and organic NS, reporting 18.1-19.7 kg/m^2 of sprouts at 16 days. Finally, Tomalá (2021) observed a 2.38 kg yield with the use of mineral NS.

The germination process is unleashed when the seed is moisturized (watering). When roots and the first leaves appear, the plant is able to obtain nutrients from its surroundings and other essential elements for the synthesis of its own sustenance through photosynthesis. Therefore, the specimen must be subjected to ideal nutritional conditions, including watering processes that use mineral solutions (Gilsanz, 2007; Núñez-Torres and Guerrero-López, 2021).

CONCLUSIONS

HGF production complements commercial products used to feed cattle; it is particularly useful during drought periods, when fresh forage is scarce. Hydroponic crops are viewed as an outstanding nutrient source for animal diets; therefore, additional research must be carried out on this subject, using a greater variety of fodder plant species, to offer

more options for producers. Consequently, HGF production is an efficient alternative that guarantees a constant supply of food for the animals, significantly counteracting agroclimatic setbacks.

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Acclimation of *Agave potatorum* Zucc. micropropagated plants

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ABSTRACT

Objective: *Agave potatorum* Zucc. is an intensively exploited wild plant species. We intend to evaluate how the concentration of mineral salts (MS), indole-butyric acid (IBA), and the incubation conditions affect *in vitro*-rooted *A. potatorum* during acclimation.

Design/Methodology/Approach: We conducted 18 treatments that resulted from combining three factors: 1) MS concentrations (50%, 75%, and 100%); 2) IBA concentrations (without auxin, 0.5 mg L^{-1}, and 1 mg L^{-1}); and 3) incubation environments (fluorescent lighting in a laboratory or exposure to solar radiation in a greenhouse). Thirteen plants from each treatment were transplanted into individual pots containing a 1:1 mixture of peat moss and perlite. These were placed in the acclimation greenhouse for 150 days, exposed to changes in solar radiation, starting from 600 μmol m^{-2} s^{-1} and conditions of high relative humidity (80-90%), and reaching outdoor conditions with full solar radiation,1400±200 μmol m^{-2} s^{-1}. All *in vitro* culture plants, both in greenhouse and laboratory, underwent the same environmental and management conditions.

Results: After 150 days of acclimation, plants micropropagated in a culture medium with 0.5 to 1 mg L^{-1} of IBA, 100% MS, and incubated in a greenhouse showed better growth.

Study limitations/Implications: The *in vitro* culture protocol was (successfully) modified by providing solar radiation in a greenhouse during the rooting of *A. potatorum* sprouts.

Findings/Conclusions: Solar radiation during *in vitro* incubation of *A. potatorum* favors its acclimation.

Keywords: Solar radiation, Adaptation, *In vitro* culture, Agave.

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INTRODUCTION

Agave potatorum Zucc. is a monocotyledonous wild species that grows in the Central Valleys, the Sierra Sur, and the Mixteca regions in Oaxaca, Mexico. Its extraction is intensive and lacks any management, which reduces its population, all the more since it is the raw material to produce tobalá mezcal —a representative distilled beverage of the state (Pérez and Casas, 2007; Enríquez-del-Valle *et al.*, 2016a). Seeds are the only form

of natural reproduction for this species. This reproduction mechanism is not enough to compensate for the number of extracted plants, which is why some producers in the Sierra Sur and the Mixteca regions have established small plantations since the late 1990s (Enríquez-del-Valle *et al.*, 2008; Enríquez-del-Valle *et al.*, 2016a). However, the demand exceeds the availability of plants (Enríquez-del-Valle *et al.*, 2021; Correa-Hernández *et al.*, 2022). Micropropagation can be a strategy to address the situation and increase plant production in the short term (Enríquez-del-Valle *et al.*, 2016b).

The micropropagation technique has proved successful in rescuing and preserving threatened species. It is also an effective technique to obtain large clonal populations from selected plants and large-scale multiplication of superior genotypes. Micropropagation consists of the aseptic asexual propagation from plant cells, tissues, or organs that enables the generation of somatic tissues, cell division, and morphogenesis, as in the production of sprouts, roots, and somatic embryos (Enríquez-del-Valle *et al.*, 2016a, 2021). Acclimation is the final stage of all micropropagation schemes, and crop survival depends on it. Micropropagation has already been used in *Agave fourcroydes* (Madrigal *et al.*, 1990), *A. tequilana* (Valenzuela-Sánchez *et al.*, 2006), *A. karwinskii* and *A. potatorum* (Domínguez *et al.*, 2008; Bautista-Castellanos *et al.*, 2020; Correa-Hernández *et al.*, 2022), *A. cocui* Trelease (Salazar *et al.*, 2009), and *A. angustifolia* (Enríquez-del-Valle *et al.*, 2005; Ríos-Ramírez *et al.*, 2017, 2018). Artificial lighting was used in all incubation stages of the cited *in vitro* cultures. During acclimation in the greenhouse, micropropagated plants of *Agave Americana* var. Oaxacensis adapted using sand or perlite as a substrate, and their size related positively to the applied fertigation dose (Enríquez-del-Valle *et al.*, 2013). Modifications can be implemented in each stage of the *in vitro* propagation scheme to increase production efficiency and plant quality. Thus, it is possible to evaluate variations in the concentration of components in the culture media, particularly mineral salts and growth regulators, such as indole-3-acetic acid (AIA), naphthaleneacetic acid (NAA), and indole-butyric acid (IBA), which induce adventitious root formation and sprout growth by cell extension in the sprouts of different species (Gilroy and Trewavas, 2001; Soto *et al.*, 2006). *Agave potatorum* Zucc. is an intensively exploited wild species. We intend to evaluate how the concentration of mineral salts (MS), indole-butyric acid (IBA), and the incubation conditions affect *in vitro*-rooted *A. potatorum* during acclimation.

MATERIALS AND METHODS

The experiment was conducted in the plant-tissue culture laboratory, the acclimation greenhouse, and the nursery of the Instituto Tecnológico del Valle de Oaxaca, located in the municipality of Santa Cruz Xoxocotlán, Oaxaca. The institute is located at 96° 44' W, 17° 02' N, at an altitude of 1530 masl.

The plant material was obtained from *in vitro* cultures of *Agave potatorum* Zucc. in its rooting stage. Said material came from 165 cm^3 flasks containing 25 mL of one of nine variants of culture media to induce the formation of adventitious roots. The composition of the culture media combined the following components: 1) 0.4 mg L^{-1} thiamine HCL; 50 mg L^{-1} myo-inositol; 30 g L^{-1} sucrose; 2) inorganic salts (MS) in different concentrations (50%, 75%, and 100%); and 3) auxin of indole-butyric acid (IBA) in different concentrations

(0, 0.5, and 1 mg L^{-1}), with a pH of 5.8 and 5.6 g L^{-1} of agar. In addition, we used different types of incubation: 1) in laboratory conditions, with fluorescent light at 35 μmol m^{-2} s^{-1}, for a photoperiod of 16 h to 8 h of darkness, with a temperature in the range of 18 °C to 29 °C; 2) in greenhouse conditions, where plant material was exposed to solar radiation, 400 μmol m^{-2} s^{-1}, under a polyethylene cover, using the natural photoperiod, and a wide range of daily temperatures (12 °C-29 °C).

Thirteen plants in the rooting stage were transplanted per treatment, each into a 250 cm^3 pot with a 1:1 mixture of peat moss and perlite. The potted plants were then placed for 63 days in the acclimation greenhouse, exposed to solar radiation, which reached 600 μmol m^{-2} s^{-1} at midday, under conditions of high relative humidity (80%-90%) produced for a period of 10 seconds every 12 minutes by an intermittent nebulization system from 11 a.m. to 3 p.m. Every day, after mist irrigation, plants were fertigated at the substrate level with 10 mL $plant^{-1}$ of Steiner (1984) nutrient solution diluted to 20%.

From day 64 to day 90, the plants were placed in the nursery under shade mesh, where they were exposed to solar radiation, which reached 900 μmol m^{-2} s^{-1} at midday, under low relative humidity (60%-70%) outdoors. From day 91 to day 150, the plants were exposed to full solar radiation, which reached 1400±200 μmol m^{-2} s^{-1} at midday. In the nursery, the plants were irrigated with water at the substrate level. Twice a week, fertigation was conducted with the same nutrient solution formulation, except the nutrient concentration was increased to 100%. Throughout acclimation, all plants from the 18 *in vitro* culture treatments underwent the same environmental and management conditions in the greenhouse and nursery. Our experiment used a completely randomized design with a 3×3×2 factorial arrangement. The 18 treatments consisted of variations in the culture media and *in vitro* culture incubation conditions in which plants were obtained. The experimental unit was a potted plant, and 13 replications per treatment were conducted. After 150 days of ex vitro growth, 10 plants from each treatment were randomly selected to assess the following variables: number of leaves, stem diameter, foliar volume, root volume, foliar area, and total dry weight. The data were used to conduct analyses of variance and means comparisons (Tukey, 0.05).

RESULTS AND DISCUSSION

Acclimation of micropropagated agave plants

After 150 days of acclimation in the greenhouse/nursery, all the plants obtained from the various *in vitro* culture conditions adapted and grew. However, although all plants were managed similarly in the greenhouse and nursery, differences in size persisted due to their distinctive *in vitro* culture environments. The *in vitro* culture conditions affected the characteristics of the plants and their performance during the acclimation stage. The analyses of variance (Table 1) show that MS concentration in the culture medium had a highly significant effect ($P \leq 0.01$) on foliar volume, root volume, foliar area, and total dry weight. The concentration of IBA in the culture medium had a significant effect ($P \leq 0.05$) on root volume and a highly significant effect ($P \leq 0.01$) on foliar area, foliar volume, and total dry weight. The *in vitro* incubation environment had a significant effect ($P \leq 0.01$) on the number of leaves, foliar volume, foliar area, and total dry weight.

Table 1. Variance analysis of the characteristics of micropropagated plants of *Agave potatorum* after 150 days of acclimation.

Source	DF	Mean squares and significance					
		NL	SD (cm)	VS (cm^3)	RV (cm^3)	FA (cm^2)	TotDW (g)
Treat	17	2.92 **	6.09 ns	17.30 **	8.36 **	6061.47 **	1.27 **
Sal	2	3.44 ns	9.13 ns	42.44 **	16.22 **	9117.10 **	3.88 **
IBA	2	1.81 ns	6.38 ns	17.61 **	12.07 *	4919.37 **	2.20 **
Inc	1	15.02**	2.59 ns	76.05 **	3.76 ns	42331.04**	2.87 **
Sal×IBA	4	1.71 ns	6.47 ns	7.59 *	4.88 ns	3113.37**	0.41 ns
Sal×Inc	2	4.24 *	4.23 ns	18.72 **	20.24 **	5180.08**	1.54 *
IBA×Inc	2	2.04 ns	5.86 ns	4.02 ns	0.29 ns	1680.11ns	0.52 ns
Sal×IBA×Inc	4	1.21 ns	5.98 ns	5.53 ns	5.32 ns	1616.77ns	0.22 ns
Error	162	1.31	6.18	3.09	2.6	796.1	0.34
Total	179						

VS=variation source; DF=degrees of freedom; IBA=indole-butyric acid; Inc=incubation; NL=number of leaves; SD=stem diameter; FV=foliar volume; RV=root volume; FA=foliar area; TotDW=total dry weight. **=highly significant F value (P≤0.01); *=significant F value (P≤0.05); ns=not significant F value (P>0.05).

The agave plants obtained in culture media with MS concentrations at 100% and 50% had 7.8 and 7.4 leaves, a stem diameter of 1.7 and 1 cm, 7 and 5.4 cm^3 of foliar volume, 4.5 and 3.5 cm^3 of root volume, 145.6 and 122 cm^2 of foliar area, and 5.91 and 4.39 g of total dry weight respectively. In each case, the magnitudes observed were significantly different (Tukey, 0.05). Previous studies of *Agave potatorum* in its rooting stage showed that a higher concentration (100%) of indole-butyric acid (IBA) and auxins increased the number of roots and the stem diameter compared to plants grown in a culture medium with a lower MS concentration (75%) (Bautista-Castellanos *et al*., 2020). Similarly, a higher MS concentration in the rooting stage has a positive effect: it increases the size, number of leaves, foliar volume, foliar area, and total dry weight (Enríquez-del-Valle *et al*., 2021). The same results were obtained for *Agave americana* var. Oaxacensis (Miguel-Luna *et al*., 2013).

Auxin-type growth regulators have been widely studied and used to root various plant species, such as cuttings of *Caesalpinia echinata* Lam. and *Malvaviscus arboreus* Cav., rooted with indole-3-butyric acid (IBA) (Endres *et al*., 2007; Loss *et al*., 2009). Said regulators are also used in *in vitro* culture media for various species. For instance, when cultivating *A. angustifolia in vitro*, IBA is used for the multiplication of sprouts and rooting of plants since it reduces the time required for roots and adventitious sprouts to emerge (Enríquez-del-Valle *et al*., 2005). Miguel-Luna *et al*. (2013) report similar data for *in vitro* cultures of *Agave americana* var. Oaxacensis. The same happens with other species, such as *A. potatorum* (Bautista-Castellanos *et al*., 2020; Enríquez-del-Valle *et al*., 2021). The previous results concur with our research, in which plants from a culture medium with an IBA content of 1 and 0 mg L^{-1} obtained values of 7.7 and 7.5 leaves, 1.1 and 1 cm in stem diameter, 6.6 and 5.5 cm^3 in foliar volume, 4.2 and 3.4 cm^3 in root volume, 139.99 and 122.05 cm^2 in foliar area, and 5.7 and 4.6 g of total dry weight respectively (Table 2). In each case, significantly different magnitudes were observed (Tukey, 0.05).

Table 2. Characteristics of *Agave potatorum* Zucc. plants obtained under different *in vitro* culture conditions after 150 days of *ex vitro* development.

Factor	Characteristics of plants		
	NL (cm)	**SD (cm^2)**	**FV (cm^3)**
MS Salt (%)			
50	7.4±1.22[a]	1.0±0.29[a]	5.4±1.81[b]
75	7.4±1.21[a]	1.0±0.29[a]	5.7±1.7[b]
100	7.8±1.16[a]	1.7±4.26[a]	7.0±2.41[a]
IBA dosage (mg L^{-1})			
0	7.5±1.1[a]	1.0±0.3[a]	5.5±1.9[b]
0.5	7.6±1.3[a]	1.6±4.2[a]	6.1±2.0[ab]
1	7.7±1.1[a]	1.1±0.3[a]	6.6±2.1[a]
Incubation environment			
lab	7.2±1.11[b]	1.1±0.5[a]	5.4±1.77[b]
gh	7.8±1.23[a]	1.4±0.3[a]	6.7±2.22[a]
Factors	Characteristics of plants		
MS Salt (%)			
	VR (cm^3)	AF (cm^2)	PSToT(g)
50	3.5±1.7b	122±32.26b	4.39± 1.80 b
75	3.6±1.4b	127.6±29.98b	5.26±1.84ab
100	4.5±2.03[a]	145.6±41.02[a]	5.91± 2.36 a
AIB dosage (mg L^{-1})			
0	3.4±1.4b	122.05±35.1[a]	4.6±1.95 b
0.5	4.0±1.9ab	133.20±37.2[a]	5.3±2.27 ab
1	4.2±1.8[a]	139.99±33.7[a]	5.7± 2.35 a
Incubation environment *			
lab	3.7±1.53[a]	116.4±29.72b	4.88± 2.01 b
gh	4.0±1.98[a]	147.0±35.32[a]	5.60± 2.20 a

IBA=indole-butyric acid; gh=greenhouse; lab=laboratory; NL=number of leaves; SD=stem diameter; FV=foliar volume; RV=root volume; FA=foliar area; TotDW=total dry weight. Means with identical letters in the same column within each block of rows are not statistically different (Tukey 0.05). Each mean is followed by its standard deviation. *TotDW=comparison in incubation environment; means with the same letter in the same column are not significantly different (t, 0.05).

The results of our study show that after 150 days of *ex vitro* growth, the plants from *in vitro* cultures incubated in a greenhouse exceeded the values of plants obtained from *in vitro* cultures incubated in the laboratory by, on average, 7.7% in number of leaves, 21.42% in stem diameter, 19.4% in foliar volume, 7.5% in root volume, 28.8% in foliar area, and 12.85% in total dry weight (Table 2). The above values suggest that, at the end of the rooting stage, the plants obtained from *in vitro* cultures incubated in the greenhouse developed leaves with morphology and physiology closer to those of plants considered adapted to the *ex vitro* environment. Consequently, these plants suffered less stress and growth arrest when compared to plants from *in vitro* cultures incubated in the laboratory.

It is possible that exposure to solar radiation (400 μmol m^{-2} s^{-1}) during incubation in the greenhouse caused the in vitro cultures in their sprout-rooting stage to make gradual adaptations. Hence, this period serves as a pre-adaptation stage, as Teixeira-da Silva *et al.* (2005) described for the micropropagation of *Musa* and *Cymbidium*.

The characteristics of plants obtained from *in vitro* cultures incubated in the greenhouse and those obtained from *in vitro* cultures incubated in the laboratory coincide with the description of Miguel-Luna *et al.* (2013), who reported morphological variation in the in vitro rooting stage of *Agave americana* var. Oaxacensis incubated in two environments: a laboratory with fluorescent lighting and a greenhouse exposed to solar radiation. However, they did not evaluate the ex vitro performance of the micropropagated plants. In our study, we prove that the morphological characteristics of *Agave potatorum* plants resulted from the composition of the culture medium and the physical incubation environment and that said morphological characteristics affected the plants' *ex vitro* growth.

CONCLUSIONS

Agave potatorum Zucc. sprouts established in culture media with 1 mg L^{-1} of IBA produced plants with wider leaves, greater total dry weight, and larger stem diameter when compared to plants obtained in culture media without IBA. As the concentration of MS in the culture medium increased, the plants obtained were larger. The *in vitro* sprouts incubated in a nursery environment produced plants with larger stem diameters than the *in vitro* sprouts incubated in a laboratory environment. After five months of *ex vitro* development, plants from sprouts rooted in culture media with 0.5 to 1 mg L^{-1} of IBA, 100% concentration of inorganic salts (MS), and incubated in a greenhouse reached a larger size than plants from *in vitro* cultures incubated in the laboratory.

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Evaluation of homeopathic treatments for the control of moniliasis (*Moniliophthora roreri* Samson & Benny), in cacao (*Theobroma cacao* L.)

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ABSTRACT

Objective: To evaluate low- and high-dilution homeopathic products in the incidence of *Moniliophthora roreri* in cacao plants.

Design/Methodology/Approach: The plantation was studied using a randomized block design with three repetitions and six treatments: Homeopathic nosode 12C and 200C, Cacao homeopathic combination remedy 12C and 200C, water control, and control sample, with applications every 15 days. The studied variables were: Number of flowers, number of healthy and diseased cherelles, number of healthy and diseased pods, ripe pod weight, fresh grain weight, and leaf color.

Results: A significant difference was observed in the number of flowers with Cacao combined remedy 200C during November-December 2018. A significant difference was observed in leaf color with Cacao combined remedy 200C during October 2018-January 2019 and with Cacao combine remedy 12C in October 2018. No significant difference was found in treatments for number of healthy and diseased cherelles, number of healthy and diseased pods, ripe pod weight, and fresh grain weight.

Study limitations/Implications: The treatment application period was short. In order to obtain more convincing results, a one-year application period prior to evaluation is suggested.

Findings/Conclusions: The Cacao combined remedy 200C had a positive effect on the number of flowers during the period in which flowering decreases due to environmental conditions and on leaf greenness during four consecutive months of evaluation. Cacao homeopathic combination remedy 12C showed a significant difference for the latter variable only in October 2018. This suggests that the low-dose homeopathic medication and a high centesimal potency promotes a better response of the cacao plant to an acute disease such as moniliasis.

Keywords: Agrohomeopathy, *Moniliophthora roreri*, Tabasco.

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INTRODUCTION

The cultivation of cacao (*Theobroma cacao* L.) has cultural, social, economic, and environmental importance in Mexico. Tabasco is the leading producer nationwide with 68% and 61% of crop area and production respectively (SIAP, 2018). Mexico is the thirteenth worldwide producer with less than 1%, with yields of 0.62 to 0.46 tons/ha. This country showed a 27% decrease in planted area during 2003-2019 (CDRESSA, 2020)

due to old plantations, incidence of diseases and pests, and change in land use caused by low grain prices (Espinosa-García *et al.*, 2015). One of the main problems that impacts yield by up to 80% is moniliasis, caused by the fungus *Moniliophthora roreri* (Cif. and Par.) (Evans *et al.*; Tirado-Gallego *et al.*, 2016), which was first reported in Mexico in 2005 and mainly impacts fruits with less than a two-month development (Hernández *et al.*, 2015). Fruit ripening takes place approximately 140-205 days after pollination (Castelán, 2010; De Sousa *et al.* 2018). Moniliasis infection in fruits varies with fruit maturity: in 20- to 60-day-old cherelles, the infectious cycle lasts 40 days and fruits present malformations or humps; in 60- to 80-day-old pods the cycle lasts 55-75 days and causes premature ripening and malformations; 120- to 160-day-old pods do not present external symptoms and the seeds are not impacted (Evans, 2016). In Tabasco, this disease is controlled mainly through copper-based fungicides and cultural practices such as pruning, shade control, elimination of diseased fruits, and drainage (Arvelo *et al.*, 2017). Ortíz-García *et al.* (2015) achieved a 79% decrease in the incidence of moniliasis in cacao by using a chemical fertilizer, monocrotophos to control *Selenotrips rubrocinctus*, and fungicides azoxystrobin and copper hydroxide. However, this method entails risks of environmental pollution and health damage (Muhammetoglu *et al.*, 2010; Londoño-Franco *et al.*, 2016). Another way to deal with moniliasis has been the replacement of cacao plants from seeds (ungrafted) with resistant clones (grafts); however, this practice has impacted the biodiversity of plantations in Tabasco (Ramírez-Guillermo *et al.*, 2018). A harmless alternative in the control of pests and diseases in crops is agrohomeopathy, which consists in using homeopathic remedies in infinitesimal doses obtained by repeated dilutions. According to Da Silva *et al.* (2012), the use of homeopathic treatments in plants induces resistance to pathogenic organisms. There are few studies on the impact of agrohomeopathic medication on perennial crops. Narváez-Martínez *et al.* (2014) assessed the impact of homeopathic medication on the incidence of *Neoleucinodes elegantalis* in *Solanum quitoense* (known as "naranjilla" or "lulo" in Spanish-speaking countries), with a decrease in eggs, larvae, and damage to the plant. Pérez-Fernández *et al.* (2016) described the experience of farmers in Teocelo, Veracruz, with the use of agrohomeopathic agents to control stem rust in coffee with good results. Chávez-García and Castelán-Estrada (2019) reported that with agroecological management, which included the use of homeopathic medication for at least one year, farmers estimated a decrease of over 50% in moniliasis in 85% of the plantations studied; as well as an increase in the number of flowers, healthy pods, and leaf greenness (Chávez, 2019). In light of this, the objective of the present study was to evaluate the effects of two homeopathic preparations, in low and high dilutions, on the incidence of moniliasis in cacao plants.

MATERIALS AND METHODS

The study was carried out in an 8-year-old cacao plantation, with no history of agrochemical use, located in Miahuatlán 2a Sección, Cunduacán, Tabasco, Mexico (18° 00' 39" N, 93° 18' 23" W). We used a complete randomized block design with three repetitions and six treatments: Homeopathic nosode 12C, Homeopathic nosode 200C, Cacao homeopathic combination remedy (known as *Polifármaco Cacao* in Spanish) 12C, Cacao homeopathic combination remedy 200C, water control, and control sample.

Homeopathic nosode was prepared from 0.05 g of monilia spores, diluted first in grinded sugar and then in 50% alcohol, in 12 and 200 centecimal dilutions (C-potency). The Cacao combination remedies (potencies 12C and 200C) were prepared by the Centro Nacional de Investigación en Agrohomeopatía of the Universidad Autónoma de Chapingo, that safeguards the production formula and provided the preparations for the present study. All homeopathic products were prepared using the Hahnemannian centesimal method (Ruiz-Espinoza, 2015). To prepare the applications, a drop of homeopathic medication was added to a liter of water, which was then succussed (vertically shaken and then hit against a soft surface) for two minutes, with two minutes of rest; this dilution was placed in a new spray pump, in a 1:99 proportion in water. The whole plant was sprayed with this preparation every 15 days, from August to December 2018. The variables assessed were number of flowers, number of healthy and diseased cherelles, number of healthy and diseased pods, ripe pod weight, fresh grain weight, and cacao leaf color. The analysis of variance (ANAVA) and means test (Tukey $\alpha=0.05$) were conducted with InfoStat 2018.

RESULTS AND DISCUSSION

The variable number of flowers presented a significant positive difference with the Cacao homeopathic combination remedy 200C treatment during November-December 2018, in spite of the diminished flowering due to the beginning of the windy season and its concurrent decrease in temperature (Figure 1). According to Saénz and Cabezas (2007), cacao flowering decreases with average monthly temperatures lower than 20 °C, which occurs during the months of November-January in Tabasco. This drop in temperature and flowering marks two fruit harvest seasons as seen in Figure 2: the longest and more plentiful harvest period takes place in December-March, while the shortest and less copious one occurs during May-July (Ortiz *et al.*, 2010; SIAP, 2019). The flowering recorded in the present study corresponded to the December-March harvest (Figure 1). Higher arithmetic

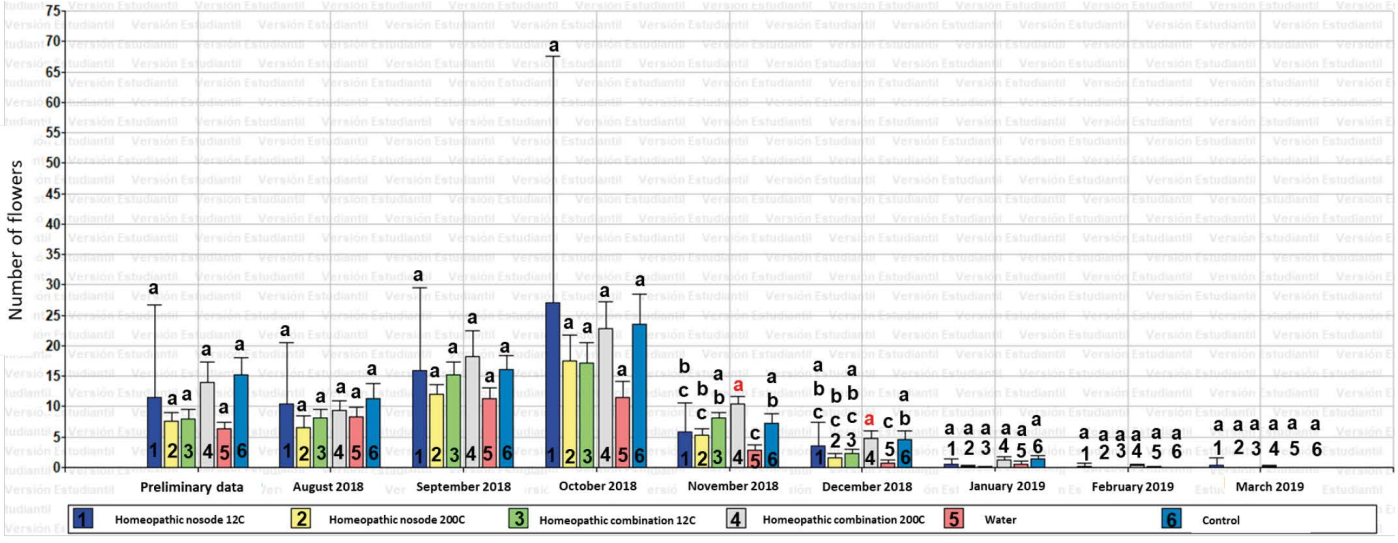

Figure 1. Variance analysis of treatments for number of flowers produced from August 2018 to March 2019 in the cacao plantation of Miahuatlán 2a Sección, Cunduacán, Tabasco. Tukey $\alpha=0.05$.

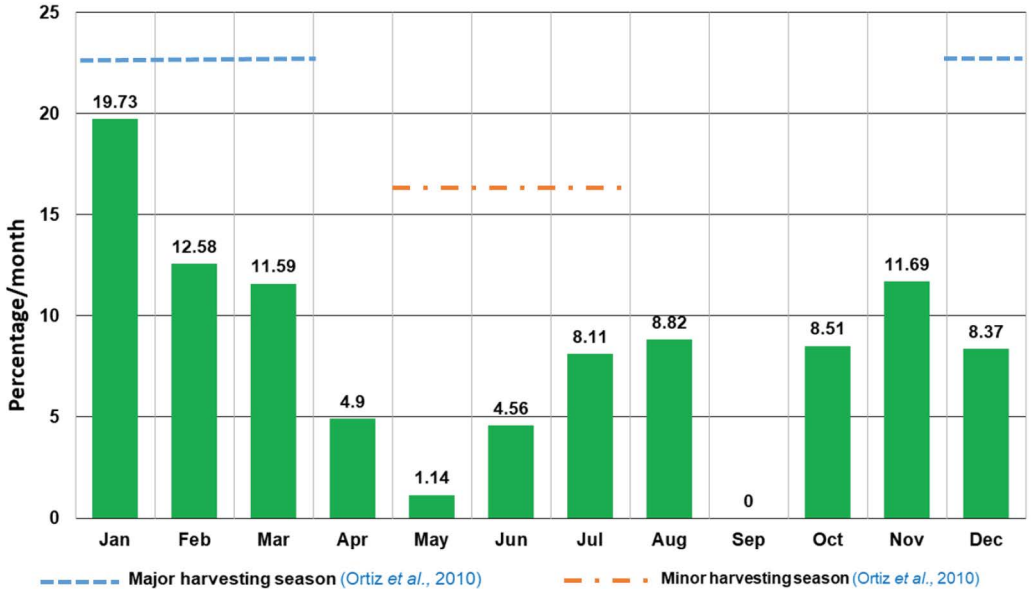

Figure 2. Monthly percentages of the annual flow of dry cacao bean production in Tabasco. Modified from SIAP (2019).

values were recorded with the Cacao homeopathic combination remedy 200C treatment during September 2018 and January 2019; similarly, higher values were observed with Homeopathic nosode 12C in October 2018 and in February 2018-March 2019. The control sample, however, also presented a higher arithmetic value in August 2018 and January 2019, which makes more statistical support necessary.

Figure 3 shows the results for the leaf color variable, with a significant difference for the Cacao homeopathic combination remedy 200C treatment during October 2018-January

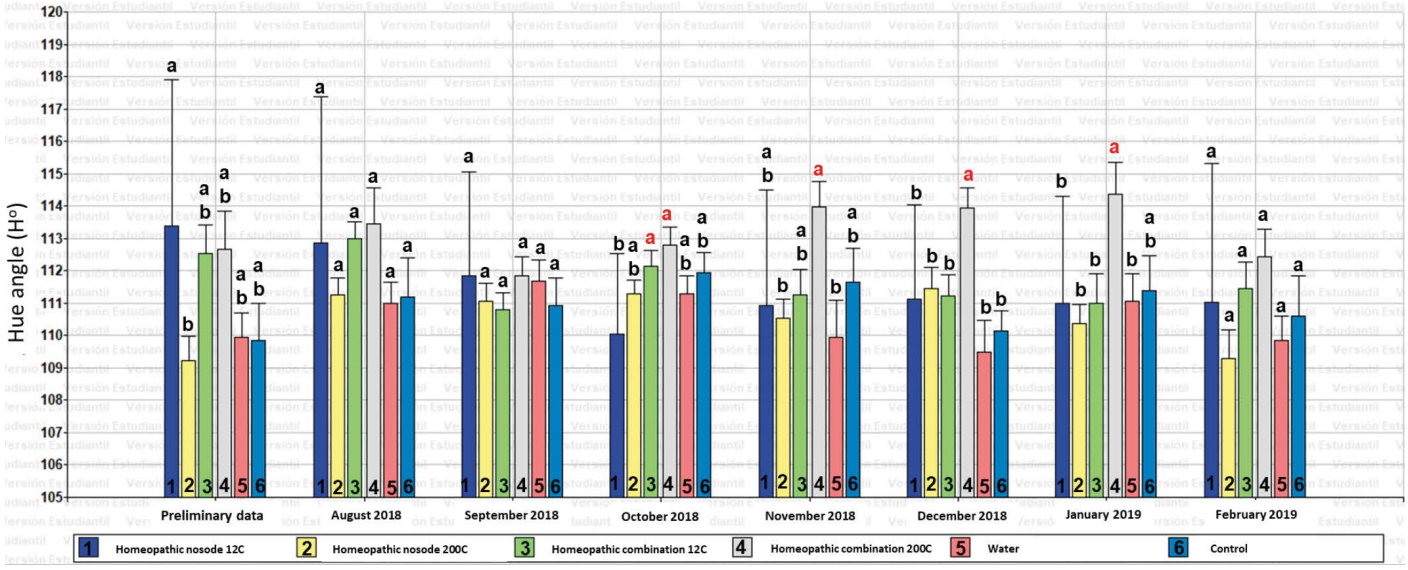

Figure 3. Variance analysis of treatments for leaf color (hue angle H°) during August 2018-February 2019 in a cocoa plantation in Miahuatlán 2a Sección, Cunduacán, Tabasco. Tukey $\alpha = 0.05$

2019. This same treatment showed higher arithmetic values during August 2018 and February 2019.

The Cacao homeopathic combination remedy 12C treatment showed a significant difference for this same variable in October 2018. As Chávez (2019) reported, the results showed that the farmers of Chontalpa, Tabasco, observed greater leaf greenness in cacao plants after applying the agrohomeopathic 200C medication in their plantations. Homeopathic preparations in low doses, with a large number of dilutions and high centesimal potencies, as in the case of the Cacao homeopathic combination remedy 200C, are recommended in situations of acute morbidity when the disease appears suddenly, with rapid evolution and resolution, as in the case of moniliasis. On the contrary, homeopathic remedies in high doses, with a low number of dilutions and low centesimal potencies, as in the case of the Cacao homeopathic combination remedy 12C, are recommended for chronic problems that impact an organism's health slowly and progressively until death (Ruiz-Espinoza, 2015; Anuja and Kshipra, 2018). Our results show that the low-dose Cacao homeopathic combination remedy 200C contributed to flowering and leaf greenness in cacao plants to a greater extent than the Cacao homeopathic combination remedy 12C, which only showed a significant difference for leaf greenness over a short period. This could suggest a better response of the cacao plant to low doses of the homeopathic preparation in the face of an acute disease such as moniliasis, that appears suddenly when favored by conditions of high humidity and temperature, and impacts the good development of fruits but without risking the life of the plant.

No significant difference was found in any of the treatments applied for the variables number of healthy and diseased cherelles, number of healthy and diseased pods, ripe pod weight, and fresh grain weight. According to Chávez-García and Castelán-Estrada (2019), the farmer estimate reporting less damage by moniliasis in cacao fruits under agroecological management with the use of the agrohomeopathic 200C medication came from plantations with at least one year under treatment. Although to date different hypothetical models have been proposed to explain the mechanism whereby homeopathic remedies work (Anuja and Kshipra, 2018), one of them suggests that they generate a self-defense reaction, like a vaccine, caused by infinitesimal doses of the pathogenic agent in the body (Da Silva *et al.*, 2012; Deboni, 2019). As a result, we consider that longer periods of application of the studied homeopathic preparations should be considered, at least one year prior to monitoring, to favor a possible autoimmune response that can be expressed more clearly and conclusively in the statistical analysis.

CONCLUSIONS

Of all the treatments applied and variables considered, the Cacao homeopathic combination remedy 200C had a higher positive impact on the number of flowers during the period in which flowering decreases due to a lower environmental temperature. It also favored a greater leaf greenness for four consecutive months in the evaluation period. The Cacao homeopathic combination remedy 12C also showed a significant difference for this same variable in October 2018 only. This could suggest a better response of the cacao plant to low doses (high centesimal potency) of the homeopathic preparation for an acute

disease such as moniliasis, which appears suddenly when favored by conditions, without putting the life of the plant at risk. We suggest considering longer application periods of the homeopathic preparations, at least one year prior to evaluation, to have more statistically conclusive results.

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Lamb feeding preference for maize, bean, and broad bean stubble treated with silage effluent, urea-molasses, or phosphoric acid

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ABSTRACT

Objective: To determine the acceptability of several stubbles: maize stubble treated with urea-molasses (MaUM), silage effluent with phosphoric acid (MaEflAcP), and control (MaTes); bean stubble treated with silage effluent (FrEfl), effluent silage with urea-molasses (FrEflUM), and control (FrTes); and broad bean stubble treated with silage effluent and urea-molasses (HaEflUM), urea-molasses and phosphoric acid (HaUMAcP), and control (HaTes).

Design/Methodology/Approach: Six Dorper/Katahdin lambs of 21 kg (LW) were used to carry out the feeding preference test. In the morning, lambs were fed with a mixture of the three treatments of each stubble (70% of the dry matter (DM) requirement/40 min); meanwhile, in the evenings, they were offered each treatment in independent feeders (10% of the DM requirement/20 min). This process lasted 7 days. Intake was measured in grams (offer minus rejection). Once the ordinal qualitative variables of consumption frequency (1=low, 2=medium, and 3=high) were classified, the data were analyzed using the ordinal logistic regression model. The results were expressed as the estimated proportion of incidence of the frequencies (1, 2, or 3).

Results: The proportion estimated for the high consumption frequency recorded higher results for MaUM, HaUMAcP, and FrEflUM, reaching 95.24, 73.44, and 43.49%, respectively. The proportion estimated for the low consumption frequency for HaTes, MaTes, and FrTes was 95.12, 88.11, and 48.77%, respectively.

Study Limitations/Implications: The diets with different quality and flavors did not meet the assumptions of normality and homoscedasticity; however, the results provide valuable information for the improvement of dry matter intake and animal production.

Finding/Conclusions: Urea-molasses (UM) promoted consumption preference both alone or in combination with other ingredients, in all the stubbles tested.

Keywords: Food preference test, lambs, treated stubbles.

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INTRODUCTION

The scarcity of high-quality forages limits the physical development of ruminants and meat and dairy production. Consequently, improving the nutritional quality of crop residues used in production systems of the Mexican plateau or dry tropic (Montossi *et al.*, 2013) is fundamental to reduce the competition among species for access to grains grown for human consumption (Giraudo *et al.*, 2014; Crosby *et al.*, 2018).

Maize (RMa), bean (RFr), and broad bean (RHa) stubbles provide few nutrients to ruminants (Chegeni *et al.*, 2013); however, nutrition and animal productivity can be enhanced, if these stubbles are treated to improve their digestibility. Therefore, considering the demand *vs.* nutrient concentration interaction, the feeding preference of ruminants and the potential benefits should be evaluated (Favreau-Peigné *et al.*, 2012). Authors Ginane *et al.* (2011) researched the sweet, salty, and bitter flavors of feeds and classified the level of flavor acceptance among ruminants as low, medium, and high. Their conclusion was that sweet flavors were highly accepted by cows and goats, but not by sheep. Salty flavors were positively or negatively accepted (depending on the mineral needs of the body), while bitter flavors recorded very negative values. Villalba *et al.* (2019) evaluated the feeding preferences of lambs and concluded that palatability increased the consumption of medusahead (*Taeniatherum caput-medusae* (L.) Nevski), common wheat (*Triticum aestivum* L.), and *quebracho* (*Schinopsis quebracho-red*) dry matter (DM) with 17, 2.4, and 9.9 g DM/kg LW condensed tannins, respectively. Taking for granted the sensorial characteristics of the animals, as well as the palatability and acceptance of feed leads to tedious diets that reduce livestock production, alters animal welfare, and damages the economy of farmers (Catanese *et al.*, 2012; Ginane *et al.*, 2011). Therefore, the objective of this study was to evaluate the feeding preferences of lambs, using RMa, RFr, and RHa treated with UM, Efl, and AcP combinations, in order to determine which combination is the favorite of lambs. This information will lay the foundations for the improvement of supply management.

MATERIALS AND METHODS

The stubbles were produced during the 2021 agricultural cycle in Tlachichuca, Puebla, Mexico (19° 01' 36" and 19° 19' 54" N and 97° 10' 24" and 97° 30' 18" W, at 2,603 m.a.s.l). According to the Köppen-Geiger climate classification, the area has a BSk (cold and semi-arid) climate, with a 16.7 °C mean annual temperature and a 513 mm mean precipitation (Climate-data.org., 2022; Municipios.mx., 2023). The feeding preference of lambs was evaluated in this locality.

Supplies

The stubbles were obtained from the local native varieties and their production was carried out according to the local traditions and customs. Maize was fertilized using the 138-00-00 formula using urea as a source of nitrogen; meanwhile, the leaves of bean and broad bean were sprayed with a 1 kg/ha Micromin® 20-30-10 (Mezclas y fertilizantes S.A. de C.V.) fertilizer during flowering.

The effluent (Efl) was obtained from maize silage in its milky-doughy phenological stage. It had 23% DM, with 42, 37, and 21% leaf, stem, and grain, respectively. A commercial urea (Agrogen S. A. de C. V. México) was used with 46% nitrogen. Liquid molasses (without brand) was used. The phosphoric acid (Greenhow S. A. de C. V. México) had a 79% purity and 57% phosphorous oxide. The DM percentage of Rma, RFr, and Rha was 90.4%. The feeding preference test was carried out with six male Dorper/Kattadin sheep, with 21.0±1.7 kg live weight and 100 days of age. They had a homogeneous physiological state and were used for zootechnical purposes. They

were handled according to the *Reglamento para uso y cuidado de animales destinados a la investigación* (Guidelines for the use and welfare of animals used in research works) of the Colegio de Postgraduados (COLPOS, 2019).

Stubble treatments

The doses used consisted of 2% urea, 6% molasses, and 0.57 mL/kg phosphoric acid (AcP), mixed with 30% water. The percentages and figures were based on the amount of DM and the doses were homogeneously sprayed on the stubbles. Efl was obtained from the maize silage process: ~25% of stubble (RMa, RFr, or RHa) was added to the bottom of a silo bag and ~75% of the silage fodder was placed in the upper part of the bag (volume:volume). The silage bags were completely sealed in order to achieve fermentation and they were opened at 160 days.

Feeding preference consumption test

This evaluation was based on the procedures reported by Oliviera *et al.* (2015), Costes-Thire *et al.* (2018), and Pedernera *et al.* (2020). The diet of the lambs was balanced to meet their development nutritional requirements (NRC, 2007) and it was supplemented with 16% of crude protein (CP). The lambs were fed twice a day, at 8:00 am and 6:00 pm; they also had free access to water and to a mineral salt block.

During the mornings, lambs were fed with a mix of the three different stubble treatments for 40 minutes (70% of the ration). Afterwards, three feeders (each filled with 10% of the rest of the stubble treatments) were placed in an individual metabolism crate. The containers were randomly placed in each session to eliminate biases in the resulting data. Using this configuration, each lamb selected and consumed *ad libitum* the stubble treatment of their preference for 20 minutes. Afterwards, consumption was measured as the difference between the weight of the offer and the rejection. Finally, all the rejected feeding was mixed with the concentrate and fed again to the sheep for their total consumption at the communal stable. During the evenings, a mixture of the concentrate and the stubble treatments was offered at the communal stable. This process was carried out for seven days in a row, for each of the stubble treatments. The adaptation period lasted five days.

Experimental design

The experiment was carried out using a completely random block design. Each lamb was considered as a block in which all the treatments were evaluated. Given that the assumption of normality and homoscedasticity were not satisfactorily met, the data were analyzed using the ordinal logistic regression model. This model establishes and estimates the statistical significance of the factors (stubbles) in face of the success probability response (feeding preference) for each combination of the factor levels (treatments). This significance is established through the inverse logarithm of the probability ratio, according to the lineal prediction (Heredia *et al.*, 2012), using the expression (logit function):

$$\ln\left(0_i\right) = \alpha_i + t_i$$

Where: α_i is the intercept associated with the equation that models the probability ratio of the i category; t_i is the effect of the i treatment; 0_i is the odds ratio associated with the i category of the dependent variable, that used the ratio expression

$$0_i = \frac{P(\text{if the value } \leq i \text{ category /value of } x)}{P(\text{if the value } > i \text{ category /values of } x)}$$

Data were classified as the ordinal qualitative variables observed in each evaluation session. Consumption frequency was classified as 1 (low), 2 (medium), and 3 (high). The results were analyzed according to the different stubble treatments, because the feeding preference was tested using the three different treatments of the same stubble, during the same period. The analysis was carried out using the R 4.1.1 statistical package (R Core Team, 2020). The response variable (feeding preference) was adjusted to the ordinal regression model using the R 4.1.1 statistical package. The compliance of the data with the assumption of proportionality was verified.

The relationship between the parts (ratio) was taken into account. The analysis of variance used the incidence (the times when a feeding frequency was recorded) of the feeding preference (1, 2, or 3) as a measure unit. The percentage observed between the ratios was used to develop the graphs.

RESULTS AND DISCUSSION

Preference per treatment depending on the type of stubble

There were differences in the estimated feeding preference of RMa (p=0.001, Figure 1). The most popular feeding option was MaUM, which reached a high (3) consumption frequency ratio (95%). The second option was MaEfAcP, which recorded a medium (2)

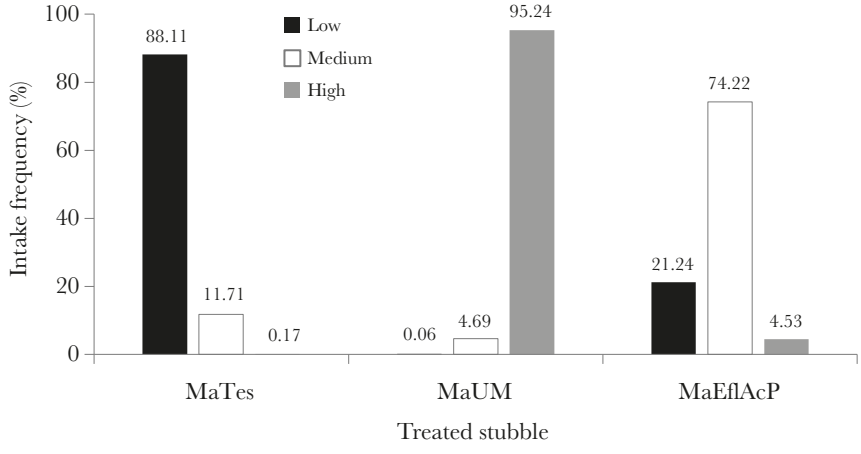

Figure 1. Estimated feeding preference of maize stubble among lambs. Low: low consumption frequency; Medium: medium consumption frequency; High: high consumption frequency; MaTes: maize stubble control; MaUM: maize stubble mixed with urea-molasses; MaEflAcP: maize stubble mixed with silage effluent and phosphoric acid.

frequency ratio (74%), while Tes obtained a low (1) frequency ratio (88%) and, consequently, was the less attractive option in the feeding preference test.

Figure 2 shows differences (p=0.001) regarding the estimated RFr consumption; however, the differences were narrower than the results obtained by RMa and RHa. The high (3) consumption frequency of FrEflUM was higher (43.49%), which proves the influence of this treatment in the feeding preference of lambs. The results of the medium (2) consumption frequency were closer between the FrTes (33%), FrEfl (36%), and Fr EflUM (35%) treatments. However, the low (1) consumption frequency recorded differences (p=0.001): FrTes had the highest values (48.77%), while FrEfl and FrEflUM obtained medium and low values, respectively.

There were differences (P=0.001) regarding the estimated consumption frequency of the RHa treatments. The UMAcP treatment recorded a high (3) frequency ratio (73%), while the EflUM treatment reached a medium (2) frequency consumption (70%) (Figure 3). Meanwhile, HaTes recorded a low (1) frequency consumption (95%) and, consequently, was the least popular options among lambs.

Van-Den-Berg *et al.* (2016) concluded that the feeding preference of ruminants is influenced by nutritional quality and palatability: pleasant flavors and aromas encourage a higher DM consumption. In this study, the UM and EflAcP mixed with RMa recorded a higher incidence in the high consumption frequency than Tes.

This preference is a consequence of flavor and nutritional quality improvement. In this regard, Abera *et al.* (2018) pointed out that the RMa mixed with urea (4%) and molasses (10%) treatment had 4.1% more CP content than the control, while it recorded a 5.5, 4.6, and 1.2% reduction of the NDF, ADF, and ADL concentration, respectively. In addition, they recorded a 538 g and a 771 g DM/d consumption of RMa Tes and RMa mixed with urea and molasses, respectively.

Catanese *et al.* (2015) mentioned a feeding preference test with sheep, using alfalfa and oat hay. These treatments recorded an 820 and 205 g DM/d consumption of alfalfa and oat, respectively. They concluded that the feeding preferences of animals are determined

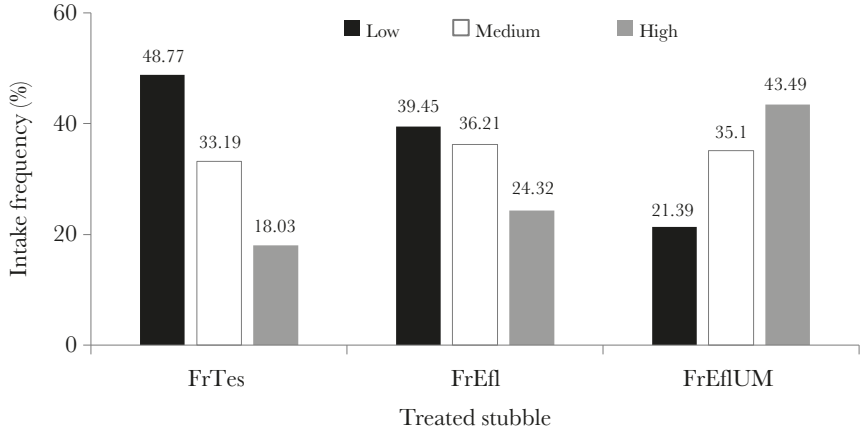

Figure 2. Estimated feeding preference of bean stubble among lambs. Low: low frequency consumption; Medium: medium frequency consumption; High: high frequency consumption. FrTes: bean stubble control; FrEfl: bean stubble mixed with silage effluent; FrEflUM: bean stubble mixed with urea-molasses.

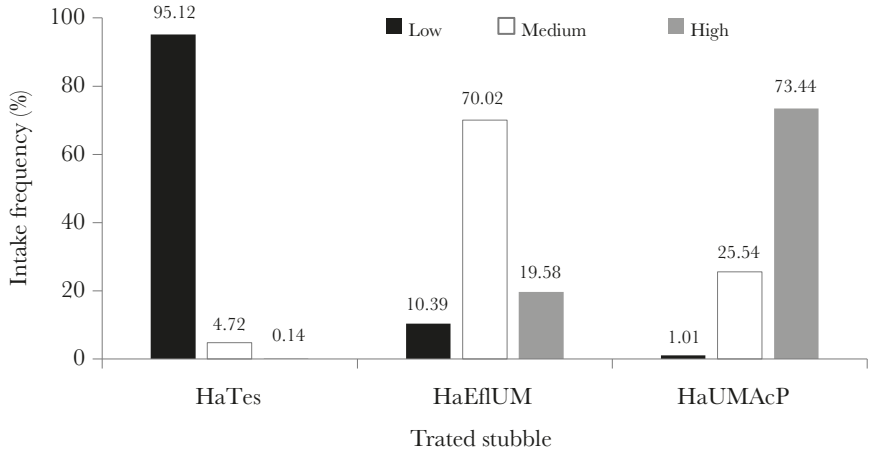

Figure 3. Feeding preferences of broad bean stubble among lambs. Low: low frequency consumption; Medium: medium frequency consumption; High: high frequency consumption. HaTes: broad bean control; HaEflUM: broad bean stubble mixed with silage effluent and urea-molasses; HaUMAcP: broad bean stubble mixed with urea-molasses and phosphoric acid.

mainly based on nutrition. Likewise, Meagher *et al.* (2017) studied the feeding preferences of Holstein calves, using the following combinations: 1) Timothy grass and alfalfa hay; 2) tall fescue and orchard-grass hay; 3) chopped rye straw; and 4) a mixture of all these ingredients. They concluded that No. 4 was the most palatable treatment for the animals, due to its high nutrient concentration. Consequently, the calves ate an average of 554 out of 600 seconds.

Therefore, the feeding behavior of ruminants indicates the nutritional quality of the forages, which decreases as the fiber components increases (Neave *et al.*, 2018). In their report about treatments with RFr and 4% urea, Rodríguez *et al.* (1985) pointed out that, compared with control, NDF decreased by 1.65%, ADF increased 3.18%, and ADL remained the same (11.07%). In addition, they concluded that RFr had a high ADL content.

The results of this study match the findings of Cardoza and López (2016), who emphasized the ADL concentration, pointing out that lignin content influenced the feeding preference of goats. Their study shows that *Acacia pennatula* mixed with 7.78% ADL recorded a higher consumption than *Gliricidia sepium* mixed with 18.44% ADL. These results also match the findings of Monllor *et al.* (2020), who studied the feeding preference of Murcia-Granada goats, using a silage made up of bracts of artichoke (*Cynara scolymus*) with 48% NDF, 33% ADF, and 5% ADL. These treatments were compared with broccoli (*Brassica oleracea*, var. Italica) silage byproducts with 49% NDF, 36% ADF, and 7% ADL. The Murcia-Granada goats consumed 113 g DM/d of bracts of artichoke and 13 g DM/d of broccoli byproducts.

CONCLUSIONS

Sheep differentiated between the MaTes, FrTes, and HaTes treatments, without rejecting them. The stubble treatments influence the feeding preferences of sheep, as a result of their attractive flavors and improved nutritional quality. The most prefered treatment was the addition of urea-molasses.

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Competitiveness of Mexican vanilla (*Vanilla* spp.) in the international market

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ABSTRACT
Objective: To analyze the competitiveness of Mexican vanilla in the international market.
Design/Methodology/Approach: The relative trade balance (RTB) and the revealed comparative advantage index (RCAI) were calculated to analyze the competitiveness of the Mexican vanilla in the international market. Both crushed and whole vanilla beans were evaluated.
Results: Around 1% of the total vanilla area is harvested in Mexico, representing 6% of the world production volume. The Mexican market is competitive in the international whole vanilla beans market, but not in the case of crushed or ground vanilla.
Study Limitations/Implications: The SIAVI portal was discontinued. Therefore, the international vanilla trade data could only be retrieved for the period ending on November 2021.
Findings/Conclusions: The Mexican market has potential for the development of vanilla production. However, current production is practically absorbed by the local market.

Keywords: relative trade balance, revealed comparative advantage index, production analysis.

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INTRODUCTION

Vanilla (*Vanilla* spp.) is part of the Mexican plant heritage: 9 of the 15 species of Mesoamerican vanillas are found in the country and *V.* × *tahitensis* is the second most produced hybrid in the world (UV, 2018; SAGARPA, 2017). Totonacapan is the main vanilla production region in Mexico. It consists of 20 municipalities of the state of Veracruz and 19 municipalities of the state of Puebla (Luis *et al.*, 2020). According to the Agricultural and Fisheries Information Service (SIAP), more than a thousand hectares of vanilla were harvested in Mexico in 2021, with an average yield of 0.58 tons per hectare. These results imply that 610 tons of this orchid were produced. Since, the average rural price was $95.5 thousand pesos per ton, the value of vanilla production was approximately $58.2 million pesos (SIAP, 2023). Comparing the domestic and international production volumes, the Mexican market had a 15.19% participation in the year in question, since the worldwide production amounted to a 6,888 tons volume (FAOSTAT, 2023).

In 2021, the main importing countries of vanilla were the USA, France, and Germany, with 1,806.8, 1,080.9, and 710.6 t, respectively. Meanwhile, Madagascar was the main exporter with 2,534.3 t of vanilla (FAOSTAT, 2023). The objective of this research was to analyze the competitiveness of Mexican vanilla in the international market, with the purpose of determining if it has a comparative advantage regarding the rest of the world.

MATERIALS AND METHODS

A cross-sectional analysis was developed to determine the participation of the Mexican market in the global vanilla production; data from a single year were collected to describe the variables and analyze their incidence and interrelation at a given time (Hernández, Fernández and Baptista, 2010). The share of planted area and volume of vanilla production in Mexico were estimated with relation to the international market. The data were taken every five years, from 1980 until 2020; the Sistema de Información Agroalimentaria de Consulta (SIACON) and the statistical database of the Food and Agriculture Organization of the United Nations (FAOSTAT) were consulted to obtain the Mexican and global data, respectively. The percentage estimate was obtained using the following formula:

$$r_{\%} = \left(\frac{v_n}{v_m}\right)100$$

Where: $r_{\%}$ is the participation percentage of the Mexican market in the world market; v_n is the value of the variable in the domestic market; and v_m is the value of the variable in the international market. On the one hand, if the value of $r_{\%}$ tends to zero, the implication is that the domestic market is not significant in the international market; on the other hand, if the indicator approaches 100, the national market plays an important role in the world.

The variables figures researched for the longitudinal or evolutionary analysis of the Mexican vanilla market from 2012 to 2021 (SIACON) were: planted and harvested area, yield, production volume, average rural price, and value of the production of the crop. The growth rate of each variable (*i.e.*, the accumulated increase or decrease in each one in the last ten years) was estimated using the following formula (Rivera *et al.*, 2020; Pérez *et al.*, 2010):

$$r_{t,0} = \left(\frac{x_t - x_0}{x_0}\right)100$$

$r_{t,0}$ is the growth rate (percentage) of each variable from 2012 to 2021; x_t is the value of each variable in 2012; x_0 is the value of each variable in 2021.

To analyze the Mexican market competitiveness in relation to the international market, the volume and value variables of vanilla exports and imports were obtained. The 09051001 "whole vanilla beans" and 09052001 "crushed or ground vanilla" tariff fractions were

used for the same purpose (SIAVI, 2023). Finally, the competitiveness of Mexican vanilla was analyzed from 2013 to 2021 for each of the fractions, using the concepts and formulas detailed below.

The relative trade balance (RTB) indicator was calculated to measure the relationship between the exports and imports of a product from a country with regards to the international market or a specific market. The indicator is interpreted as an index of competitive advantage that determines if the country is a net exporter or importer of the product (Luquez *et al.*, 2022; García, 1995). It was estimated using the following formula:

$$RTB_{ij} = \frac{X_{ij} - M_{ij}}{\left|X_{ij} + M_{ij}\right|}$$

Where: RTB_{ij} is the relative trade balance of Mexico in relation to vanilla; X_{ij} is the volume of vanilla that Mexico exports to the international market; and M_{ij} is the volume of vanilla that Mexico imports from the international market. If the RTB index is between zero and one, the country is a net exporter of the product and has a competitive advantage; however, if the value is between minus one and zero, the country is a net importer of the product and lacks a competitive advantage.

The revealed comparative advantage index (RCAI) was used to confirm if Mexico has a competitive advantage in the international vanilla market, based on trade specialization taking a reference point (Ramírez-Padrón *et al.*, 2018; Balassa, 1965). The index was calculated for the international market using the following formula (ECLAC, 2008):

$$RCAI = \frac{x_{iw} - m_{iw}}{\left|x_{iw} + m_{iw}\right|}$$

Where: *RCAI* is the revealed comparative advantages index; x_{iw} is the value of vanilla exports from Mexico to the international market; and m_{iw} is the value of vanilla imports from the international market into Mexico. If the RCAI value is negative, the country is not competitive in the analyzed market; meanwhile, if the index is positive, the country has a competitive advantage in such market.

RESULTS AND DISCUSSION

Vanilla is a crop that has a designation of origin. However, the share of the domestic vanilla market in relation to the global harvested area (HA) has historically been very low (>5%). Regarding production (P), Mexico has had a maximum participation of 13% in the international vanilla market (Figure 1). Mexico is an important producer of this crop.

From 2012 to 2021, the area planted and harvested in the country has had a negative trend; however, the implementation of public policies for the promotion of vanilla

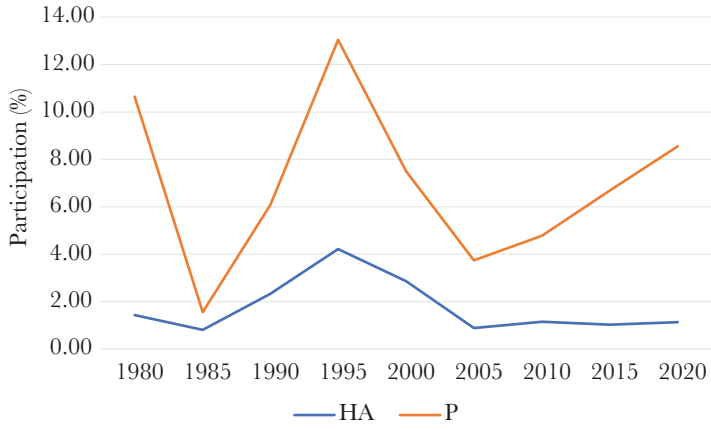

Figure 1. Participation of Mexican vanilla in the world. Source: Figure developed by the authors based on data from SIACON and FAOSTAT.

production —such as its incorporation into the Proyecto Nacional de Plantas Nativas para la Alimentación y la Agricultura (SADER, 2020)— has improved these variables.

During the analysis period, the yield per hectare of this orchid increased from 0.35 tons (2012) to 0.58 tons per hectare (2021); meanwhile, the volume of domestic vanilla production increased from 390 to 610 tons (Figure 2). The variation in the production level of vanilla is related to its yield, therefore, Mexico has conditions to promote intensive production in controlled environments, as an option for the development of the activity.

The average rural price of vanilla has recorded an upward trend, increasing from $53,000 pesos per ton (2012) to $95,500 pesos per ton (2021). The value of vanilla production in Mexico increased in the analyzed period, reaching more than $58 million pesos. Vanilla cultivation has the economic potential to promote the development of the Totonacapan region.

The RTB analysis shows that Mexico is competitive in the international whole vanilla beans market. Figure 3 shows that the value of this comparative advantage indicator was

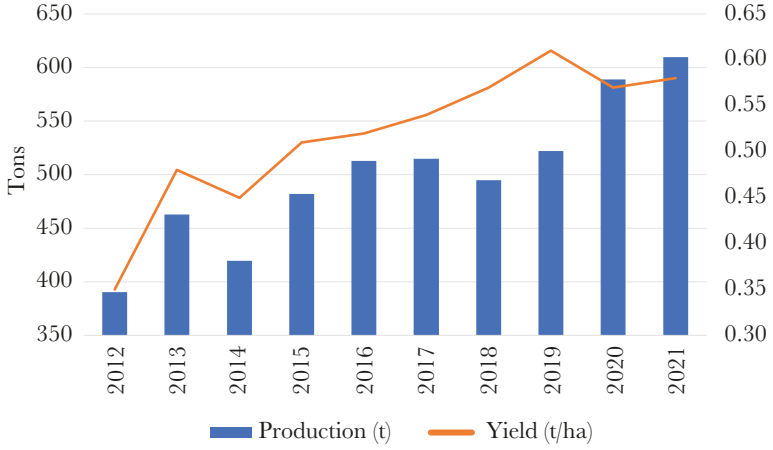

Figure 2. Yield and volume of Mexican vanilla production. Source: Figure developed by the authors with data from SIACON.

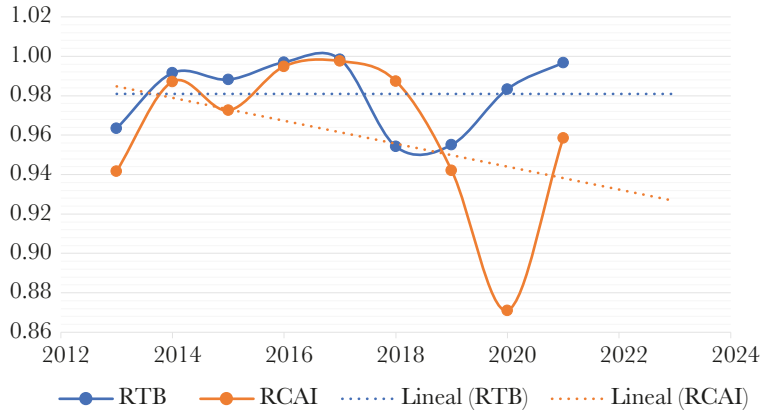

Figure 3. Competitiveness rates for whole vanilla beans in Mexico. Source: Figure developed by the authors with data from SIAVI.

close to one during the analysis period, while the linear projection of that variable shows that the trend is constant (*i.e.*, close to one). However, if this indicator is compared with the RCAI (although this indicator is also positive and close to one), a downward trend is recorded in the analysis period —*i.e.*, the competitiveness of the value of the Mexican whole vanilla beans market has decreased due to the low price that exporters have obtained in recent years. The specialization and promotion of the activity in the region with a designation of origin would enhance the position of Mexico in the international trade of this orchid, generating foreign currency for the country and greater income for the producers and marketers of this crop.

In the case of crushed or ground vanilla, the RTB recorded overall negative values during the analysis period, implying that Mexico is not competitive in the international market; meanwhile, the linear projection of the variable showed a negative trend (Figure 4). However, the RCAI of the exports and imports of this type of vanilla records positive values, which implies that the value of the Mexican market is competitive in relation to this product, despite the likewise downward trend.

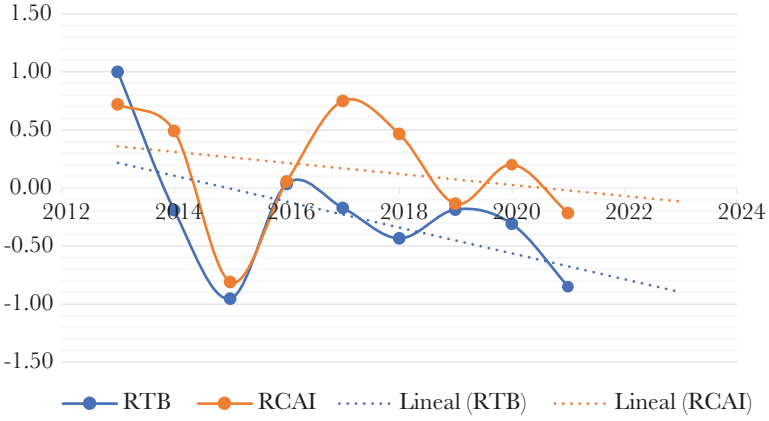

Figure 4. Competitiveness rates for whole vanilla beans in Mexico. Source: Figure developed by the authors with data from SIAVI.

Consequently, the Mexican market is competitive in the vanilla market as a result of the quality of its product, while the agro-industry uses the lower quality pods to produce essences and other byproducts.

CONCLUSIONS

The yield achieved by vanilla in Mexican soil has a comparative advantage. The increase in vanilla production in recent years is related to the incorporation of the crop into the Proyecto Nacional de Plantas Nativas para la Alimentación y la Agricultura. Mexico has the potential to increase the production of this crop with promotion policies that enable the producers to develop the activity.

The Mexican market has a comparative advantage regarding the international market for whole vanilla beans. However, the export volume amounted to less than 30 tons in most of the years of the analysis period. Therefore, the current production is practically absorbed by local market. In conclusion, to increase vanilla production, an additional plan is required to encourage internal consumption and to position Mexican vanilla in foreign markets.

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Assessment of sustainability attributes of traditional and conventional maize cultivation in farmer agricultural systems

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ABSTRACT

Objective: To carry out a comparative analysis of the sustainability of traditional and conventional maize cultivation systems in Cuanacaxtitlán.

Design/Methodology/Approach: The proposal was implemented through a mixed method approach in which a qualitative research predominated. A sustainability assessment for the 23 farmer systems of maize production in Cuanacaxtitlán was carried out, according to the Indicator-Based Sustainability Assessment Framework (Marco para la Evaluación de Sistemas de Manejo Incorporando Indicadores de Sustentabilidad: MESMIS).

Results: The sustainability level between the two types of agroecosystems does not differ much. According to the chosen indicators, TMS is more sustainable in the environmental and economic aspect, while CMS is more sustainable in the social aspect. The average profitability of TMS is better as a result of the milpa profits.

Study Limitations/Implications: Family Production Units (FPUs) do not own their lands, which limits the improvement of profitability and sustainability in maize production. To this end, land distribution or support programs for landless farmers is important.

Findings/Conclusions: Among the 23 systems under analysis, critical values were obtained for two environmental indicators: fertilizer application and pesticide use. Therefore, improving the use of these inputs is an important task.

Keywords: farmer systems, MESMIS, sustainability, agroecology.

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INTRODUCTION

Farmer agricultural systems produce food for more than 70% of the world's population [1]. Farmer mode of production is characterized by family farming focused on self-sufficiency, with a diversification strategy that follows an ecological rationality aimed at sustainable energies [2]. Sustainability is an important concept, because ecological principles guide production systems, determining the aim and type of agriculture. It also refers to the capacity of an agroecosystem to maintain its productivity, despite the ecological, social, and economic factors that may impact it [3]. Modern agricultural production lacks economic, social, and environmental sustainability. It is characterized by the use of packages that include improved seeds, pesticides, and synthetic fertilizers.

This production model —as a business controlled by transnational corporations— has strong economic connotations that threaten food security. People's right to healthy food is impacted by policies that break the relationship that farmers have with nature, encouraging dependence on the market. By displacing diversification-based ancestral methods, this dependency has a negative effect on the food self-sufficiency of farmer families. Therefore, the preservation of agroecological practices in farming ecosystems must be promoted [4]. These practices define productivity, stability, resilience, reliability, adaptability, equity, and self-management as basic attributes of a sustainable management system that must be capable of renewing itself while maintaining its productivity.

In conclusion, the objective of this study was to analyze and compare the sustainability of traditional and conventional maize management systems in Cuanacaxtitlán, Guerrero.

MATERIALS AND METHODS

The study was carried out in Cuanacaxtitlán, a Mixtec town in the municipality of San Luis Acatlán, Guerrero. A mixed method approach was used to develop the project, with a predominance of qualitative research. A sustainability assessment for 23 maize production systems was carried according to the MESMIS proposed by Masera *et al.* (4), which includes the following six steps:

1. **Determination of the subject of the assessment**. The characterization of the 23 farming systems took into account their socio-environmental context, the internal and external flows affecting them, and their components and interactions. Ten maize production systems were identified with a predominance of Traditional Management Systems (TMS) and 13 with a predominance of Conventional Management Systems (CMS). These systems were classified according to the type of seed used, workforce, production objective, and technology employed. Subsequently, TMS and CMS were established as the reference management and as an alternate system, respectively.
2. **Determination of critical points**. This step consisted of identifying weaknesses and strengths of the management system. The determination of critical points was the result of the workshop "Problemáticas presentadas en la producción de maíz" ("Problems faced in maize production"), which included an analysis of the weaknesses, threats, strengths, and opportunities observed by the producers.
3. **Selection of indicators**. The information gathered in step 2 was assessed. Once the diagnostic criteria and indicators about sustainability properties and their social, economic, or environmental aspects were condensed, diagnostic criteria and indicators were defined based on critical points of the two production systems.
4. **Measurement and monitoring of indicators**. After establishing the indicators, a semi-structured questionnaire was applied to obtain information about crop management, including production, total costs, commercialization, organization, and environmental awareness. Subsequently, field visits were made to nearby plots and information was collected and processed in Excel.
5. **Presentation and integration of results**. A radial chart (known as a "spider" or "amoeba") was drawn, identifying critical aspects of both agroecosystems. The

results were presented to the producers during a workshop where an agreement was reached regarding actions for the improvement of maize production.

6. **Conclusions and recommendations**. Based on the assessment of indicators, a Comprehensive and Sustainable Action Plan (CSAP) was developed, defining priority actions, producer involvement, etc. The work plan is linked to the different stages of agroecological transition, which, according to López [5], is proposed in a non-linear sequence, generated according to the needs and motivations of the producers. Six training workshops were developed to implement the CSAP, addressing the following subjects: rescue and improvement of native maize, infiltration trenches, bokashi production, foliar fertilizers based on poultry manure and ant manure, natural insecticides, and vermiculture. These subjects were discussed in information exchange workshops focused on the farmer-to-farmer extension approach, which is "a participatory way of promoting and improving farmer production systems" [6].

RESULTS AND DISCUSSION

An amoeba graph was developed according to the results of each indicator, showing critical aspects of both agroecosystems and detecting the points that should be strengthened (see Figure 1). Each indicator was assigned a value from 0 to 100, where 0 is the worst value and 100 is the optimum value of an agroecosystem. Optimal values (100%) are located in the periphery, while points that need to be strengthened are above 50% and critical values are shown below 50%.

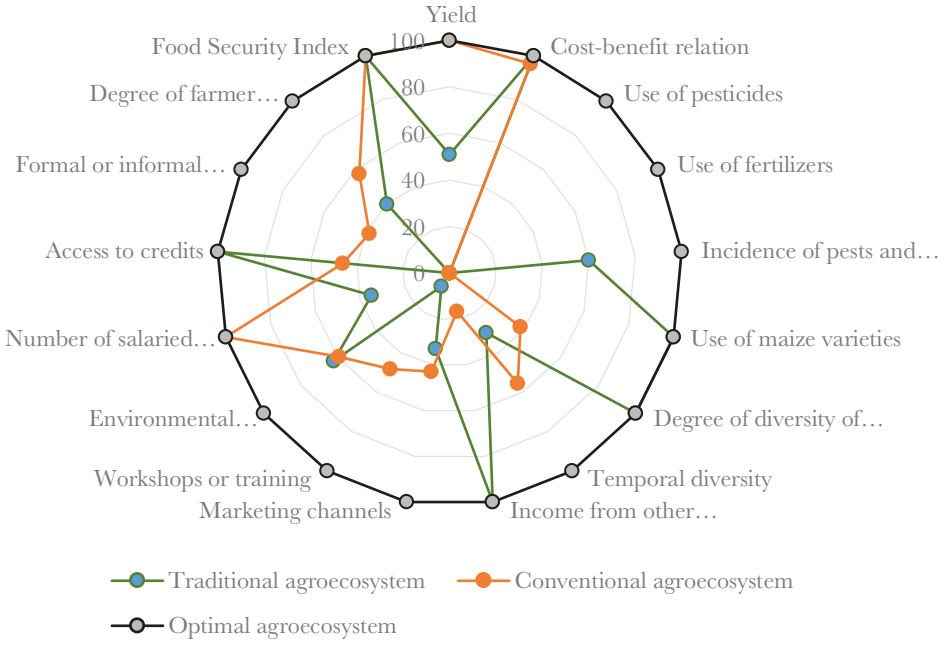

Figure 1. Amoeba graph for the comparison of the sustainability indicators in two maize management systems in Cuanacaxtitlán, Guerrero.

The results obtained were classified according to their sustainability attributes: productivity; stability, resilience, and reliability; adaptability indicators; and equity.

Productivity. The transition from the traditional to the conventional production system has been encouraged by such factors as the low yield of native maize, the difficulty in commercializing surpluses (as a consequence of the preference for hybrid maize), and intermediation as the main commercialization channel. Low yields observed in these systems may be associated with the intensity of soil management and the lack of crop rotation and organic matter application, as well as the unbalanced fertilization in each production cycle [7]. Fertilizer application on steep slopes generates runoff losses, while low organic matter favors leaching and soil acidity reduces the nutrient cycling. In the TMS, the average cost of production per hectare was $5,648 MXN, while in the CMS it was $15,815 MXN. In both production systems the highest cost is generated by the workforce, who are mainly hired for two reasons:

1) Production is done by hand using rustic tools; the low mechanization is related to the reduced areas that are sown and the steepness of the slopes.
2) Since Cuanacaxtitlán is a migrant community, the average family size is four people, which results in a limited availability of family workforce.

In this context, TMS had a slightly higher average profitability than CMS. Therefore, the milpa is a strategy that farmers can use to improve the profitability of their production, since the cultivation of different species and varieties reduces risks in the event of external changes [8].

Stability, resilience, and reliability. Both systems use commercial chemical products to control weeds. In TMS, the producers carry out a manual control of weeds before sowing and complement this practice with the application of herbicides, using an average of 6 liters per hectare. In CMS, an average of 9 liters of herbicides were used per hectare. In both systems, a small percentage of farmers used Foley® to control codling moth and Arrivo® against bean slugs. An excessive use of herbicides and pesticides may be associated with farmers' lack of knowledge about the correct handling of these products and the environmental and health damage they cause; therefore, attention should be paid to proper weed control and pesticide use. Fertilization in the systems analyzed is unbalanced: some systems over-fertilize, while others under-fertilize. In this context, managing and improving the use of inputs is necessary to positively impact the profitability of the crop. Native maize —such as olotillo, maizón, toro, sapo, and combinations thereof— are sown in TMS. Producers have preserved native seeds, as a result of their nutritional and cultural importance, as well as the lack of resources to switch to conventional production. Therefore, the preservation of native seeds is directly related to the poverty of family units, as well as to their cultural values. Toro maize is sown specifically to prepare pozole, a traditional and widely-recognized Mexican dish. Tecomache or cuarenteño maize, a short cycle variety, is one of the lost varieties mentioned by the producers. P4082W, P3966W, P4028W, and other Pioneer® hybrid maize varieties are sown in SMCs, which makes producers dependent on this input. Purchase of seeds represents an average expenditure

of $2,491.52 MXN: 16.79% of the total cost of maize production per hectare. Therefore, the use of native seeds has certain advantages over hybrid seeds, as they have adapted to the location and are resistant to pests and diseases, while their production does not involve high costs, since they can be used in the next cycle. In the CMS, the native seed has been lost and producers only sow hybrid seeds as a monoculture or in association with other crops. Consequently, the use of improved varieties leads to the erosion of native seeds [9]. The simplification of diversity is one of the many consequences of the implementation of modern agricultural practices in rural communities. According to Toledo [10], the modernization process displaces the rural way of life, replacing it with specialization in all its dimensions, with its production aimed exclusively at the market. In Cuanacaxtitlán, farmer agricultural systems used to perform agroecological practices such as polycultures, manual weeding, living fences, sowing of native seeds, and conservation of medicinal and ornamental weeds; however, given the market exclusivity of hybrid maize, farmer systems have begun a transition process, gradually abandoning their traditional practices. Consequently, the food that makes up their diet is replaced by products from the market, which they can only acquire after the sale of their produce [11]. In Cuanacaxtitlán, the abandonment of the milpa has entailed the loss of an entire gastronomic legacy.

Adaptability indicators. The participation of farmers from both production systems in the workshops or training was low. A high percentage of farmers only speak Mixtec and consequently had difficulty understanding the indications given in the sessions. Additionally, the training was very theoretical regarding the technical handling of information and lacked practical support; therefore, the farmers prefer to continue sowing as they learned from their predecessors. In both production systems, farmers recognize that conventional practices pollute their resources, which backs up the decision to change their farming practices.

Equity. The high number of wage-earning workforce that participate in the production process represents a source of employment in the community. In 50% of the systems, expenditure on this heading accounts for more than 50% of their total expenses; additionally, migration limits the workforce, which demands increasingly higher wages, increasing costs and decreasing the net income obtained per producer. Dependency on external workforce puts the farmer system at a disadvantage, establishing an inequitable cost-benefit ratio. In CMS, high production costs generate dependency on financial resources, reducing net income. In CMS, the degree of satisfaction with the production is slightly higher than in TMS; producers consider that sowing hybrid maize should result in a much higher yield. The CMS has higher values in the FFSI; less than 10% is destined for self-consumption. In the TMS, more than 80% of the total production is destined for self-consumption; 50% of the producers do not have food security, which matches the conclusions of Damián *et al.* [12], who report that most maize producers do not have FFS. In Cuanacaxtitlán, the low FFS level in the TMS can be explained by the low yields obtained and the small area sown. Achieving food self-sufficiency is vital in the pursuit of sustainable agriculture; therefore, everyone involved must work on agroecological practices that optimize space and maintain acceptable yields in the long term. Given the socio-economic and environmental benefits of sowing maize under polyculture,

traditional management systems are slightly more sustainable than conventional ones. The SMC are mixed agroecosystems where traditional practices (*e.g.*, crop association, crop rotation, and manual weeding during land preparation) are still practiced. These systems required the sowing of hybrid maize as an adaptation and innovation strategy for production and as a response to market demands to improve the profitability of the crop. The latter objective has not been achieved, as the profitability of CMS and TMS are similar. Two indicators which influence yield and production costs were reported in both agroecosystems: the application of fertilizer and the use of pesticides. Therefore, work must be done on the adequate management of inputs.

CONCLUSIONS

The two types of agroecosystems have similar levels of sustainability. According to the selected indicators, TMS is more sustainable in the environmental and economic aspect, while CMS is more sustainable in the social aspect. In the 23 systems analyzed, critical values were obtained for two environmental indicators: fertilizer and pesticide use. CMS has a better average profitability due to the benefits of the milpa. The implementation of lore exchange workshops based on the farmer-to-farmer extension approach is very effective in the transmission of knowledge, promotes the exchange of experiences, and generates motivation and conviction about sustainable production.

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Colegio de Postgraduados

Characterization of native Ya'ax ik chili pepper (*Capsicum annuum* L.) in the Yucatan Peninsula

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ABSTRACT

Objective: To morphologically characterize the Ya'ax ik chili pepper: wild chili (*Capsicum annuum*), native variety from the Yucatan Peninsula.

Design/methodology: Ya'ax ik chili pepper seeds were sown in 200-cavity polystyrene trays, and then the plants were placed in bags for hydroponics and were morphologically characterized using the *Capsicum* descriptor of the International Institute of Genetic Resources.

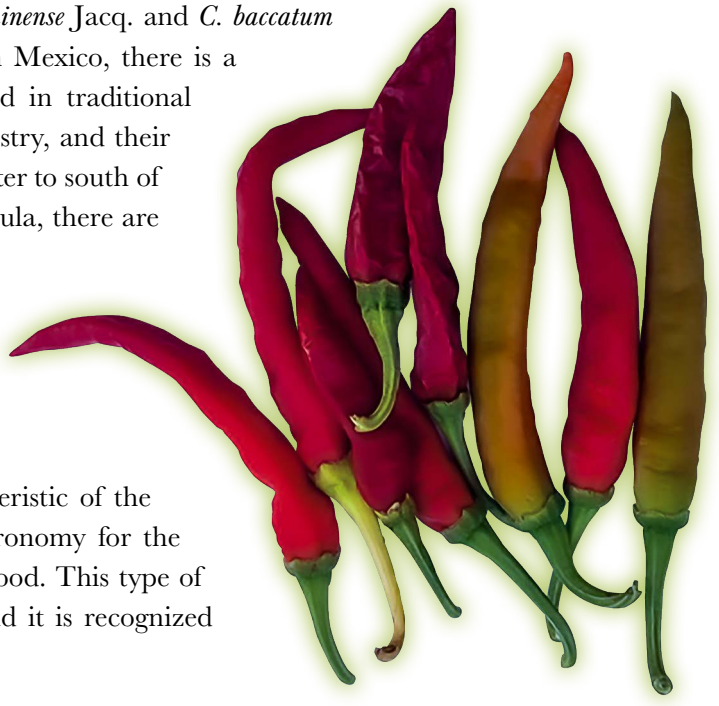
Results: The plant showed 118 cm and 34.8 cm of height and ramification width and width of the intermediate plant, hanging white flowers and green fruits in the early stage and red at maturity with 7.42 and 2.32 cm length and width. Additionally, tolerance to the virus caused by whiteflies could be observed.

Findings and conclusions: Plant with morphological characteristics susceptible to be genetically improved for its extensive farming, given the market acceptance of the fresh and dried fruit, supported by market prices.

Keywords: Morphological characterization, *Capsicum annuum*, native.

INTRODUCTION

Chili pepper (*Capsicum* spp.) was one of the first domesticated plants in the American Continent (Hernández-Verdugo *et al.*, 2012); the genus is constituted by around 30 species, which have a large assortment of fruit shapes, colors and sizes, of which the following have been domesticated: *C. annuum* L., *C. frutescens* L., *C. pubescens* (Ruíz & Pav.), *C. chinense* Jacq. and *C. baccatum* L. (Bosland and Votava, 2012). In Mexico, there is a great variety of chili peppers used in traditional cuisine, as well as in the food industry, and their diversity is found mainly in the center to south of the country. In the Yucatan Peninsula, there are different types of chili peppers that are characteristic of the region, such as habanero, chile dulce, chile Xcat ik. However, there are other types of native chili peppers grown in a smaller scale such as Ya'ax ik, which is characteristic of the region and used in the local gastronomy for the elaboration of pastes for regional food. This type of chili pepper is called wild chili and it is recognized



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for its rusticity and tolerance to drought, pests and diseases. It is a herbaceous plant with height of 0.40 to 1.0 m, red fruits in maturity, and *Capsaicin* content of 13.52 and 11.77 mg/ of dry weight (González-Estrada *et al.*, 2010), of which there is reference of seven ecotypes differentiated by the coloring of its mature fruit, which could be found in association with corn crops (Bautista-Parra, 2023, personal communication). Despite the importance of this type of chili pepper, there is little information about it, so the research study conducted was focused on the morphological characterization *ex situ* of the Ya'ax ik chili pepper.

MATERIALS AND METHODS

The morphological characterization of the Ya'ax ik chili pepper was part of the research project, "Collection, Morphological Characterization, Molecular Typifying, Conservation and Sustainable Use of the *Capsicum* Genus in the State of Campeche", study carried out in the Lol'ic (chili pepper flower) nursery located in the former Mérida-Motul highway, in front of the San Antonio Holactun Hacienda in the community of Conkal, Yucatán. Sowing was done on August 22, 2023, in polystyrene trays with 200 chambers using Peat Moss as substrate. The transplant was carried out 60 days after sowing in plastic bags for hydroponics with 600 caliber, 50 cm wide and 40 cm long, when the plants reached a height greater than 20 cm. The substrate used was 40% nitosol soil + 40% rendzine soil + 10% agrolite, mixture recommended for agricultural purposes. The plants remained under protected system conditions for three months, to later be taken to open air conditions. Integrated management of fertilization and pest and disease control was done, taking as reference the recommendations from Soria *et al.* (1999). The morphological characterization was carried out considering 10 plants, randomly arranged with three repetitions. Characteristics of the seedling, plant, flower, fruit and seed were evaluated through the *Capsicum* descriptor of the International Plant Genetic Resources Institute (IPGRI, 1995). The results from the morphological characterization of quantitative variables were found through the use of mean descriptive statistics, standard deviation, and coefficient of variation.

RESULTS AND DISCUSSION

The morphological characterization of the Ya'ax ik chili pepper determined represents one of the different ecotypes from this native chili pepper, according to producers from the rural communities; this agrees with what was cited by Aguilar-Rincón *et al.* (2010).

Seedling

The characterization of the seedling stage of the Ya'ax ik chili pepper does not exist, even though it represents an important stage for nursery keepers who determine the purity of the material reproduced and the most adequate conditions for plant material production, for its later establishment in the field. The Ya'ax ik chili pepper seedling presented an average emergence period of 8 to 10 days, which confers it similar germination characteristics to those of a native cultivated variety such as habanero chili pepper (*Capsicum chinense*) or chile dulce (*Capsicum annuum*), and at the same time differentiates it from chili peppers such as Maax (*Capsicum annuum*) and Pico paloma (*Capsicum frutescens*), which present germination

Figure 1. Plantation of Ya'ax ik chili pepper in the rural communities of the state of Yucatán.

and tiered emergence of the plant through the substrate, requiring more time for the establishment of the seedlings in the trays.

The Ya'ax ik chili pepper seedlings showed green intermediate coloring, oval shape of the cotyledon leaf, green color of the coleoptile with scarce pubescence, and length and width of 12.13±1.71 mm and 2.03±0.41 mm, respectively.

Plant

At the plant stage, the Ya'ax ik chili pepper could be established as a plant of annual life cycle, rustic and upright growth. Based on what was observed, it could be established that the life cycle of the plant and its duration is in function of the agronomic management and the environmental conditions. For the time which the plants remained under a protected system, they showed a more accelerated growth, while the growth speed decreased when they were taken out to open air conditions. Under the conditions of study, tolerance to the appearance of virosis transmitted by white fly was observed, for the time when damage to the plant was observable such as curling of the leaves (seven months). The Ya'ax ik chili plant was characterized by scarce tillering, angular stem, green, scarce pubescence, with presence of anthocyanins in the knot during the initial stages of growth, which disappeared as the plant grew.

The characteristic of the stem in angular shape differs from what was described by Aguilar-Rincón *et al.* (2010), who described a stem with cylindrical shape, which can be because of the different variants of this type of chili pepper in the region. The plants presented an average height and width of 118±9.40 cm and 68.5±14.33 cm, respectively, a slightly greater height than what was reported by González *et al.* (2014), who reported a height range of 0.40 to 1.0 m. This behavior is attributed to the management conditions granted under a protected system. Under growth conditions in rocky terrains, a plant with lower height has been observed. The scarce pubescence found in the stems of plants was considered to be a morphological characteristic of plants with a certain degree of

Figure 2. Shape of the plant stem and absence of anthocyanins in the knots.

domestication, which reduces its physiological and adaptive growth capacity under the environmental conditions of regulation of high radiation, which is related to hydric economy and gas diffusion, as pointed out by Molina-Montenegro (2008).

The density of ramification and leaves was intermediate, with an oval leaf shape of undulated margin, opaque green coloring, and scarce pubescence, which agrees with what was described by González-Estrada *et al.* (2014). The length of the mature leaf was 9.64±2.20 cm and the leaf width 3.48±0.62 cm.

The leaf density and ramification was intermediate, which can partly explain the plant's tolerance to drought, from the reduction of the leaf surface exposed to transpiration and therefore the water loss, in addition to the smaller size of its leaves, compared to the leaf size of chile dulce (11.35/5.72 cm) and Rosita habanero chili (14.78/77.64 cm), regarding the length and width rate of the leaf. This type of chili pepper under field conditions is cultivated in various types of soil, even in stony soils, where it adapts very well and the morphological characteristics of the plant make it adaptable to conditions of association crops and at the same time it can modify its expression with another type of management.

Figure 3. From left to right: Ya'ax ik chili pepper plant under protected system and open air.

Figure 4. Position of the hanging flower and Ya'ax ik chili pepper flower.

Flower

The flowers of the Ya'ax ik chili pepper are characterized by being solitary flowers (one per knot), white, with hanging position, medium size, annular constriction of the absent calyx, and round shape of the corolla. Quantitatively, the size of the corolla was 1.57±0.25 cm. The flowers presented a green bluish anther color, with white filament color, and exserted stigma. The flowers presented anther length of 2.95±0.54 mm and style length of 5.93±0.63 mm. Regarding the calyx, absence of pigmentation, an intermediate margin and absence of annular constriction were found.

One of the important characteristics of the flowers was that it was not lost by the plant, with the consequent low production of fruit and flowers with strong floral pedicels adhered to the flower and the stem, which confers the plant a characteristic of survival from the consequent production of fruit.

Fruit

The Ya'ax ik chili pepper fruits showed absence of anthocyanin spots, fruit color in intermediate orange stage, high setting, red coloring, without flowering vestiges. Long fruits with semi-coarse epidermis with placenta distributed along the fruit were observed, green in the early stage and red in maturity; the shape of the fruit in union with the obtuse

Figure 5. Ya'ax ik chili pepper fruits in mature state.

pedicel, persistent pedicel to the fruit and stem, mostly with two loculi, and mild transversal wrinkling of the stem, which agrees with what was described by Aguilar-Rincón *et al.* (2010). The average length and width were 7.42 and 1.03 cm, values within the range was established by González-Estrada *et al.* (2014).

The length of the fruit found was 7.42±0.55, with a width and weight of the fruit of 1.03±0.34 cm and 2.38±0.56 g, respectively, values that are within the range established by González-Estrada *et al.* (2014). The thickness of the pericarp of the fruit was 1.26±0.25 mm, with 15.33±8.16 seeds per fruit.

CONCLUSIONS

The morphological characterization of the Ya'ak ix chili pepper allowed a greater knowledge of the plant and its parts, which will allow producers to make decisions regarding the crop, and will support geneticists in the creation of programs of genetic improvement that satisfy the market demands in terms of yield and quality.

The morphological data obtained must be taken as preliminary, because of the existence reported of different ecotypes of the chili pepper, as well as the possible modifications from the environmental effect that can happen in the expression of the different morphological characteristics of the plant.

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Colegio de Postgraduados

In vitro nutritional assessment and estimation of methane emissions from Kikuyu grass pastures overseeded with rye

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ABSTRACT

Objective: To determine the *in vitro* digestibility and gas production, and to estimate the methane emissions from Kikuyu grass pastures, and Kikuyu grass overseeded with rye.

Design/methodology/approach: Two pastures were assessed. One was the subtropical grass Kikuyu (*Cenchrus clandestinus*) (KY), and the other was Kikuyu grass plus overseeding with rye (*Secale cereale*) (KYCEN), both associated with white clover (*Trifolium repens* cv. Ladino). Sample collection was in June and July 2021. *In vitro* digestibility of dry matter (MS), organic matter (MO), and Neutral Detergent Fibre (FDN), as well as the methane emissions were estimated. The *in vitro* digestibility and gas production variables were analysed with a split-plot experimental design, and the methane emission variables were analysed with a doble cross-over design.

Results: There were no significant differences between treatments for dry matter (MS), organic matter (MO) or Neutral Detergent Fibre (FDN) *in vitro* digestibility, nor in methane emissions ($P>0.05$).

Limitations on study/implications: The *in vitro* assessment of digestibility, gas production and the estimation of methane emissions of Kikuyu grass pastures and Kikuyu plus rye enable the implementation of feeding strategies for small-scale livestock production systems that do not only benefit the farmers but also the environment.

Findings/conclusions: It is concluded that Kikuyu grass pastures and Kikuyu with rye are a viable feeding option for small-scale dairy systems.

Keywords: Kikuyu grass, rye, gas production, methane.

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INTRODUCTION

Agricultural production faces new challenges worldwide, such as the greenhouse gases it generates. Livestock farming contributes 14.5% of these gases [1], including methane —a greenhouse gas (GHG) with 28 times greater global warming potential than carbon dioxide [2] and a 10-year average lifetime in the atmosphere.

Since 35% of enteric methane production comes from pasture systems [3], feeding strategies that can reduce CH_4 emissions should be considered. In small-scale dairy

systems, the feed is mainly based on pasture grazing. The proper management of these systems potentially improves their profitability and sustainability, enhances the quantity and quality of the forage consumed by animals, and even reduces CH_4 emissions [4].

Kikuyu grass (*Cenchrus clandestinus*) is a subtropical grass of African origin, well adapted to forage-based dairy systems in Latin America (Colombia, Brazil, and Mexico), Oceania (Australia and New Zealand), and South Africa. Properly used, it has moderate-good quality and high yield potential [4,5].

Rye (Secale cereale) is a small grain cereal, with a short growth cycle; consequently, it requires less water, is resistant to frost, and can be used for grazing, silage, or grain harvesting [6]. Small grain cereals have good forage yields and, given the current situation (low availability of irrigation, plus low precipitation and changes in rain patterns, due to increasing climate change [7]), rye is a viable option for these production systems.

The production systems that benefit from the use of these forages include small-scale dairy systems, which are considered a feasible instrument to stimulate economic growth and reduce poverty; additionally, they contribute 37% of the domestic milk production [8]. However, there are more systems that benefit from their use, such as sheep production systems.

The *in vitro* gas production technique is a method that has been widely used to assess the effect of different forages: it simulates the ruminal environment (temperature, pH, anaerobiosis, and mineral intake) to assess the fermentation of different substrates or additives [9]. The equations that estimate methane emissions have been used because they are less expensive than other *in vivo* methods [10]. Therefore, objective of this work was to assess the *in vitro* digestibility and gas production, as well as to estimate methane emissions, from Kikuyu grass pastures and Kiyuyu overseeded with rye.

MATERIALS AND METHODS

Location of the study site

The study was carried out in a small-scale dairy farm located in the municipality of Aculco in the Estado of México (between 20° 06' and 20° 17' N and 99° 40' and 100° W), at 2,440 meters above sea level. The site has a temperate-subhumid climate, a rainy season from May to October, and frost from November to February. The average annual temperature is 13.5 °C and the average annual precipitation ranges from 700 to 1,000 mm [11].

Experimental development and treatments

Two 1-ha pastures were assessed. One pasture was naturally invaded by a Kikuyu subtropical grass (*Cenchrus clandestinus*) (KY). The other one featured Kikuyu pasture which was overseeded with rye (*Secale cereale*) (KYCEN) on April 9, 2021. Both pastures were associated with white clover (*Trifolium repens*) cv. Ladino, among other unidentified grass species eaten by grazing dairy cows.

Samples were collected in June and July, 2021, during the rainy season. Three forage samplings were carried out at 14-day intervals. The experiment followed the guidelines of rural participatory research [12].

Variables assessed

Ruminal fermentation kinetics and *in vitro* digestibility

The simulated grazing technique was used to collected 200-g forage samples in different sites of the assessed pastures. The samples were then placed in an extraction oven at 55 °C until a constant weight was achieved. Subsequently, they were ground to 2.0 mm and processed to determine the digestibility, metabolizable energy, and ruminal fermentation kinetics, using the in vitro gas production technique.

The ruminal fluid from two cows was used to determine the variables of the ruminal fermentation kinetics of pasture forage. The diet of these cows was composed of grazing, maize silage, and commercial concentrate. The fluid was extracted through a nasogastric tube. According to the procedure described by [13], 990±0.01 mg of dry forage samples from each pasture were weighed and subsequently placed in 120 ml glass bottles with crimp caps. Ninety ml of buffer solution and 10 ml of ruminal fluid were added in a 9:1 (vol/vol) ratio. The solution had been previously gassed with CO_2 for 20 minutes to generate anaerobiosis.

Subsequently, the samples were incubated at 39 °C and gas production was measured using a pressure transducer (DELTA OHM, Manometer, 8804) at 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 72, 84, and 96 hours. Each sample was analyzed in sextuplicate with 96 h incubation in two repeated courses at different periods.

After 96 hours of incubation, the residues of each sample were analyzed to determine the DM, OM, and NDF digestibility [14]. In the case of the *in vitro* digestibility of neutral detergent fiber (IVDNDF), the residues from the other three bottles were removed with 50 ml of NDF solution, placed in an autoclave at 105 °C for one hour, filtered in Schott Duran® No. 1 filter crucibles, and placed in the muffle at 450 °C for 4 hours. The IVDDM was calculated based on the weight difference between the DM of the initial sample and the DM of the gas production residue, while the IVDNDF was calculated using the NDF digestibility values of the sample already incubated, divided between the NDF content of the initial sample. The ash content of the samples after 96 h incubation was used to determine the residual organic matter (OM) and the *in vitro* digestibility of organic matter (IVDOM) following the micro technique proposed by [15].

The results obtained were used to determine the *in vitro* fermentation parameters, which were estimated through the adjustment of the accumulated gas volume obtained from each bottle to the mathematical model developed for this study [16], using the following equation in the GraFit Data Analysis Software (V3) [17]:

$$PG = B\left(1 - \exp - c\left(t - lag\right)\right)$$

Where: PG=total gas production (ml gas/100 mg DM); B=asymptotic gas production from the fermentation of the neutral detergent fiber; c=degradation rate of gas production (per hour); lag=time elapsed before the beginning of the fermentation of structural carbohydrates [18].

The ether extract (EE) was determined by the immersion solvent extraction method [19], while the gross energy (GE) required to estimate methane emissions was calculated according to [10].

Enteric methane emissions

Methane emissions were estimated following the model proposed by [20]. Data from the research carried out [21] —with eight multiparous Holstein cows, similar numbers of days in milk, daily milk yield, and live weight— were used with a double cross-over design. The following equation was used:

$$\mathrm{CH}_4\left(g/day\right) = -60.5 + \left(12.4 \times DMI\right) - \left(8.78 \times \%EE\right) + \left(2.10 \times \%NDF\right) + \left(16.1 \times \%\,fat\ in\ milk\right) + \left(0.148 \times LW\right)$$

Where: *DMI*=dry matter intake (kg/cow/day), *EE*=ether extract of the diet, *NDF*=neutral detergent fiber of the diet, and *LW*=live weight (kg/cow).

The correction factor for methane Ym (ratio of gross energy lost as methane) was calculated based on [22].

$$Ym = 100 \times \left(\mathrm{CH}_4\left(MJ/day\right)/GE\ consumed\left(MJ/day\right)\right)$$

Where: *GE*=gross energy.

The metabolizable energy of the forages was estimated based on the digestible organic matter in the dry matter, using the following equation [23]:

$$ME = 0.16 * DOMD/10$$

Where: *DOMD*=digestible organic matter in dry matter.

Experimental design and statistical analysis

A split-plot design was used for the gas production and DM, OM, and NDF digestibility variables with the following statistical model:

$$Y_{ijkl} = \mu + T_i + E_j + P_k + Tp_{ij} + e_{ijk}$$

Where: μ=general mean; T=effect of the main plot (i=1, 2); E=experimental error of the main plot; P=effect of the assessment periods (k=1, 2, 3); Tp=effect of the interaction between the main plot (crops) and the split plot (assessment periods); E=residual variation.

For the methane emissions estimation variables, a double cross-over design was used with the following model:

$$Y_{ijkl} = \mu + S_i + C_{(i)j} + P_k + T_l + e_{ijkl}$$

Where: Y_{ijkl}=response variable; μ=general mean; S_i=effect of the sequence (i=1 and 2); $C_{(i)j}$=effect of the cow within the sequence (j=1... 4); P_k=effect of the experimental periods (k=1...3); T_l=effect of treatments (l=1 and 2); e_{ijkl}=experimental error [24].

RESULTS AND DISCUSSION

Table 1 includes the *in vitro* digestibility of DM, OM, and NDF results, as well as the estimation of the metabolizable energy content of the forages from the two experimental pastures. There were no significant differences (P>0.05) for any of the variables assessed.

Since the study was carried out during the rainy season (the optimal time for forage growth), both pastures had very similar results. Kikuyu obtained good digestibility results, due to its subtropical origin and its growing season (spring-summer).

The results are higher than those obtained by [25] in the same study area, with Kikuyu pastures, *Festulolium* cv. Spring Green, *Lolium perenne* cv. Pay Day, and *Lolium arundinaceum*

Table 1. Average *in vitro* digestibility (g/kg DM) and metabolizable energy (MJ ME kg^{-1} DM) of forage from experimental pastures during different sampling periods (PI, PII, PIII).

VARIABLE	PERIODS			Mean TX	SEMTx	P-Value	SEMExP	P-Value
	PI	PII	PIII					
IVDMD (g/kg DM)								
KY	783.83	773.42	773.16	776.80	0.13	0.947^{NS}	2.44	0.422^{NS}
KYCEN	772.55	774.75	783.70	777.00				
Mean for Periods	778.19	774.08	778.43					
Interaction SEMTx*ExP					3.16	0.06^{NS}		
IVOMD (g/kg DM)								
KY	835.93	825.68	826.42	829.34	5.35	0.114^{NS}	3.23	0.467^{NS}
KYCEN	836.78	834.12	839.85	836.92				
Mean for Periods	836.36	829.90	833.13					
Interaction SEMTx*ExP					1.82	0.479^{NS}		
IVNDFD (g/kg DM)								
KY	835.17	757.06	830.71	807.65	11.95	0.406^{NS}	22.78	0.231^{NS}
KYCEN	814.84	823.35	835.45	824.55				
Mean for Periods	825.01	790.21	833.08					
Interaction SEMTx*ExP					12.86	0.242^{NS}		
ME (MJ/kg DM)								
KY	10.65	10.51	10.51	10.56	0.002	0.929^{NS}	0.03	0.385^{NS}
KYCEN	10.50	10.53	10.65	10.56				
Mean for periods	10.58	10.52	10.58					
Interaction SEMTx*ExP					0.04	0.053^{NS}		

KY=Kikuyo; KYCEN=Kikuyo+rye; IVDMD=*in vitro* dry matter digestibility; IVOMD=*in vitro* organic matter digestibility; IVNDFD=*in vitro* neutral detergent fibre digestibility; ME=metabolizable energy; SEMTx=standard error of the mean for pasture treatments (main plots); SEMExP=standard error of the mean for experimental periods (split plot); SEMTx*ExP=standard error of the mean for the interaction between treatments and experimental periods; NS=(P>0.05).

cv. TF-33, in the rainy season. They obtained average results of 700.70 gr, 637.90 gr, and 744.80 gr for IVDDM, IVDOM, and IVDNDF, respectively.

Some authors [26] mention that DM digestibility is an important indicator of forage quality: good quality forage has a digestibility of ≥700 g/kg DM. In this work, both pastures recorded higher values than 700 g/kg DM and therefore can be considered good quality material [14]. These authors assessed small grain cereals (including rye), obtaining higher results than 700 g/kg DM; however, metabolizable energy was higher (11.6 MJ) than in this work (10.56 MJ on average, for both pastures).

The IVDOM and IVDNDF were higher than those reported by [14], who carried out an experiment with small grain cereals (including rye), at a more advanced phenological stage, recording an average of 730 and 618.6 g/kg DM for IVDOM and IVDNDF, respectively. Likewise, these results are higher to those found by [25], who reported 637.90 g/kg DM for IVDOM and 744.80 g/kg DM for IVDNDF in Kikuyu grass pastures.

Metabolizable energy depends on the nutritional quality of forage: it is more stable in the growth period and later decreases as grain formation begins (in the case of cereals) and nutrients are mobilized towards the grain [14]. For this study, an average of 10.56 MJ was determined, similar to the results of [25] who obtained 10.34 MJ.

Table 2 shows the results of *in vitro* gas production where no significant differences were observed (P>0.05). *In vitro* gas production is a suitable indicator for the prediction of the carbohydrate degradation of forages [27]. This gas production is caused by the

Table 2. Averages of *in vitro* gas production parameters resulting from the fermentation of pasture forage assessed in three sampling periods (PI, PII, PIII).

VARIABLE	PERIODS			Mean TX	SEMTx	P-Value	SEMExP	P-Value
	PI	PII	PIII					
B (ml gas g^{-1} DM)								
KY	227.35	229.96	230.68	229.33	3.37	0.059^{NS}	3.06	0.123^{NS}
KYCEN	232.54	234.99	234.76	234.10				
Mean for Periods	229.95	232.47	232.72					
Interaction SEMTx*ExP					1.51	0.186^{NS}		
cB (g h^{-1})								
KY	0.03	0.03	0.03	0.03	0.002	0.253^{NS}	0.001	0.68^{NS}
KYCEN	0.03	0.03	0.03	0.03				
Mean for Periods	0.03	0.03	0.03					
Interaction SEMTx*ExP					0.0008	0.68^{NS}		
Lag (h)								
KY	6.66	6.67	6.01	6.45	0.26	0.583^{NS}	1.21	0.067^{NS}
KYCEN	6.10	8.20	6.01	6.77				
Mean for Periods	6.38	7.44	6.01					
Interaction SEMTx*ExP					0.1	0.896^{NS}		

KY=Kikuyo; KYCEN=Kikuyo+rye; B=gas production potential (ml gas/g DM) based on the insoluble but potentially degradable fraction; cB=rate of fermentation of fraction b; lag=lag time (h) before fermentation of NDF; SEMTx=standard error of the mean for pasture treatments (main plots) ; SEMExP=standard error of the mean for experimental periods (split plot); SEMTx*ExP=standard error of the mean for the interaction between treatments and experimental periods; NS=(P>0.05).

fermentation of carbohydrates and their transformation into acetate, propionate, and butyrate; consequently, any change in carbohydrate fractions will be reflected in gas production.

The accumulated gas production (B) reached 231.75 ml, lower than the result found by [14], who obtained 258.72 ml in rye pastures, but higher than that results of [25], who obtained 209.64 ml in Kikuyu pastures. For their part, [28] assessed the nutritional value of forage species in the central Mexican Plateau and observed a higher content of accumulated gas production (215.66 ml) in Kikuyu. According to them, Kikuyu has a high hemicellulose content and a low cellulose content; therefore, hemicellulose is the NDF fraction that is completely fermented by microorganisms.

The degradation rate (c) is related to the fermentation of the substrate, which in turn is related to the type of structural carbohydrates that may indicate that there is more or less cellulose available for ruminal microorganisms [18]. This study recorded no difference between the treatments, with an average c of 0.03 for both pastures. For their part, [25] reported a fermentation rate of 0.02 for Kikuyu, as a result of the higher content of lignified cell walls, characteristics of the subtropical and tropical C4 grasses. However, in this study degradation rate was higher, due to the association between several species, which improves the nutritional quality of the pastures.

Lag (h) indicates the time in which microorganisms begin to degrade structural carbohydrates. The content of rapid degradation carbohydrates (*e.g.*, sugars, starch, and pectin) increases the lag time [18]. Lag time is important in digestibility because the presence of high amounts of fermentable carbohydrates diminishes its duration [29]. In this study, a Lag time of 6 hours was reported. Period II was the longest (7 hours), perhaps as a result of the maturation of the pastures, which is directly related to the fiber content that, as has been previously reported [21] increases over time. However, in the case of Kikuyu over seeded with rye, there was a decrease in period III, perhaps due to the increase in rains as this period approached, therefore, there was a greater growth of forage, resulting in a new decrease in Lag time. This result is similar to that found by [28] in Kikuyu grass pastures, with an average of 6 hours.

The estimated enteric methane emission is shown in Table 3. There were no significant differences per treatment for any of the variables. Nevertheless, KYCEN obtained higher numerically values for methane production (CH_4 g/kg DM) and the percentage of gross energy lost as methane (Ym).

The estimated average production of CH_4 was 298.54 g/cow/d, higher than the value reported by [8] in small-scale dairy systems (an average of 216.12 g/cow/d). In their research, [8] assessed four feeding strategies: CC=cut and carry, CC+CS=cut and carry plus maize silage, CIG=continuous intensive grazing; and CIG+CS=continuous intensive grazing plus maize silage. The farms that implemented pasture grazing as source of quality fresh forage (CIG) generated less methane than farms that implemented cut and carry and maize silage.

According to the abovementioned information, the estimated average production of CH_4 was 298.54 g/cow/d, lower than the value reported by [30] in small-scale dairy systems in the central Mexican Plateau. In their research, [30] obtained 335 g/cow/d in optimized

Table 3. Average values of estimated enteric methane emissions from Kikuyu pasture and Kikuyu overseeded with rye in small-scale dairy systems.

Variable	Treatment			P-Value	Experimental periods			SEMExP	P-Value
	KY	KYCEN	SEMTx		I	II	III		
CH_4 g/cow/day	296	301.08	4.59	0.184^{NS}	297.5	301.5	297.6	4.59	0.783^{NS}
CH_4 MJ/cow/day	16.34	16.66	0.25	0.184^{NS}	16.43	16.65	16.43	0.25	0.783^{NS}
CH_4 g/kg milk	18.72	18.28	0.49	0.994^{NS}	16.89	18.03	20.58	0.49	0.000*
CH_4 g/kg ECM	18.53	17.62	0.41	0.885^{NS}	16.7	17.62	19.92	0.41	0.000*
CH_4 g/kg DMI	23.62	23.86	0.22	0.178^{NS}	22.93	23.33	24.91	0.22	0.000*
Ym (% GE intake)	6.94	7.00	0.06	0.178^{NS}	6.74	6.85	7.32	0.06	0.000*

KY=Kikuyo; KYCEN=Kikuyo+rye; ECM=Energy-corrected milk production; DMI=Dry matter intake; GE=Gross energy. SEMTx=Standard error of the mean for pasture treatments; SEMExP=Standard error of the mean for experimental periods.

diets with a feeding mainly based on good quality forage with a metabolizable energy of 11 MJ. For their part, [10] used questions to estimate 283 g/cow/d for temperate regions in Mexico and 319.1 g/cow/d for tropical regions (results similar to the ones determined in this study), using Kikuyu grazing associated with other grass species found in the temperate regions of Mexico. Kikuyu is a plant with a subtropical origin with nutritional quality similar to temperate grasses. It has adapted very well to temperate zones [31]; therefore, the result obtained in this study is very similar to that obtained in such areas. The estimated mean emissions of CH_4 are within the normal range (77 to 447 g/cow/d) reported by [32].

The average methane emission intensity was 18.07 g CH_4/kg of ECM (energy-corrected milk). This figure is higher than the intensity recorded by [8], who obtained 15.1 g CH_4/kg of ECM. However, it is very similar to the results recorded by [33] for the Latin American region (19.9 g CH_4/kg of ECM) and with the figures estimated by [30] in the same study area (18.2 g CH_4/kg of ECM). For his part, [34] mentions that a greater intake of highly digestible foods reduces the generation of CH_4 (average of 23 g kg^{-1} DMI (dry matter intake)).

About 6-10% of the total gross energy consumed by dairy cows is converted into CH_4, which is released into the atmosphere through respiration [35]. Meanwhile, this study recorded than an average of 6.97% of the energy is converted into methane.

[36] identified that the incorporation of high-quality fresh forages can reduce CH_4 emission by 15% [8]. They found that the supply of higher quality fresh forage through grazing favored CH_4 emission per animal per day by 8.9%, compared with the cut and carry system [30]. They found that methane emissions diminish by 2% when associated pastures are used instead of single-grass pastures. Therefore, methane emissions per kg of milk produced can be reduced through the use of better feeding strategies, based mainly on good quality forage, grown in the same farm.

CONCLUSIONS

Given the lack of significant differences between both treatments, Kikuyu grass pastures and Kikuyu grass overseeded with rye are a viable feeding option for small-scale dairy systems, during the rainy season.

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Colegio de Postgraduados

Identification of fungal disease in a *Vanilla planifolia* Jacks plantation in the central zone of the state of Veracruz, Mexico

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ABSTRACT

The state of Veracruz is the main producer of vanilla in Mexico. In recent years, this crop has been seriously affected by severe phytosanitary problems caused mainly by fungal pathogens. For this reason, it was proposed to develop this study with the objective of determining the causal agents of the disease, incidence, and severity observed in a vanilla plantation located in the locality "El Palmar," Municipality Emiliano Zapata, Veracruz. A census sampling was carried out on one hectare of the plantation to determine the incidence of the disease. Disease severity per plant was determined using a 4-grade scale. Leaves and stems with disease symptoms were collected for morphological description of the pathogenic agents by scanning electron microscopy. The results showed the presence of two species of phytopathogenic fungi, *Fusarium oxysporum* f. sp. vanillae and *Puccinia sinanoemea*, known to cause root rot and rust diseases in vanilla. The infection resulted in the death of most individuals in the plantation. The incidence was classified as severe (a grade of 4), with 80% infestation by both pathogens. This study contributes to the understanding of the phytosanitary problems this crop faces due to mixed infections of fungal pathogens.

Keywords: phytopathogens, incidence, rust, *Fusarium*.

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INTRODUCTION

Vanilla (*Vanilla planifolia* Jacks.) is an orchid native to Mexico of great economic importance because vanillin, the second most expensive aromatic spice in the food industry after saffron, is extracted from pods of this plant (Anilkumar, 2004). Because of its great commercial value, both in the confectionery industry and in cosmetology, it is grown commercially in the "Totonacapan region" of the state of Veracruz, México, which accounts for 70% of national vanilla production (Hernández, 2011).

In recent years, this crop has suffered serious phytopathogenic effects. Among the main diseases that reduce production in this crop, there are the root rot disease caused by *Fusarium oxysporum* f. sp. vanilla and the basal rot caused by *Phytophtora* spp. Basal rot of stems and roots. These can affect vanilla production per unit area and even cause premature death of the plants (Santa-Cardona *et al.*, 2018).

Other fungal pathogens that attack vanilla are anthracnose (*Colletotrichum* sp.) and rust (*Uromyces joffrini*) (Hernández, 2011). All these pathogens, especially those caused by *Fusarium* spp., are causing serious losses worldwide (FAOSTAT, 2017; Ramírez-Mosqueda *et al.*, 2019). Although, in the state of Veracruz, Mexico, the greatest

affectations caused mainly by *Fusarium* spp. have been reported, as the causal agent of basal rot (Santa-Cardona *et al.*, 2018), affectations caused by other phytopathogenic fungi such as rust have recently been detected in vanilla-growing areas. However, there is not enough information on the effects of this disease on this valuable crop. For this reason, it was proposed to determine the causal agents of the disease, its incidence, and its severity on a commercial vanilla plantation located in the central zone of the state of Veracruz.

MATERIALS AND METHODS

A census-type sampling was carried out on one hectare of vanilla (*Vanilla planifolia* Jacks.) plantation established in the "Palmar region", Municipality of Emiliano Zapata, Veracruz. The studio area has average annual temperatures of 23.4 °C and average annual precipitation of 122.2 mm; the warmest months are from April to September with average maximum temperatures between 26-28 °C. The plantation was established for approximately 8 years under shade canopy conditions. Disease incidence was determined. For this purpose, a diseased plant showed symptoms of necrotic lesions on leaves and stems, typical of fungal diseases.

In a plantation established in the "Palmar region", Municipality of Emiliano Zapata, Veracruz, the incidence of the disease was determined. For this purpose, a plant was considered infected when it showed symptoms of necrotic lesions on leaves and stems, which are typical of fungal diseases.

Ten leaves with pustules were collected from each diseased plant and taken to the laboratory for fungal isolation, determination of the causal organism, and morphological description of the pathogen using the keys of the *Fusarium* Laboratory Manual (Leslie and Summerell, 2006) and for Puccinia, the Keys from Uredinales (rusts) from Mexico: Puccinia were used (Gallegos and Baker 2018, Sandoval-Sánchez *et al.*, 2020). Subsequently, using the scale proposed by Vidal-Martínez *et al.* (2011), the percentage of disease was determined for both phytopathogens based on the damage caused in each plant according to the census carried out; so, the degree of infection was determined for each plant. The infection was measured according to the following grades: grade 0: no presence of the disease; grade 1: 10% incidence; grade 3: 26-50% incidence; grade 4: >50% incidence (Figure 1). Using the methodology of Ortega-Centeno (2009), leaves with yellow-orange pustules were visually detected and collected.

Subsequently, infected leaves were selected and cut from the base; they were placed on absorbent paper in zip-lock bags to be transferred to the laboratory. Once the plants were collected, they were taken to the Institute of Biotechnology and Applied Ecology of the Universidad Veracruzana (INBIOTECA) laboratory.

To carry out the study of infections, the following methodology was used: with a sterilized toothpick, the leaves were infected by placing 1×10^6 spores as inoculum for infection of the fungi *Puccinea sinamonea* and *Fusarium oxysporum*; infected leaves were checked every day for five days. Young leaf cuts were placed on filter paper, sealed, and sprayed with distilled water to form a humid chamber. Four Petri dishes were placed at room temperature (22-24 °C), and other 4 Petri dishes were placed in an oven at 28 °C, the infected leaves were

checked every day for five days. The small leaf cuts were made and placed on filter paper, sealed, and sprayed with distilled water to form a humid chamber.

To carry out microscopic studies of rust, samples of the field collection were taken from approximately 1 cm^2 of the base of the rust-infected leaf and placed in a humid chamber with absorbent paper in a zip-lock bag. The samples were transferred to the laboratory to be observed the following day in a scanning electron microscope (FEI QUANTA 250 FEG), in the advanced microscopy unit of the BioMimic Cluster of Instituto de Ecología A.C. By scraping with a sterilized needle, the uredospore was extracted from the infected leaves and placed in sterile water to be observed under the electron microscope. In the case of *F. oxysporum*., samples were taken from the stems stored in the humid chambers in a similar way as in the case of rust, and the samples were prepared for observation under the scanning electron microscope. The samples obtained were photographed with a JEOL model JSMIT300 scanning electron microscope.

RESULTS AND DISCUSSION

Two pathogens, *Fusarium oxysporum* f sp. vanillae and *Puccinea sinamonea*, were found as the almost destruction of the vanilla plantation in the locality of "El Palmar". The *Fusarium* observed symptoms were chlorotic rings on the stems and basal rot. (Figure 1A); in the case of rust, there were pustules on the leaves that were yellowish to reddish brown (Figure 1C). Of the total number of plants tested, 80% were found to be severely deteriorated with necrotic stems and leaves (Figure 1A), and 15% showed low levels of infection by both fungi with isolated spots or pustules without reaching necrosis on stems and leaves (Figure 1C). Five percent of the plants were healthy.

Regarding the incubation of the humid chambers, those at ambient temperature (22-24 °C) and those exposed to a temperature of 28 °C, both conditions resulted in the development of fungal infection; we found a more significant (about 10%) infection in the leaf segments. It was observed that the severity of these fungi increased exponentially due to the predominant environmental conditions of the site, characterized by abundant humidity and predominant temperatures between 28-30 °C. It has been proposed that the increase in temperatures is one of the factors that have most influenced the spread of diseases in plants since it impacts more significant quantities of fungal spores that increase the inoculum and give rise to more substantial infection, increasing the probable rate of genetic changes and thus a more rapid evolution of these pathogens (Hamada and Ghini 2011; SAGARPA 2012).

On the other hand, some predictions consider that there could be an increase of up to 1.5 °C by the middle of this century, increasing the probability of imbalance in all ecosystems, especially for Mexico; which, due to its geographical location and diversity of climatic and orographic conditions, could have severe problems for the cultivation of different species in our agricultural fields (Zamora-Martínez, 2015). The results of rust incidence and damage sampling revealed a 100% incidence, with disease incidence ranging from grade 2 to grade 4.

In the initial stages, yellowish-green leaves with slight disease scores on the underside (grade 2) were observed. Then the leaves turned yellowish, with an increase in the presence

of yellow-orange pustules to brown pustules that later became necrotic (grade 4). Similar symptomatology was also observed by Álvarez-Morales and Salazar-Yepes (2014) in lemon grass (*Cymbopogon citratus* DC.) affected by rust. Samples placed in Petri dishes showed that no rust was present because no mycelium developed. In the case of *Fusarium oxysporum*., the symptoms were a yellowish-brown halo with cottony mycelium (Figure 1A).

F. oxysporum is a pathogenic fungus whose growth is characterized by growing by forming colonies of various colors (white, pale pink, red, orange, purple, light blue, and olive green) in a moderate to rapid manner (Jiménez-Quesada *et al.*, 2015). The results of microscopic observation showed that *Fusarium* has many septate hyphae, and *Fusarium conidia* were observed, as reported by Robles Yerena *et al.* (2017). In this study, *Fusarium hyphae* could be seen intermingled with rust hyphae (Figure 1B).

According to our microscopic observations, it was found that rust infection begins on the underside of the leaf, penetrating through the stomata. Then, infection spreads through the mesophyll until it colonizes the entire leaf tissue with hyphae, and subsequently hyphae exit through one or more stomata on the upper side of the leaf. Thus, the pathogen manages to completely expand uredospores to the outside and spread spores to infect other leaves (Figure 1D).

Microscopic studies showed that rust spores are strawberry-shaped with many regularly arranged dorsal and lateral spines. The results found demonstrate the presence of two pathogens that invaded the vanilla plantation under study. These were *F. oxysporum*, of

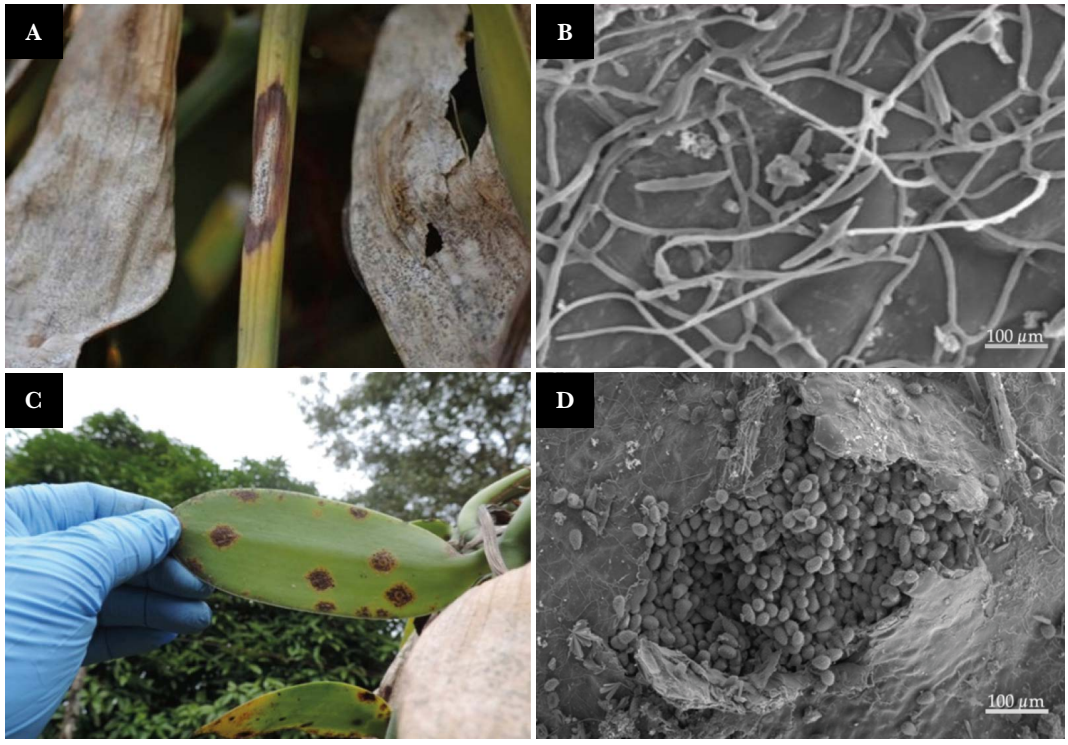

Figure 1. *Fusarium oxysporum* leaf and stem damage (A). Micrograph of hyphae and conidia of *Fusarium oxysporum* (B). Presence of rust pustules on the leaves of *Vanilla planifolia* (C). Micrograph showing the invasion of rust in the mesophyll and epidermis of vanilla leaves (D).

which the main symptom observed was the necrosis of the stems until separated from the roots. This fungus is the devastating causal agent causing stem and root rot in vanilla.

The symptoms of rust (*Puccinea sinamonea*) are yellowing of the leaves and the presence of orange pustules, mainly on the underside of the leaves. The presence of rust has also been observed on the new leaves of lemongrass (*Cymbopogon citratus* (DC.) (Alarcón, 2011). The results obtained in this study contribute to the phytopathological study of *Vanilla* as a crop, considering that to date there has been no adequate diagnosis and control of these pathogens in the *Vanilla* growing areas of Veracruz (Borbolla-Pérez *et al*., 2016). It is expected to continue with the characterization of the rust strains observed in the field and to carry out the corresponding molecular studies.

CONCLUSIONS

The presence of rust and fusarium wilt in the vanilla area of the locality of Emiliano Zapata was confirmed. These pathogens were identified as *Puccinea sinamonea* and *Fusarium oxysporum*. These organisms are reported as devastating agents of the vanilla crop. Fungi incubated in humid chambers at 28 °C showed significant proliferation and greater infection damage in young vanilla leaves, coinciding with the predominant high humidity and temperature conditions in the fields from May to September. The highest infestation (80%) grade 4 observed in the field corresponds to plants with both fungal infestations.

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Colegio de Postgraduados

Analysis of the structure of the global avocado (*Persea americana* Mill) trade network

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ABSTRACT

Objective: To analyze the structure of the global avocado trade network and to understand the dynamics of commercial interaction between the leading countries in the market, using the social network analysis approach.

Design/Methodology/Approach: One-hundred thirty-three countries that established 989 commercial exchange relationships were analyzed. This exchange accounted for 99% of the volume of the global avocado market for the year 2021.

Results: The significant diversification in the import sources and export destinations of countries such as Netherlands and Spain suggests a robust and diversified trade strategy. Network indicators give a valuable insight into the centrality and importance of countries in the global avocado trade network. Mexico plays an important role in the network that goes beyond the quantity produced.

Study Limitations/Implications: The most current analyzed data available to date belong to the year 2021.

Findings/Conclusions: The analysis reveals the complex dynamics of interaction between the leaders of the global avocado trade.

Keywords: global trade network, avocado export, eigenvector, Social Network Analysis, agricultural markets.

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INTRODUCTION

Mexico is the main avocado producer and exporter. The current value of the "green gold" global market exceeds $18 billion dollars, distributed between the Americas (59%), Europe (22%), and Asia (11%) (Rabobank, 2023). Considered a superfood, avocado is recognized for its contribution of vitamins, minerals, and monounsaturated fats (Bhuyan *et al.*, 2019). International avocado trade mainly takes place between the United States and Mexico in the context of the United States-Mexico-Canada Agreement (USMCA). In this scenario, Mexico has an outstanding capacity to produce avocado throughout the year, as a consequence of the 226,534 hectares planted in the state of Michoacán. The decrease in production during June and July is complemented by Peru. The preference for Mexican avocado over other US suppliers plays a relevant role in this dynamic (Williams and Hanselka, 2022).

This intense commercial activity and the relationships between avocado-producing and -consuming countries can be analyzed through a Social Network Analysis (SNA).

SNA has emerged as a fundamental tool for deciphering social and structural phenomena in various disciplines. This approach provides a unique perspective to understand the structure and interactions of the global avocado trade network, identifying key actors, trade flows, and patterns of influence (Wasserman and Faust, 1994). In the context of agricultural markets, international trade networks take shape as manifestations of a complex combination of diverse underlying factors, both natural and social (Shutters and Muneepeerakul, 2012). The SNA has helped to unravel this complexity in various contexts, addressing everything from the corn trade (Aguirre-López *et al.*, 2021; Wu and Guclu, 2012, 2013) to the impact of aflatoxins in the pistachio trade (Bui-Klimke *et al.*, 2014), raw materials networks (Nobi *et al.*, 2020), textile value chains (Shepherd, 2017), and global electricity network (Ji *et al.*, 2016), among other fields of study.

Considering the worldwide importance of avocado, this study aims to analyze the structure of the global avocado trade network through the SNA approach, to understand the dynamics of commercial interaction between the leading countries in the market.

MATERIALS AND METHODS

The predominant avocado exporters and importers during the year 2021 were selected. The countries were identified through the ISO3 code (https://www.fao.org/faostat/es/#definitions), composed of three alphanumeric characters (for example, Mexico's ISO3 code is MEX). Countries with less than one ton (t) in exchanges were not considered. The information used comes from the FAOSTAT avocado trade database (2023). One-hundred thirty-three countries that established 989 commercial exchange relationships were analyzed; those nations accounted for 99% of the volume of the global avocado market for the year 2021.

The data collected was organized and structured in Microsoft® Excel. An asymmetric matrix was developed to establish relationships between importing and exporting countries based on their exchange volumes. Subsequently, Gephi software version 0.10.1 was used to develop a graphic representation of the structure of the global avocado trade network and to calculate its indicators. Color attributes were assigned to countries on the same continent to visualize interactions between continents. Similarly, the size of the nodes (countries) in the network and the thickness of their links (trade relations) were characterized through the exchange volume (tons).

Different social network indicators were used to analyze the dynamics of global avocado trade. A graphic representation of the indicators was developed using the DataWrapper software. These indicators help to understand the structure and interactions in the trade network, as well as to identify the key actors and their influence on the global avocado exchange (Table 1).

RESULTS AND DISCUSSION

Avocado producers and exporters

The global avocado market during 2021 amounted to 3,202,009 t, out of which 3,200,812 t were analyzed, with a value of $8.407 billion dollars. The leading producers

Table 1. SNA indicators employed.

Indicator	Definition	Interpretation in avocado trade
Network size	Total nodes.	Total number of countries that participate in the commercialization of avocado.
Number of links	Relationship between network nodes.	Trade relations established between countries.
Degree	Links of a node.	Commercial relations that a country has, there is no difference between export or import.
Out Degree	Outbound links from a node.	Countries to which a country exports avocado. Breadth of commercial relationships.
In Degree	Inbound links of a node.	Countries from which a country imports avocado. Supply dependency.
Eigenvector centrality (Nodes)	Relative importance of a node in the network.	Influence of a country considering both its direct connections and the position of the countries with which it is related.
PageRank Distribution (Network)	Importance of a node based on its position and connections.	Relevance of a country considering both the quantity and quality of its network connections.

are Mexico (MEX), Colombia (COL), Peru (PER), Dominican Republic (DOM) and Chile (CHL). Most of these countries are also major exporters. Mexico is the largest producer and exporter of avocado, with 2,442,945 t produced on 226,534 hectares, with an average yield of 10.8 $t \bullet ha^{-1}$.

Some producing countries do not appear as exporters, since they allocate their production to domestic consumption. These countries include Indonesia (IDN) with 669,260 t, Brazil (BRA) with 300,894 t, and Haiti (HTI) with 248,135 t.

Figure 1 highlights the exporting countries based on their exportation volumes. Mexico exported 1,405,117 t to 44 countries, which represents 44% of the global market. The USA imported 77% of Mexico's production —1,085,353 t of the said volume (with a value greater than $2.858 billion dollars)—, followed by Canada (CAN) and Japan (JPN) with 103,449 t (7.4%) and 64,473 t (4.6%), respectively.

The second exporter (PER), with 18% of the world supply, exported 569,458 t to 45 countries, mainly to the Netherlands (NLD), ESP, USA, CHL, and the United Kingdom (GBR). The third exporter (NLD) supplied 11% of the world demand, exporting 337,156 t to 41 destinations; for this purpose, it imported 457,136 t from 33 nations. This situation makes this European nation the most important country in the structure of the global avocado trade network, given the commercial size of its interaction, combined with the number of countries with which it exchanges avocado (*i.e.*, its interconnectivity in the grid). The fourth place (ESP) covered 4% of the global market (50 countries); for this purpose, it exported 133,377 t to 28 nations. It imported 213,908 t to which it added its own production (116,770 t), which positioned it as the main European producer.

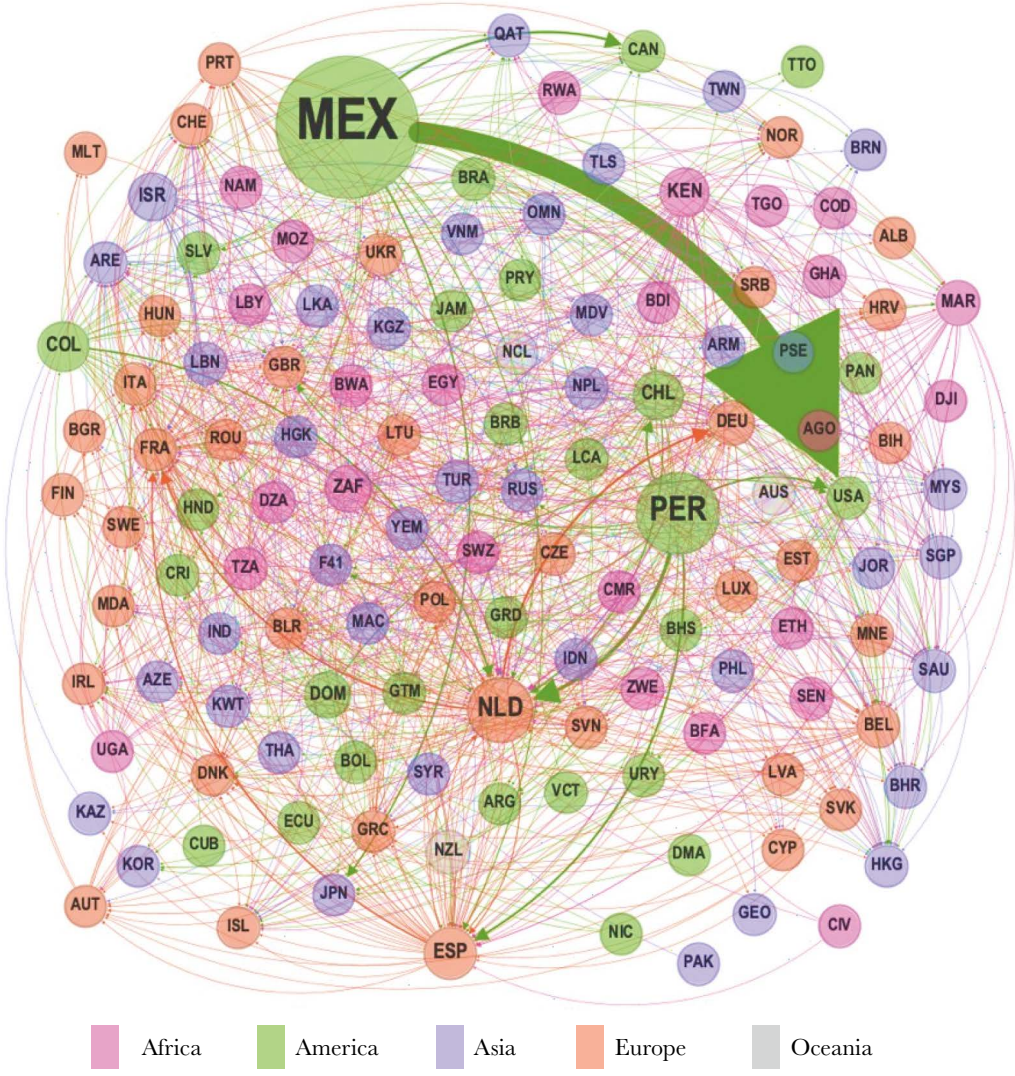

Figure 1. Global Network of Avocado Exporters (2021).
Note: The size of the node and its relationships is a function of the exported t.

Avocado import destinations

The main buyer of avocado is the USA with 1,213,412 t, which represents 38% of the world production ($3.13 billion dollars). It imports avocado from 5 countries and exports this produce to 23 countries, among them the Republic of Korea (KOR), JPN, and Singapore (SGP), with 2,017, 1,242, and 809 t, respectively.

The European Union —made up of 27 countries with great commercial connectivity— is the second destination for avocado exports in the world. The EU members include NLD and ESP, the second and third importers, respectively. France holds the fourth position, with 181,288 t purchased from 32 countries; however, it exports 19,293 t to 30 international destinations (Figure 2).

Finally, Chile is the country whose imports have the highest growth rate, increasing from 1,000 t in 2012 to 59,000 t in 2022 (Rabobank, 2023). Together with the USA, Chile

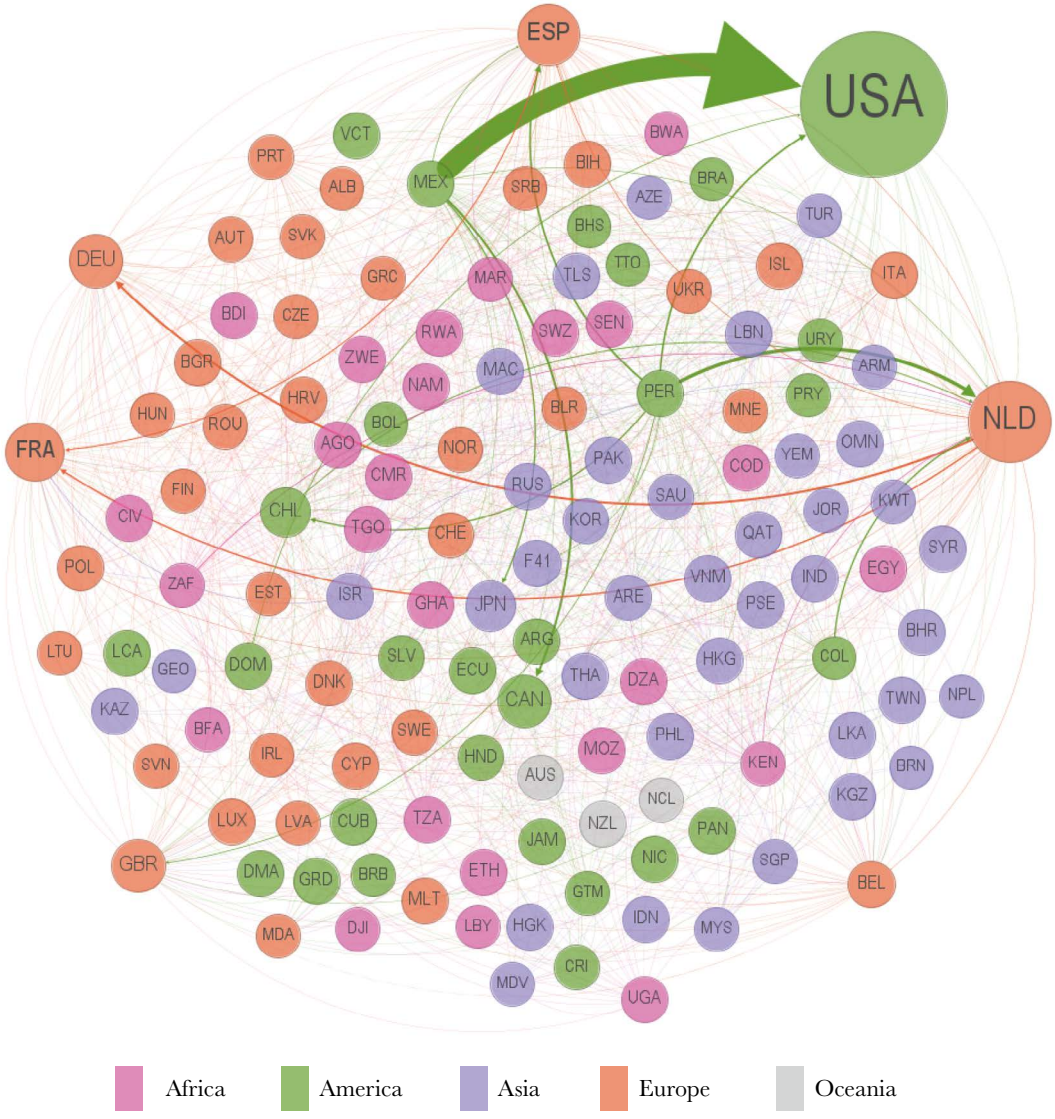

Figure 2. Global network of avocado importers (2021).
Note: The size of the node is a function of the imported t: likewise, the thickness of its relations is proportional to the quantity (t) exported and imported between two nodes.

is the only American country whose exports do not only consists of its own production. CHL imports from 5 countries and exports 104,347 t to 33 destinations.

The structures of the global avocado export and import networks prove the dominance of MEX as the main exporter and the USA as the main importer. However, these structures minimize the role of the European Union as an importer, as its destinations are spread among the 27 countries that make up this unique economic bloc.

Avocado intermediary countries

Setting aside the traded volumes, Figure 3 emphasizes which countries have greater international connectivity, measured by their degree as an SNA variable that adds the

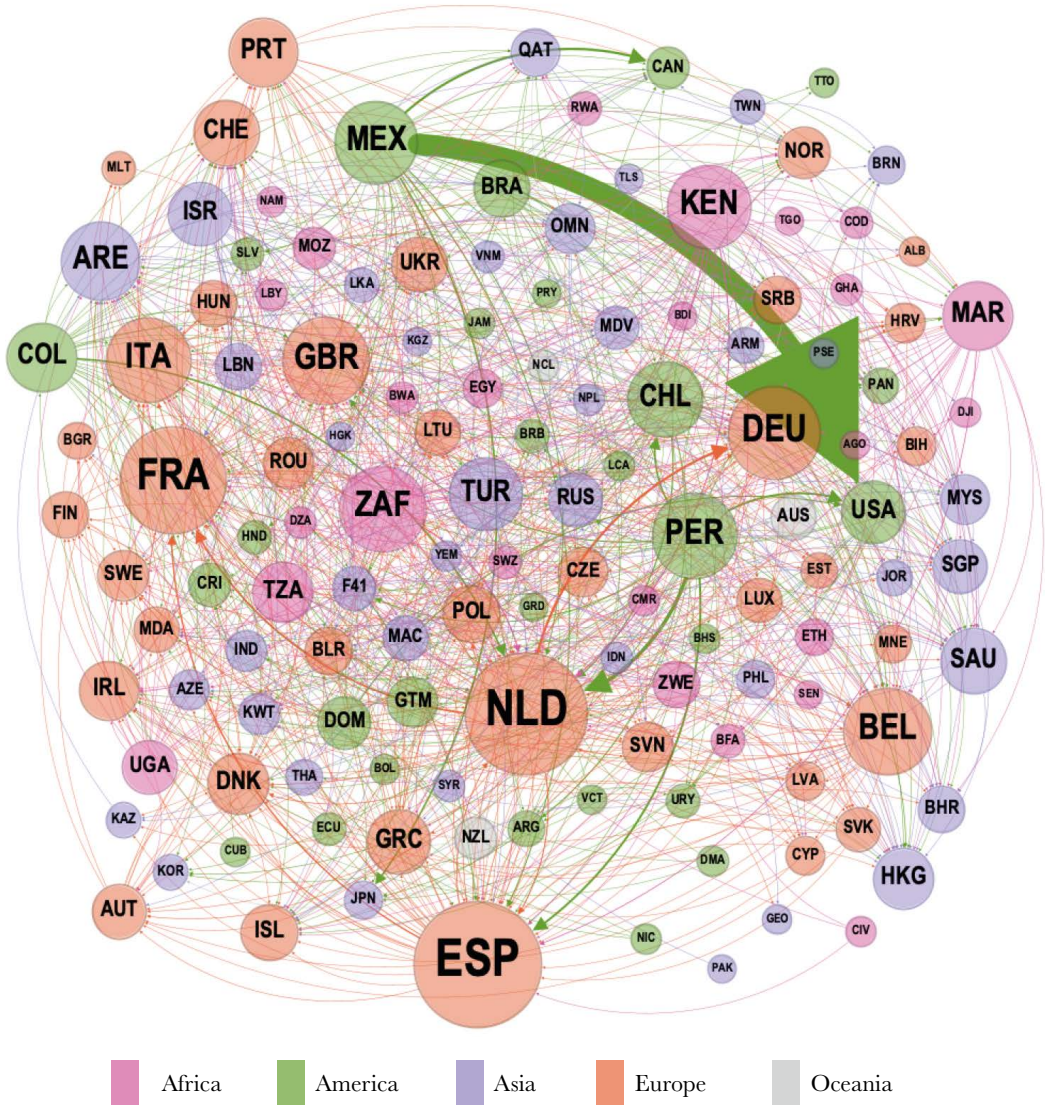

Figure 3. Avocado Global Connectivity Network (2021).
Note: The size of the node is a function of its degree (*i.e.*, the sum of the number of imports and exports).

entries (imports) and exits (exports) of a given country, highlighting the international intermediaries, which import not only for consumption, but also for exportation purposes.

Figure 3 show that European countries play a major role as international commercial intermediaries that increase the reach of avocado destinations in the world, as is the case of ESP, NLD, FRA, DEU, and BEL, which exchange avocado with 78, 74, 62, 51 and 47 countries, respectively. GBR is a lesser commercial intermediary which imports from 36 countries and exports to 11, despite not producing avocados.

Although they are not large producers, they have an intense commercial activity in the global avocado market. They are key suppliers for the same European countries, importing mainly from American and African countries. This reflects their economic and logistical capacity to supply the European Union.

Figure 4 shows the role of ESP as one of the main distributors in Europe, resulting from its intense commercial activity as an intermediary. It imports from 28 countries (entry level) distributed in the 5 continents, including PER, MEX, NLD, Morocco (MAR), COL, Kenya (KEN), and CHL. It exports to 50 countries (exit level), particularly to NLD, with which it has an intense commercial interaction, since they supply avocados to each other. In Figure 4, the countries located to the left of ESP are its 28 suppliers, while those located to its right are its 50 destinations.

It seems to be a pattern that most exporters are low-income countries and that exports represent an important share of their GDP, while importing countries (especially commercial intermediaries) have a better economic development, as confirmed by their GDP.

Indicators of the global avocado network

Figure 5 shows a detailed view of the position and importance of different countries in the global avocado trade network. Network indicators provide valuable information about the structure and dynamics of this network, which helps to understand trade interactions between countries in the avocado market.

Therefore, although ESP has a higher exit level and is the main supplier of the European Union, the role of NLD as a major supplier stands out, as a result of its commercial relations with other countries that also play an important role in the avocado trade.

The PageRank indicator evaluates the importance of a country based on the quantity and quality of links it receives from other countries. On the one hand, NLD has the highest PageRank value (0.08) —*i.e.*, it receives a high number of links from other countries with high centrality in the network—, as a result of its role as an important distribution center

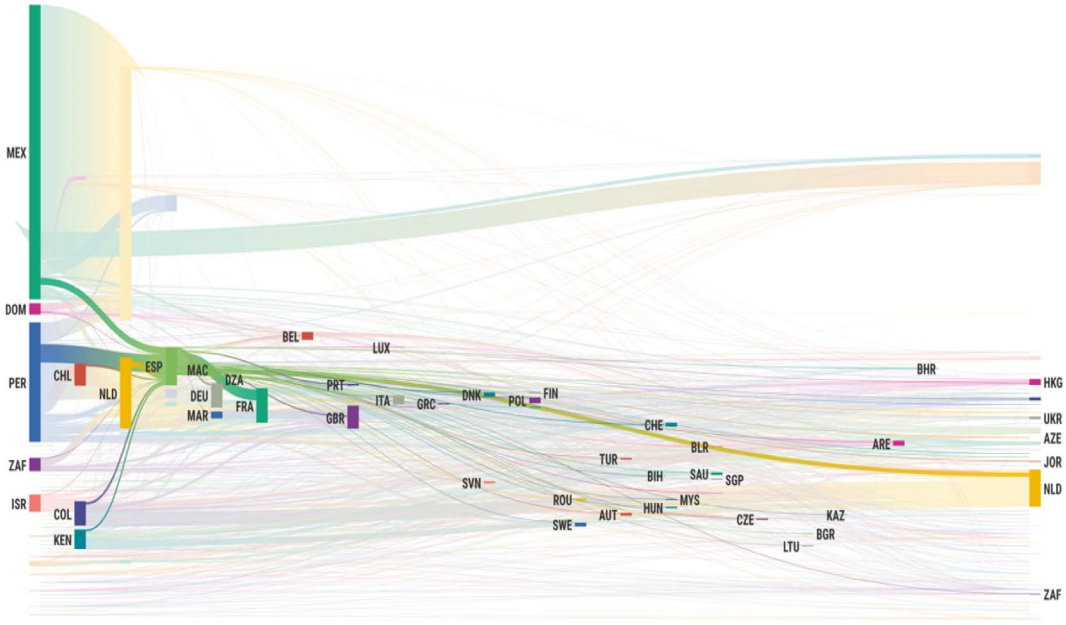

Figure 4. Spain as the main international intermediary in the avocado trade. Note: For more information, please consult the interactive graph: https://tinyurl.com/Sanky-Aguacate.

Country	Production (t)	Exports (t)	Imports (t)	Out-Degree	In-Degree	PageRank	Eigenvector
NLD	0	337,156	457,136	41	33	0.08	1.00
FRA	1,100	19,293	181,288	30	32	0.06	0.96
ESP	116,770	133,377	213,908	50	28	0.04	0.93
GBR	0	523	114,272	11	36	0.03	0.89
DEU	0	37,577	121,647	26	25	0.04	0.86
ITA	0	2,542	41,654	24	21	0.03	0.73
BEL	0	15,980	39,277	23	24	0.02	0.59
ARE	0	2,814	23,667	9	31	0.02	0.42
USA	136,750	8,817	1,213,412	23	5	0.01	0.01
COL	979,618	116,796	395	32	1	0.00	0.00
KEN	416,803	94,016	143	43	1	0.00	0.00
DOM	634,368	55,735	0	20	0	0.00	0.00
IDN	669,260	210	0	3	0	0.00	0.00
PER	777,096	569,458	0	45	0	0.00	0.00
MEX	2,442,945	1,405,117	0	44	0	0.00	0.00

Figure 5. Top 5 leading countries in avocado production, exports, imports, and network indicators (2021).

in Europe. On the other hand, MEX, the largest producer and exporter of avocado, nevertheless has a low PageRank value, which suggests that its position in the network is not based solely on the volume of its avocado production and exportation. This situation is explained by the proximity of MEX to the USA, to which it exports 77% of its production.

The Eigenvector indicator evaluates the importance of a country, taking into consideration the importance of the countries with which it is connected. NLD (1.0) is the most important country in the Global Avocado Network, followed by FRA (0.96) and ESP (0.93). These values indicate their connections to important countries on the network, which are related to their role as important avocado exporters. MEX, despite its high production, has a relatively low Eigenvector value, which suggests that its importance in the network may be influenced by other factors beyond its avocado production, such as its export concentration focused on the USA.

CONCLUSIONS

The analysis of the structure of the global avocado trade network has provided a detailed understanding of the dynamics of commercial interaction between the leading countries in this market. Network indicators (*e.g.*, exit level, entry level, PageRank, and Eigenvector) have revealed crucial aspects about the centrality and importance of countries in this network. Countries like NLD and ESP stand out for the diversification of both their import sources and export destinations, suggesting a robust trade strategy.

MEX and the USA are the most important countries in the global avocado trade network, in relation to their exchanged volumes. However, based on their connectivity, the most important countries are found in Europe: NLD, FRA, and ESP.

Meanwhile, the importance of Mexico in the network, despite being the largest producer and exporter of avocado, goes beyond the quantity it produces.

This study lays the foundation for future research about seasonal supply, productivity, and price fluctuations in different destinations, as well as for the identification of the attractive growth rate of its imports.

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The effect of the *Stevia rebaudiana* Morita II on the warts caused by fowl pox in *Meleagris gallopavo* as model study

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ABSTRACT
Objective: to evaluate the effect of leaves of *Stevia rebaudiana* Morita II on swelling caused by fowl pox in backyard turkeys of the *Meleagris gallopavo* species.
Design/methodology/approach: Different percentages of ground stevia leaves (5.5, 15, 20 and 25%) combined with ground corn were evaluated and the reduction of avian pox in turkeys was evaluated.
Results: The diet with 5% stevia leaves with corn was the most accepted combination by the turkeys; it also leads to immunomodulation to eliminate avian pox in a period of 30 days.
Limitations on study/implications: One of the main limitations is the production of stevia matter, however, due to the required amount, it has the possibility of being applied in the feeding of these animals, this study has applications in animal health and food safety in turkeys.
Findings/conclusions: The use of stevia in combination with corn could represent a natural alternative for the treatment of fowl pox and be profitable enough to prevent the death of turkeys.

Keywords: fowl pox, turkey, *Stevia rebaudiana*, morita II.

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INTRODUCTION

Backyard breeding is a common activity in indigenous communities worldwide. In Mexico rural communities, this activity is mainly focused on the production of poultry and pigs, although cattle, sheep, goats and rabbits also be found (Elkashef *et al.*, 2016; González Ortiz *et al.*, 2014; Nelson, 1998). Animals breeding in this fashion is mainly focused on feeding them with products and by-products obtained from the same site, milpa and kitchen waste. No veterinary intervention is common in backyard breeding, only including cultural practices as feeding with citric products and medicinal herbs (Nelson, 1998).

Domestication of the turkey (*Meleagris gallopavo* Linn) by the Mesoamerican civilizations allowed the development of an incipient poultry farming before the arrival of European civilizations. Currently, backyard turkeys are raised by women, who use broken corn, soaked tortilla and vegetable waste to feed them. For families dedicated to raising this species, it serves mainly as a source of food (García-Flores & Guzmán-Gómez, 2016; González Ortiz *et al.*, 2014).

The breeding of *Meleagris gallopavo*, in the center and south of Yucatan is carried out in 86% by country Mayan women who manage a backyard production system, and who also feed their turkeys with commercial feed; whole corn and kitchen waste. It is known that corn kernels and their nixtamalization are used to feed backyard turkeys in some communities in southern Yucatán (Domínguez May *et al.*, 2021). For producers, this activity generates an additional income (Canul *et al.*, 2011). However, backyard turkeys are frequently sickened by fowl pox, and most producers do not use pharmacological treatments to control this disease (Canul et al., 2011).

Fowl pox is a disease caused by the Avipoxvirus virus, it infects several species of wild and domestic birds worldwide. This virus causes respiratory problems and lesions in the integumentary system of birds. Fowl pox dry form produces skin warts or scabs (mainly on un-feathered parts of the body). The diphtheritic form shows cankers in the mouth, oesophagus or trachea. Other symptoms include blindness, feed refusal, lowered egg production, facial swelling (Umar *et al.*, 2021). Although worldwide the economic losses of commercial birds have been reduced by immunization programs against the virus, fowl pox continues to prevail on the planet (Bertalmio *et al.*, 2017).

Medicinal plants are a source of bioactive compounds used to treat diseases all around the world (Fallah Huseini *et al.*, 2006; Rafieian-Kopaei *et al.*, 2013). *Stevia rebaudiana* Bertoni is considered one of the species that provides suitable sweeteners since they do not generate calories (Haida & Hakiman, 2020). Moreover, it is a very interesting medicinal plant because it is used in the treatment of various diseases such as diabetes, hypertension, inflammation and cancer. In a study carried out in rats, it was shown that steviosides from *S. rebaudiana* improved the response of the immune system, increasing phagocytic activity and proliferation of B cells and T cells (Sehar *et al.*, 2008). Furthermore, a study in broiler chickens showed a positive effect of *S. rebaudiana* based sweetener included in bird diet on body weight gain and immune response to New castle virus vaccination (Molina *et al.*, 2021). Therefore, the use of *S. rebaudiana* as a food supplement could be of great help in the treatment of smallpox in backyard turkeys of rural communities.

The objective in this research was to evaluate the effect of leaves of *Stevia rebaudiana* Morita II on swelling caused by fowl pox in backyard turkeys of the *Meleagris gallopavo* species.

MATERIAL AND METHODS

Survey of people who domesticate the species *Meleagris gallopavo*

We applied a closed-response, multiple-choice survey with 12 items applied to 42 inhabitants of the communities of Cankab and Xohuayan, in Southern Yucatan. Survey was applied to families that raise backyard turkeys of the *Meleagris gallopavo* species.

The survey was divided into two sections: socioeconomic aspects and poultry raising practices. For the application of the surveys, home visits were made, collecting the opinion of the inhabitants verbally. Due to the high proportion of Mayan-speaking inhabitants, a Spanish-Mayan translator was required for adequate communication with the respondents.

The data collected were processed in the IBM SPSS® statistical package using a descriptive approach to identify the frequencies and percentages of responses.

Preparation of the materials

S. rebaudiana Morita II plants were grown in a greenhouse of Instituto Tecnologico Superior del Sur de Yucatan (ITSSY), in the community of Oxcutzcab, Yucatan, Mexico. Leaves from three-month old plants were sun dried and ground with a blender. *Zea mays* grains were obtained from local market and ground with a manual grain mill. Ground material were storage in sealed bags until use (Figure 1). Corn and stevia mixtures were prepared in different proportions, as described in Table 1. The feed mix was offered on demand to backyard-raised adult turkeys. The acceptance of the food was evaluated every day by the owner.

Figure 1. Feed generated with *Stevia rebaudiana* Morita II and *Zea mays*. A) Three-month old stevia plants. B) Dried leaves of stevia plants. C) *Zea mays* grains (corn). D) Mixture of ground Morita II leaves and ground corn kernels.

Table 1. Evaluated percentages of ground leaves of *Stevia rebaudiana* Morita II.

Ingredients of the food product	Serving (%)			
	1st test	2nd test	3rd test	4th test
Ground Stevia	25	20	15	5.5
Ground corn	75	80	85	94.5

Evaluation of fowl pox symptoms in turkeys feed with modified diets

In total 30 turkeys were evaluated, 15 with fowl pox symptoms and 15 without symptoms. Presence of skin warts and scabs (characteristic symptoms of fowl pox) was evaluated every day after the start of treatment. Control turkeys fed only grounded corn were set.

RESULTS AND DISCUSSION

Of the 42 people surveyed, 13 are from the Cankab community, and 29 from Xohuayan. The majority are women with 61.9% (26) and 38.1% (16) are men, with 72.58% being married. The elementary education level is the majority with 42.8% (18), while only 19% (8) have studies at the primary school level.

All of surveyed inhabitants includes corn in growth and fattening diets for backyard-raised turkeys, either in the form of ground grains, dough or nixtamalized tortillas. Additionaly, 83.3% includes also commercial balanced food from CAMPIPAVO Brand and 16.7% add vegetable residues (Figure 2).

Effect of stevia-enriched diets on fowl pox symptoms in turkeys

Turkeys included in this study did not consume enriched diets with high proportions of stevia leaves (Table 2). Only a poor acceptance was observed with 15% stevia leaves, while the food with 6.5% stevia was consumed in a similar way to the control diets.

On the other hand, a change in the coloration of dermal warts was observed after the third day of consumption of diets with stevia. After 15 days, the warts began to decrease in size and fall off. After 30 days of consumption, no obvious symptoms of fowl pox were observed in all turkeys fed stevia and corn (Figure 3A-D).

Figure 2. raising backyard turkeys. A) Turkeys in a wire mesh cage without a roof. B) Turkeys in a cage with a roof. C) Turkeys before being fed. D) Girl feeding turkeys. E) Turkeys eating nixtamalized corn mixed with CAMPI brand (commercial food). F) Mix of nixtamalized corn and commercial food.

Table 2. Consumption response of the ground leaves of Morita II in combination with ground corn.

25 g in 75 g of ground corn	–
20 g in 80 g of ground corn	–
15 g in 85 g of ground corn	+
5.5 g in 94.5 g of ground corn	+++

(–) Negative; (+) poor; (++) good; (+++) same as control.

Figure 3. Effect of *Stevia rebaudiana* Morita II against fowl pox. A) Color change of some bumps on the third day; B) Change of color to brown of most of the bumps, approximately after two weeks; C) Turkey eating the food product in the fourth week of treatment. D) Total removal of bumps on the turkey's skin, after 30 days of treatment.

The results suggest that *Stevia rebaudiana* Morita II leaves could have immunomodulatory activity, as observed by Sehar and coworkers (2008) on mice fed stevioside from *S. rebaudiana* Bertoni (Sehar *et al.*, 2008) In the same way, *S. rebaudiana* based sweetener included in broiler chicken diets showed an increase on body weight gain and immune response to New castle virus vaccination (Molina *et al.*, 2021).

A more detailed study would require several biometric tests on the turkeys before and after eating, in order to see the levels of leukocytes in the blood, which indicate the presence of an infection (López Farré & Macaya Miguel, 2009; Salvador-Reyes *et al.*, 2014). With this experiment, the antimicrobial and antiviral properties of the *S. rebaudiana* Morita II leaves could be demonstrated.

CONCLUSIONS

For the turkeys could consume the product derived from the combination of *Stevia rebaudiana* Morita II with corn, the maximum percentage of ground leaves had to be 5.5%, this is due to the degree of sweetness of the Morita II leaves. The sick turkeys that consumed this mixture had positive effects, their warts were dried and reduced, and after 30 days of treatment scabs disappeared.

The consumption of *Stevia rebaudiana* Morita II as a food supplement could be a good alternative in the treatment of fowl pox. The high cost of synthetic drugs for the treatment of fowl pox has caused many poultry farmers of the Cankab and Xohuayán communities to be affected in the raising of turkeys of the species *Meleagris gallopavo*. Turkeys that are infected with bird flu often die because poultry farmers do not have enough financial resources to pay for the costs of the medicines or because they do not know natural remedies. The use of this plant as a natural medicine could be a good option, since its production is economic; therefore, it may be a very promising alternative to improve the response of the immune system of *Meleagris gallopavo* against the fowl pox. With this ecologic alternative, losses of specimens of this species could be avoided.

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Colegio de Postgraduados

State of poeciliid fishes in the international market

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ABSTRACT
Objective: To make a review about the knowledge of the fish of the family poecilidade in Mexico in the main areas of knowledge.
Design/methodology/approach: The present review was carried out through the search and bibliographic compilation of literature, as well as in the consultation of articles in different databases, for its subsequent analysis.
Results: Poeciliid fish are the most widespread group of ornamental fish internationally, they are found in all international markets, leading productions for aquarium purposes, and therefore have great economic importance within the trade of aquatic organisms for commercial purposes. Ornamental.
Limitations on study/implications: The Poeciliids are a well-known group of fish, however information on marketing and specific sales data is limited.
Findings/conclusions: The most sold fish is the Guppy (*P. reticulata*), followed by mollies, platies and swordtails, which constitute the main internationally traded poeciliids.

Keywords: Ornamental fish, pet trade, exotic species, aquarism.

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INTRODUCTION

Currently, the ornamental fish trade has grown considerably, due to its great acceptance and good development prospects (Lango-Reynoso *et al.*, 2012; Evers *et al.*, 2019) being one of the markets with the highest profits worldwide, which reach 30 billion dollars annually, Within the international field of aquarism, aquarium hobby has had an annual growth of 14% since the 70's and in this area, freshwater species, mainly fish, are those that have the greatest demand, since total market represent 90%, in contrast to 10% of marine species (Evers *et al.*, 2019; Ruíz, 2022). Among the most commercialized freshwater species are poeciliid fish, which are small fish with a maximum length of 200 mm, belonging to the Poeciliidae family, characterized by presenting lecithotrophic viviparity and their males

have a gonopodium. Within the aquarium hobby, they are highly valued fish for their variety of shapes and striking colors, as well as for being highly fertile, which makes them quite attractive (Miller *et al.*, 2009; Hedrick & Hurt, 2012; Gavriloaie *et al.*, 2016).

MATERIALS AND METHOD

The selection of bibliographic material was carried out by searching different online databases and scientific search engines, such as: Elsevier-Scopus, SCIELO and Google academic, data from the FAO and official pages were also consulted as well as digital repositories from different universities and book chapters.

RESULTS AND DISCUSSION

Below are the main marketing markets for poecilids and their situation worldwide:

Asian market

Asia is the region with the largest production and marketing of ornamental fish in the world. The countries that lead the trade in ornamental fish and especially poecilids are: Singapore, Thailand, China, Indonesia and Sri Lanka (Monticini, 2010; Hernández-López & Luna-Vivaldo, 2021; Kabir & Hawkeswood, 2021). The most traded species in these countries are: gupies (*Poecilia reticulata*), swordtails (*Xiphophorus helleri*), platys (*Xiphophorus maculatus*) and mollies (*Poecilia* sp.) (Wijesekara & Yakupitiyage, 2001; Arévalo-Rivera *et al.*, 2010; Evers *et al.*, 2019; Kabir & Hawkeswood, 2021). Within these countries, Singapore stands out, as it is the largest exporter of poecilids worldwide, distributing this group of fish to more than 60 countries. Within its total production of ornamental fish species, poecilids represent 30%, among which stand out: los Platys (*X. maculatus*), mollies (*Poecilia* sp.) y los guppies (*P. reticulata*); However, when the total production is analyzed in terms of specimens and not species, it is clear that of the total number of specimens produced and distributed around the world, guppies alone represent more than 80% of the total number of specimens sold (Monticini, 2010; Kabir & Hawkeswood, 2021), Another of the countries in which there is a specific record of this group of ornamental fish is Sri Lanka, a country in which the majority of fish exported are poeciliids: Mollies, platys, swordtails and guppies, together constituting more than 80% of the total fish exported, of which guppies (*P. reticulata*) stand out as they represent 65% of its international sales (Wijesekara & Yakupitiyage, 2001), Within the Asian region, not only do poecilids stand out in terms of export, they also happen to be the most demanded group of fish in local Asian markets, where poecilids represent around 49% of their sales and in the case of Japan only gupies represent 28% of imported fish (Monticini, 2010; Dey, 2016).

American market

The poeciliid fish market in America is mainly made up of the United States and Mexico. In the United States, its trade is dominated by a few species, highlighting the guppies (*P. reticulata*) and the swordtails (*Xiphophorus* sp.) (Tamaru *et al.*, 2001; Evers *et al.*, 2019). These fishes have had crossbreed between congeners over the years to accentuate colors and shapes, so in many cases they are not pure species (Pãsãrin & Petrescu-Mag, 2011), within

this market, poecilids are a group of fish that stand out for their high demand to the extent that *P. reticulata*, *X. maculatus*, *X. helleri* and *Poecilia* sp., are known as the "group of four", since they are the four fish that are sold the most in all of America and that are easiest to find at points of sale (Hellweg, 2016; Evers *et al.*, 2019). Mexico is a country that, in terms of ornamental fish production, is mainly a producer and exporter of poecilids, with its neighboring country, the United States, being the main destination, In Mexico, the most produced ornamental fish is the Gupy, followed by *P. sphenops*, *X. helleri* and *X. maculatus*, which although they are native to Mexico, are cultivated in their ornamental varieties (Devezé *et al.*, 2004; Maya *et al.*, 2007; Scotto, 2020) Although there are fish with higher prices in the market, poecilids have a greater margin in terms of profits because they do not need very specialized infrastructure for their production unlike other fish, which is why more than 52% of farms producing ornamental fish in Mexico raise poecilids, of which gupies along with carp (*Carassius auratus*) represent 88% of the total fish sold in this country (Espinosa *et al.*, 2011; Ruíz, 2022).

European market

Within Europe, poeciliid fish have great relevance in terms of their marketing. In this market, the best-selling fish is the guppy (*P. reticulata*), which together with the neon tetra (*Paracheidoron inessi*) make up 25% of total ornamental fish sales; therefore, they have great economic importance. In European countries such as Spain and Romania, poecilids are of great commercial importance and are countries that stand out for their cultivation. The Czech Republic is a country that has recently been on the rise in the production of gupies, mainly due to its proximity to other European countries (Monticini, 2010; Vivas, 2019; Novák *et al.*, 2020), while other European countries such as the United Kingdom, France, the Netherlands and Germany are mainly importing countries of poecilids (Novák *et al.*, 2020).

In the European market, Germany stands out, because unlike other international markets, it is characterized by the fact that it is the largest importer of poeciliid species in the world, which is why they are importers of species that are normally not so requested in other countries such as: *Poecilia vivípara*, *Gambusia holbrooki*, *Phalloceros caudimaculatus*, *Cnesterodon carnegidi*, *Poecilia sphenops*, *Girardinus uninotatus*, *Gambusia affinis*, *Cenesterodon decenmaculatus*, *Poecilia latipina*, *Poecilia branneri*, *Gambusia nicaraguensis*, *Girardinus metalllicus*, *Phallichthys amates*, *Poecilia caucana*, *Phalloptychus januarius*, *Poecilia mexicana*, *Poecilia vittata*, *X. maculatus*, *X. helleri*, *Poecilia dominicensis*, *Poecilia melanogaster*, *P. reticulata* and *Belonesox belizanus*). Therefore, Germany can be considered the importing country of the greatest diversity of poecilid species internationally (Evers *et al.*, 2019; Novák *et al.*, 2020).

Although there is no specific record of the imports of these species, mainly of their origin and specific quantities, it is suspected that they come from regions of America, in which poecilids are native and in many cases extracted from their natural habitat, without a well-regulated trade in ornamental fish, whose main destination has been traced to the European market (Hignette, 2003; Vaca & Quirce, 2005; Mancera & Reyes, 2008; Zúñiga, 2010).

Other markets

India

The Indian market for ornamental fish has been growing significantly in recent years. Although this region is rich in diversity of freshwater species, its trade focuses on non-native species (Pandey & Mandal, 2017), and poecilids dominate the market due to their high demand, mainly: *P. reticulata*, *Poecilia* sp., *X. variatus* and *X. helleri*, which have higher sales in volume compared to other species (Raja *et al.*, 2014). Although more native species are beginning to be cultivated, the Indian market has grown mainly because some countries cannot meet the demand for poecilids that they have, Thus, India's main export destination for poecilids is Singapore and the United States (Abalika *et al.*, 2003; Monticini, 2010; Raja *et al.*, 2019).

CONCLUSIONS

Poecilids are a group of fish of utmost importance in international trade, they constitute most sales of freshwater ornamental fish, highlighting the guppy fish (*P. reticulata*) in first place, followed by mollies, platys and sword tails. Nowadays, more and more countries actively participate in the trade of these species that, although they may seem to have little unit value, in volume they become the species with the greatest demand in the international aquarium market, Therefore, poecilids are a group of highly profitable fish to market.

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Acute toxicity induced by treated sewage to zebrafish (*Danio rerio*)

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ABSTRACT

Objective: Acute toxicity markers in zebrafish caused by exposure to treated sewage from two sewage treatment plants were evaluated in order to obtain accurate and relevant information on the potential environmental health risks associated with these waters.

Design/methodology/approach: The acute toxicity assessment was performed according to the guidelines proposed by the Organization for Economic Cooperation and Development (OECD). Five proportions of each type of water treated with dechlorinated tap water (20, 40, 60, 60, 80 and 100%) were tested to determine the Median Lethal Dilution ($LDil_{50}$). The dechlorinated tap water was the negative control and as a positive control potassium dichromate at Mean Lethal Concentration (0.065 mg/L). The description of the teratogenic effects was carried out using the spine biomarker, which consists of dividing the fish into three sections to identify the damaged area.

Results: The results revealed that the mixture of treated sewage (mCl) and treated sewage disinfected with sodium hypochlorite (NaClO) caused toxic effects in zebrafish embryos. On the other hand, treated sewage from the Macroplanta de Bahía de Banderas (BBdClO) was not toxic at 96 hours of exposure.

Limitations on study/implications: It would be advisable to characterize the treated sewage from these two sewage treatment plants.

Findings/conclusions: These findings underscore the importance of assessing the toxicity of treated sewage and its short- and long-term effects on aquatic organisms, providing valuable information for water resource management and environmental protection.

Keywords: *Danio rerio*, toxicity, treated sewage.

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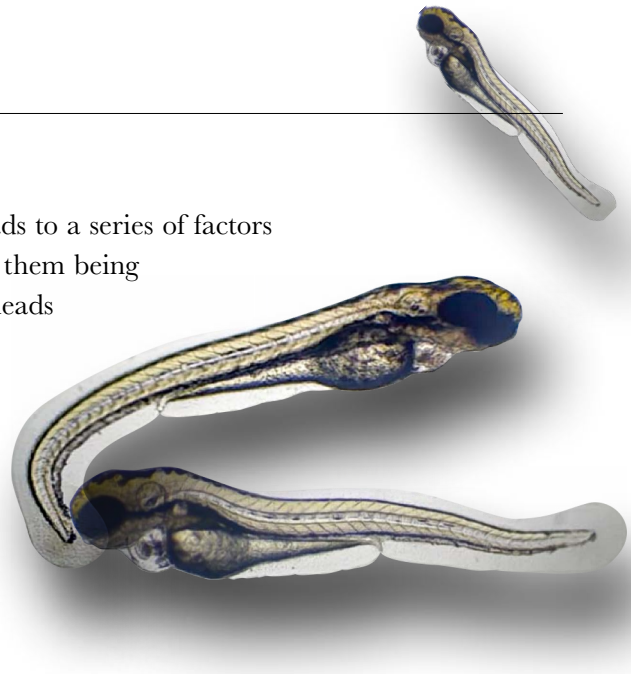
INTRODUCTION

The increase in human population leads to a series of factors that contribute to water pollution, one of them being the excessive use of drinking water that leads to the generation of sewage that ends up in treatment plants and is subsequently discharged into the environment (Sayo *et al.*, 2020). Sewage treatment plants (WWTPs) were designed to treat sewage from urbanization;



however, they do not completely remove all pollutants (Papa *et al.*, 2016). The removal efficiency of these pollutants is going to depend on each type of treatment, even so, the removal efficiency of the treatments is minimal, due to the fact that the WWTPs are not well equipped (Deblonde *et al.*, 2011; Zhou *et al.*, 2019).

Treated sewage contains two major groups of pollutants, called traditional pollutants and emerging pollutants (Feng *et al.*, 2022). Traditional pollutants are those whose toxic effects are known and studied; within this group we find heavy metals with toxicity potential. Emerging pollutants are those whose presence is recent in the environment and their toxic effects are not fully known; for example, pharmaceuticals and personal hygiene products, surfactants, pesticides, among others (Ahmed *et al.*, 2017; Von Sperling, 2007; Zhou *et al.*, 2019). Most of these xenobiotics and their active metabolites can cause adverse effects on aquatic organisms, a part of these compounds accumulates in aquatic tissues and pose a threat to both the ecosystem and humans (Babić *et al.*, 2017; Brar *et al.*, 2010). The persistence of these compounds in the environment depends largely on their chemical properties such as liposolubility, volatility, adsorption and low biodegradation, these characteristics also prevent sewage treatments from being more efficient for their removal (Robledo Zacarías *et al.*, 2017).

Moreover, treated sewage contains high concentrations of ammonium from human urine and fecal matter, and WWTPs use chlorine in the form of sodium hypochlorite and/or chlorine gas for water disinfection (Albolafio *et al.*, 2022). By-products are also generated from the reaction of chlorine with compounds found in water and are sometimes more toxic than the original compounds (Huang *et al.*, 2021).

Treated sewage has been shown to induce various toxic effects in zebrafish embryos (Garcia-Cambero *et al.*, 2019). Zebrafish is a very reliable model for toxicity assessment of treated sewage due to characteristics such as sensitivity, transparent embryonic development, well-characterized genetics, and its ability to represent the effects of toxicants found in aquatic systems (Nagel, 2002). The use of this model can provide information on potential risks to human health and the environment associated with treated sewage (Porretti *et al.*, 2022).

In Mexico, the composition of sewage discharges is regulated by the official Mexican standard NOM-001-SEMARNAT-2021, which establishes the permissible limits of pollutants in these discharges into receiving bodies owned by the nation. The treated sewage used in this study meets the requirements of the aforementioned standard. It is important to point out that the main economic activity in Puerto Vallarta is tourism, while manufacturing and transformation industries are absent. Therefore, there are no heavy metals, cyanide, arsenic and hexavalent chromium in the sewage (Ponce-Palomera *et al.*, 2022).

This work represents one of the first efforts in evaluating the toxicity of treated sewage in the region, markers of acute toxicity in zebrafish caused by exposure to treated sewage from two WWTPs were evaluated in order to obtain accurate and relevant information on the potential environmental health risks associated with treated sewage, which in turn guides decision-making in water resource management and environmental protection.

MATERIALS AND METHODS

Source of secondary treated sewage

The treated sewage was obtained from the Norte-II sewage treatment plant in Puerto Vallarta, Jalisco, and from the Macroplanta in Bahía de Banderas, Nayarit. The Norte-II has two operating units and one disinfects sewage with sodium hypochlorite (NaClO) and the other with gaseous chlorine (dCl_2), and a mixture (mCl) of both with a ratio of 30% and 70%, respectively, is discharged into the Ameca River. Macroplanta, it only disinfects the water with sodium hypochlorite (BBdClO). Spot sampling was conducted in August 2022 for mCl, April 2023 for NaClO and May 2023 for BBdClO. For toxicity tests, free chlorine was measured with a specific multiparameter ion meter (Hanna HI 83200®) and removed with a 4% sterile sodium thiosulfate solution to avoid chlorine toxicity.

Cultivation and collection of embryos

To obtain embryos, nine females and six males (3:2 ratio) were separated for one week. One night before spawning, they were placed in an oviposition tank to avoid cannibalism towards the embryos. Spawning occurred at dawn and embryos were collected at one hour post fertilization (hpf) with a glass siphon and rinsed with Hank's solution (Westerfield, 2007). Using a stereo transmission microscope, embryos were selected from one hpf of development and unfertilized eggs were separated.

Toxicity test

The evaluation of acute toxicity was performed according to the guidelines proposed by the OECD (Organization for Economic Cooperation and Development) in section #236 entitled: Fish Embryo Acute Toxicity (FET) Test. Plastic well plates were replaced by glass Petri dishes to avoid adsorption to polystyrene (OECD, 2013). Three types of treated water were tested: NaClO, mCl and BBdClO. To determine the Median Lethal Dilution ($LDil_{50}$), five proportions of each type of treated water were analyzed with dechlorinated tap water (20, 40, 60, 60, 80 and 100%). The dechlorinated tap water was the negative control and as a positive control 3,4-dichloroaniline was substituted with potassium dichromate at the Median Lethal Concentration (0.065 mg/L). Ten milliliters of each proportion of treated sewage and each control were placed in triplicate in Petri dishes containing twenty embryos each. Mortality was recorded every twelve hours for 96 hours and was defined as embryo coagulation or loss of heartbeat. The test was carried out in an incubator at 28 °C.

The description of teratogenic effects was performed using the spine biomarker, which consists of dividing the fish into three sections to identify the damaged area (Castillo-Salas *et al.*, 2022; Gaytán-Oyarzún *et al.*, 2008; Olivares *et al.*, 2021). This description was only performed for mCl, 150 embryos were placed in Petri dishes in triplicate and the $LDil_{50}$ (88.13%) was added, observed at 72 hpf using a stereoscopic microscope and malformations were recorded.

Data analysis

The $LDil_{50}$ and its standard error were determined through the calculation of the Median Effective Dose (ED_{50}) described by Miller & Tainter, (1944). Pearson

correlation analyses were performed to evaluate the relationship between $LDil_{50}$ and hours of exposure.

RESULTS

$K_2Cr_2O_7$ showed toxicity from 12 hours of exposure and the LC_{50} decreased over time. The LC_{50} of $K_2Cr_2O_7$ showed a negative logarithmic relationship with exposure time $R^2=0.994$, $p<0.05$ (Figure 1).

$LDil_{50}$ of mCl and NaClO were calculated, and $LDil_{50}$ at 96 hours were 88.13±9.55% and 36±3%, respectively. The $LDil_{50}$ data show an exponential relationship with exposure time $R^2=0.8948$, $p<0.05$ (Figure 2) and $R^2=0.950$, $p<0.05$ (Figure 3). On the other hand, BBdClO did not show toxicity at 96 h of exposure.

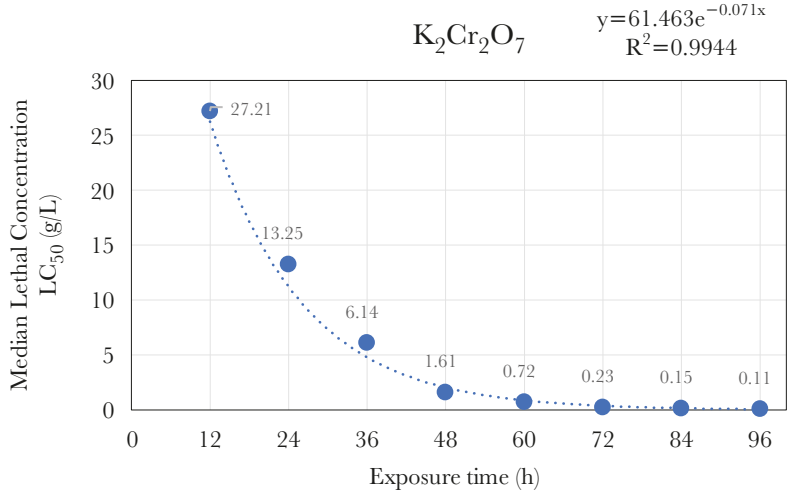


Figure 1. Median lethal concentration of potassium dichromate ($K_2Cr_2O_7$) for zebrafish embryos in relation to exposure time.

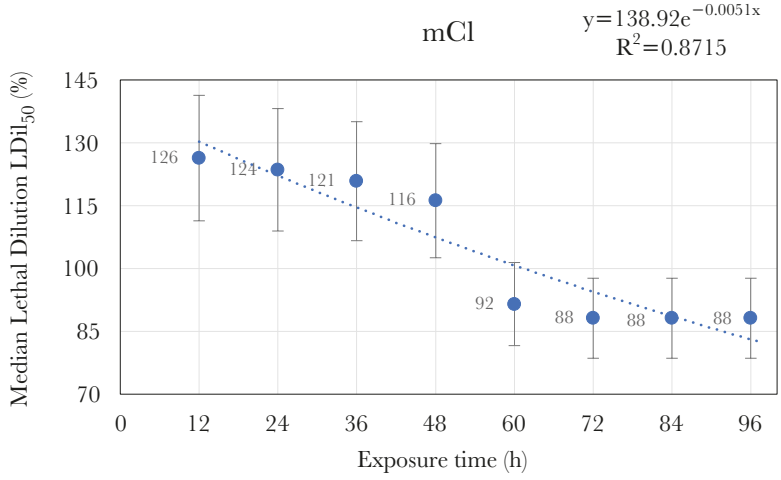


Figure 2. Median lethal dilution of mCl for zebrafish embryos in relation to exposure time. mCl: mixture of treated sewage disinfected with chlorine gas and sodium hypochlorite.

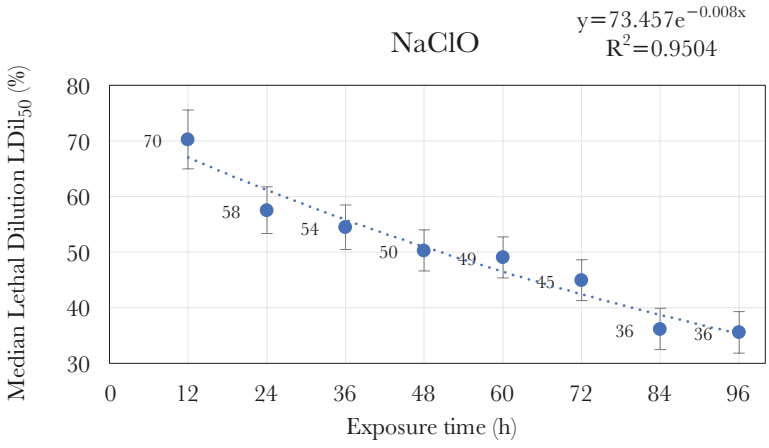


Figure 3. Median lethal dilution of NaClO for zebrafish embryos in relation to exposure time. NaClO: treated sewage disinfected with sodium hypochlorite.

The proportion of malformations was calculated (Figure 4) taking into account the spine biomarker. The results showed early and late malformations, as well as cardiac alterations at 72 hpf. For this, only $LDil_{50}$ 88.13% of mCl was taken and a very low proportion of these events was observed; total malformations represented 12 %. Only one type of early malformations was observed, which was the hook malformation and represented a proportion of less than 3%. As for late malformations, all those proposed by Sánchez-Olivares *et al.* (2021) were found, the most frequent being caudal fin malformation and double malformation, which represented 2 and 1.56%, respectively. Regarding cardiac alterations, a higher proportion of pericardial edema was found with 3.78%, which was the most prevalent effect and no yolk sac edema was observed. The control group had a normal development.

On the other hand, embryos exposed to half of the $LDil_{50}$ (22.5%) of NaClO showed low proportions of malformations. As for early malformations, only hook malformation was found with less than 1%. Late malformations were simple and curve malformations

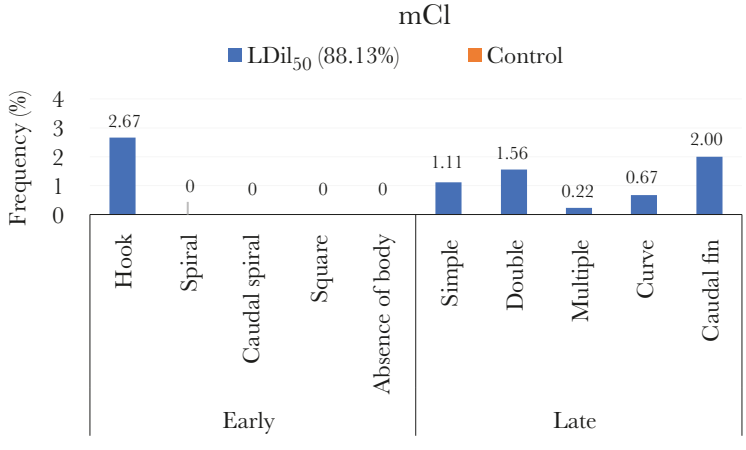


Figure 4. Frequency of malformations at 72 hours post-fertilization after exposure to the treated water mixture (mCl).

representing 3.33% and 2.2% respectively. As for cardiac alterations, they were found in a higher proportion compared to mCl. It is worth mentioning that, although it is not a malformation, almost half of the exposed embryos did not hatch at 72 hours. Total malformations represented 12.5%. The control group had normal development. Finally, 100% BBdClO did not present malformations in zebrafish development at 72 hours of exposure.

Photographs were taken of the malformations of zebrafish embryos exposed to mCl. Figure 5 shows the early malformations and cardiac alterations. Hook malformation (Figure 5A) which was the only early malformation observed, normal embryo development (Figure 5B) and pericardial edema (Figure 5C) consisting of water entering the pericardial zone.

Figure 6 shows the late malformations, the single malformation (Figure 6A) consists of a single malformation of the spine in the cephalic area, double malformation (Figure 6B) with two malformations of the spine in the cephalic and central area, and multiple malformation (Figure 6C) consisting of several malformations of the spine in the three divisions of the fry's body. Curved malformation (Figure 6D), the fry's body turns to one side or the other preventing correct swimming, and finally caudal fin malformation (Figure 6E), in which the caudal fin is affected and bends upwards, downwards or to the sides.

DISCUSSION

For this study, the Median lethal concentration (LC_{50}) of potassium dichromate ($K_2Cr_2O_7$) at 72 hours was taken for zebrafish as a positive control; the LC_{50} was obtained from the study by Sánchez-Olivares *et al.* (2021), which shows that 0.065 mg/L of $K_2Cr_2O_7$ causes the death of half of the exposed organisms. However, when used in this study, this concentration had no effect on the organisms. For this reason, the LC_{50} of $K_2Cr_2O_7$ was calculated and an LC_{50} of 234.08±168.32 mg/L at 72 hours was obtained. The OECD establishes that acute toxicity tests must be carried out up to 96 hours; for this reason, the LC_{50} was changed to 96 hours as a positive control and was 110.25±68.03 mg/L. On the

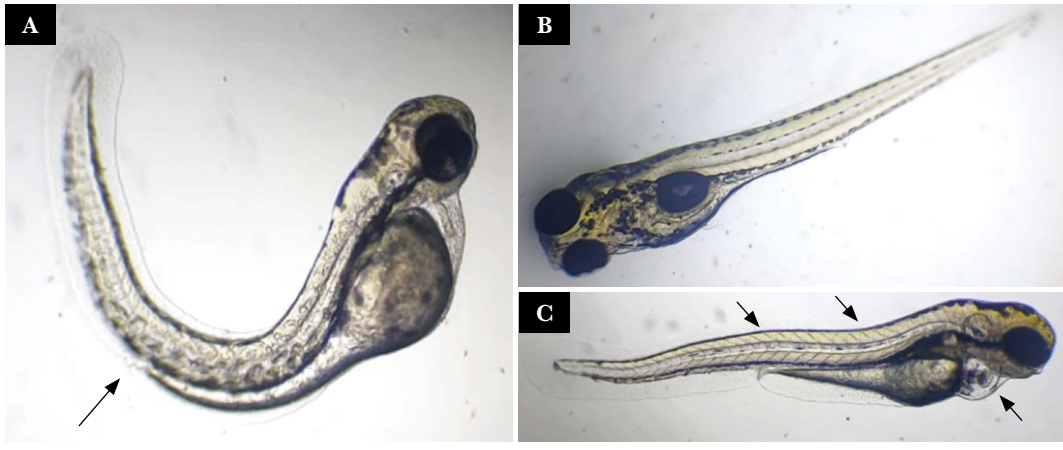


Figure 5. Early malformations and cardiac alterations in zebrafish embryos exposed to mCl. A) hook, B) control, C) pericardial edema.

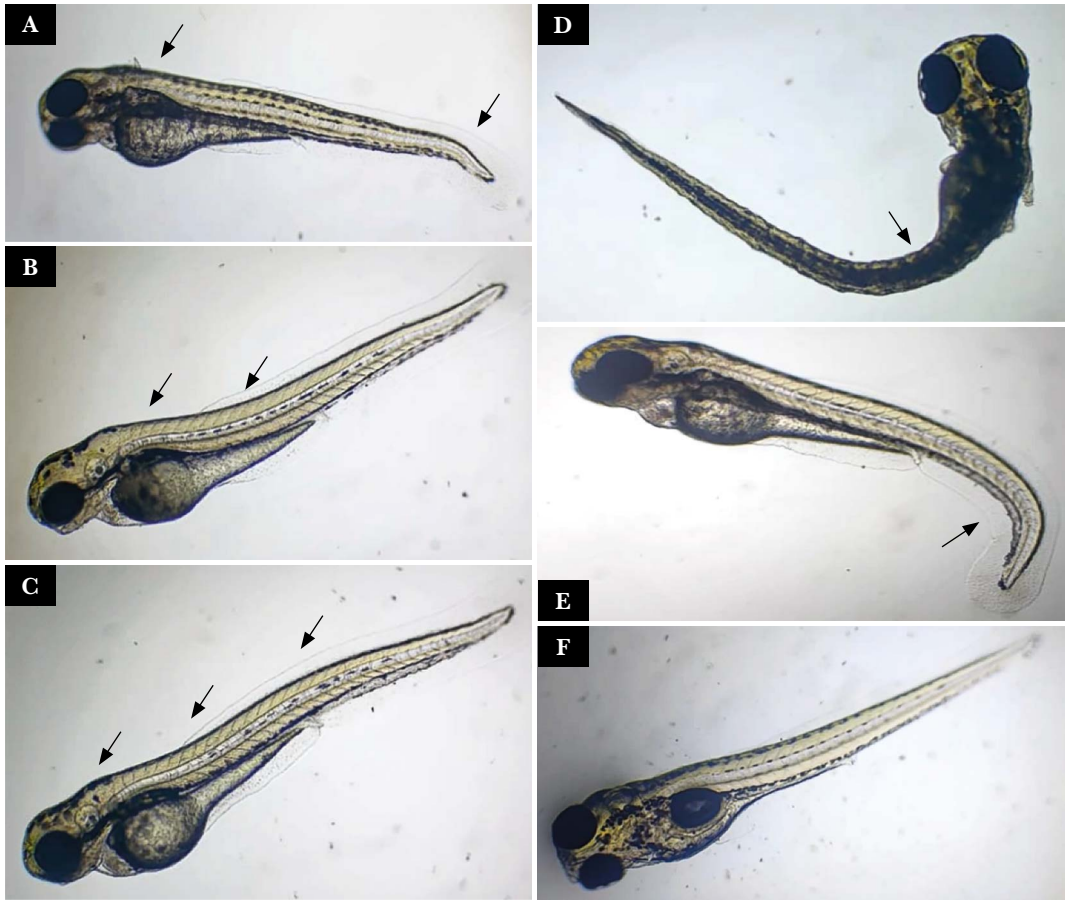


Figure 6. Late malformations in zebrafish embryos exposed to mCl. A) single, B) double, C) multiple, D) curve, E) caudal fin, F) control.

other hand, Menares Toro, (2017) obtains a LC_{50} of 651 mg/L of this same compound at 96 hours, which is almost six times higher than that obtained in this work. The author mentions that $K_2Cr_2O_7$ does not induce toxic effects on zebrafish at 48 hours of exposure, because the chorion, due to its selective permeability, protects the embryo from the entry of this compound and proposes to perform toxicity tests after hatching, however, in the present study there is mortality of organisms from 12 hours of exposure, suggesting the entry of $K_2Cr_2O_7$ into the embryo.

da Silva *et al.* (2021) obtains a LC_{50} of 32.5% in zebrafish embryos exposed to municipal treated sewage from Aguascalientes. Babić *et al.* (2017) demonstrates that mortality of embryos exposed to treated water starts after 24 hours and increases with exposure as occurs in this study. Guerrero-Jiménez *et al.* (2017) finds an annual LC_{50} of 60.71% in the rotifer *Lecane quadridentata* and does not find toxicity in the cladoceran *Dahpnia magna*, therefore, the toxicity of sewage is specific due to metabolic and physiological differences in each species. In this study BBdClO did not present acute toxicity to zebrafish embryos, so the toxicant removal process could be adequate for this WWTP.

Galus *et al.* (2013) found high percentages of deformity in embryos when exposed to treated urban sewage. These malformations are very common in zebrafish embryos

when exposed to various pollutants separately, such as, for example, hexavalent chromium (Olivares *et al.*, 2021), microplastics (De Marco *et al.*, 2022), pharmaceuticals (Pohl *et al.*, 2019), polycyclic aromatic hydrocarbons such as benzo(a)pyrene (Elfawy *et al.*, 2021), organophosphate pesticides (Pamanji *et al.*, 2016) and endocrine disrupting substances such as ethinylestradiol (Ramírez-Montero *et al.*, 2022). All these substances are found in significant concentrations in treated effluents and perhaps are the ones that give rise to these malformations. It has been shown that some of these substances such as benzo(a) pyrene and profenes delay hatching in zebrafish, probably some of these compounds are involved in the delay in hatching of embryos exposed to NaClO in the present work, but further studies would be needed to know which pollutant is the cause of this effect.

CONCLUSIONS

This study highlights the importance of analyzing the toxicity of treated sewage and its impact on aquatic organisms, as well as its relevance to human and ecosystem health. The results indicate that treated sewage cause acute adverse effects in zebrafish. These findings support the need for improved sewage treatment processes. It is of concern that toxicity was observed in the embryonic development of zebrafish, the malformations and cardiac alterations suggest that contaminants present in the water may interfere with critical processes in the development of aquatic organisms. On the other hand, constant monitoring of water quality in receiving bodies in order to know the impact of these treated sewages on aquatic organisms is required.

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Colegio de Postgraduados

Fungi diversity in roots of *Guarianthe skinneri* in urban condition: velamen *versus* cortex

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ABSTRACT

Objective: To Identify the endophytic fungal community of the *G. skinneri* root in urban populations, showing differences in composition between velamen and cortex to better understand the fungus-root interaction in these orchids.

Design/Methodology/Approach: We collected tissues from velamen and root from five specimens of *G. skinneri* growing on urban trees in Tapachula, Chiapas. We extracted DNA, PCR amplified the ITS marker, sequenced on the Illumina platform followed by diversity analyses and taxonomic assignment.

Results: We detected 845 OTUs that were assigned to the Fungi kingdom. Velamen and cortex share 403 OTUs, 402 were found exclusively in velamen and 40 in cortex. Besides orchid mycorrhiza forming fungi, we detected other species (*e.g Alternaria* sp., *Beauveria* sp., *Fusarium* sp., *Glomus* sp. and *Tricoderma* sp.) that could be involved in root physiology during development and defense against pathogens and predators.

Study Limitations/Implications: Metagenomic studies provide substantial amounts of data that go beyond conventional studies. However, the information generated is still limited regarding the role of each endophyte.

Findings/Conclusions: Despite these limitations, our work fills a knowledge gap because we detected endophytes that were previously unknown for *G. skinneri*, leading to new research questions about root-endophyte relationships.

Keywords: non-mycorrhizal endophytic fungi, orchid mycorrhiza fungi, metagenomics, NOM-059-2010.

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INTRODUCTION

Orchidaceae is one of the most species rich plant families, it includes around 31,000 to 35,000 species (Śliwiński *et al.*, 2022). Seventy percent of orchids are epiphytes (Emeterio-Lara *et al.*, 2021) and they are distributed in tropical and subtropical regions (Zarate-García *et al.*, 2020). There are approximately 1,250 orchid species in México; in Chiapas State inhabit 723 species (Martínez-Meléndez *et al.*, 2017), with 325 species found in the Soconusco region, representing 26% of the national orchid richness (Solano-Gómez *et al.*, 2016). *Guarianthe skinneri* (Bateman) Dressler & W.E. Higgins (Orchidaceae), is an epiphytic orchid distributed naturally from Southern Mexico to Panama (Bertolini *et al.*, 2016). It is used in religious celebrations and as an ornamental plant in Socunusco, Chiapas (Coutiño-Cortés *et al.*, 2018). Currently, *G. skinneri* is listed in the Mexican Norm for species protection (Norma Oficial Mexicana 059-2010; SEMARNAT, 2010), under the 'threatened' category due to strong anthropogenic pressure caused by illegal extraction and trade, as well as land use change and biodiversity loss.

Roots are an important feature for epiphytic orchids. Their anatomy is composed of several layers, they are, in order from the exterior to the interior: the velamen, parenchyma

(cortex), endodermis and vascular cylinder (Deepthi and Ray, 2018; Joca *et al.*, 2017; Moreira and Isaias, 2008). Roots serve for anchorage on the phorophyte. The velamen, a dead and spongy tissue, absorb water and nutrients, reduces water loss (Joca *et al.*, 2017; Zotz and Winkler, 2013), provides mechanical protection for the living cortex layers, protects from UV-B radiation and is an ecological niche for diverse microorganisms, including bacteria and endophytic fungi, that would colonize the cortex as mycorrhizal symbionts (Chomicki *et al.*, 2015; Bhargava *et al.*, 2019; Tedersoo *et al.*, 2020). Orchid seeds necessarily depend on orchid mycorrhizal fungi (OMF) for successful germination. OMF supplement the lack of nutrients in the seed endosperm and cotyledons (Bidartondo *et al.*, 2004; Hossain *et al.*, 2013; Tian *et al.*, 2021). OMF penetrate the seed parenchyma cells during germination, a stage known as protocorm, they also penetrate root tissues of seedlings and adult plants. Once OMF are inside the plant cells, intracellular packages of supercoiled hyphae (*i.e.* pelotons) are formed (Dearnaley *et al.*, 2016).

Symbiotic germination processes in orchids have been studied extensively (Alghamdi, 2019; Dearnaley, 2007; Izuddin *et al.*, 2019; López-Chávez *et al.*, 2016), showing that the *phylum* Basidiomycota form relationships with different epiphytic orchid species (Petrolli *et al.*, 2021; Rammitsu *et al.*, 2021). However, there is a knowledge gap regarding the velamen role as microorganism filter (Chomicki *et al.*, 2015; Joca *et al.*, 2017; Zotz and Winkler, 2013) and the fungal diversity that it harbors. Herrera *et al.* (2010) reported that members of the *phylum* Ascomycota colonize this structure. To better understand the ecological complexity and evolution of the mutualistic relationship between orchids and fungi, it is important to uncover the fungal diversity present in the velamen and cortex. Most of the endophytic fungi of orchids are not mycorrhizal and their function is not well studied. However, understanding its diversity, ecological and physiological roles could help in the conservation of the orchid family (Yuan *et al.*, 2009).

To generate a knowledge baseline regarding the adaptation of *G. skninneri* in an urban environment, we aim to determine the fungal diversity of roots in both velamen and cortex. Understanding biotic interactions with root endophytic fungi is essential for the conservation and ecological restauration of endangered species (Ortega-Larrocea and Rangel-Villafranco, 2007).

MATERIALS AND METHODS

Biological material collection

We selected three main roots from plants growing in the streets of Tapachula, Chiapas, Mexico in July 2021. Samples were transported in plastic bags in a cooler to the laboratory of Ecology and Sustainable culture of Orchids in El Soconusco in El Colegio de la Frontera Sur, Tapachula, for DNA extraction.

Sample processing

Roots were rinsed with sterile water to wash away dirt from the surface. Velamen was removed entirely with a scalpel. The cortex was cut into 0.5 cm fragments. Then, velamen and cortex fragments were transferred separately to 1.5 ml tubes for DNA extraction.

DNA extraction

DNA was extracted from the velamen and cortex fragments separately with the Soil Microbe DNA MiniPrepTM Kit (Zymo Research Cat. No. 6001). DNA quality was verified running a 1% agarose gel electrophoresis.

DNA sequencing and Bioinformatic analysis

Amplicon library preparation for the 18S-ITS1-5.8S-ITS2 region, Illumina 250 bp pair-end sequencing and bioinformatic analysis was outsourced to CD-GENOMICS (Shirley, NY_USA).

Raw reads were demultiplexed according to their corresponding index. Demultiplexed reads were trimmed to remove low quality nucleotides, index and primer sequences. Sequences were paired with the software FLASH (V1.2.7) to produce clean reads for analysis with QIIME (V1.9.1). Chimera sequences were removed by comparing sequences to the reference database using the UCHIME algorithm to obtain effective tags (Nilsson *et al.*, 2018). The resulting sequences were grouped at 97% similarity with the algorithm UCLUST in QUIIME (versión 1.8.0) to obtain Operational Taxonomic Units (OTUs). OTUs were then taxonomically annotated with the database UNITE. The FUNGuild platform was used to assign an ecological function to the taxonomically identified OTUs. A Venn diagram was drawn to know the number of shared OTUs between velamen and cortex.

Histograms were created to show the distribution of taxa at different taxonomic levels (phylum, class, order, family and genus). Three indices for alpha diversity were calculated: Shannon, Simpson, Chao 1 and ACE (*abundance coverage estimator*). To compare indices among samples, sequence number was standardized. A rarefaction plot was created to explore if sequencing was deep enough to comprehend diversity in cortex and velamen samples. Data were selected randomly from the cortex and velamen samples separately, then the number of species in each sub-sample was determined. A curve rank-abundance was plotted to show species relative abundance, richness and evenness. Finally, an accumulation curve was plotted to show a relationship between sample number and number of genera assigned.

RESULTS AND DISCUSSION

845 OTUs were taxonomically assigned with UNITE (Nilsson *et al.*, 2018). The Venn diagram shows that there are 403 OTUs shared between the cortex and velamen samples, 402 OTUs were found exclusively in velamen, whereas 40 OTUs were exclusive to cortex (Figure 1).

Most of the taxonimically assigned OTUs belonged to the *phylum* Ascomycota (Figura 2 y 3).

Among the OTUs shared between velamen and cortex, 3 phyla, 21 classes, 62 orders, 115 families, 169 genera and 198 species were assigned (Table 1a, supplementary material). Five phyla, 24 classes, 59 orders, 106 families, 168 genera and 187 species were among the OTUs exclusively found in velamen (Table 1b, supplementary material). In cortex, 2 phyla, 9 classes, 19 orders, 25 families, 29 genera and 29 species were identified (Table 1c, supplementary material).

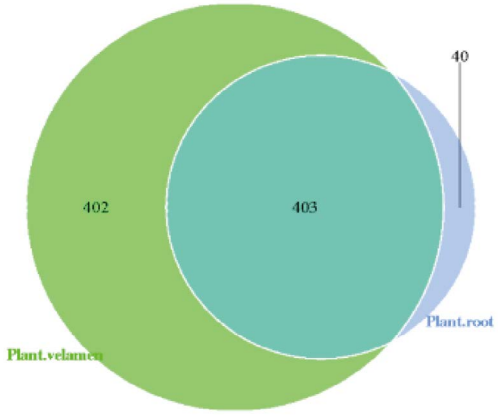

Figure 1. Venn diagram showing number of OTUs detected in velamen and cortex samples of *G. skinneri*.

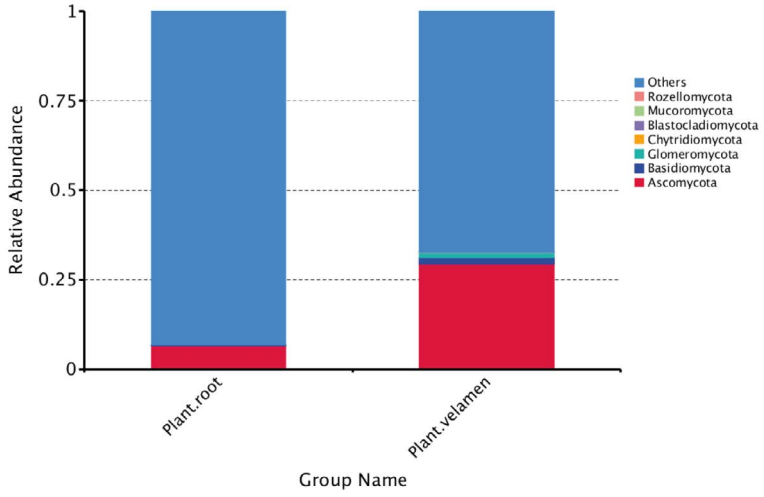

Figure 2. Relative abundance of phyla found in cortex and velamen. 'Others' includes sequences that could not be assigned to any phylum.

Ascomycota phylum shows higher abundance in both sample types. At the class level, Dothideomycetes is the most abundant in both samples, whereas the order Pleosporales is the most abundant in cortex samples (root) and Capnodiales in velamen. The families unidentified *Capnodiales* sp., and Didymellaceae were the most prevalent in cortex and velamen, respectively. At the genus level, *Ectophoma* in cortex and unidentified *Capnodiales* sp. in velamen were the most abundant. The species *Ectophoma pomi* and *Capnodiales* sp. were the most abundant in cortex and velamen, respectively.

Three biodiversity indices were evaluated, all showed that velamen has more fungal species richness than the cortex (Table 1).

According to beta diversity, there is more diversity among the velamen samples than among the cortex samples. The PCoA analysis based on Weighted Unifrac metric, also indicates that the composition of cortex and velamen fungal communities are different (Figure 4).

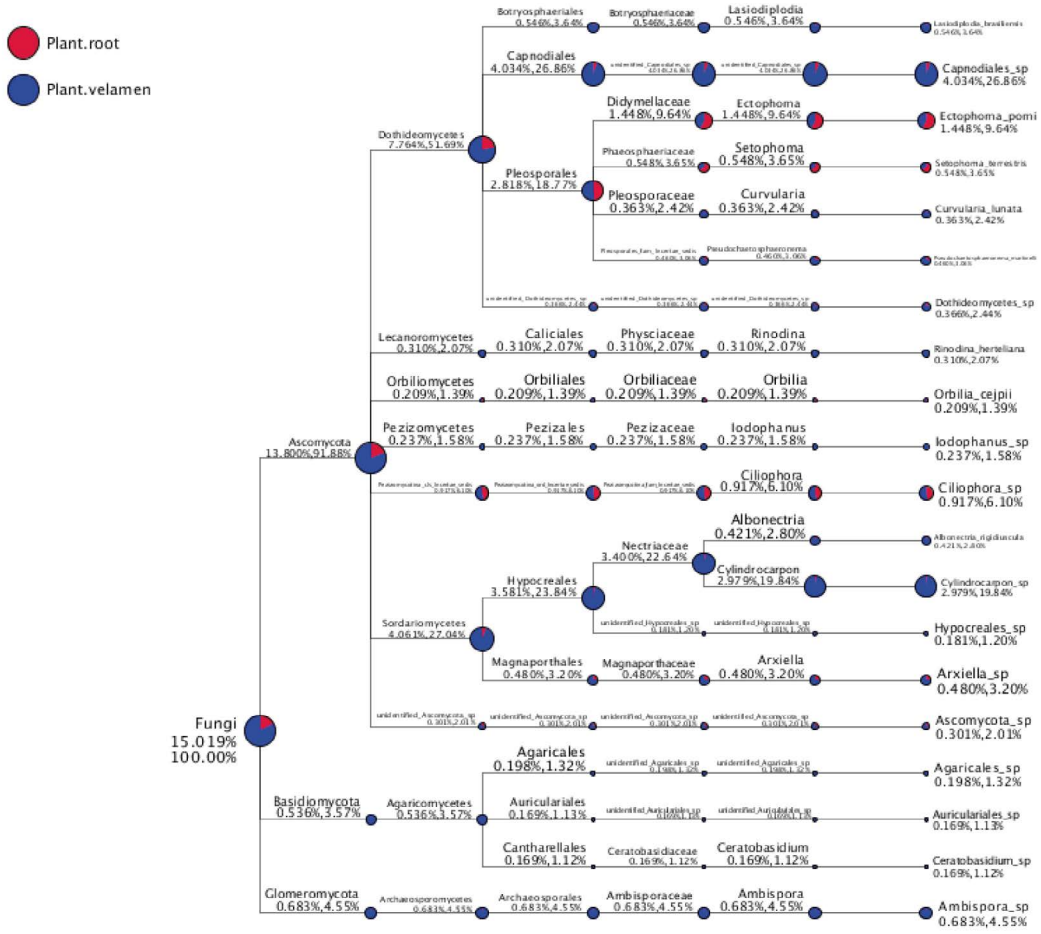

Figure 3. Phylogenetic tree showing total number of OTUs totales (cortex+velamen) and their relative percentage in each shown in color.

Table 1. Shannon, Simpson, Chao1 and ACE indices calculated based on OTUs detected in cortex and velamen samples.

Sample	Species observed	Shannon	Simpson	Chao1	ACE
Cortex	177	0.658	0.132	197.501	201.888
Velamen	424	2.952	0.581	452.078	458.034

The rarefaction curve (Figure 6) shows that sequencing depth was enough to sample the diversity harbored by cortex and velamen tissues. Indeed, the sampling effort is representative of the fungal diversity found in the *G. skinneri* roots in an urban environment.

The cortex slope shows that with 1,000 sequences around 100 species were observed, and with 6,000 sequences, 150 species were observed. The velamen slope shows that with 1,000 sequences, approximately 250 species were observed. Whereas with 6,000 sequences 420 species can be observed approximately. The abundance rank curve demonstrates that cortex samples (root) harbor less species diversity than the velamen samples, with a species rank of around 150 species in cortex and 420 species in velamen (Figure 7).

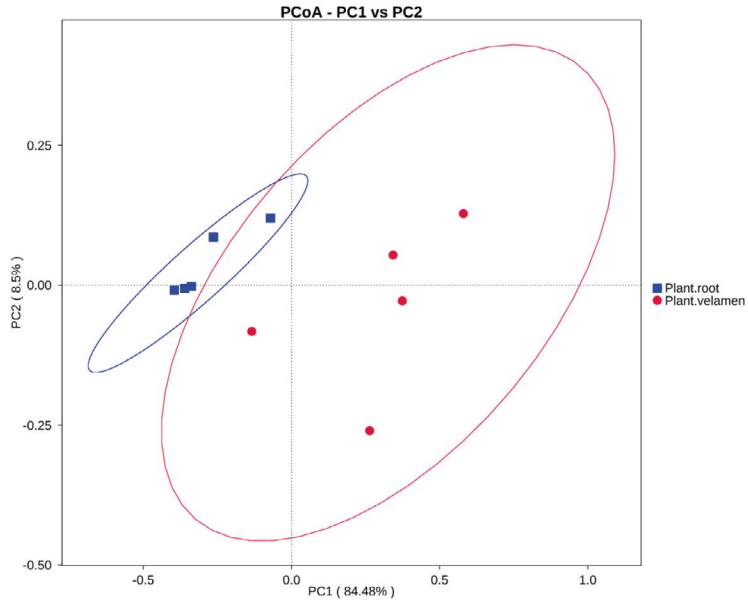

Figure 4. PCoA analysis based on Weighted Unifrac metric.

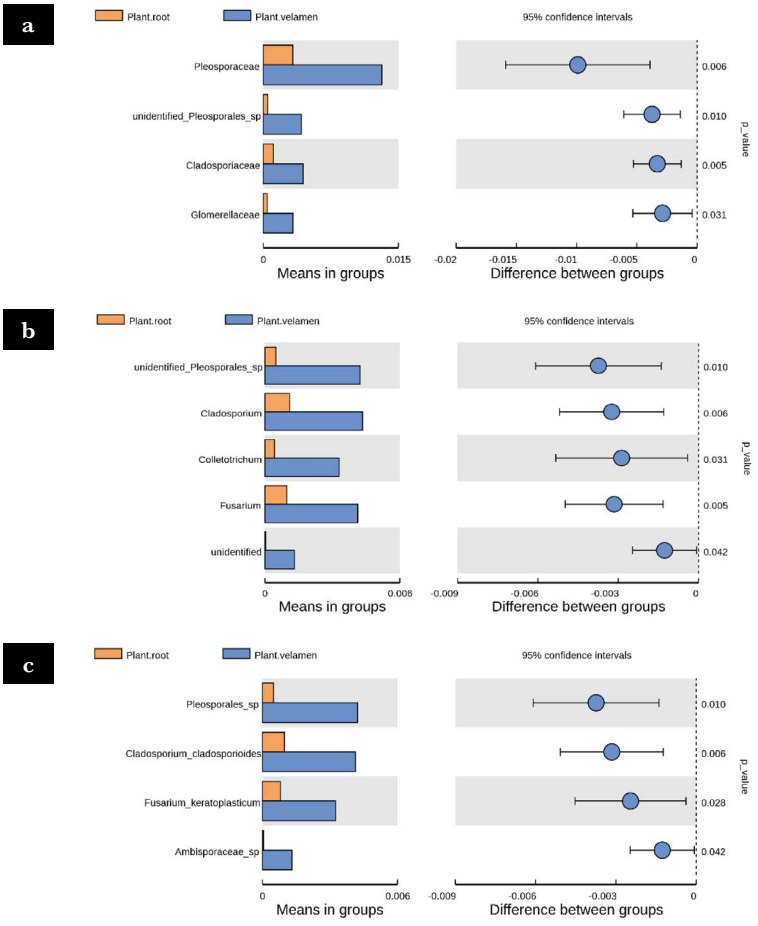

Figure 5. T test analysis comparing relative abundances of OTUs in root and velamen samples at a) family, b) genus and c) species level, showing taxa with significantly different values for each condition.

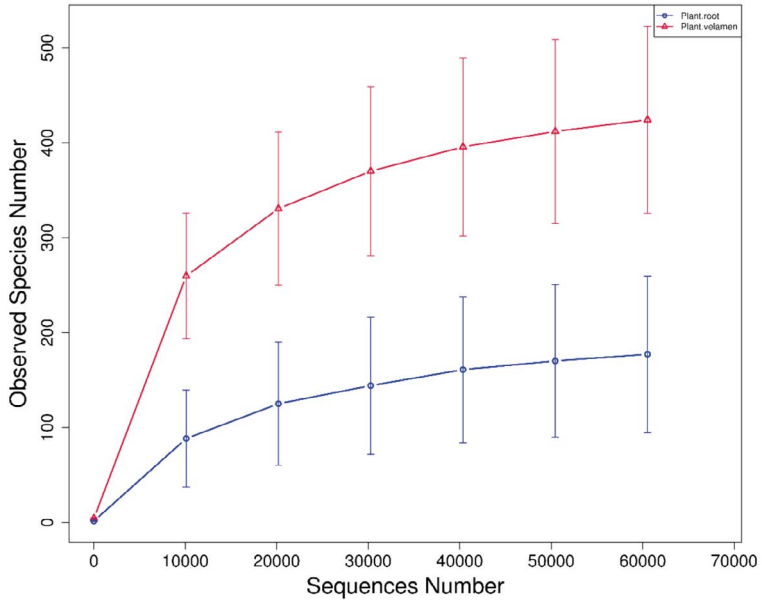

Figure 6. Rarefaction curve showing sequences number *vs.* observed species number for (a) velamen, (b) cortex.

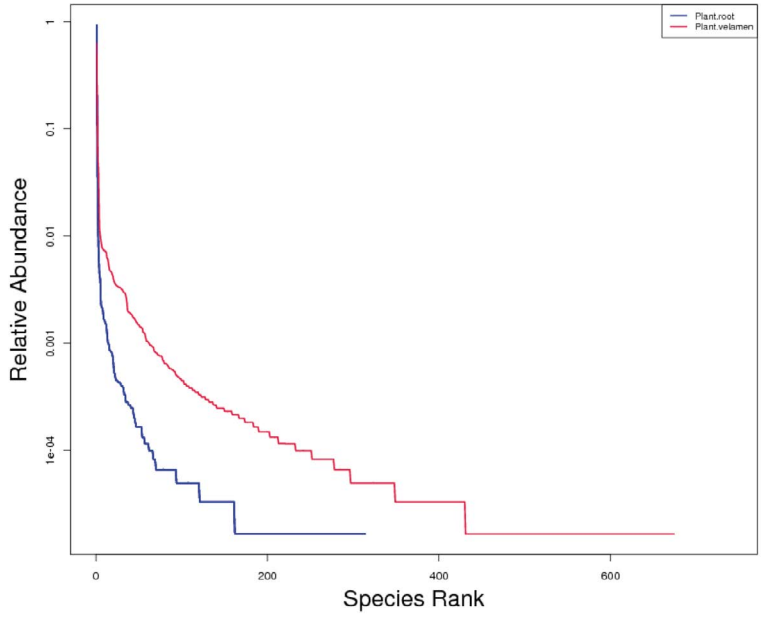

Figure 7. Rank curve of abundance for all (a) velamen and (b) cortex samples. Each curve represents each of the samples plotted as relative abundance on Y axis and abundance rank on X axis.

Velamen is the abiotic interphase between the root and the phorophyte bark (Herrera *et al.*, 2010). It is a sponge composed of dead cells that provide a microhabitat for microscopic life, serving as an abiotic filter for endophytes. It is followed by the exodermis that together with the cortex, select biotically the microorganisms that can enter to become endophytes. Thus, it is logical to think that the numerous taxa that can inhabit the abiotic velamen would reduce their frequency when colonizing

living tissue that applies defense and interaction mechanisms. Although this study is fungi-centered it is necessary to consider that there are root interactions with bacteria (Aguilar *et al.*, 2018), meaning thar there are complex interactions. There are statistically significant differences between the two sample groups, showing that velamen has more fungal diversity than the cortex. Interestingly, there are 40 fungi that were found in the cortex but not in velamen. This may be linked to mycorrhizal colonization of epiphytic orchids occurring at a certain time in the year: rain season, when environmental conditions lead to fungi proliferation and root growth, favoring the contact between root surface and the fungi that would enter the velamen and then the cortex (Bertolini *et al.*, 2014). These 40 OTUs likely belong to fungi that could access the cortex prior to sample collection and that disappeared from the velamen due to unfavorable environmental conditions but could survive within the cortex. Our study shows that orchids living in urban environments can still establish mutualistic relationships with orchid mycorrhizal fungi.

Interestingly, we detected a high number of non-mycorrhizal endophytic fungi in velamen and cortex. Beltrán-Nambo *et al.* (2018) reported 17 genera belonging to Ascomycota with four *Trichoderma* species and two *Fusarium* species among other in orchids from southeast Mexico. Although *Fusarium* is a pathogen, previous studies show that it can aid seed germination (Sisti *et al.*, 2019). Similarly, Xue *et al.* (2022) recorded *Thricoderma*, *Fusarium*, *Alternaria* among the non-mycorrhizal fungi in terrestrial orchids in China. There is no doubt that our results showing the presence of *Glomeromycota* sp., *Tricoderma* sp., *Beauveria* sp., *Fusarium* sp., *Alternaria* sp., *Glomus* sp., among others, are novel for scientific advancement and lead to future research to explain the root-microorganism relationships to better understand ecological processes occurring in these interactions and in turn provide efficient conservation protocols.

CONCLUSIONS

Our results are pioneers in providing evidence for statistically significant differences between the fungal composition of velamen and cortex of *G. skinneri*, confirming that velamen harbors a richer community than the cortex. At the same time, we identify beneficial fungi *Beauveria*, *Glomus* y *Trichoderma*, that deserve more specific studies to understand their role in root physiology, development and health of *G. skinneri*.

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SUPPLEMENTARY MATERIAL

Supplementary material can be found in https://www.dropbox.com/scl/fi/m21z8kkn96nqmxnbut98l/material_suplementrario_Ochoaetal2024.xlsx?rlkey=m4zkvi8yeepybwt1i54qk380e&dl=0

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